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Mutagenic Effectiveness and Efficiency of Chemical Mutagens in Barnyard millet (*Echinochloa frumentacea*)

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ABSTRACT: Mutagenic effectiveness and efficiency of individual treatments of Ethyl Methane Sulphonate (EMS) and Sodium Azide (SA) were assessed in Barnyard millet. The treatments included three doses each of EMS and SA calculated based on their Lethal Dose (LD_{50}). Different types of chlorophyll mutants namely *albino, xantha, chlorina, striata, alboviridis, xanthoviridis* and *albomaculata* were observed in the M_2 population. Based on plant survival and chlorophyll mutation frequency in M_2 generation, the effectiveness and efficiency were calculated for both the chemical mutagens. Mutagenic effectiveness and efficiency increased with the decrease in the concentration of mutagen. Hence, lower doses of chemical mutagens have proved to be more effective and beneficial in inducing mutations in barnyard millet.

Keywords: Barnyard millet, Chemical mutagens, Mutagenic effectiveness and efficiency, Mutation breeding, Ethyl Methane Sulphonate, Sodium Azide.

INTRODUCTION

Barnyard millet (*Echinochloa* sp.), an ancient domesticated crop is highly nutritious and widely cultivated in the Semi-Arid tropics of Asia and Africa. It has a species diversity of around 35, of which two are cultivated: *Echinochloa esculenta* (Japanese barnyard millet) and *Echinochloa frumentacea* (Indian barnyard millet) which are grown as minor cereals across Japan, Korea, north-eastern parts of China, India, Pakistan and Nepal (Yabuno, 1987). It is not only grown for human consumption as a Nutri-cereal, but also as an excellent fodder cereal crop (Vetriventhan, 2020). Barnyard millet straw is superior to rice, oat or Timothy straw in terms of protein and calcium content (Obara, 1936). Also, it has good agronomic features, high nutritional value and tolerance to various biotic and abiotic stresses (Kim *et al.,* 2011). Despite having several advantages, the cultivable area of barnyard millet in India is only 0.146 m ha with an average production of 0.151 m t. It is cultivated only across the southern and north-western districts of Tamil Nadu (Nirmalakumari and Vetriventhan, 2009). To widen their area of cultivation genetic improvement is a necessity. Species *Echinochloa* is highly autogamous with small florets and due to earlier and slight opening of flowers, pollen availability is less which makes emasculation and artificial hybridization difficult.

Spontaneous beneficial mutations occur seldom in any crop. Induced mutagenesis is the best alternative breeding method suggested. To generate variability, it is essential to select effective and efficient mutagens to recover a high frequency and range of desired mutations. Mutagens may be chemical or physical and among them, the common one is Ethyl Methane Sulfonate (EMS) and Sodium Azide (SA) which

induces random, point mutations across the genome. Among the chemical mutagens, EMS is the most strong and effective, and it is highly successful in generating mutations (Priyadharshni *et al.,* 2020). Mutagenic effectiveness is measured in terms of mutations induced per unit dose of mutagen, while efficiency is the ratio of the frequency of mutations induced concerning the biological damage (Vinithasri *et al.,* 2020). Both mutagenic effectiveness and efficiency are the deciding factors in generating desirable mutations by any mutagen (Konzak *et al.,* 1965, Nilan *et al.,*1965).

To determine the efficacy of the mutagens chlorophyll mutations are one of the main criteria that may be beneficial in understanding different physiological activities and biochemical reactions. Although mutagens induce alterations in the nucleotide sequence of DNA, each mutagen has a unique mode of action. Furthermore, a mutagen may efficiently cause mutations, but the associated undesired consequences like mortality or sterility may reduce its effectiveness.

The barnyard millet crop is still unexplored and limited varieties had been released so far. Among those, the variety MDU 1 released from Agricultural College and Research Institute, Madurai during 2017 maturing at 90-95 days is occupying a larger area in the southern zone of Tamil Nadu. To ensure proper cultivation under any cropping system, early maturing varieties have to be developed. In this regard without altering the existing yielding potential, early mutants of the variety MDU 1 have to be isolated. Considering these aspects, the present study was taken up to evaluate the effectiveness and efficiency of the chemical mutagen EMS and SA to recover a high frequency and spectrum of beneficial mutations in MDU 1 barnyard millet.

MATERIALS AND METHODS

Barnyard millet variety MDU 1 which is a popular high yielder widely cultivated across various districts of Tamil Nadu was subjected to mutagenic treatments with EMS and SA. Seed materials for the experiment were collected from the Department of Plant Breeding and Genetics, Agricultural College and Research Institute, TNAU, Madurai where the variety had been evolved. Moisture content (12%) and purity of the seeds had been ensured and 400 well-filled seeds for each treatment had been selected. The selected seeds were pre-soaked in distilled water for 12 hours at room temperature. The imbibed seeds were taken out after decanting the excess water and dried in shade for 6 hours. After shade drying, the seeds were soaked with a fresh solution of Ethyl Methane Sulphonate (pH 7.0) and Sodium Azide (pH 3.0) prepared in phosphate buffer at different concentrations (05, 10, 20, 30, 40, 50 and 60 mM). After ensuring uniform treatment of the seeds, the seed material was washed with running tap water to remove the excess chemical. Control seeds were soaked in buffer solution alone. The chemical treated seeds (M_0) along with control were sown immediately in a roll paper towel for germination and also in the field at the research plot of the Department of Plant Breeding and Genetics, Agricultural College and Research Institute, Madurai. Each M_1 plant was harvested individually and grown as a plant to progeny row to raise the M_2 population. Different kinds of chlorophyll mutants were scored from emergence till the age of four weeks in M_2 generation. Chlorophyll mutants were used to evaluate the mutagenic effect. During the first four weeks after germination, the M_2 generation was tested for lethal chlorophyll mutations, whereas viable chlorophyll and morphological viable mutations were scored throughout the crop. The mutant characterisation was carried out following categorization given by Gustafsson, (1940). The distinct chlorophyll mutants (*albino, xantha, chlorina, striata, alboviridis, xanthoviridis and albomaculata*) were observed in the M_2 generation of MDU 1 Barnyard millet were

Chlorophyll Mutants	Description
Albino	The entire seedling is devoid of chlorophyll pigment and has white leaves. It does not survive beyond 10-12 days after germination
Xantha	Yellow-coloured leaves with no chlorophyll pigment (survived for up to 10 to 12 days after sowing)
Chlorina	The leaves are light green, and the majority of them drop off within 20 days. Few vigorous seedlings will survive.
Striata	They have green and yellow-white longitudinally striped leaves that are viable.
Xantha-Viridis	Seedlings are distinguished by the presence of both viridine green and brilliant yellow colours on the same leaf.
Alboviridis	Initially white and later becomes normal plants
Albomaculata	White dots on green leaves

Using Konzak's (1965) formula, mutagenic effectiveness and efficiency were calculated using the parameters collected in M_1 and M_2 generation.

Mutagenic effectiveness and efficiency

Chemical mutagen

$$\text{Mutagenic effectiveness} = \frac{\text{Mutation frequency (Mf)}}{\text{Concentration of mutagen (\%)} \times \text{Time (Hours)}}$$

Where,

Mutation frequency (Mf) – Frequency of chlorophyll or viable mutations on M_1 and/or M_2 plant basis.

Mutagenic efficiency

$$\text{Mutagenic efficiency} = \frac{\text{Mutation frequency (Mf)}}{\text{Per cent Injury (I) or Per cent Lethality (L) or Per cent Sterility (S)}}$$

Where

Mf – Frequency of viable/cholorophyll mutants in M_1/ M_2 generation

I – Per cent of seedling injury in M_1 / M_2 generation

L – Per cent of lethality in M_1 / M_2 generation

S – Per cent of pollen sterility in M_1 / M_2 generation

Mutation rate

$$\text{Mutation rate} = \frac{\text{Sum of values of efficiency or effectiveness of particular mutagen}}{\text{No. of treatments of particular mutagen}}$$

RESULTS AND DISCUSSIONS

The biological damages and chlorophyll mutants obtained in the M_1 and M_2 generations can be used to assess mutagen's efficiency. The most efficient method for assessing mutagen-induced genetic alterations in target plant genotypes is to measure the chlorophyll spectrum in M_2 generation (Eswaramoorthy *et al.*, 2021). The effectiveness and efficiency of Ethyl Methane Sulphonate and Sodium Azide on MDU 1 barnyard millet were investigated in this study (Table 1). Hence the efficiency of a Sodium Azide was better than the EMS concerning lethality. It implies that the higher the amount of mutagen, the greater the effect on the genome of organisms. It increases the possibility of structural abnormalities and indels formation in the genome. However, the odds of acquiring a higher frequency of beneficial mutation are unaltered by the higher abnormalities caused in the genome. Even small point mutations induced by low dosages of mutagen have yielded amazing results in mutation breeding. As a result, it is vital to investigate the efficacy and efficiency of mutagens in the M_2 generation based on chlorophyll mutants.

Table 1: Chlorophyll mutation frequencies in M_2 generation.

Mutagens (Dose/Conc.)	No. of M_1 plants		No. of M_2 seedlings		Mutation frequency (%)	
	Plants forwarded	Segregating	Studied	Chlorophyll mutants	M_1 plant basis	M_2 seedling basis
Control	**60**	**0**	**619**	**0**	**0**	**0**
EMS						
10 mM	191	42	2381	98	21.98	4.11
20 mM	173	30	1798	71	17.34	3.94
30 mM	155	23	1236	53	14.83	4.28
EMS Mean					**18.05**	**4.11**
Sodium Azide						
10 mM	184	39	2148	83	21.19	3.86
20 mM	167	26	1567	64	15.56	4.08
30 mM	143	21	1134	46	14.68	4.05
Sodium Azide Mean					**17.14**	**3.99**

The maximum reduction in plant height on the 30th day was noticed (47.09 cm in EMS and 45.45 cm in SA) for both the chemical mutagen. Plant height retardation may be attributed to the inhibitory action of enzymes during the early growth phase, the formation of growth retardants, changes in enzyme specificity, a delay in the commencement of first meiosis (Natarajan, 1958), inhibition of cell division, and a reduction in IAA level (Miura *et al.*, 1974). Survival rate on the 30th day was observed in both the chemical mutagens and compared with the control. In treatment (50 mM) of both the chemical mutagen exhibited a greater reduction in survival rate. Inhibition of mitosis chromosomal aberrations and inhibition of auxin synthesis (Skoog, 1935) results in the reduction of survival percentage over control. By increasing the dose of mutagens, the seed fertility of MDU 1 Barnyard millet gets affected. It is caused mainly due to point mutation, physiological disturbance, chromosomal aberrations and also upset in the genetic equilibrium (Ramya *et al.*, 2014). The reduction in seed fertility was ranged from 87.4 (10 mM) to 69.2 (50 mM) for EMS and Sodium Azide ranged from 86.8 (10 mM) to 67.6 (50 mM) respectively.

The chlorophyll mutants were ineffective in the crop improvement/breeding programme, but they were a significant measure of mutagenic efficacy and efficiency in M_2 generation. Chlorophyll deficit types can emerge as a result of cytoplasmic gene modification and the majority of them are deleterious throughout crop growth (Prasanth, 2019). The majority of the chlorophyll mutants perished at an early stage, with only a handful exhibiting impaired growth and development. Polygenes at many loci regulated the occurrence of chlorophyll mutants (Bind *et al.*, 2016). Only a few chlorophyll mutants can outgrow and

develop normally. Several studies found a dose-dependent increase in chlorophyll mutations (Barshile, 2006), whereas others found a dose-dependent decrease (Wani *et al.*, 2011).

The spectrum of chlorophyll mutants was diverse in MDU 1 Barnyard millet, consisting of *albino, xantha, chlorina, striata, alboviridis, xanthoviridis and albomaculata* were obtained and depicted in (Fig. 1). *Albino* followed by *Xantha* were the most predominant chlorophyll mutants and *Albomaculata* is the less predominant mutant in the present study. *Albino* was characterized by white leaves of seedling and it survived for 10 - 12 days after germination. The order of spectrum of chlorophyll mutants in MDU 1 Barnyard millet is *Albina* (80.81) > *Xantha* (66.91) > *Chlorina* (57.06) > *Striata* (40.00) > *Xanthoviridis* (26.5) > *Alboviridis* (25.55) > *Albomaculata* (9.08) for EMS and for Sodium Azide *Albino* (83.22) > *Xantha* (66.84) > *Chlorina* (63.61) > *Striata* (31.66) > *Alboviridis* (26.38) > *Xanthoviridis* (15.64) > *Albomaculata* (12.87) respectively (Table 2 and Fig. 2).

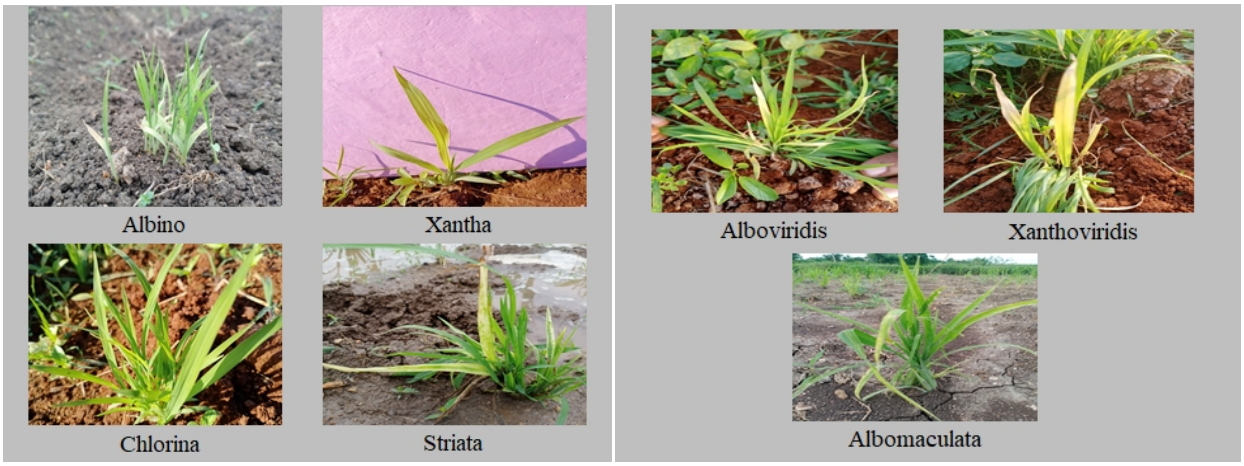

Fig. 1. Chlorophyll mutants obtained in M_2 generation of Barnyard millet.

Table 2: Frequency of different types of chlorophyll mutants in M_2 generation.

Mutagens (Dose/Conc.)	Total chlorophyll mutants	Relative percentage of chlorophyll mutants (%)						
		Albino	Xantha	Chlorina	Striata	Alboviridis	Xanthoviridis	Albomaculata
Control	0	0	0	0	0	0	0	0
EMS								
10 mM	98	28.57	19.38	15.30	12.24	8.16	14.28	2.04
20 mM	71	23.94	21.12	16.90	12.67	9.85	8.45	7.04
30 mM	53	28.30	26.41	18.86	15.09	7.54	3.77	-
EMS Mean	**74**	**26.93**	**22.30**	**17.02**	**13.33**	**8.51**	**8.83**	**4.54**
Sodium Azide								
10 mM	83	26.50	21.68	15.66	12.04	10.84	7.22	6.02
20 mM	64	28.12	23.43	21.87	10.93	4.68	6.25	4.68
30 mM	46	28.26	21.73	26.08	8.69	10.86	2.17	2.17
Sodium Azide Mean	**64.33**	**27.62**	**22.28**	**21.20**	**10.55**	**8.79**	**5.21**	**4.29**

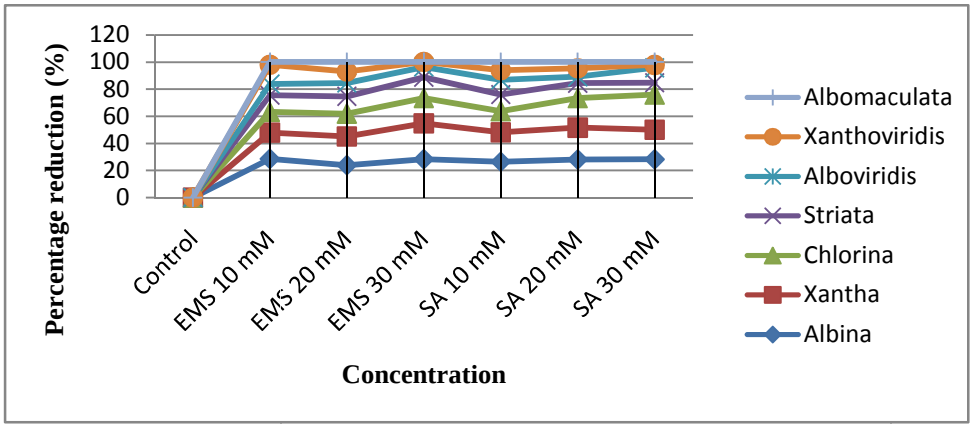

Fig. 2. Frequency and spectrum of chlorophyll mutants in M_2 generation of MDU 1 Barnyard millet.

Based on the several types of chlorophyll mutants acquired from a single plant progeny of the M_1 generation, it was categorized into one type, two types, and three types of mutations (i.e., single and multiple mutations). The occurrence of multiple mutational events may result in the change of several genes, resulting in the alteration of various enzymes and proteins, culminating in the pleiotropic effect. The highest number of two type and three type mutants were observed in 10 mM of Ethyl Methane Sulphonate (Table 3).

Table 3: Estimates of M_1 plants segregating for single and multiple chlorophyll mutations in M_2 generation.

Mutagens (Dose/Conc.)	Number of M_2 plant progenies Studied	Number of M_2 plant progenies segregating	M_1 plant progenies segregating for mutation					
			Frequency			Percentage		
			One Type	Two Type	Three Type	One Type	Two Type	Three Type
EMS								
10 mM	2381	98	72	19	7	73.46	19.38	7.14
20 mM	1798	71	58	11	2	81.69	15.49	2.81
30 mM	1236	53	39	9	5	73.58	16.98	9.43
Sodium Azide								
10 mM	2148	83	69	10	4	83.13	12.04	4.81
20 mM	1567	64	54	7	3	84.37	10.93	4.68
30 mM	1134	46	35	8	3	76.08	17.39	6.52

Mutagenic effectiveness and efficiency based on seedling injury, lethality and sterility were calculated on M_1 and M_2 generation. Ethyl Methane Sulphonate was found to be more effective than Sodium Azide. In M_1 generation, the maximum mutagenic effectiveness was recorded at 10 mM of EMS (54.95) and the minimum was recorded at 30 mM of Sodium Azide (12.23). The maximum mutagenic effectiveness was recorded at 10 mM of EMS (10.27) and the minimum was recorded at 30 mM of Sodium Azide (3.37) in M_2 generation. A similar pattern of findings was recorded by Yadava *et al.* (2003) in Kodo millet.

In M_1 generation, based on lethality (L) the highest efficiency was recorded in 10 mM of EMS (112.77) and the lowest mutagenic efficiency was recorded in 30 mM of SA (23.98). Based on injury (I), the maximum efficiency was observed at 10 mM of EMS (232.10) and the minimum mutagenic efficiency was observed at 30 mM of SA (54.16) (Table 4 and Fig. 3). Based on sterility (S), the mutagenic efficiency was maximum in 10 mM of EMS (664.04) and minimum in 30 mM of SA (80.61). In M_2 generation, based on lethality, the highest efficiency was observed in 10 mM of EMS (52.69) and the lowest mutagenic efficiency was observed in 30 mM of SA (5.50). Based on injury, the maximum efficiency was recorded at 10 mM of EMS (108.44) and the minimum mutagenic efficiency was recorded at 30 mM of SA (12.43) (Table 5 and Fig. 4). Based on sterility, the mutagenic efficiency was maximum in 10 mM of EMS (310.27) and minimum in 30 mM of SA (18.50).

The frequency of chlorophyll mutants was found to be dose-dependent in this experiment. The effectiveness was highest in EMS (10 mM) followed by SA (10 mM), while efficiency based on chlorophyll mutants was high in EMS than SA. From an overall perspective, the results revealed that the MDU 1 Barnyard millet responded well to both the chemical mutagens. The chemical mutagen Ethyl Methane Sulphonate is the effective and efficient one for inducing chlorophyll mutants in the variety MDU 1 Barnyard millet. It showed the sign of generating more variability in the segregating population for various quantitative characters.

Table 4: Mutagenic Effectiveness and Efficiency (Chlorophyll mutants) on M_1 plant basis.

Mutagens (Dose/ Conc.)	Percent survival reduction on 30th day (Lethality)	Percent height reduction on 30th day (Injury)	Seed fertility reduction (Sterility)	Mutants 100 M_1 Plants (Mp)	Effectiveness (%)	Efficiency (%)		
						Lethality	Injury	Sterility
EMS								
10 mM	19.49	9.47	3.31	21.98	54.95	112.77	232.10	664.04
20 mM	46.21	16.29	9.95	17.34	21.67	37.52	106.44	174.27
30 mM	57.40	21.97	13.93	14.83	12.35	25.83	67.50	106.46
Sodium Azide								
10 mM	22.76	15.47	6.34	21.19	52.97	93.10	136.97	334.22
20 mM	48.48	19.63	10.87	15.56	19.45	32.09	79.26	143.14
30 mM	61.20	27.10	18.21	14.68	12.23	23.98	54.16	80.61

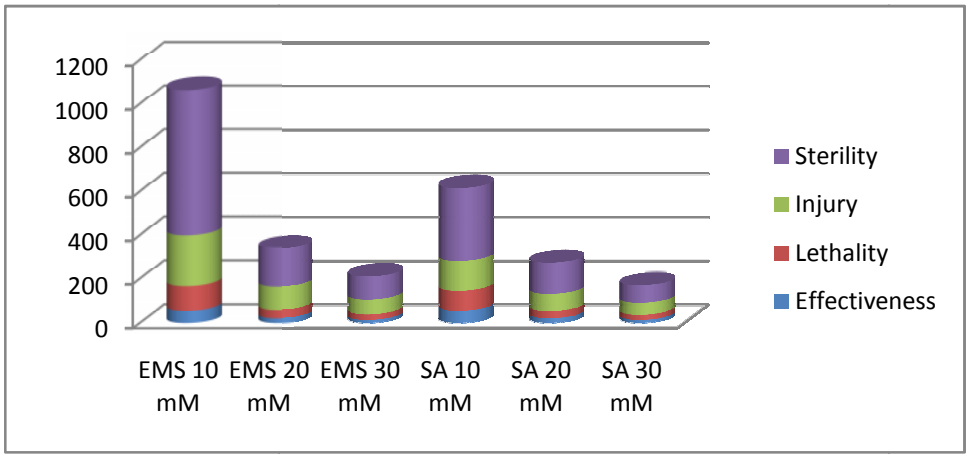

Fig. 3. Mutagenic Effectiveness and Efficiency (Chlorophyll mutants) on M_1 plant basis.

Table 5: Mutagenic Effectiveness and Efficiency (Chlorophyll mutants) on M_2 plant basis.

Mutagens (Dose/Conc.)	Percent survival reduction on 30th day (Lethality)	Percent height reduction on 30th day (Injury)	Seed fertility reduction (Sterility)	Mutants 100 M_2 Plants (Mp)	Effectiveness (%)	Efficiency (%)		
						Lethality	Injury	Sterility
EMS								
10 mM	19.49	9.47	3.31	4.11	10.27	52.69	108.44	310.27
20 mM	46.21	16.29	9.95	3.94	4.92	10.64	30.20	49.44
30 mM	57.40	21.97	13.93	4.28	3.56	6.20	16.20	25.55
Sodium Azide								
10 mM	22.76	15.47	6.34	3.86	9.65	42.39	62.37	152.20
20 mM	48.48	19.63	10.87	4.08	5.1	10.51	25.98	46.91
30 mM	61.20	27.10	18.21	4.05	3.37	5.50	12.43	18.50

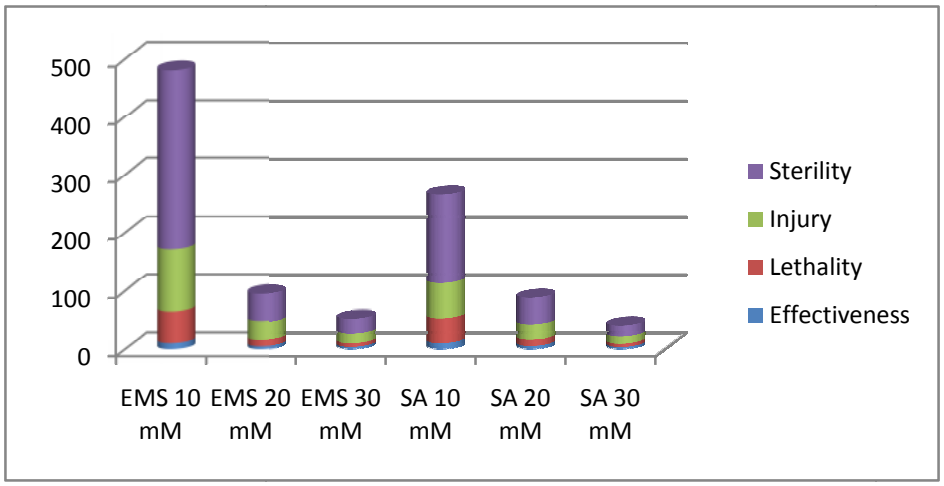

Fig. 4. Mutagenic Effectiveness and Efficiency (Chlorophyll mutants) on M_2 plant basis.

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Conflict of Interest. None.

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