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Research Article

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Impact of electron beam and ethyl methane sulphonate on chlorophyll mutations in rice genotypes ASD 16 and Norungan

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ABSTRACT

Induced mutagenesis facilitates the creation of novel gene combinations within a plant genome, preserving its basic structure. This study investigates the impact of electron beam radiation and ethyl methane sulphonate (EMS) on chlorophyll mutations in rice genotypes, ASD 16 and Norungan. The seeds were irradiated with five different doses of electron beam and EMS during *rabi* 2021-22. The M_1 generation was assessed for seedling survival, seedling height and spikelet fertility followed by an identification of chlorophyll mutants in the M_2 generation. At specific mutagen doses, ASD 16 and Norungan exhibited the genotypic difference for chlorophyll mutants. Various chlorophyll mutations, such as *albino*, *chlorina*, *xantha*, *striata*, *viridis*, *albomaculata*, *alboviridis* and *xanthoviridis* were observed. ASD 16 was more sensitive to both mutagens, while Norungan showed a broader response. EMS proved to be a more effective in inducing mutations than the electron beam. Lower and moderate mutagen doses demonstrated higher efficiency indicating the importance of optimizing mutagenic conditions. This study illuminates the significance of chlorophyll mutants genetic makeup varietal differences. The strong and diverse response observed in Norungan underscores its suitability for mutation breeding programmes. These findings contribute to the efficient utilisation of mutagenesis in improving rice traits providing practical implications for elevating crop quality and promoting genetic diversity in rice cultivation.

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INTRODUCTION

The deliberate creation of beneficial mutations in rice (*Oryza sativa* L.) has generated significant interest in increasing its genetic diversity during the past five decades. The key to achieving this objective is to introduce both quantitative and qualitative variations into the crop. Physical and chemical mutagens have greater effectiveness in increasing mutation rates compared to natural or spontaneous mutations. Mutation serves as a weak source for altering allele frequencies but a strong source for introducing new alleles (Jadhav *et al.*, 2023). Utilising spontaneous mutations in crop breeding programmes is challenging because they occur rarely and randomly. Induced mutation techniques generate new variations in multiple traits, which can function as a new cultivar or serve as a donor in hybridisation (Oladosu *et al.*, 2015). A total of 873 rice mutants have been released as varieties from 30 different nations and India contributed 64 of these mutant varieties (FAO/IAEAMVD, 2022).

The impact of mutations can vary from alterations in single nucleotide sequences to significant changes *viz.*, deletions or rearrangements of chromosomes. Alterations in chlorophyll accurately reflect genetic changes in the plant ideotype caused by mutagens (Bordoloi *et al.*, 2023). Electron beam radiation can cause gene mutations through single strand breaks (SSB) and double strand breaks (DSB) with minimal effects on the plasma membrane and protein function; electron beam radiation causes 5.7 times more double strand breaks than gamma rays. Ethyl methane sulphonate (EMS) is a well known mutagenic organic compound that only induces point mutations through nucleotide substitution (Lalitha *et al.*, 2020).

The utility of mutagens depends not only on their potency but also on their efficacy, which is determined by their mutagenic effectiveness and efficiency. Mutagenic effectiveness and sensitivity measure the extent of mutations in response to its mutagen. The dosage assesses the sensitivity of a particular genotype to higher mutagen doses while the mutagenic efficiency estimates the frequency of resulting mutations from the damage caused by the mutagen (Raina *et al.*, 2022). This study examined the rate and range of chlorophyll mutations in M_2 generations of the ASD 16 rice cultivar and Norungan landrace. In addition, the mutagenic effectiveness and efficiency of electron beams and ethyl methyl sulphonate in inducing chlorophyll mutations were also assessed.

MATERIALS AND METHODS

The experiment was conducted at the Department of Plant Breeding and Genetics in Agricultural College and Research Institute, Madurai during *Rabi*, 2022-23. Two rice genotypes, ASD 16, an indica rice variety with high productivity and short bold grains and Norungan, an indica landrace with drought tolerance were chosen for the study. This study aimed to examine the impact of mutagens on these genotypes and evaluate their response to the mutagens. Well filled dried seeds of ASD 16 and Norungan (500 seeds per dosage) with 12 per cent moisture content were exposed to irradiation doses *viz.*, 100 Gy, 200 Gy, 300 Gy, 400 Gy and 500 Gy of electron beam in a 10 MeV electron beam accelerator facility at Bhabha Atomic Research Centre, Kharghar, Navi Mumbai, India. For EMS treatment, matured seeds of ASD 16 and Norungan were treated with varying concentrations *viz.*, 30 mM, 40 mM, 50 mM, 60 mM and 70 mM. The seeds were initially soaked in double distilled water for 12 hours to boost moisture absorption and trigger active cell division. Then 300 seeds were placed in the EMS solution and exposed to controlled conditions with rotary shaking for six hours to ensure even distribution of mutagen. Then, the seeds were thoroughly rinsed under running tap water to eliminate residual EMS and they were left to air dry for 30 minutes. Seeds that were presoaked in distilled water for 18 hours were used as control. Immediately after treatment, both treated and control seeds were sown in raised nursery beds in the field.

Seed germination was noticed on the seventh day, considering seeds with radicle emergence beyond 2 mm as germinated. The seedling injury was determined based on the seedling height on 14th day. The M_1 generation assessment included lab study to evaluate the influence of various concentrations on seeding height, lethality and spikelet fertility assessment was done as per SES and IRRI (2013).

Spikelet fertility percent =

$$\frac{Number\,of\;fertile\,spikelets}{Total\,number\,of\;spikelets\,observed}\times 100$$

All viable M_1 plants were harvested separately. Two hundred primary panicles from 200 plants were forwarded to the next generation for each dose of electron beam and EMS treatments. In the M_2 generation, a total of 58,400 seedlings resulting from all treatments were carefully monitored for the presence of chlorophyll mutants. Visual inspections for chlorophyll mutants were carried out on the 14th day after germination. Mutant characterisation was carried out using the classification of chlorophyll mutants given by Gustafsson (1940).

The *albino* and *xantha* mutants were lethal and did not survive (lethal), whereas *viridis*, *alboviridis* and *xanthoviridis* mutants were viable, indicating their ability to survive. The frequency of chlorophyll mutants was determined by calculating on M_2 seedling basis. Mutagenic effectiveness calculates the frequency of mutations caused by a specific mutagen dose, whereas efficiency provides information about the biological damage experienced. The formula proposed by Konzak *et al.* (1964) was adapted to compute mutagenic effectiveness and efficiency, considering the mutation frequency of chlorophyll mutants.

Mutation effectiveness =

$$\frac{Mutagenic\,frequency}{Dose(Gy)\,or\,(Concertation(c)\,x\,time(t))}$$

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

Biological damage produced by mutagens includes injury (reduction in height), lethality (percentage survival) at 30 DAS in the nursery and spikelet sterility during the maturity stage.

Mutation rate =

$$\frac{sum\,of\;values\,of\;effectiveness\,or\,efficiency\,of\;particular\,mutagen}{Numbers\,of\;treatments\;of\;a\,particular\,mutagen}$$

RESULT AND DISCUSSION

The use of physical and chemical mutagens has proven to be more effective in increasing mutation rates compared to natural or spontaneous mutation. Understanding the mutation and its practical value will help in realizing the genetic, physiological and biochemical basis of rice traits. The findings of the mutation research in rice will be highly applicable to the crops of *poaceae* due to its high quality gene sequences and synteny within the process (Viana *et al.*, 2019). Chlorophyll mutations can occur due to a reduction in chlorophyll biosynthesis activity, degradation of chlorophyll content, deficiency of carotenoids and alterations in chlorophyll development genes (Lalitha *et al.*, 2020). There are many different forms of alternations in chlorophyll, ranging from viable to non-viable, making them a dependable and informative tool for evaluating the genetic effects of mutagens (Awan *et al.*, 2021).

Frequency of Chlorophyll Mutants

The assessment of the chlorophyll spectrum in the M_2 generation is a reliable method for evaluating mutagen induced genetic alterations in the target plant genotype (Vinithashri *et al.*, 2020). The frequency and spectrum of chlorophyll mutations have typically served as valuable indicators for assessing the mutagenic effectiveness and efficiency of mutagens. These indicators reveal the dosage necessary to cause mutations in a specific genotype. In Table 2, ASD 16 exhibited the highest number of 144 chlorophyll mutants at 300 Gy electron beam and 152 mutants at 50 mM EMS while Norungan showed more chlorophyll mutants (157 at 300 Gy electron beam and 163 at 50 mM EMS); however, a greater proportion of mutations occurred in ethyl methane sulphonate (EMS) treatment. The mutagenic frequency ranged from 1.73 to 11.08 for electron beam and 1.74 to 3.94 for EMS treatment in ASD 16 whereas for Norungan, it ranged from 1.51 to 6.46 for electron beam and 1.28 to 10.19 for EMS. Considering the two genotypes, it was noted that 500 Gy of electron beam and 70 mM of EMS were known to induce a higher frequency of chlorophyll mutants in both ASD 16 and Norungan.

Chlorophyll variegation, as indicated in Table 1, may be evident throughout the entire plant or localized to specific leaf areas (Imam *et al.*, 2019). In ASD 16, electron beam induced chlorophyll mutants resulted in *albino*, *xantha*, *chlorina*, *striata* and *xanthoviridis* while EMS also generated *alboviridis* mutants in addition to those produced by the electron beam. In Norungan, electron beam treatment results in *albino*, *xantha*, *chlorina*, *striata*, *viridis* and *xanthoviridis* mutants while EMS yielded an additional set of chlorophyll mutant including *albomaculata*, *alboviridis* and *xanthoviridis* (Figure 1). The same mutagen exhibited diverse range of chlorophyll mutants due to varietal differences (Raina *et al.*, 2022). Chlorophyll biosynthesis genes located near the centromere and proximal segment undergo mutagenic effects resulting in varied chlorophyll mutations and observed varietal changes (Vinithashri *et al.*, 2020). These genetic regions regulate chlorophyll production and determine its characteristics and susceptibility to mutations. Chlorophyll mutants function as markers for genetic, physiological and biochemical studies. To gain a comprehensive understanding of mutation attributes, it is valuable to isolate and analyse chlorophyll mutants at the molecular level due to the distinct influence on chlorophyll production and characteristics resulting from genetic variations.

Table 1: Classification of chlorophyll mutants

Albino	White coloured seedlings without chlorophyll that died within a week
Xantha	Yellow to yellowish white seedlings which after a week
Chlorina	Uniform light green-coloured seedlings and lot of them die in 20 days. Few vigorous seedlings will survive to maturity
Striata	Longitudinal strips of different colours and they are viable
Viridis	Uniform light yellow green leaves started drying within 10-15 days of seedling
Alboviridis	Initially white and later become normal plants
Xanthoviridis	Initially yellowish green and later become normal plants
Albomaculata	White dots on green leaves

ASD 16 exhibited a higher prevalence of *albino* mutants under electron beam (48.05 per cent) and EMS (39.00 per cent), while in Norungan, 37.88 per cent albino mutants in EMS and 36.39 per cent in electron beam were observed. In the electron beam, the relative percentage of *albino* was higher at 500 Gy and 70 mM EMS for ASD 16 and 100 Gy and 40 mM for Norungan. In the M_2 generation, the *albino* mutants were the most common chlorophyll mutation observed in all doses of mutagens. Mutation studies on rice showed a higher occurrence of *albino* mutants using physical and chemical mutagens (Hasib *et al.*, 2022). The M_2 generation exhibited an increase in the frequency and quantity of *albino* mutations based on seedlings. The *albino* mutants are more commonly observed among the chlorophyll mutations in aromatic rice cultivars (Chakravarti *et al.*, 2017). Higher prevalence of *albino* mutants in the two genotypes for two mutagens suggests a consistent occurrence of *albino* mutants. The polygenic regulation of chlorophyll

Table 2: Frequency of chlorophyll mutation induced by Electron beam and Ethyl Methane Sulphonate in M_2 generation of ASD 16 and Norungan

Mutagenic treatment	ASD 16					Norungan				
	No. of M_1 plants raised	No. of M_1 plants forwarded	No. of M_2 seedlings studied	No. of chlorophyll mutants observed in M_2 seedlings	Mutation frequency	No. of M_1 plants raised	No. of M_1 plants forwarded	No. of M_2 seedlings studied	No. of chlorophyll mutants observed in M_2 seedlings	Mutation frequency
Control	50	0	1556	0	0	50	0	2251	0	0
Electron beam										
100 Gy	148	38	4572	79	1.73	173	73	5360	81	1.51
200 Gy	122	47	2789	116	4.16	165	69	4480	108	2.41
300 Gy	96	55	1688	144	8.53	152	51	3890	157	4.04
400 Gy	38	12	1292	92	7.12	109	32	2410	112	4.65
500 Gy	26	7	975	108	11.08	46	10	1501	97	6.46
Overall	430	159	11316	539	32.62	645	235	17641	555	19.07
Ethyl Methane Sulphonate										
30 mM	134	41	4780	83	1.74	127	75	7430	95	1.28
40 mM	117	35	4125	127	3.08	105	72	5890	121	2.05
50 mM	88	38	3963	152	3.84	81	64	3920	163	4.16
60 mM	23	11	2682	105	3.91	29	21	2180	154	7.06
70 mM	17	6	1876	74	3.94	14	8	1089	111	10.19
Overall	379	131	17426	541	16.51	356	240	20509	644	24.75

Figure 1: Chlorophyll mutants isolated in M_2 generation of ASD 16 and Norungan. a) Albino @ 50 mM EMS in ASD 16, b) Albino @ 50 mM EMS in ASD 16, c) Xantha @ 40 mM EMS in ASD 16, d) Striata @ 70 mM EMS in Norungan, e) Viridis @ 500 Gy Electron beam in Norungan and f) Albomasculata @ 70 mM EMS in Norungan

formation and the high mutability of related genes account for the presence or absence of mutants at various doses (Naveena *et al.*, 2020).

Spectrum of Chlorophyll Mutants

The spectrum of chlorophyll mutants in the M_2 generation induced by electron beam and EMS in ASD 16 and Norungan were presented in Tables 3 and 4. In ASD 16, the order of the chlorophyll mutant spectrum for the electron beam was *albino* (48.05) > *xantha* (25.97) > *chlorina* (21.89) > *striata* (2.96) > *xanthoviridis* (1.11). For EMS, the order is *albino* (39.00) > *chlorina* (24.76) > *xantha* (23.84) > *striata* (8.87) > *alboviridis* (2.58) > *xanthaviridis* (0.92). In Norungan, the spectrum for electron beam was *albino* (36.39) > *xantha* (25.94) > *chlorina* (27.74) > *striata* (5.58) > *viridis* (3.96) > *xanthaviridis* (0.36) and for EMS, it was *albino* (37.88) > *xantha* (24.53) > *chlorina* (15.21) > *striata* (9.93) > *viridis* (5.27) > *albomasculata* (1.24) > *alboviridis* (3.57) > *xanthaviridis* (2.32).

The occurrence or absence of mutants at different doses may be attributed to the polygenic regulation of chlorophyll formation, along with the high mutability of genes related to chlorophyll (9). Mutants of the *viridis* and *xanthoviridis* types were rarely induced and their frequency was also observed to be low, ranging from 1.73 to 11.08 for electron beam and 1.74 to 3.94 for EMS in ASD 16, while for Norungan, the ranges were 1.51 to 6.46 for electron beam and 1.28 to 10.19 for EMS. Considering the two genotypes, 500 Gy of electron beam and 70 mM of EMS are known to induce a higher frequency of chlorophyll mutants in both ASD 16 and Norungan.

In moderate doses, both rice genotypes displayed the highest number of chlorophyll mutants suggesting no clear correlation between mutagen dose and mutant occurrence (Gautam *et al.*, 2021). Chlorophyll deficiency primarily caused by genetic alterations in the genes responsible for the synthesis of photosynthetic pigments. In addition, chlorophyll deficient mutants lack the well defined grana structure in chloroplasts (Benedict & Ketring, 1972). Chimeric regions arise as a consequence of DNA modifications in chloroplasts (Li *et al.*, 2019).

Mutation Effectiveness and Efficiency

The highest mutagenic effectiveness was noted at 500 Gy for electron beam (0.055) and at 40 mM and 50 mM (0.077) for EMS in ASD 16 and in Norungan, the highest effectiveness was observed at 100 Gy (0.015) and 70 mM (0.146). Ethyl methane sulphonate surpasses the electron beam in terms of mutagenic effectiveness. In ASD 16, the efficiency was calculated based on spikelet sterility and it was highest for the electron beam at 300 Gy (0.394) and EMS at 30 mM (0.258), followed by seedling injury at 300 Gy (0.220) and 60 mM (0.440) and lethality at 500 Gy (0.337) and 40 mM (0.091). In Norungan, the efficiency was highest at 100 Gy for spikelet sterility (0.215) and 30 mM (0.378), seedling injury at 100 Gy (0.354) and 70 mM (0.221) and lethality at 100 Gy (0.136) and 70 mM (0.198) (Table 5 and 6). Similar to effectiveness, the efficiency was generally higher at moderate and lower mutagenic doses. Mutation rate was higher in ASD 16 for EMS (0.067) and in Norungan also it was higher for EMS (0.088) than electron beam in terms of effectiveness. In terms of efficiency, the mutation rate for Norungan was highest in EMS (0.290) compared to the electron beam (0.195) based on sterility criteria.

Recessive gene mutations can arise from deficiencies, duplications or point mutations caused by mutagens. The absence of chlorophyll mutants in the M_1 generation and their manifestation in the M_2 generation demonstrates the recessive nature of these mutations. The mutagenic effectiveness and efficiency provide valuable information on the usefulness of mutagens in crop enhancement. A linear relationship was observed between the dose of the mutagen and the percentage of lethality, sterility and seedling injury with higher concentrations leading to increased levels of these effects (Raina *et al.*, 2022). The utilization of chemical mutagens was more effective in inducing chlorophyll mutants in rice compared to physical mutagens, which implies that chemical mutagens are more adept at causing genetic changes related to chlorophyll in rice plants (Usharani & Kumar, 2015). Among the mutagens studied, EMS demonstrated the most effective followed by gamma rays

Table 3: Spectrum of chlorophyll mutants obtained in M_2 generation of ASD 16 by electron beam and EMS

Mutagen	No. of M_2 seedlings raised	No. of chlorophyll mutants observed in M_2 seedlings	Classes of chlorophyll mutants in M_2 seedlings					
			Albino	*Xantha*	*Chlorina*	*Striata*	*Alboviridis*	*Xanthaviridis*
Electron beam								
100 Gy	4572	79	38 (48.10)	22 (27.85)	19 (24.05)	0	0	0
200 Gy	2789	116	54 (46.55)	32 (27.59)	27 (23.28)	3 (2.59)	0	0
300 Gy	1688	144	71 (49.31)	39 (27.08)	26 (18.06)	6 (4.17)	0	2 (1.39)
400 Gy	1292	92	41 (44.57)	23 (25.00)	25 (27.17)	2 (2.17)	0	1 (1.09)
500 Gy	975	108	55 (50.93)	24 (22.22)	21 (19.44)	5 (4.63)	0	3 (2.78)
Total	11316	539	259 (48.05)	140 (25.97)	118 (21.89)	16 (2.96)	0	6 (1.11)
Ethyl methane sulphonate								
30 mM	4780	83	34 (40.96)	19 (22.89)	21 (25.30)	8 (9.64)	1 (1.20)	0
40 mM	4125	127	46 (36.22)	31 (24.41)	34 (26.77)	11 (8.66)	3 (2.36)	2 (1.57)
50 mM	3963	152	58 (38.16)	38 (25)	36 (23.66)	14 (9.21)	5 (3.29)	1 (0.66)
60 mM	2682	105	41 (39.05)	20 (19.05)	27 (25.71)	11 (10.48)	4 (3.81)	2 (1.90)
70 mM	1876	74	32 (43.24)	21 (28.38)	16 (21.62)	4 (5.41)	1 (1.35)	0
Total	17426	541	211 (39.00)	129 (23.84)	134 (24.76)	48 (8.87)	14 (2.58)	5 (0.92)

Figures in parenthesis indicates the percentage of chlorophyll mutants

Table 4: Spectrum of chlorophyll mutants obtained in M_2 generation of Norungan by electron beam and EMS

Mutagen	No. of M_2 seedlings raised	No. of chlorophyll mutants observed in M_2 seedlings	Classes of chlorophyll mutants in M_2 seedlings							
			Albino	*Xantha*	*Chlorina*	*Striata*	*Viridis*	*Albomaculata*	*Alboviridis*	*Xanthaviridis*
Electron beam										
100 Gy	5360	81	37 (45.67)	21 (25.92)	18 (22.22)	3 (3.70)	2 (2.46)	0	0	0
200 Gy	4480	108	45 (41.66)	33 (30.55)	23 (21.29)	4 (3.70)	3 (2.77)	0	0	0
300 Gy	3890	157	53 (33.75)	42 (26.76)	49 (31.21)	8 (5.09)	5 (3.18)	0	0	0
400 Gy	2410	112	35 (31.25)	21 (18.75)	40 (35.71)	9 (8.03)	7 (6.25)	0	0	0
500 Gy	1501	97	32 (32.98)	27 (27.83)	24 (24.74)	7 (7.21)	5 (5.15)	0	0	2 (2.06)
Total	17641	555	202 (36.39)	144 (25.94)	154 (27.74)	31 (5.58)	22 (3.96)	0	0	2 (0.36)
Ethyl methane sulphonate										
30 mM	7430	95	34 (35.78)	21 (22.10)	18 (18.94)	11 (11.57)	6 (6.31)	1 (1.05)	2 (2.10)	2 (2.10)
40 mM	5890	121	51 (42.14)	24 (19.83)	17 14.04)	15 (12.39)	7 (5.78)	1 (8.26)	3 (2.47)	3 (2.47)
50 mM	3920	163	62 (38.03)	48 (29.44)	21 (12.88)	16 (9.81)	7 (4.29)	1 (6.13)	5 (3.06)	3 (1.84)
60 mM	2180	154	59 (38.31)	39 (25.32)	24 (15.58)	15 (9.74)	6 (3.89)	2 (12.98)	6 (3.89)	3 (1.94)
70 mM	1089	111	38 (34.23)	26 (23.42)	18 (16.21)	7 (6.30)	8 (7.20)	3 (27.02)	7 (6.30)	4 (3.60)
Total	20509	644	37.88 (244)	24.53 (158)	15.21 (98)	9.93 (64)	5.27 (34)	1.24 (8)	3.57 (23)	2.32 (15)

Figures in parenthesis indicates the percentage of chlorophyll mutants

Table 5: Mutation effectiveness and efficiency based on chlorophyll mutants in M_2 generation of ASD 16

Mutagen	Per cent survival reduction (Lethality)	Per cent Seedling height reduction (Injury)	Per cent Spikelet Sterility (sterility)	Mutation frequency (Mp)	Mutagenic effectiveness	Efficiency		
						(Mp×100)/L	(Mp×100)/I	(Mp×100)/S
Electron beam								
100 Gy	31.87	9.31	10.84	1.73	0.017	0.054	0.186	0.160
200 Gy	54.68	31.54	16.9	4.16	0.021	0.076	0.132	0.246
300 Gy	74.64	38.8	21.63	8.53	0.043	0.114	0.220	0.394
400 Gy	81.71	45.43	27.10	7.12	0.036	0.087	0.157	0.263
500 Gy	87.21	54.13	33.21	11.08	0.055	0.127	0.205	0.334
Overall	330.11	179.21	109.68	32.62	0.172	0.459	0.899	1.396
Mutation rate				6.52	0.034	0.092	0.180	0.279
Ethyl methane sulphonate								
30 mM	21.21	15.89	6.74	1.74	0.058	0.082	0.074	0.174
40 mM	33.92	18.27	14.31	3.07	0.077	0.091	0.076	0.166
50 mM	63.43	14.07	17.85	3.84	0.077	0.061	0.106	0.243
60 mM	67.98	8.9	24.27	3.91	0.065	0.058	0.117	0.223
70 mM	80.84	9.92	33.57	3.94	0.056	0.049	0.170	0.260
Overall	267.38	67.05	96.74	16.501	0.333	0.339	0.542	1.066
Mutation rate				3.30	0.067	0.068	0.108	0.213

Mp - Mutation frequency, L - Lethality, I - Seedling injury, S - Spikelet sterility

Table 6: Mutation effectiveness and efficiency based on chlorophyll mutants in M_2 generation of Norungan

Mutagen	Per cent survival reduction (Lethality)	Per cent seedling height reduction (Injury)	Percent spikelet sterility (Sterility)	Mutation frequency (Mp)	Mutagenic effectiveness	Efficiency		
						(Mp×100)/L	(Mp×100)/I	(Mp×100)/S
Electron beam								
100 Gy	11.12	4.26	7.02	1.51	0.015	0.136	0.354	0.215
200 Gy	20.45	8.11	14.21	2.41	0.012	0.118	0.297	0.170
300 Gy	33.18	16.64	19.62	4.04	0.013	0.122	0.243	0.206
400 Gy	46.15	31.59	25.39	4.64	0.012	0.101	0.147	0.183
500 Gy	54.96	38.94	31.91	6.46	0.013	0.118	0.166	0.202
Overall	165.86	99.54	98.15	19.06	0.065	0.593	1.207	0.976
Mutation rate				3.81	0.013	0.119	0.241	0.195
Ethyl methane sulphonate								
30 mM	6.73	11.12	3.39	1.28	0.043	0.190	0.115	0.378
40 mM	15.61	18.65	10.84	2.05	0.051	0.132	0.110	0.190
50 mM	27.54	31.59	16.51	4.16	0.083	0.151	0.132	0.252
60 mM	39.00	41.55	22.87	7.06	0.118	0.181	0.170	0.309
70 mM	51.52	46.09	31.87	10.19	0.146	0.198	0.221	0.320
Overall	140.40	149.00	85.48	24.75	0.440	0.852	0.748	1.448
Mutation rate				4.95	0.088	0.170	0.150	0.290

Mp - Mutation frequency, L - Lethality, I - Seedling injury, S - Spikelet sterility

and nitrosoguanidine (Das *et al.*, 2021). This study revealed that electron beam and EMS showed increased effectiveness at moderate doses. However, a decline in mutagenic impact was observed at higher doses, indicating that the rise in the mutational rate did not correspond proportionally with the increase in mutagenic doses (Goyal *et al.*, 2019). It was observed that the proportion of chlorophyll mutants did not increase with higher mutagen doses; rather, a descending trend was noticeable at elevated doses. This result could be explained by the mortality of mutated plants or possibly by the diplontic selection (Raina *et al.*, 2022).

Mutagenic efficiency was generally found to be higher at a moderate dose in ASD 16 while lower in Norungan. This is due to biological damage tends to increase more rapidly with rising mutagens doses than the number of mutations. It was observed that the mutagenic efficiency was minimal at higher doses for both genotypes with the two mutagens. Higher efficiency was shown at lower and moderate concentrations compared to higher concentrations. Similar results were observed for *Cicer arietinum* L. by Wani (2018), *Vigna mungo* L. by Usharani and Kumar (2015), *Vigna unigulata* L.by Raina *et al.* (2022).

The mutation rate in terms of efficiency was highest in Norungan for EMS, which was estimated based on spikelet sterility criteria. Various factors, such as genetic makeup of the genotype, cell cycle stage, physiological state of the seed material and the nature of mutagen, all contributed for determining the effectiveness and efficiency of the mutagen. It is crucial to consider both the effectiveness and efficiency of a mutagen to achieve a high frequency of desirable mutants (Viana *et al.*, 2019). In this study, the chlorophyll mutant in Norungan was found to be more diverse than ASD 16 and Norungan surpassed ASD 16 in all criteria, *i.e.*, seedling lethality, seedling injury and spikelet sterility. This study indicated that Norungan showed a notable response to the electron beam and EMS than ASD 16.

CONCLUSION

The study investigated the impact of electron beam radiation and ethyl methane sulphonate (EMS) on chlorophyll mutations in two rice genotypes, ASD 16 and Norungan. Both mutagens caused varying frequencies of chlorophyll mutants and significant differences were observed between the two genotypes. The results revealed that the highest segregation of chlorophyll mutations occurred at 300 Gy electron beam and 50 mM EMS in both the cultivars. ASD 16 exhibited higher sensitivity to both mutagen with peak mutation frequencies at 500 Gy for the electron beam and 70 mM of EMS. Norungan exhibited a more diverse response, with higher mutation frequency and variability compared to ASD 16.

The chlorophyll mutants observed included *albino*, *xantha*, *chlorina*, *striata*, *viridis*, *albomaculata*, *alboviridis* and *xanthoviridis*, each displayed unique characteristics and survival rates. This study highlighted the varietal differences in occurrence of chlorophyll mutants induced by the same mutagen, underscoring the importance of understanding the genetic makeup of specific genotypes. The highest mutagenic effectiveness was noted at specific doses for each mutagen in both ASD 16 and Norungan. EMS proved to be more effective than the electron beam in terms of mutagenic effectiveness. Lower and moderate doses of mutagens demonstrated the higher mutagenic efficiency, emphasizing the importance of optimising mutagenic conditions for desired outcomes. This study highlighted the need to consider both the effectiveness and efficiency of mutagens to achieve a high frequency of desirable mutants in crop improvement programmes.

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