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Assessment of the Effectiveness and Efficiency of Ethyl Methane Sulphonate, Sodium Azide and Hydroxylamine Hydrochloride in the Induction of Chlorophyll Mutations in Cowpea (*Vigna unguiculata* L. Walp)

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ABSTRACT

Ethyl Methane Sulphonate, Sodium Azide and Hydroxylamine Hydrochloride were assessed through chlorophyll mutation analysis in the second mutant generation for spectrum, frequency and their corresponding potential in successful inducement of useful mutations in cowpea. The results showed that EMS produced the highest number of mutations followed by SA and HH in that order. The mutagen efficiency was strongest in Ethyl Methane Sulphonate at dose 0.1% for all the genotypes treated as well as for all the genotypes treated with Sodium Azide and Hydroxylamine Hydrochloride. Sodium Azide on the other hand produced the best mutagen effectiveness at dose 0.01%. The average mutagen effectiveness was higher in Sodium Azide treated cowpea than Ethyl Methane Sulphonate and Hydroxylamine Hydrochloride explants. Ethyl Methane Sulphonate had better average mutagen efficiency than Sodium Azide and Hydroxylamine Hydrochloride. It is therefore concluded that considering the mutagens and doses deployed in this study, Sodium Azide is more effective which indicates that it might produce more mutations than Ethyl Methane Sulphonate and Hydroxylamine Hydrochloride. However, Ethyl Methane Sulphonate is more efficient as its stronger efficiency indicates less deleterious effects in addition to its production of much more chlorophyll mutations. This makes it a better mutagen than Sodium Azide and Hydroxylamine Hydrochloride for chemical mutagenesis in cowpea.

Keywords: Chlorophyll, Effectiveness, Efficiency, Mutations, Cowpea, Ethyl Methane Sulphonate, Sodium Azide, Hydroxylamine Hydrochloride.

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INTRODUCTION

Cowpea [*Vigna unguiculata (L)* Walp] is one of the most important food and forage legumes in the semi-arid tropics and a very valuable and dependable commodity crop for farmers and grain traders (Singh, 2005; Timko and Roberts, 2007). The crop is well adapted to the drier regions of the tropics, where other food legumes do not perform well. It is grown mostly by poor farmers in developing countries, with over 75% of production occurring in the dry savannas of tropical West Africa (Singh, 2005). As a leguminous crop, cowpea improves soil fertility through its ability to fix atmospheric nitrogen through its root nodules (Becker *et al.*, 2023) (Rajput *et al.*, 2024). The crop plays a considerable role in the nutritional balance and economy of the rural population in West Africa sub-region (Krasova-Wade *et al*., 2006). West- Africa is the epicentre of cowpea production which accounts for about 60% of the world production (Nkomo *et al.*, 2021) with Nigeria alone producing about a third of the world's output with approximately 2.14 million metric tonnes (Horn and Shimelis, 2020). Chlorophyll mutations manifest as variations in leaf color, such as albino, chlorina, or xantha phenotypes which can be detected at the seedling stage, making them convenient for early screening in mutation breeding programs. Albino and other severely chlorophyll-deficient mutants may not survive beyond the seedling stage, limiting their use in advanced breeding. While chlorophyll mutations themselves may not directly contribute to agronomic traits, their presence signals the likelihood of other concurrent mutations in the genome as the relevance of chlorophyll mutations lies in their correlation with useful traits (Mishra & Chaudhary, 2011). These accompanying mutations might affect traits such as yield, disease resistance, drought tolerance or nutrient content and identified chlorophyll mutants may become the focus of further screening for desirable agronomic traits, such as improved pod yield, pest resistance, or enhanced nutritional profiles. Higher chlorophyll levels are usually associated with higher yield (Ghimire *et al.,* 2015). Chlorophyll-deficient mutants are useful in studying genes related to photosynthesis and pigment biosynthesis because they lead delayed reproductive maturity and variable plant height, suggesting a complex relationship between chlorophyll and growth traits (Khangura *et al.,* 2022) and stress responses, providing insights into the plant's biology. It is however important to note that the response to mutagens can vary across cowpea genotypes, influencing the frequency and type of chlorophyll mutation observed. Chlorophyll mutations provide an accessible and effective tool for monitoring genetic variability induced by mutagenesis in cowpea (Raina *et al.,* 2022). While these mutations are not agronomically significant on their own, they serve as reliable indicators of the mutagenic process and help identify potentially beneficial genetic changes. Coupled with advanced screening and selection strategies, they play a pivotal role in cowpea improvement programs.

Ethyl Methane Sulphonate, Sodium Azide and Hydroxylamine Hydrochloride are potent mutagens which are commonly used to induce genetic mutations in cowpea which and all have the potential to produce chlorophyll mutations. There is however insufficient information on the assessment of the three mutagens and their capacity to induce these chlorophyll related gene alterations which have been positively correlated to the production of useful mutations. This study therefore employs the assessment of the cowpea chlorophyll apparatus after induced genetic changes to ascertain the suitability of each mutagen for the generation of useful mutations in cowpea.

MATERIALS AND METHODS

Experimental location

A field experiment was carried out between August and November of the year 2022 at the Teaching and Research Farm of the Federal University of Agriculture, Makurdi located at latitude 7.41^0 N and longitude 8.39^0 E. and at the elevation of 97m above sea level with an average of 1150 mm of rain fall.

Source of experimental materials

The genotypes used in the study are FUAMPEA 1, IFE BROWN and SAMPEA 14. FUAMPEA 1 was obtained from the University of Agriculture Makurdi seed centre. IFE BROWN and SAMPEA 14 were obtained from the Institute for Agricultural Research (IAR), Ahmadu Bello University, Zaria. FUAMPEA 1 is resistant to striga, electra and it is high yielding (1.9tons/ha), IFE BROWN is brown seeded and has a short cooking time while SAMPEA 14 is also resistant to striga, electra and it is high yielding (2.6 tons/ha). The genotypes were chosen for their differences in yield potential. None of these genotypes were photo-sensitive.

Experimental grade Ethyl Methane Sulphonate (EMS), Sodium Azide (SA), Hydroxylamine Hydrochloride (HH), Phosphate buffer, and distilled water were obtained from a reputable laboratory chemical distributor.

Experimental design treatment designations and field dimensions

The experiment was a 3x3x4 Factorial (genotype, mutagen, dose) with 36 treatment combinations, replicated three times and laid out in a Randomized complete Block Design (RCBD). The experimental field measured 71m x 14m. The distance between the three blocks was 3m for each separation. Each plot measured 3m x 1m with inter plot spacing of 1m, had two rows of cowpea separated by 0.75m while intra row spacing was 0.20m.

Induction of mutagenesis in cowpea seeds

Viable seeds were presoaked in distilled water which is up to 10 times their volume for 3 hours. HH solutions were prepared with concentrations of 0.01 %, 0.05 % and 0.1 % using distilled water. The seeds were then removed from the distilled water by draining the water from the seeds with the aid of a sieve. The seeds were air dried under the shade for three hours till there was no extra moisture on the surface of the seeds.

Seeds were soaked in the mutagen solutions according to their treatment combinations which was up to 10 times the volume of the seeds for three 3 hours. 10 ml of a 0.1 M phosphate buffer were added to stabilize the pH and keep it as close to 7 as possible. The solutions were stirred at fifteen minutes intervals to maintain homogeneity. When the mutation time is complete, the treatment was stopped by draining the mutagenic solutions from the seeds using a sieve. The seeds were washed separately under running water for one hour to remove excess mutagens from the seeds after which the seeds were immediately taken to the field for planting. Mutagen effectiveness and efficiency were estimated by the following formulae;

$$\text{Mutagen effectiveness} = \frac{MF}{C \times T}$$

Where MF=Mutation Frequency, C= Dose of Mutagen, T=Time

$$\text{Mutagen efficiency} = \frac{MF}{M}$$

Where MF=Mutation Frequency, M= Mortality

Cultural practices

The experimental site was carefully chosen for physical assessment of soil type relatively flat elevation and absence of stubborn weeds. The area was properly cleared of weeds and the seed beds were made.

At the second week after planting, Nitrogen Phosphorus Potassium (NPK) fertilizer to the ratio of 15:15:15 was applied to the mutant seedlings at the rate of 100kg/ha (Omoigui *et al.,* 2020) and earthing up was done at week four. The field was weeded manually at two weeks after planting with a hand hoe. The process was repeated at week 5-6 after planting and subsequently as the need arose (Omoigui *et al.,* 2020). Lamda-cyhalothrin was applied at the rate of 0.8 L/ha and was used to eliminate insect pests, pod sucking bugs and caterpillars (Omoigui *et al.,* 2020). Imidacloprid was sprayed on the plants with the rate of 0.6 L/ha in the case of aphid infestation (Omoigui *et al.,* 2020).

Its effect was preventive as well. At harvest, the pods were harvested according to their treatments and taken to the lab. These applications and processes were repeated for the second mutant generation (M2) where the data for the amount and spectrum of chlorophyll mutations were observed and recorded.

RESULTS

Chlorophyll mutation spectrum and frequency in chemically induced m2 cowpea mutants

Table 1 shows chlorophyll mutation spectrum and frequency in chemically induced M2 cowpea mutants. The result showed that, the M2 produced a total of 66 chlorophyll mutations which included Maculata, Chlorina and Semi-Albina with 27, 18 and 10 mutations respectively. Maculata showed yellow or whitish dots on the leaves. Chlorina had abnormally light green colour of leaves and Semi albina had white leaves with faint green colouration. Viridis had reduced leaflet size which were viridine green (light-yellow green) while Xantha plants were bright yellow. Table 2 indicates that Ethyl Methane Sulphonate treated FUAMPEA 1 (G1) at 0.1% produced the highest chlorophyll mutation frequency (8) followed by Ethyl methane sulphonate FUAMPEA 1 (G1) at 0.05% (7). Mutagen effectiveness in the Ethyl Methane Sulphonate treatments performance varied among the genotypes. EMS treated FUAMPEA 1 (G1) had its best mutagen effectiveness performance at dose 0.05%, IFE BROWN (G2) at 0.1% and SAMPEA 14 (G3) at 0.01%. Sodium Azide treatments showed a decrease in effectiveness with an increase in dose in FUAMPEA 1 and IFE BROWN. Hydroxylamine Hydrochloride treatments also performed in decreasing fashion in relation to an increase in dose except in SAMPEA 14 where mutagen effectiveness peaked at dose 0.05% and fell with a further increase in dose.

The Mutagen Efficiency was mostly strongest at Ethyl Methane Sulphonate Dose 0.1% for all the genotypes treated while for Sodium Azide and Hydroxylamine Hydrochloride treatments, the strongest Mutagen Efficiency was either at the lowest dose (0.01%) or at the highest dose (0.1%) except in the Hydroxylamine Hydrochloride treated SAMPEA 14 (G3) where the dose with the strongest efficiency was at 0.05%.

Sodium Azide produced the highest mutagen effectiveness at dose 0.01%. The average Sodium Azide effectiveness was 48.99, Ethyl Methane Sulphonate was 36.66 and Hydroxylamine Hydrochloride was 19.25 (Tables 3 and 4).

DISCUSSION

Chlorophyll mutation spectrum, frequency, mutagen effectiveness and efficiency

Since the works of Muller (1927) a significant amount of genetic variability has been induced using different mutagens. This includes chlorophyll mutations in the M2 being one of the most reliable criteria for the evaluation of the quality of genetic alteration which can result from mutagenic treatments (Reena *et al.,* 2014) (Rakhi *et al.,* 2019). The capacity of the mutagens involved to produce these results in non-targeted mutation experiments are usually indicated by changes and patterns in the leaf colour (Devmani *et al.,* 2016) that are easy to identify. The M2 is much desirable for this effect because in the M1, only dominant mutations show up which are usually few in number. In the M2, segregation occurs leading to the formation and expression of homozygotes for both recessive and dominant alleles thus creating a chance for the identification of many more genotypes. For this to happen, genes which control the functions and expression of the chlorophyll apparatus must be distributed along the chromosome, located on several chromosomes (Rakhi *et al.,* 2019) as well as also on plastid chromosomes.

Chlorophyll mutations are for the most part lethal and do

Table 1: Chlorophyll Mutation Spectrum and Frequency in Chemically Induced M2 Cowpea Mutants. NIL: No recorded mutation.

Treatment	Chlorina	Maculata	Viridis	Xantha	Semi- Albina	Mutation Freq.	Segregation ratio
G1E0.01		1				1	1
G1E0.05	1	4	2			7	1:4:2
G1E0.1	3	3	2			8	3:3:2
G2E0.01		3				3	3
G2E0.05		1				1	1
G2E0.1	1	3		1		5	1:3:1
G3E0.01	1	1				2	1:1
G3E0.05	1					1	1
G3E0.1		1			2	3	1:2
Total	7	17	4	1	2	31	
G1S0.01	2		2		1	5	2:2:1
G1S0.05	1	1				2	1:1
G1S0.1		1				1	1
G2S0.01		2				2	2
G2S0.05		2				2	2
G2S0.1	1			1	2	4	1:1:2
G3S0.01					2	2	2
G3S0.05						Nil	Nil
G3S0.1						Nil	Nil
Total	4	6	2	1	5	18	
G1H0.01	1					1	1
G1H0.05		2		1		3	2:1
G1H0.1	2		1			3	2:1
G2H0.01	1					1	1
G2H0.05	1					1	1
G2H0.1	1					1	1
G3H0.01		1				1	1
G3H0.05		1			3	4	1:3
G3H0.1	1		1			2	1:1
Total	7	4	2	1	3	17	66

Table 2: Mutagen Effectiveness and Efficiency of Ethyl Methane Sulphonate on Cowpea in the Second Mutant Generation.

Treatment	Mutation Freq.	Percentage Mutation Frequency	Percentage of Chlorophyll Mutated Progeny	Percentage Mortality (30th Day)	Mutagen Effectiveness MF/CxT	Mutagen Efficiency MF/M
G1E0.01	1	3.22	2.08	26.15	33.33	0.05
G1E0.05	7	22.58	16.66	35.38	46.67	0.30
G1E0.1	8	25.81	13.33	7.69	26.67	1.60
G2E0.01	3	9.67	4.34	8.00	100.00	0.50
G2E0.05	1	3.22	2.03	36.00	6.66	0.03
G2E0.1	5	16.13	9.09	26.66	33.33	0.25
G3E0.01	2	6.45	6.89	67.41	66.66	0.03
G3E0.05	1	3.22	2.04	44.94	6.66	0.02
G3E0.1	3	9.67	3.40	1.12	10.00	3.00
Total	31	46.97	6.64(Av)	28.15(Av)	36.66(Av)	0.64(Av)

Av: Average

not bestow any known advantage on the plant except that their frequency can be used as markers for the estimation of important mutation indices such as mutagen effectiveness and efficiency. On a treatment basis of the M2 generation, chlorophyll mutation frequency generally increases with a corresponding increase in dose to a certain concentration beyond which it decreases (Rakhi *et al.,* 2019) as observed in Fuampea 1 (G1) treated with Ethyl Methane Sulphonate (EMS) and Sampea 14 (G3) treated with Hydroxylamine Hydrochloride (HH).

Chlorina was produced at higher doses in only the EMS treatments while maculata was produced by all the doses

Table 3: Mutagen Effectiveness and Efficiency of Sodium Azide on Cowpea in the Second Mutant Generation.

Treatment	Mutation Freq.	Percentage Mutation Frequency	Percentage of Chlorophyll Mutated Progeny	Percentage Mortality (30th Day)	Mutagen Effectiveness MF/CxT	Mutagen Efficiency MF/M
G1S0.01	5	27.78	6.17	10.00	166.66	0.56
G1S0.05	2	11.11	3.27	32.22	13.33	0.06
G1S0.1	1	5.55	1.96	43.33	3.00	0.02
G2S0.01	2	11.11	2.12	5.05	66.66	0.40
G2S0.05	2	11.11	5.40	62.62	13.33	0.03
G2S0.1	4	22.22	5.70	30.30	13.33	0.13
G3S0.01	2	11.11	2.29	5.43	66.66	0.40
G3S0.05	Nil	Nil	Nil	Nil	Nil	Nil
G3S0.1	Nil	Nil	Nil	Nil	Nil	Nil
Total	18	27.27	27.27(Av)	26.99(Av)	48.99(Av)	0.22(Av)

Av: Average, NIL:No recorded mutation

Table 4: Mutagen Effectiveness and Efficiency of Hydroxylamine Hydrochloride on Cowpea in the Second Mutant Generation.

Treatment	Mutation Freq.	Percentage Mutation Frequency	Percentage of Chlorophyll Mutated Progeny	Percentage Mortality (30th Day)	Mutagen Effectiveness MF/CxT	Mutagen Efficiency MF/M
G1H0.01	1	5.88	3.70	60.29	33.33	0.02
G1H0.05	3	17.65	5.88	25.00	20.00	0.17
G1H0.1	3	17.65	5.45	19.11	10.00	0.23
G2H0.01	1	5.88	2.08	40.74	33.33	0.03
G2H0.05	1	5.88	2.22	44.44	6.66	0.02
G2H0.1	1	5.88	2.13	41.98	3.33	0.02
G3H0.01	1	5.88	2.47	14.29	33.30	0.40
G3H0.05	4	23.53	8.51	55.36	26.66	0.03
G3H0.1	2	11.76	2.46	27.68	6.66	0.13
Total	17	25.76	25.76(Av)	36.32(Av)	19.25(Av)	0.07(Av)

Av: Average

for all the genotypes. In the SA and HH treatments, they were produced at both high and low doses while xantha was produced at only high doses. Inspite of the genotype, maculata was the dominant mutation across EMS and SA treatments while chlorina was prevalent in the HH treatments. The dominance of chlorina among the HH treatments was consistent with the work of Wani (2011) and Bhat *et al.,* (2007) who observed the trend among cowpea and *Vicia faba* genotypes treated with EMS, Hydrazine Hydrate (HZ), SA and Methyl Methane Sulphonate (MMS). The effectiveness of EMS in inducing chlorophyll mutations above SA and HH was clear as it induced more than half of the total number at 30 mutations (64.87%). It was followed by 28.12% for SA and HH was least with 25% of the total mutations observed. This was not surprising as among chemical mutagens EMS is reported to induce the highest frequency of chlorophyll mutation (Kozgar, 2014). SA also underperformed in the production of chlorophyll mutations when compared to Ethyl Methane Sulphonate (MMS) and Hydrazine hydrate (HZ) in cowpea (Wani, 2020). In the current study however, Hydroxylamine Hydrochloride (HH) performed slightly below Sodium Azide (SA). Conversely, SA produced high mutation frequencies in rice in a study carried out by Awan *et al.* (1980). This disparity could be due to external factors or procedural conditions under which the mutagenic treatment was done such as the time of mutagenesis, concentration of the buffer used or dose range used. It could most definitely be as a result of a difference in the type of experimental material or genotype used because different genetic materials from different plants respond differently to various mutagens. In the current study, this was substantiated as out of the thirty mutations observed in the EMS treatments, more than half (16) were observed in Fuampea 1, which left Ife Brown (G2) and Sampea 14 (G3) with 9 and 5 mutations respectively. This outstanding performance of EMS in inducing chlorophyll mutations is linked not only to its ability to induce point mutations much like other chemical mutagens which results in the simple impairment of genes to varying degrees, but also in its capacity to effect chromosome breaks resulting in chromosomal aberrations affecting a significant amount of coding regions along the chromosome, culminating in a greater number of mutations just as Ghatnekar (1964) observed translocations and inversions in his EMS treated material. EMS was also observed to be more effective in inducing chlorophyll mutations than gamma rays in several investigations and a progressive increase in mutation frequency was observed to improve with an increase in dose (Devmani *et al.,* 2016). It is noteworthy that for both EMS and HH treatments in the study, Fuampea 1 consistently had the high frequency of mutation occurrence. SA treatments had Fuampea 1 and Ife Brown share the highest number of mutations (8) while Sampea 14 had the least with 2 mutations all from the 0.01% dose. Sampea 14 showed the highest number of mutations among the HH treatments with 8 mutations followed by Fuampea 1 with 7 mutations.

The mutation frequency showed dose related changes only in Fuampea 1 and Sampea 14 EMS treatments. This linear relationship was also reported by Kharkwal (1998) in chickpea, John (1999) in cowpea, Das and Kundagrami (2000) in grasspea and in cowpea Wani *et al.,* (2011). In the SA treatments, Fuampea 1 decreased while Ife Brown increased in a dose related fashion. In the HH treatments, Fuampea 1 and Sampea 14 were observed to have mutation frequencies that peaked at dose 0.05% and decreased at 0.1%. This trend which depicted higher frequencies of chlorophyll mutations with lower doses of the deployed mutagen was also reported by (Shahwar *et al.,* 2023) in lentil. In the present study however, dose 0.1% however, had the highest mutation frequency at 40.62%, 32.81% at 0.05% and 26.56 at 0.01%.

The spectrum of chlorophyll mutations in EMS induced treatments were observed to be widest in Fuampea 1 at dose 0.05% and 0.1% each having three mutation types namely chlorina, maculata and viridis while EMS induced Ife Brown at 0.1% produced chlorina, maculata and xantha. There were two SA treatments with a three mutation spectrum which were G1S0.01 and G2S0.1 which also both had chlorina, viridis, semi-albina and chlorina, xantha and semi-albina respectively. The spectrum of mutations that EMS induced in Fuampea 1 were also observed by Devmani *et al.,* (2016) who reported xantha, chlorina and viridis as three out of four mutations identified in the EMS treatment of cowpea and also that the frequency of these mutations observed increased with an increase in dose (Rakhi *et al.,* 2019) as seen in the current study. The inducement of chlorophyll mutations in a wide range of plants could be traced to diminished chlorophyll biosynthesis, extra degradation of chlorophyll and the consequent decolorization due to deficiency of carotenoids and the obvious preferential action of the mutation process on chlorophyll related development genes (Reddy *et al.,* 1993).

Wani (2020) also stated that the high rate of chlorophyll mutations in mutagenized populations could be due to its propensity to affect certain regions of chromosomes. The reason for this could be that, the active coding regions which contain genes that control chloroplast formation are rich in G-C combinations making it a generally guanine rich zone and making impairment more widespread (Wani 2020) as a result of the affinity of the base substitution action of the chemical mutagens to guanine. Ethyl Methane Sulphonate displayed the highest capacity to induce chlorophyll mutations followed by Sodium Azide and Hydroxylamine Hydrochloride was the least. The strongest mutation dose used in the study (0.1%) also produced the highest number of total chlorophyll mutations followed by 0.05% while 0.01% produced the least. Fuampea 1(G1) succumbed to chlorophyll mutations as it showed the least resistance to mutagen attack which also indicates its suitability as an experimental subject for the production and identification of useful mutations in segregating generations. These differences in effect could arise due to differences in DNA sequences and composition of the genotypes used in the study (Kr *et al.,* 2013), the varying efficiency of DNA repair mechanisms between the genotypes and epigenetic modifications such as DNA methylation (Ashapkin *et al.,* 2016) and histone modification.

Mutagen effectiveness and efficiency

Mutagenic effectiveness is a measure of the frequency of mutations induced by a unit dose of a mutagen (Konzak *et al.,* 1965) while mutagenic efficiency indicates the quantification of undesirable biological effects like lethality and sterility caused by the mutagen (Konzak *et al.,* 1965) which leads to cytological and cryptic structural changes (Girija and Dhanavel, 2009). Acquiring strong effectiveness and efficiency is based on the nature of the created mutation and must significantly exceed cytological disturbances such as chromosomal aberrations as well as other toxic and physiological effects which decrease post mutation survival and consequently eliminating the mutation (Girija and Dhanavel, 2009). The behavior of the mutagen effectiveness in the current study changed with the type of mutagen. SA and HH generally obeyed the decreased effectiveness with an increase in dose for all the genotypes just as Girija and Dhanavel found in their 2009 study of mutagen effectiveness and efficiency. EMS on the other hand indicated a decrease in effectiveness with an increase in dose only in Sampea 14 (G3). The highest dose of EMS had the lowest mutagen effectiveness in Fuampea 1 and 0.05% had the highest. Ife Brown (G2) on the other hand recorded its least mutagen effectiveness at 0.05% and its best at the least dose of 0.1%.

The efficiency of the EMS did not show any dose related change among the genotypes used in this study. It was however observed that there was a direct relationship between the mortality at 30 Days after Planting (DAP) and the mutagen efficiency which meant that the dismal survival figures had a cause-and-effect relationship with the posting of poor mutagen efficiency values especially in the SA and HH treatments. This observation could be occasioned by undesirable effects brought about by mutagens (as indicated by the efficiency) such as induced chromosomal mutations which are lethal and could affect post emergence physiology, survival and thereby increasing mortality. Since mutagen efficiency is the rate of production of these deleterious effects during the mutagenic process, it could then be safely inferred that Ethyl Methane Sulphonate out of the mutagens used in this study produces the least unwanted effects. This could also mean that even though Sodium Azide could produce a larger number of mutations, Ethyl Methane Sulphonate produced more useful mutations due to its much higher efficiency.

In conclusion, Sodium Azide and Ethyl Methane Sulphonate have their unique advantages of higher mutagen effectiveness and higher mutagen efficiency respectively. The researcher would have to choose what serves the interest of their research best. However, given that Ethyl Methane Sulphonate was outstanding in the number of chlorophyll mutations produced which has been positively and significantly correlated with the production of useful mutations, Ethyl Methane Sulphonate should be chosen over Sodium Azide as the mutagen of choice within the parameters of this study

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