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Maleic Hydrazide Induced Variability in Fenugreek (Trigonella Foenum-Graecum L.) Cultivars CO1 and Rmt-1

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ABSTRACT

Trigonella foenum graecum (fenugreek), a leguminous annual herb with tremendous medicinal and therapeutic importance. Pre-soaked seeds were treated with different concentrations- 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09 and 0.10% of maleic hydrazide for 6 hours duration each. The mutagen treatments shifted the mean values of all quantitative and bio-physiological traits in positive as well as negative direction as compared to control. The total chlorophyll content in both the cultivars showed a considerable negative shift from their control at all the treatment conditions. Seed germination, pollen fertility and plant survival reduced in a progressive manner with the increasing mutagen. The treatments were found to be appropriate for breeding objectives focused on productivity, considering the lower biological damages with a higher percent of the variance in quantitative traits. A cconsiderable increase in variance was recorded for all the traits studied including morphological traits. The novel morphological mutations induced in the present study would also be useful in botanical studies of fenugreek. The present experimentation reflected that the induced variation with 0.06% could be applied for the generation of new cultivars of fenugreek. The mutation breeding approach could be used in fenugreek to develop cultivars with improved seed yield, early seed maturity, better seed quality and determinate growth habit.

INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L.) is an annual forage legume crop, believed to be native to the Mediterranean region however, it is grown as a spice in most parts of the world. India is the leading producing country in the world and is currently responsible for more than 68% of world total production [1]. Fenugreek is regarded as the oldest known medicinal plant in recorded history (Lust 1986). Its seed and leaves have tremendous medicinal value and have been used to reduce blood sugar and lower blood cholesterol in humans and animals [2,3]. Fenugreek is also used as an herb (dried or fresh leaves), spice (seeds), and vegetable (fresh leaves, sprouts, and microgreens). Sotolon is the chemical responsible for fenugreek's distinctive sweet smell. Both biotic and abiotic factor serve as a limiting factor for the production of Trigonella, the major constraints are biotic stresses such as pod borer, aphids cutworms, powdery mildew, rust and wilt are the major pest and diseases affecting Fenugreek production. Legumes are rich in nitrogen and phosphorus due to their ability of fixing nitrogen, this property attracts insects' pest and diseases. Induced mutagenesis has been widely used as a potent method of enhancing variability for crop improvement [4,5].

Fenugreek is traditionally assumed and purportedly consumed as a medicinal plant since prehistoric time and is undoubtedly considered safe to human health. Its nutritional value and biologically active compound profile are unquestionably appreciated

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by medical science. Moreover, drought, saline and heavy metal tolerability, wide adaptability to various climatic regions and marginal lands are the potentialities of this crop to hold a righteous place in agricultural systems. However, unfortunately just a few advances have been made for crop improvement till now. Hence, a huge gap still exists particularly in varietal development and more specifically in biotechnologically facilitated breeding.

Though fenugreek cultivation is concentrated mainly in some countries of Africa and Asia; however, it is being cultivated throughout the world under diverse environmental conditions. Widespread cultivation of fenugreek requires that we solve the seed production problem through proper understanding of the agronomic complexities of growing this crop and select new early maturing and high yielding germplasm. Since fenugreek is a self-pollinated crop, mutation breeding is considered to be the best method to generate novel mutations for effective selection of mutants with a determinate growth habit that mature earlier than indeterminate types and have high seed yield and quality. The main objectives of present research work was carried out to assess the effect of treatment doses versus duration of MH on the intensity of mutation induction and to study the magnitude of variability induced in various morphological, physiological and quantitative traits of fenugreek.

MATERIALS AND METHODS

Experimental plant material selected for the present investigation was fenugreek verities (CO 1 and RMt-1), commonly known as Methi. The chemical mutagen "Maleic hydrazide" (MH) was used in the present investigation to induce genetic variability in the selected plant materials for possible selection. Maleic Hydrazide (MH): $C_4H_4N_2O_2$ is an alkylating agent and a carcinogen.

A fresh aqueous stock solution of Maleic hydrazide (1% v/v) was prepared in phosphate buffer at pH 7.0. The pH of the solution was maintained by using buffer tablets. From this stock, working solutions of 0.01%, and 0.10% concentrations of MH were prepared **(Table 1)**.

Table 1. Details of the variety used in the present investigation.

Variety	Parentage	Characters
CO 1	Reselection from TG 2336	Duration of the crop is 110 days. Dual purpose quick growing, suited for intercropping, high seed protein 20-23%. Yield per ha 685kg grain, 4.51 of green.
RMt-1	Pure line selection from Nagpur type	Duration of the crop is 145 days. High yield, moderately branched, moderately tolerant, to root rot and powder mildew. Seed protein: 21%. Yield per ha 1500 kg grain

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.

$$Germination\ (\%)\frac{\text{No. of seeds germinated}}{\text{No. of seeds sown}}\times 100$$

Seedling Height

Seedling height was recorded after 14 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.

Estimation of Chlorophyll and Carotenoid Contents

The chlorophyll contents of leaves on which measurement were made was estimated by the method of MacKinney (1941). The chlorophyll contents present in the extracts of leaves was calculated according to the equation given by Arnon (1949).

$$Total\ chlorophyll\ \left(mg\ g^{-1} leaf\ fresh\ mass\right) = (20.2(\text{OD}\,645)+8.02(\text{OD}\,663))\times\frac{v}{1000\times w}$$

Where, OD_{645}, OD_{663}=Optical densities at 480 and 663 nm respectively; V=Volume of an extract; W=Mass of leaf tissues.

Plant Survival

The surviving plants in different treatments and control were counted at the time of maturity and the survival was computed as percentage of the germinated seeds in the field.

The following formula was used to calculate the percentage of inhibition, injury or reduction.

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$$\begin{aligned}&Percentage\ inhibition\\&(or)\\&Percentage\ injury=\frac{CONTROL-TREATED}{CONTROL}\\&(or)\\&Percentage\ reduction\end{aligned}$$

Pollen Fertility

Pollen fertility was estimated from fresh pollen samples. From mature anthers, some amount of pollen was dusted on the slide containing a drop of 1% acetocarmine solution. Pollen grains which took stain and had a regular outline were considered as fertile, while empty and unstained ones as sterile. Pollen fertility inhibition was measured in terms of reduction in % pollen fertility with respect to control.

Quantitative Traits

The eight quantitative traits namely Plant height (cm), branches per plant, Pods per plant, seeds per pod, 100 seed weight (g), yield per plant (g) were thoroughly studied in M_2 generation. Data collected for quantitative traits in M_2 generation were subjected to statistical analysis in order to assess the extent of induced variation.

Coefficient of variation (C.V.) measures the relative magnitude of variation present in observations relative to magnitude of their mean. It is expressed as the percentage ratio of standard deviation to corresponding mean, i.e.

$$C.V.\ (\%)=\frac{Standard\ Deviation}{Mean}\times 100$$

EXPERIMENTAL RESULTS

The mutagenic effects of maleic hydrazide (MH) doses and comparative effect of their treatment durations were studied on seed germination and percentage inhibition, seedling height and seedling injury, total chlorophyll content, morphological changes and certain quantitative parameters of fenugreek. The results are being presented below:

Seed Germination, Plant Survival and Pollen Fertility

The results for seed germination, plant survival and pollen fertility are given in **Figure 1**. Seed germination was counted in control as well as in various mutagen treatments within 1 to 6 days. The percentage of seed germination was more in var. RMt-1 then Var. CO1. Seed germination was reduced and delayed with the increasing mutagen doses/treatment durations.

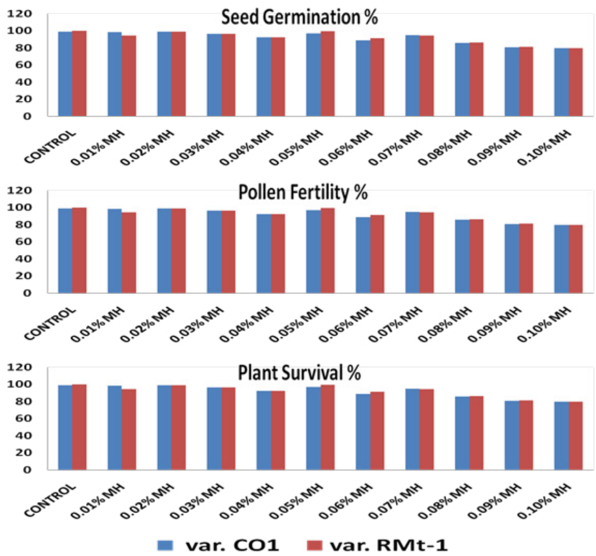

Figure 1. Estimates of mean ($\overline{X}$) seed germination (%), plant survival at maturity (%) and pollen fertility (%) in M_1 generation of Fenugreek (*Trigonella Foenum-graecum L.*) var. CO1 and var. RMt-1.

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In var. CO1 it decreased from 100% (control) to 53.33% (0.10% MH) whereas in var. RMt-1 it decreased from 93.37% (control) to 46.67% (0.10% MH). Percentage inhibition increased with the increase in concentration of mutagen treatments. It was highest at 0.10% MH in var. CO1 (46.67%) and var.RMt-1(46.66%). Data recorded for seedling height of 14 days old seedling raised in Petri plates in B.O.D. incubator. Data showed all mutagen treatments conditions brought about reduction in seedling height. It decreases from 8.67 and 10.33 cm (controls) to 3.33 and 3.67 cm (0.08% MH) of var. CO1 and var. RMt-1 respectively. The percent inhibition was more drastic at the higher concentrations of mutagens. Data on plant survival in M_1 generation recorded at maturity in the pots kept in net house were presented. Percentage of plant survival decreased gradually with the increase in doses and duration of mutagenic treatments. The highest plant survival was observed in the controls (93.33% and 93.33%) of var. CO1 and var. RMt-1 respectively, which then showed progressive decline in highest dose of 0.10% MH to 46.67% and 40.00% in var. CO1 and var. RMt-1 respectively. Present study revealed that the degree of sterility of pollen grains was substantially reduced (19.00% and 20.00%) in the 0.10% MH treatment for variety var. CO1 and RMt-1, respectively. It was observed during the present study that the higher treatment duration of MH brought about significant reduction in biological parameters compared to their respective treatments at lower duration in both the cultivars.

Total Chlorophyll Contents

The results for total chlorophyll content in the control and treated plants of the fenugreek are given in **Table 2**. It was observed that total chlorophyll content of leaves in both the cultivars showed a considerable negative shift from their parents at all the treatment conditions. In var. CO1, it was 1.03 mg/g in control which increased maximum to 1.07 mg/g in 0.10% MH, whereas in RMt-1 it increases from 1.092 mg/g (control) to 1.06 mg/g (0.10% MH).

Table 2. Estimates of Mean, Standard Deviation (S.D.), shift in mean ($\bar{X}$) and Coefficient of Variation (C.V. %) for total chlorophyll content (mg g^{-1} leaf fresh mass) of leaves in M_1 generation of Fenugreek (*Trigonella Foenum-graecum* L.) var. CO1 and var. RMt-1.

Treatments	Variety C01					Variety RMt-1				
	Range	Mean ± S.E.	Shift $\bar{X}$	S.D.	C.V. %	Range	Mean ± S.E.	Shift in $\bar{X}$	S.D.	C.V. %
Control	1.03-1.03	1.03 ± 0.000	0.00	0.000	0.00	1.00-1.03	1.02 ± 0.010	0.00	0.017	1.70
0.01% MH	0.98-1.00	0.99 ± 0.007	-0.04	0.012	1.16	0.99-1	1.00 ± 0.003	-0.02	0.006	0.58
0.02% MH	0.85-0.89	0.87 ± 0.012	-0.16	0.021	2.38	0.86-0.86	0.86 ± 0.000	-0.16	0.000	0.00
0.03% MH	0.99-1.00	0.99 ± 0.003	-0.04	0.006	0.58	0.99-1	0.99 ± 0.003	-0.03	0.006	0.58
0.04% MH	0.93-0.95	0.94 ± 0.007	-0.09	0.012	1.22	0.94-0.95	0.95 ± 0.003	-0.07	0.006	0.61
0.05% MH	0.83-0.85	0.84 ± 0.007	-0.19	0.012	1.37	0.84-0.85	0.85 ± 0.003	-0.17	0.006	0.68
0.06% MH	0.87-0.88	0.88 ± 0.003	-0.15	0.006	0.66	0.87-0.88	0.88 ± 0.003	-0.14	0.006	0.66
0.07% MH	0.83-0.83	0.83 ± 0.000	-0.20	0.000	0.00	0.82-0.84	0.83 ± 0.007	-0.19	0.012	1.39
0.08% MH	0.87-0.89	0.88 ± 0.007	-0.15	0.012	1.32	0.89-0.89	0.89 ± 0.000	-0.13	0.000	0.00
0.09% MH	0.95-0.99	0.97 ± 0.012	-0.06	0.020	2.06	0.95-0.97	0.96 ± 0.007	-0.06	0.012	1.21
0.10% MH	1.06-1.09	1.07 ± 0.010	0.04	0.017	1.62	1.06-1.07	1.06 ± 0.003	0.04	0.006	0.54

Morphological Variants

Different types of morphological variants affecting almost all parts of the plant were observed in moderate and higher doses of mutagens. The phenotypic assessments of the mutagenized population was carried out in M1 generation. Variations in morphology observed in present fenugreek varieties induced by MH were represented in **Figures 2 and 3**. Variation in the plant height include tall and dwarf, and growth habits such as erect, semi-erect and horizontal importantly bushy and prostrate, alteration in pod sizes viz; long pod, narrow pods were noticed frequently in treated plants. The leaf morphological variations recorded were foliage colour such as light, medium and dark and leaflet size viz., small, medium and large and shape such as narrow and broad. In higher concentration of mutagens seedlings revealed stunted growth and excessive vegetative growth with degree of sterility. Generally, 0.05% MH for var. CO1 and 0.06% for RMt-1 induced higher frequencies of variants compared to other mutagen treatments. In the present study, different types of chlorophyll variants were recorded in the pot experiments and amongst all the different mutagen treatments, 0.04% MH showed the higher number of chlorophyll variants in both the varieties.

Quantitative Traits

Data on various quantitative traits were presented in **Tables 3 and 4** for *Trigonella Foenum-graecum* L. vars. CO1 and RMt-1, respectively. Height of mature plants generally decreased with the increasing concentrations in both the cases. The average height reduced from 45.33 cm (control) to 40.00 cm (0.10% MH) in var. Co1, while it decreased from 45.33 cm (control) to 40.00 cm (0.10 MH) in RMt-1. The coefficient of variation was minimum 0.00% (var. CO1) and 1.27% (RMt-1) in control. Compared to the controls and other treated populations the C.V. increased in random trend. Being maximum in 0.02% MH (4.12%) and 0.01% MH (3.39%) in Var. CO1 and RMt-1 respectively. Numbers of primary branches were counted after flowering in control as well as treated plants and it found to decrease from controls (3.67 and 4, 00 in var. CO1 and RMt-1 respectively) in all the mutagen treatments. The minimum numbers of branches were observed to be 2.67 and 2.33 in 0.09% MH in var. CO1 and RMt-1 respec

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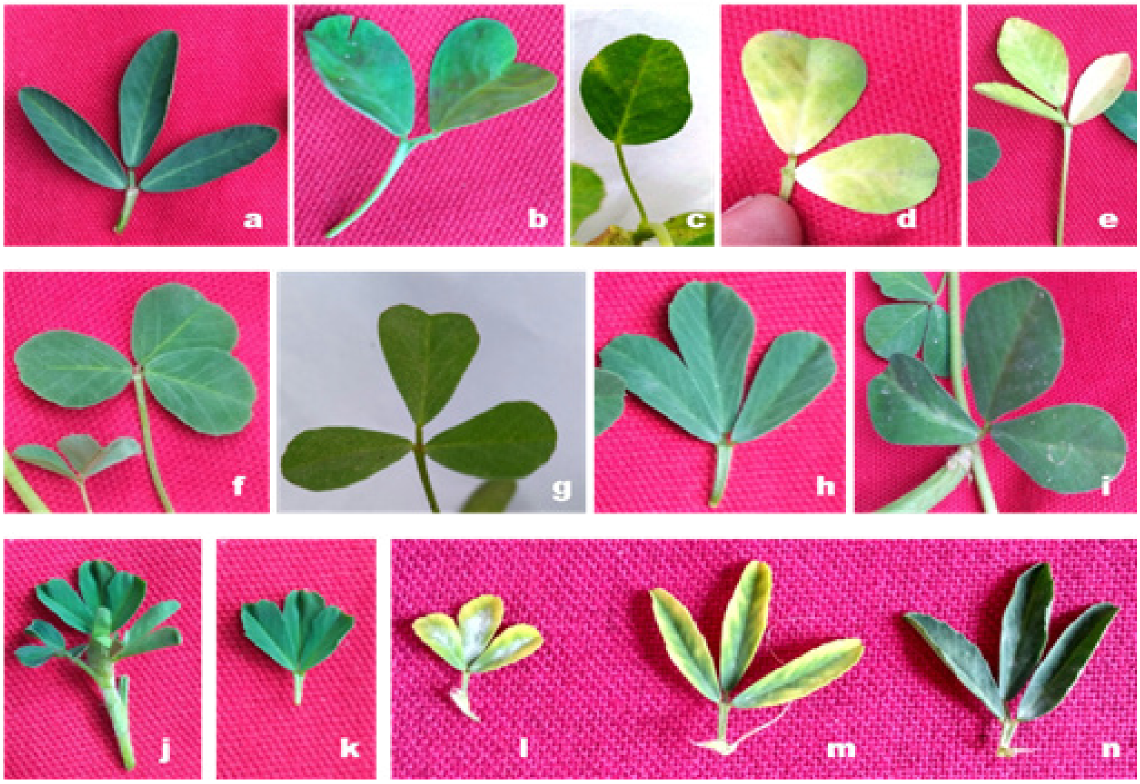

Figure 2. Isolated morphological and chlorophyll variations of leaf in mutagen treated populations of fenugreek varieties. a: Trifoliate leaf with elliptical leaflets. b: Bifoliate leaf with oblanceolate and obcordate leaflets. c: Leaf with single ovate leaflet. d: Bifoliate chlorotica leaf with ovate leaflets. e: Trifoliate leaf with albino and albino-terminalis leaflets. f: Trifoliate leaf with round oval leaflets. g: Trifoliate light green leaf with oblanceolate and cuneate leaflets. h: Trifoliate green leaf with oblanceolate and oblong leaflets. i: Trifoliate leaf with round oval leaflets and whitish midribs. j: Green leaf with bunch small leaflets. k: Trifoliate leaf with small overlapping leaflets. l: Trifoliate with small chlorine leaflets. m: Trifoliate with mariginata leaflets. n: Trifoliate dark green leaf with rolled leaflets.

Figure 3. Plant height and growth habit variants in treated M_1 generation of fenugreek varieties. a: Plants with dwarf growth habit. b: Plants with tall growth habit. c: Plants with more branching and long internodes. d: Plants with normal growth habit. e: Variations in pod size.

Table 3. Estimates of Mean, Standard Error (S.E.) and Coefficient of Variation (C.V. %) for Plant height (cm), branches per plant, Pods per plant, seeds per pod, 100 seed weight (g), yield per plant (g) in M_2 generation of Fenugreek (*Trigonella Foenum-graecum* L.) var. CO1.

Treatments	Plant height (cm)		branches per plant		Pods per plant		seeds per pod		100 seed weight (g)		yield per plant (g)	
	Mean ± S.E.	C.V. %	Mean ± S.E.	C.V. %	Mean ± S.E.	C.V. %	Mean ± S.E.	C.V. %	Mean ± S.E.	C.V. %	Mean ± S.E.	C.V. %
Control	45.33 ± 0.00	0.00	3.67 ± 0.33	15.75	18.67 ± 0.33	3.09	12.00 ± 0.00	0.00	0.75 ± 0.00	0.00	1.89 ± 0.01	0.61
0.01% MH	46.33 ± 0.00	0.00	3.67 ± 0.33	15.75	20.00 ± 0.00	0.00	13.67 ± 0.33	4.22	0.73 ± 0.00	0.79	1.91 ± 0.01	0.60
0.02% MH	41.33 ± 1.00	4.12	4.67 ± 0.33	12.37	21.00 ± 0.00	0.00	13.00 ± 0.58	7.69	0.73 ± 0.00	0.79	1.88 ± 0.01	0.62
0.03% MH	49.00 ± 0.33	1.19	3.67 ± 0.33	15.75	18.33 ± 0.33	3.15	12.00 ± 0.58	8.33	0.75 ± 0.00	0.77	1.89 ± 0.00	0.00
0.04% MH	54.33 ± 0.33	1.06	3.00 ± 0.00	0.00	19.67 ± 0.33	2.94	13.00 ± 0.00	0.00	0.74 ± 0.00	0.78	1.90 ± 0.00	0.30
0.05% MH	53.33 ± 0.33	1.08	4.00 ± 0.58	25.00	22.00 ± 0.58	4.55	15.67 ± 0.33	3.69	0.71 ± 0.01	1.62	1.96 ± 0.01	0.59
0.06% MH	57.67 ± 0.33	0.99	5.00 ± 0.00	0.00	21.00 ± 0.00	0.00	14.33 ± 0.33	4.03	0.75 ± 0.00	0.77	1.97 ± 0.02	1.63
0.07% MH	43.67 ± 0.33	1.35	3.00 ± 0.00	0.00	18.33 ± 0.33	3.15	12.00 ± 0.00	0.00	0.77 ± 0.00	0.00	1.79 ± 0.00	0.32
0.08% MH	40.00 ± 0.33	1.46	4.33 ± 0.33	13.32	21.00 ± 0.00	0.00	14.33 ± 0.33	4.03	0.80 ± 0.00	0.72	1.87 ± 0.00	0.31
0.09% MH	56.00 ± 0.67	2.09	2.67 ± 0.33	21.65	21.00 ± 0.00	0.00	13.33 ± 0.33	4.33	0.76 ± 0.00	0.76	1.99 ± 0.01	0.77
0.10% MH	43.00 ± 0.58	2.44	3.67 ± 0.33	15.75	18.00 ± 0.00	0.00	14.33 ± 0.33	4.03	0.75 ± 0.00	0.00	2.00 ± 0.00	0.29

Table 4. Estimates of Mean, Standard Error (S.E.) and Coefficient of Variation (C.V. %) for Plant height (cm), branches per plant, Pods per plant, seeds per pod, 100 seed weight (g), yield per plant (g) in M_2 generation of Fenugreek (*Trigonella Foenum-graecum* L.) var. RMt-1.

Treatments	Plant height (cm)		branches per plant		Pods per plant		seeds per pod		100 seed weight (g)		yield per plant (g)	
	Mean ± S.E.	C.V. %	Mean ± S.E.	C.V. %	Mean ± S.E.	C.V. %	Mean ± S.E.	C.V. %	Mean ± S.E.	C.V. %	Mean ± S.E.	C.V. %
Control	45.33 ± 0.33	1.27	4.00 ± 0.00	0.00	19.00 ± 0.00	0.00	13.00 ± 0.58	7.69	1.28 ± 0.00	0.00	2.48 ± 0.02	1.16
0.01% MH	46.33 ± 0.88	3.30	3.00 ± 0.00	0.00	20.33 ± 0.33	2.84	14.00 ± 0.00	0.00	1.30 ± 0.00	0.45	2.35 ± 0.00	0.25
0.02% MH	41.33 ± 0.33	1.40	4.00 ± 0.00	0.00	21.00 ± 0.58	4.76	12.67 ± 0.67	9.12	1.30 ± 0.00	0.00	2.43 ± 0.02	1.19
0.03% MH	49.00 ± 0.00	0.00	4.67 ± 0.33	12.37	18.00 ± 0.00	0.00	13.00 ± 0.00	0.00	1.25 ± 0.00	0.00	2.43 ± 0.01	1.04
0.04% MH	54.33 ± 0.67	2.13	3.33 ± 0.33	17.32	19.00 ± 0.00	0.00	13.00 ± 0.00	0.00	1.26 ± 0.00	0.00	2.17 ± 0.33	26.43
0.05% MH	53.33 ± 0.33	1.08	3.00 ± 0.00	0.00	22.00 ± 0.00	0.00	15.33 ± 0.67	7.53	1.29 ± 0.00	0.45	2.54 ± 0.00	0.00

0.06% MH	57.67 ± 0.33	1.00	4.00 ± 0.00	0.00	21.00 ± 0.00	0.00	14.33 ± 0.33	4.03	1.23 ± 0.00	0.47	2.53 ± 0.00	0.23
0.07% MH	43.67 ± 0.67	2.64	3.67 ± 0.33	15.75	18.33 ± 0.33	3.15	12.33 ± 0.33	4.68	1.23 ± 0.01	0.81	2.66 ± 0.01	0.38
0.08% MH	40.00 ± 0.00	0.00	4.33 ± 0.33	13.32	20.00 ± 0.00	0.00	15.00 ± 0.00	0.00	1.21 ± 0.00	0.48	2.68 ± 0.00	0.22
0.09% MH	56.00 ± 0.00	0.00	2.33 ± 0.33	24.74	20.67 ± 0.67	5.59	13.33 ± 0.33	4.33	1.22 ± 0.00	0.47	2.59 ± 0.00	0.22
0.10% MH	43.00 ± 0.00	0.00	3.67 ± 0.33	15.75	19.67 ± 0.33	2.94	15.33 ± 0.33	3.77	1.20 ± 0.00	0.00	2.71 ± 0.01	0.64

tively. Similarly, number of fertile branches also showed decreasing trend with increasing treatments and durations. Normally, the average number of pods in the control population was decreased in the treated populations of both the varieties. It increased from control (18.67) to treatment 0.05% MH (22.00) in Var. CO1 while in var. RMt-1(19.00) it increased to 22.00 in 0.05% MH. Normally, the average number of pods in the control population decreased in most of the differently treated populations of both the varieties. It increased from control (12.00) to treatment 0.05% MH (15.67) in var. CO1 while in var. RMt-1(13.00) it increased to 15.67 in 0.05% MH. Average weight of 100 seeds in controls of the two cultivars namely Var. CO1 and RMt-1 were 0.75 g and 1.28 g, respectively. The treatment 0.08% MH increased 100 seed weight in var. CO1 only, while 0.02% MH increased in both the varieties of fenugreek in M_1 generation. Average yield per plant showed treatment specific random increase with different treatments of mutagen. It was in the range of 2.00 g to 1.79 g in var. CO1, while 2.71 g to 2.17 g in Rajendra kanti. The high CV % observed in lower doses confirmed the high variation at the genetic level in the mutagenized population in both the varieties.

DISCUSSION

In the present chapter the comparative response of cultivars fenugreek (*Trigonella foenum-graecum* L.) towards treatments of maleic hydrazide (MH) were discussed.

Bio-Physiological Damages

Micke suggested that the study of mutagenic sensitivity is imperative for enhancement of genetic variability. Biological damage caused by mutation to seed germination, seedling height, pollen sterility and survival at maturity may be considered as an indication of mutagenic sensitivity [6]. Analysis on biological damages caused by mutagen doses is an imperative tool in any mutation breeding experiment which can be well assessed from the effect of mutagenic treatments on seed germination inhibition and seedling injury [7,8]. In the present study, the seed germination generally decreased with increasing intensity of mutagen doses. The maximum germination inhibition (46.67% and 46.66%) in var.CO1 and RMt-1, respectively was recorded in 0.10% MH treatment. The reduction due to inhibitory effect of MH as observed in fenugreek has also been reported by several workers, such as Verma et al. in *Trigonella foenum-graecum,* Vandana and Dubey, Ashour and Abdou, Kumar et al. in *Vicia faba*; Ansari and Siddiqui in *Ammi majus*; Mitra and Bhowmik in *Nigella sativa* [9]. The reduction in germination may be due to destruction of the activity of gibberellic acid, following the radiation treatment and metabolic disturbances during germination [10,11]. Griffith and Johnson and Srivastava reported that reduction in germination percentage was due to weakening and disturbances of growth process, regulated in early elimination of seedlings. Krishna et al. considered that the inhibition of germination may be due to interaction between mutagen and the seed cell system. It may be due to mutational changes at genetic or chromosomal level due to toxicity of mutagens as the reduction in germination corresponds with the increasing chromosomal aberrations [12]. It may also be due to toxicity of mutagens followed by mutational changes at genic or chromosomal level because the reduction in germination corresponds with the increasing chromosomal aberrations.

The diminution of pollen fertility and viability observed in the present study can be attributed to abnormal meiosis forming abnormal or unequal gametes because the structure and physiology of the pollen grains is under genetic control and irregular or abnormal meiosis may cause significant changes in the pollen properties. A dose-dependent reduction in pollen fertility was recorded in mutagen treated plants that was correlated with other biological damages. This is in agreement with earlier studies that reported dose dependent decrease in pollen fertility. Increase in meiotic abnormalities is known to cause increased pollen sterility [13]. The pleiotropic effects of the mutant gene(s) like breakage, stickiness and spindle abnormalities also contribute to pollen sterility. Reduction in pollen fertility observed in treated population is attributed to the vast array of meiotic aberrations that were induced by mutagens leading to the formation of aberrant pollen grains [14]. Mutagen induced gene mutation or invisible deficiencies may also be the probable reasons of pollen sterility. Pollen grains of plants developed from mutagen treated seeds showed germination inhibition at different treatments. The stored RNA, protein, and bioactive small molecules in mature pollen allow rapid germination and tube growth. These are the products of sporophytic gene expression, arising from the tapetal layer of the anther wall, and gametophytic gene expression from the vegetative and generative nuclei [15]. Mutagens directly act on genetic material and also affect their expression, thus possibly affecting germination and tube growth. Reductions in percent germination and inhibition in the treated population could be the result of altered protein composition or error in protein signal recognition required for pollen development. It may also be due to the abnormal micros orogenesis resulting into unfertile gametes with faulty proteins or may be due to the damage of nuclei or cytoplasm. All these and other unknown factors may contribute to poor pollen germination observed in plants grown from MMS doses of different strength in both the lentil varieties.

Assessment on the chlorophyll contents of treated plants showed considerable increase in in the treated populations, which also indicates the enhance photosynthetic efficiency of the mutagenized population of both the varieties compared to their untreated parental population. Estimation of chlorophyll content in the present study showed a wide variation among populations treated with different mutagen doses. Chlorophyll content is one of the reliable indices of photosynthetic activity [16]. Normally chlorophyll content progressive decreases from control to higher mutagen doses. The highest chlorophyll content in plants occurs at the outset of the flowering phase, and chlorophyll is believed to take part in the process of organogenesis. Nitrogen concentration in green vegetation is related to chlorophyll content, and therefore indirectly influence basic plant physiological processes: photosynthesis [17,18]. Nitrogen is a structural element of chlorophyll and protein molecules, and it thereby affects formation of chloroplasts and accumulation of chlorophyll in them.

Morphological Variants

Generally the frequency of morphological variants increased with the increasing concentrations and duration of mutagen, the maximum useful being in 0.8% MH for Var. CO1 and 0.010% MH for Var. RMt-1. The variation in leaf morphology of seedlings and in mature plants, such as unequal, rudimentary, thickening and poor development of leaflets, stunted and poorly branched mature plants were commonly observed in higher doses. Growth habit variants like tall plant with increased internodes length and bushy plants with increased primary branching and reduced number of nodes or internodes length were also observed. The study investigates the relationship among morphological parameters and with seed yield for 167 fenugreek accessions including two checks (Methi and Methray) under field at (NARC) Islamabad Pakistan. Augmented field design was used with an inter row distance of 45 cm and intra row distance of 1 m was maintained. The results revealed that there was positive and significant correlation of seed yield with leaf length, days to flower and days to maturity. These characters might be enhanced instantaneously and given earlier stress for indirect selection of high yielding fenugreek accessions. PCA analysis showed that the presence of genetic diversity among the valued germplasms. Four out of the 21 principal components with an eigenvalue of ≥ 1.0 accounted for 53.37% of the overall differences found among 167 genotypes of fenugreek. Contribution of the first three PCs were 20.07%, 12.29% and 11.29%, accessions indicating that these accessions may be used in breeding programs for obtaining promising lines. The presence of considerable genetic differences, highly significant, positive interrelated parameters can be utilized in breeding programmes for the development of new fenugreek varieties Similar abnormalities have also been reported in *Chloris guana*, in *Vicia faba,* in *Vigna radiata* L [19-22]. by MMS and gamma ray treatments. The leaf abnormalities may also be due to actual mutation processes which are most easily induced in leguminous plants or due to chromosomal alterations [23,24].

Quantitative Parameters

Use of mutations for obtaining early maturing varieties has been a frequent breeding objective. The data obtained on days to maturity resulted in a consideranlegain in reducing the maturity period with treatment 0.09% MH in Var.CO1 and 0.07% MH in Var. RMt-1. Fenugreek, a winter season crop, is exposed to chilling and freezing temperatures during vegetative and reproductive growth. Cold stress during reproductive phase is unfavorable to flowering and pod set in fenugreek. Early maturity would be ideal for fenugreek to avoid severe cold and attain maximum production. Yaqoob and Rashid in mungbean, Wang et al. in soybean, Shamsuzzaman et al. in chickpea, Singh et al. in lentil Arulbalachandran et al. in urdbean and Khursheed et al. in faba bean reported a significant reduction in days to maturity after mutagenic treatments [25].

The average height of mature plants vary with different concentrations of the mutagenic treatment except 0.06% MH in Var. CO1 where 13.33 positive shift was observed. The maximum reduction in average height was recorded in 0.08% MH and for varieties Pant Var.CO1 and Var.RMt-1 respectively. Reduction in growth as seen in fenugreek has also been observed by many workers such as Verma et al. Krishna et al. in Rhodes grass, Khan et al. in mungbean, Vandana and Dubey and Kumar et al. in faba bean and Ansari and Siddiqui in Ammi majus. Several workers have explained the causes of decreasing plant height due to mutagenic treatments Laskar et al. [26]. The structural changes in the constitution of chromosomes or chromosomal damage may be major factors in growth inhibitions [27-29]. On the other hand, growth inhibition may arise from interference of mutagens with the cell elongation (Sparrow and Sparrow, 1965) or injury caused to the meristematic cells. Some other aspects, such as auxin reduction, physiological disorder or metabolic disturbances may also play important role in the reduction of height in the treated plants [30].

Seed yield in fenugreek is a complex trait and is influenced by many other quantitative traits such as pods bearing branches, pods per plant, seeds per pod and 100 seed weight. The increase in yield contributing characters like of pods per plant and 100 seed weight as observed in Trigonella foenum-graecum in the present study, was also reported [31-34]. *Vicia faba*, mungbean, *Oryza sativa*, *Hordeum vulgare*, *Triticum durum, Cicer arietinum*, *Vigna unguiculata*, *Cajanus cajan*, *Vigna mungo* and *Vigna radiate* [35-38]. The decrease in number of pods and 100 seed weight at higher concentrations of MMS as observed in *Lens culinaris* in the present study, was also reported by Verma et al. by gamma rays, Amer and Fara by carbamate pesticide in *Vicia faba*, Reddy and

Rao in *Capsicum annum* by herbicides, Lakshmi et al. in *Capsicum annum* by insecticides, Vandana and Dubey in *Vicia faba* by EMS and DES, and Kumar et al. in *Vicia faba* by gamma rays and DES. These reports confirmed the importance of induced mutagenesis in crop improvement. The decrease or increase in the number of pods and 100 seed weight occurred due to induced mutation in meiotic cycle which affected the frequency of normal microspores up to greater extent and the megaspore to a lesser extent and hence the fruit set was directly affected [39-41].

CONCLUSION

The induction of mutation has been accepted as a useful tool for increasing the productivity in the plant breeding programme. The success in plant improvement programmes, however, depends basically on controlling and directing the induced mutation process for the production of desired mutation. In the present investigation, mutagenic effect of maleic hydrazide (MH) was carried out on two varieties of *Trigonella foenum-graecum* namely var. CO1 and var. RMt-1. Fenugreek, being an economically and nutritionally important self-pollinating crop with narrow genetic base, induced mutagenesis provides much needed genetic variations for breeding and selecting mutants with higher yield and wider adaptability in addition to increase in nutritional parameters. It was observed in the present study that the induction of genetic variability using MH in the fenugreek varieties depends on the mutagen doses. The treatments were found to be appropriate for breeding objectives focused on productivity, considering the lower biological damages with higher rate of variation in quantitative traits and inducing unique morphological variations with higher rate in qualitative traits.

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