Research Article

A Study on EMS and Gamma Mutagenesis of Clusterbean [*Cyamopsis tetragonoloba* (L.) Taub]

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Abstract

Dry and pure seeds of two clusterbean cultivars (RGC 936 and HGS 365) were treated with gamma rays (10, 20, 30 and 40 kR) and EMS (0.1, 0.2, 0.3 and 0.4 per cent). Gradual reduction in germination and subsequent survival of the treated population were observed with the increased mutagen dose in both cultivars. Four different types of morphological mutants were observed with the maximum mutation frequency of spreading types in case of higher doses of gamma rays. Positive association was observed between mutagen dose and mutation frequency of morphological traits in M_2 generation. Three different types of chlorophyll mutant viz. *chlorina*, *xantha* and *albina* were observed: chlorophyll mutants induced with gamma rays had higher frequency than EMS induced mutants. Fifty best performing progenies, identified in M_3 on the basis of earliness and higher seed yield, were further tested and fifteen of them were selected in M_4 generation.

Introduction

Clusterbean (*Cyamopsis tetragonoloba* L. Taub) is a main arid legume crop, traditionally grown as a system sustaining crop under poorly endowed conditions with minimal management. It is grown over an area of 3.0 million hectares in India, mostly in the northwestern parts of the country, such as the states of Rajasthan (80% of the total acreage), Haryana and Gujarat. However, it is of great concern that the total area of cultivation and productivity levels have been fluctuating over the years (area: 1.5-3.0 million hectare and productivity: 50-500 kg), since it is grown as a rainfed crop and the distribution of rainfall (less than 100 mm to more than 500 mm) is so erratic and uneven in this region.

Mutagenesis is rewarding in situations where naturally existing genetic variability is low (either over all diversity or variability for specific traits) and where simply inherited defects need to be rectified in an otherwise agronomically superior cultivar (Chopra and Sharma, 1985). Mutation breeding is a potentially powerful tool for clusterbean improvement, since it has very limited exploitable and useful genetic variation. The creation of variability through hybridization is very difficult and cumbersome owing to very small and delicate flower structures, which often result in very poor seeds setting in the manually hybridized buds and higher frequency of flower drop during and after crossing (Arora and Pahuja, 2008). There is also limited variability for characters of economic importance in existing cultivars. In this study, chemical and physical mutagenesis was employed for creation of variability in two widely grown clusterbean cultivars.

Materials and methods

Mutagenic treatment

The seeds of two clusterbean cultivars, RGC 936 and HG 365, were treated with gamma rays and ethyl methane sulphonate (EMS). Gamma irradiation was made for dry seeds at doses of 10, 20, 30 and 40 kR in the Indian Agricultural Research Institute, New Delhi, with a ^{60}Co source. The EMS treatment was performed in freshly prepared phosphate buffer (pH 7.0) after pre-soaking the seeds for 6 h at doses of 0.1, 0.2, 0.3 and 0.4 per cent for 6 h followed by post-treatment washing of 1 h under gentle flow of running tap water.

Handling of M_1 and M_2 plants

The data on germination of M_1 seeds and subsequent survival of the M_1 plants till maturity were recorded. M_1 plants were individually harvested to raise the M_2 generation following the plant to row method. The M_2 generation was screened for morphological and chlorophyll mutations. Chlorophylle mutants were determined and grouped by their type in M_2 generation using classifications of Priilin *et al.,* (1976). Growth pattern of M_2 plants were compared with their respective parental cultivars for the selection of morphological mutations, i.e. spreading, dwarf, mating and vigorous plant types, together with agronomic and yield related mutants for early maturity, higher yield, more number of branches, clusters and pods, plant height, number of seeds per pod and pod length. Mutation frequency was calculated on the basis of 100 M_2 plant rows according to Gaul's (1964) method. M_2 plants with superior performance were selected and their performances were assessed in M_3 and M_4 generations.

Assessment of M_3 and M_4 lines

Fifty M_3 progenies were selected on the basis of their *per se* performance for certain characteristics of economic importance. In M_4 generation the 50 M_3 progenies were further evaluated to assess the stability of the promising mutants. These progenies were sown in 4 rows of 4 m length each spaced 30 cm apart along with checks.

Proper agronomic practices were followed for raising a health crop in all the generations (M_1 M_4) and grown strictly under rainfed conditions. The observations were recorded on 5 randomly selected plants in each progeny. The statistical analysis was performed as per the standard statistical procedures.

Results

Effect of mutagenic treatment on germination and survival

In M_1 generation the initial damage caused by the mutagenic treatments is judged by the reduction in germination and further survival of the plants up to maturity. Gradual reduction in germination and subsequent survival of the treated population was observed with the increase of mutagen dose in both cultivars (Fig. 1). Higher dose (40 kR) gamma rays caused the maximum reduction of germination in cultivar HGS 365 while the damage was comparable at lower doses in both cultivars. The decline in survival rate was minimum at lower dose (10 kR) of gamma rays in both the cultivars, while higher doses were much more detrimental. Lower concentration of EMS reduced germination more drastically in RGC 936 while at higher concentration was detrimental to HGS 365, but at 0.3% concentration, the damage was comparable in both cultivars. The survival rate of the germinated individuals was lower in HGS 365 than RGC 936.

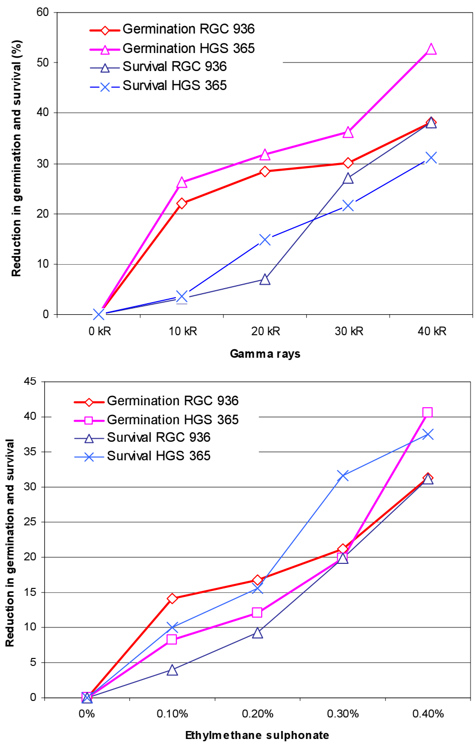

Figure 1. Effect of gamma rays (upper) and EMS (bottom) on germination and survival of clusterbean.

Mutation frequency in the M_2 generation

Chlorophyll deficiency

Three different types of chlorophyll deficiency mutant viz. *chlorina*, *xantha* and *albino* were observed. The mutation frequency of *albino* type was always the highest in all treatments, while those of *xantha* and *chlorina* varied between cultivars and among doses (Table 1). In general, the mutation frequency of *albino* type induced by gamma rays was higher than by EMS in both cultivars while no definite pattern was observed for *chlorina* and *xantha* (Table 1). The cultivars HGS 365 (21.8% & 23.5%) had higher overall chlorophyll mutation frequencies than RGC 936 (16.6% & 12.2%) treated either with gamma rays or EMS (Table 1).

Morphological and agronomic traits

Frequencies of various morphological mutations were also computed and presented in Table 1. Four different types of morphological trait, namely spreading (branched from base with upright behaviour), dwarf, mating (branches spread on ground like turf) and vigorous, were observed. Spreading and vigorous mutants were more frequently observed with gamma treatments in both cultivars than EMS, but this trend was not observed for dwarf and mating mutants (Table 1). Except for the spreading traits, HGS 365 had higher mutation frequencies than RGC 936 in both gamma and EMS mutagenesis, which is consistent with the chlorophyll deficiency mutations (Table 1).

Performance of M_3 and M_4 progenies

In M_3 generation fifty progenies were selected on the basis of their *per se* performance for agronomic traits (Table 2). Twenty promising progenies were found superior for earliness, twenty for grain yield per plant, five for plant height, nine for pods per plant and ten for seeds per pod over the respective parent (control). Some of the progenies had shown superiority for more than one trait (yield, pods per plant and seeds per pod). These progenies were evaluated in M_4 generation for their yield performance and other agronomic traits (Table 3). Mutant progeny RGC 936-30-2 matured in just 65 days with higher grain yield (8.02 g) and maximum number of seed bearing pods (9.7) closely followed by RGC 936-1-17, which matured in 68 days with almost the same yield level (7.93 g) with maximum branches (5.9), clusters per plant (12.9), pods per plant (27.1) and seeds per pod (9.7). This mutant progeny showed superiority for all the agronomic traits except maturity with almost the same yield level (7.93 g) than that of the best yielder. The other mutant progeny of RGC 936 also showed superiority for more than one trait.

Eight promising mutants were identified in cultivar HGS 365 on the basis of their agronomic performance and superiority over parental control. Mutant progeny HGS 365-15-5 was found superior with higher yield per plant (9.95g), branches per plant (6.3), clusters per plant (13.7), pods per plant (34.8) and seeds per pod (9.1). Besides yield, other mutants of HGS 365 had also shown superiority for other agronomic traits.

Table 1. Frequency of morphological and chlorophyll mutations in clusterbean

Cultivars/ Treatment	Morphological mutations (%)					Chlorophyll mutations (%)			
	Spreading	Dwarf	Mating	Vigorous	Total	Albino	Chlorina	Xantha	Total
RGC 936, Gamma rays									
Control	-	-	-	-	-	-	-	-	-
10 kR	0.87	-	0.43	0.40	1.70	1.14	0.57	0.85	2.56
20 kR	2.42	0.85	1.30	0.76	5.33	1.78	0.88	1.78	4.44
30 kR	3.65	4.56	5.87	2.05	16.13	3.23	1.29	0.00	4.52
40 kR	16.72	8.18	3.09	4.21	32.20	3.39	1.69	0.00	5.08
RGC 936, EMS									
0.1%	-	0.62	0.43	-	1.05	0.85	0.42	0.40	1.67
0.2%	2.15	0.62	0.40	0.25	3.42	1.03	0.68	0.65	2.36
0.3%	4.54	2.12	2.02	0.46	9.14	1.71	1.14	1.15	4.00
0.4%	6.77	3.42	5.84	1.21	17.24	2.75	1.38	0.00	4.13
HGS 365, Gamma rays									
Control	-	-	-	-	-	-	-	-	-
10 kR	1.16	0.28	0.57	-	2.01	1.34	0.67	1.34	3.35
20 kR	2.39	1.92	1.14	1.58	7.03	2.70	1.08	1.08	4.86
30 kR	4.66	3.72	10.31	4.47	23.16	2.86	2.10	2.11	7.07
40 kR	15.18	8.96	7.34	16.76	48.24	5.88	0.00	2.35	8.23
HGS 365, EMS									
0.1%	0.36	0.28	0.67	0.59	1.90	1.09	0.54	0.82	2.45
0.2%	1.71	0.80	1.26	2.23	6.00	2.50	1.00	1.50	5.00
0.3%	3.28	1.74	10.65	2.42	18.09	2.86	1.90	1.90	6.66
0.4%	4.76	16.35	8.98	4.39	34.48	5.13	0.00	2.56	7.69

Table 2. Selected promising progenies for agronomic traits in M_3 generation

SN	Agronomic trait	RGC 936		HGS 365	
		Mutants*	Parent	Mutants*	Parent
1	Early maturity (days)	< 66 (10)	72	< 65 (10)	73
2	High yielding (g/plant)	> 6.0 (10)	5.23	> 6.5 (10)	5.58
3	Plant height (cm)	> 45.0 (2)	38.0	> 50.0 (3)	42.0
4	Number of pods per plant	> 24.0 (5)	18.0	> 29.0 (4)	19.0
5	Number of seeds per pod	> 9.0 (4)	8.2	> 9.0 (6)	8.4

* Figures in parentheses show the number of progenies selected for the trait. These numbers exceed fifty because some progenies were common for more than one trait.

Table 3. Agronomic performance of promising M_4 progenies

Mutants	Yield per plant (g)	Days to maturity	Plant height (cm)	Branches per plant	Clusters per plant	Pods per plant	Seeds per pod
RGC 936-1-5	6.89	67.5	42.7	5.7	12.3	25.5	9.2
RGC 936-1-17	7.93	68.0	46.0	5.9	12.9	27.1	9.7
RGC 936-5-4	7.90	65.5	43.7	5.9	12.7	24.5	8.8
RGC 936-12-11	6.81	66.5	43.4	5.6	11.8	22.6	8.5
RGC 936-16-2	7.54	66.5	44.8	5.8	12.2	23.8	9.0
RGC 936-20-22	7.48	68.5	41.9	5.7	11.9	22.2	8.9
RGC 936-30-2	8.02	65.0	43.0	5.5	12.6	22.9	9.7
RGC 936 (C)	***4.26***	***71.5***	***38.1***	***4.5***	***9.2***	***18.1***	***7.9***
HGS 365-2-7	7.82	67.5	44.6	5.4	11.9	24.6	8.8
HGS 365-3-10	7.35	68.0	44.3	5.4	12.0	24.6	9.0
HGS 365-7-5	7.74	69.0	50.0	5.5	11.8	25.1	8.8
HGS 365-10-2	7.84	65.0	49.3	4.8	10.2	25.8	9.1
HGS 365-12-6	9.16	67.0	46.7	4.7	12.1	33.6	8.5
HGS 365-15-2	8.47	68.0	49.0	4.9	13.4	28.2	9.0
HGS 365-15-5	9.95	69.0	48.8	6.3	13.7	34.8	9.1
HGS 365-18-4	9.50	63.5	45.9	6.2	10.4	30.6	8.8
HGS 365 (C)	***4.55***	72.3	***36.3***	***4.4***	***9.2***	***18.3***	***8.3***

Discussion

In the present study, gradual reduction in germination and subsequent survival of treated population were observed with corresponding increase in concentration/dose of both mutagens. These findings are consistent with the earlier findings of Rao and Rao (1982) in clusterbean. However, Singh and Chowdhary (1972) did not observe significant effect up to 40 kR on germination, yet there was a considerable effect at 100 and 150 kR doses of gamma irradiation in clusterbean. Frequencies of morphological and chlorophyll mutations were observed in M_2 generation since all the variants are expected to be expressed in M_2 generation. Higher frequency of certain plant types at higher doses might be due to considerable changes at DNA level by the mutagens while lack of any pattern of certain morpho-types or chlorophyll mutants might be due to random nature of mutations. It was observed that both the cultivars manifested differential reactions to the treatment with gamma rays and EMS. Differential behaviour of various mutagens and selective response of cultivars might be due to the mode of action of the mutagens employed and genetic variability among the cultivars used in the study. These findings are consistent with the findings of Badami and Bhalla (1992) in clusterbean and others in pulses (Kothekar 1989, Waghmare and Mehra 2001 and Gaikwad and Kothekar 2004).

On the basis of mean performance and variance, selected M_2 plants were advanced to M_3 generation and agronomically superior mutant progenies, and were tested for their stability in M_4 generation. The purpose of the present study was to isolate early maturing and high yielding mutant lines. Fifteen mutants, seven derived from RGC 936 and eight from HGS 365 were found to be superior for earliness as well as higher grain yield. Yadav *et al.*, (2004) had also isolated mutants in M_3 generation from clusterbean cultivar RGC 197 through gamma irradiation.

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