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Studies on the Mutagenic Effectiveness and Efficiency of Gamma Rays, EMS and Combined Treatment in Sesame (*Sesamum indicum* L.) var.TMV3.

Anbarasan K, R Rajendran* and D Sivalingam.

Department of Botany, Annamalai University, Annamalainagar-608 002, Tamil Nadu, India

ABSTRACT

The present investigation deals with the assessment of effectiveness and efficiency of EMS, gamma rays and their combination on M_1 and M_2 generations of sesame. The seeds of sesame var.TMV3 were treated with different doses of gamma rays such as 30, 40, 50, 60 and 70KR; EMS (0.6, 0.8, 1.0 1.2 and 1.4mM) and the combined treatments of both EMS and gamma rays like 30KR +0.6mM, 40KR + 0.8mM, 50KR + 1.0mM, 60KR + 1.2mM and 70KR + 1.4mM. The treated and untreated (control) seeds were sown in randomized block design with three replications. The effects of mutagens were analyzed in M_1 and M_2 generations. In M_1 generation, the seed germination percentage on 15th day, seedling survival (lethality) on 30th day and seedling height (Injury) on 30th day were recorded. In M_2 generation, the chlorophyll mutants like chlorina, xantha, viridis and albino were observed from 10 to 15th days and the mutation frequency was calculated based on their occurrence. The results showed a gradual reduction in germination, survivability and seedling height in M_1 generation. The mutation effectiveness and efficiency were gradually decreased with increasing doses /concentrations of mutagens used.

Keywords: Sesame, mutation, EMS, gamma rays, frequency, effectiveness, efficiency.

**Corresponding author*

INTRODUCTION

Sesame (*Sesamum indicum* L.) is one of the world's important oil seed crops and belongs to the family Pedaliaceae. It is self pollinated annual shrub grown in tropical, sub-tropical and southern temperate areas of the world, especially in India, China, South America and Africa. Sesame is commonly known as "Queen of the oil seeds", because among the oil crops, it contains highest oil content and protein. The oil and fatty acid compositions are determined by genetic and environmental factors and the oil content of sesame ranges from 34 to 63% [1-[3]. Sesame contains high per cent of antioxidants such as sesamol, sesamin, sesamolin and sesaminol and four main fatty acids like palmitic, stearic, oleic and linoleic and also rich in minerals like vitamin E, calcium, magnesium and phosphorus [4, 5]. The sesame oil is used to reduced blood cholesterol, high blood pressure and prevent the a atherosccolesterol, heart diseases and cancers [6, 7]. The oil cake of sesame has high protein content with essential amino acids like methionine and tryptophan. The oil cake is also used as fodder.

Mutation is a sudden heritable change in amount and arrangement of DNA of an organism. A number of physical and chemical agents are used to induce genetic variation in crop plants artificially and the agents are known as mutagens and the process is called induced mutation. Induce mutagenesis is considered an effective and potential method to create genetic variability especially in crop plants [8]. Gamma rays belong to ionizing radiation and produced by decaying of some radio isotopes like, C^{14} and Co^{60}. Gamma rays are directly penetrating the plant tissues and partially ionizing. Depending upon the level of radiation, it affects the cellular structure and metabolism of plants like deletion of thylakoid membranes, alteration in photosynthesis, modulation of the antioxidative system and accumulation of phenolic compounds [9] [10]. Nowadays gamma rays are commercially used for induction of mutation and a great majority of mutant varieties (64%) were developed by the use of gamma rays [11].

EMS (Ethyl Methane Sulphonate) is a mutagenic organic chemical. it induces mutations in genetic materials by nucleotide substitution, typically quanine alkylation and it produces only point mutation [12] and it is reported as the most effective and powerful mutagen when compared to other mutagenic agents [13]. Gamma rays and EMS could be fruitfully applied to develop new varieties with high yield and other improved agronomic traits [14]. Many new cultivars have been directly or indirectly released in the world through induced mutation and it allowing to isolate mutants with desirable characters of economical importance such as high productivity, early flowering and maturity [15], modified plant architecture, closed capsules, disease resistance [4, 16] seed retention, large seed size, desirable seed color and high oil content [17].

MATERIALS AND METHODS

Two hundred well matured dry seeds of sesame were pre-soaked in distilled water for two hours and transferred to freshly prepared different concentrations of EMS (0.6, 0.8, 1.0, 1.2 and 1.4mM) for four hours with regular shaking. After completion of treatment, the treated seeds were carefully removed from the EMS solutions and washed in tap water for four to five times. Two sets of two hundred healthy dry seeds of sesame were baked in moist germinating paper and treated with various doses of gamma rays like 30, 40, 50, 60 and 70KR. One set was used for analysis of individual effect of gamma rays, another set was used to combined studies, i.e., gamma rays + EMS. The gamma irradiated seeds were pre- soaked in distilled water and the filtered seeds were directly added to the freshly prepared EMS solutions, i.e., 30KR+0.6mM, 40KR+0.8mM, 50KR+1.0mM, 60KR+1.2mM and 70KR+1.4mM for four hours. Untreated seeds were soaked in distilled water for six hours and used as control. The treated and untreated seeds were sown in the field with three replications to raise M_1 generation. The total number of seeds germinated on 15^{th} day after sowing was counted in each treated population and expressed in percentage and total number of seedling survival and height of seedling were measured on 30^{th} day and it was expressed in percentage and cm. All the recommended cultural practices like irrigation, weeding and plant protection methods were carried out during the period of crop growth and all the survived plants of M_1 generation were harvested separately and the seeds were collected carefully. The M_1 generation seeds were sown in a randomized block design (RBD) with three replications for raising M_2 generation. The M_2 generation seedlings were screened from 10 to 20 days to record various types of chlorophyll mutants periodically. Identification of chlorophyll mutants was done based on the nomenclature adopted by Gustafson [18]. The mutation frequency was estimated on the basis of number of chlorophyll mutants and the effectiveness and efficiency of mutagens were worked out by using the formulae suggested by Konzok *et al*.[19].

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Table 1: Effect of mutagens on seed germination (15th day), seedling survival and seedling height (30th day)

Doses / Concentrations of mutagens		Seed germination on 15th day		Seedling survival on 30th day		Seedling height on 30th day		
		Mean ± SE (%)	Per cent over control	Mean ± SE (%)	Per cent over control	Range	Mean ± SE (%)	Per cent over control
Gamma rays (KR)	Control	84.19±2.52	00.00	79.01±2.37	00.00	25.02 - 40.06	37.33 ± 1.11	00.00
	30	81.05±2.43	-3.73	68.29±2.04	-13.57	23.06 - 39.01	32.03 ± 0.96	-14.19
	40	78.84±2.36	-6.36	61.92±1.85	-21.63	20.07 - 37.04	30.53 ± 0.91	-18.21
	50	70.29±2.10	-16.52	53.11±1.59	-32.78	20.00 - 36.04	28.63 ± 0.85	-23.30
	60	66.48±1.99	-21.04	49.20±1.47	-37.72	18.08 - 34.62	27.13 ± 0.81	-27.32
	70	60.77±1.82	-27.82	44.38±1.33	-43.82	16.07 - 31.05	25.83 ± 0.77	-30.80
EMS (mM)	0.6	74.00±2.22	-12.11	66.30±1.98	-16.08	24.02 - 37.01	31.02 ± 0.93	-16.90
	0.8	70.03±2.10	-16.82	60.22±1.80	-23.78	21.01 - 34.02	28.92 ± 0.86	-22.52
	1.0	68.09±2.04	-19.13	56.83±1.70	-28.07	20.05 - 33.05	27.91 ± 0.83	-25.23
	1.2	63.33±1.89	-24.74	48.02±1.44	-39.22	18.07 - 32.01	24.02 ± 0.72	-35.65
	1.4	59.97±1.79	-28.77	41.16±1.23	-47.90	15.07 - 30.02	22.99 ± 0.68	-38.41
Combined (Gamma rays + EMS)	30+0.6	75.65±2.26	-10.15	58.86±1.76	-25.50	22.02 - 36.03	28.64 ± 0.85	-23.27
	40+0.8	74.58±2.23	-11.42	52.21±1.56	-33.91	20.04 - 35.04	26.33 ± 0.78	-29.46
	50+1.0	69.74±2.09	-17.17	46.68±1.40	-40.91	17.04 - 33.04	23.54 ± 0.70	-36.94
	60+1.2	62.82±1.88	-25.39	44.03±1.32	-44.27	16.07 - 31.03	22.93 ± 0.68	-38.57
	70+1.4	57.62±1.72	-31.56	41.03±1.23	-48.06	15.03 - 28.03	21.24 ± 0.63	-43.10

Table 2: Mutation frequency of chlorophyll mutants in M_2 generation

Doses / Concentrations of Mutagens		Gamma rays (KR)					EMS (mM)					Combined (Gamma rays + EMS)				
		30	40	50	60	70	0.6	0.8	1.0	1.2	1.4	30+0.6	40+0.8	50+1.0	60+1.2	70+1.4
No. of plant studied		515	560	605	551	520	506	533	605	578	542	506	522	603	624	547
Chlorophyll Mutants	Chlorina	1	2	2	2	1	2	2	3	1	-	1	2	2	2	1
	Albino	3	4	5	3	3	2	3	6	4	2	3	4	6	5	3
	Xantha	1	1	1	1	1	1	1	1	1	3	1	1	1	1	1
	Viridis	2	1	2	1	-	2	2	1	1	1	3	2	3	2	2
	Total	7	8	10	6	4	7	8	11	7	6	8	9	12	10	7
	Frequency	1.35	1.42	1.65	1.08	0.76	1.38	1.50	1.81	1.21	1.10	1.58	1.72	1.99	1.60	1.27

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Table 3: Mutagenic effectiveness and efficiency of mutagens in M_2 generation

Doses / Concentrations of mutagens		Survival Reduction (L)	Height Reduction (I)	Mutation Frequency	Effectiveness	Efficiency	
						M /L	M/I
Gamma rays (KR)	30	13.57	14.19	1.35	0.34	0.099	0.095
	40	21.63	18.21	1.42	0.20	0.065	0.077
	50	32.78	23.30	1.65	0.15	0.049	0.052
	60	37.72	27.32	1.08	0.07	0.028	0.039
	70	43.82	30.80	0.76	0.04	0.017	0.024
EMS (mM)	0.6	16.08	16.90	1.38	0.37	0.085	0.081
	0.8	23.78	22.52	1.50	0.25	0.063	0.66
	1.0	28.07	25.23	1.81	0.17	0.052	0.053
	1.2	39.22	35.65	1.21	0.16	0.030	0.033
	1.4	47.90	38.41	1.10	0.13	0.022	0.028
Combined (Gamma rays + EMS)	30+0.6	25.50	23.27	1.58	0.39	0.061	0.067
	40+0.8	33.91	29.46	1.72	0.24	0.056	0.058
	50+1.0	40.91	36.94	1.99	0.18	0.053	0.055
	60+1.2	44.27	38.57	1.60	0.11	0.036	0.041
	70+1.4	48.06	43.10	1.27	0.07	0.026	0.029

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CHLOROPHYLL MUTANTS

Chlorina (1.0mM)

Chlorina (70 KR)

Chlorina (1.2 mM)

Chlorina, Virds (70 KR + 1.4 mM)

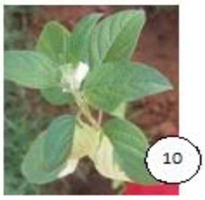
Virids (1.0mM)

Virids (1.2mM)

Virids (50KR)

Virids (40 KR + 0.8mM)

Virids(70KR)



Albino(50KR)

Albino (0.8mM)

Albino (1.4mM)

Albino (60KR +1.2mM)

Albino (70KR +1.4mM)

Xantha (70KR)

Xantha (0.8mM)

Xantha (1.4mM)

Chlorina (40KR + 0.8mM)

RESULTS AND DISCUSSION

The present study revealed that the seed germination percentage decreased progressively as the doses/ concentrations of mutagens treatment increased and the highest percentage of reduction in seed germination was observed at 70KR+1.4mM of combined treatment(31.56) followed by 1.4mM of EMS (28.77) and 70KR gamma rays (27.82) (Table-1). There are many early reports on dose depended inhibition of seed germination in sesame given by Gual,[20], Pugalendi,[21]; Dhanavel *et al*. [22] in cowpea, Kulkarni Ganesh, [23] in horse gram. The reduction in seedling survival (lethality) and seedling height (injury) was observed in all the mutagenic treatments.

It was progressively increased with lower to higher doses/ concentrations of mutagens and the considerable reduction was observed at 70KR+1.4mM of combined treatment (48.06; 43.10), 1.4mM of EMS (47.90; 38.41) and 70KR of gamma rays (43.82; 30.80) (Table-1). This inhibitory effect was also reported by Gaul [20] in sesame, Pavadai and Dhanavel [24] in soybean and Velu *et al*.[25] in cluster bean.

In the present investigation, the spectrum of chlorophyll mutants like chlorina, viridis, albino and xantha were observed in individual and combined treatments (Figs. 1-18). Among these chlorophyll mutants albino was more abundance in all individual and combined treated progenies (Table -2).

Mutation frequency

The mutation frequency was estimated based on the number of chlorophyll mutants observed in M_2 generation. It was gradually increased with lower to higher doses/ concentrations of mutagens. The maximum mutation frequency was observed at 50KR + 1.0mM of combined treatment (1.99), 1.0mM of EMS (1.81) and 50KR of gamma rays (1.65) (Table -2). The combined treated progenies showed highest frequency than individual treatment of EMS and gamma rays.

Mutagenic effectiveness and Efficiency

Mutagenic effectiveness is a measure of the frequency of mutation induced by unit mutagen. Whereas mutagenic efficiency gives an indication of the proportion of mutation in relation to undesirable changes like lethality, injury and sterility. In the present study, the combined treatment showed more effective than EMS and gamma rays in inducing mutations. The highest mutation effectiveness and efficiency was observed at lower doses / concentrations of mutagens, *i.e*., 30KR+0.6mM of combined treatment (0.39), 0.6mM of EMS (0.37) and 30KR of gamma rays (0.34) (Table- 3). In the present study, the effectiveness and efficiency was gradually decreased with increasing doses or concentrations of mutagens. Similar observations have been reported earlier by Jayakumar and Selvaraj [26] in sun flower, Solanki [27] in lentil and Sharma *et al*.[28] in urd bean.

CONCLUSION

Based on the observation, the sesame genotype TMV3 was treated with gamma rays and EMS individually and also the combination of both. In M_1 generation, seedling survival (lethality) and seedling height reduction (Injury) were increased with increasing doses / concentrations of mutagens. The maximum chlorophyll mutants and frequency was recorded in combined treatment than individual treatment of gamma rays and EMS and also the combined treatment showed more effective than other mutagenic treatments.

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