EMS INDUCED GENOMIC DISORDERS IN SESAME (*SESAMUM INDICUM* L.)

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The genotoxicity of EMS (Ethyl Methane Sulphonate) was evaluated at various parameters of sesame (*Sesamum indicum* L. var. IC413205). Seeds of sesame were treated with 0.5% solution of EMS for 3 durations, *i.e.* 3, 5, and 7 h. During the present investigation many chromosomal anomalies, namely precocious movements, stickiness, univalents, bridges, laggards, multivalents etc, were induced in inbred line of sesame. Higher frequencies of chromosomal anomalies were displayed at the maximum dose (7 h) of treatment. From the present study it can be concluded that the mutagenic effectiveness increased with the increase in the dose/treatment. This work investigates the effects of EMS on germination, plant height, seed yield and its correlation with meiotic behavior of Sesame in M1 generation. Although Sesame is not a model system for cytological studies, but we found that it is possible to conduct some significant cytogenetic studies on this species. Though some modifications in the standard methods for meiotic studies were necessary to obtain satisfactory results.

Key words: EMS, chemical mutagenesis, Sesame, chromosomal anomalies.

INTRODUCTION

Sesame (S*esamum indicum* L.) is one of the most important crops in the world according to some archaeological findings (Nayer, 1984; Bedegian and Harlan, 1986), cultivated in Asia from ancient times. Sesame is an important source of high quality edible oil and protein food for poor farmers of major sesame growing countries such as Sudan, Nigeria, Ethiopia, Uganda, Mexico, Venezuela, India, China, Pakistan, Turkey, and Myanmar. India, with 2.5 million hectares cultivated to this crop, is a major sesame producer. The seeds of sesame (*Sesamum indicum*) are rich in protein, fat, calcium, phosphorus and a fair source of B vitamins, a substantial amount of trace elements and fatty acids (FAO, 1988).

Sesame is generally unimproved and many collections have been made of land races, with little or no genetic information that can lead to its utilization in breeding programmes. A number of factors affecting sesame improvement programmes have been identified. Firstly, the germplasm of sesame is not as large as in other crops (Ashri, 1982). Secondly, the architecture of sesame is poorly

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adapted to modern farming system because of its indeterminate growth habit, sensitivity to wilting under intensive management and seed shattering at maturity (Cagirgan, 1994, 2001; Uzun and Cagirgan, 2006). This study aimed at investigating the effect of EMS on germination, growth and cytology of sesame is a strategy in its improvement through mutation breeding programmes.

MATERIALS AND METHODS

Seeds of inbred lines of sesame, *i.e.* IC413204, IC413205, IC413206, IC413208, IC413209 were obtained from NBPGR regional station Akola, Maharastra, India. The seeds of inbred IC413205 inbred line were selected to obtain M_1 generation. Then seeds of IC413205 were treated with a 0.5% solution of EMS for three durations, *i.e.* 3, 5 and 7h, respectively. The seeds were then washed thoroughly in running tap water for 12 h and excess moisture was blotted off. Three replicates were maintained for each dose of treatment and ten seeds were used in each replication. Suitable control set was maintained in distilled water and then the seeds were sown under natural conditions to raise the population. At the time of flowering, young floral buds were fixed in 1:3 acetic acid: absolute alcohol solutions for 24 h, after which they were transferred to 70% alcohol and stored at 4 °C. For cytological analysis, slides were prepared using the anther squash technique with 2% acetocarmine stain.

RESULTS

The mutagenic treatment resulted in a number of meiotic abnormalities in the PMCs while meiosis was perfectly normal in the control plants (n = 13). A dose based increase in meiotic abnormalities was observed in EMS treated sets. Although a wide range of abnormalities was present in the treated sets but stickiness, precocious movements, secondary associations and laggards were in higher frequency. Other abnormalities recorded in the treated sets were disorientation, fragmentation, multivalent formation, bridges, univalents, scattering, non-synchronous divisions, cytoplasmic connections, micronuclei, multipolarity, nucleolar divisions, etc. The frequency of individual as well as total abnormality percentage of abnormal cells varied considerably.

The most important abnormality induced by EMS was stickiness of chromosomes (Fig. C) at metaphase (I/II) and anaphase (I/II) where chromosomes formed a compact mass and identity of individual chromosome was lost. It was

recorded to be the highest at 7 h duration of EMS treatment at metaphase and at anaphase.

Precocious movement of chromosomes was the second most frequent abnormality observed in the treated sets. Multivalents and bridges, not visible at 3 h treatments, were however recorded with a considerable frequency at 7 h, treatment dose. Fragmentation of chromosome was also not observed at 3 h. Disturbed polarity was negligible at 3 h while it was absent at 5 h but at 7 h treatment duration high frequency of disturbed polarity was observed.

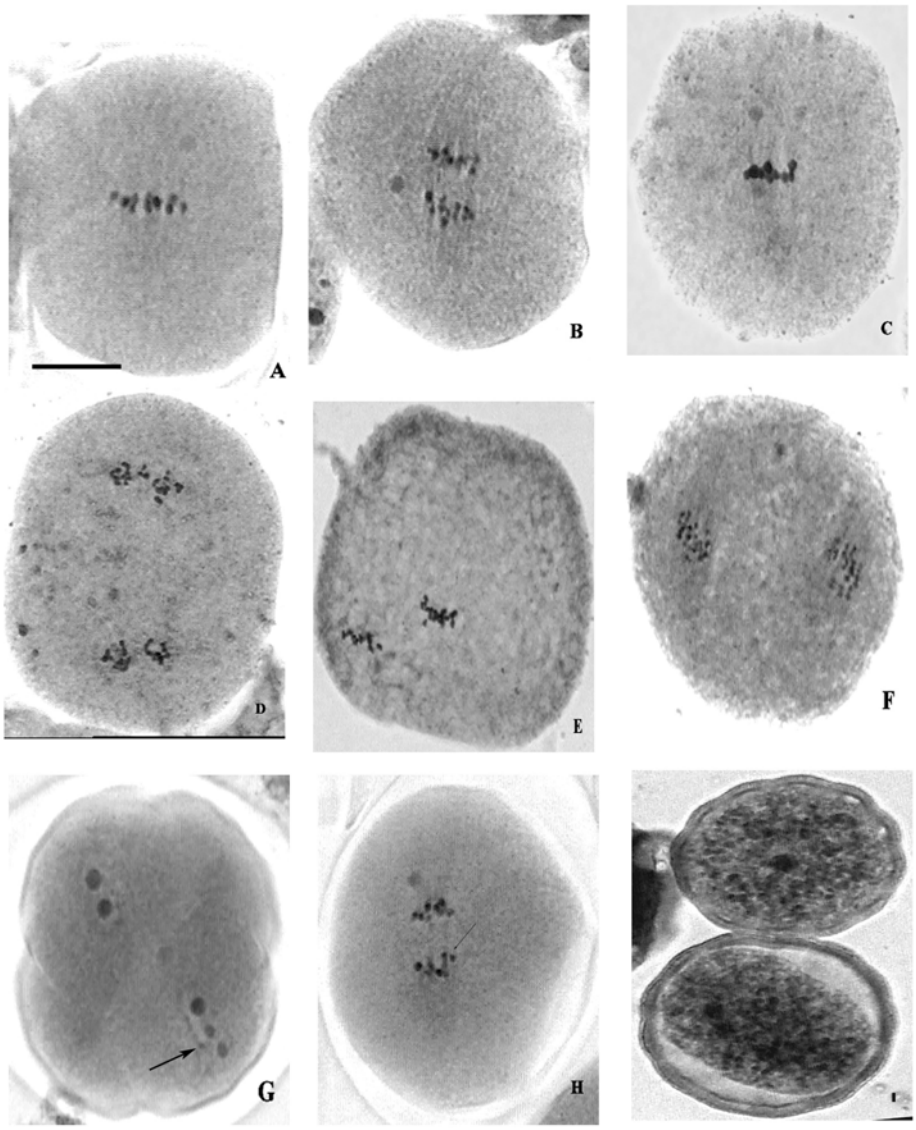
Unorientation (Fig. E) of bivalents at the equatorial plate was observed at a lower frequency in the treated sets but at the highest dose of 7 hrs. EMS treatment was recorded to be greater in number as compared to other dose durations. Persistent nucleolus, micronuclei (Fig. G) and nucleolar divisions were also observed in few cases at the highest treatment duration of EMS. The number of nucleoli varies from two to many. Multinucleolar conditions were observed at diakinesis and persistent nucleolar bodies of varying size were also recorded at different meiotic stages. Though the cytoplasmic connections between some of the PMCs were observed in treated sets but no case of true chromosome transfer among cells was observed. Univalents, scattered bivalents and non-synchronous divisions were also observed in few PMCs.

Total abnormality percentage showed an exponential increase along with the dose. In the treated sets percentage abnormality was recorded as 5.45 % at 3 h treatment, which rose sharply to 19.71 % at 7 h duration of treatment.

Mutagenic treatment also affected the morphological parameters of treated sets. Germination percentage was found to be significantly reduced at all the durations of the treatment (86.08 % at 3 h, 74.52 % at 5 h, and 63.56 % at 7 h) as compared to control where it was 94.26 %. Percentage reduction in germination over control ranged from 8.18 % at 3 h, treatment to 30.70 % at 7 h. Survival percentage also displayed similar results, as compared to control. There was also a delay observed in germination of seeds in treated sets.

Plant height was also found to be significantly reduced at higher doses of mutagenic treatment but some of the plants at lower doses respond positively to mutagen and recorded a slight increase in plant height.

The number of capsules per plant displayed a negative relationship with the treatment dose. It decreased considerably along with the increase in dose and was recorded approximately 48.62 capsules per plant at 7 h as compared to control in which approximately 68.64 capsules per plant were formed. Seed weight also showed similar results. Pollen fertility registered a marked decrease along with increasing treatment durations. Pollen fertility observed in the control was 98.08 % and the lowest recorded was 89.14 % at 7 h.



A. Normal Metaphase I. **B**. Normal Anaphase I. **C**. Sticky Metaphase I.
D. Laggard at Anaphase II. **E**. Unorientation at Anaphase I.
F. Fragmentation at Metaphase II. **G**. Micronuclei at Telophase II.
H. Laggard at Anaphase I. **I**. Fertile and Sterile Pollen Grains.

Table 1

Percentage of different types of abnormalities occurring in different meiotic stages of sesame as affected by 0.5 % EMS in inbred line

Treatment	Total no. of PMCs studied	No. of abnormal PMCs	% of abnormal PMCs	Abnormality (%) at metaphase I/II	Abnormality (%) at anaphase I/II
Control	231	–	–	–	–
3h	220	12	5.454	3.60	1.85
5h	233	33	14.163	8.43	5.69
7h	213	42	19.718	7.08	12.64

Table 2

Effect of EMS on certain growth and yield parameters in inbred line of sesame

Parameters	Control	3h	5h
	7h		
Germination (%)	94.26±1.65	86.08±1.34	74.52± 2.41
	63.56±1.62		
Survival (%)	90.35±0.57	80.83±1.19	69.34±1.6
	55.74±2.33		
Plant height (cm)	120.60±0.73	126.04±2.16	117.31±1.47
	112.55±1.44		
Pollen fertility (%)	98.08±3.12	95.73± 1.83	93.00±1.26
	89.14±2.91		
No. of capsule/Plant	68.64± 2.52	65.25±1.95	59.45±1.43
	48.62±1.74		
No. of seeds/Capsule	75.00±3.09	69.00±2.76	63.00±1.84
	52.00±2.36		
1000 Seed weight (g)	3.162±0.39	3.179±1.05	3.110±1.61
	3.035±2.16		

DISCUSSION

The frequency and spectrum of aberrations observed during the present investigation clearly displayed that EMS is a very potent mutagen for *Sesamum indicum* L. The results also showed a co-linearity between the duration of treatment and the percentage of chromosomal anomalies. Chemical mutagen induced chromosomal variations that have been widely investigated from the point of view of understanding the mechanics of EMS induced damage and biological dosimetry in *Sesamum indicum* L. Enhancement in the frequency of meiotic chromosomal anomalies is wide and included a high proportion of stickiness and secondary association and moderate frequency of laggards and multivalents. EMS induced

chromosomal stickiness has also been reported by Kumar and Singh (2003) in *Hordeum vulgare* L. It implies that the chemical mutagen may have brought some alterations in the pattern of organization of chromosomes. Similar results were also found by Kumar and Rai (2006) and Sharma and Kumar (2003).

The phenotypic manifestation of stickiness may vary from mild, when only a few chromosomes of the genome are involved, to intense, with the formation of pycnotic nuclei that may involve the entire genome, culminating in chromatin degeneration. Chromosome stickiness has been documented to be due to genetic or environmental factors. Genetically induced stickiness has been reported by Golubovskaya (1995), Caetano-Pereira *et al*. (1995), and Kumar and Rai (2006), while stickiness has also been reported in other crops like *Glycine max* L., *Hordeum vulgare* L., pearl millet, and wheat. Gaulden (1987) postulated that sticky chromosomes might result from the defective functioning of 1 or 2 types of specific non-histone proteins involved in chromosome organization, which are needed for chromatid separation and segregation.

The observed precocious chromosome migration to the poles may have resulted from univalent chromosomes at the end of prophase I or precocious chiasma terminalization at diakinesis or metaphase I. Univalents may originate from an absence of crossing over at pachytene or from synaptic mutants. Precocious migration of univalents to the poles is a very common abnormality among plants, which was also evident in our case. Unorientation and scattering of chromosomes may be due to either the inhibition of spindle formation or the destruction of spindle fibers formed. The behavior of these and of the laggard chromosome is characteristic in that they generally lead to micronucleus formation. Laggards and disturbed polarity might have appeared due to improper spindle functioning. Bridges seem to be a result of non-separation of chiasma due to stickiness. In many studies, chromosome cluster, fragments, laggard, chromatin bridges, and micronuclei were observed as the effects of physical and chemical mutagens.

Chromosomal damages may be the prominent causes of reduced seed germination and decreased yield as compared to controls. The reduction in germination percentage might have been due to the effect of mutagen on meristematic tissues of the seed. The mutagenic treatments also delayed the germination process. Kleinhofs *et al*. (1978) reported a delay in the initiation of metabolism following germination, resulting in uniform delay in mitotic activity, seedling growth, and ATP and DNA synthesis. Although all the doses of mutagen elicited a reducing effect on plant height, some of the plants at 3h treatment duration displayed an increase in plant height, which may be due to the mutation in major or minor genes. Fertility depends on the efficiency of the meiotic process. Studies on different plant species have shown that the decline in seed production is correlated with meiotic irregularities. Reduction in pollen fertility also supports a decrease in seed production due to the meiotic anomalies. Similar results were also

obtained by Kumar and Rai (2006). Ehrenberg (1959, 1960) and Kawai (1969) stated that the highest mutation rates also induce a high degree of lethality, sterility, and other undesirable effects. From the practical breeding point of view, the mutagenic treatments that induce high mutation rates with the least accompanying deleterious effects are desirable. During the present investigation, through EMS, many chromosomal anomalies were induced. The genetic structure of our material was highly affected, favoring new genetic changes in the following generations. From the present study, it is quite evident that EMS is very efficient mutagen for creating genetic variability in the natural gene pool of *Sesamum indicum* L.

CONCLUSION

Over the years, man relied upon spontaneously occurring variants, coming from mutations, to improve the yield and quality of crop plants (Herper 1999). In the first two decades of the twentieth century, ionizing radiation and chemical substances were used to effectively induce mutations in plants (Poehlman, 1979). The use of chemical mutagen in crop improvements has been reported in a number of species (Ojomo and Chheda 1972, Sander and Muchlbever 1977, Biswas and Dutta 1988).

Acknowledgments. The authors are grateful to NBPGR regional station Akola, Maharastra, India for providing inbred seeds of Sesame (*Sesamum indicum* L.). Sincere thanks to all the members of the Plant Genetics Laboratory for their encouragement and support. Thanks are also due to the Head, Department of Botany, University of Allahabad, Allahabad, UP for providing the necessary facilities.

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