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# EMS induced changes on cytology of Methi (*Trigonella foenum-graecum* L.)

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**ABSTRACT**

The present studies were carried out to have an insight about chromosomal abnormalities by the use of EMS as radiomimetic agents in *Trigonella foenum-graecum* L, viz. methi. Mutation breeding in crop plants such as fenugreek is a successful approach in change of product having narrow genetic base. This study is to the determine the effect of physical mutagen (EMS) were used. The seeds were treating with different doses/concentration of EMS. The objectives of the present were:- (i) To study the effect of mutagenic treatments on various biological parameters. (ii) To investigate the chromosome behaviour of treated populations with respect to controls.

**Keywords:** Methi; EMS; Treatment; Mutagen; Chromosome.

## INTRODUCTION

Fenugreek (*Trigonella foenum-graecum*) is a legume and it has been used as a spice throughout the world to enhance the sensory quality of foods. Fenugreek is commonly known as methi plant (2n=16). According to Bentham and Hooker, (1862 -1883) this plant is placed as follows:

Kingdom- Plantae,
Division- Magnoliophyta,
Class- Magnoliopsida,
Order- Fabales,
Family -Fabaceae,
Sub-family- Faboideae,
Genus - *Trigonella*
Species - *foenum-graecum*

It is known for its medicinal qualities such as antidiabetic, anticarcinogenic, hypocholesterolemic, anti-fertility, anti-cancer, antimicrobial, antioxidant, and immunological activities. Beside its medicinal value, it is also used as a part of various food product developments as food stabilizer, adhesive, and emulsifying agent. Fenugreek is an important cultivated crop in parts of Europe, Northern Africa, West and South America and Australia (Acharya *et al*., 2006). Major fenugreek producing countries are Russia, India,

Pakistan, Germany, Argentina, Egypt, Canada, Iran, Canada, USA and China (Basu, 2008). India is the largest producer of fenugreek in the world where Rajasthan, Gujarat, Uttaranchal, Uttar Pradesh, Madhya Pradesh, Maharashtra, Haryana and Punjab are the major fenugreek producing states (Debranjan and Tara, 2010). The young plants serve as vegetable for human consumption seeds as a spice or as herbal medicine (Petropoulos, 2002). Fenugreek leaves and seeds have been used extensively to extracts and powders for medicinal uses.

The biological and pharmacological properties of fenugreek are attributed to the variety of its constituents, namely; steroids, N -compounds, polyphenolic substances, volatile constituents, amino acids etc. Fenugreek seed contains 45-60% carbohydrates, mainly fibre (galactomannans), 20-30% proteins high in lysine and Tryptophan, 5-10% fixed oils (lipids), Pyridine alkaloids, mainly trigonelline (0.2 0.38%), choline (0.5%), free amino acids, such as 4-hydroxyisoleucine (0.09%), arginine, histidine and lysine, calcium and iron, saponins (0.6 1.7%), glycosides yielding steroidal sapogenins on hydrolysis (diosgenin, yamogenin), cholesterol sitosterol, vitamin A, B,C and nicotinic acid (Budavari, 1996; Kavina *et al*., 2020).

Plant breeding is often regarded as an important one of the branches among applied genetics. It forms the most important breakthroughs in the history of genetics that led to the discovery of experimental mutagenesis in the early 21st century. Mutation techniques can generate genetic variation and increase the desired characters significantly in plants of new cultivars. The application of mutagenesis in agriculture for improving the crop plants presented a new departure from the conventional breeding methods. In conventional breeding methods, the store of natural variability present either in the base population initially or introduced through hybridization, is subjected to recombination and selection so as to increase the frequency of favourable combinations of genes in the selected line.

During the past 70 years, more than 2543 mutant cultivars from 175 plant species including ornamentals, cereals, oilseeds, pulses, vegetables, fruits and fibers have been officially released in 50 countries all over the world. Mutation breeding helps in greater magnitude of variability in various plant traits in a shorter time. Mutation induction offers significant increase in crop production and the possibility of inducing desired attributes that either cannot be found in nature or have been lost during evaluation. Mutation breeding has been widely used for the improvement of plant characters in various crops. It is a powerful and effective tool in the hands of plant breeders especially for autogamous crops having narrow genetic base (Kharkwal, 1998).

Mutation is a sudden heritable change in an organism and generally a structural change in genes. Mutation produced by changes in the base sequences of genes are known as gene or point mutations. Some mutations produced by change in chromosome structure, or even in chromosome number are known as chromosomal mutations. The induced mutations are caused artificially by mutagenic factors. The agents that induce mutations are called mutagens and mutagens mainly consist of two different kinds; radiation (physical) and certain chemical mutagens. Practicing of induced mutation for crop improvement is known as mutation breeding. Treatments with mutagens alter genes or break the chromosomes. Gene mutations occur naturally as errors in DNA replication. Most of these errors are repaired but some may pass on to the next cell division to become established in the plant offspring as spontaneous mutations. Gene mutations without phenotypic expressions are usually not recognized. Consequently, genetic variation appears rather limited and breeders have to resort to mutation induction. Mutagenic agents have been used to induce useful phenotypic variations in plants for more than seventy decades (Anitha *et al*., 2005).

Ethyl methane sulphonate (EMS) is a chemical mutagen of the alkylating group and has been commonly used in plant breeding because it can cause high frequency of gene mutations and low frequency of chromosome aberration. EMS alkylates guanine residues, producing O6-ethyl guanine, which pairs with T but not with C (Chandra Mohan *et al.,* 2016). As a result, replication of un-repaired alkylation damage will effectively replace the G/C base pair with an A/T. This mechanism predicts a strong G/C to A/T bian in EMS induced mutations, as observed in numerous mutagenic studies. In fenugreek, several have tried for artificial induction of mutations through the use of mutagens (Basu *et al.,* 2008) [9]. Despite the release of different cultivars, fenugreek production has not increased to any noticeable extent over the last

decades. The present work is therefore, designed to evaluate the morphological and cytological effects of chemical mutagens in fenugreek with the main objective of inducing changes in the genotype to enhance genetic variability in this plant as to broaden its genetic base for selection of desirable genotypes for commercial cultivation (Chandra *et al.,*2016).

## MATERIALS AND METHODS

For the present study a local variety of dry and dormant seeds of the fenugreek (*Trigonella foenum-graecum*) were purchased from the organic stores and treated with EMS. The following study was carried out in Pachaiyappa College Botanical Gardern.

**Mutagen used:** The seeds of fenugreek were treated with different treatment of chemical mutagen. The chemical mutagens used were Ethyl Methane Sulfonate [EMS ($CH_3$ $OSO_2$ $C_2H_5$)]. The chemical was obtained from HI-MEDIA laboratories, Mumbai, having a half life period of 30 hours with a molecular weight of 124.16 and density of 1.20.

Two sets containing 400 healthy seeds were selected for treatment. To determine the LD50 value, fenugreek seeds were pre soaked in double distilled water for 6 hours followed by EMS 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9% and 1.0% concentrations freshly prepared solution for 3 hours. After the EMS treatment, the treated seeds were washed thoroughly in running tap water to remove the residual effect of the mutagenic chemicals. After the completion of the treatment treated seeds were sown immediately in the field along with their respective controls to raise the $M_1$ generation in a randomized block design with three replications. The seedling height reduction (I) in different M1 generation was studied following Nilan *et al.,* (1965) and Velu *et al.* (2007). The plant survival (L) was computed as the percentage of plants surviving till maturity. The biological damage (lethality/ injury) was computed as the reduction in plant survival and plant height. The respective control and treatment progenies were screened several times for morphological mutations throughout the crop duration.

**Cytological observation:** The root tips collected from control and treated seedlings were fixed in 1:3 acetic ethanol. The root tip squashes were made by using Iron alum Haematoxylin squash technique (Marimuthu and Subramanian, 1960). The root tips were hydrolyzed in 0.1N HCl for 5 to 10 minutes at 60ºC and then they were thoroughly washed in distilled water and transferred to 4% iron alum for 3 minutes. The root tips were then washed in distilled water and transferred to ripened dilute haematoxylin stain and kept for 3 hours. The root tips were thoroughly washed in distilled water and then they were treated in 45% acetic acid for 1 minute to soften the tissues. Acetic acid being a de-staining agent, the time of study in haematoxylin had to be adjusted to the time required for softening in acetic acid. One or two root tips were placed on a clean slide and squashed by using a cover slip and the slide was sealed and mounted in DPX solution and then examined. The normal and abnormal mitotic stages were photographed.

**Evaluation of $M_1$ generation:**

**Seed germination:** The data on seed germination was recorded right from the emergence of first shoot in each treatment including control. After recording the data, percentage of seed germination was calculated by using the formula,

*Germination (%) = No of seeds germination ÷ total No of seeds × 100*

**Seedling height (cm):** Seedling height was estimated on 20th day of germination by measuring root and shoot lengths of 15 randomly selected seedlings from each treatment as well as control. Seedling injuries as measured by the reduction in root and shoot length and calculated in terms of percentage of root and shoot injury.

*Seedling Injury % = control ÷ treated plants × 100*

**Plant survival:** The surviving plants in different treatments were counted at the time of maturity and the survival percentage and percent lethality were calculated by the following formula.

*Survival (%) =Number of plants atmaturity x100 ÷ Number of seed.*

## RESULTS AND DISCUSSIONS

The present investigation was undertaken in order to study the artificial inducement of mutation in fenugreek local by using EMS mutagens through the

biological changes in $M_1$ generation. This was aimed to find out the economic potentialities of the viable mutant and the nature of induced variability in the qualitative and quantitative traits in all generation.

**$M_1$ GENERATION**

**LD $_{50}$ for EMS**

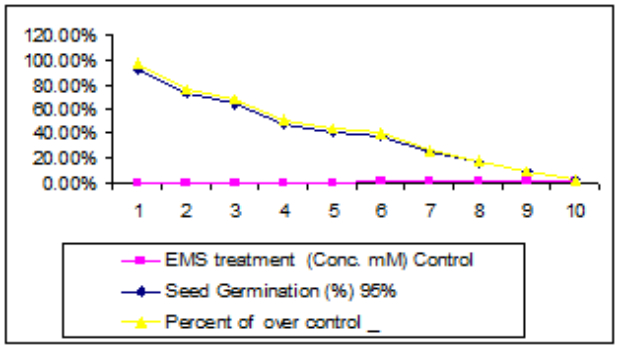
Data on the effect of mutagens on germination, expressed as per cent control and $LD_{50}$ is presented in Table 1. The untreated seeds of genotype had 100 per cent germination. The germination percentage decreased with increase in the dose/conc. of the treatment. Fenugreek seeds were treated with EMS, showed reduction in germination at higher concentration. Lowest germination percentages (2.1.%) were observed at 1.0% of EMS on 7$^{th}$ day after the germination. Based on the germination studies, 50% lethality was observed at 0.4% of EMS (Graph-1).

**Cytological studies:**

Cytological analysis with respect to their mitotic behaviour is considered to be one of the most dependable indices to estimate the potency of mutagen. Cytological studies provide information regarding the response of Fenugreek genotypes to a particular mutagen and provide greater chances for the selection of desired characters. Root mitotic studies revealed a wide range of chromosomal aberration such as nullisome, anaphasic bridge with laggard, anaphasic multiple bridges and laggards, anaphasic bridge, late anaphase, clumping of chromosome and precocious movement of chromosomes. Chromosome laggards were observed for all mutagenic treatments. In the present study, the aberrations caused by mutagens are due to partial or complete failure of spindle mechanism. Maximum chromosome aberrations were observed in 0.7% of EMS when compared to control (Pic: 1,2,3). (Table-1).

**Chemical mutagen (EMS)**

Root tip squash was carried out in the different concentration of 0.1% , 0.2%, 0.3%, 0.4%, 0.5% 0.6% 0.7% of EMS treated seedlings. In the present study, some of the cytological behaviour likes normal metaphase, anaphasic laggards, anaphasic bridge and stickiness with precocious movement of chromosome, anaphasic and nullisomic chromosomes were also observed (Pic: 4,5).




**Graph- 1:** Determination of LD50 value for EMS in Fenugreek.

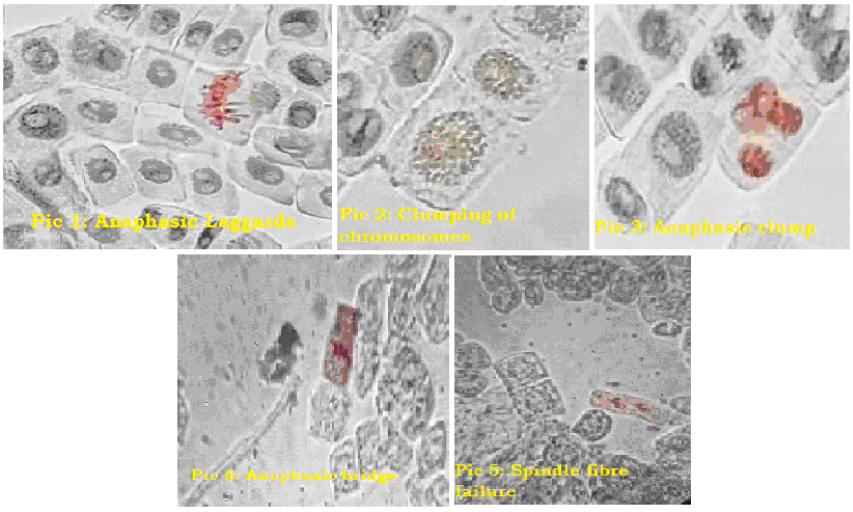


**Table-1: Effect of Ethyl methane sulphonate on mitotic cell division of fenugreek**

| Control | Number of cell observed | Number of abnormal cell | | | Total number of abnormal cell | % of abnormal cell frequency |
|---|---|---|---|---|---|---|
| | | Bridge | Laggard | Stickiness | | |
| 0.1 % | 70 | 8 | 6 | 4 | 18 | 25.7 |
| 0.2 % | 65 | 7 | 5 | 5 | 17 | 26.1 |
| 0.3 % | 68 | 12 | 8 | 10 | 30 | 44.1 |
| 0.4 % | 73 | 16 | 8 | 14 | 38 | 50.0 |
| 0.5 % | 76 | 17 | 12 | 11 | 40 | 66.0 |
| 0.6 % | 61 | 20 | 17 | 13 | 55 | 70.5 |
| 0.7 % | 78 | 22 | 18 | 20 | 60 | 76.9 |

**Table 2: Effect of Ethyl methane sulphonate on seed germination in $M_1$ generation of fenugreek**

| EMS treatment (Conc. mM) | Seed Germination (15th day) | | |
|---|---|---|---|
| | Total no of seeds | Mean | % over control |
| Control | 20 | 18 | - |
| 0.1 % | 20 | 17 | 94.4% |
| 0.2 % | 20 | 16 | 88.8% |
| 0.3 % | 20 | 15 | 83.3% |
| 0.4 % | 20 | 10 | 50.0% |
| 0.5 % | 20 | 8 | 44.4% |
| 0.6 % | 20 | 6 | 30% |
| 0.7 % | 20 | 4 | 22.2% |

**Table-3: Effect of Ethyl methane sulphonate on seedling survival in $M_1$ generation of fenugreek**

| EMS treatment (Conc. mM) | Seedling survival (20th day) | | |
|---|---|---|---|
| | Range | Mean | Percent over control |
| Control | 20 | 18 | 100% |
| 0.1 % | 20 | 15 | 83.3% |
| 0.2 % | 20 | 13 | 72.2% |
| 0.3 % | 20 | 11 | 61.6% |
| 0.4 % | 20 | 9 | 50.2% |
| 0.5 % | 20 | 7 | 38.2% |
| 0.6 % | 20 | 5 | 27.1% |
| 0.7 % | 20 | 2 | 11.1% |

**Field studies**

**Seed germination on 15th day**

A general reduction in seed germination was recorded due to all mutagenic treatments as shown in table-3. The reduction in seed germination ranged from 22.20 to 94.4 per cent in 0.7% of EMS (Table 2).

## DISCUSSION

Mutation breeding in crop plants is an effective tool in hands of plant breeders especially in crops having narrowgenetic base. Many mutants have been identified as donors of desirable traits in breeding program. Mutation breeding has contributed significantly to plant improvement, resulting in release of at least 2250 varieties of different crops. In India, at least 300 cultivars have been developed in at least 55 plant species (Kharkwal *et al*., 1998). By mutagen treatment which breaks the nuclear DNA during the process of DNA repair mechanism, new mutations are induced randomly. The changes can occur also in cytoplasmic organelles, and also results in chromosomal or genomic mutations and that enable plant breeders to select useful mutants such as flower colour, flower shape, disease resistance, early flowering types (Jayakumar and Selvaraj, 2003). The low level of genetic diversity in black gram, mutation induction constitutes a valuable strategy to create genetic variability, which in turn reduces the time required to breed new varieties compared with traditional methods (Gaul, 1964).

**$M_1$ generation**

**$LD_{50}$ Value**

In the present study to find out optimum dose/conc. of the mutagens, germination percentage of the seeds was calculated with effect of chemical mutagens of fenugreek. Among the mutagenic doses/conc., of the $LD_{50}$ (optimum) value was recorded at 0.5% of EMS (51.43%, 51.05%) and the maximum reduction of germination percentage were noted at 0.8% of EMS showed more lethal effect of fenugreek. Similar results were noted in sesame (Mensha *et al*., 2007) and *Lepidium sativum* (Majeed and Muhammad, 2010). The availability of efficient seed germination system after the mutagenic treatment is crucial in achieving successful mutagenesis. The higher exposure of gamma rays may cause injury in seeds and usually show inhibitory effects on seeds of Angiosperms and Gymnosperms. Compared to physical mutagen, the germination of seeds reduced more under chemical mutagen that they damage the biological material as reflected in the quantitative parameters (Gaul, 1964).

**Cytological Studies**

In the present study, fenugreek contains 8 bivalents (2n = 16) which are small in size and recognizable at higher magnification (10x, 100x). Lower concentration of mutagens like 0.2% of EMS revealed more or less normal pairing like that of control and mutagenic treated seeds. However, a consistent increase in the frequency of various types of chromosomal abnormalities was observed with increasing concentration of mutagens at 0.6% of EMS. Chromosomal abnormalities included the formation of anaphase bridges, laggards; multiple bridges, late anaphase, and precocious movement of chromosomes, unequal separation of chromosomes, clumping of chromosomes were also observed. Among the 0.6% of EMS, the maximum abnormalities, both structural and behavioural were induced in both the varieties. Dose dependent increase in frequency of different chromosomal aberrations has also been reported in Cowpea (Dhanavel *et al*., 2008).

Low frequency of anaphasic bridges was observed with 0.2% of EMS in fenugreek. They were produced due to the sub-chromatid exchanges, unequal exchange or by formation of dicentric chromosomes. The occurrence of breaks at the same locus and their lateral fusion leads to the formation of dicentric chromosome which is pulled equally to both the poles forming a bridge. The precocious separation of chromosomes at metaphase was observed at higher concentration at 0.6% of EMS only. It might have resulted due to the disturbed homology for chromosome pairing or disturbed spindle mechanism. Besides the precocious separation of univalents, the bivalents were also observed to move ahead and seemed as stray chromosome, this may move to one pole resulting into unequal distribution of chromosome or loss of a complete bivalent at metaphase stage (Diouf *et al*., 2000).

**Seedling survival percentage**

Gradual reduction of seedling survival percentage and plant height was observed in different doses/concentrations on 20$^{th}$ day (field condition) by the effect of EMS mutagen. This mutagen was effective in reducing the survival percentage and plant height of $M_1$ plants. The phenotypic response varied with respect to mutagenic treatments. 0.6% of EMS recorded highest reduction in survival percentage. This probability reflects the organ specific action of the mutagens. The gamma rays may be affecting shoot initials more than the root initial (Waghmare and Mehra, 2000). Similar results were reported in mung bean (Singh *et al*., 1997) and cowpea (Dhanavel *et al*., 2008) and Millet (Yadava *et al*, 2003).

**SUMMARY**

In order to find out optimum dose/concentration of the mutagen, germination percentage was calculated withlocal variety of fenugreek. Based on the seed germination, 50% lethality ($LD_{50}$) was determined in EMS (0.4%). Root tip mitotic studies revealed a wide range of chromosomal aberrations such as stickiness, precocious movement, anaphasic bridges and laggard, anaphasic multiple bridges, late anaphase, clumping of chromosome**.** The chromosome studies were made in treated plants such as EMS. Maximum chromosome aberrations were observed in 0.7% of EMS. Seedlings survival on 20$^{th}$ day after sowing was counted. The maximum survival reduction was observed at 0.7% of EMS compared to other doses.

**Conflict of Interest**

The author declares that there is no conflict of interest.

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