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Mutagenic damage by gamma rays and Ethylmethane Suphonate in two cultivars of Faba bean

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Abstract
For improvement of different crop plants, mutation breeding is most reliable technique. Mutation breeding widely used for the improvement of plant characters and has been a powerful and effective tool in increasing variability especially for self-pollinated crops with narrow genetic base. Assessment of biological damage induced by different doses of physical and chemical mutagens is an important aspect during mutation breeding programmes. The current experiment was conducted to assess the biological damage induced by different doses/concentrations of gamma rays and ethylmethane sulphonate in two cultivars of faba bean, an underutilized crop viz., vikrant and PRT-12. Both single and combination treatments of gamma rays and EMS were used in this experiment. Biological damage through seed germination and percentage injury was calculated in both laboratory and field. A gradual decrease was noticed in seed germination in both B.O.D. petriplates experiment and in field with increasing doses of single and combined treatments of gamma rays and EMS in both the varieties (Vikrant and PRT-12) of faba bean. Variety Vikrant showed more reduction in seed germination than variety PRT-12. The reduction in seed germination was highest in gamma rays+EMS combined treatments followed by EMS and gamma rays.

Keywords: mutation breeding, gamma raysn ethylmethane sulphonate, faba bean

Introduction
The continuous increase in population, urbanization and climate changes are the major causes of food insecurity in India. The FAO (2013) has reported that mankind is facing worse problems of food insecurity and consistent malnutrition and that this might be reduced through interventions in agricultural research policies. Different strategies and work plans are needed to increase the food production. Conventional and modern plant breeding strategies are being adapted to enhance the production levels of crops (WHO, 2005; Kozgar *et al*., 2014) [22], Whereas modern methods of breeding have significantly increased crop yields over the past 50 years, the future potential of these methods is constrained by the limitations in the natural diversity of trait genotype within the crop species and sexual-compatibility boundaries between crop types (WHO, 2005) [22]. In Indian agriculture pulses play an important role in maintaining soil fertility and supplying protein to the large population of the country which consumes pulses. Pulse crops are endowed with unique property of fixing atmospheric nitrogen in their root nodules and improving soil physical property.
So the current experiment was conducted to assess the biological damage induced by different doses/concentrations of gamma rays and ethylmethane sulphonate in two cultivars of faba bean, an underutilized crop viz., vikrant and PRT-12 in M_1 generation.

Materials and Methods
Varieties used
Two varieties of faba bean (*Vicia faba* L.) namely Vikrant and PRT-12 were used in the present study. Seeds of both the varieties were obtained from the Indian Agricultural Research Institute (IARI), New Delhi, India. Both the varieties are well adapted to agroclimatic conditions of Uttar Pradesh (including the site of study). A brief description of both the varieties is given below:

Variety Vikrant
Released at Haryana Agricultural University, Hisar as a pure line selection from local material of Meerut (Uttar Pradesh), matures in 140-144 days, plant erect having an average height of 65-70 cm, seeds smooth and small size.

3.2.1.2. Variety PRT-12
Local collection from a village of Faizabad district (Uttar Pradesh), matures in 145-148 days, plant erect having an average height of 60-65 cm, seeds medium bold.

Mutagens used
Seeds of both the varieties of faba bean were treated with gamma rays, EMS and their combination treatments as detailed below:

Gamma rays (γ rays)
Dry seeds of each variety, with moisture content 12%, were irradiated with 100, 200, 300 and 400 Gy of gamma rays with a radioisotope ^{60}CO source (Gamma chamber Model-900 supplied by Bhabha Atomic Research Centre, Mumbai, India) at the National Botanical Research Institute, Lucknow, Uttar Pradesh, India.

Chemical mutagen: Ethyl methanesulphonate (EMS)
EMS ($CH_3SO_3C_2H_5$), a monofunctional alkylating agent is manufactured by Sissco Research Laboratories Pvt. Ltd., Mumbai, India. For EMS treatments, healthy seeds of uniform size of each variety were presoaked for 9 hours in distilled water and treated with 0.01, 0.02, 0.03 and 0.04 % of EMS for 6 hours with intermittent shaking at room temperature of 22±1°C. The solution of EMS was prepared

in the phosphate buffer of pH 7. Only freshly prepared solutions were used for all the treatments. The pH of the solution was maintained by using buffer tablets manufactured by MERCK manufactures, Mumbai, India. After treatment, the seeds were thoroughly washed in running tap water to remove the excess of mutagen.

Combination treatment: Gamma rays + EMS

For combination treatments, dry seeds of each variety were first irradiated with gamma rays at 100, 200, 300 and 400 Gy doses and then treated with 0.01, 0.02, 0.03 and 0.04 % EMS. (i.e. 100 Gy+0.01% EMS, 200 Gy+0.02% EMS, 300 Gy+0.03% EMS and 400 Gy+0.04% EMS). The procedure adopted was similar to that for the individual treatment.

Experimental procedures

350 seeds were used for each treatment and control. For each variety, 350 pre-soaked seeds were again soaked in phosphate buffer for 6 hours to serve as controls.

Handling and selection of the treated material in different generations

M_1 generation

Three replications of 100-seeds each, were sown for every treatment and control in each variety in a randomized complete block design (RCBD) at the Agriculture Farm, Aligarh Muslim University, Aligarh. The spacing was maintained at 30 cm (seed to seed in a row) and 60 cm (between the rows) in the field. The experiment was conducted during rabi (winter) season of 2012. Recommended agronomic practices were employed for preparation of field, sowing and subsequent management of the population of faba bean.

The remaining lot of 50 seeds was grown on moist cotton in petriplates for determining percentage of seed germination and measuring the seedling height i.e. root and shoot lengths. For this, the petriplates were kept in the B.O.D. incubator at 25±1ºC temperature.

Seed germination

After recording germination counts, the percentage of seed germination was calculated on the basis of total number of seeds sown in the petridishes and in the field.

Seedling height

Seedling height was recorded after 10 days by measuring the root and shoot lengths for each treatment and control. Seedling injury was measured in terms of reduction in seedling height with respect to control.

Results

Data recorded on seed germination in laboratory (B.O.D. petriplates experiment) and in field are given in Tables 1 & 2, Figs. 1 & 2, Plates- I & II. A gradual decrease was noticed in seed germination in both B.O.D. petriplates experiment and in field with increasing doses of single and combined treatments of gamma rays and EMS in both the varieties (Vikrant and PRT-12) of faba bean. Variety Vikrant showed more reduction in seed germination than variety PRT-12. The reduction in seed germination was highest in gamma rays+EMS combined treatments followed by EMS and gamma rays. Seed germination started the fourth day (B.O.D. petriplate experiment) and ninth day (in field) after sowing in controls of both the varieties. Germination was delayed by three days in higher treatments of gamma rays and EMS alone or in combination in both B.O.D. petriplate experiment and in field.

Percentage germination and reduction (In B.O.D. petriplates experiment)

In variety Vikrant, the control showed 98% seed germination, while the percentage germination in different mutagen treatments ranged between 80-96. The percentage germination was maximum (96) in 100 Gy gamma rays, whereas it was minimum (80) in combination treatment of 400 Gy gamma rays+0.04% EMS. Percentage reduction ranged between 2.04-18.26 in various mutagen treatments. It was highest (18.36%) with the combined 400 Gy gamma rays+0.04% EMS treatment. Variety PRT-12 behaved more or less identically.

Percentage seed germination and reduction (In field)

In variety Vikrant, the percentage seed germination was 96.66 in control. Seed germination ranged from 79.66-94.00 in the treatments of gamma rays and EMS employed alone or in combination. The minimum percentage reduction (2.75) was recorded in 100 Gy gamma rays and the maximum was (17.58) in 400 Gy gamma rays+0.04% EMS treatment. In variety PRT-12, the minimum reduction in seed germination was (1.39) in 100 Gy of gamma rays, while it was maximum (18.06) in 400 Gy gamma rays+0.04% EMS treatment.

Table 1: Effect of single and combination treatments of gamma rays and EMS on seed germination of *Vicia faba* L. Var. Vikrant in M_1 generation.

Treatment	In Laboratory Seed germination (%)		In Field Seed germination (%)	
	Actual	%age reduction	Actual	%age reduction
Control	98.00	-	96.66	-
100 Gy γ rays	96.00	2.04	94.00	2.75
200 Gy γ rays	94.00	4.08	92.66	4.13
300 Gy γ rays	90.00	8.16	86.66	10.34
400 Gy γ rays	88.00	10.20	84.00	13.09
Mean	92.00	6.12	89.33	7.57
0.01% EMS	94.00	4.08	93.33	3.44
0.02% EMS	92.00	6.12	91.66	5.17
0.03% EMS	88.00	10.20	84.33	12.75
0.04% EMS	86.00	12.24	83.66	13.75
Mean	90.00	8.16	88.24	8.77
100 Gy γ rays+0 .01% EMS	94.00	4.08	92.66	4.13
200 Gy γ rays+0.02% EMS	90.00	8.16	89.33	7.58
300 Gy γ rays+0.03% EMS	84.00	14.28	83.33	13.79
400 Gy γ rays+0.04% EMS	80.00	18.36	79.66	17.58
Mean	87.00	11.22	86.24	10.77

Table 2: Effect of single and combination treatments of gamma rays and EMS on seed germination of *Vicia faba* L. Var. PRT-12 in M_1 generation.

Treatment	In Laboratory Seed germination (%)		In Field Seed germination (%)	
	Actual	%age reduction	Actual	%age reduction
Control	98.00	-	96.00	-
100 Gy γ rays	96.00	2.04	94.66	1.39
200 Gy γ rays	94.00	4.08	93.33	2.78
300 Gy γ rays	90.00	8.16	87.66	8.68
400 Gy γ rays	88.00	10.20	85.66	10.77
Mean	92.00	6.12	90.32	5.90
0.01% EMS	94.00	4.08	93.66	2.43
0.02% EMS	92.00	6.12	92.66	3.47
0.03% EMS	90.00	8.16	85.33	11.11
0.04% EMS	86.00	12.24	85.00	11.45
Mean	90.50	7.65	89.16	7.11
100 Gy γ rays+0 .01% EMS	94.00	4.08	93.66	2.43
200 Gy γ rays+0.02% EMS	90.00	8.16	89.66	6.60
300 Gy γ rays+0.03% EMS	86.00	12.24	83.66	12.85
400 Gy γ rays+0.04% EMS	80.00	18.36	78.66	18.06
Mean	87.50	10.71	86.41	9.98

Fig 1: Comparative effect of 100 Gy γ rays, 0.01% EMS and 400 Gy γ rays+0.04% EMS on seed germination and seedling growth of *Vicia faba* L. var. Vikrant (B.O.D. petriplates experiment).

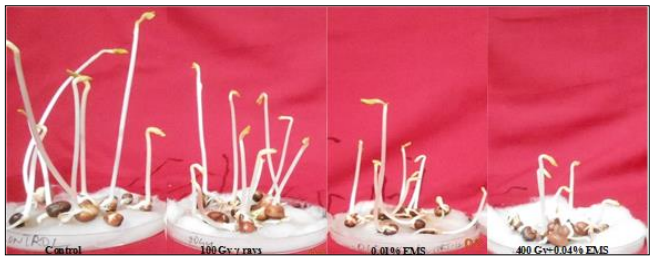

Fig 2: Comparative effect of 100 Gy γ rays, 0.01% EMS and 400 Gy γ rays+0.04% EMS on seed germination and seedling growth of *Vicia faba* L. var. PRT-12 (B.O.D. petriplates experiment).

Seedling height (cm)

Seedling height of both varieties, calculated after 10 days of germination in B.O.D. petriplate experiment, showed that the seedling height decreased with increasing doses of single and combined treatments of mutagens (Tables 3 & 4). The decrease in height was more in variety Vikrant than in variety PRT-12. The reduction was more pronounced in combination treatments in comparison to individual mutagenic treatments of gamma rays and EMS. The maximum reduction (6.33 cm) in seedling height was observed in combination treatment of 400 Gy gamma rays+0.04% EMS in the variety Vikrant.

The percentage injury in seedling height increased with mutagen concentration. In the variety Vikrant, the seedling injury ranged from 3.14-16.78% in gamma rays treatments and 7.03-20.67% in EMS and 23.39-33.57% in combination treatments. In the variety PRT-12, the seedling injury ranged from 2.33-16.53% in the treatments of gamma rays and 6.38-22.92% in the EMS treatments. The injury was more drastic in the combination treatments of gamma rays and EMS, ranging from 13.48-34.78%.

Table 3: Effect of single and combination treatments of gamma rays and EMS on seedling height of *Vicia faba* L. var. Vikrant in M_1 generation (B.O.D. petriplate experiment).

Treatment	Length in cm		%age injury
	Shoot length (Root length)	Total length $\bar{x} \pm$ S. E	
Control	6.76 (2.76)	$9.53^{a} \pm 0.29$	-
100 Gy γ rays	6.60 (2.63)	$9.23^{ab} \pm 0.26$	3.14
200 Gy γ rays	6.36 (2.40)	$8.80^{abc} \pm 0.23$	7.66
300 Gy γ rays	6.13 (2.23)	$8.36^{abcd} \pm 0.20$	12.27
400 Gy γ rays	5.86 (2.06)	$7.93^{bcde} \pm 0.16$	16.78
0.01% EMS	6.36 (2.50)	$8.86^{abc} \pm 0.28$	7.03
0.02% EMS	6.16 (2.26)	$8.43^{abcd} \pm 0.23$	11.54
0.03%EMS	5.96 (2.06)	$8.03^{bcde} \pm 0.17$	15.73
0.04% EMS	5.66 (1.90)	$7.56^{cdef} \pm 0.13$	20.67
100 Gy γ rays+0.01% EMS	5.03 (2.26)	$7.30^{def} \pm 1.40$	23.39
200 Gy γ rays+0.02% EMS	5.93 (2.06)	$8^{bcde} \pm 0.26$	16.05
300 Gy γ rays+0.03% EMS	4.86 (1.86)	$6.73^{ef} \pm 0.20$	29.38
400 Gy γ rays+0 .04% EMS	4.56 (1.76)	$6.33^{f} \pm 0.20$	33.57

Different letters show significant difference at $p \leq 0.05$. Means with the same letter are not statistically different. Figures in parenthesis represent root length.

Table 4: Effect of single and combination treatments of gamma rays and EMS on seedling height of *Vicia faba* L. var. PRT-12 in M_1 generation (B.O.D. petriplate experiment).

Treatment	Length in cm		%age injury
	Shoot length (Root length)	Total length $\bar{x} \pm$ S. E	
Control	6.96 (2.90)	$9.86^{a} \pm 0.35$	-
100 Gy γ rays	6.83 (2.80)	$9.63^{ab} \pm 0.32$	2.33
200 Gy γ rays	6.66 (2.63)	$9.36^{abc} \pm 0.28$	5.07
300 Gy γ rays	6.36 (2.43)	$8.86^{bcde} \pm 0.23$	10.14
400 Gy γ rays	5.96 (2.26)	$8.23^{efg} \pm 0.32$	16.53
0.01% EMS	6.60 (2.63)	$9.23^{abcd} \pm 0.26$	6.38
0.02% EMS	6.33 (2.43)	$8.76^{cde} \pm 0.24$	11.15
0.03%EMS	5.90 (2.26)	$8.16^{efg} \pm 0.24$	17.24
0.04% EMS	5.53 (2.06)	$7.60^{gh} \pm 0.34$	22.92
100 Gy γ rays+0.01% EMS	6.20 (2.33)	$8.53^{def} \pm 0.16$	13.48
200 Gy γ rays+0.02% EMS	5.70 (2.10)	$7.80^{fg} \pm 0.11$	20.89
300 Gy γ rays+0.03% EMS	4.80 (2)	$6.90^{hi} \pm 0.05$	30.02
400 Gy γ rays+0 .04% EMS	4.63 (1.80)	$6.43^{i} \pm 0.14$	34.78

Different letters show significant difference at $p \leq 0.05$. Means with the same letter are not statistically different. Figures in parenthesis represent root length.

Discussion

The breeding potential of crop plant is to exploit the existing genetic variability through selection or create new variability. Mutation breeding can constitute a valuable tool to the conventional breeding methods in widening the genetic base of crop plants through creation of useful mutants and has played a significant role in the development of many crop varieties (Tomlekova, 2014; Gulfishan *et al*., 2016) [11, 9].

The effect of single and combined treatments of gamma rays and EMS were studied on biological parameters like seed germination, seedling height and injury in two varieties of faba bean in M_1 generation. Seed germination and seedling height decreased with the increasing mutagen treatments. However, the extent of decrease differed in both the varieties treated with different doses/concentrations of mutagens and their combination treatments. Combination treatments were found to be more effective than the single treatments. Seed germination and seedling height reduced more in the variety Vikrant.

Several workers have shown the adverse effects of mutagens on biological parameters and explained the causes responsible for inhibition (Salve and More, 2014; Khursheed *et al*., 2015) [16, 10]. Percentage seed germination was decreased with increasing doses/concentrations of both single and combined mutagen treatments. The percentage reduction of seed germination due to mutagens depends on both the type of mutagen and a particular genotype. Different genotypes show different sensitivity towards mutagens (Khursheed *et al*., 2015) [10]. The metabolic processes affected by mutagens at the embryonic level are responsible for differential sensitivity (Ashri and Herzog, 1972). Several workers have reported different justifications regarding decrease of seed germination with increasing doses/concentrations of mutagens. Khan and Goyal (2009) [8] in mungbean reported the reduction of seed germination due to the alteration in the activity of enzymes involved in the germination by the mutagens. Inhibition of growth by high dosage gamma irradiation may be due to the arrest of cells in G_2/M phase of mitosis and/or genomic damage (Preussa and Britta, 2003) [14]. Strickberger (1976) [19] reported that the gamma rays induced disturbance in the base pair relationship may be probable reason for the reduction in seed germination. Some workers have reported that the high gamma irradiation dose induced chromosomal aberrations in cells lead to stoppage of cell division thus, leading to the reduction in seed germination (Singh *et al*., 1997) [17]. Yadav (1987) [24] described reduction in seed germination due to the delayed onset of mitosis. Singh and Singh (1989) [17] described reduction of seed germination due to the severe distortion of dividing phase of cell cycle due to increase in concentrations/dosages of mutagens. Roychowdhury and Tah (2011) [15] attributed that the decrease in the percentage of seed germination can be due to the effect on the meristematic tissues of the seedsdue to mutagens. The inhibition in seed germination was slightly more in EMS in comparison to gamma rays treatments. Similar results have also been reported earlier by Dube *et al*. (2011) [5] in *Cyamopsis tetragonoloba*.

In the present study EMS treatments were given to the pre-soaked seeds which might increase the sensitivity to chemical mutagen. The greater reduction in percentage seed germination in the two varieties of faba bean caused by EMS might be due to the change in metabolic condition of the cells during pre-soaking. Reduced seed germination due to single and combination treatments of gamma rays and EMS in the present study may be the result of the delay or the inhibition of metabolic activation necessary for seed germination or due to inhibition of mitotic process.

Many workers have reported low dose promoting effects by mutagens on physiological parameters as reported by Alikamanoglu *et al.* (2011) [2] in *Glycine max*. Abdel-Hady *et al*. (2008) [1] postulated stimulatory effect of gamma rays on seed germination and attributed that this effect is due to the activation of RNA or protein synthesis during the early stages of germination. A similar postulation regarding the low dose gamma irradiation induction of growth stimulation was given by Wi *et al*. (2007). They reported that it occurs due to the increase in the anti-oxidative capacity of cells and by the change in the hormonal signaling network. Ariraman *et al*. (2014) [3] are of the view that the percentage stimulation/reduction in seed germination is due to the effect on meristematic tissues of seeds by the mutagens. Majeed and Mohammad (2010) [13] reported increase in percentage germination at higher doses of gamma rays in *Lepidium sativum*.

Seedling height decreased with the increasing doses of mutagens. Different reports are available to explain the

reduction in seedling growth. Inhibition of auxin synthesis (Goud and Nayar, 1968) [7], physiological injury in the seeds and seedlings (Usuf and Nair, 1974) [21], chromosome damages and/or inhibition of cell divisions (Salve and More, 2014) [16] were correlated with reduction in seedling height after mutagen treatments. Depression in seedling growth may be due to the uneven damage to the meristematic cells as a consequence of genetic injury. Inhibition of seedling growth was higher in the combined mutagen treatments compared to individual mutagen treatments of gamma rays and EMS. This supports the earlier findings of Laskar and Khan (2014) [12] in lentil.

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