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Response of Mutagens on spectrum and frequency of Chlorophyll and other viable mutations in Sesame (*Sesamum indicum* L.)

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Abstract
Sesame is an important edible oil yielding crop grown worldwide. Due to its self-pollinated nature and lack of variability, conventional breeding techniques have not so fruitful results in development of improved genotypes. An alternate solution is use of mutagenesis. Therefore, an experiment was conducted at the research farm of RBS College, Bichpuri, Agra to study the response of mutagens on spectrum and frequency of chlorophyll and other viable mutations in sesame. In M2 generation, the highest chlorophyll mutations were recorded in 25kR followed by 0.2%HA, 0.2%HA+ 0.2%MH and 0.2%MH.The leathery leaves had high frequency followed by xantha, albina and chimera. The stem types mutations were recorded highest in 0.1%HA+0.1%MH followed by 0.2%HA+0.2%MH, 0.2%MH, 20kR and 0.1%HA. The lowest frequency was recorded in 25Kr. Bushy stem were observed highest and lax branched as lowest. The leaf type's mutants have highest frequency in20kR followed by 15kR+0.1%HA, 30kR and 60kR.Narrow leaf mutants were found highest followed by thick leaves, elongated leaves bunchy and long petiole. The highest frequency of flower and capsule type mutants were recorded in 30kR followed by 60kR, 20kR+0.1%HA, 0.1HA and 45kR. The mutations rate was maximum in 60kRfollowed by 20kR+0.1%HA, 30kR and 20kR (reduced with dose of mutagens) 0.1%HA+0.1%MH was observed as most effective mutagens followed by 0.1%HA, 0.2%MH and 0.1% MH. Moreover, 0.1% HA+0.1%MH also found most efficient and the efficiency of 0.2% HA+0.2%MH, 60kR, 15kR+0.1%MH and 20kR+0.1%HA was observed in decreasing order.

Keywords: Mutation, Sesame, Gamma rays, Maleic Hydrazide, Hydroxylamine

Introduction
Among the oilseeds, sesame (*Sesamum indicum* L.) is an important and ancient oil-yielding crop. It is also known as Til, Gingerly, Benni seeds, Sim sim, Tilli nuvrula vellvor and Rasi. Sesame is rich source of edible oil containing about 46 to 52 percent. Fats are highly stable and do not develop rancidity in its oil as compared to other oils, leading to loss of flavor and vitamins. Due to presence of linoleic acid and tocopherol, it is a remarkable antioxidant. Seeds are highly rich in quality protein and essential amino acids especially methionine which is considered as rejuvenative and anti-aging for human body.
In India, it was grown on 1850 hectare of land with the production of 750 metric tons during 2010 with the average yield of 405 kg/ha (FAO, 2011). As sesame is self-pollinated crop species. So, there is a lack of genetic variability. It has also been grown under poor farm management practices. Thus, the yield of sesame is very low in our country. To generate variability attempts have been made by crossing to some extent, but desirable success could not have been attained although. The conventional breeding methods use the natural genetic variability due to the spontaneous mutation and / or hybridization. Furthermore, mutagenesis is the best tool to improve genetic architecture of plant within a short time.
In recent years, induced mutants have been directly released as improved varieties on wide groups of crops plants. During the period 1962-96, the release of about 127 mutants (Kharakwal, 1996) had clearly indicated the increasing the popularity of adopting mutation techniques for crop improvement. Besides, other uses of induced mutations, which have a great relevance in modern plant breeding are reconstruction of plant ideotype (Swaminathan, 1961), incorporation of one or two desirable attributes in other wise well adopted varieties (Verughese and Swaminathan, 1969) [16], upgrading of protein quality (Mossberg, 1969) [16], induction of disease resistance (Friesliben & Lein, 1942 and Konzak, 1959) [9] and getting

transgressive variants coupled with conventional breeding methods (Hagberg 1959, Gaul, 1963 and Aastvert, 1966) [6]. Four types of chlorophyll mutants namely xantha viridis were observed in maximum proportion followed by chlorina and striata (Sheeba, *et al.* 2003) [12]. Frequency of chlorophyll mutants increased with an increase in gamma ray dosage and EMS concentration in mutagen treated population (Begum and Dasgupta, 2005).

The foremost objective in breeding of sesame is to evolve high yielding, high oil content and short duration cultivars. Other important objectives are

(i) development of varieties resistant to biotic and abiotic stress,
(ii) to minimize the oxalic acid content and to increase the lysine content for quality meal,
(iii) to incorporate non-photo and thermo sensitiveness and
(iv) to incorporate nitrogen responsive coupled with stiff stem, non-shattering and senescence.

The present investigation was under taken to judge the response of mutagens on spectrum and frequency of chlorophyll and other viable mutations in sesame (*Sesamum indicum*). The study is also concerned with the comparative evaluation of effect of physical mutagen (gamma rays) chemical mutagen (maleic hydrazide and hydroxylamine) and combination of both the mutagens on Gujarat Til-1 variety of sesame (*Sesamum indicum*).

Material and Methods

The experiment was conducted in *kharif* season atAgricultural Research Farm, R.B.S. College, Bichpuri, Agra, U.P., situated 11 Km from Agra in West on Agra-Bharatpur Road, at 27.2º Nlatitude and 72.9º E longitude and 163.4 m above the mean sea level (MSL).

The experimental material consisted of Gujarat Til-1 variety of sesame, which was procured from National Seed Corporation, as a certified seed. The variety produces white seeds and much popular among the farmers of western Uttar Pradesh. The seeds of sesame were got treated with gamma rays doses of 15, 20, 25, 30, 45 and 60 kR at CIMAP Lucknow, U.P. The source of gamma rays was gamma chamber containing ^{60}CO and for combinations treatments of radiation and chemical, the seeds treated with 15 & 20 kR gamma rays were soaked in 0.1 and 0.2 recent aqueous solutions each of hydroxylamine and maleic hydrazide and in combination of both for 6 hrs. Volume of solutions was kept 10 times approximately to the seed volume.

Table 1: Single and combined treatments of radiation and chemicals

S. No.	Treatments		
	Single treatment of radiation & chemicals	**Combined treatments of radiation & chemical**	**Combined treatment of chemicals**
1.	Dry Control	15 kR + 0.1% HA	0.1% HA + 0.1% MH
2.	15 kR	15 kR + 0.1% MH	0.2% HA + 0.2% MH
3.	20 kR	20 kR + 0.2% HA	
4.	25 kR	20 kR + 0.2% MH	
5.	30 kR		
6.	45 kR		
7.	60 kR		
8.	Wet control		
9.	0.1% HA		
10.	0.1% MH		
11.	0.2% HA		
12.	0.2% MH		

A detailed account of treatments is tabulated in table 1.

Raising the M_1 generation:

To raise the M_1 generation the above treated 200 seed of each of the treatment were sown at first shower of monsoon at Agricultural Research Farm of R.B.S. College, Bichpuri, Agra in Randomized Block Design with three replications, each accommodating 5 rows of 4 meter length and spacing of 45X10 cm. Before sowing, seeds were treated with chemicals and then thoroughly washed in running water for 30 minutes. Untreated dry seeds were used as dry control and untreated seeds soaked in water for six hours as wet control.

Raising of M_2 generation:

To grow M_2 generation seeds were collected from all the competitive M_1 plants of 3 middle rows were grown to raise the M_2 in succeeding in Randomized Block Design (RBD) trial with 4 replications. Each row of 4 m length and the spacing was maintained as 45X15 cm.

Observations in M_2 generation:

i. Macro Mutations

In M_2 generation, observations were recorded on chlorophyll and other viable mutations to study their spectrum, frequency, efficiency and effectiveness of each of the treatments.

ii. Effectiveness and Efficiency of treatments

Viable mutations in M_2 generation/ chlorophyll mutants.

Effectiveness of treatment = MF/Dose kR/Tc

Where,

MF Mutation rate/ Frequency of treatment

kR = Dose of physical mutagen

TC = Concentration of chemical mutagen

Efficiency of treatment = MF/L

Where

MF= Mutation frequency of treatment

L = Lethality percent of treatment

Result and Discussion

Chlorophyll mutations:

Table 2 shows that in M_2 generation in total 28 chlorophyll mutations were observed out of which 5 mutants in 25 kR, 4 mutants in 15 kR + 0.1% MH and 4 mutants in 0.1% MH shows high frequency of mutations. Leathery type mutants (06) followed by xantha (05) albino (04) and chimera (04) were observed. The chimera and virids show low spectrum and frequency. Similar findings were also reported by *Sheeba et al.* (2003) [12].

Table 2: Spectrum and Frequency of Chlorophyll Mutations in M_2 Generation

S.No.	Treatment	Total Population	Chlorophyll Mutations							Total
			Albino	Xantha	Chlorine	Virids	Chimera	Leathery Leaves		
								In Seedlings	In Plant	
1	Dry Control						01 (0.13)			
2	15 kR	778	01 (0.14)	02 (0.27)						01 (0.13)
3	20 Kr	738	02 (0.25)							03 (0.41)
4	25 kR	802							03 (0.37)	05 (0.62)
5	30 kR	946						02 (0.21)		02 (0.21)
6	45 kR	623								
7	60 kR	427								
8	Wet Control									
9	15 kR + 0.1 HA	816		01 (0.12)			01 (0.12)			02 (0.24)
10	15 kR + 0.1 MH	925	01 (0.11)			03 (0.32)				04 (0.43)
11	20 kR + 0.2 HA	568								
12	20 kR + 0.2 MH	653								
13	0.1 HA	1043								
14	0.1 MH	835		02 (0.24)			02 (0.19)			02 (0.19)
15	0.2 HA	587							02 (0.24)	04 (0.48)
16	0.2 MH	428						02 (0.47)		02 (0.47)
17	0.1 HA + 0.1 MH	169			2 (1.18)			2 (1.18)		
18	0.2 HA + 0.2 MH	208							01 (0.53)	01 (0.48)
Total		10546	04 (0.04)	05 (0.05)	02 (0.02)	03(0.03)	04 (0.04)	04 (0.04)	06 (0.06)	28 (2.65)

Viable mutations

Viable mutations studied in M_2 were stem type, leaf type, flower and capsule type and these are presented in tables 3 the results are being discussed as follow:

a. Stem Type Mutants

A perusal of table 3 reveals that out of total 46 stem type mutants. Maximum (12) were tall stem type followed by busty type (08), dwarf (07), fascinated (06) and thick stem (06). The minimum mutants were lax branch type (03). The table 3 shows that highest stem type mutants were induced by 0.1% HA (07) followed by 20 kR (05), 15 kR (04) and 0.1% HA + 0.1% MH. The minimum mutants were observed (01) in 25kR. (Kar and Swain also observed dwarf mutants using 70kR gamma rays and Datta *et al*. 2006) [3] got. Fascinated mutants by the use of 30kR gamma rays in their study which are in support the present investigation.

Table 3: Spectrum and Frequency of Stem Type Mutations in M_2 Generation

S.No.	Treatment	Total Population	Stem Type Mutations							Total
			Dwarf Stem	Tall Stem	Fascinated Stem	Thick Stem	Un-branched	Lax Branched	Bushy	
1	Dry Control									
2	15 kR	778		04 (0.51)						04 (0.51)
3	20 kR	738				03 (0.41)	02 (0.27)			05 (0.68)
4	25 kR	802						01 (0.21)		01 (0.12)
5	30 kR	946		03 (0.32)						03 (0.32)
6	45 kR	623								
7	60 kR	427						02 (0.47)		02 (0.47)
8	Wet Control									
9	15 kR + 0.1 HA	816							02 (0.36)	04 (0.48)
10	15 kR + 0.1 MH	925	01 (0.12)							03 (0.32)
11	20 kR + 0.2 HA	568			03 (0.32)					
12	20 kR + 0.2 MH	653				03 (0.46)				03 (0.46)
13	0.1 HA	1043	04 (0.38)		03 (0.29)					07 (0.67)
14	0.1 MH	835					02 (0.24)			02 (0.24)
15	0.2 HA	587		02 (0.34)						02 (0.34)
16	0.2 MH	428		03 (0.70)						03 (0.70)
17	0.1 HA + 0.1 MH	169	02 (1.18)						02 (1.18)	04 (2.36)
18	0.2 HA + 0.2 MH	208							03 (1.44)	03 (1.44)
Total		10546	07 (0.067)	12 (0.11)	06 (0.056)	06 (0.056)	04 (0.038)	03 (0.028)	08 (0.076)	46 (0.44)

b. Leaf type mutants

Data on leaf type mutants are given in table 4, a perusal of which reveals that the largest spectrum 7 plants (3 narrow and 4 thick) leaf type mutants were observed in 20 kR. However, maximum frequency (0.70%) (03 long petiole) was observed in 60 kR. The occurrence of narrow leaf (10) was most frequent as compared to elongated (07) and thick (09) leaf mutants. Chowdhury and Datta (2008) also observed similar type of leaf mutants in their study by the use of gamma rays treatments.

Table 4: Spectrum and Frequency of Leaf Type Mutations in M_2 Generation

S.No.	Treatment	Total Population	Leaf Type Mutations						
			Narrow Leaf	Elongated	Brunchy	Thick	Long Petiole	Ternate	Total
1	Dry Control								
2	15 kR	778						01 (0.13)	01 (0.13)
3	20 kR	738	03 (0.41)			04 (0.54)			07 (0.95)
4	25 kR	802		03 (0.37)					03 (0.37)
5	30 kR	946	02 (0.21)		02 (0.21)				04 (0.42)
6	45 kR	623				02 (0.32)			02 (0.32)
7	60 kR	427		01 (0.23)			03 (0.70)		04 (0.93)
8	Wet Control								
9	15 kR + 0.1 HA	816		02 (0.25)	04 (0.49)				06 (0.74)
10	15 kR + 0.1 MH	925	01 (0.11)						01 (0.11)
11	20 kR + 0.2 HA	568				03 (0.53)			03 (0.53)
12	20 kR + 0.2 MH	653							
13	0.1 HA	1043	03 (0.29)						03 (0.29)
14	0.1 MH	835	01 (0.12)						01 (0.12)
15	0.2 HA	587		01 (0.17)					01 (0.17)
16	0.2 MH	428							
17	0.1 HA + 0.1 MH	169						01 (0.59)	01 (0.59)
18	0.2 HA + 0.2 MH	208					02 (0.96)		02 (0.96)
Total		10546	10 (0.094)	07 (0.066)	06 (0.057)	09 (0.085)	05 (0.047)	02 (0.019)	39 (0.37)

c. Flower type mutations

A perusal of table 5 reveals that highest mutants (10) were recorded for early flowering in 20kR + 0.2% HA treatment while late flowering mutants were also observed (05) in 0.1% HA, (04) in 45 kR and (03) in 0.2% MH treatments small sized (04) and color less flower (01) were recorded in 60 kR treatments.

Table 5: Spectrum and Frequency of Flower and Capsule Type Mutations in M_2 Generation

S.No.	Treatment	Total Population	Flower Type Mutations						Capsule Type Mutations					Grand total
			Early Flowering	Late Flowering	Small Flowers	Colorless Flowers	Pigmented	Total	Large	Small	Pattern	High in Number	Total	
1	Dry Control													
2	15 kR	778								04 (0.51)	02 (0.26)	02 (0.26)	08 (1.02)	08 (1.02)
3	20 kR	738												
4	25 kR	802												
5	30 kR	946	04 (0.42)					04 (0.42)		03 (0.32)			03 (0.32)	07 (0.74)
6	45 kR	623		04 (0.64)				04 (0.64)						08 (1.28)
7	60 kR	427			04 (0.94)	01 (0.23)		5 (1.17)		04 (0.94)			04 (0.94)	09 (2.11)
8	Wet Control													
9	15 kR + 0.1 HA	816								03 (0.36)			03 (0.36)	03 (0.36)
10	15 kR + 0.1 MH	925												
11	20 kR + 0.2 HA	568	10 (1.76)					10 (1.76)						10 (1.76)
12	20 kR + 0.2 MH	653												
13	0.1 HA	1043		05 (0.48)								04 (0.38)	04 (0.38)	09 (0.86)
14	0.1 MH	835												
15	0.2 HA	587												
16	0.2 MH	428		03 (0.70)										03 (0.70)
17	0.1 HA + 0.1 MH	169												
18	0.2 HA + 0.2 MH	208												
Total		10546	14 (0.13)	12 (0.11)	4 (0.037)	1 (0.00P)				14 (0.13)	02 (0.026)	06 (0.05)		

Figures in parentheses are in percentage

d. Capsule type mutations

Table 5 showed that small sized capsule were recorded (04) in 15 kR and 60 kr both and (03) in 15 kR + 0.1% HA. However, 03 plants recorded as sterile. 02 mutants in 15 kR showed the special pattern of capsule arrangement on the stem (6 capsules at one axil point). The capsules were short and closely attached. The treatment 0.1% HA showed high number of capsule per plant. Sorour (1999) [14], Sarvor and

Haq (2005) [10] and Ibrahim et al. (2010) had also observed high number of capsules per axil due to gamma-ray and EMS treatments. Diouf (2010) [4] also got closed capsule mutants in their study using 30 and 40 kR gamma rays.

e. Total Mutation Rate

A perusal of table 6 reveals that the highest mutated families (4.14%) in 0.1% HA + 0.1% MH treatment being followed by 60 kR (3.74%) and 0.2% HA + 0.2% MH (2.88). It is because of higher number of mutants in low survival population to higher mutagen dose. Sengupta & Dutta (2004) [13] due to high EMS doses were also able to get high frequency of viable mutations in sesame.

f. Effectiveness and Efficiency

Effectiveness and Efficiency of different treatments are presented in Table 6, which reveals that the application of chemical treatments and gamma rays and their combination with gamma rays (treatment 0.1% HA + 0.1% MH) showed high effectiveness along with efficiency. The effectiveness is followed by 0.2 HA+0.2 MH and efficiency being followed by 60 kR treatment to gamma rays. It is due to higher dose of mutagens resulting greater biological changes (injury, lethality and sterility). These results get support from the findings of Girjesh Kumar and Yadav (2010) [7] who observed increasing effectiveness and efficiency with increased dose of mutagens.

Table 6: Effectiveness and Efficiency of Mutagenic Treatments

S No.	Treatment	Total Population	Mutation rate		Effectiveness	Efficiency
			Total no. of mutants	Mutation rate/ frequency (MF)		
1	Dry Control					
2	15 kR	778	14	1.79	0.12	0.018
3	20 kR	738	15	2.05	0.10	0.020
4	25 kR	802	09	1.12	0.04	0.012
5	30 kR	946	20	2.11	0.07	0.022
6	45 kR	623	10	1.60	0.04	0.017
7	60 kR	427	16	3.74	0.06	0.049
8	Wet Control					
9	15 kR + 0.1 HA	816	15	1.83	1.22	0.024
10	15 kR + 0.1 MH	925	08	0.86	0.57	0.042
11	20 kR + 0.2 HA	568	13	2.29	0.57	0.032
12	20 kR + 0.2 MH	653	03	0.46	0.12	0.0066
13	0.1 HA	1043	21	2.01	20.10	0.025
14	0.1 MH	835	07	0.84	8.40	0.009
15	0.2 HA	587	03	0.51	2.55	0.0077
16	0.2 MH	428	08	1.87	9.35	0.027
17	0.1 HA + 0.1 MH	169	07	4.14	414.00	0.0735
18	0.2 HA + 0.2 MH	208	06	2.88	72.00	0.042
Total		10546	175	1.66		

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