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Effect of mutagens on morphological characters of fenugreek (*Trigonella foenum-graecum* L.)

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Fenugreek (*Trigonella foenum-graecum* L.) is one of the oldest medicinal plants, originating in India and Northern Africa. An annual plant, fenugreek grows to an average height of two feet. The leaves and seeds, which mature in long pods, are used to prepare extracts or powders for medicinal use. In recent time mutagen have become important tools in crop improvement. These mutagens are being used to produce resistance in various susptible crops to improve their yield and quality trait against harmful pathogens. In the present work, two different mutagenic agents *viz.,* physical *i.e.* UV radiation and chemical *i.e.* ethidium bromide of various doses were used and seeds were also treated with both mutagens. Total 10 parameters were observed and compared with control. The screening for best mutagen was done by comparing these 10 parameters, it is clear from the obtained data that the seed treated with ethidium bromide showed better result than seed treated with ultra violet radiation and both ethidium bromide and UV.

Key words : Mutagen, Fenugreek, Ethidium bromide, Ultra violet

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INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L.) is a flowering annual plant, with autogamous flowers. This crop is native to an area extending from Iran to northern India and widely cultivated in China, India, Egypt, Ethiopia, Morocco, Ukraine, Greece, Turkey, etc. (Petropoulos, 2002; Polhil and Raven, 1981). Fenugreek leaves and seeds are consumed in different countries around the world for different purposes such as medicinal uses (anti-diabetic, lowering blood sugar and cholesterol level, anti-cancer, anti-microbial, etc.), making food (stew with rice in Iran, flavour cheese in Switzerland, syrup and bitter run in Germany, mixed seed powder with flour for making flat bread in Egypt, curries, dyes, young seedlings eaten as a vegetable, etc.), roasted grain as coffee-substitute (in Africa), controlling insects in grain storages, perfume industries, etc. (Basch *et al.,* 2003). Fenugreek can be a very useful legume crop for incorporation into short-term rotation (Moyer *et al.*, 2003) and for hay and silage for livestock feed, for fixation of nitrogen in soil and its fertility, etc. (Sadeghzade *et al.*, 2009). The production of this crop is affected by environmental stress such as: drought, salinity, chilling.

In *Trigonella foenum graecum* L. Raghuvanshi and Singh (1974) studied the mutagenic effect of colchicines and gamma rays. Laxmi *et al.* (1983), studied a green seed coat colour of mutant in *Trigonella foenum-graecum* L., followed with treatment of 0.6 per cent MMS. Jain and Aggrawal (1987) treated the seeds of *Trigonella corniculata* and *Trigonella foenum graecum* L. with different concentration of EMS, MMS and SA (NaN3) separately to study the effect on the level of ascorbic acid. Devi and Reddy (1990) studied sensitivity to chemical mutagens like, ethyl methane sulphonate (EMS), diethyl sulphonate (DES) and ethylene imine (EI).

Plant breeding requires genetic variation of useful traits for crop improvement. Often, however, desired variation is lacking. Mutagenic agents, such as radiation and certain chemicals, then can be used to induce mutations and generate genetic variations from which desired mutants may be selected. Mutation induction has become a proven way of creating variation within a crop variety.

In present study the objective is to screen the mutagens for better improvement of spinach.

RESEARCH METHODOLOGY

The M47 variety was selected for the experiment; the

seeds were obtained from the local market. Before start of experiment the seeds were sterilized with 10 per cent sodium hypochlorite for 10 min. then rinsed 3-4 times with sterilized distilled water and air dried, these seeds were treated various mutagenic agent (Table 1).

This experiment was carried out by three replicates. The seeds were treated with two mutagens

– Physical mutagen:
  Total four sets of 60 seeds were treated with UV radiation for 20 sec, 30 sec, 50 sec, and 60 sec. and coded as U_1, U_2, U_3 and U_4.
– Chemical mutagen:
  Total four sets of 60 seeds were treated with ethidium bromide as 1 per cent, 0.75 per cent, 50 per cent and 0.25 per cent for one hour and coded as E_1, E_2, E_3 and E_4, respectively.
– Another four sets of 60 seeds were treated with both mutagens as UV radiation and ethidium bromide as given in (Table 1) and coded as B_1, B_2, B_3 and B_4.

The experiment was conducted in Petri plated on filter paper beds to check the germination percentage. 10 seeds were sown in Petri plats and germinated on paper imbibed in distilled water. These Petri plates were incubated at room 27 ± 3^0 C (technique given by Kaymakanova).

Remaining 50 seeds were sown in field by making furrows and field area was marked with codes. These plants watered and observed daily. The data regarding number of leaves and leaf surface area was obtained from field plants.

RESEARCH FINDINGS AND ANALYSIS

The experimental findings of the present study have been presented in the following sub heads:

Germination velocity :

Germination velocity calculated for different mutagens on 2nd day given in Table 1. Germination velocity was found maximum in control, U_1,U_2, E_1, E_2,E_4, B_2,B_3, B_4 simultaneously (5,5,5,5,5,5,5,5,5,5), whereas, the lowest germination velocity was observed in B_1 (3.5), growth velocity was calculated by using a modified Timson's index of germination velocity Bo Guan *et al.* (2010).

$$\text{Germination velocity} = \sum \frac{\text{Number of germinated seeds}}{\text{Days of count}}$$

Seed vigour :

The principle object of a seed vigour test was to differentiate a range of quality levels for example high, medium and low vigour seed. Seed vigour is obtained by germination percentage and length of stem and radical (Maisurias and Patel, 2009). However, as shown in Table 1 a significant difference was observed among genotypes with respect to mutagen.

The seed vigour of U_4 was highest (next to control) 7.36 while, it was observed lowest *i.e.* 0.18 in B_1.

Seed vigour index= Length of shoot+length of root x germination%

Seed germination percentage :

Seed germination percentage was calculated at 2, 4 and 6 days, significant differences were obtained for two consider factor is type of mutagen and seed germination. Highest percentage of seed germination was observed on second day in E_2 and B_4 *i.e.* 40 per cent. Least germination percentage was observed 0 per cent for B_1. On 4th day the highest germination percentage was observed in U_2, E_2 and E_4 *i.e.* 100 per cent. The least germination percentage was observed in B_1 30 per cent,

Table 1: Various parameters applied to mutagen

Code	Mutagenic agent		G.V.	S.V.	G% A2D	G% A4D	Shoot length (Mean)	Root length (Mean)	No. of leaves after (mean)		Leaf surface area after (mean)	
	Uv (sec)	EtBr %							20 days	40 days	20 days	40 days
Control	Nil	Nil	5	843	100	100	5.22	3.21	3.9	9.1	5.1	6.0
U_1	20 sec	Nil	5	396	100	100	1.78	2.18	4	7.7	4.9	6.6
U_2	30 sec	Nil	5	380	100	100	2.08	1.72	3.9	7.4	5.9	5.6
U_3	50 sec	Nil	4.5	362	90	100	1.73	1.89	3.2	7.3	5.5	6.5
U_4	60 sec	Nil	4.5	423	90	100	2.42	1.81	3.7	6.8	5	2.4
E_1	Nil	1%	5	736	100	100	5.32	2.04	4	5.9	3.9	3.3
E_2	Nil	0.75%	5	271	100	100	1.16	1.55	3.2	6.5	5.1	4.0
E_3	Nil	0.50%	4.5	344	90	100	1.39	2.05	3.8	6.1	4.4	3.0
E_4	Nil	0.25%	5	654	100	100	4.58	1.96	3.5	6.5	4.6	*2.3
B_1	20 sec	1%	*3.5	*194	*70	100	*0.56	*1.38	*3	*2.9	3.6	3.0
B_2	30 sec	0.75%	5	290	100	100	1.00	1.90	3.6	7.1	*3.3	4.0
B_3	50 sec	0.50%	5	262	100	100	1.19	1.43	3.7	6.8	8.5	9.6
B_4	60 sec	0.25%	5	307	100	100	1.55	1.52	3.7	7.6	5.2	3.0

Bold values Indicate highest observation *Marked values indicate lowest observation

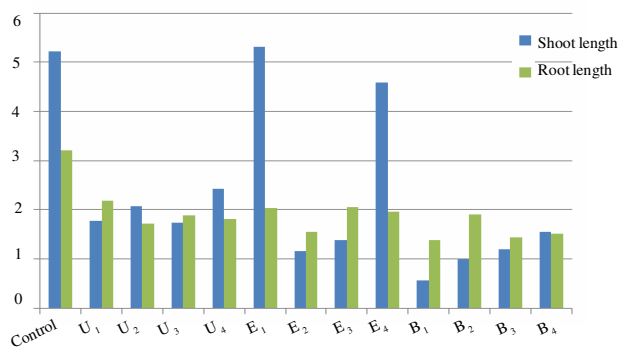

Fig. 1 : Shoot/root length

germination was also observed on 6th day and it was found that the highest germination percentage was calculated in U_2, E_2 and E_4 *i.e.* 100 per cent. It is minimum 50 per cent in B_1, B_3 and E_3 (Table 1, Fig. 2).

Seed germination was calculated by using following formula (Rajurkar *et al.*, 2011)

$$\text{Seed germination} = \frac{\text{Number of seed germinated}}{\text{Total number of seed planted}}$$

Effect of mutagen on length of shoot and root :

Shoot length and root length (Table 1, Fig. 1) was calculated after 6 days, significant results were obtained. Highest shoot length (mean) was found in U_4 *i.e.* 5.08 cm where as it measured 2.75 cm in control. The lowest shoot length was found to be 0 cm in B_1 on the other hand highest root length was found in U_4 (2.28 cm), E_4 (1.87 cm). The lowest root length was observed in 0.18 cm in B_1.

Leaf surface area :

Leaf surface area was calculated after 20 days, it was found that the greater area was calculated 4.9 cm^2 for U_1 mutagen. In control it was found to be 2.3 cm^2. The lowest area was observed in B_2 *i.e.* 2.3 cm^2. Leaf surface was calculated after 40 days, it was found that the greater area was calculated 19.6 cm^2 for E_4 mutagen. In control it was found to be 7.55 cm^2. The lowest area was observed in B_2 *i.e.* 4.25 cm^2.

Number of leaves per plant after 20 days :

Number of leaves per plant after 20 days, number of leaves was calculated and mean was prepared by using data (Table 1). It was found that 3.6 was the mean value which was maximum in U_2, control showed 3.3 mean value. The lowest

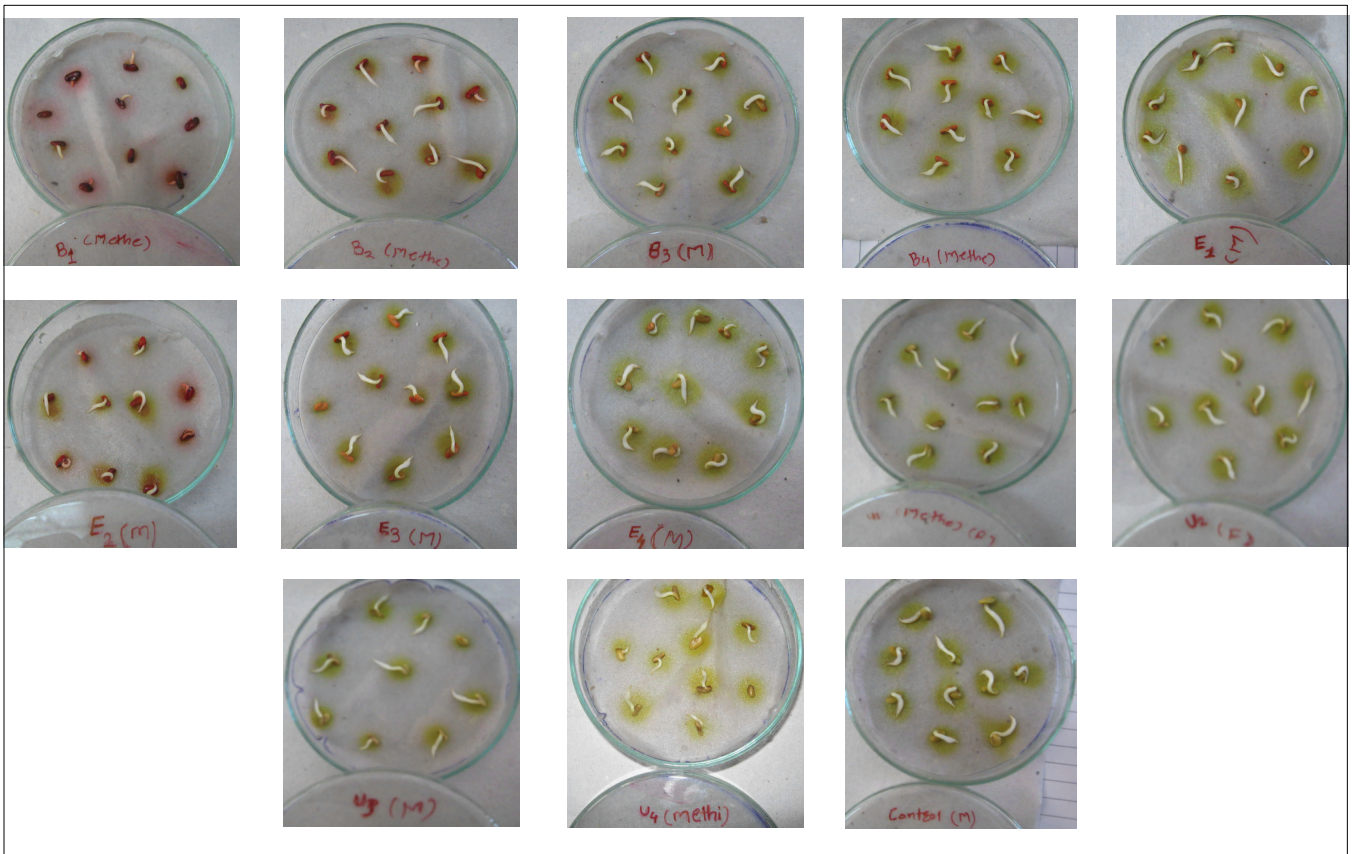

Fig. 2: Seed germination after two days

value was calculated was 2.5.

Number of leaves per plant after 40 days :

Number of leaves was calculated and mean was prepared by using data (Table 1). It was found that 6.27 was the mean value of which was highest in E_1, control showed 6 mean value. The lowest value was calculated was 3.17.

Screening for best mutation :

The parameter studied germination percentage (%) germination velocity, germination percentage after 2 days, germination percentage after 4 days and germination percentage after 6 days. Seed vigour, shoot length, number of leaves, leaf area showed variable data. All the data obtained were compared with control, total 11 parameters screened for best mutation.

From observation (Table 1) it is clear that application of single mutation was significant and it also clear that application of ethidium bromide was more significant (E_4 *i.e.* 0.25% EtBr). Out of 11 parameters 8 parameters showed significant result when compared to control. On the other hand U_2 (ultra violet light for 30 sec.) showed significant result for 6 parameters as compared to control.

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