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Effect of Short Duration EMS Treatments on the Frequency and Spectrum of Induced Mutations in Greengram*

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ABSTRACT

Two greengram (*Vigna radiata.* (Wilezek) varieties, viz. DT. 9-E and TT. 9-E were treated with 0.25 % EMS for a period of one hour under reduced atmospheric pressure, after pre-soaking the seeds from 11.00 to 13.00 hours at halff an hour intervals. The M_1, M_2 and M_3 generations were grown in the field and macro-mutants picked out in the latter two generations. The M_2 generation gave higher frequency and spectrum of mutations than M_3. Wider spectrum of chlorophyll, foliar and agronomic mutations occurred in treatments after different periods of pre-soaking showing time dependance of different loci for replication. EMS induced more of agronomic mutations than chlorophyll and foliar mutations. Maturity in greengram seems to be controlled by two groups of genes, one group tending towards earliness and the other towards lateness.

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INTRODUCTION

Possibility of increasing mutation frequency in greengram by EMS treatment was reported by Santos (1969). Pre-soaking seeds before mutagenic treatment was found to be very effective in increasing mutation frequency (Blixt, 1967; Sevin *et al.* 1968; Delgado de la Flor, 1972; Sahoo and Samolo, 1973 and Brunner, 1977). Swaminathan and Sharma (1968) and Singh and Singh (1977) gave short duration chemical mutagen treatments on barley and reported an increase in the spectrum of mutations. They also observed time dependance of certain loci for expression of mutant characters. Samolo (1971) gave short duration chemical mutagen treatment on wheat and observed time dependence of certain mutant characters for their expression and widening of spectrum of mutations. The present investigation in greengram was taken up to find out the effect of short duration treatments with EMS on the frequency and spectrum of mutations in M_2 and M_3 generations.

MATERIAL AND METHODS

Seeds of greengram varieties DT. 9-E and TT. 9-E were pre-soaked in distilled water for different durations from 11.00–13.00 hours at half an hour

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intervals before EMS treatment. Excess moisture from the seeds was blotted out before treating the seeds with 0.25% EMS at 27° C for one hour under reduced atmospheric pressure. The control was pre-soaked for 12.00 hours and was also kept under the same reduced pressure. After treatment, the seeds were thoroughly washed under running tap water for one hour and then sown in the field. Seeds from this generation (M_1) was harvested plantwise and were grown on plant to row basis in the M_2 generation. The cholorophyll, foliar and agronomic mutations were picked out from this generation. The seeds from M_2 plants (excluding macro-mutants) were bulked line-wise to grow the M_3 generation. The M_3 lines were also scored for mutations to know the trend of spectra in later generations. The frequency and spectrum of both the varieties have been considered together for the comparison of M_2 with M_3 generation.

RESULTS AND DISCUSSION

There was a gradual rise in mutation frequency from 11.00 to 12.30 hours of pre-soaking in M_2 generation and upto 13.00 hours in M_3 generation (Table 1). The peak in mutation frequency observed in the treatment after 12.30–13.00 hours of pre-soaking indicates that majority of loci in majority of cells replicate at that time. This provides more scope for the mutagen to act upon more number of loci. Savin *et al.* (1968) observed high mutation frequency in barley after 16 hours of pre-soaking and interpreted that this is due to synchronization of treatment time with S-phase of DNA synthesis. Sahoo and Samolo (1973) from long duration treatment studies suspected the mid S-phase in greengram perhaps correspond to a period around 12 hours of pre-soaking. On similar analogy this study pin points to the period of 12.30–13.00 hours after pre-soaking to correspond to the mid-synthetic phase.

The frequency of mutations observed in M_3 generation was less than that in M_2 generation in all pre-soaking hours. The occurrence of mutations in the M_3 generation indicates delayed effects of EMS. This might also be due to more number of genes controlling a character which gets expression in later generation. Lusina (1970) observed 12.5% decrease in chlorophyll mutations in M_3 generation of peas.

The occurrence of more agronomic mutations than chlorophyll and foliar mutations in the M_2 generation clearly indicates that EMS was an effective mutagen for increasing frequency of beneficial mutations (Table 1). Chlorophyll mutations had wider spectrum after 11.30 hours of pre-soaking, whereas, the foliar mutations had wider spectrum after 12.30 hours of pre-soaking in this generation. On the other hand the agronomic mutations had wider spectrum after 12.00 hours of pre-soaking. The effect of the mutagen is perhaps time dependent for different loci. This is due to differences in the replication time of different groups of loci. Similar results were reported by Swaminathan and Sharma (1968) in barley and Samolo (1971) in wheat.

In the M_3 generation also such difference existed. The widest spectrum of chlorophyll mutations occurred after 12.30 hours, foliar mutations after 13.00 hours and agronomic mutations after 12.00 hours of pre-soaking. This change

TABLE 1

Spectrum and frequency of mutations in M_2 and M_3 generations (in percentage).

Type of Mutations	*M_2 Generation*					*M_3 Generation*				
	Hours of pre-soaking before EMS treatment					*Hours of pre-soaking before EMS treatment*				
	11.00	*11.30*	*12.00*	*12.30*	*13.00*	*11.00*	*11.30*	*12.00*	*12.30*	*13.00*
Chlorophyll										
Xantha	0.05	0.16	0.06	0.17	—	—	0.14	0.14	0.09	—
Chlorina	0.29	0.59	0.25	0.17	0.49	0.27	0.32	0.35	0.62	0.65
Pale green	2.10	2.26	2.23	2.45	1.70	1.21	1.44	1.43	2.24	1.84
Viridis	0.49	0.43	0.70	0.61	0.49	0.34	0.25	0.38	0.26	0.65
Sectorial chimaera	0.93	0.81	0.64	0.79	0.97	0.30	0.22	0.35	0.31	0.28
Total (Chlorophyll)	3.86	4.25	3.88	4.19	3.65	2.12	2.37	2.65	3.52	3.42
Foliar										
Mono-cotyledonary	0.34	0.38	0.51	0.09	—	0.23	0.29	0.07	0.04	—
Tri-cotyledonary	—	—	0.32	0.35	0.49	—	—	0.35	0.44	0.46
Uni-foliata	—	0.22	0.06	0.26	—	—	0.32	0.14	0.22	—
Bi-foliata	0.59	0.75	0.32	0.44	0.97	0.57	0.68	0.66	0.53	0.18
Quadri-foliata	0.93	0.22	0.70	1.57	1.70	1.21	1.04	1.29	1.23	1.94
Penta-foliata	0.20	0.11	—	0.35	—	0.38	0.39	0.52	0.26	0.09
Lobed-loaf	0.20	0.48	0.38	0.35	0.24	0.23	0.18	0.28	0.18	0.37
Obtuse apex	0.39	0.38	0.51	0.52	0.24	0.19	0.04	0.10	0.22	0.28
Waxy leaves	0.98	1.02	1.02	1.40	0.49	0.38	0.25	0.38	0.48	0.65
Total (Foliar)	3.63	3.56	3.82	5.33	4.13	3.19	3.19	3.79	3.60	3.97
Agronomic										
Late	1.56	2.20	1.65	1.48	0.97	0.95	1.40	1.60	1.23	0.92
Early	1.42	1.72	1.91	1.48	2.18	0.61	0.75	0.73	0.66	0.92
Tall	0.68	0.86	1.15	1.14	0.73	0.19	0.22	0.28	0.22	0.37
Dwarf	0.34	0.59	0.83	0.87	0.49	0.30	0.32	0.28	0.40	0.09
Terminal inflorescence	0.29	0.48	0.70	0.52	0.77	0.15	0.32	0.31	0.18	0.28
More branched	0.20	0.48	0.57	0.17	—	0.23	0.11	0.17	0.26	0.18
Long podded	0.15	0.22	0.32	0.17	0.24	0.11	0.07	0.03	0.04	—
Short podded	0.15	0.16	0.06	0.35	—	0.11	—	0.07	0.09	0.09
More podded	0.05	0.11	—	—	—	0.04	0.04	0.03	0.09	0.09
Bold podded	0.10	—	0.19	0.26	—	0.11	0.11	0.03	0.04	—
Fodder type	—	0.11	0.06	—	0.24	0.04	0.04	0.03	—	—
Male sterile	—	0.05	0.13	0.52	0.73	—	0.07	0.14	0.18	0.46
Total (Agronomic)	4.94	6.98	7.57	6.96	6.55	2.84	3.45	3.70	3.39	3.40
Mutation frequency	12.43	14.79	15.27	16.48	14.33	8.15	9.01	10.14	10.51	10.79
Total plants scored	2048	1860	1571	1145	412	2639	2786	2874	2276	1085

in the timing of chlorophyll and foliar mutations may be due to delayed effects of EMS.

The palegreen, quadrifoliata, late, early, tall and dwarf mutants had wider spectrum of mutations in both the generations. These are semi-quantitative characters and are perhaps affected to different degrees and get expressed in different generations. It is also interesting to note that tri-cotyledonary mutations appeared only in treatments after 12.00 to 13.00 hours pre-soakings, whereas, uni-foliata mutatns appeared only in 11.30 to 12.30 hours of pre-soaking. The genes controlling these characters were perhaps affected in the said particular pre-soakings. Singh and Singh (1977) reported occurrence of particular chlorophyll mutations in particular pre-soaking periods in barley.

Widest spectrum of early mutants occurred after 13.00 hours of pre-soaking, whereas, the same for late maturing mutants was observed in the treatment after 11.30 and 12.00 hours of pre-soaking in both the generations. A time gap between these two mutation peaks indicate that genes controlling maturity character were affected by the mutagen at two different times. Thus it can be suspected that maturity in greengram is controlled by two groups of genes, one group tending towards earliness and the other towards lateness.

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