



Cytological Abnormalities in *Trigonella* induced by EMS, MMS and MES

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ABSTRACT

The present studies were carried out to have an insight about chromosomal abnormalities by the use of EMS, MMS and MES as radiomimetic agents in *Trigonella foenum-graecum* L, viz. *desi* and *kasuri methi*. Among two varieties *desi methi* was found to be more sensitive than *kasuri methi*. Higher doses of EMS, MMS and MES decreased mitotic index.

Key Terms: Fenugreek, radiomimetic agents

INTRODUCTION

Trigonella (*Trigonella foenum-graecum* L.) also known as Fenugreek, is a multi-purpose annual autogamous crop grown as spice, fodder and leafy vegetable belongs to family Fabaceae (Bentham and Hooker, [1]). The seeds and leaves are rich source of vitamin A, vitamin C, protein, carbohydrates and minerals especially organic iron, phosphorus and calcium etc. The seeds of fenugreek contain alkaloid, colin, bitter material, fatty acid, diastase which are excellent remedies for dysorexia and weakness resulting from emaciation. It improves the appetite, increase the number of red blood cells. It is also used as very popular flavoring media in bakery (Makai *et al.*, [2]). Seeds are used in the indigenous system of medicine (Chopra *et al.*, [3]). Fournier [4], Paris *et al.* [5], Sauvaire *et al.* [6] Jain *et al.* [7]. Variation in chromosome number in few pollen mother cells may be due to cytomixis, which is considered a source of production of aneuploid and polyploid gametes (Koul, [8]; Yen *et al.*, [9]; Bhat *et al.*, 2006b [10]). Abdul-Barry *et al.* [11] has confirmed the antidiabetic actions of *Trigonella*.

There are many physical and chemical agents used now a day to increase yield of the crop or to develop high yielding crop cultivars. Among the various agents radiomimetic agents are important one. These radiomimetic agents have bi-functional alkyl reactive groups that react with DNA, causes extensive cross linkage of DNA, chromosome breakage, chromosome mutations and gene mutation. Therefore, the aim of present study was to evaluate the cytological abnormalities occurred in *Trigonella* with the use of EMS, MMS and MES as radiomimetic agents.

MATERIALS AND METHODS

Seeds of two varieties of *Trigonella foenum-graecum* L. viz. *desi methi* and *kasuri methi* were procured from Jawahar Lal Agriculture farm Eintkhedi, Berasia Road, Bhopal (M.P.). Three mutagens EMS, MMS and MES which are radiomimetic agents, were used in present investigation. Three concentrations of each mutagen i.e., 0.1%, 0.2% and 0.3% were selected on the basis of preliminary experiment, LD-50 dose.

Methodology

Fully mature and healthy seeds of uniform size free from mould and mechanical injury were selected for different concentration of mutagenic treatment. To determine the effective range of mutagens pilot experiment were conducted in preceding year with the two varieties, *desi methi* and *kasuri methi* by way of employing wide dose range. Period of presoaking the seeds making them vulnerable to the action of different mutagens was also ascertained through preliminary experiments.

For mitosis study from each treatment root tips of approximately 8.5 mm long were collected in 1:3 aceto alcohol. After 24 hours of fixation root tips were transferred to 70% alcohol separately and stored in a refrigerator. Haematoxyline squashes of root tips were prepared after hydrolyzing with 5N HCl, at 60°C for chromosomal studies. Mitotic index was calculated using the following formula

$$\text{Mitotic Index} = \frac{\text{Total number of dividing cells}}{\text{Total number of cells studied (Dividing + undividing)}} \times 100$$

For study of meiosis, flower buds were fixed in 1:3 aceto alcohol containing traces of ferric acetate for 24 hours. Material thus fixed was preserved in 70% alcohol for cytological studies. Anthers were smeared in 1% acetocarmine and iron-alum haematoxyline. Photomicrographs of some important nuclear stages of mitosis and meiosis were taken.

RESULT AND DISCUSSION

It is now generally accepted that nucleus is the major site of injury caused by radiomimetic agents EMS, MMS and MES in a wide variety of plants. Thus cytological anomalies are considered as one of the dependable measure for estimating the effect of chemicals on immediate generation. Results in present investigation revealed that higher doses of all the three radiomimetic agents were significantly effective for causing the chromosomal anomalies.

In Desi methi highest mitotic index observed in control 22.00. The lowest mitotic index observed under 0.3% EMS was 10.00. In higher doses of all the three radiomimetic agents the mitotic index decreased (Table-1). In case of Kasuri methi highest mitotic index was observed in control 21.42, while lowest mitotic index 0.3% MES was 13.17. As the dose of three radiomimetic agents increased, mitotic index decreased (Table-2). Number of mitotic abnormal cells in both varieties increased as the dose of three radiomimetic agents increased (Table 1 & 2; Fig. 1, 2,).

Table1. The effect of various doses of radiomimetic agents EMS (Ethyl Methane Sulphonate), MMS (Methyl Methane Sulphonate) and MES (Methyl Ethane Sulphonate) on Mitotic Indices and Abnormality in Root Tip Cells (RTCs) in *Trigonella foenum-graecum* L. (*Desi methi*) in M₁ generation.

S. No.	Radiomimetic agents	Doses (%)	Root tips Cells				
			Number of cells observed	Number of dividing cells	Number of Non-dividing cells	Mitotic Index	Number of abnormal cells
1.	—	Control	150	34	116	22.00	—
2.	EMS	0.1	192	33	159	17.00	20
3.		0.2	140	16	124	11.00	26
4.		0.3	112	12	100	10.00	24
5.	MMS	0.1	170	24	146	14.00	20
6.		0.2	110	20	90	18.00	26
7.		0.3	185	30	155	16.00	27
8.	MES	0.1	130	20	110	15.00	19
9.		0.2	110	16	94	14.00	28
10.		0.3	90	12	76	13.00	29

Table 2. The effect of various doses of radiomimetic agents EMS (Ethyl Methane Sulphonate), MMS (Methyl Methane Sulphonate) and MES (Methyl Ethane Sulphonate) on Mitotic Indices and Abnormality in Root Tip Cells (RTCs) in *Trigonella foenum-graecum* L. (*kasuri methi*) in M₁ generation.

S. No.	Radiomimetic agents	Doses (%)	Root tips Cells				
			Number of cells observed	Number of dividing cells	Number of non-dividing cells	Mitotic index	Number of abnormal cells
1.	—	Control	140	30	110	21.42	—
2.		0.1	182	32	150	17.58	20
3.	EMS	0.2	138	22	116	15.94	24
4.		0.3	100	14	86	14.00	28
5.		0.1	162	25	137	15.43	24
6.	MMS	0.2	156	23	133	14.74	29
7.		0.3	140	19	131	13.57	32
8.		0.1	180	33	147	18.33	22
9.	MES	0.2	172	25	147	14.53	25
10.		0.3	167	22	145	13.17	29

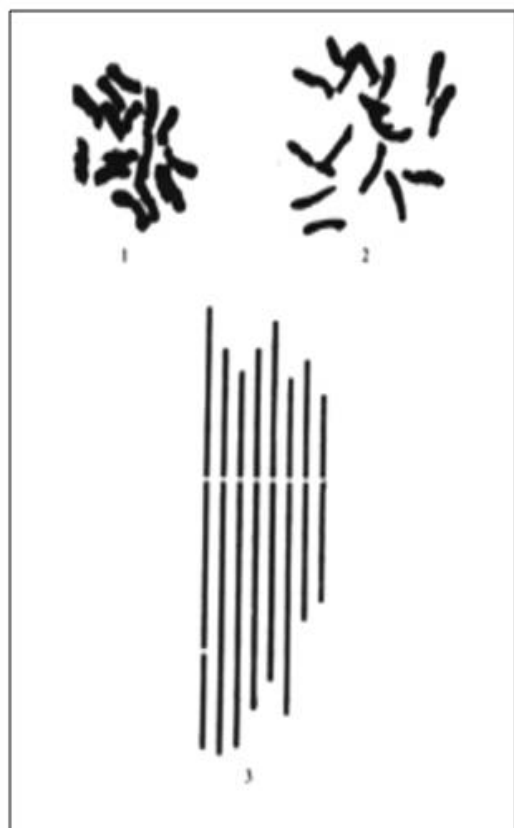


Figure 1

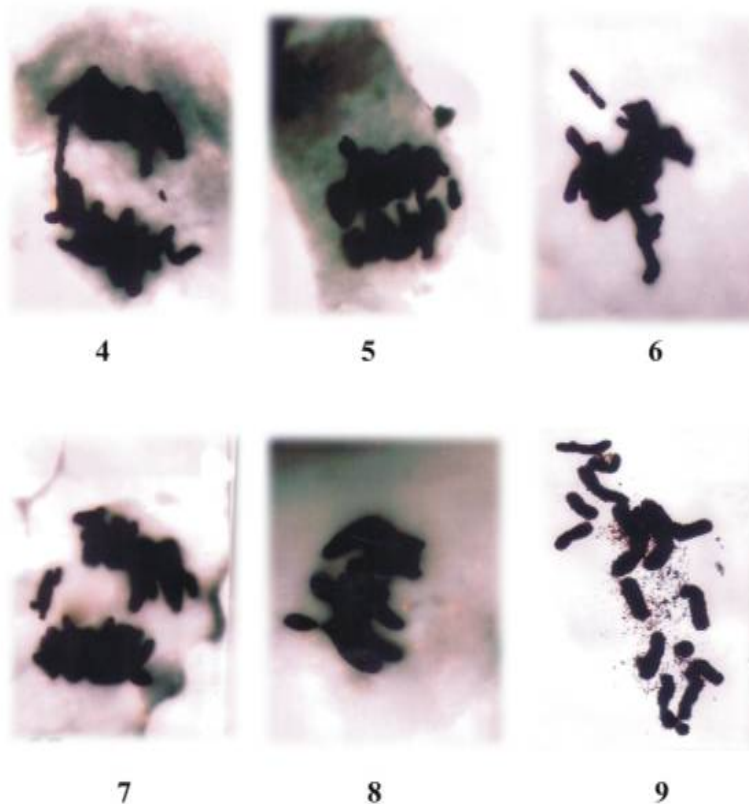


Figure 2

Meiotic studies were carried out to have an insight about chromosomal abnormalities in both the varieties. Diakinesis and metaphase-I exhibited various types of anomalies, the prominent among these were the chain and ring translocations appearance of univalent, tetravalent, bridge, laggard etc. which are recorded in (Fig.3; Table-3 & 4). Occurrence of meiotic abnormalities proved that all the treatments of three radiomimetic agents employed were effective.

Table 3. The effect of various doses of radiomimetic agents EMS (Ethyl Methane Sulphonate), MMS (Methyl Methane Sulphonate) and MES (Methyl Ethane Sulphonate) on Meiosis Pollen Mother Cells in *Trigonella foenum-graecum* L. (*desi methi*) in M₁ generation.

Radiomimetic agents	Doses (%)	Pollen Mother Cells				
		Number of cells studied	Number of cells dividing stage	Number of cells showing abnormalities	% of dividing cells	% of abnormal cells
—	control	1540	970	—	62.98	—
	0.1	1200	680	198	56.66	16.50
EMS	0.2	1050	540	192	51.42	18.28
	0.3	925	480	190	51.89	20.54
	0.1	980	420	191	42.85	19.48
MMS	0.2	678	260	154	38.34	22.71
	0.3	550	196	141	35.63	25.63
	0.1	1050	567	190	54.00	18.09
MES	0.2	930	480	180	51.61	19.35
	0.3	890	421	178	47.30	20.00

Table 4. The effect of various doses of radiomimetic agents EMS (Ethyl Methane Sulphonate), MMS (Methyl Methane Sulphonate) and MES (Methyl Ethane Sulphonate) on Meiosis Pollen Mother Cells in *Trigonella foenum-graecum* L. (*kasuri methi*) in M₁ generation.

S. No.	Radiomimetic agents	Doses (%)	Pollen Mother Cells				
			Number of cells studied	Number of cells dividing cells stage	Number of cells showing abnormalities	% of dividing cells	% of abnormal cells
1.	—	Control	1500	968	—	64.53	—
2.		0.1	1050	650	187	61.90	17.80
3.	EMS	0.2	940	520	174	55.31	18.51
4.		0.3	890	430	180	48.30	20.22
5.		0.1	970	450	186	46.39	19.17
6.	MMS	0.2	892	340	198	38.11	22.19
7.		0.3	590	200	148	33.89	25.08
8.		0.1	1040	592	185	56.92	17.78
9.	EMS	0.2	920	456	190	49.59	20.65
10.		0.3	840	410	178	48.80	21.19

Examination of pollen cells at late diakinesis, metaphase and anaphase revealed a good deal of chromosomal aberrations in both varieties *desi methi* and *kasuri methi* of *Trigonella foenum-graecum*. A linear relationship was found between frequency of occurrence of abnormalities and dose rate. Data collected indicates that more anomalies are induced with increased dose level. Such types of abnormalities have been also reported by Zuk and Swietlinska [12] in *Vicia faba*; Gupta and Gupta [13] in *Crotalaria juncea*; Soheir, [14] in *Allium cepa*; Zeerak [15] in brinjal (*Solanum Melongena* L.); Kar *et al.* [16] in *Sesamum indicum* L.; Consolaro *et al* [17] in Brazilian populations of (*L.*) Urban (Umbelliferae); Zaman and Saleh [18] in wheat; Talukdar and Biswas [19] in grass pea; Kumar and Gupta [20] in black gram; Goyal and Khan [21] in *Vigna mungo* (L.) Hepper; Mohd Gulfishan [22] in *Vicia faba* var. *major*.

A comparison of sensitivity of varieties *desi methi* and *kasuri methi* to different doses of EMS, MMS and MES, reveal that former variety is more sensitive while later rather resists the effect of radiomimetic agents. Such a differential sensitivity and response of chromosomes of two varieties may be attributed to biochemical contents present in the cell of the two varieties *i.e.*, which differentially react with three radiomimetic agents and products of their reaction differentially influence the chromatin. As the three radiomimetic agents EMS, MMS and MES are alkylating it is conceivable that they behave like ionizing

radiation in hitting the specific target of the genetic material. Larger number of laggards may be the broken fragments of univalents. Supporting the view of Mc-clintock [23] the occurrence of multinucleate nuclei may be attributed to the suppression of organizing capacity of nucleolar chromosomes due to particular genotypic change induced by alkylating agents employed.

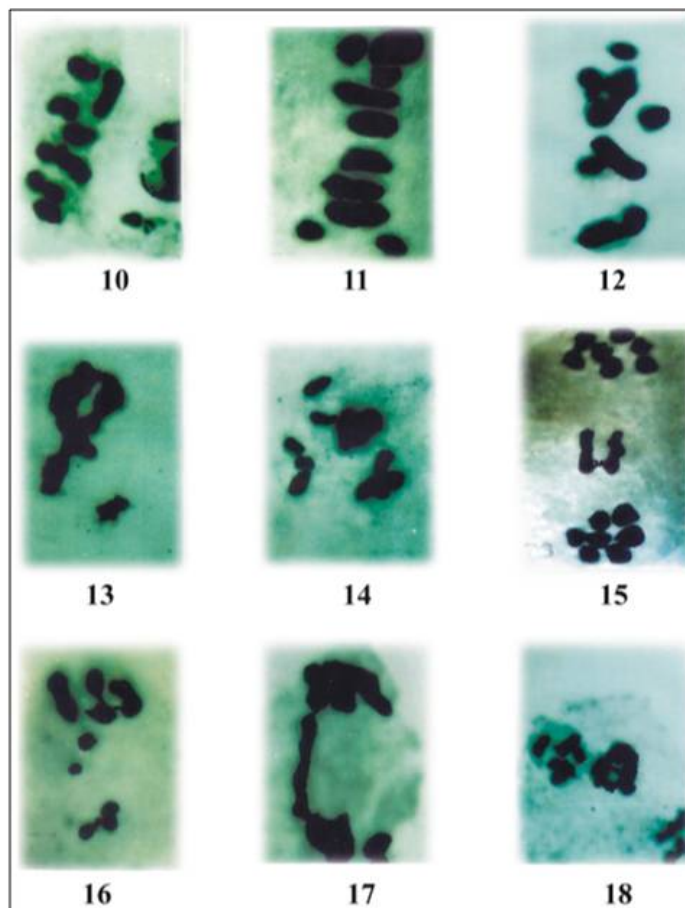


Figure 3

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