Mutagenic sensitivity of Gamma rays, EMS and Sodium azide in *Trigonella foenum-graecum* L.

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ABSTRACT

This study was performed by exposing the seeds of fenugreek (*Trigonella foenum-graecum* L.) to gamma rays, ethyl methanesulphonate [EMS] and sodium azide [SA]. The observations were made on seed germination, seedling height, plant survival and pollen fertility. The study revealed that germination percentage, seedling height, percent survival and pollen fertility decreased with an increase in dose/concentration of the mutagens. The results conclude that treatments of gamma rays were more effective in reducing germination and survival percentage and seedling height as compared to EMS and SA (ϒ-rays>EMS>SA). On the other hand EMS treatments were more superior to gamma rays and SA in inducing pollen sterility (EMS>ϒ-rays>SA). Lower treatments of all the three mutagens have influenced less biological damage and would be suitable for inducing desirable mutations in fenugreek.

Key words: Fenugreek, mutagen, germination, pollen fertility, Injury

INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L.) commonly known as methi is a well known economic herb of the family Fabaceae. The young plants serve as vegetable for human consumption seeds as a spice or as herbal medicine (Petropoulos, 2002). Physical and chemical mutagens induce physiological damages (injury), gene mutations (point mutations) and chromosomalaberrations in the biological material in M_1 generation (Gaul, 1970). Gamma rays, an energetic form of electromagnetic radiations are known to be the most popular mutagens for their simple application, good penetration, reproducibility, high mutation frequency and less disposal problems (Chahal and Ghosal, 2002). Ethyl methane sulphonate (EMS), a chemical mutagen of the alkylating group has been reported to be the most effective and powerful mutagen and usually causes high frequency of gene mutations and low frequency of chromosome aberrations in plants (Van Harten, 1998; Khatri *et al*., 2005). Sodium azide (NaN3) is the least dangerous and the most efficient mutagen and has been reported to be mutagenic in several crop species (Adamu and Aliyu, 2007; Mostafa, 2011). The mutagenicity of sodium azide is arbitrated through the formation of an organic metabolite which enters the nucleus, interacts with DNA and generates point mutations in the genome. According to Nilan *et al* (1977), SA is relatively safe to handle, inexpensive and non-carcinogenic as compared to other mutagens.

In mutation breeding studies, it is important to determine a suitable dose/concentration of mutagen for a crop plant which can be employed for inducing maximum variability through point mutations. Seed germination, seedling growth, pollen sterility and chromosomal aberration are the commonly used criteria for studying radio-sensitivity in plants (Kon *et al*., 2007; Lal *et al*., 2009; Sangle *et al*., 2011; Sheikh *et al*., 2012).The aim of present study was to determine the response of fenugreek seeds to gamma rays, EMS and sodium azide based on germination and survival percentage, root-shoot length, and pollen fertility with the main aim of identifying appropriate dose/conc. of these mutagens for induction of desirable mutations.

MATERIALS AND METHODS

Certified seeds of *Trigonella foenum-graecum* L.were procured from Sher-i-Kashmir University of Agricultural Sciences and Technology (SKAUST-K). One physical mutagen (gamma rays) and two chemical mutagens (EMS and SA) were employed in the present study. Seeds were exposed to different doses/concentrations of gamma rays (100Gy, 200Gy, 300Gy and 400Gy) from ^{60}Cobalt source at the Baba Atomic Research Centre (BARC) Zakura, Srinagar, ethyl methanesulphonate (0.1%, 0.2%, 0.3% and 0.4%) and sodium azide (0.1%, 0.2%, 0.3% and 0.4%). Prior to the chemical mutagenic treatment, the seeds were presoaked in distilled water for a period of 12h in order to make them relatively more sensitive to mutagenic action. Each dose/treatment comprised of 350 seeds. Out of the 350 seeds, 300 seeds were sown in the field in a Completely Randomized Block Design (CRBD) to raise the M_1 generation, whereas the remaining set of 50 seeds in each treatment were allowed to germinate on moist cotton in Petri-plates for measuring root-shoot length. Observations were recorded on various parameters viz., seed germination, seedling height (root-shoot length), plant survival and pollen fertility.

Root-shoot length was recorded from 15 randomly selected seedlings on 10th day after sowing in the petri-dishes. Seed germination was recorded on alternate days from 6th day after sowing in the field. The number of seeds showing emergence of the cotyledon were counted. Pollen fertility was determined from 15 randomly selected plants from each treatment including control. The pollen grains from freshly dehisced anthers were stained with 1% acetocarmine. Dark stained and normal size pollen grains were counted as fertile, while the empty, partially stained and shriveled ones were counted as sterile. For plant survival the number of plants reaching maturity in the field were noted and expressed as percentage.

RESULTS AND DISCUSSION

The data presented in the Table 1 shows reduction in seed germination, plant survival and pollen fertility with the increase in mutagenic treatments (Figs. 1-3). Seed germination was about 95% in control. Among mutagenic treatments, the maximum seed germination was noticed at 0.1%SA (78%) whereas the minimum seed germination was recorded at 400Gy (42%). The pooled mean values indicate gamma rays to be most effective followed by EMS and sodium azide (ϒ-rays>EMS>SA). Plant survival tended to decrease with the increase in dose/concentration of mutagens except in case of 300Gywhere it was slightly higher (75%) as compared to 200Gy (74.07%). The pooled mean values depicted minimum survival percentage in case of gamma rays (74.27%) followed by EMS (79.29%) and SA (80.88%) (ϒ-rays>EMS>SA). Similarly the higher doses of all mutagens reduced the pollen fertility, maximum reduction being observed at 0.4%EMS (59.95%) as compared to 95.42% in control. The order of effectiveness in reducing the pollen fertility was, however, EMS>ϒ-rays> SA.Data recorded on seedling height measured in terms of root-shoot length is presented in Table 2, fig.4. It is evident that seedling height decreased with an increase in dose/concentration of mutagens. Control populations exhibited the highest seedling height of 7.78cm. Among the mutagenic treatments maximum injury was recorded in 400Gy treatment (74.29%) followed by 0.4% SA treatment (63.75%). The pooled mean values for seedling height and percent injury indicated that gamma rays were more effective followed by EMS and SA treatments (ϒ-rays>EMS>SA).

Many workers have reported the adverse effects of physical and chemical mutagens on various biological parameters (Kon *et al*., 2007; Lal *et al*., 2009; Dhakshanamoorthy *et al.*, 2010; Sangle *et al*., 2011). These workers have observed dose dependent reduction of the above mentioned biological parameters. The decrease in seed germination induced by mutagenic treatments may be the result of damage of cell constituents at molecular level or altered enzyme activity (Khan and Goyal, 2009; Chowdhury and Tah, 2011). Micco *et al.,* (2011) have correlated seed germination with abnormalities in mitotic cycles and in metabolic pathways of the cells.). The reduction in plant survival is attributed to cytogenetic damage and physiological disturbances (Sato and Gaul, 1967). Srivastava *et al.,* (2011) in wheat suggested the reduction in seedling survival due to the hindrance caused by the mutagen on different metabolic pathways of the cells. Similar findings have also been reported in wheat (Rachovska and Dimova, 2000) and sunflower (Mostafa, 2011).

The decrease in pollen fertility with the increase in mutagenic treatments may be attributed to an increase in chromosomal aberrations as well as physiological damages. These results are in accordance with the findings of other workers (Muthusamy and Jayabalan, 2002; Mensah *et al*., 2007).

Table 1. Effect of Gamma rays, EMS and sodium azide on seed germination, plant survival and pollen fertility in M1 generation of *Trigonella foenum-graecum* L.

Treatments		Germination (%)	Plant survival (%)	Pollen fertility (%)
Control		95.00	93.68	95.42
ϒ- rays				
	100Gy	72.00	86.11	75.53
	200Gy	54.00	74.07	67.04
	300Gy	48.00	75.00	55.80
	400Gy	42.00	61.90	43.94
	Mean	**54.00**	**74.27**	**60.58**
EMS				
	0.10%	75.00	88.00	78.67
	0.20%	80.00	83.75	68.76
	0.30%	73.00	78.08	52.30
	0.40%	49.00	67.35	40.07
	Mean	**69.25**	**79.29**	**59.95**
SA				
	0.10%	78.00	91.02	81.68
	0.20%	72.00	87.50	71.96
	0.30%	73.00	79.45	65.35
	0.40%	61.00	65.57	57.60
	Mean	**71.00**	**80.88**	**69.14**

At least some part of the pollen sterility induced by EMS and SA might be due to gene mutations as also reported by Sato and Gaul (1967) in barley subsequent to EMS treatments. The reduction in seedling height in the treated populations may be attributed to the variation in auxin level (Goud and Nayar, 1968), change in the specific activity of quite a few enzymes (Cherry *et al*., 1962) and physiological injury induced in the seeds and seedlings (Ignacimuthu and Babu, 1988). Evans and Sparrow (1961) suggested that the chromosomal damage and inhibition of cell division are the chief causes of reduced seedling growth. Blixt (1970) opined that the inhibition in seedling growth might be due to the gross injury caused at cellular level either due to gene controlled biochemical processes or acute chromosomal aberrations or both. The observations on biological parameters in the present study revealed that gamma rays were more superior to EMS and SA in reducing seed germination, seedling height and plant survival. However, EMS in turn was more effective than gamma rays and SA in inducing pollen sterility. In general, the reduction in seed germination, plant survival and pollen fertility was more at the higher dose/concentration levels, which indicate the greater sensitivity of fenugreek due to occurrence of more genic, chromosomal and physiological disturbances at these concentrations.

Table 2. Effect of Gamma rays, EMS and sodium azide on seedling height (root–shoot length) in *Trigonella foenum-graecum* L.

Treatment		Root length (cm)	Shoot length (cm)	Total length (R+S)	Percent Injury
Control		3.36	4.42	7.78	0.00
ϒ- rays					
	100Gy	2.96	4.30	7.26	6.68
	200Gy	2.40	3.46	5.86	26.37
	300Gy	1.76	2.14	3.90	49.87
	400Gy	0.98	1.02	2.00	74.29
	Mean	**2.02**	**2.73**	**4.75**	**39.30**
EMS					
	0.10%	2.92	4.02	6.94	10.80
	0.20%	2.62	3.18	5.80	24.45
	0.30%	2.24	2.34	4.58	41.13
	0.40%	1.64	1.30	2.94	62.21
	Mean	**2.35**	**2.71**	**5.06**	**34.65**
SA					
	0.10%	3.14	4.26	7.40	4.88
	0.20%	2.62	3.50	6.12	21.34
	0.30%	2.02	2.32	4.34	44.21
	0.40%	1.38	1.44	2.82	63.75
	Mean	**2.29**	**2.88**	**5.17**	**33.54**

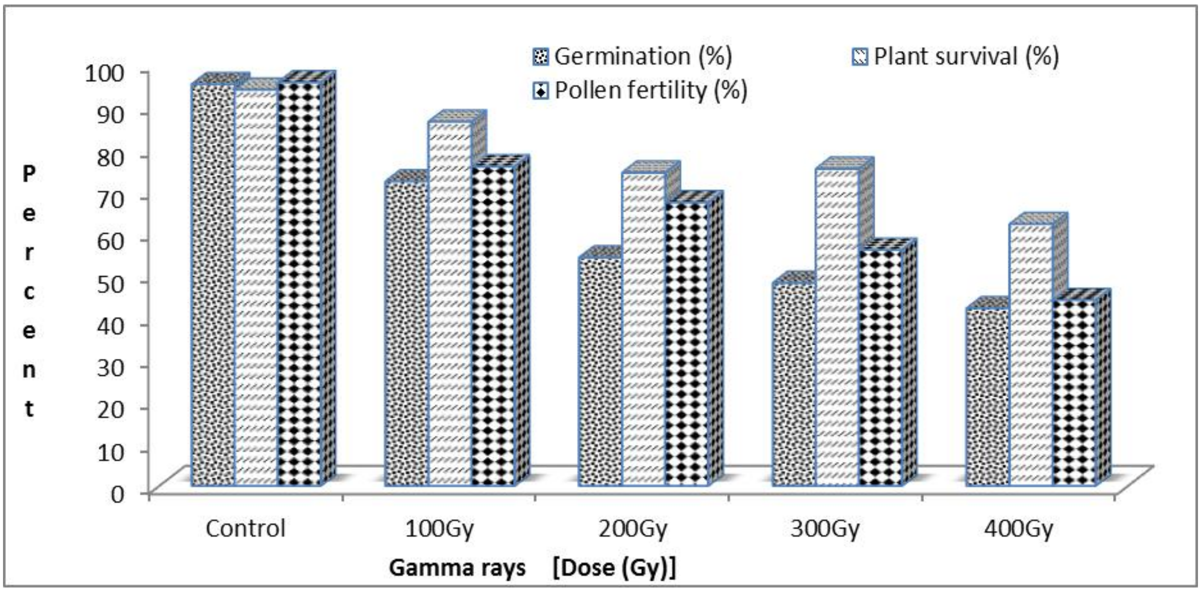

Fig.1: Comparative effect of Gamma rays on seed germination, plant survival and pollen fertility in *Trigonella foenum-graecum* L.

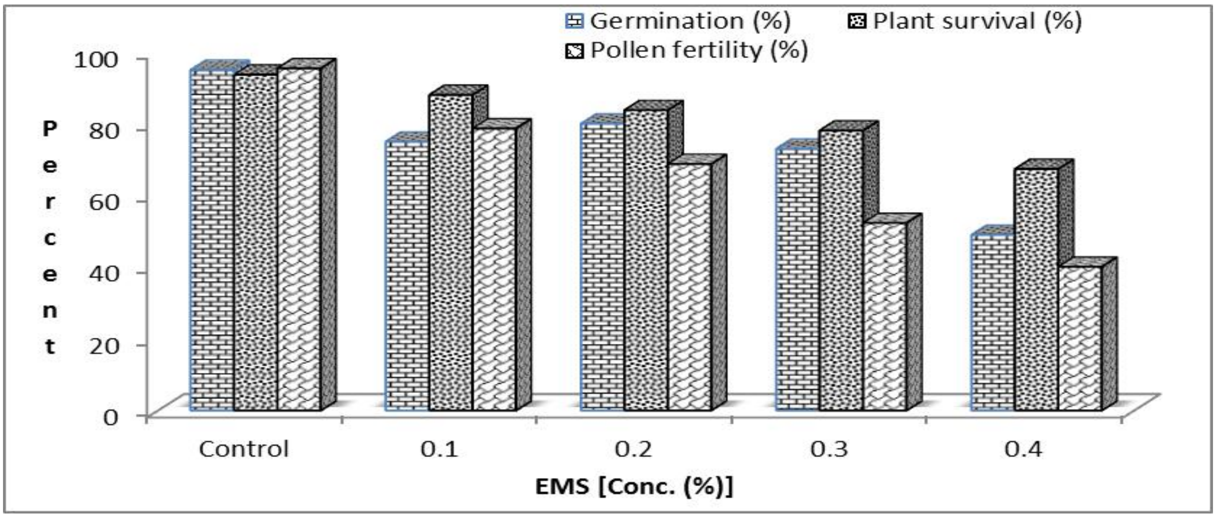

Fig. 2: Comparative effect of EMS on seed germination, plant survival and pollen fertility in *Trigonella foenum-graecum* L.

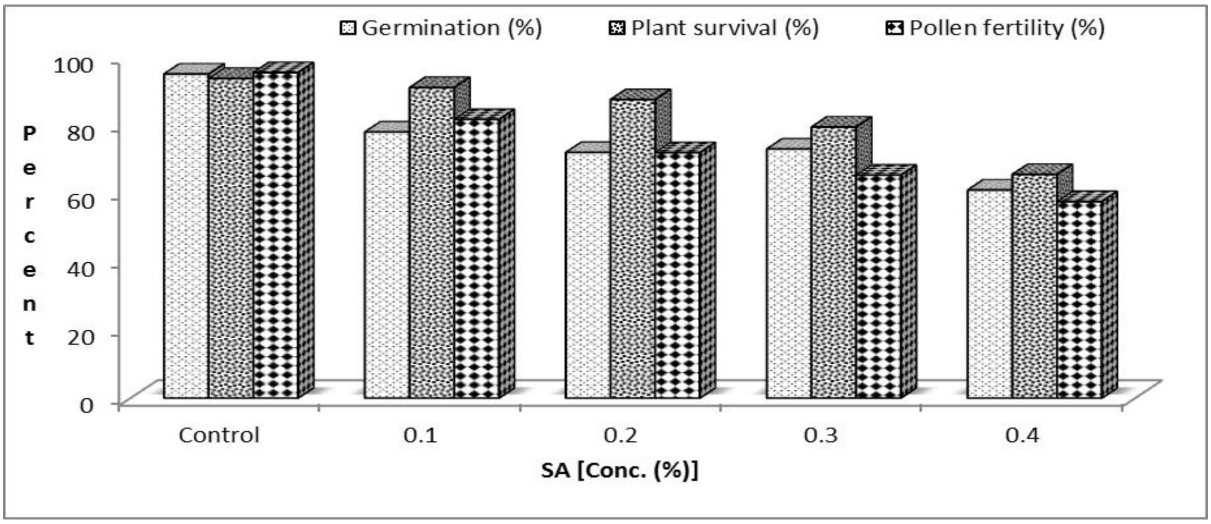

Fig.3: Comparative effect of SA on seed germination, plant survival and pollen fertility in *Trigonella foenum-graecum* L.

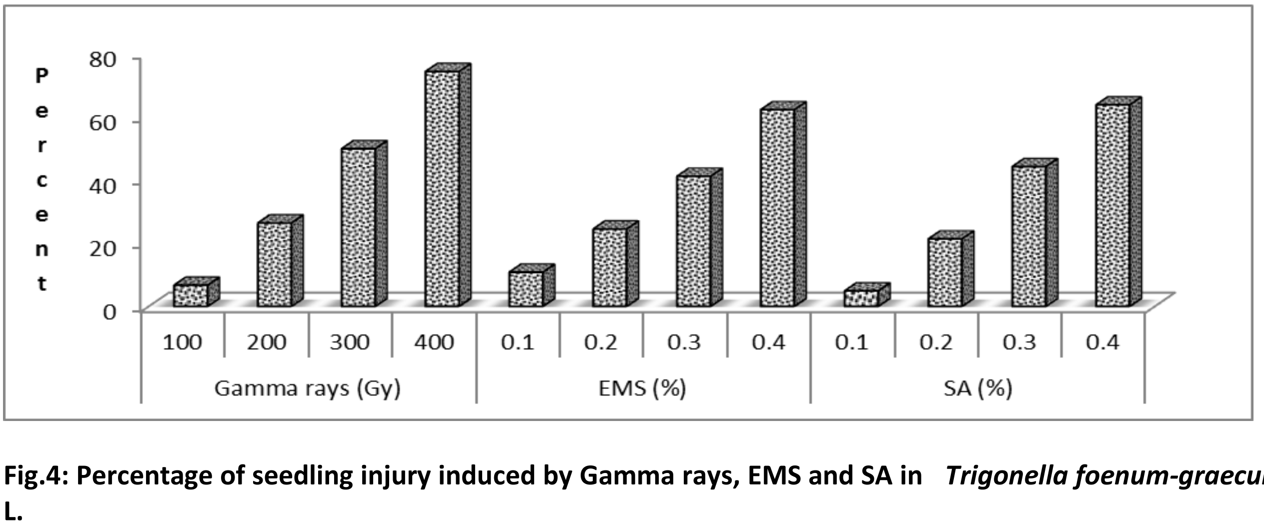

Fig.4: Percentage of seedling injury induced by Gamma rays, EMS and SA in *Trigonella foenum-graecum* L.

The above findings indicate a general superiority of gamma rays over EMS and SA and provide a guideline for selecting suitable mutagenic treatments for inducing desirable mutations in fenugreek. The lower treatments of these mutagens used in the present study could therefore be successfully employed in enhancing genetic variability in this crop plant.

ACKNOWLEDGEMENT

We are thankful to the Director BARC, Zakura, Srinagar for providing gamma rays facility.

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How to Cite this Article:

Shagufta Bashir, Aijaz A Wani and Irshad A Nawchoo, 2013. Mutagenic sensitivity of Gamma rays, EMS and Sodium azide in Trigonella foenum-graecum L. *Sci. Res. Rept,* **3**(1):20-26.