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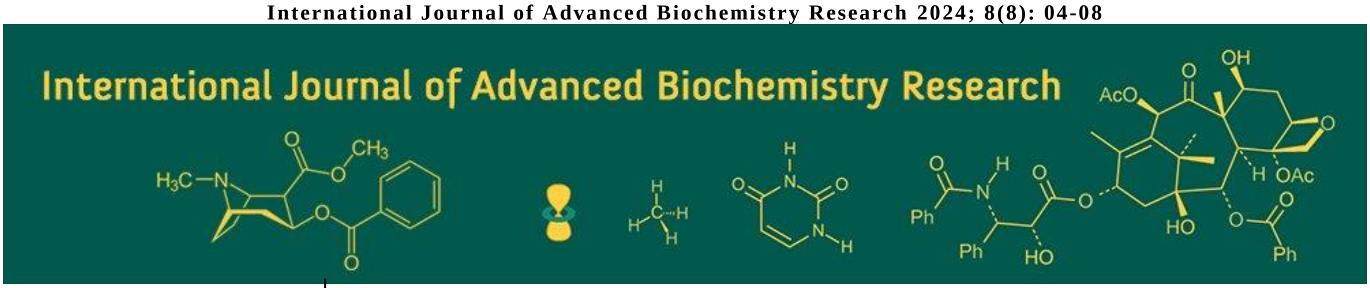

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Chlorophyll mutation spectrum and effectiveness in M_2 generation of sesame (*Sesamum indicum* L.)

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Abstract

In the TKG-55 variety of sesame (*Sesamum indicum* L.), ten desirable macro mutants were identified following treatments with NaN_3 and a combination of EMS chemical mutagenesis. The identified mutants include viridis (characterized by seedling color and increased seed protein content), Albino (white), Xantha (yellow), Chlorina (light green), Albomaculata (white spots on green leaves), Alboviridis (initially white, later turning normal), and Xanthaviridis (initially yellow, later turning normal). All mutant traits were recessive compared to normal, with viridis exhibiting digenic inheritance while the others showed monogenic inheritance. Additionally, these macro mutants were initially evaluated for germination, survival, and lethality percentages.

Keywords: *Sesamum indicum*, mutagenesis, desirable macro mutants, chemical mutagenesis

Introduction

Sesame (*Sesamum indicum* L.) is recognized as an ancient oilseed crop (Brar and Ahuja, 1979) [2]. As noted by Oplinger *et al.* (1990) [26], it is a vital source of high-quality edible oil and protein, particularly benefiting low-income farmers in major sesame-growing regions such as Sudan, Ethiopia, Uganda, Nigeria, Mexico, Venezuela, India, China, Pakistan, Turkey, and Myanmar. Sesamum, a diploid species ($2n = 2x = 26$), has a basic chromosome number of $X = 8$ or 13, with some variants being tetraploid or octaploid (Kobayashi, 1991) [15]. It belongs to the Pedaliaceae family and is one of the oldest oilseeds known, playing a significant role in human nutrition (Komivi *et al.*, 2017) [14].

Mutations serve as valuable tools for geneticists, enabling the study of gene function and plant development, and providing raw materials for the genetic enhancement of economically important crops. Mutation breeding offers an alternative to conventional breeding methods for crop improvement. Induced mutations can swiftly create variability in both quantitatively and qualitatively inherited traits (Maluszynski *et al.*, 1995; Muduli and Mishra, 2007) [12, 9]. Mutagenesis has been successfully utilized to induce genetic variability in numerous crops, leading to the isolation of mutants with desirable traits such as increased seed yield, early maturity (Wongyai *et al.*, 2001) [1], modified plant architecture, closed capsules, disease resistance (Cagirgan, 2001) [25], seed retention, larger seed size, preferred seed color, and high oil content.

Chemical mutagens are agents that alter the genetic material, typically DNA, by increasing mutation rates beyond natural levels. Mutagens have significantly contributed to the development of crop varieties with beneficial mutations, resulting in notable increases in food production. Sodium azide, a potent mutagen in plants, has demonstrated efficiency dependent on concentration and treatment duration (Al-Qurainy and Khan, 2009) [17]. It has been successfully employed in the improvement of rice, barley, and oats (Awan *et al.*, 2000) [29].

Materials and Methods

In this experiment, 100 well-filled seeds of the TKG-55 variety were pre-soaked for four hours in distilled water before being subjected to various concentrations of EMS and NaN3 in double-distilled water. The mutagenic solution's pH was adjusted to seven using a phosphate buffer. After soaking, the seeds were blotted to remove excess water and then immersed in the mutagenic solutions for three hours with intermittent shaking to ensure even absorption. The volume of the mutagen solution was ten times that of the seed volume, and the treatment was conducted at room temperature. Post-treatment, the seeds were rinsed thoroughly with tap water ten times. Control seeds included untreated dry seeds and seeds soaked in distilled water for seven hours.

For the M_2 generation study, the method suggested by Ganguli (1991) [22] was followed. Healthy plants from the M_1 generation were selected and sown in family rows with specific spacing in December-March 2023 to study both micro and macro mutations. Chlorophyll mutants were classified based on the system by Gustafsson (1940) [19] and Blixt and Gottascalk (1975) [27], including types like viridis, albino, xantha, chlorina, albomaculata, alboviridis, and xanthaviridis. The color of the first leaf was used for scoring the mutants, and the different types were recorded from the seventh to fifteenth day. Data were analyzed for mutant effectiveness, mutation frequency, and the spectrum of mutants.

Results and Discussion

Higher doses of E.M.S. and combined treatments with NaN3 significantly reduce germination and survival rates while increasing injury and lethality percentages, demonstrating dose-dependent toxicity. Lower concentrations of these chemicals are less harmful compared to higher concentrations.

Germination Percentage

As the concentration of E.M.S. increases from 0.1% to 0.4%, the germination percentage decreases. For example, at 0.1% E.M.S., the germination percentage is 76%, but it drops to 56% at 0.3% E.M.S. This trend indicates that higher E.M.S. concentrations inhibit seed germination. A similar pattern is observed in combination treatments. For instance, the germination percentage at a combination of 0.1% NaN3 + 0.1% E.M.S. is 85%, but it declines to 49% at a combination of 0.4% NaN3 + 0.4% E.M.S. This suggests that the presence of NaN3 exacerbates the negative effects of E.M.S. on germination.

Injury Percentage

Injury percentage increases with higher doses of E.M.S. At 0.1% E.M.S., the injury percentage is 4%, while at 0.4% E.M.S., it rises to 6.2%. This indicates that higher concentrations of E.M.S. cause more physical damage to the plants.

The injury percentage is higher in combination treatments compared to E.M.S. alone. For instance, at 0.4% NaN_3 + 0.4% E.M.S., the injury percentage reaches 14.5%, showing that NaN3 significantly increases the injurious effects of E.M.S.

Lethality Percentage

Lethality percentage also increases with E.M.S. concentration. At 0.1% E.M.S., the lethality percentage is 15%, but it rises to 32% at 0.3% E.M.S. This demonstrates a clear dose-dependent increase in plant death due to E.M.S., lethality percentage is generally higher in combination treatments. For example, at 0.1% NaN3 + 0.1% E.M.S., the lethality percentage is 34%, which increases to 37% at 0.4% NaN3 + 0.4% E.M.S. This suggests that the combined effect of NaN3 and E.M.S. is more lethal than E.M.S. alone.

Survival Percentage

Survival percentage decreases with increasing E.M.S. concentration. At 0.1% E.M.S., the survival percentage is 85%, but it drops to 68% at 0.3% E.M.S. This indicates that higher concentrations of E.M.S. reduce the overall survival rate of the plants. Survival percentage is generally lower in combination treatments. For instance, at 0.1% NaN3 + 0.1% E.M.S., the survival percentage is 66%, which decreases further to 63% at 0.4% NaN3 + 0.4% E.M.S. This indicates that the combination of NaN3 and E.M.S. has a synergistic negative effect on plant survival.

The data indicate that both E.M.S. and its combination with NaN3 negatively impact plant growth traits such as germination, injury, lethality, and survival. The detrimental effects are more pronounced at higher concentrations, with the combination treatments showing greater negative impacts compared to E.M.S. alone. This suggests that while E.M.S. is toxic to plants, its combination with NaN3 exacerbates these toxic effects, leading to lower germination and survival rates and higher injury and lethality percentages. This finding was in agreement with the report of Ganesh kumar *et al.* (2001) [23] in sesame. The decrease in germination due to mutagenic treatment observed in the present study was also in conformity with the earlier reports of, Anitha Vasline (1998) [30], Radhakrishnan *et al.* (2001) [7] in sesame; Jegadeeswaran (1989) [18] in groundnut; Shamsi (1981) [3] in sunflower; Ahmed John (1996) [31], Barela *et al.*, (2022) [28] in green pea and Deepalakshmi (2000) [24] in black gram (table1, fig 1&2).

Efficiency and effectiveness

Higher doses of E.M.S. and combinations with NaN3 generally increase injury and lethality, while decreasing survival. E.M.S. at 0.2% (M=3.98) and the combination at 0.2% NaN3 + 0.2% E.M.S. (M=6.00) show high effectiveness. Efficiency values indicate that combined treatments, especially at 0.2%, result in higher injury and lethality percentages. Control groups show no injury, lethality, or survival impact, confirming the negative effects are due to the treatments. The combination treatments show greater negative effects compared to E.M.S. alone. The table.2 shows the effectiveness and efficiency of various treatments on plant traits.

Effect of mutagenic treatments in M_2 Generation

Frequency of chlorophyll mutants

The frequency of chlorophyll mutations, in general, were low in this crop thus it may be attributed to the fact that oil seed crops are resistant to induced chlorophyll mutations as reported by Rajan (1969) [6] and Rangaswamy (1982) [5].

E.M.S. Treatments: At 0.1% E.M.S., two mutants (Chlorotica and Vario-maculata) were observed. At 0.2%, four mutants were found, including Xantha, Chlorina, Auera, and Terminalis. The 0.3% dose resulted in three mutants (Albina, Variegata, and Terminalis), and 0.4% resulted in one mutant (Albina).

Combination Treatments (E.M.S.+NaN3): The combinations show varying results. The 0.1% NaN3 + 0.1% E.M.S. resulted in three mutants (Albina, Chlorotica, and

Terminalis). The 0.2% combination yielded six mutants, including Xantha, Chlorotica, Viridis, and Auera. The 0.3% and 0.4% combinations produced two mutants each, with the latter having the highest effectiveness value (M = 6.00).

Effectiveness (M): The highest effectiveness is observed with the 0.2% NaN3 + 0.2% E.M.S. combination (M = 6.00), while the lowest effectiveness values are associated with higher E.M.S. doses alone (e.g., 0.4% E.M.S., M = 1.00).

The table highlights that both E.M.S. alone and in combination with NaN3 induce various chlorophyll mutations, with combinations generally showing higher mutation frequencies and effectiveness. The 0.2% NaN3 + 0.2% E.M.S. combination appears particularly effective in inducing mutations, while higher concentrations of E.M.S. alone tend to reduce effectiveness and frequency. These results suggest that combined treatments of NaN3 and E.M.S. are more potent in generating chlorophyll mutations than E.M.S. alone.

Spectrum of chlorophyll mutation

The highest mutation frequencies in the M_1 generation are observed in the Xantha mutant type (10.00) and the lowest in Chlorina, Viridis, and Marginata (2.00 each). Similarly, in the M_2 generation (table.3), Xantha again shows the highest frequency (0.219), indicating a more persistent mutation through generations, while the lowest frequencies (0.044) are observed in Chlorina, Viridis, and Marginata. This suggests that the Xantha mutation is more stable and frequent across generations compared to other mutant types. The total mutation frequency values highlight that the majority of mutations decrease in frequency from the M_1 to M_2 generations, reflecting the natural selection pressures and possibly the viability of these mutations. (Table 3). The reason for the appearance of greater number of Xantha viridis type may be attributed to involvement of polygenes in chlorophyll formation (Gaul, 1964) [21]. According to the greater efficiency of low dose of mutagens appeared in relation to the fact that lethality and injury increased with increase in dose at faster rate than the useful mutations.

Chlorophyll mutants Efficiency

The efficiency was calculated on lethality, injury and sterility basis. In TGM 55, the efficiency on lethality basis showed a declining trend as dose increase up to 0.1% in NaN3 treated dose and the maximum value obtained on lethality basis was 34 per cent in NaN3 @ 0.4% (Table1). The seedling injury causes lethality varied from 3% NaN3 @ 0.1% to 7.8% per cent NaN_3 @ 0.4% for sodium azide and from 3.4% (NaN3 + E.M.S. (0.2+0.2)) to 11.23 per cent (NaN3 + E.M.S. (0.3+0.3)) for combination treatment of NaN3 & EMS in TGM 55. Among all chemical treatments, the treatment NaN3@ 0.4 with the value of 1.03 in TGM 55 was found to be efficient. (table 3).

Table 1: Effect of mutagens in M_2 generation

S. No.	Treated Dose (in %)	NST	G%	IJ%	LT%	SL%
1	E.M.S. (0.1)	50	94	4	25	75
2	E.M.S. (0.2)	50	84	7.8	29	71
3	E.M.S. (0.3)	50	76	10	34	66
4	E.M.S. (0.4)	50	68	6.2	46	55
5	Control 1	50	100	0	0	100
6	NaN3 + E.M.S. (0.1+0.1)	50	96	3.9	32	68
7	NaN3 + E.M.S. (0.2+0.2)	50	80	9.87	39	61
8	NaN3 + E.M.S. (0.3+0.3)	50	56	5.1	38	62
9	NaN3 + E.M.S. (0.4+0.4)	50	64	14.5	50	50
10	Control 2	50	100	0	0	100

Here, NST (Number of seeds treated), G% (Germination), LT% (Lethality), IJ% (Injury), SL% (Survival)

Table 2: Effect of mutagens on effectiveness and efficiency in M_2 generation

S. No.	Treated Dose (in %)	M (Effectiveness)	Efficiency		
			Injury	Lethality	Survival
1	E.M.S. (0.1)	2.00	50.00	13.33	2.35
2	E.M.S. (0.2)	3.98	51.03	14.21	5.53
3	E.M.S. (0.3)	3.00	30.00	9.38	4.41
4	E.M.S. (0.4)	1.00	16.13	3.45	1.41
5	Control 1	0.00	0.00	0.00	0.00
6	NaN3 + E.M.S. (0.1+0.1)	3.00	76.92	8.82	4.55
7	NaN3 + E.M.S. (0.2+0.2)	6.00	60.79	33.33	7.32
8	NaN3 + E.M.S. (0.3+0.3)	2.00	39.22	4.65	3.51
9	NaN3 + E.M.S. (0.4+0.4)	2.00	13.79	5.41	3.17
10	Control 2	0.00	0.00	0.00	0.00

Table 3: Chlorophyll mutation spectrum in M_2 generation

S. No.	Mutant Characters	Treated dose (in %)										Total Mutants	Mutation Frequency	
	Type	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}		M_1 Plant basis	M_2 Plant basis
1	Albina	0	0	1	0	0	1	0	0	1	0	3	6	0.131291
2	Xantha	0	1	0	1	0	0	2	1	0	0	5	10	0.218818
3	Chlorina	0	1	0	0	0	0	0	0	0	0	1	2	0.043764
4	Chlorotica	1	0	0	0	0	1	0	1	0	0	3	6	0.131291
5	Viridis	0	0	0	0	0	0	1	0	0	0	1	2	0.043764
6	Auera	0	1	0	0	0	0	2	0	0	0	3	6	0.131291
7	Variegata	0	0	2	0	0	0	0	0	0	0	2	4	0.087527
8	Marginata	0	0	0	0	0	0	0	0	1	0	1	2	0.043764
9	Vario-maculata	1	0	0	0	0	0	1	0	0	0	2	4	0.087527
10	Terminalis	0	1	0	0	0	1	0	0	0	0	2	4	0.087527
	Total	2	4	3	1	0	3	6	2	2	0	23	46	1.006565
	M_2 POPULATION	267	201	109	111	563	213	158	72	34	557			
Mutation spectrum	M_1	4.0	8.0	6.0	2.0	0.0	6.0	12.0	4.0	4.0	0.0			
	M_2	0.75	1.99	2.75	0.90	0.00	1.41	3.80	2.78	5.88	0.00			
	M (EFFECTIVENESS)	2.00	3.98	3.00	1.00	0.00	3.00	6.00	2.00	2.00	0.00			

Here, T_1- (E.M.S. (0.1), T_2-E.M.S. (0.2), T_3- E.M.S. (0.3), T_4 - E.M.S. (0.4), T_5- Control 1, T_6- NaN3 + E.M.S. (0.1+0.1), T_7- NaN3 + E.M.S. (0.2+0.2), T_8- NaN3 + E.M.S. (0.3+0.3), T-9 NaN3 + E.M.S. (0.4+0.4), T_{10}- Control 2

Conclusion

In the M_2 generation, the frequency of chlorophyll and viable mutants was calculated as a percentage based on M_1 plants and M_2 seedlings. No clear pattern emerged for the number of chlorophyll mutants in treatments involving EMS and the combination of EMS and NaN3 in TGM 55. Among the treatments, TGM 55 showed higher frequencies compared to the others. The chlorophyll mutants observed included xantha, albina, and viridis. NaN3 produced the highest number of xantha mutants, except at an EMS concentration of 0.2%. The combination of NaN3 and EMS yielded the highest number of marginata mutants. Generally, there were more mutants of one type than those showing two or three types. Combination treatments proved to be more efficient and effective than EMS alone for inducing chlorophyll mutants, with both mutagens being most effective in the TGM 55 treatment.

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