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Studies on mutagenic effectiveness and efficiency in Fenugreek (*Trigonella foenum-graecum* L.)

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A comparative study on effect of different dose/concentration of gamma rays, ethyl methanesulfonate (EMS) and sodium azide (SA) on various biological parameters (seed germination, seedling height, plant survival, pollen fertility and chromosomal aberrations) in M_1 generation and spectrum and frequency of macro-mutations (chlorophyll and other morphological mutations) induced in the M_2 generation was carried out in fenugreek in the present investigation. Mutagenic effectiveness decreased with the increase in dose/concentration of the mutagen where as the efficiency of mutagens showed variable trend depending on the criteria selected for its calculation. The lower or intermediate treatments of all the mutagens were found more efficient in causing less biological damage and inducing maximum macro-mutations. The order of mutagenic efficiency was EMS > SA > Ɣ- rays.

Key words: Fenugreek, biological damage, morphological mutations, gamma rays, EMS, SA.

INTRODUCTION

The presence of genetic variability is necessary for the crop improvement. The variability available to the breeders comes from spontaneous or artificially induced mutations. The artificial induction of mutation in a crop species is achieved through the use of physical and/or chemical mutagens that enlarge the mutation frequency, when compared to the spontaneous occurrence. Almost all mutagens have the property of reacting with DNA and thereby bringing about changes in nucleotide sequences. However, the mode of action of each mutagen is distinct. Besides, a mutagen may effectively bring about mutations, but the accompanying undesirable effects like lethality or sterility may decrease its efficiency. Thus, in order to exploit induced mutagenesis for crop improvement, the basic studies on effectiveness and efficiency of a mutagen in a crop are necessary to recover high frequency of desirable mutations (Kumar and Mani, 1997; Badere and Chaudhary, 2007). Mutagenic effectiveness is an index of the response of a genotype to the increasing doses of the mutagen, whereas mutagenic efficiency indicates the extent of genetic damage recorded in the M_2 generation in relation to the biological damage caused in M_1 (Gaul et al., 1972; Khan et al., 2009; Wani, 2009). According to Kumar et al. (2003), M_1 test data along with the frequency and spectrum of morphological mutations in M_2 provide good guidelines for determination of efficient mutagenic treatments. Fenugreek (*Trigonella foenum-graecum* L.), commonly known as methi, is an important legume crop widely used as vegetable and fodder. It is one of the oldest known medicinal plants in the recorded history and its leaves and seeds have been used extensively for medicinal purposes (Basch et al., 2003). The available genetic variability in the crop has been almost exploited

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Abbreviations: Mf/Me, Mutation rate based on meiotic aberrations; **Mf/L,** lethality; **Mf/I,** injury; **Mf/S,** sterility.

Table 1. Mutagenic effectiveness and efficiency of Gamma rays, EMS and SA in *Trigonella foenum-graecum* L.

Treatment	Lethality (% L)	Pollen sterility (%S)	Seedling injury (%I)	Chromosomal abnormalities (%M)	Percent M_2 plants mutated (Mf)	Mutagenic effectiveness Mf/dose	Mutagenic efficiency			
							Mf/L	Mf/S	Mf/I	Mf/M
Gamma rays (Gy)										
100	8.08	21.33	6.68	6.01	1.46	0.146	0.181	0.068	0.128	0.243
200	20.93	31.24	26.37	9.05	2.23	0.111	0.106	0.071	0.084	0.246
300	19.94	47.70	49.87	11.87	3.42	0.114	0.171	0.072	0.069	0.288
400	33.92	59.93	74.29	15.12	3.47	0.087	0.102	0.058	0.047	0.229
EMS (%)										
0.1	6.06	24.47	10.80	4.58	1.45	2.417	0.239	0.059	0.134	0.316
0.2	10.60	32.96	24.35	7.47	3.04	2.533	0.287	0.092	0.124	0.407
0.3	16.65	44.20	41.13	10.07	4.31	2.394	0.259	0.097	0.105	0.428
0.4	28.11	56.06	62.21	13.08	2.31	0.962	0.082	0.041	0.037	0.177
SA (%)										
0.1	2.84	18.32	4.88	3.77	0.36	0.600	0.127	0.019	0.074	0.095
0.2	6.60	28.04	21.33	5.45	2.59	2.158	0.392	0.092	0.121	0.475
0.3	15.19	34.69	44.21	7.92	3.17	1.761	0.208	0.091	0.072	0.401
0.4	30.01	42.40	63.75	10.50	1.29	0.537	0.043	0.030	0.020	0.123

for improvement by conventional breeding methods. The present investigation was undertaken with the aim to study the effects of gamma rays, ethyl methanesulfonate (EMS), and sodium azide (SA) on the frequency and spectrum of macro-mutants and to evaluate the relative effectiveness and efficiency of these mutagens with the main aim of identifying suitable mutagenic treatments that can induce maximum frequency of mutations in this crop.

MATERIALS AND METHODS

The seed material chosen for the present study was the local variety of *Trigonella foenum- graecum* L., obtained from Sher-i-Kashmir University of Agricultural Sciences and Technology (Skaust-K), Srinagar. Dry and healthy seeds of fenugreek were treated with different doses of gamma rays (100, 200, 300 and 400Gy) and various concentrations of EMS (0.1, 0.2, 0.3 and 0.4%) and SA (0.1, 0.2, 0.3 and 0.4%). Gamma irradiation treatment was carried out at Babha Atomic Research Centre (BARC), Srinagar from ^{60}Cobalt source. For chemical mutagenesis (EMS and SA) seeds were presoaked in distilled water at room temperature for a period of 12 h. Thereafter, the presoaked seeds were treated with 0.1, 0.2, 0.3 and 0.4% concentrations of EMS and SA, respectively. After the chemical treatment, the seeds were thoroughly washed in running tap water to remove the excess chemicals from the seed surface. The treated seeds were then immediately sown in the field along with the control to raise the M_1 generation. Data on various biological parameters such as seed lethality, seedling injury, pollen sterility and meiotic aberrations were recorded in M_1 generation. Seeds from each M_1 plant were harvested separately in treated as well as control populations. Thereafter, the seeds in each treatment were bulked and a random sample of 300 seeds was selected from the bulk for raising the M_2 generation in three replicates in a completely randomized block design. The treated as well as control populations were carefully screened for morphological mutations throughout the growth period of plants in the field. For classification of different types of chlorophyll mutations, Gustafsson (1940) method was used. The effectiveness and efficiency of the mutagens in inducing mutations were estimated by adopting the formula suggested by Konzak et al. (1965). Mutation frequency was calculated by the following method.

$$\text{Mutation frequency (\%)} = \left(\frac{\text{Number of mutated plants}}{\text{Total number of plants}}\right) \times 100$$

Mutagenic effectiveness

Physical mutagen:

$$\text{Effectiveness} = \left(\frac{\text{Mutation frequency (Mf)}}{\text{Dose in Kilo roentgen (Kr)}}\right) \times 100$$

Chemical mutagen:

$$\text{Effectiveness} = \left(\frac{\text{Mutation frequency (Mf)}}{\text{(Conc. of mutagen)} \times \text{(time of treatment)}}\right) \times 100$$

Mutagenic efficiency

$$\text{Efficiency} = \frac{\text{Mutation frequency (MF)}}{\text{\% lethality (L) or \% injury (I) or \% sterility (S) or \% meiotic abnormalities (M)}}$$

RESULTS AND DISCUSSION

Mutagenic effectiveness was calculated to assess the frequency of mutations induced by each dose/concentration of the mutagens. The major trend pertaining to this

Table 2. Mutation frequency of gamma rays, EMS and SA in relation to biological damage (lethality, sterility, injury and chromosomal abnormalities) in *Trigonella foenum-graecum* L.

Mutagen	Mf/L	Mf/S	Mf/I	Mf/Me	Pooled mean
Gamma rays	0.140	0.067	0.082	0.252	0.135
EMS	0.217	0.072	0.100	0.332	0.180
SA	0.192	0.058	0.072	0.273	0.149

Mf/Me, Mutation rate based on meiotic aberrations; Mf/L, lethality; Mf/I, injury; Mf/S, sterility.

parameter influenced by different mutagens can be understood through a critical perusal of Table 1. All the biological parameters such as lethality, pollen sterility, seedling injury and meiotic aberrations increased with the increase in mutagenic dose/conc. The treatments of gamma rays were more effective in reducing plant survival (lethality), seedling height (injury) and inducing maximum frequency of meiotic aberrations as compared to EMS and SA (Ɣ-rays>EMS>SA). On the other hand EMS treatments were more superior to gamma rays and SA in inducing pollen sterility (EMS>Ɣ-rays>SA). In M_2 generation of fenugreek, the numerical values of effectiveness gradually reduced at the higher doses/conc. of all the three mutagens. In case of gamma rays the highest effectiveness value (0.146) could be seen at 100 Gy which decreased beyond this dose. Among EMS treatments effectiveness increased up to 0.2% but decreased at the higher treatments (0.3 and 0.4%). In case of SA the highest effectiveness value (2.158) could be seen at 0.2% and lowest value (0.537) at 0.4%. Among the three mutagens EMS proved to be more effective in inducing mutations as compared to gamma rays and SA. The gamma rays were least effective in this regard. The order of mutagenic effectiveness was EMS> SA> Ɣ-rays.

The mutagenic efficiency is the ratio of frequency of mutations induced in M_2 generation to various biological damages induced in M_1 generation. The data on efficiency of mutagens in relation to various biological damages is presented in Table 1. The efficiency of mutagens showed variable trend depending on the criteria selected for its calculation and the degree of efficiency of various mutagens also showed variation. Among the four different criteria selected, the highest efficiency was recorded in terms of meiotic abnormalities followed by lethality as compared with that of injury and sterility. In gamma rays efficiency increased with the enhancement of dose when estimated on the basis of meiotic abnormalities and pollen sterility up to 300Gy but decreased at the 400Gy. Efficiency recorded in terms of lethality showed a variable trend. Higher values were observed at 100Gy (0.181) and 300Gy (0.173). On the basis of injury, efficiency showed decreasing trend with increase in the dose. In case of EMS and SA, efficiency was more at the intermediate treatments whereas, it decreased at the higher treatments. In general, EMS proved to be the most efficient and gamma rays to be the least efficient mutagen for all the criteria used (Table 2).

Effectiveness and efficiency of mutagens have been worked out by a number of workers (Shah et al., 2008; Girija and Dhanavel, 2009; Shirsat et al., 2010, Wani et al., 2011; Giri and Apparao, 2011; Mahamune and Kothekar, 2012). Some researchers found that chemicals are more effective and efficient in inducing mutations than gamma rays (Solanki, 2005; Rekha and Langer, 2007; Basu et al., 2008; Dhanavel et al., 2008; Ganapathy et al., 2008; Wani, 2009). In the present study, the degree of effectiveness and efficiency varied among different mutagens.

In general lower or intermediate doses proved to be most effective in inducing mutations. The decrease in effectiveness at higher dose/treatments may be attributed to the failure in proportional increase of mutation frequency induced at higher treatments. Similar findings were obtained by Singh and Chaturvedi (1980) in *Vigna radiate* and Wani (2009) in chickpea. Mutagenic efficiency calculated on the basis of lethality, sterility, injury and meiotic aberrations with respect to induced morphological mutations in M_2 population basis showed variation depending upon the criterion selected for its estimation. In general, the intermediate dose treatments proved to be most efficient on the basis of all the criteria used. Similar conclusions have been drawn by several workers (Sudha, 1990; Reddy and Annadurai, 1991; Solanki and Sharma, 1994; Wani et al., 2011). The higher efficiency obtained at lower and intermediate doses of mutagens might be due to the fact that the lethality, injury, sterility etc increases with mutagen concentration at a rate faster than the frequency of mutations (Blixt, 1964). In general, mutation rate based on meiotic aberrations (Mf/Me) was highest followed by lethality (Mf/L), whereas, it was lowest in case of sterility (Mf/S). EMS proved to be efficient followed by SA and Gamma rays. Variations obtained on the basis of criteria used in the mutagenic efficiency have also been reported in sunflower (Kumar and Ratnam, 2010); clusterbean (Bhosle and Kothekar, 2010) and lentil (Dixit and Dubey, 1986).

Conclusion

The result of present study indicates that lower or intermediate concentrations of mutagens (EMS, SA and gamma rays) are more effective in induction and recovery

of mutations for crop improvement of fenugreek.

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