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Effect of Selfing in M_1 on the Realization of Mutation Frequency in M_2 and M_3 Generations in Greengram.

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Three greengram cultures viz. TT9-E, DT 9-E and DT 12-E were treated with 0.25% EMS for one hour under reduced pressure, after different periods of pre-soakings. The mutation frequency was recorded in M_2 and M_3 generations in selfed and unselfed progenies. The peak of mutation frequency occurred in the treatment after 12.30 hours of pre-soaking in the M_2 generation. The selfed progenies had higher mutation frequencies than unselfed progenies. The lower mutation frequencies in unselfed progenies indicated definite occurrence of certain amount of outcrossing in the M_1 generation due to induction of M_1 pollen sterility. Even in a self pollinated crop like greengram upto 2.63% mutants could not get expression due to induction of pollen sterility and outcrossing.

Seed treatment with mutagens induce pollen sterility in M_1 to certain extent in different crop plants. Sahoo and Samolo (1973) treated greengram with EMS and reported induction of 26.1% pollen sterility. Heringa (1964) reported a sterility of 70-80% in EMS treated populations of peas. Induction of pollen sterility is expected to favour cross-pollination and thus vitiate the frequency of observed mutations. As there is no data collected yet to show the differences in expression fo mutations in selfed and unselfed progenies, the present investigation was taken up in greengram using EMS as the treatment chemical.

METERIAL AND METHODS

Seeds of three greengram (*Vigna radiata* (L.) Wilczek) cultures, viz. TT 9-E, DT 9-E and DT 12-E, were pre-soaked in water for 11·00 to 13.00 hours at half an hour intervals before treatment with 0.25% EMS for one hour under reduced pressure. Seeds were thoroughly washed in water before sowing in field. Flower buds from the first inflorescence of 5 random plants were collected in each treatment and about 1000 pollen grains were studied for pollen sterility. The second inflorescence from each M_1 plant was bagged in butter paper to ensure self pollination. The pods of selfed and unselfed inflorescences were harvested separately. Those seeds were grown in plant-to-row basis in M_2 generation. Different chlorophyll, foliar and agronomic mutations were scored both in selfed and unselfed progenies in this generation All the chlorophyll and viable mutations (foliar and agronomic) were together considered for mutation frequency Seeds from M_2 plants (excluding macro-mutants) were bulked linewise to grow the progenies of selfed and unselfed populations in M_3 generation.

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In M_2 also, different mutants were scored as was done in M_2 generation. The paired 't' test was utilised for testing the differences between the frequency of mutations in the selfed and unselfed progenies in M_2 and M_3 generations.

RESULTS AND DISCUSSION

The M_1 Pollen sterility in treated populations varied from 4.39 to 14.94% (Table-1). An increase in pollen sterility in treatments from 11.00 to 12.30 hours of pre-soaking was found to be associated with an increase in M_2 mutation frequency. Such association of sterility with mutation frequency was reported in barley (Ehrenberg *et al.*, 1961; Kunzel and Scholz, 1971 and Arnasan and Satpathy, 1971) and greengram (Sahoo and Samolo, 1973).

The mutation frequency at different pre-soaking hours followed the same trend in selfed and unselfed progenies in M_2 and M_3 generations. The occurrence of a peak in mutation frequency in the treatment after 12.30 hours of pre-soaking indicates that majority of loci in a large proportion of cells perhaps replicate at that time. Hence 12.30 hours of pre-soaking before mutagenic treatment in greengram is the best for increasing the frequency of mutations.

A comparision of M_2 mutation frequency in selfed and unselfed progenies (Table-1) clearly shows that selfed progenies invariably have higher frequencies than unselfed progenies. DT 9-E scored the highest average frequency of 17.63% mutations followed by 14.94% in DT 12-E and 13.52% in TT 9-E in selfed progenies. Similar was the trend in unselfed progenies but with lower frequencies of mutation. The mutation frequencies in unselfed progenies in different treatments were 0.62 to 2.63% lower than the frequencies in selfed progenies. The differences were significant at 1% level in all the three cultures. This indicates that there is a definite reduction in the recovery of mutations in unselfed progenies due to natural outcrossing in M_1 generation.

In the M_3 generation also the progenies of selfed populations had higher mutation frequencies than the progenies of unselfed population. But the differences were less than those in M_2 generation. The differences were significant at 1% level in the culture TT 9-E, at 5% level in DT 9-E and nonsignificant in DT 12-E. The narrowing of differences between selfed and unselfed M_3 families may be due to natural outcrossing in M_2 generation where, selfing was not practised. This confirms the above finding that more of outcrossing in the M_1 generation if not selfed, causes reduction in realization of mutants.

Thus the lower mutation frequency in unselfed progenies can be interpreted to be because of masking of some mutated genes by normal dominant genes due to outcrossing in the EMS treated populations. So artificial selfing of M_1 plants is essential, even in a predominantly self pollinated crop like greengram, for proper evaluation and complete realization of mutation frequency.

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TABLE-1 M_1 pollen sterility and M_2 and M_3 mutation frequency in selfed and unselfed unselfed progenies of greengram

Name of the culture	Pre-soaking before treatment (hours)	M_1 pollen sterility (%)	M_2 Mutation frequency (%)			M_3 mutation frequency (%)		
			selfed	Unselfed	Difference	Selfed	Unselfed	Difference
TT 9-5	11.00	6.59	11.83	10.33	1.59	11.20	10.53	0.67
	11.30	7.81	12.65	12.03	0.62	12.28	11.81	0.47
	12.00	7.26	12.93	11.83	1.10	12.83	11.75	1.08
	12.30	12.18	15.12	14.43	0.69	12.73	11.64	1.09
	13.00	14.94	15.00	12.77	2.23	12.43	11.84	0.59
	Average	9.75	13.52	12.27	1.246**	12.29	11.51	0.780**
	S. E.				0.301			0.129
DT 9-2	11.00	4.39	15.74	14.24	1.50	8.40	8.14	0.28
	11.30	9.51	17.42	15.21	2.21	8.18	8.92	0.26
	12.00	10.76	17.33	16.10	1.23	7.67	7.50	0.17
	12.30	10.67	20.00	18.53	1.47	9.45	8.38	1.07
	13.00	9.32	17.67	16.39	1.28	8.80	8.24	0.56
	Average	9.13	17.53	16.09	1.538**	7.58	7.94	0.464**
	S. E.				0.176			0.165
DT 12-5	11.00	5.44	13.04	12.16	0.98	8.13	8.31	-0.18
	11.30	8.28	14.29	12.50	1.79	7.25	7.14	0.11
	12.00	10.18	16.92	14.29	2.63	9.09	8.41	0.68
	12.30	11.95	16.67	15.25	1.42	8.47	8.11	0.36
	13.00	14.07	13.79	12.33	1.46	7.23	6.10	1.13
	Average	9.98	14.94	13.31	1.636**	7.43	7.01	0.426**
	S. E.				0.288			0.227

** - Significant at 5% and 1% level respectively