

MUTAGENIC POTENTIAL OF EMS AND SODIUM AZIDE IN *VIGNA RADIATA* L. VAR. NARENDRA MUNG-1

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Mung bean (*Vigna radiata* L.) has a very important position among all the pulses which are grown in India. It is a rich source of various types of nutrients. Several workers have conducted experiments on mutation breeding in this species. The present investigation analyses relative effects of the treatments of Ethyl Methane Sulphonate (EMS) and Sodium Azide (SA) on meiosis in M_1 generation of *V. radiata* L. var. Narendra Mung-1. All the treatment doses of both the chemical mutagens, elicited a dose based increase in various chromosomal abnormalities. Major cytological abnormalities were stickiness or clumping, precocious movement, univalent formation, laggards, unorientation, secondary association, bridges etc.

Keywords: Chromosomal abnormalities; Mutation; *Vigna radiata*.

Introduction

For a mutation breeder, it is important to ascertain the most effective mutagen for his crop, to obtain maximum results using mutagenesis. Induction of chromosomal abnormalities has been regarded as one of the most dependable parameters for estimating the mutagenic efficiency of a mutagen, as these abnormalities are the primary basis of phenotypic or morphological variation.

The present study has been undertaken to assess the relative effectiveness of 0.5% Ethyl Methane Sulphonate (EMS) and Sodium Azide (SA) at three different durations viz. 3h, 5h and 7h on var. Narendra Mung-1 of *V. radiata* L.

Materials and Methods

The seeds of *V. radiata* L. Var. Narendra Mung-1 obtained from Narendra Dev University of Agriculture and Technology, Kumarganj, Faizabad, were treated with 0.5% concentration of EMS and SA for three different durations viz. 3h, 5h and 7h. The treated seeds were grown along with controls, in randomized block design in field to raise M_1 generation.

For cytological analysis, young flowering buds were fixed in 1:3 acetic alcohol and later preserved in 70% alcohol. Slides were prepared using standard acetocarmine squash technique.

Results and Discussion

Meiosis was perfectly normal ($n=11$) in the control sets (Figs. 1,2). The plants of EMS and SA treated sets showed various types of chromosomal abnormalities at different meiotic stages. PMCs of both the treated sets exhibited a dose based increase in the meiotic abnormalities. A comparative account of various anomalies induced by EMS and SA has been presented in Table-1.

Stickiness or clumping (Fig. 5) of chromosomes was the most common metaphase abnormality and could be observed almost at all the treatment doses of both the

mutagens. The maximum frequency of stickiness was observed at 7h duration of 0.5% EMS (3.21%) while it was 2.75% at the same duration of SA treatment. Precocious movement (Fig.4) was another frequent anomaly and it registered a maxima at 7h duration of 0.5% SA treatment (1.3%) as against 0.71% at 7h duration of EMS treatment. Unorientation of chromosomes could also be seen at all the treatment doses of both the mutagens but was highest (1.07%) at 7h duration of EMS, whereas it was 0.78% at the same dose of SA. Secondary associations were more common in SA treated sets being present at all the 3 durations while it was observed only at 5h and 7h durations in EMS treated sets. Multivalents (Fig. 3) were present at all the three doses of EMS but had a low frequency. They were absent at 3h duration of SA.

Anaphasic abnormalities followed a similar trend with common occurrence of laggards, bridges, unequal separation, multipolarity, clumping and stickiness. EMS was more potent in the induction of laggards (Fig. 6) which were observed almost at all the durations. Highest frequency of laggards could be seen at 7h duration of EMS (2.50%). Other anaphasic abnormalities were also more frequent in EMS treated sets in comparison to SA treated sets. Bridges exhibited the highest frequency (1.25%) at 7h duration of EMS treatment which was more or less similar to 7h SA treated sets (1.17%). Multipolarity was observed in both the treatment sets but only at the higher durations. Unequal separation was another common abnormality observed at all the three doses of each of the treatment sets. The frequency of clumping (Fig. 7) was high at anaphase like that of metaphase I and II (Fig.8). It was highest at 7h EMS treated sets (1.96%). Formation of one or more micronuclei (Fig. 9) was also observed at telophase of both the treatment sets..

The total abnormality percentage was 15.68% and 15.11% at the highest durations of EMS and SA,

Table 1. Comparative analysis of mutagenic effects of Ethyl Methane Sulphonate and Sodium Azide on the PMCs of *Vigna radiata* L. Var Narendra Mung-1

Dose	No. of PMCs Scored	Metaphase I/II Abnormalities (%)				Anaphase I/II Abnormalities (%)							No. of Ab PMCs	% of Ab
		UN	PM	MV	SA	UV	CL	LG	BR	US	MP	CL		
0.5% EMS														
3h	540	0.18	-	0.37	-	-	2.09	1.85	-	0.37	-	1.1	32	5.96
5h	555	0.54	0.36	0.18	0.36	0.18	2.34	1.98	0.38	0.18	-	1.60	45	8.10
7h	560	1.07	0.71	0.53	0.53	0.71	3.21	2.50	1.25	1.07	2.14	1.96	88	15.68
0.5% SA														
3h	536	0.37	0.18	-	0.37	-	1.67	1.5	-	0.56	-	0.75	29	5.40
5h	545	0.18	0.73	0.18	0.55	0.37	2.08	1.83	0.37	0.37	-	1.28	43	7.94
7h	510	0.78	1.37	0.39	0.99	0.59	2.75	2.35	1.17	0.99	1.97	1.77	77	15.11
Control	520	-	-	-	-	-	-	-	-	-	-	-	-	-

UN-Unorientation; PM-Precocious Movement; MV-Multivalents; SA-Secondary association; UV-Univalents; CL-Clumping; LG-Laggards; BR-Bridge; US-Unequal separation; MP-Multipolarity

respectively. In general, all the doses of EMS registered higher abnormality percentage than the corresponding SA doses.

During the present investigation, both the chemical mutagens elicited almost similar types of meiotic abnormalities but the frequencies of the total abnormalities induced, were different in the two treatment sets. It shows the differential mutagenic potential of both the mutagens on Narendra Mung-1. The most dominant abnormality i.e. stickiness of chromosome is a result of partial dissociation and altered pattern of organisation of nucleoproteins¹. Stickiness may also be due to the disturbance in nucleic acid metabolism in the cells². Sticky meiosis in tomato has also been reported by Rao and Rao³ which according to them is the manifestation of a dominant gene mutation induced by mutagens.

Gaulden⁴ attributes chemically induced stickiness to direct action of mutagens on the histone proteins leading to improper folding of DNA.

Multivalent formation at metaphase has also been reported in plants like barley⁵, *Trigonella*⁶ and Lentil⁷. The occurrence of lagging chromosome may be explained on the basis of abnormal spindle formation⁸.

Chromosome bridge may arise due to stickiness or due to the formation of dicentric chromosomes by breakage and reunion⁹. Bridges were also observed by Dempong and Maxwell¹⁰ in *Tradescantia* as a result of treatment with nongalamycin.

Secondary association of chromosomes, in many diploid species, has been interpreted as a result of modified chromosome arrangement due to duplication, interchanges

or stickiness¹¹. Formation of univalent may be a result of decrease in chiasma frequency. Similar results were obtained by Sadanandam and Subhash¹² in *Capsicum*, following treatment with chemical mutagens.

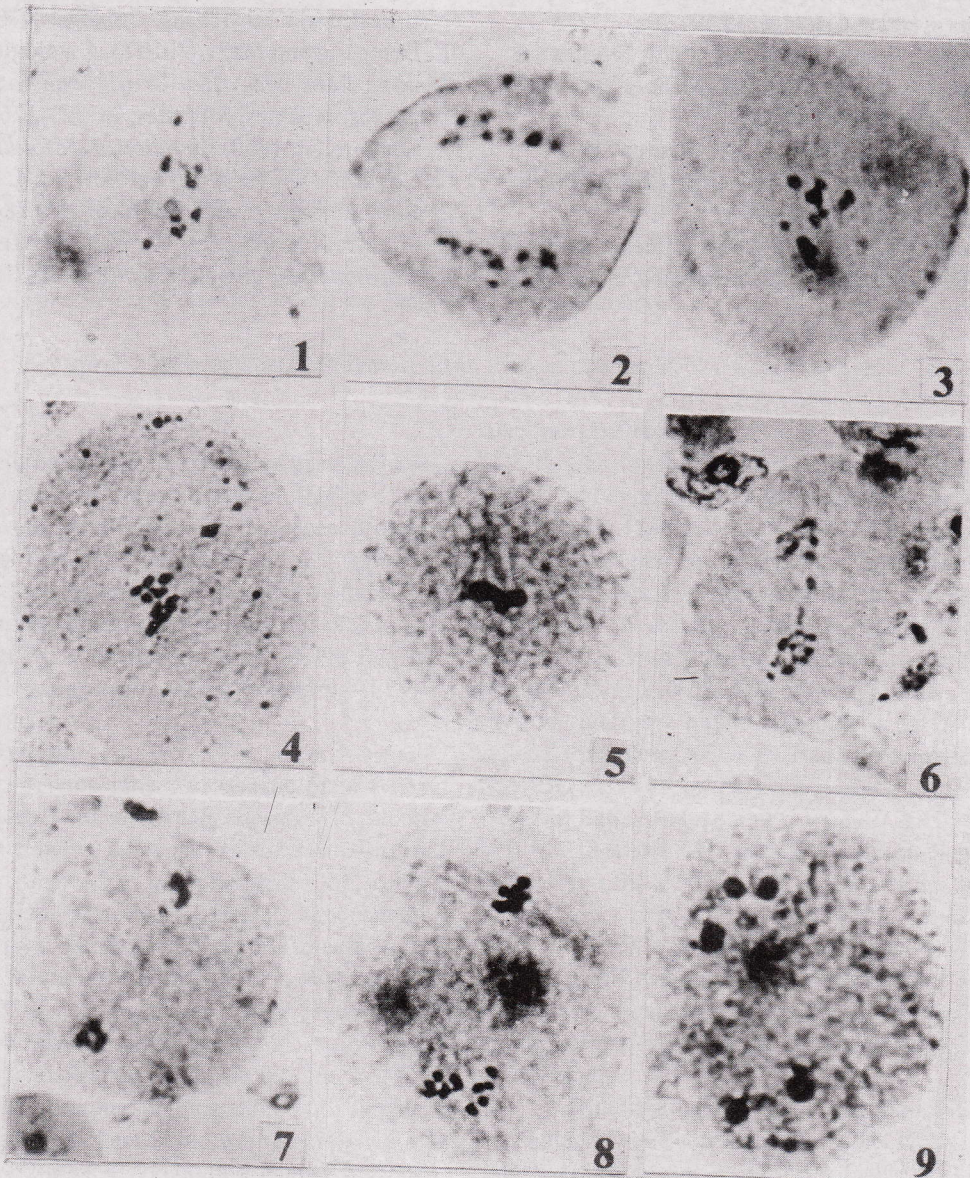
Any type of mutation needs instability of genome, for its occurrence. Therefore a mutagen which elicits maximum instability to the genome is more effective in bringing about mutations. Abnormalities are a good indicator of instability of the genome. Taking this parameter it is evident from the above study that EMS and SA, both are very effective mutagens for *V. radiata* var Narendra Mung-1. It may also be stated that lower doses of the mutagens may be better for mutagenesis since high doses cause more lethality and disallow the mutations to be inherited.

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References

1. Evans JH 1962, Chromosome aberration induced by ionizing radiations. *Int. Rev. Cytol.* **13** 221-232.
2. Darlington CD 1942, Studies in *Prunus* I and-II. *J. Genet.* **19** 215-221.
3. Rao PN and Rao RN 1977, Gamma Ray induced meiotic chromosome stickiness in tomato. *Theo. Appl. Genet.* **50** 247-252.
4. Gaulden ME 1987, Hypothesis: Some mutagens directly alter specific chromosomal protein to produce



Figs. 1-2 Normal stages in meiosis of *V. radiata* L. Var Narendra Mung-1
1. Normal Diakinesis (n=11)
2. Normal Anaphase I (11:11)
Figs. 3-9 Meiosis in mutagen treated *V. radiata* L. Var Narendra Mung-1.
3. Multivalent at Diakinesis
4. Precocious movement at Metaphase I
5. Clumping of chromosomes at Metaphase I
6. Lagging chromosomes at Anaphase I
7. Stickiness at Anaphase I
8. Scattering at one pole at Metaphase II
9. Micronuclei at Telophase I

- chromosome stickiness. *Mutagenesis* **2** 357-365.
5. Burnham CR, White FH and Livers R 1954, Chromosomal interchanges in barley. *Cytologia* **19** 191-202.
 6. Raghuvanshi S S and Singh AK 1974, Studies on effect of gamma rays on *Trigonella foenum-graecum* L. *Cytologia* **39** 473-482.
 7. Gupta PK, Kumar S, Tyagi BS and Sharma SK 1999, Chromosome interchanges in lentil (*Lens culinaris* Med.). *Cytologia* **64** 387-394.
 8. Tarar JL and Dnyansagar VR 1980, Effect of gamma rays and EMS on growth and branching in *Turnera ulmifolio* Linn. *J. Cytol. Genet.* **14** 118-124.
 9. Raj SA and Rao R 1972, Cytological studies in *Vicia faba* L. treated with lathyrogens. *Cytologia* **37** 245-246.
 10. Dempong and Maxwell 1973, Cytological effects of nongalamycin in *Tradescantia paludosa* microsporocytes. *Mut. Res.* **21** 323-326.
 11. Stebbins GL 1950, *Variation and evolution in plants*. Columbia University Press New York.
 12. Sadanandam A and Subhash K 1984, Effect of chemical mutagens on chiasma frequency in *Capsicum annum* L. *Cytologia* **49** 415-419.