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Research Article

Analysis of induced genetic variability for morphological and floral characters with male sterility in sesame (*Sesamum indicum* L.)

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Abstract
Male sterility can be useful to obtain high yielding sesame hybrids with reduced cost and labour. It can be induced artificially into the released cultivars by using chemical mutagens. Hence the present study was undertaken to induce male sterility in a released sesame genotype cv. B 67 by three different concentrations of Ethyl methane sulphonate (0.25%, 0.50% and 0.75%) and Nitroso-guanidine (0.01%, 0.02% and 0.04%). The treated seeds were sown to obtain M_1 generation of mutants. This population was further advanced by progeny row method to M_4 generation where thirty five stable mutants along with parent were screened for abnormal phenotypic and floral characters. The observation indicated generation of large variability for seven morphological characters and five floral characters among the mutants while male sterility could be achieved only up to 56.79%. These male sterile plants were characterized by low plant height, polypetalous and irregular or cleistogamous flowers. The present study implies that the above chemical mutagens can be used to develop useful male sterile mutants in sesame.

Key words
Sesame, mutagens, genetic variability, male sterility, macromutant

Introduction

Sesame (*Sesamum indicum* L., 2n=26), commonly known as 'Til' is a self pollinated crop with 2x = 2n = 26 chromosomes. It is one of the ancient and most important oilseed crop cultivated mostly in the states of Gujarat, Rajasthan, Madhya Pradesh, Tamil Nadu, Odisha, Andhra Pradesh and Chhattisgarh. World production of sesame seeds was 6.2 million tonnes, led by Tanzania, India, and Sudan. It is cultivated in an area of about 1.95 million ha with a production of 8.5 lakh tones and productivity of about 436 kg/ha (SOPA, 2016). It is a short duration crop grown throughout the year and fits well into various cropping sequences/systems. Easiness in crossing through a massive manual hybridization technique was reported by Yadav and Mishra (1991) due to which exploitation of heterosis in this crop gained momentum. At present sesame hybrids can only be produced by the conventional method of hand emasculation and pollination which involves huge amount of labour and cost. Use of stable source of male sterile line will be cost effective method for production of hybrids for its commercial cultivation.

The male sterility system becomes an effective tool for hybrid seed production. The existing genetic male sterile (GMS) materials are not good. Their male sterility rates are low (less than 50%) and their agronomic characters are not ideal. Induction of mutation is an efficient method for creating new breeding materials, so research was carried out to create new male sterile breeding material that would be ideal and be used for heterosis breeding directly (Savant, 2016). The effect of male-sterile genes can occur in some of the strictly regulated stages of pollen development (Kaul, 1988; Horner and Palmer, 1995). Earlier male-sterile (MS) mutants (Kaul, 1998; Nilton *et al.*, 2005) and their cytological studies (Nilton *et al*, 2005) have been reported in many species of higher plants as the result of both spontaneous and induced mutations. There are numerous reports of induction of male sterility in many crop species by physical and chemical mutagens (Kaul, 1988). Prakash *et al.* (2001) reported that different chemicals affect male sterility system in sesame. In sesame, improvement in different morphological character (Hoballah, 1996; Sengupta and Animesh, 2004) as well as biochemical parameter (Cigdem *et al.,* 2007; Savant *et al.,* 2009; Savant and Kothekar, 2011) traits has been reported following artificial induced mutagenesis. Hence efforts were taken up to induce mutations affecting floral traits resulting in male sterility in sesame. The present research describes some of the agronomic traits and floral characters of true breeding induced macro mutants in B-67 cultivar of *S. indicum* with an objective to screen male sterile mutants.

Materials and Methods

In the present study dry and uniform seeds of sesame cv. B 67 were treated with 3 different concentrations of Ethyl methane sulphonate (EMS) and Nitroso-guanidine (NG). The effective viable concentrations of the chemicals were determined by pilot experiments (EMS: 0.25%, 0.50% & 0.75% and NG: 0.01%, 0.02% & 0.04%).

Each treatment consisted of 100 healthy sesame seeds. The seeds were pre-soaked in distilled water for 6 hours followed by treatment with different concentrations of the chemical mutagen for eight hours with intermittent shaking. The volume of mutagen solution was about 10 times the volume of seeds. All the treatments were carried out at room temperature (27 ± 1^{o} c) with intermittent shaking. The seeds treated with chemical mutagens were thoroughly washed under tap water for 30 minutes to leach out the residual chemicals adsorbed to the treated seeds and the seeds were dried on blotting paper.

Seeds from all the treatments along with the control were sown to raise M_1 generation and the seeds harvested from individual M_1 plants were used to raise M_2 generation in progeny row method. The seeds of individual M_1 plants which had more than 80% pollen sterility were sown in progeny rows. The M_2 seedlings thus raised were critically examined from seedling to maturity to spot out sterile mutants. In the sterile plants, flowers of one branch were selfed by bagging with butter paper bags (Fig 1) and in another branch flowers were allowed to open pollinate. The remaining branches were sib-mated with the normal fertile sister plants and the seeds were collected separately. Selfed seeds of the macro mutants were sown at M_3 and subsequently true breeding mutants were raised at M_4. Screening for viable mutants was done at the time of flowering and examined for pollen fertility. Based on this, thirty five viable mutants were obtained which were tested further for seven quantitative and five floral characters.

The 35 mutants along with their parent, B-67 were grown in randomized block design with 3 replications. Each entry was represented by a single row plot of 2 m. in each replication with spacing of 50 x 10 cm. All the recommended package of practices was followed to raise a healthy crop. Observations were taken from a sample of 3 randomly chosen plants in each replication for the 7 quantitative traits, *viz.*, plant height (cm), number of branches per plant, number of capsules per plant, capsule length (cm), seed number per capsule, seed weight per capsule (g) and 1000-seed weight (g). Analysis of variance was carried out for all the characters studied.

To identify male sterile mutants the floral characters were studied with respect to stigma position in the flower (below/ above anthers), anther colour (green/yellow/brown/white), anther size (small/large), anther shape (flat/ round) and pollen sterility (dark/light stained pollen). The unopened flower bud in one branch was bagged with butter paper bag for selfing and capsule formation was recorded to determine pollen sterility (Figure 1). Also pollen sterility was assessed by the standard acetocarmine stain technique. The matured anthers collected from unopened flowers were smeared and stained with 1:1 acetocarmine : glycerine solution and were observed under microscope. The fully dark stained and filled pollen grains were counted as fertile while empty, shrivelled and unstained/light pollen grains were counted as sterile. The mutant genotypes were classified into three groups for these qualitative floral characters basing on index score method (Singh and Chaudhary, 1996).

Result and Discussion

The results indicated highly significant differences among the 35 mutants (M) and the parent (P) for all the characters except for seed weight per capsule and thousand seed weight (Table 1). This indicates the presence of wide genetic variability among the mutants. High magnitude of variation in the experimental material was reflected by high values of mean and range for most of the characters among the mutants (Table 2). Plant height among the mutants varied from 25.60 cm to 141.80 cm. Five mutants recorded medium plant height between 95.14 cm to 101.97 cm and 12 mutants less than 95.14 cm. For 19 mutant genotypes it was higher than 101.97 cm (Table 3). This clearly indicates the height shows considerable variability among the mutants. Number of branches per plant ranged from 1.0 to 7.0 with a mean value of 4.02. Number of capsules per plant showed a greater variation ranging from 2.0 to 85 with a mean value of 31.13. Length of capsule showed variation ranging from 1.84 cm to 3.02 cm with a mean value of 2.42 cm. The seed number per capsule ranged from 34.0 to 71.60 with a mean value of 58.49. The seed weight per capsule ranged from 0.07g to 0.74 g with a mean value of 0.91 g. Nine mutants recorded high seed weight per capsule above 0.17g and seventeen mutants recorded low seed weight per capsule below 0.17g while ten mutants recorded medium seed weight per capsule. Thousand seed weight ranged from 1.86 g to 3.90 g with an overall mean of 2.73 gm. Twenty one mutant genotypes recorded lower seed weight (<2.69 g), one medium seed weight (2.69 to 2.76 g) and fourteen mutants genotypes recorded high thousand seed weight (>2.76 g). It is an important yield component which contributes positively towards economic yield.

High values of PCV and GCV were noted for capsule number per plant (C/P) and seed weight per capsule (SW/C). This indicates relatively higher contribution of these characters towards genetic variability. For all characters PCV was greater than GCV estimates because former includes variation due to interactions. The narrow difference between PCV and GCV for all the traits indicated that these characters were less affected by environment. Further high GCV for capsule number per plant (C/P) and seed weight per capsule (SW/C) indicates presence of better scope of genetic improvement in these traits which could be achieved using simple selection procedures. The heritability estimates ranged from 38.84 per cent in capsule length to 99.41 per cent in branch number per plant indicating varied seasonal effect on character expression. High estimates of heritability (>80 per cent) was obtained for all characters except for capsule length and seed number per capsule indicating predominance of heritable components of variation suggesting effectiveness of selection on the basis of phenotypic expression of the traits. The genetic gain (as percentage of mean) was higher for capsule length and seed number per capsule thus points to the predominance of additive effects and can be taken as unit characters for effective selection. High heritability estimates coupled with high genetic advance obtained in case of capsule number per plant indicated the presence of additive gene effect for this character and phenotypic selection in the desired direction might be quite effective for this character.

The present study indicated presence of floral abnormalities in some of the 35 mutants screened (Table 4). The position of stigma below the anthers is desirable for normal pollination to occur. In the present study the position of stigma within the flowers ranged from 1.0 (below anthers) to 2.0 (above anthers). Five mutants exhibited normal flowers with stigma positioned below the anthers while 31 mutant genotypes exhibited stigma positioned above the anthers. The anthers of the mutants exhibited a range of colour from green, yellow and brown, while one mutant exhibited white anthers. The size of anthers was small in 4 mutant genotypes while 21 mutant genotypes exhibited large anthers. About seven mutant genotypes exhibited round anthers.
Reduced pollen fertility of mutant plants is a reliable parameter indicating the effectiveness of mutagenic treatment (Kivi, 1962). On microscopic examination the pollen sterility ranged from 0 to 85.19% with a mean value of 14.05%. Four mutants were found to be above 56.79% male sterile. These mutants were found to produce small round anthers. Out of these 4 mutants, two mutants did not form pollen grain or had only 2-3 pollen grains in their anthers. Also they did not produce any capsules on selfing. The androeciums of these floral mutants were shriveled with thin pollen density. The male sterile types did not differ in habit from the normal plant except for the presence of either shrunken or green anthers and reduction in the size of the anthers.
In the present investigation a number of variations were induced by chemical mutagens for phenotypic characters like plant height, flower morphology as well as pollen count and stainability (Figure 2-12). Mutants to improve seed yield by using chemicals in sesame has also been reported by Nura *et al* (2013), Savant (2016) and Dey (2017). Decrease in fertility with increasing dose of mutagen has been reported by many researchers (Ganesan, 1995). Rangaswamy and Rathinam (1982) observed male sterile plants in sesame with shrivelled anthers containing no pollen following gamma irradiation. Brar (1982) also obtained male sterile plants in sesame which had shortened filaments of anthers, lack of viable pollen and failure of anthers to dehisce at maturity.

The present results indicate that the chemical mutagens *i.e* Ethyl methane sulphonate and Nitroso-guanidine continue to be a powerful tool to develop heterotic hybrids using induced mutants and also to induce male sterile mutations. In this present investigation male sterility in sesame could be achieved only up to 56.79% through induced mutation. However more desirable result can be achieved by refining the concentration/dose. These male sterile plants were characterized by low plant height, polypetalous, irregular or cleistogamous flowers.

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Table 1. Variability, heritability and genetic advance for seven morphological characters in sesame mutants

Characters	Range		MEAN	SE	CV %	Coefficients of variability		Heritability (h2 %)	GA	GA% over Mean
	Minimum	Maximum				GCV	PCV			
PH	25.60	141.80	98.55	3.416	6.004	22.64	21.83	92.97	42.73	43.36
B/P	1.00	7.00	4.02	0.079	3.410	44.25	44.11	99.41	3.64	90.60
C/P	2.00	85.00	31.13	1.213	6.751	53.79	53.36	98.42	33.95	109.06
CL	1.84	3.02	2.42	0.113	10.474	13.39	8.35	38.84	0.26	10.72
SN/C	34.00	71.60	58.49	2.276	8.700	14.75	11.91	65.19	11.58	19.80
SW/C	0.07	0.74	0.19	0.020	23.632	59.69	54.82	84.33	0.20	103.70
TSW	1.86	3.90	2.73	0.032	2.009	18.69	18.58	98.84	1.04	38.06

B/P (Branches per plant), C/P (Capsules per plant), PH (Plant height in cm), CL (Capsule length in cm), SW/C (seed weight per capsule (g), SN/C (Seed number per capsule), TSW (Thousand seed weight (g), CV%: Coefficient of variation, GCV: Genotypic coefficient of variation, PCV: Phenotypic coefficient of variation, h^2 : heritability, GA: Genetic advance

Table 2. Morphological comparison of induced sesame mutants with parental genotype

Characters	Induced mutants	Parent variety (B 67)
	Range, (mean)	Range, (mean)
Plant height (cm)	25.60 - 141.80 (98.2)	100.0 - 123.0 (111.0)
Branch number /plant	1 - 7 (4.04)	3.0 - 4.0 (3.3)
Capsule number /plant	2 - 85 (30.77)	38 - 49 (43.7)
Capsule length (cm)	1.84 - 3.02 (2.42)	2.3 - 2.5 (2.4)
Seed number per capsule	34.0 - 71.60 (58.75)	48 - 50 (49.2)
Seed weight per capsule (g)	0.07 - 0.74 (0.19)	0.17 - 0.21 (0.19)
Thousand seed weight (g)	1.86 - 3.90 (2.73)	2.2 - 2.8 (2.5)

Table 3. Performance ranking of 35 induced sesame mutants and parent genotype for morphological characters

Characters	Levels of performance of mutants and parent genotype		
	Low	Medium	High
Plant Height (cm)	12	5	19
Branch number per plant	17	5	14
Capsules per plant	16	3	17
Capsule length (cm)	13	14	9
Seed number per capsule	14	7	15
Seed weight per capsule (g)	17	10	9
Thousand seed weight (g)	21	1	14

Table 4. Frequency of 35 induced sesame mutants on performance ranking for floral characters

Characters	Range of means	Levels of Performance of mutants and parent genotype		
		Low	Medium	High
Stigma position	1.0 - 2.0	5	0	31
Anther colour	1.0 - 3.0	20	7	9
Anther size	1.0 - 2.0	4	11	21
Anther shape	1.0 - 1.67	0	29	7
Pollen sterility	0 - 85.19	28	4	4

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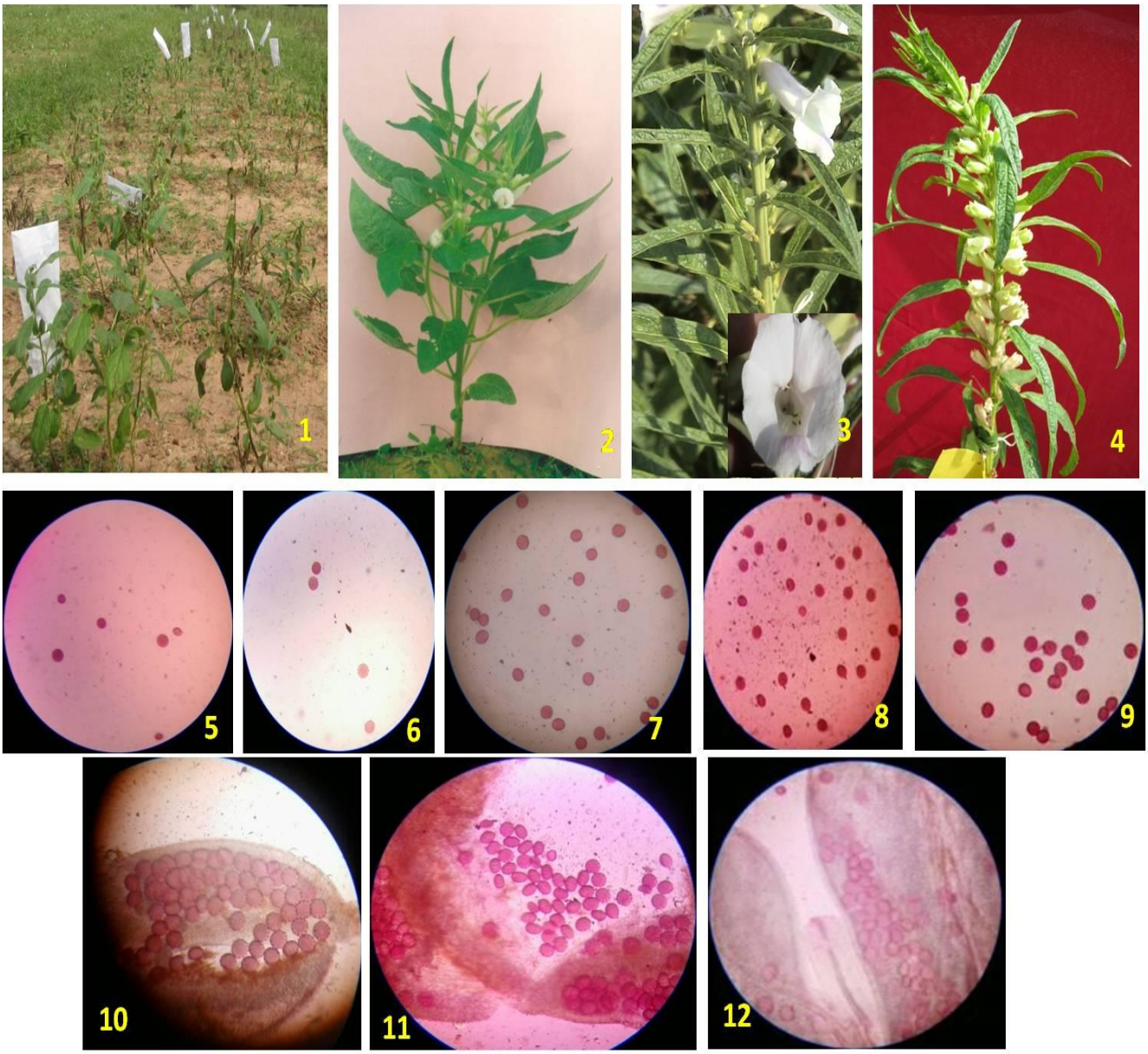

Fig 1 : Bagging of the mutants for purity,
Fig 2 : A mutant for plant height,
Fig 3 : Cleistogamous flower but sterile,
Fig 4 : Sterile flowers
Fig 5: Very low pollen count but darkly stained,
Fig 6: Very low pollen count but lightly stained,
Fig7: high pollen count but lightly stained
Fig 8-9: very high pollen count but darkly stained,
Fig 10: lightly stained sterile pollen remain enclosed within the anther,
Fig 11: Darkly stained sterile pollen remain enclosed within the anther,
Fig 12: Lightly stained sterile pollen with low count and remain enclosed within the anther