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Induced mutagenesis in sesame (*Sesamum indicum* L.)

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Abstract
Three sesame entries *viz.* Gujarat Til-4 (GT-4), Gujarat Til-10 (GT-10) and Patan-64 were treated with 0.5%, 1.0%, 1.5%, 2.0% and 2.5% doses of Ethyl Methane Sulphonate (EMS). In M_1 generation the mutagenic effect of EMS on seed germination with respect to different doses was studied. In M_2 generation analysis of variance, estimation of genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability and genetic advance (GA) were undertaken. Characters like plant height, number of pods per plant, 1000 seed weight and yield per plant exhibited high values of GCV and PCV whereas high heritability coupled with high genetic advance as percent of mean were observed for plant height, number of capsules per plant and yield per plant.

Keywords: *Sesamum indicum*, EMS, seed germination, mutation, coefficient of variation, heritability

Introduction
Sesame (*Sesamum indicum* L.) 2n = 2*x* = 26 is a self pollinated oilseed crop, of the family Pedaliaceae. It is called the "Queen of oil seeds" because of its excellent qualities of the seed, oil and meal. Brown or black seeded are valued more for oil whereas, white seeded are rich in iron. India is a major producer of sesame (Anon., 2017) [4]. The major sesame producing states in India are West Bengal, Madhya Pradesh, Rajasthan, Gujarat and Uttar Pradesh. In Gujarat, there is a total production of about 0.10 million tonnes and an average productivity of about 564 kg/ha (Anon., 2015) [3]. Since there is a limitation of available area due to urbanization, increasing productivity is the only approachable way to augment sesame production in India. This will eventually need variability for selection or hybridization for breeding high yielding varieties, however germplasm of sesame is not as large as in other crops (Ashri, 1982) [5] secondly, the genetic architecture of sesame is poorly adapted to mechanized farming system due to its indeterminate growth habit, sensitivity to wilting under intensive management and seed shattering at maturity (Uzun and Cagirgan, 2006) [12]. Hence, creation of variation for improvement of one or two traits becomes a necessity for this crop. Mutation breeding is an effective tool for crop improvement and an efficient mean to supplement existing germplasm for cultivar improvement in breeding programmes. It has been employed successfully to foster additional variability for qualitatively and quantitatively inherited traits in a number of crop plants e.g. rice (Talebi *et al.*, 2012) [11], sesame (Begum and Dasgupta, 2014) [6] etc. Therefore the present investigation was undertaken with the objective to study the effect of chemical mutagen (EMS) on three genotypes which eventually will help plan further breeding programmes for improvement of sesame, to work out GCV and PCV and to estimate heritability and genetic advance (GA) for assessing the heritable portion of total variation.

Materials and Methods
The present study was under taken at agronomy farm, B. A. College of agriculture, Anand Agricultural University, Anand during summer season (2016) for M_1 generation and *kharif* 2016 for M_2 generation. The farm is located in Agro-climatic zone-III (Middle Gujarat) of Gujarat state. Geographically Anand is situated at 22° 35' N Latitude and 72° 55' E longitude with an altitude of 45.1 meters above the mean sea level. Three sesame genotypes were used namely Gujarat Til-4 (V_1), Gujarat Til-10 (V_2) and Patan 64 (V_3). A chemical mutagen Ethyl Methane Sulphonate (EMS) with concentrations of 0.5%, 1.0%, 1.5%, 2.0% and 2.5% along with a control were also used for inducing mutation in this study.

Preparation of EMS and process of application
Seeds of GT-4, GT-10, Patan 64 were soaked overnight in distilled water at room temperature. Next morning, seeds were removed from water and treated with EMS solution (0.5%, 1.0%, 1.5%, 2.0%, 2.5%) prepared in phosphate buffer of pH-7 for 5 hrs. Intermittent shaking,

followed by decantation of the EMS and rinsing with tap water and later by distilled water for ten times were carried out.

Table 1: Dose and symbols in M_1 and M_2 generation

Variety	Dose and symbols in M_1 generation					
GT-4	V_1D_1 (0.5%)	V_1D_2 (1.0%)	V_1D_3 (1.5%)	V_1D_4 (2.0%)	V_1D_5 (2.5%)	V_1D_6 (Control)
GT-10	V_2D_1 (0.5%)	V_2D_2 (1.0%)	V_2D_3 (1.5%)	V_2D_4 (2.0%)	V_2D_5 (2.5%)	V_2D_6 (Control)
Patan 64	V_3D_1 (0.5%)	V_3D_2 (1.0%)	V_3D_3 (1.5%)	V_3D_4 (2.0%)	V_3D_5 (2.5%)	V_3D_6 (Control)

V = Variety, D =Dose, Control = For each variety, seeds were pre soaked in distilled water for 6 hours to serve as control.

Variety	Dose and symbols in M_2 generation				
GT-4	V_1D_1 (0.5%)	V_1D_2 (1.0%)	V_1D_3 (1.5%)	V_1D_4 (2.0%)	V_1D_5 (Control)
GT-10	V_2D_1 (0.5%)	V_2D_2 (1.0%)	V_2D_3 (1.5%)	V_2D_4 (2.0%),	V_2D_5 (Control)
Patan 64	V_3D_1 (0.5%)	V_3D_2 (1.0%)	V_3D_3 (1.5%)	V_3D_4 (2.0%)	V_3D_5 (Control)

V = Variety, D =Dose, Control = non treated seeds harvested in bulk from M_1.

In M_1 generation

The treated and controlled seeds were spread over moist germinating paper in petriplates and germination per cent of seeds was observed on 7th day and 15th day. The observed data was then analyzed by appropriate statistical procedures as suggested for Completely Randomized Design (Factorial) by Steel and Torrie (1960) [10].

In M_2 generation

In M_2 generation the data recorded during the experiment were analyzed statistically following the analysis of variance for 2 factors experiment in FRBD (Gomez and Gomez, 2010) [9]. The data were collected on individual plant basis from five randomly selected plants from each replication and averaged. Mean value was calculated for plant height, number of primary branches, number of capsule per plant, number of seeds per capsule, 1000 seed weight and seed yield per plant. GCV and PCV were calculated by the formula suggested by Burton and Devane, (1953) [8]. Heritability and genetic advance (GA) were estimated by adopting the procedure suggested by Allard (1960) [1].

Results and Discussions

M_1 Generation

Analysis of variance for seed germination per cent revealed that the variance due to variety, dose and interaction between variety and dose were highly significant which indicated the existence of variability in the experimental material.

Table 2: Analysis of variance for germination per cent in M_1 generation of sesame.

Source of Variation	DF	Mean square at 7th day	Mean square at 15th day
Variety (V)	2	85.99**	26.01**
Dose (D)	5	3263.78**	10790.00**
VXD	10	17.20**	14.79**
Error	36	0.18	0.49

* Significant at 5% level of significance, ** Significant at 1% level of significance

From the table of Mean data (Table 3 and 4) it is evident that the seed germination per cent in the genotype subjected to treatment with different doses of EMS is less than those of their respective controls. It clearly indicates that treatment with EMS have exerted an inhibitory effect on seed germination. It was observed that out of 18 treatments only 15 treatments were studied in M_2 because the dose D_5 (2.5% EMS) did not germinate in any of the variety in field as well as in laboratory condition. Hence, D_5 (2.5% EMS) was removed while carrying out study in M_2 generation. A dose dependent reduction of germination was clearly evident from the values recorded on 7th and 15th day. Similar findings were earlier reported by Boranayaka *et al.* (2010) [7] and Anabarasan *et al.* (2013) [2].

Table 3: Mean values of genotypes for sesame in M_1 Generation (7th day).

Variety	Dose						
	D_1	D_2	D_3	D_4	D_5	D_6	Mean
V_1	46.00	34.00	28.00	18.00	0.00	54.00	30.00
V_2	48.00	40.00	34.00	18.00	0.00	56.00	32.66
V_3	42.00	34.00	26.00	20.00	0.00	48.00	28.33
Mean	45.33	36.00	29.33	18.66	0.00	52.66	10.11
For comparing the means of :-		SEm±			CD		
Variety (V)		0.10			0.29		
Dose (D)		0.14			0.41		
VXD		0.24			0.71		
CV%		4.19					

Table 4: Mean values of genotypes for sesame in M_1 generation (15th day).

Variety	Dose						
	D_1	D_2	D_3	D_4	D_5	D_6	Mean
V_1	86.00	82.00	76.00	68.00	0.00	96.00	68.00
V_2	90.00	84.00	76.00	70.00	0.00	98.00	69.66

V_3	84.00	78.00	74.00	74.00	0.00	94.00	67.33
Mean	86.66	81.33	75.33	70.66	0.00	96.00	22.77
For comparing the means of :-		SEm±			CD		
Variety (V)		0.16			0.47		
Dose (D)		0.23			0.67		
VXD		0.40			1.17		
CV%		3.07					

M_2 Generation

The analysis of variance for different characters studied in M_2 generation (table 5) revealed significant differences of all the characters which indicated the existence of variability in the experimental material.

In sesame generally medium height (75-125 cm) is preferred but from the results it was found that for variety GT-4, D_2 (1.00% EMS) and control (D_5) showed desirable height, as for variety GT-10, D_1 (0.5% EMS), D_3 (1.5% EMS) and D_4 (2.00% EMS) along with control (D_5) showed desirable height indicating the positive outcome from mutagenesis. The genotype GT-10 with D_5 *i.e* control recorded maximum number of primary branches while the genotype GT-4 with dose D_1 *i.e.* 0.5% EMS had minimum number of primary branches.

For number of capsule per plant and number of seed per capsule treatment V2D3 (1.5% EMS) recorded high values suggesting their superiority than control which was a positive result from mutagenesis. GT-10 with control dose (D_5) recorded maximum 1000 seed weight while Patan 64 with dose D_4 *i.e.* 2.00% EMS had the least 1000 seed weight. The genotype GT-10 with control dose (D_5) recorded maximum yield per plant while Patan 64 with dose D_4 *i.e.* 2.00% EMS recorded the least yield per plant.

Table 5: Analysis of variance for various characters in M_2 generation of sesame.

Source of variation	D.F.	Mean squares for different characters					
		Plant height	Number of primary branches	Number of capsule per plant	Number of seeds per capsule	1000 seed weight	Yield per plant
Replication	2	51.75	0.02	33.09	7.52	0.09	0.09
Treatment	14	988.51**	0.35**	224.55**	85.62**	0.86**	20.44**
Variety	2	2956.28**	1.33**	665.47**	255.97**	1.92**	14.16**
Dose	4	729.71**	0.21*	141.87**	89.27**	1.28**	60.09**
Var X Dose	8	625.97**	0.19*	155.66**	41.22*	0.40**	2.19**
Error	28	60.76	0.06	10.68	17.84	0.10	0.10

* Significant at 5% level of significance, ** Significant at 1% level of significance

Table 6: Mean values of genotypes for different characters of sesame in M_2 generation.

Treatments	Plant height (cm)	Number of primary branches	Number of capsule per plant	Number of seeds per capsule	1000 seed weight (g)	Yield per plant (g)
	1	2	3	4	5	6
Varieties						
V_1	63.97	2.48	18.39	28.37	1.81	2.20
V_2	89.99	3.05	29.76	33.57	2.39	3.58
V_3	67.84	2.63	18.07	25.41	1.73	1.71
Min	63.97	2.48	18.07	25.41	1.73	1.71
Max	89.99	3.05	29.76	33.57	2.39	3.58
SEm±	2.01	0.066	0.84	1.09	0.08	0.08
CD (5%)	5.83	0.18	2.44	3.15	0.24	0.23
Dose						
D_1	72.70	2.67	20.04	29.33	1.87	1.32
D_2	66.57	2.64	18.16	25.82	1.92	1.14
D_3	70.73	2.78	23.44	34.24	1.98	1.89
D_4	70.11	2.56	20.40	27.64	1.54	1.05
D_5	89.54	2.96	28.31	28.56	2.58	7.08
Min	66.57	2.56	18.16	25.82	1.54	1.05
Max	89.54	2.96	28.31	34.24	2.58	7.08
SEm±	2.59	0.08	1.08	1.40	0.10	0.10
CD (5%)	7.52	0.24	3.15	4.07	0.31	0.30
VXD						
V_1D_1	61.66	2.13	14.00	25.67	1.27	0.45
V_1D_2	79.00	2.47	23.20	29.80	1.80	1.42
V_1D_3	43.93	2.53	10.53	30.07	1.73	0.61
V_1D_4	51.93	2.53	12.47	26.47	1.67	0.67
V_1D_5	83.33	2.73	31.73	29.87	2.60	7.84
V_2D_1	84.70	3.00	27.60	32.93	2.33	2.29
V_2D_2	66.70	2.87	17.80	27.67	2.33	1.45
V_2D_3	93.64	3.13	39.40	40.87	2.27	3.79
V_2D_4	89.47	2.67	31.73	30.93	2.27	2.16
V_2D_5	115.43	3.60	32.27	35.47	2.73	8.24

V_3D_1	71.75	2.87	18.53	29.40	2.00	1.21
V_3D_2	54.00	2.60	13.47	20.00	1.63	0.57
V_3D_3	74.63	2.67	20.40	31.80	1.93	1.28
V_3D_4	68.93	2.47	17.00	25.53	0.68	0.34
V_3D_5	69.87	2.53	20.93	20.33	2.40	5.17
Mean	73.93	2.72	22.07	29.12	1.97	2.49
Min	43.93	2.13	10.53	20.00	0.68	0.34
Max	115.43	3.60	39.40	40.87	2.73	8.24
	**	*	**	*	**	**
SEm±	4.50	0.14	1.88	2.43	0.18	0.18
CD (5%)	13.03	0.42	5.46	7.06	0.54	0.52
CV(%)	10.5	9.3	14.8	14.5	16.3	12.4

* Significant at 5% level of significance, ** Significant at 1% level of significance

The estimates of variance component revealed that the characters plant height, number of capsules per plant, 1000 seed weight, yield per plant, showed larger contribution of genotypic variance to total variance and also expressed high magnitude of heritability, which revealed less influence of environmental factors on the expression of these characters. Rest of the characters showed moderate heritability, indicating high influence of environmental factors on expression of these characters. Extent of variability in the experimental material was high for characters like plant height, number of capsules per plant, 1000 seed weight, yield per as indicated by high estimates of GCV; while rest of the characters had moderate GCV per cent.

The estimates of genetic advance as per cent of mean were high coupled with high heritability for plant height, number of capsules per plant; yield per plant indicating the predominance of additive gene action in the inheritance of these traits, hence, simple selection would be effective for genetic improvement of the characters in desired direction. For number of seed per capsule genetic advance as per cent of mean were high coupled with moderate to low heritability indicating limited scope of improvement.

Table 7: Estimates of genotypic variance (σ^2_g), phenotypic variance (σ^2_p) genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability in broad sense (h^2_b) and genetic advance (GA) in M_2 generation of sesame.

Character	σ^2_g	σ^2_p	GCV	PCV	h^2_b (%)	GA (%)
Plant height (cm)	309.25	370.01	23.79	26.02	83.00	47.79
Number of primary branches	0.09	0.15	11.47	14.79	60.00	17.59
Number of capsule per plant	71.29	81.97	38.26	41.02	87.00	73.49
Number of seeds per capsule	22.59	40.43	16.32	21.84	55.00	25.13
1000 seed weight (g)	0.25	0.35	25.54	30.33	70.00	0.49
Yield per plant (g)	6.78	6.88	104.26	105.00	98.00	213.84

Conclusion

The findings of present investigation lead to the following summarisation

In M_1 generation there was a gradual reduction in germination per cent with the increase in dose indicating the inhibitory effect on seed germination by EMS.

In M_2 generation the characters *viz* plant height, number of capsules per plant, yield per plant displayed substantial variability, high heritability and high genetic advance. Hence, these characters could be improved by selection. The characters like number of primary branches, number of seed per capsule showed moderate magnitude of heritability and moderate to high genetic advance, hence there would be little scope for improvement of these characters through selection. For characters like 1000 seed weight the per cent genetic advance was low accompanied by high heritability which indicated predominance of non-additive gene action in the inheritance of character. Hence for the improvement of this character, population improvement approach would be remunerative.

Thus from the present investigation it can therefore be concluded that the different effects of the EMS on the three genotypes and that the induction of mutation by EMS did create variability but did not give improved result with respect to yield in M_2 generation. However, selection of mutants can only begin in the M_2 generation as the mutant genes mostly are present in heterozygous condition and it needs many generations of selfing to achieve homozygosity. It is also possible that mutations may result in chromosomal aberrations leading to deletions and inversions; this in turn may cause loosing of important genes which would have contributed to high yield. This can be one of the reasons for the fact that mutations generally do not enhance yield but can be a tool for achieving special traits of importance.

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