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STUDY THE EFFECT OF PHYSICAL AND CHEMICAL MUTAGENS ON BIOLOGICAL PARAMETERS IN M_1 GENERATION OF *Trigonella foenum-graecum* L.[#]

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Abstract

Trigonella foenum-graecum L.is commonly known as fenugreek, belongs to family Fabaceae. Plant is autogamous, annul and having multiple significant properties. It is used as spice, fodder and leafy vegetable. The seeds and leaves are rich source of Vitamin A, Vitamin C, proteins, carbohydrates and minerals especially organic ions, phosphorous and calcium etc. *Trigonella* has been extensively studied for its role in treatment of diabetes and hypercholesterolaemia. The present work was carried out to study various biological parameters of *Trigonella* with the treatment of physical, chemical mutagens and their combinations. The seeds were treated with physical mutagens like Gamma rays individually using different concentrations/doses (240Gy, 300 Gy, 360 Gy and 420 Gy). Chemical mutagen used was EMS (0.025%, 0.050%, 0.075% and 0.1%) and the combination of both (240Gy+0.1%EMS, 300Gy+0.075%EMS, 360 Gy+0.050%EMS, 420 Gy+0.025%EMS). Treated seeds were sown to raise M_1 generation. Germination percentage, seedling height and mitotic index found to be increased with decreasing concentration of mutagens while seedling injury, pollen sterility increased with increasing concentration. Different morphological changes in leaf (chimeras) were observed like leaf xantha, chlorina, albino etc. Margins of leaflets were notched to form bilobed and trilobed leaflets. Change in colour of flower i.e white to pale yellow and yellow was observed.

Key Words: EMS, Gamma rays, Mutagens, Seedling injury and height, chimeras etc.

[#]Short Communication

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Introduction

Trigonella foenum-graecum L. commonly known as *Trigonella* belongs to family Leguminosae. The genus *Trigonella* is one of the largest genera of the tribe Trifoliatae in the family Fabaceae and sub-family Paplionoideae (Balodi and Rao, 1991). *Trigonella* has been used as a medicinal herb both in Indian Ayurvedic and Traditional Chinese Medicines (Tiran, 2003). According to Lust (1986) is one of the oldest known medicinal plants in the recorded history. *Trigonella* leaves and seeds have been used extensively to extracts and powders for medicinal uses (Basch et al., 2003).

Trigonella foenum-graecum L., plant is widely distributed throughout the world. The yields can be significant increase in quantity and quality through the suitable management of cultivation, irrigation and harvesting. The plant contains active constituents such as alkaloids, flavonoids, steroids, Saponins etc. It is an old medicinal plant. It has been commonly used as a traditional food and medicine. *Trigonella* is known to have hypoglycemic, and hypocholesterolaemic, effects, anti-inflammatory effects. Research has identified *Trigonella* as a valuable medicinal plant with potential for curing diseases and also as a source for preparing raw materials of pharmaceutical industry, like in steroidal hormones. Since *Trigonella* is a self-pollinated crop, a mutation breeding method can be used to generate mutants with a determinate growth habit. Irradiation and chemical mutagens can be used to produce point mutations in *Trigonella*.

Origin and History of *Trigonella*

Trigonella is an herb native to southeastern Europe, northern Africa, and western Asia but is widely cultivated in other parts of the world. Its botanical name is *Trigonella foenum-graecum* L; its English name comes from two Latin words meaning Greek hay. *Trigonella* is an annual plant that grows 2–3 ft (0.6-0.9 m) tall, with a strong odour and small pale yellow flowers. The seed of the *Trigonella* plant contains many active compounds with pharmaceutical applications. The seeds are collected in the autumn. The chemical components of *Trigonella* seed include iron, vitamin A, vitamin B1, vitamin C, phosphates, flavonoids, saponins, trigonelline, and other alkaloids. The seed is also high in fiber and protein.

Material and Methods

Mutagens Used

Physical Mutagens: Gamma rays, Chemical mutagens: Ethyl Methanesulphonate (EMS) and combination of Gamma Rays and Ethyl Methanesulphonate (EMS)

Modes of Treatment

Gamma Radiation

Healthy, uniform sized dry seeds of the *Trigonella foenum-graecum* L. variety Phule Kasturi, were packed in polythene bags and sealed the bags separately for Gamma radiation. Electromagnetic, ionizing radiation were applied from CO^{60} source of irradiation. Gamma radiation was carried out at Nuclear Chemistry Division, Department of Chemistry, University of Pune, Ganeshkhind, Pune – 411007. The seed samples were exposed to doses of 240 Gy, 300 Gy, 360 Gy and 420 Gy of Gamma rays.

Ethyl Methanesulphonate (EMS)

Ethyl methanesulphonate (EMS) was obtained from Spectrochem Pvt. Ltd. Mumbai (India) with a molecular weight 124.16 g/mol and density 1.20g/cm3. To determine the lethal dose (LD_{50}) and suitable concentration of mutagens for the further studies. Chemical mutagenic treatments were administered at room temperature of 25±2°C. Healthy and dry seeds of the *Trigonella foenum-graecum* variety (Phule Kasturi) having uniform size were selected for the treatment. Seeds were surface sterilized with 0.1% mercuric chloride solution for about one to two minutes than washed thoroughly and soaked in distilled water for 6 hours for pre-soaking of seeds. Pre-soaking made the seed coats permeable for the mutagenic treatment. Prior to the treatment, fresh, aqueous solutions of mutagen were prepared. The different concentrations of chemical solutions were used for mutagenic treatments were 0.025 %, 0.050%, 0.075% and 0.1%.

Combine Treatment of Gamma Rays And EMS

For the combine treatments, seeds irradiated with Gamma rays were used. After the physical mutagenic treatment, the chemical mutagenic treatment of EMS was given to the same seed samples. Combination of physical and chemical mutagens used like 240Gy + 0.1% EMS, 300Gy + 0.075% EMS, 360Gy + 0.050% EMS and 420Gy + 0.025% EMS. For each treatment, a batch of 500 seeds were used. 100 seeds from each were plotted between the folds of filter paper, kept in dark room at room temperature.

Mitotic Index

Mitotic index is a measure of cellular proliferation. It can be defined as the percentage of cells undergoing mitotic division in a given population of cells. Mitosis is the type of cell division occurs in somatic cells, resulting in to two daughter cells. Duration of the cell cycle and mitosis may be different as per cell types. An elevation in mitotic index shows more cells are dividing and lower mitotic index indicates less cells are dividing**.** From each treatment/dose treated with mutagen about one hundred seeds were kept in moist filter paper at room temperature (25 + 2°C). About 1 to 1.5cm root tips length were fixed in 1:3 Carnoy's fluids fixative (1 part of Glacial acetic acid and 3 parts of alcohol) for 12 hours. They were later on transferred to 70% ethanol and stored in the refrigerator. Before preparation of slide, the root tips were hydrolyzed in IN HCL at 60°C in water bath for 7-10 minutes and then wash with water and stained in 1% Haematoxylin by using 4% ferric alum as a mordent. They were later on squashed in a drop of 45% acetic acid. At

least 20-25 slides were prepared for each concentration /dose and the mitotic index was recorded. Mitotic index was calculated by using following formula:

$$MI = \frac{\text{Number of dividing cells} \times 100}{\text{Total numbers of cells in population (N)}}$$

Pollen Sterility

Pollen sterility was determined in 25 randomly selected plants from each treatment. The pollen grains from freshly dehisced anthers were stained with 1% Acetocarmine. The pollen grains which stained were counted as pollen fertile and partially unstained was considered as pollen sterile.

Flower Colour Mutations

In induced mutation breeding program the flower colour mutations are always expected.

Survival of Plants

Survival percentage was recorded by counting the number of surviving seedlings at 21 days after 50% emergence.

Results and Discussion

In the present investigation, the seeds of variety Phule Kasturi of *Trigonella foenum-graecum* L. were treated with Gamma rays, EMS and combination of Gamma rays and EMS. The experimental results were recorded and discussed below.

Mitotic Index

The mitotic index in the control of *Trigonella* was 19.26%. Mitotic index decreases with the gradual increase in the concentration/ doses of EMS, Gamma rays and combination treatment. The mitotic index ranged from 17.29% to 11.24% in EMS, 19.14% to 13.45% in Gamma rays and 17.50% to 11.29% in combination treatments. The highest mitotic index was 19.14% in Gamma rays and the lowest mitotic index was 11.24 at 0.1% of EMS. The number of cells with various anomalies has been scored at different stages of mitosis (Kumar and Dubey, 1997).

Fig.: 1. Effect of Mutagens on Mitotic Index in M_1 Generation of *Trigonella foenum-graecum* L.

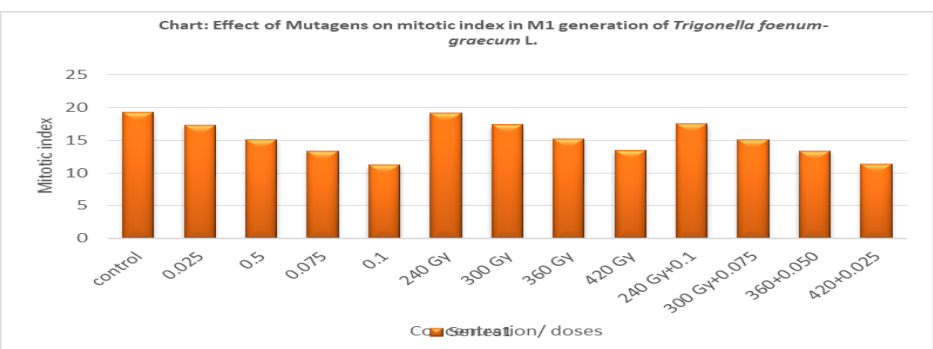
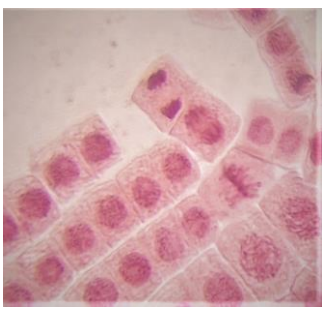


Table: 1. Effect of Mutagens on Mitotic Index in M_1 Generation of *Trigonella foenum-graecum* L.

Mutagens	Dose/Concentration	Mitotic Index	+_ S.E.
Control	-	19.26	3.05
EMS	0.025%	17.29	2.74
	0.050%	15.10	2.4
	0.075%	13.30	2.11
	0.1%	11.24	1.79
Gamma Rays	240Gy	19.14	3.03
	300Gy	17.38	2.76
	360Gy	15.24	2.42
	420Gy	13.45	2.15
Gamma rays +EMS	240GY + 0.1%	17.50	2.77
	300Gy + 0.075%	15.11	2.4
	360Gy + 0.050%	13.33	2.11
	420Gy + 0.025%	11.29	1.81



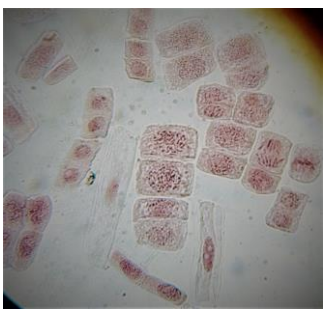


Plate: 1 and 2 showing Prophase, Metaphase, Anaphase and Telophase.

Pollen Sterility

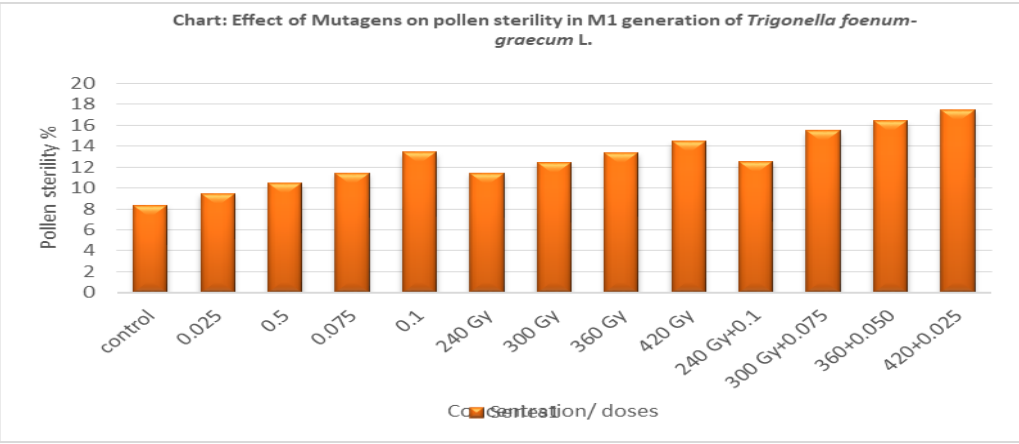
The pollen sterility in the control was 8.36%. The percentage of pollen sterility was increase with the increasing concentration /dose of the chemical and physical mutagens. The maximum sterility could be observed at the highest concentration of the various mutagens, while the Combination treatment was produced the highest pollen sterility. The maximum pollen sterility induced was 17.49% at 420 Gy+0.025% of Combination treatment. The minimum sterility was 9.48% at 0.0% of EMS. The pollen sterility varies from 9.48 % to 13.45% in EMS, in Gamma rays, 11.43% to 14.46% and 12.55% to 17.49% in combination treatments.

Siddique et.al. 1999 studied the effect of Methyl Methane sulphonate (MMS) on pollen sterility in the mungbean. A direct relationship of pollen and ovule sterility with higher doses of gamma rays and EMS in *Vigna Mungo* has been recorded by Gautam et al. (1992). The EMS treatment was found to cause higher sterility than gamma rays in chick pea (Kharakwal, 1981). Combined treatments also showed greater reduction in the survival of seedling than the individual treatment. Bhatnagar (1984) recorded the harmful effects of combined treatments on germination and survival of plants in chick pea. The pollen sterility increased in combined treatments indicating the additive or synergistic effect.

Table: 2. Effect of Mutagens on Pollen Sterility in M_1 Generation of *Trigonella foenum graecum* L

Mutagens	Dose/Concentration	% Pollen Sterility	+_ S.E.
Control	-	8.36	1.3246
EMS	0.025%	9.48	1.5
	0.050%	10.47	1.6591
	0.075%	11.45	1.8123
	0.1%	13.45	1.732
Gamma rays	240Gy	11.43	1.808
	300Gy	12.44	1.9695
	360Gy	13.39	2.1189
	420Gy	14.46	2.2896
Gamma rays +EMS	240GY + 0.1%	7512.55	1.9871
	300Gy + 0. 075%	15.51	2.4547
	360Gy + 0.050%	16.43	2.6
	420Gy + 0.025%	17.49	2.768

Fig 2- Effect of Mutagens on Pollen Sterility In M_1 Generation of *Trigonella foenum graecum* L.



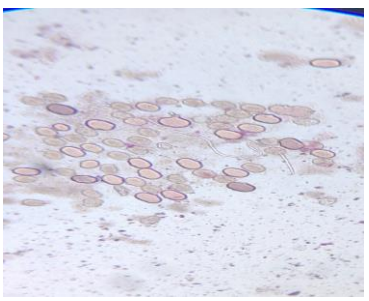
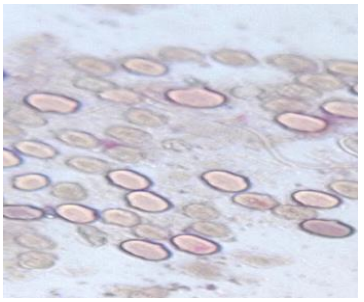



Plate: 3. Pollen sterility in 360 Gy Photoplate 4- Pollen sterility in 0.1 % EMS

Flower colour mutations

In induced mutation breeding program the flower colour mutations are always expected. In present investigation two flower colour mutants were obtained in the plant population of *Trigonella* like white and light yellow to bright yellow colour. In control plants have white coloured flowers.


(6)

(7)

Plate: 6. Mutation in flower colour (yellow)
Plate: 7. Normal white coloured flower

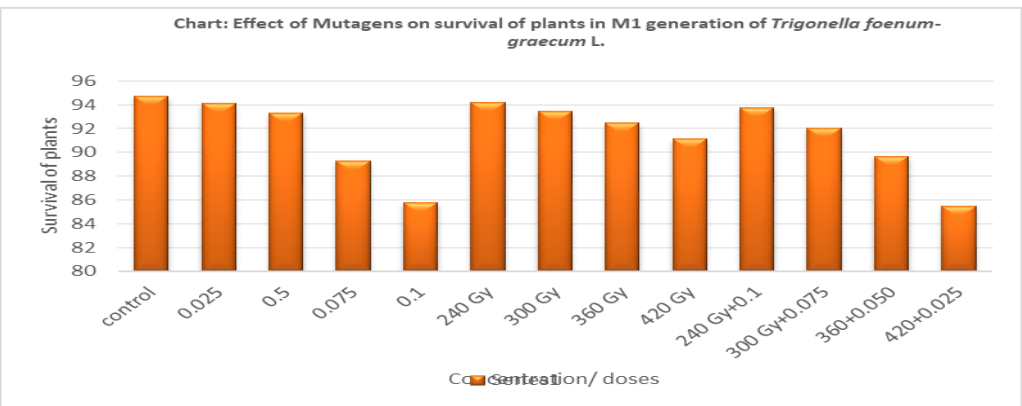
Survival of Plants

The survival of plants at maturity showed gradual increase with the concentrations/doses of different mutagenic treatments. In control the survival of plants at maturity was 94.75%. In EMS the survival ranged from 94.13% to 85.78% at different concentrations. In gamma rays, the survival ranged from 94.20% to 91.11%. In combination treatments the survival ranged from 93.78% to 46.99%. Almost all treatments show the quite satisfactory results about the survival of plants at maturity. These findings are close agreement with the earlier reports of Wang and Yu (1988), Solanki and Sharma (1999), Kumar and Selvaraj (2003), Solanki and Phogat (2005), Geeta and Wakode (2011).

Table 3-Effect Of Mutagens on Survival of Plants in M_1 Generation of *Trigonella foenum-graecum* L.

Mutagens	Dose/Concentration	Survival of plants at maturity	% Survival of plants	+_ S.E.
Control	-	90.29	94.75	1.98
EMS	0.025%	80.21	94.13	1.88
	0.050%	71.14	93.34	1.75
	0.075%	58.52	89.31	1.12
	0.1%	48.29	85.78	1.56
Gamma Rays	240Gy	81.83	94.20	1.89
	300Gy	71.08	93.42	1.77
	360Gy	61.35	92.46	1.61
	420Gy	51.3	91.11	1.4
Gamma rays +EMS	240GY + 0.1%	75.41	93.78	1.82
	300Gy + 0.075%	70.73	92.07	1.55
	360Gy + 0.050%	58.91	89.65	1.17
	420Gy + 0.025%	46.99	85.45	1.15

Fig: 3. Effect of Mutagens on Survival of Plants In M_1 Generation of *Trigonella foenum-graecum* L.



Conclusion

From present study it can be concluded that both mutagens showed an inhibitory effect on mitotic index, survival of plants and pollen sterility percentage. Also it revealed a

wide range in flower colour mutation. The concentration/dose used in present study will be effective in induction of wide range of mutants.

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