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REGULAR ARTICLE

COMPARISON OF INDUCED CHLOROPHYLL MUTATIONS AND SPECTRUM IN TWO VARIETIES OF *TRIGONELLA FOENUM- GRAECUM* L.

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SUMMARY

The present study was carried out to compare the induced chlorophyll mutations and spectrum in two varieties of *Trigonella foenum-graecum* L. viz., desi and kasuri methi. It was observed that the highest chlorophyll mutation frequency was obtained with 0.3% MES & highest chlorophyll spectrum with 0.3% MMS in desi methi. The highest chlorophyll mutation frequency was obtained with 0.3% EMS & chlorophyll mutation spectrum with MMS in kasuri methi, but the mutation spectrum was broader in desi methi as compared to kasuri methi.

Key words: Mutation, EMS, MMS, MES, *Trigonella foenum-graecum* L.

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1. Introduction

The *Trigonella foenum-graecum* L. is cultivated as a multipurpose annual autogamous crop of central India belongs to family Fabaceae. It is used as leafy vegetable as well as fodder also. Seeds are also used as spices in cooking and medicinal purposes. There are many physical and chemical agents used now a days to increase yield of the crop or to develop high yielding crop cultivars. Among the various agents radiomimetic agents are important one. These agents modify the bases or phosphates by alkylating them. These radiomimetic agents have bi-functional alkyl reactive groups that react with DNA, causes extensive cross linkage of DNA, chromosome breakage, chromosome mutations and gene mutation. Different such types of radiomimetic agents are tested by various workers time to time on various crops. The higher frequencies of chlorophyll and other viable mutations are obtained in treatments with chemical mutagens than radiations, Blixt *et al* (1958). High frequency of chlorophyll and morphological mutations in emmer wheat (*Triticum dicoccum* var. Khapli; 2n = 28) was observed by Swaminathan *et al* (1962). Chopra and Swaminathan (1967) observed higher chlorophyll and viable mutation frequency in M_2 under EMS treatment during the comparative study of EMS, hydroxylamine and their combination. Similarly, Jacob (1970) studied the comparative mutagenic effects of alkylating agents and gamma rays, and observed that EMS induced highest chlorophyll mutation frequency as comparison to MMS, MNG, BMS and gamma rays. The following order of efficiency of various mutagens recorded was EMS > MNG > MMS > gamma rays > BMS in the case of *Arabidopsis thaliana*. Khan (1988) studied the effect of gamma rays and EMS in single and combination treatments on frequency and spectrum of chlorophyll mutations in M_2. Effect of diethyl sulphate on chickpea, *Cicer arietinum* (Samiullah and Wani 2005) and genetic variability for frequency and spectrum of chlorophyll mutations on recombinant of desi x kabuli chickpea introgression genotype (Shah *et al.* 2006) have also been reported. Vasu and Hasan (2009) were studied the effect of radiomimetic agents like MES, EMS and MMS in *Trigonella* to induce plant height and pod number. The present investigations were undertaken to compare the induced

mutation frequency & spectrum in two varieties of *Trigonella* viz., desi and kasuri methi. (*Trigonella foenum-graecum* L.).

2. Material and Methods

Seeds of Two varieties of *Trigonella foenum-graecum* L. *viz.* desi methi and kasuri methi were procured from Jawahar Lal Agriculture farm Eintkhedi, Berasia Road, Bhopal (M.P.). Three radiomimetic agents (mutagens) EMS, MMS and MES, were used. Three concentrations of each mutagen *i.e.*, 0.1%, 0.2% and 0.3% were selected on the basis of preliminary experiment, LD-50 dose.

Fully mature and healthy seeds of uniform size free from mould and mechanical injury were selected for different concentration of mutagenic treatment. To determine the effective range of mutagens pilot experiment were conducted in preceding year with the two varieties, *Desi methi* and *Kasuri methi* by way of employing wide dose range. Period of presoaking the seeds making them vulnerable to the action of different mutagens was also ascertained through preliminary experiments.

Data collected in M_2 generations were subjected to statistical analysis to assist the extent of individual variations. The mutation frequency was calculated with help of following formula.

(i) M_2 family basis (% of mutated progenies in M_2 generation)

$$\text{Mutation Frequency} = \frac{\text{Number of mutated progenies}}{\text{Total number of M2 progenies}} \times 100$$

(*ii*) M_2 population basis (% mutants)

$$\text{Mutation Frequency} = \frac{\text{Number of mutated plants}}{\text{Total number of } M_2 \text{ plants}} \times 100$$

Mutation frequency and spectrum in M_2 generations

3. Result and Discussion

In present investigation, it was observed that the induced viable mutation spectrum was broader in variety *Desi methi* in comparison of variety *Kasuri methi*. Mutation frequencies and spectrum were estimated in M_2 populations on the basis of chlorophyll distributions abnormalities and other viable mutations.

In the present course of investigations, it was observed that the mutation induced by three radiomimetic agents were higher in *Desi methi* in comparison of *Kasuri methi*. Thus variety *Desi methi* was proved more sensitive to the effect of radiomimetic agents (EMS, MMS and MES) than variety *Kasuri methi*. Among three radiomimetic agents tested, EMS caused more mutations in comparison to MMS and MES in variety *Desi methi* than in variety *Kasuri methi*.

Chlorophyll mutation frequencies and spectrum in variety *Desi methi* are given in (Table-1 and Table-3). Highest chlorophyll mutation frequency was obtained with 0.3% MES treatment which was 35% while the lowest (22.36%) was recorded with 0.1% EMS (Table-1). In this variety highest chlorophyll spectrum was observed with 0.3% MMS and lowest with 0.1% MES (Table-3). For both the varieties, EMS and MMS have been found to be a potent mutagen which induced highest chlorophyll mutation frequency followed by MES.

Chlorophyll mutation frequency and spectrum in variety Kasuri methi are summarized in (Table-2 and Table-4). Highest frequency (37.93%) was observed under 0.3% EMS treatment while lowest under 0.1% MMS, which was 19.11% (Table-2). Highest chlorophyll mutation spectrum in variety Kasuri methi was observed with 0.3% MMS and lowest was found with 0.1% MES (Table-4).

Table 1. Frequency of Chlorophyll mutation in M_2 generation of *Trigonella foenum-graecum* L . (*Desi methi*) with the treatment of Radiomimetic agents EMS (Ethyl Methane Sulphonate), MMS (Methyl Methane Sulphonate) and MES (Methyl Ethane Sulphonate)

S. No.	Radiomimetic agents	Doses (%)	Number of M_2 progenies	Number of M_2 plants	Chlorophyll Mutations			
					Number of families segregating	Number of Mutations	Frequency % of segregating M_2 families	% of mutations in M_2 population
1.	–	Control	97	1924	–	–	–	–
2.		0.1	76	1352	17	82	22.36	6.06
3.	EMS	0.2	65	1290	21	91	27.63	7.05
4.		0.3	60	1050	20	98	33.33	9.33
5.		0.1	70	1210	16	99	22.85	8.18
6.	MMS	0.2	65	1030	17	102	26.15	9.90
7.		0.3	55	940	13	105	23.60	11.17
8.		0.1	74	1370	18	78	24.32	5.69
9.	MES	0.2	65	1190	20	81	30.76	6.80
10.		0.3	60	1041	21	102	35.00	9.79

Table 2. Frequency of Chlorophyll mutation in M_2 generation of *Trigonella foenum-graecum* L . (*Kasuri methi*) with the treatments of Radiomimetic agents EMS (Ethyl Methane Sulphonate), MMS (Methyl Methane Sulphonate) and MES (Methyl Ethane Sulphonate)

S. No.	Radiomimetic agents	Doses (%)	Number of M_2 progenies	Number of M_2 plants	Chlorophyll Mutations			
					Number of families segregating	Number of mutated progenies	Frequency % of segregating M_1 families	% of mutation in M_2 population
1.	–	Control	95	1898	–	–	–	–
2.		0.1	75	1292	16	80	21.33	6.19
3.	EMS	0.2	65	1182	20	92	30.76	7.78
4.		0.3	58	1038	22	102	37.93	9.82
5.		0.1	68	1021	13	91	19.11	8.91
6.	MMS	0.2	61	989	17	99	27.86	10.01
7.		0.3	52	978	18	105	34.61	10.73
8.		0.1	78	1198	17	79	21.79	6.59
9.	MES	0.2	64	1090	19	91	29.68	8.34
10.		0.3	62	1013	21	97	33.87	9.57

From both varieties four types of chlorophyll mutations *i.e.*, Albina, striata, xanthoalba and chlorina were isolated in the following order of relative proportion i.e. Xanthoalba > Straita > Albina > Chlorina (Table 3 & 4). Along with chlorophyll mutation frequencies the rate of morphological mutations also increased in both varieties with the increase in dose of mutagens which is quite in conformity with results of other workers on other crops. Bozzini and Scarascia Muncozza (1970) studied the relative frequency of chlorophyll to morphological and sterility mutations

induced in durum wheat by radiation and chemicals. Rai and Das (1975) studied gamma ray induced chlorophyll mutation in linseed, Kar et al (1995) in *Sesamum indicum* L.; Yaqoob and Rasheed (2001); Singh and Singh (2007) in mungbean (*Vigna radiata*) L.(Wilczek).

Result of the present work revealed that variety Desi methi is more mutable, than Kasuri methi as comparatively higher mutation frequency was obtained in variety Desi methi. Highest mutation frequency was recorded with MMS and EMS treatments. The data obtained suggested that induced mutability is governed by the genetic architecture of the material used. Gustafsson (1947) reported that varieties of spring barley of non-hybrid origin were less mutable than the hybrid ones. Later a genotypic control of mutation process was reported by several workers, Davies (1962) in *Lycospersicum* and *Melandrium*, Swaminathan et al. (1968) in wheat; Mandal (1974) in gram; Choudhary (1978) in wheat. Enkan (1967) reported that the frequency and spectrum of mutation is largely affected by the genotype of a variety. He further observed that as closer the genotype of varieties more similarities are likely to be found in the frequency and spectrum of induced-mutations.

Table 3. Relative frequency and spectrum of different types of Chlorophyll mutation in M_2 generation of *Trigonella foenum-graecum* L. (*Desi methi*) treated with Radiomimetic agents EMS (Ethyl Methane Sulphonate), MMS (Methyl Methane Sulphonate) and MES (Methyl Ethane Sulphonate)

S. No.	Types of Chlorophyll mutations	EMS					
		0.1 %		0.2 %		0.3 %	
		Total	%	Total	%	Total	%
1.	Albina	00	00	00	00	04	5.33
2.	Straita	08	18.18	09	20.00	18	24.00
3.	Xaithoalba	13	29.54	15	33.33	19	25.33
4.	Chlorina	09	20.45	12	26.66	21	28.00
5.	Others	14	31.81	09	20.00	13	17.33
	Grand Total	44		45		75	
S. No.	Types of Chlorophyll mutations	MMS					
		0.1 %		0.2 %		0.3 %	
		Total	%	Total	%	Total	%
1.	Albina	00	00	02	4.16	10	11.76
2.	Straita	06	17.64	08	16.66	14	16.47
3.	Xaithoalba	09	26.47	12	25.00	19	22.35
4.	Chlorina	11	32.35	16	33.33	24	28.23
5.	Others	08	23.52	10	20.83	18	21.17
	Grand Total	34		48		85	
S. No.	Types of Chlorophyll mutations	MES					
		0.1 %		0.2 %		0.3 %	
		Total	%	Total	%	Total	%
1.	Albina	00	00	04	9.52	08	12.12
2.	Straita	04	15.38	09	21.42	13	19.69
3.	Xaithoalba	05	19.23	11	26.19	15	22.72
4.	Chlorina	09	34.61	13	30.95	18	27.27
5.	Others	08	30.76	05	11.90	12	18.18
	Grand Total	26		42		66	

Table 4. Relative frequency and Spectrum of different types of Chlorophyll mutation in M_2 generation of *Trigonella foenum graecum* L. (Kasuri methi) treated with Radiomimetic agents EMS (Ethyl Methane Sulphonate), MMS (Methyl Methane Sulphonate) and MES (Methyl Ethane Sulphonate)

S. No.	Types of Chlorophyll mutations	EMS					
		0.1 %		0.2 %		0.3 %	
		Total	%	Total	%	Total	%
1.	Albina	00	00	00	00	01	1.69
2.	Straita	07	18.42	07	17.50	12	20.33
3.	Xaithoalba	11	28.94	13	32.50	15	25.42
4.	Chlorina	08	21.05	14	35.00	16	27.11
5.	Others	12	31.57	06	15.00	15	25.42
	Grand Total	38		40		59	
S. No.	Types of Chlorophyll mutations	MMS					
		0.1 %		0.2 %		0.3 %	
		Total	%	Total	%	Total	%
1.	Albina	00	00	02	4.65	05	6.84
2.	Straita	07	19.44	08	18.60	13	17.80
3.	Xaithoalba	10	27.77	10	23.25	18	24.65
4.	Chlorina	12	33.33	14	32.55	21	28.76
5.	Others	07	19.44	09	20.93	16	21.91
	Grand Total	36		43		73	
S. No.	Types of Chlorophyll mutations	MES					
		0.1 %		0.2 %		0.3 %	
		Total	%	Total	%	Total	%
1.	Albina	00	00	00	00	03	5.17
2.	Straita	06	20.68	08	22.22	12	20.68
3.	Xaithoalba	07	24.13	11	30.55	16	27.58
4.	Chlorina	10	34.48	12	33.33	17	29.31
5.	Others	06	20.68	05	13.88	10	17.24
	Grand Total	29		36		58	

Four types of chlorophyll mutations were detected in present investigation. The induction of similar type of mutations in both varieties provides an excellent example of parallelism regarding genetic variability in the two varieties of *Trigonella foenum-graecum* L. as suggested earlier Vavilov (1935). Similar results were reported by Nayar (1969), in *Sesamum*, Marki and Bianu (1970) in flax; Lysshenko and Ulitcheva (1971) in sunflower; Hussein *et al*. (1974) in Peas and Tsukuda *et al*. (1977) in rice. Recently, Bhosle and Kothekar (2010) studied the Mutagenic effectiveness and efficiency of EMS, SA and Gamma rays on two varieties of cluster bean (GE-36 and HR) and found that SA proved to be effective in two varieties and EMS proved to be more efficient.

4. Conclusion

Marked varietal differences were present in the expression of induction of chlorophyll mutations at different doses/concentrations of mutagens due to genetic differences existing among the two varieties. Among the mutagens, highest chlorophyll mutation frequency was obtained with 0.3% MES & highest chlorophyll spectrum with 0.3% MMS in desi methi. The highest chlorophyll mutation frequency was obtained with 0.3% EMS & chlorophyll mutation spectrum with MMS in kasuri methi, but the mutation spectrum was broader in desi methi as compared to kasuri methi. So, EMS (Ethyl Methane Sulphonate), MMS (Methyl Methane Sulphonate) and MES (Methyl Ethane Sulphonate) doses induced reasonable chlorophyll mutations, hence all

these treatments could be used in mutation breeding programs for inducing viable mutations.

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