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Short Communication

Frequency and spectrum of induced mutations and mutagenic effectiveness and efficiency in fenugreek (*Trigonella foenum-graecum* L.)

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Fenugreek (*Trigonella foenum-graecum* L.) is an important legume as well as spice crop. A very limited attempt has been made on genetic improvement of crops like, fenugreek due to narrow genetic base present in the germplasm. A systematic and comparative study of mutagenic effectiveness and efficiency of gamma rays, ethyl methane sulphonate (EMS) and sodium azide (employed singly and in combination) based on frequency and spectrum of chlorophyll and macromutations in M_2 generation in fenugreek were under taken.

The three samples, each comprising 300 uniform, healthy and dry seeds (10-12% moisture) of fenugreek variety "RMt-1" were exposed to 40, 50 and 60 kR doses of gamma rays (source ^{60}Co) at IARI New Delhi. For chemical treatments, six samples, each comprising of 300 seed were pre-soacked in distilled water for 6hr. and then treated with ethyl methane sulphonate (0.45, 0.65 and 0.80% concentration) and sodium azide (0.80, 1.60 and 2.50 mM) for 6 hr. in freshly prepared 0.1 molar phosphate buffer (pH 7.0 and 3.5 respectively) at 25°C ± 1°C or at room temperature. In case of combinations, two samples, each comprising 300 seed were first irradiated with 40kR dose of gamma rays and then treated with 0.45% EMS and 0.80 mM sodium azide solution in the same manners as described above. The treated seeds were dibbed in 5 perecent aqueous solution of sodium thio sulphate for 10 minutes, to stop the action of mutagens. After that, treated samples were provide intermittent sacking throughout the period of treatment to maintain uniform concentration provi of mutagens and then washed thoroughly in running water for remove the traces of chemical from the seed surface. A total of 12 treaments including control (untreated seed of Rmt^{-1}) were immediately sown in RBD with three replications at the spacing of 10 × 30 cm. during *rabi* 1998-99 to raise M_1 generation. Each M_1 plants from each treatment were harvested in individualy, and M_2 progeny raised in separate row during *rabi* 1999-2000. Chlorophyll and macro mutations were scored and classified as per Singh *et al.* [1]. Mutation frequency was calculated as percentage of M_2 plants and mutagenic effectiveness and efficiency were also calculated as per Kharkwal [2, 3].

Table 1. Spectrum and frequency of induced chlorophyll mutations in M_2 generation in fenugreek

Treatments	Dose	Total plants observed	Per cent chlorophyll mutations				Total frequency
			Xantha	*Chlorina*	*Virescent*	Others	
Control	-	1400	-	-	-	-	-
Gamma rays	40kR	1204	0.24	-	-	-	0.24
	50kR	896	-	0.11	0.11	-	0.22
	60kR	712	-	-	0.14	-	0.14
EMS	0.45%	1110	0.18	-	0.18	-	0.36
	0.65%	789	-	-	-	0.13	0.13
0.80%	744	-	-	0.14	-	0.14	0.14
S. azide	0.80mM	993	-	-	0.10	0.10	0.20
1.65mM	744	-	-	0.13	-	0.13	0.13
	2.50mM	656	0.15	-	-	-	0.15
Combinations	40kR+0.45%%EMS	953	-	-	0.10	0.10	0.20
	40kR+0.80mM S. azide	820	-	-	0.12	-	0.12

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In the present study four types of chlorophyll mutations, *viz.*, *Xantha, Chlorina*, virescent and others were identified. Table 1 indicated that, lower concentration of mutagens were more effective in inducing greater frequency of chlorophyll mutations. The highest frequency of chlorophyll mutation (0.36%) was observed in 0.45% EMS concentration and in totality, the frequency of chlorophyll mutation were also high (0.63%) in EMS. Simillar results were reported in chickpea [2] and urdbean [1]. The combined treatments of gamma rays & EMS (40kR + 0.45% EMS) was also effecfive for this respect. The spectrums of macromutations were of six types, *viz.*, tendriller, bunchy, creeper, giant leaf, sterile flower and thin and elongated leaves (Table 2). The highest mutation frequency (0.97%) was observed in 0.45% EMS treatment followed by 40 kR gamma rays (0.83%). However, in over all, gamma-radiation proved to be most effective mutagens for inducing viable or macromutations than chemicals and the quantum of macromutations decreased with increase in concetration of mutagen.

Table 2. Spectrum and frequency of morphological mutations in fenugreek

Treatments	Dose	Total plants observed	% mutation frequency						Total mutation frequency
			tendriller	bunchy	creeper	giant leaf	sterile flower	thin and elongated leaves	
Control		1400	-	-	-	-	-	-	-
Gamma rays	40kR	1204	-	-	-	-	0.83	-	0.83
	50kR	896	-	-	0.21	-	0.11	-	0.32
	60kR	712	0.14	-	-	-	0.14	-	0.28
EMS	0.45%	1110	0.09	0.45	-	0.17	0.27	0.09	0.97
	0.65%	789	-	-	0.18	-	-	-	0.18
	0.80%	744	-	-	-	0.13	-	-	0.13
S. azide	0.80mM	993	-	0.10	-	0.20	-	-	0.30
	1.65mM	744	0.13	-	-	-	0.13	-	0.26
	2.50mM	656	-	0.15	-	-	-	-	0.15
Combinations	40kR+0.45%EMS	953	-	0.10	-	-	-	0.10	0.20
	40kR+0.80mM S. azide	820	-	-	-	0.12	0.12	-	0.24

Table 3. Mutagenic effectiveness and efficiency of physical and chemical mutagen in fenugreek

Treatment	Percentage of M_2 family segregating for mutation (Mf)	Pollen sterility percentage in M_1(S)	Mutagenic effectiveness Mf/t.c.	Mutagenic efficiency Mf/S
Gamma rays				
40kR	6.0	18.3	0.15	0.33
50kR	6.0	20.0	0.12	0.30
60kR	6.0	25.3	1.00 (0.12)	0.24 (0.29)
Ethyl methane sulphonate				
0.45%	12.0	25.3	4.44	0.47
0.65%	4.0	31.7	1.02	0.13
0.80%	4.0	29.3	0.83 (2.10)	0.14 (0.24)
Sodium azide				
0.80mM	6.0	30.3	192.3	0.20
1.65mM	4.0	31.0	64.10	0.13
2.50mM	6.0	25.3	61.54 (106.0)	0.24 (0.19)
Combinations				
40kR+ 0.45% EMS	4.0	27.3	0.04	0.15
40kR+0.80mM S.azide	6.0	29.3	4.81	0.20

The data presented in Table 3 indicated that, effectiveness of treatment 0. 80 mM sodium azide was found most effective whereas, 0.45 per cent EMS found most efficient as compare to other treatments. Overall, higher effectiveness and efficiency of mutagens were recorded in sodium azide and gamma rays, respectively. In general, effectiveness and efficiency decreases with the increase in doses or concentration of mutagens. Simillar, results were recorded in chickpea [3], lentil [4]. The effectiveness and efficiency showed differential behaviour in order to their relationship with mutation rate (positive relation in physical mutagen i.e. gamma rays and negative relation in chemical mutagens i.e. EMS and sodium azide).

References

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