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Insights into Mutagenesis Induced by Gamma radiations and Ethyl Methanesulphonate (EMS) in Fenugreek (*Trigonella foenum-graecum* L.)

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Abstract
To achieve global food security, we need innovative methods to ensure food safety by 2050 to feed estimated 9 billion populations. The major hurdle is climate change with adverse effect on sustainable production of food crops. Mutation breeding with the help of new technologies has been proven successful for the production of improved cultivars worldwide. Fenugreek (*Trigonella foenum-graecum* L.) is a leguminous herb with various food and medicinal values as food, fodder, spice, condiment and seed oil. The present study aims to create genetic variability using gamma radiations, EMS and combined treatments in two varieties of fenugreek *viz.*, 'Desi methi' and 'Metha' for the improvement of qualitative and quantitative traits. Results showed inhibition in, seed germination, pollen fertility and plant survival while lower doses of mutagens showed improved yield and its attributing traits. Hence, it was observed that mutations successfully induced in fenugreek and putative mutant plants can be selected for establishment of cultivars by subsequent generations.

Keywords: Fenugreek (*Trigonella foenum-graecum* L.), ethyl Methanesuphonate, gamma rays, induced mutations

1. Introduction
Climate change is detrimental to sustainable food production whilst human population is supposed to reach 9 billion by 2050. Food security demands global strategy with innovative cost-effective methods to support agricultural (FAO, 2012) [7]. In present scenario mutation breeding has proven advantageous in improving specific character of crops by generating multiple putative mutants which are then subsequently screened (Toker *et al.*, 2007) [26]. Raina *et al.* (2016) [19] stated that released mutant varieties contribute to global food security for the improvement of livelihoods across the world. Mutant variety database (https://mvd.iaea.org) [18] recorded 3,362 mutant varieties till 2020, developed through mutation breeding programme across the world.

Fenugreek (*Trigonella foenum-graecum* L.), a semiarid crop is being used as an herb since ancient time in China and various part of Asia. It is said to be originated in east Mediterranean region (Vavilov, 1926) [27] and widely grown in India, Egypt, Ethiopia and Turkey. Plant bears leaves which are trifoliate and flowers are white and become yellow at maturity. Seeds are hard, reddish brown and lie side by side in thin elongated beak shaped pods. Seeds are smooth exteriorly with a prominent groove which delineates it in two unequal divisions (Acharya *et al.*, 2006; Srinivasan 2006 [1, 23]). Being a legume crop, it helps in fixing nitrogen and reduces the cost of nitrogen fertilizer. Fenugreek is used traditionally because it is having immense therapeutic values. The leaves are source of minerals like calcium, zinc, iron and phosphorous and vitamins like Vitamin B1, B2, B3 and vitamin C while seeds consists of protein 28.55%, fats 4% and carbohydrates 46.25% (Rao 2004 ; Sulieman *et al.*, 2008 [20, 25]). Pharmaceutical effects of fenugreek includes immunomodulatory, anti-carcinogenic, anthelmintic, antioxidant, anti-ulcer, anti-obesity, anti-diabetic and hypocholesterolemic effects and help to regulate blood circulation and therefore energize the body (Kumar *et al.*, 2013; Sudha & Mathangi, 2013 [12, 24]).

The present study was conducted to evaluate mutations in fenugreek var. Metha and var. Desi methi. Mutations are induced by gamma radiations, EMS and combined treatments in M_1 generation to evaluate primary mutagenic effects.

2. Materials and Methods
The fresh and healthy seeds of fenugreek var. 'Metha' and 'Desi methi' were used. These varieties are suitable to climatic conditions of experimental site. Both gamma rays and EMS as well as their combined treatments (gamma rays +EMS) were used. Seeds were treated with gamma rays (using ^{60}Co gamma radiation chamber (Model GC-5000, BRIT Mumbai) at IARI Pusa, New Delhi, India while chemical mutagen EMS manufactured by Sisco Research Laboratories Pvt. Ltd., Mumbai, India, were used to induce mutations. Seeds were presoaked for 8 hours prior to treat with EMS. Duration of EMS mutagen treatment was 6 hours. Seeds were washed to eliminate excess mutagen and sown at agricultural field of Aligarh Muslim University, Aligarh, India. A set of 300 seeds for each treatment including control was used to raise M_1 generation. All suggested agronomic practices were carefully conducted throughout the experimental study at field. The treatment doses/concentrations of mutagens are as follows.

Table 1

Mutagenic Doses/Concentrations		
Physical	**Chemical**	**Combination**
50 Gy γ rays	0.01% EMS	50 Gy γ rays + 0.01% EMS
100 Gy γ rays	0.02% EMS	100 Gy γ rays + 0.02% EMS
150 Gy γ rays	0.03% EMS	150 Gy γ rays + 0.03% EMS
200 Gy γ rays	0.04% EMS	200 Gy γ rays + 0.04% EMS

2.1 Seed germination, pollen fertility and plant survival

Total number of seeds germinated, was counted within 6-10 days after sowing and percentage seed germination (%) was calculated as seed germinated divided by total number of seed sown.

Pollen grains were dusted from flower and stained with 1% acetocarmine stain. Pollen grains were identified on the basis of shape, stain and outline. Fertile pollen grains are with regular outline and stain while sterile are irregular and non-stained. Percentage of pollen fertility was calculated by dividing number of fertile pollens by total number of pollen grains.

Plant survival percentage was calculated at the time of maturity. Plant survival percentage (%) of M_1 plants was calculated at maturity by dividing total number of germinated

2.2 Estimation of total Chlorophyll, Carotenoid Contents and Nitrate Reductase Activity

To determine total leaf Chlorophyll and carotenoid contents method of MacKinney (1941) [17] was followed. Leaf tissue (500 mg) was homogenized in 20 ml of acetone (80%) and centrifuged at 5000 rpm for 5 minutes. Absorbance was recorded at 645 and 663 nm for Chl *a*, Chl *b*, and 480 and 510 nm for carotenoids, respectively.

The calculation of total chlorophyll and carotenoid contents present in leaves of fenugreek was done by the equation given by Arnon (1949) [4].

Total chlorophyll (mg g^{-1}leaf fresh mass) = (20.2 (OD645) + 8.02(OD663)) x $\frac{V}{1000 \times W}$

Carotenoid (mg g^{-1}leaf fresh mass) = $\frac{7.6\,(OD\,480) - 1.49\,(OD510)}{d \times 1000 \times W} \times V$

The activity of nitrate reductase was determined by using fresh leaf sample of fenugreek. The method described by Jaworski (1971) [9] was used to calculate nitrate reductase activity.

2.3 Quantitative traits in M_1 generation

Plants were observed for various quantitative traits like days to flowering, days to maturity, plant height (cm), fertile branches per plant, pods per plant, length of pods (cm), seeds per pod, 1000 seed weight (g), seed yield per plant (g) in M_1 generation.

3. Results & Discussion

3.1 Seed germination, pollen fertility and plant survival

Results showed increased inhibition in seed germination and pollen fertility in higher dose while plant survival showed dose independency. Inhibition from control was found in percentage of seed germination, pollen fertility and plant survival in all treatments (Fig. 1). In treated population maximum seed germination was recorded as 92.33% (0.01% EMS) while lowest as 72.00% (200 Gy γ rays + 0.04% EMS) in var. Metha. Same pattern was followed in var. Desi methi. Seed germination (%) inhibition caused by abnormal mitotic process and enzyme activity due to mutagenic action (Ananthaswamy *et al.*, 1971[3]; Kurobane *et al.*, 1979 [13]).

Pollen fertility decreased with the increased dose of mutagens. In var. Metha, it ranged from 91.00 to 79.40 whereas in var. Desi methi, it ranged from 91.20 to 77.40. Abnormal microsporogenesis led decreased pollen fertility. As for plant survival rate, it also decreased in treated populations. Same observation was also reported by Bashir *et al.* (2013) [5].

3.2 Estimation of total Chlorophyll, Carotenoid Contents and Nitrate Reductase Activity

Photosynthetic pigments chlorophyll and carotenoids showed increment in lower doses while in higher doses it decreased. Chlorophyll content showed positive shift from control (1.103 mg/g) to 0.01% EMS (1.253 mg/g) in var. Metha whereas it increased up to 1.703 mg/g (0.01% EMS) from control (1.570 mg/g). Same pattern was followed for carotenoid content and NRA activity. Additionally it was observed that chlorophyll content and nitrate reductase activity was higher in var. Desi methi as compared to var. Metha but carotenoid content was higher in var. Metha than var. Desi methi (Table 1). chlorophylase, an enzyme which is supposed to degrade chlorophyll and reduction in chlorophyll content in higher doses may be considered as increased activity of this enzyme due to mutagenic action (Reddy and Vora, 1986) [21]. Enhancing effects on physiological parameters of lower doses also reported by Shereen *et al.* (2009) [22], Alikamanoglu *et al.* (2011) [2] and Bashir *et al.* (2013) [5].

3.3 Quantitative traits in M_1 generation

Results for quantitative traits studied in present experiment were indicated in table 2 and 3. Days of flowering showed negative shift from control and lowest flowering days were observed in highest dose of combination treatment (200 Gy γ rays + 0.04% EMS) and same phenomenon was observed for days to maturity. However, there were significant differences in mean days to flowering and maturity in both varieties. Var. Metha showed early flowering and early maturity as compared to var. Desi methi. Plant height reduction was observed in all treatments. Plant height reduction caused by induced mutations has been reported by Luo *et al.* (2012) [16] and Laskar and Khan (2017b) [15].

As for quantitative traits, yield attributing traits such as fertile branches/plant, pods/plant, seeds/pod and seed yield/plant showed increment in lower doses of all mutagenic treatment and EMS showed maximum positive shift followed by gamma rays and combination treatments. Yield attributing traits such as fertile number of branches, pods/plant, seeds/pod and seed yield/plant showed positive shift in lower doses of mutagen. EMS showed improvement in these traits as compared to gamma rays and combination treatments. Lower doses of mutagen showed positive shift to yield and same trend have been recorded in *Trigonella foenum graecum* (Jabee *et al.*, 2007) [8] and in lentil (Laskar and Khan (2017a) [14]. Although decrease in yield parameters was also reported by Choudhary *et al.* (2012) in fenugreek [6]. Mutagenic effects on growth regulating hormones may lead to the enhancement of yield attributing characters due to mutation in polygenic traits (Kothekar, 1983) [11]. Overall damage in M_1 generation caused by mutagenic action may be attributed to DNA disruption which resulted in abnormal enzyme synthesis and other metabolic activities (Kirtane & Dhumal 2004) [10].

Table 1: Effects of gamma rays EMS and their combinations on chlorophyll, carotenoid contents and nitrate reductase (NRA) activity in the leaves of two varieties of fenugreek in M_1 generation.

Treatment	var. Metha			var. Desi methi		
	Cholorophyll (mg g^{-1} FW) ± S.E	Carotenoid (mg g^{-1} FW) ± S.E	NRA (nmol. h-1.g-1FW) ± S.E	Cholorophyll (mg g^{-1} FW) ± S.E	Carotenoid (mg g^{-1} FW) ± S.E	NRA (nmol. h-1.g-1FW) ± S.E
Control	1.103 ± 0.029	0.513 ±0.010	289.60± 0.92	1.570± 0.066	0.350± 0.005	385.20±0.73
50 Gy γ rays	1.203± 0.060	0.525 ±0.028	291.20± 1.39	1.620± 0.068	0.357± 0.029	386.40±1.63
100 Gy γ rays	1.163± 0.089	0.517 ±0.015	290.00± 1.51	1.163± 0.089	0.351 ± 0.016	385.60±1.02
150 Gy γ rays	1.023± 0.063	0.483 ±0.014	289.20± 1.49	1.590± 0.085	0.337± 0.014	385.20±1.24
200 Gy γ rays	1.067± 0.010	0.481 ±0.010	288.20± 1.39	1.520± 0.072	0.323± 0.008	384.40±1.50
0.01% EMS	1.253± 0.029	0.528 ±0.010	291.80± 1.06	1.703± 0.066	0.361± 0.012	387.60±1.40
0.02% EMS	1.177± 0.043	0.520 ±0.015	290.40± 1.16	1.693± 0.067	0.353 ± 0.011	386.40±1.53
0.03% EMS	1.113± 0.085	0.487 ±0.018	289.80± 1.24	1.567± 0.084	0.339 ± 0.014	385.40±1.36
0.04% EMS	1.083± 0.060	0.485 ±0.013	289.20± 1.39	1.537± 0.031	0.327 ± 0.014	384.80±1.46
50 Gy γ rays + 0.01% EMS	1.160± 0.087	0.516 ±0.015	290.00± 1.09	1.507± 0.085	0.322 ±0.005	384.20±1.06
100 Gy γ rays + 0.02% EMS	1.067± 0.076	0.506 ±0.012	286.40± 1.60	1.230± 0.180	0.345 ± 0.030	383.80±1.39
150 Gy γ rays + 0.03% EMS	0.933± 0.050	0.464 ±0.010	285.40± 1.56	1.133± 0.078	0.338 ± 0.041	383.20±1.15
200 Gy γ rays + 0.04% EMS	0.927± 0.044	0.440 ±0.013	284.80± 1.59	0.953± 0.069	0.308 ± 0.020	379.80±1.39

Table 2: Estimates of mean ($\bar{X}$) ± standard error for quantitative traits in M_1 generation of fenugreek var. Metha

Treatment	Days to flowering	Plant height (cm)	Days to Maturity	Fertile branches	Pods/ plant	Pod length (cm)	Seeds/ Pod	1000 seed weight	Yield/ plant
Control	64.60±0.50	114.40±0.51	152.00±0.54	4.60±0.40	23.80±0.58	10.12±0.20	16.00±0.54	10.89±0.08	4.13±0.03
50 Gy γ rays	61.60±0.81	116.10±0.90	150.00±0.70	5.20±0.58	25.20±0.73	10.58±0.37	16.80±0.58	10.93±0.11	4.29±0.05
100 Gy γ rays	62.20±0.73	115.70±0.95	149.90±1.07	4.80±0.73	24.60±0.67	10.48±0.55	16.60±0.74	10.84±0.15	4.26±0.05
150 Gy γ rays	62.60±0.92	115.10±0.67	149.80±0.66	4.40±0.67	23.60±0.97	10.42±0.50	16.00±0.70	10.77±0.16	3.95±0.08
200 Gy γ rays	63.00±0.54	114.00±0.82	149.00±0.70	4.40±0.92	22.80±0.86	10.10±0.33	15.80±0.66	10.71±0.09	3.85±0.05
0.01% EMS	60.00±0.70	116.30±0.93	151.40±0.92	5.40±0.50	26.00±0.89	11.15±0.51	17.40±0.67	11.34±0.11	4.33±0.04
0.02% EMS	60.80±0.86	115.90±0.91	150.40±1.20	5.20±0.66	25.60±0.92	10.98±0.52	16.80±0.58	11.09±0.15	4.27±0.04
0.03% EMS	61.40±0.92	115.40±0.88	150.00±0.94	5.00±0.70	25.20±0.66	10.46±0.76	16.20±0.66	10.83±0.13	4.01±0.14
0.04% EMS	62.00±0.70	114.10±0.58	149.20±0.96	4.80±0.86	24.80±0.86	10.38±0.55	16.00±0.83	10.75±0.14	3.89±0.07
50 Gy γ rays + 0.01% EMS	62.40±0.92	115.80±0.90	149.20±1.06	5.00±0.70	25.00±0.70	10.80±0.48	16.20±0.58	10.96±0.08	4.21±0.07
100 Gy γ rays + 0.02% EMS	62.80±0.80	114.70±0.61	149.20±0.86	4.60±0.92	24.20±0.73	10.22 ±0.33	16.00±0.70	10.63±0.10	4.02±0.07
150 Gy γ rays + 0.03% EMS	63.00±0.89	114.30±0.61	147.80±0.86	4.40±0.50	24.00±0.83	10.28±0.67	15.20±0.80	10.59±0.15	4.00±0.05
200 Gy γ rays + 0.04% EMS	63.40±0.81	113.60±0.85	147.40±0.92	4.00±0.70	23.40±0.92	10.02±0.38	14.40±0.92	10.54±0.10	3.81±0.06

Table 3: Estimates of mean ($\bar{X}$) ± standard error for quantitative traits in M_1 generation of fenugreek var. Desi methi

Treatment	Days to flowering	Plant height (cm)	Days to Maturity	Fertile branches/ plant	Pods/ plant	Pod length (cm)	Seeds/ pod	1000 seed weight (g)	Yield/ plant (g)
Control	120.60±0.67	86.68±0.91	160.80±0.66	5.40±0.40	30.2±0.86	10.72±0.31	17.40±0.67	6.96±0.08	3.89±0.09
50 Gy γ rays	117.80±0.86	89.78±1.30	158.40±0.81	6.00±0.70	33.6±1.96	10.38±0.67	18.60±0.50	6.43±0.13	4.24±0.18
100 Gy γ rays	117.00±0.70	89.40±1.15	158.60±0.92	5.60±0.67	34.8±2.47	9.58±0.50	16.60±0.92	6.21±0.12	4.17±0.16
150 Gy γ rays	116.20±0.70	85.12±1.44	157.80±1.57	5.40±0.74	30.8±2.78	10.60±0.51	15.80±0.86	6.20±022.	3.97±0.16
200 Gy γ rays	115.80±0.80	82.60±1.38	157.60±1.02	4.80±0.37	28.6±2.54	9.76±0.45	15.20±0.96	6.15±0.20	3.77±031
0.01% EMS	116.80±0.86	88.80±1.22	158.00±0.70	6.40±0.81	35.8±1.59	11.74±0.41	18.20±0.86	6.47±0.12	4.18±0.21
0.02% EMS	115.80±0.96	88.42±1.42	157.40±0.86	6.20±0.58	35.2±2.65	11.60±0.51	17.80±0.96	6.21±0.19	4.10±0.13
0.03% EMS	115.60±0.74	83.20±1.05	156.80±0.74	5.20±0.67	33.6±3.04	10.48±0.59	15.80±0.66	6.10±0.15	3.91±0.10
0.04% EMS	115.00±0.70	81.98±1.08	156.40±0.92	5.00±0.70	33.2±2.51	9.50±0.48	15.40±0.92	5.93±0.29	3.75±0.22
50 Gy γ rays + 0.01% EMS	118.00±1.02	82.42±1.24	158.20±1.68	5.80±0.37	32.2±2.35	10.44±0.30	17.80±0.86	6.10±0.13	4.02±0.23
100 Gy γ rays + 0.02% EMS	117.20±0.86	80.50±1.30	156.60±0.67	5.40±0.74	30.4±2.24	9.98 ±0.69	15.40±0.67	6.01±0.19	3.83±0.16
150 Gy γ rays + 0.03% EMS	116.60±0.92	78.46±1.34	156.20±0.87	4.80±0.58	26.2±2.28	9.32±0.40	13.60±0.60	5.99±0.12	3.43±0.32
200 Gy γ rays + 0.04% EMS	115.40±0.92	78.08±1.34	155.80±0.86	4.80±0.80	24.8±1.15	8.10±0.69	12.20±0.86	5.96±0.14	3.29±0.21

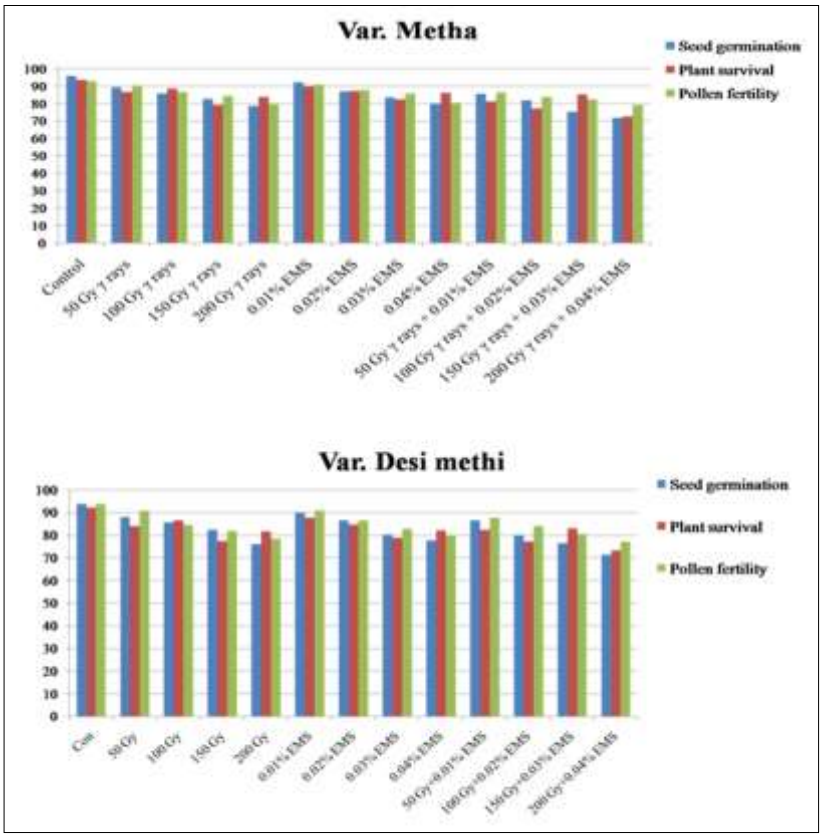

Fig 1: Percentage of seed germination, plant survival and pollen fertility of fenugreek var. Metha and Desi methi

4. Conclusion

It is concluded that fenugreek is a promising herb with numerous health benefits and recent studies on anticancerous properties of fenugreek established research interest among scientists for the food and nutritional enhancement of crop. The present study showed successful induction of mutations to create variants with desirable yield attributing characters. The selection and cultivation of useful putative mutants along with marker assisted breeding for subsequent breeding cycles to stabilize heritable changes might lead to the development and release of mutant varieties for our elite farmers.

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