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Determination of Mutagenic Effectiveness and Efficiency of Gamma Rays and EMS in Fenugreek cv. Rmt-1

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ABSTRACT: Fenugreek is a leafy vegetable as well as a spice, charged with medicinal properties. Owing to its inbreeding nature and traditional selection-based breeding methods adopted in the past, the available genetic variability available in the crop has become shrunken. Mutagenesis is a well-recognized tool to expand genetic variability. This investigation involves mutagenesis in fenugreek cv. Rmt-1 with physical (gamma rays) and chemical mutagens (EMS) and their combinations. The mutation frequency, mutagenic effectiveness and mutagenic efficiency based on lethality and injury of the seedlings were calculated for the gamma rays, EMS and their combination doses. The low and moderate doses of both gamma rays and EMS were found more effective than the higher doses. The highest doses of gamma rays and EMS with longer treatment duration resulted in the highest biological damage as lethality and injury of the seedling (220 Gy + EMS 0.30 % @ 8 hours). In the chemical mutagenic treatments, a longer duration hampered the effectiveness of the mutagens. The combination of gamma rays and EMS at lower and moderate doses expressed its worth to create higher mutation frequency by manifesting higher efficiency than the individual mutagens single-handed. The mutagenic efficiency based on lethality and seedling injury were maximum under ϒ 60 Gy + EMS 0.30% @ 6 h and EMS 0.25% @ 6 hours as 0.84 and 0.79, respectively. The interaction and influence of the individual mutagens in combinations were studied by the coefficient of interaction (k) and their interaction was found to be synergistic in nature.

Keywords: Induced mutation, Gamma rays, EMS, Fenugreek, M_2 population, mutagenic effectiveness and efficiency.

INTRODUCTION

The prevalence of genetic variability is the persistent key for crop improvement through plant breeding strategies. The genetic variability results from either spontaneous or induced mutations. Since spontaneous mutations are seldom, the induced mutation satisfies the required genetic variability within a crop's cultivated germplasms. It is brought about by the use of either physical or chemical mutagens or their combinations that potentially amplify the frequency of mutation relatively higher than that of spontaneous mutations. The physical and chemical mutagens beget mutations in a trend towards novel plant attributes catenated with wider genetic variability (Lagoda, 2007).

Nearly all the mutagens confirm to a similar reaction with DNA and consequent changes in nucleotide sequences. Nevertheless, they are unique in their mode of action (Bashir *et al.,* 2013). Amid the physical mutagens, ionizing gamma rays are the most common in use (Ashadevi *et al.,* 2017) while, among the chemical mutagens, Ethyl Methane Sulphonate (EMS) ($CH_3SO_2OC_2H_2$) is widely employed for genetic variability induction (Jayakumar and Selvaraj, 2003).

Whilst mutations are favorable for supplementing the genetic variability, they can perhaps be deleterious in their nature. Lethality, injury and sterility are the undesirable results of the induced mutations that can reduce the efficiency of the mutagens. Therefore, it

becomes crucial to analyze the mutation frequency, mutagenic effectiveness and efficiency of a particular mutagen dose for achieving a high frequency of useful mutations (Smith, 1972; Kumar and Mani, 1997).

Fenugreek (*Trigonellafoenum-graecum*) is a multipurpose crop used for its foliage and seeds as a leafy vegetable and spice, respectively, integrated with medicinal attributes (Basch *et al.,* 2003). As the cultivated germplasm of fenugreek lacks in genetic variability, many initiatives on expanding the variability have been carried off through mutation breeding. This experiment was set up to study the effect of gamma rays, EMS, and their combinations on the frequency of mutants and to investigate their mutagenic efficiency and effectiveness to recover the high frequency of advantageous mutants.

MATERIALS AND METHODS

The experiment was conducted in the Vegetable Research Center, Department of Horticulture, College of Agriculture, Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur, Madhya Pradesh. One of the commonly cultivated fenugreek varieties of Madhya Pradesh, cv. Rmt-1 was employed in the mutagenic treatments. The mutagenic treatment involves gamma rays, EMS and their combination at different doses. The treatment details are furnished in Table 1.

The well-filled and dry seeds of cv. Rmt-1 were subjected to various doses of gamma rays. The gamma irradiation facility was obtained from the Sophisticated Analytical Instrument Facility (SAIF) at ICAR-Indian Institute of Horticulture Research (ICAR-IIHR), Hessaraghatta, Bengaluru, Karnataka.

The first step in the chemical mutagenesis was presoaking of seeds in distilled water for 6 hours to activate the torpid embryo, to make cell membranes more permeable for the chemical mutagen to diffuse into the biological material thereby facilitating the uptake of the chemical mutagen (Ashadevi *et al*., 2019). The per cent solutions (0.25 per cent and 0.30 per cent) of EMS were prepared and the presoaked seeds are subjected to soaking for the different time durations (6,8 and 10 hours) (Table 1). After the treatment, the seeds are thoroughly washed to remove the mutagen residues from the seed surface. The washed seeds are immediately sown to raise the M_1 population. The crops were raised with the recommended package of practices.

Table 1: Mutagenic treatment details.

Treatments	Mutagens and their doses	Treatments	Mutagens and their doses
T_1	ϒ 60 Gy	T_{12}	60 Gy + EMS 0.30% @ 6 h
T_2	ϒ 100 Gy	T_{13}	ϒ 100 Gy + EMS 0.30% @ 6 h
T_3	ϒ 140 Gy	T_{14}	ϒ 140 Gy + EMS 0.30% @ 6 h
T_4	ϒ 180 Gy	T_{15}	ϒ 180 Gy + EMS 0.30% @ 6 h
T_5	ϒ 220 Gy	T_{16}	ϒ 220 Gy + EMS 0.30% @ 6 h
T_6	EMS 0.25% @ 6 h	T_{17}	ϒ 60 Gy + EMS 0.30% @ 8 h
T_7	EMS 0.25% @ 8 h	T_{18}	ϒ 100 Gy + EMS 0.30% @ 8 h
T_8	EMS 0.25% @ 10 h	T_{19}	ϒ 140 Gy + EMS 0.30% @ 8 h
T_9	EMS 0.30% @ 6 h	T_{20}	ϒ 180 Gy + EMS 0.30% @ 8 h
T_{10}	EMS 0.30% @ 8 h	T_{21}	ϒ 220 Gy + EMS 0.30% @ 8 h
T_{11}	EMS 0.30% @ 10 h	T_{22}	Control

Kumar *et al*. (2003) proposed that the M_1 test data and the frequency and spectrum of mutations in M_2 render a good guide for the assessment of efficient mutagenic treatments. The mutation frequency (Mf) is the magnitude of mutated plants in the total population obtained as a result of exposure to the mutagenic agent (Ashadevi *et al*., 2019).

$$\text{Mutation frequency} = \frac{\text{Number of mutated seedlings}}{\text{Total number of } M_2 \text{ seedlings}} \times 100$$

The mutagenic effectiveness is a measure of the frequentness of mutations induced by a unit of a mutagen (Konzak *et al.,* 1965) or the number of mutations invoked per unit dose of mutagen in unit time (Konzak *et al.,* 1964; Khan and Wani, 2006). It is calculated as per the formula of Konzak*et al*. (1965).

$$\text{Mutagenic effectiveness (Gamma rays)} = \frac{\text{Mutation frequency in } M_2 \text{ generation}}{\text{Dose in Gray (Gy)}}$$

$$\text{Mutagenic effectiveness (EMS)} = \frac{\text{Mutation frequency in } M_2 \text{ generation}}{\text{EMS concentration} \times \text{duration of treatment}}$$

$$\text{Mutagenic effectiveness (Gamma rays + EMS)} = \frac{\text{Mutation frequency in } M_2 \text{ generation}}{\text{Dose in Gray (Gy)} \times (\text{EMS concentration x duration of treatment})}$$

The mutagenic efficiency is the production of favorable mutations that are free from linkage with undesirable genetic alterations. This is determined as the extent of mutation frequency appertaining to the biological damage as lethality and injury (Konzak *et al*., 1965; Gaul *et al*., 1972). It gives knowledge and understanding on the proportion of mutation in regards to deleterious biological effects of the mutagen. Therefore, mutagenic efficiency is more justified than mutagenic effectiveness. Higher the mutagenic efficiency, lesser is the mutagen-induced biological damage (Jain and Khandelwal, 2009).

$$\text{Mutagenic efficiency} = \frac{\text{Mutation frequency in } M_2 \text{ population}}{\text{Lethality (per cent) or Injury (per cent) in } M_1 \text{ population}}$$

The biological damage in the M_1 population caused by a mutagenic agent is adjudged by lethality and injury. Lethality is the proportion of non-germinated seeds, indicating the acuity of the genotype to a particular mutagen dose (Boranayaka *et al.*, 2010). Some seeds germinate yet the side effects of the mutagen hamper the seedling growth and development. Injury (I) is termed as the average reduction in seedling height at 25 DAS over the control (Ashadevi *et al*., 2019).

In this study, the mutagens are used in combination and it becomes essential to study the influence of the combination doses of gamma rays and EMS on the frequency of mutation by calculating the Coefficient of interaction (k) (Sharma, 1970). If k equals 1, then the interaction between the mutagens is additive whereas if k is less or more than 1 the interaction is synergistic.

$$\text{Coefficient of interaction (k)} = \frac{(a+b)}{(a)+(b)}$$

Where,

k – hypothetical interaction coefficient

(a + b) – mutation frequency due to combined mutagen doses

(a) + (b) - mutation frequency due to individual mutagen doses

RESULTS AND DISCUSSION

The mutagenic effectiveness and efficiency are pivotal for the mutation breeding experiments. Both are observed in M_2 generation. Table 2 exhibits the mutagenic effectiveness of different mutagenic treatments. Among the different gammaray doses, maximum mutagenic effectiveness was recorded under ϒ 60 Gy (0.037), followed by ϒ 100 Gy (0.036). ϒ 220 Gy was found to be the least effective with a value of 0.11 (Fig. 1). The mutagenic effectiveness decreased with the increase in the gammaray dose. Among different EMS concentrations, highest effectiveness was registered with EMS 0.25% @ 6 hours (2.123) while the least was recorded in EMS 0.30% @ 10 hours (0.661) (Fig. 2). The mutagenic effectiveness is realized to be reduced with an increase in the dose and treatment duration. Among the different combinations of gamma rays and EMS, ϒ 60 Gy + EMS 0.30% @ 6 h was observed with the highest mutagenic effectiveness (0.044) while the least mutagenic effectiveness occurred in ϒ 180 Gy + EMS 0.30% @ 8 h (0.008) (Fig. 3). As the combination of mutagens is concerned, the gamma ray dose, EMS concentration and the treatment duration found to have a role in the induction of high frequency of useful mutation; an increase in the above-resulted decrease in the mutagenic efficiency of the mutagen combination, indicating that the increase in mutation frequency was not proportional to the increase in the mutagen doses (Khursheed *et al.,* 2018).

Table 2: Mutagenic effectiveness of gamma rays, EMS and their combination doses.

ϒ ray	Mutagenic effectiveness	EMS	Mutagenic effectiveness	ϒ + EMS	Mutagenic effectiveness
ϒ 60 Gy	0.037	EMS 0.25% @ 6 h	**2.123**	ϒ 60 Gy + EMS 0.30% @ 6 h	0.044
ϒ 100 Gy	0.036	EMS 0.25% @ 8 h	1.317	ϒ 100 Gy + EMS 0.30% @ 6 h	0.032
ϒ 140 Gy	0.029	EMS 0.25% @ 10 h	0.748	ϒ 140 Gy + EMS 0.30% @ 6 h	0.017
ϒ 180 Gy	0.020	EMS 0.30% @ 6 h	1.505	ϒ 180 Gy + EMS 0.30% @ 6 h	0.018
ϒ 220 Gy	0.011	EMS 0.30% @ 8 h	1.271	ϒ 220 Gy + EMS 0.30% @ 6 h	0.010
-	-	EMS 0.30% @ 10 h	0.661	ϒ 60 Gy + EMS 0.30% @ 8 h	0.027
-	-	-	-	ϒ 100 Gy + EMS 0.30% @ 8 h	0.016
-	-	-	-	ϒ 140 Gy + EMS 0.30% @ 8 h	0.012
-	-	-	-	ϒ 180 Gy + EMS 0.30% @ 8 h	<u>0.008</u>
-	-	-	-	ϒ 220 Gy + EMS 0.30% @ 8 h	0.009

Bold values indicate the highest mean value: Underlined values indicate the least mean value

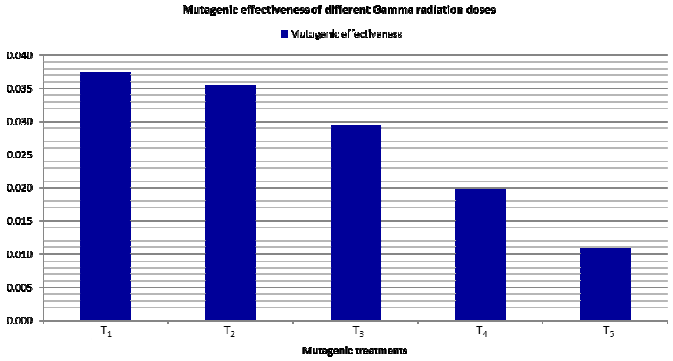

Fig. 1. Mutagenic effectiveness of different gamma radiation doses in fenugreek cv. Rmt-1.

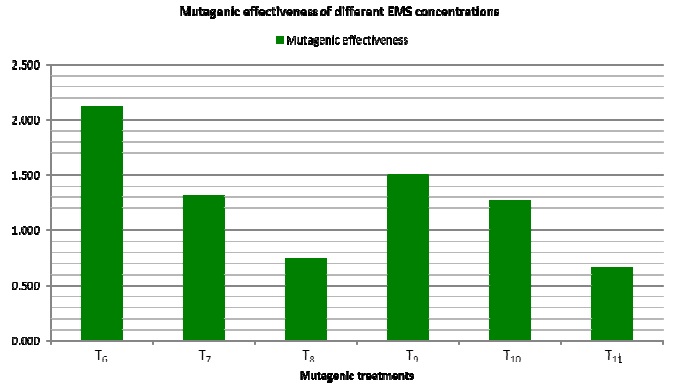

Fig. 2. Mutagenic effectiveness of different EMS concentrations.

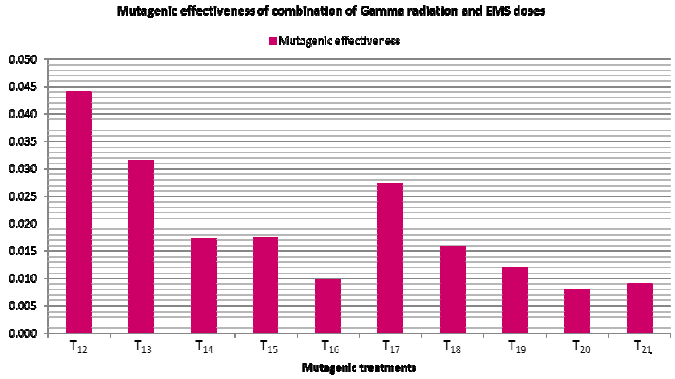

Fig. 3. Mutagenic effectiveness of combination of gamma radiation and EMS doses.

In the present investigation, the biological damage in the M_1 population is studied through lethality (per cent) and seedling injury (per cent). The lethality was the highest in T_{22} (ϒ 220 Gy + EMS 0.30% @ 8 hours) (22.67), indicating that the higher doses would be detrimental to the plant growth development. The least lethality was found in T_{12} (ϒ 60 Gy + EMS 0.30% @ 6 h) (5.33) (Table 3). This shows that the combined usage of mutagen at optimum dosage would be advantageous in generating a high frequency of useful mutations. The seedling injury has recorded the maximum in T_{22} (ϒ 220 Gy + EMS 0.30% @ 8 hours) (26.87), wherein the maximum lethality was also observed indicating the impact of higher mutagenic doses on both germination and plant growth and development.

The mutation frequency was noted the maximum with T_{15} (ϒ 180 Gy + EMS 0.30% @ 6 hours) (5.70), followed by T_{13} (ϒ 100 Gy + EMS 0.30% @ 6 h) (5.67) (Table 3) (Fig. 4). This highlights the potential of the combinations of physical and chemical mutagens to induce mutations at higher rates than the individual mutagen. The treatment T_8 (EMS 0.25% @ 10 h) recorded the lowest mutation frequency. The longer duration during the seed treatment is found to be disadvantageous regarding the biological damage in the M_1 population and mutation frequency in the M_2 population.

Table 3: Mutagenic efficiency of Gamma rays, EMS and their combinations.

Treatments	Germination (per cent)	Lethality (L) (per cent)	Seedling height @ 25 DAS (cm)	Seedling injury (SI) (per cent)	Mutation frequency in M_2(Mf)	Mutagenic efficiency		K factor
						Mf/L	Mf/SI	
T_1	94.00	6.00	25.47	5.78	2.24	0.37	0.39	-
T_2	93.33	6.67	24.77	8.37	3.55	0.53	0.42	-
T_3	92.67	7.33	24.90	7.88	4.12	0.56	0.52	-
T_4	91.33	8.67	24.30	10.10	3.56	0.41	0.35	-
T_5	90.67	9.33	22.33	17.38	2.38	0.25	0.14	-
T_6	94.00	6.00	25.87	4.30	3.18	0.53	**0.74**	-
T_7	90.00	10.00	24.50	9.36	2.63	0.26	0.28	-
T_8	85.33	14.67	21.67	19.84	1.87	0.13	0.09	-
T_9	92.00	8.00	24.63	8.87	2.71	0.34	0.31	-
T_{10}	88.00	12.00	23.67	12.44	3.05	0.25	0.25	-
T_{11}	81.33	18.67	21.17	21.69	1.98	0.11	0.09	-
T_{12}	94.67	5.33	25.23	6.65	4.77	**0.89**	0.72	0.96
T_{13}	92.67	7.33	24.60	8.99	**5.67**	0.77	0.63	0.91
T_{14}	91.33	8.67	23.00	14.91	4.37	0.50	0.29	0.64
T_{15}	87.33	12.67	22.23	17.75	5.70	0.45	0.32	0.91
T_{16}	86.00	14.00	22.00	18.61	3.89	0.28	0.21	0.76
T_{17}	92.67	7.33	24.50	9.36	3.94	0.54	0.42	0.75
T_{18}	87.33	12.67	24.07	10.96	3.78	0.30	0.34	0.57
T_{19}	83.33	16.67	21.53	20.34	4.01	0.24	0.20	0.56
T_{20}	82.00	18.00	20.83	22.93	3.50	0.19	0.15	0.53
T_{21}	77.33	**22.67**	19.77	**26.87**	4.81	0.21	0.18	0.89
T_{22}	**100.00**	-	**27.03**	-	-	-	-	-

Bold values indicate the highest mean value: Underlined values indicate the least mean value

The mutagenic efficiency was determined based on lethality and injury of the seedling in the M_1 population. The mutagenic efficiency based on the lethality was found to be the maximum in T_{12} (ϒ 60 Gy + EMS 0.30% @ 6 h) (0.89). The lowest value of the same was found to be 0.11 in T_{11} (EMS 0.30% @ 10 hours). This again proves the inefficiency of the longer treatment duration. The mutagenic efficiency based on the injury of the seedling was registered the maximum (0.74) in T_6 (EMS 0.25% @ 6 h), deliberating the high efficiency of EMS linked with lower biological damage. The lowest mutagenic efficiency based on injury was found with T_{11} (EMS 0.30% @ 10 hours) and T_8 (EMS 0.25% @ 10 hours) (0.09) (Fig. 4).Various reports on effectiveness and efficiency of different mutagens are available elsewhere as made by Koli and Ramkrishna (2002) in fenugreek, Velu *et al.* (2007) in cluster bean, Girija and Dhanavel (2009) in cowpea and Sharma *et al.* (2009) in garden pea. The above results state that the lower doses are more efficient than the higher doses. The higher mutagenic efficiency at lower doses might be because of the lower levels of biological damage (Bhosle and Kothekar, 2010). This was in similarity with the findings of Dhanavel *et al.* (2008); Dube *et al.* (2011); Khursheed *et al.* (2016).

To study the influence of the combination doses of gamma rays and EMS is essential. The Coefficient of interaction (k), formulated by Sharma (1970) is employed here to study the nature of their interaction. In all the combination treatments, the values were found to be lesser than 1 (Table 3). This indicates that their influence and interaction to effectuate the mutation are synergistic in nature. The results were in line with that of Raina and Khan (2020).

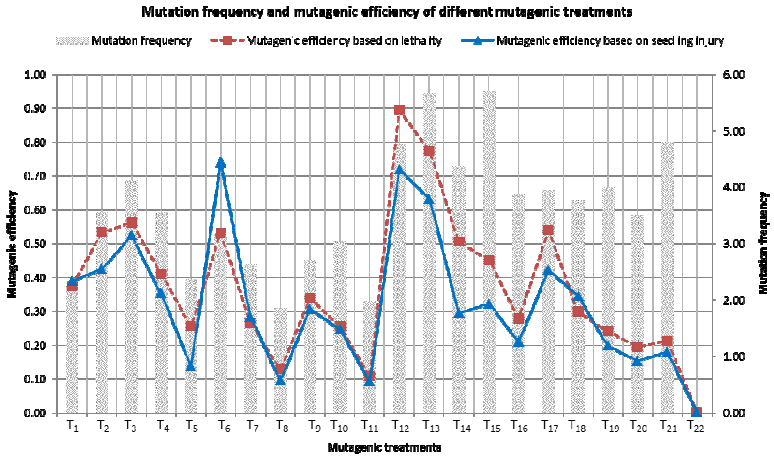

Fig. 4. Mutation frequency and mutagenic effectiveness of different mutagenic treatments.

CONCLUSION

The present investigation was carried out to study the plant response as mutagenic effectiveness and efficiency of fenugreek cv. Rmt-1. Among the gamma rays, EMS and their combinations, EMS treatments were found to be more effective in mutagenicity. The combined treatments with both physical and chemical mutagens are found highly efficient with low biological damage. The effectiveness and efficiency of the mutagens were found to be declining with an increase in the mutagen dose. The moderate doses are found more effective and efficient than the higher doses. The longer treatment duration recorded the maximum biological damage and the lower effectiveness and efficiency. Therefore, optimum treatment duration is recommended. The determination of mutational frequency, effectiveness and efficiency enables appropriate decisions on the mutagenic doses for the future works.

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Conflict of Interest. None.

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