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Studies on chlorophyll spectrum and mutation effectiveness in M_2 generation of Sesame (*Sesamum indicum* L.)

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Abstract
Ten desirable macro mutants in TKG-55 sesame (*Sesamum indicum* L.), resulting from induced NaN3 and combination treatment with EMS chemical mutagenesis, have been identified for: viridis (seedling color as marker, increased seed protein content), Albino (White), Xantha (Yellow), Chlorina (Light green), Albomaculata (White dots on green leaves), Alboviridis (Initially white and later becomes normal plants), Xanthaviridis (Initially yellow and later become normal plants). All mutant traits were recessive compared to normal, with viridis showing digenic inheritance and the others monogenic. Along with macro mutants were initially observed for germination, survival and lethality percentage respectively.

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

Introduction
Sesame (*Sesamum indicum* L.) is considered to be an ancient oil seed crop (Brar and Ahuja, 1979) [31]. According to Oplinger *et al.*, (1990) [7], it is an important oilseed to generate high quality edible oil and protein for low income peasants of major sesame growing countries including Sudan, Ethiopia, Uganda, Nigeria, Mexico, Venezuela, India, China, Pakistan, Turkey and Myanmar. Sesamum is a diploid species (2n = 2x = 26), with a basic chromosome number of X = 8 or 13, while some are tetraploids and octaploids (Kobayashi 1991) [18], and it belongs to the Pedaliaceae family. It is one of the most ancient oil seeds known to mankind and plays a major role in human nutrition (komivi *et al.*, 2018) [19].
Mutations are the tools at hand exploited by the geneticist to study the nature and function of genes which are the basis of plant growth and development, hence producing raw materials for genetic improvement of economic crops (Adamu *et al.*, 2007) [1]. Mutation breeding is one possible alternative to conventional breeding for crop improvement. Induced mutations can rapidly create variability in quantitatively and qualitatively inherited traits in crops (Maluszynski *et al.*, 1995; Muduli and Mishra, 2007) [21, 24]. Mutagenesis has been successfully used to induce genetic variability in many crops, allowing to isolate mutants with desirable characters such as increased seed yield, earliness (Wongyai et al., 2001) [32], modified plant architecture, closed capsules, disease resistance, seed retention, larger seed size, desirable seed colour and high oil content. Chemical mutagens are agents that change the genetic materials, usually the DNA of an organism by increasing the frequency of mutations above the natural background level. Mutagens have played a pivotal role in creating crop varieties with induced mutations resulting in significant increases in food production. Sodium azide is a powerful mutagen in plant and its efficiency depend on concentration and treatment duration (Al-Qurainy and Khan, 2009) [16]. It has been successfully used in the improvement of rice, barley and oats, (Awan *et al.*, 2011) [4].

Materials and Methods
Chemical mutagenic treatment hundred well filled seeds of TKG-55 variety were pre-soaked for four hours in distilled water. Seeds were treated with different concentrations of NaN_3 & EMS in double distilled water and pH of the mutagenic solution was adjusted to seven by

using phosphate buffer. The pre-soaked seeds after removal from the water were packed between the folds of blotting paper to remove excess water adhering to the surface. The seeds were immersed for three hours in the required concentrations of the mutagen with intermittent shaking. To ensure uniform absorption of the mutagen, the volume of the mutagen solution was maintained at a proportion of ten times to that of seed volume. The whole treatment was carried out at room temperature. After the treatment, the seeds were thoroughly washed with tap water for ten times. Non-treated dry seeds and pre-soaked seeds in distilled water for seven hours were used as control.

The M_2 generation study was conducted by resorting to methods suggested by Ganguli (1991) [11]. The normal good looking plants based on base population randomly selected in each treatment in the M_1 generation were advanced to M_2 generation during Aug - Nov 2023. They were sown in family rows in different blocks with a spacing of 45 cm between rows and 30 cm between plants to study the micro and macro mutations.

The chlorophyll mutants were classified as per the system proposed by Gustafsson (1940) [14] and Blixt and Gottascalk (1975) [6]. The chlorophyll mutants include the following. Mutant Described as viridis (seedling color as marker, increased seed protein content), Albino (White), Xantha (Yellow), Chlorina (Light green), Albomaculata (White dots on green leaves), Alboviridis (Initially white and later becomes normal plants), Xanthaviridis (Initially yellow and later become normal plants). The colour of the first formed leaf was taken for scoring the chlorophyll mutants. To compute the spectrum (relative percentage of different types) of mutants, the different types of seedling mutants for chlorophyll deficiency were scored separately from seventh to fifteenth day. The data were analysed for statistical parameters such as mutant effectiveness, mutation frequency for individual mutants as well as for different treatments respectively.

Results and Discussion

The seed germination percentage was reduced more under higher dose of combination treatments than NaN_3 mutagen treatments. Maximum reduction was recorded at 0.4+0.4% dose of NaN_3 + E.M.S. treatments (58.82 per cent reduction over control) and minimum of 0.1+0.1 per cent dosage in NaN_3 + E.M.S. (84 per cent reduction over control). Lethality was observed ranging minimum at 0.1% dose of NaN_3 (causes 17% lethality) to maximum at 0.3+0.3% combination dose of NaN_3 + E.M.S. (causes 47% lethality). This finding was in agreement with the report of Ganesh Kumar *et al.* (2001) [10] 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) [3], Radhakrishnan *et al.* (2001) [26] in sesame; Jegadeeswaran (1989) [15] in groundnut; Shamsi (1981) [30] in sunflower; Ahmed John (1996) [2], Barela *et al.*, (2022) [5] in green pea and Deepalakshmi (2000) [9] in black gram (table1, fig 1 & 2).

Seedling survival during early phase, the seedlings could adjust or repair themselves to eliminate the dead or unwanted cells. On the other hand, some of the seedlings were not able to overcome the radiation damage and hence, they died before they put forth any side effects. This might be due to inhibition of auxin synthesis, lack of assimilatory mechanism, inhibition of mitosis and chromosomal damages. An inverse relationship between the dosage of mutagen and survival was observed in both cases. The survival reduction ranged from 53% in NaN_3 + E.M.S. (0.3+0.3%) to 83 per cent in NaN_3 (0.1) in chemical mutagens. Many workers viz., Cagirgan (1996) [8], Govindarasu *et al*. (1998) [13], Sasikala and Kamala (1988) [29], Anitha Vasline (1998) [3] and Khin Mar Mar New (2001) [17] have reported a dose dependent decrease in plant survival in sesame.

Effect of Mutagenic Treatments in M_2 Generation

Frequency of chlorophyll mutants

The number of plants segregated for chlorophyll deficiency on the basis of M_1 plants and M_2 seedlings were computed and furnished in Table 2 (TKG 55). The chlorophyll mutants occurred in all the treatments, the frequency of chlorophyll mutation varied from 0.49 (NaN_3 0.1%) to 2.11 per cent (NaN_3 0.3%) on M_2 plant basis and from 2% (NaN_3 0.4%) to 4% per cent (NaN_3 0.3%) on M_1 seedling basis. In combination treatments, the frequency ranged from 0.93% (NaN_3 + E.M.S. (0.2+0.2%)) to 5 per cent (NaN_3 + E.M.S. (0.3+0.3%)) on M_2 plant basis and 2% (NaN_3 + E.M.S. (0.2+0.2%)) dose to 4% per cent (NaN_3 + E.M.S. (0.2+0.2%)) on M_1 seedling basis. The treatment (NaN_3 + E.M.S. (0.3+0.3%)) exhibited the maximum frequency of chlorophyll mutation for both M_1 plant basis (4%) and M_2 seedling basis (5%) (Table 2). 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) [27] and Rangaswamy (1982) [28].

Spectrum of chlorophyll mutation

The spectrum of chlorophyll mutations and the relative frequencies of different types of chlorophyll mutants are presented in Table 2 for TGM 55. Seven types of chlorophyll mutants *viz.,* Albina, Xantha, Chlorotica, Viridis, Variegata, Marginata and Terminalis were observed in M_2 seedlings. Maximum number of chlorophyll were observed in xantha mutants except in NaN_3 @ 0.3% while albina and terminalis are intermediate in its occurrence. EMS & NaN_3 exposed treatments showed the maximum xantha albina mutants. The frequency ranged from 0.04 per cent of viridis to 0.13 per cent of xantha (Table 2). The presence of occurrence of chlorophyll mutants was higher in individual treatments (from 2 per cent in NaN_3 @ 0.1% to 4.0 per cent in NaN_3 @ 0.3% while compared to combination treatments NaN_3 & EMS (from NaN_3 + E.M.S. (0.3+0.3%) and NaN_3 + E.M.S. (0.4+0.4%). The order of frequency of mutants occurrence was chlorina < Vario-maculata < Auera < Viridis < Marginata < Albina < Chlorotica < Variegata < Terminalis < Xantha on over all basis. The reason for the appearance of greater number of Xantha viridis type may be attributed to involvement of polygenes in chlorophyll formation. 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 (table 2, Fig. 3).

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 NaN_3 treated dose and the maximum value obtained on lethality basis was 34 per cent in NaN_3 @ 0.4% (Table1). The seedling injury causes lethality varied from 3% NaN_3 @ 0.1% to 7.8% per cent NaN_3 @ 0.4% for sodium azide and from 3.4% (NaN_3 + E.M.S. (0.2+0.2)) to 11.23 per cent (NaN_3 + E.M.S. (0.3+0.3)) for combination treatment of NaN_3 & EMS in TGM 55. Among all chemical treatments, the treatment NaN_3 @ 0.4 with the value of 1.03 in TGM 55 was found to be efficient. (table 1, fig.1&2).

Table 1: Effect of mutagens on single plant yield in M_2 generation

S. No.	Treated Dose (in%)	Mutant population	Germination (%)	Lethality (%)	Injury (%)	Survival (%)
1	NaN_3 (0.1)	333	74	17	3	83
2	NaN_3 (0.2)	243	79	30	4.7	70
3	NaN_3 (0.3)	234	54	25	5	75
4	NaN_3 (0.4)	153	70	34	3.4	66
5	Control 1	450	98	0	0	100
6	NaN_3 + E.M.S. (0.1+0.1)	279	84	32	2.8	68
7	NaN_3 + E.M.S. (0.2+0.2)	207	65	20	3.4	80
8	NaN_3 + E.M.S. (0.3+0.3)	126	59	47	11.23	53
9	NaN_3 + E.M.S. (0.4+0.4)	207	52	35	8.3	65
10	Control 2	450	95	0	0	100

Table 2: Chlorophyll mutation spectrum in M_2 generation

S. No.	Mutant Characters type	Treated dose (in%)										Total Mutant	M_2 pop.	Mutation Frequency	
		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	0	0	0	1	0	0	1	0	2	205	4	0.08
2	Xantha	0	1	0	1	0	0	0	1	0	0	3	134	6	0.13
3	Chlorina	0	0	0	0	0	0	0	0	0	0	0	95	0	0
4	Chlorotica	1	0	0	0	0	0	0	1	0	0	2	71	4	0.08
5	Viridis	0	0	0	0	0	0	1	0	0	0	1	441	2	0.04
6	Auera	0	0	0	0	0	0	0	0	0	0	0	160	0	0
7	Variegata	0	0	2	0	0	0	0	0	0	0	2	108	4	0.08
8	Marginata	0	0	0	0	0	0	0	0	1	0	1	40	2	0.04
9	Vario-maculata	0	0	0	0	0	0	0	0	0	0	0	70	0	0
10	Terminalis	0	1	0	0	0	1	0	0	0	0	2	427	4	0.08
	Total	1	2	2	1	0	2	1	2	2	0	13	1751	26	0.56
	M_2 population	205	134	95	71	441	160	108	40	70	427	1751			
Mutation Frequency	M_1 Plant basis	2	4	4	2	0	4	2	4	4	0	26			
	M_2 Plant basis	0.49	1.49	2.11	1.41	0.00	1.25	0.93	5.00	2.86	0.00	0.74			

Here, T_1- (NaN_3 (0.1), T_2- NaN_3 (0.2), T_3- NaN_3 (0.3), T_4 - NaN_3 (0.4), T_5- Control 1, T_6- NaN_3 + E.M.S. (0.1+0.1), T_7- NaN_3 + E.M.S. (0.2+0.2), T_8- NaN_3 + E.M.S. (0.3+0.3), T_9- NaN_3 + E.M.S. (0.4+0.4), T_{10}- Control 2

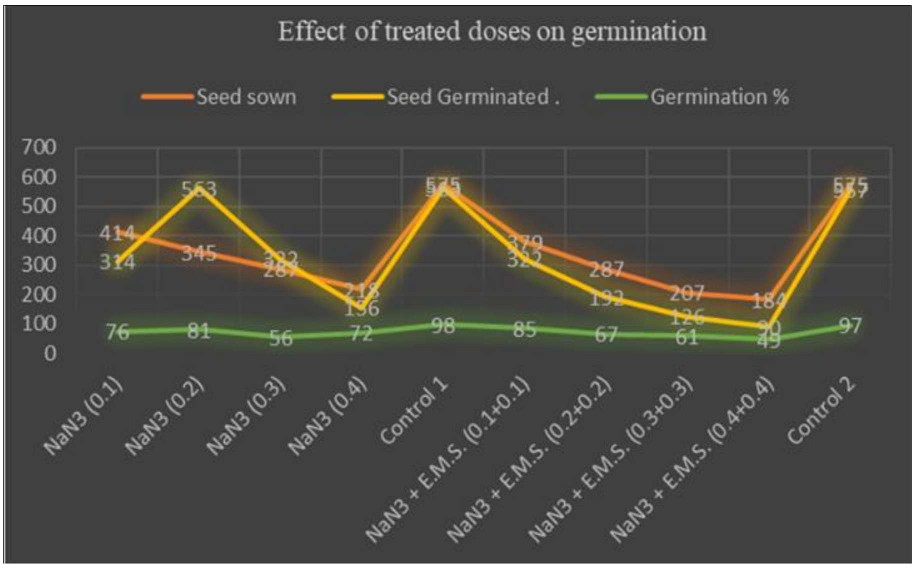

Fig 1: Effect of treated doses on seed germination

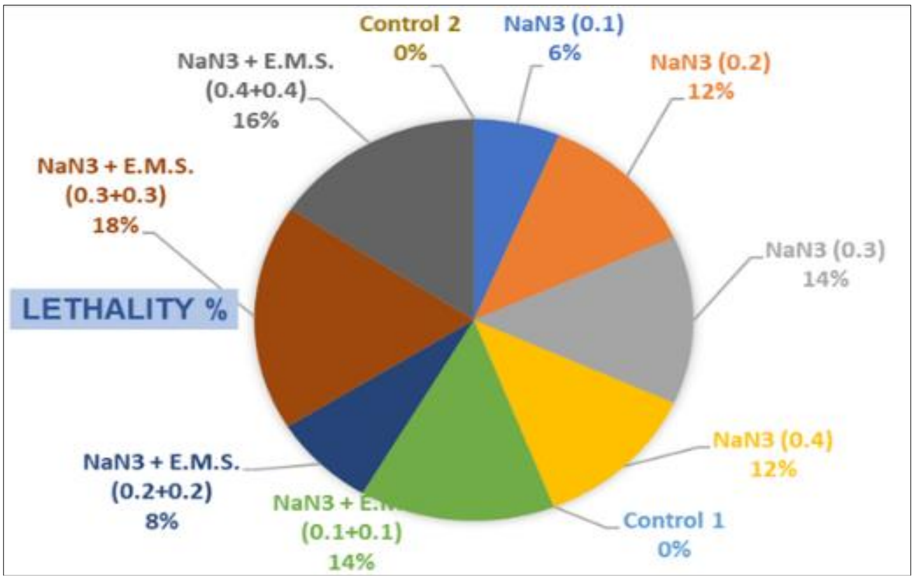

Fig 2: Effect of treated doses on population lethality

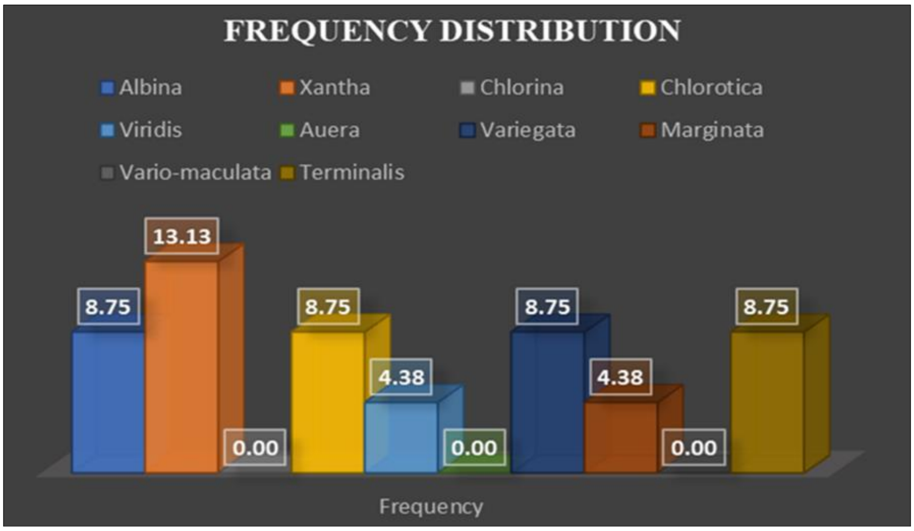

Fig 3: Spectrum of chlorophyll mutation in M_2 generation

Conclusion

In M_2 generation, the frequency of chlorophyll and viable mutants were calculated as percentage of M_1 plant and M_2 seedling basis. No clear trend could be observed for number of chlorophyll mutants in NaN_3 and combination treatment of NaN_3 & EMS in TGM 55. Among the treatments, TGM 55 registered more frequency than rest of the treatments. The spectrum of chlorophyll mutants comprised of xantha, albina and viridis. NaN3 exhibited maximum number of xantha mutants except in NaN_3 @0.1% whereas combination treatment of NaN_3 & EMS exposed treatments showed the maximum marginata mutants. The proportion of one type of mutant was more than those segregating for either two or three types. Combination dose were found to be more efficient and effective than NaN_3 for chlorophyll mutants. The effectiveness and efficiency of both the mutagens were more in TGM 55 treatment dose.

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