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Effectiveness and efficiency of mutagens and induced variability in clusterbean (*Cyamopsis tetragonoloba*)

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ABSTRACT

An experiment was conducted during 2003–06 to study the variability for adaptability over the locations of clusterbean [*Cyamopsis tetragonoloba* (L.) Taub.]. Healthy and pure seeds of 2 clusterbean cultivars ('RGC 936' and 'HG 365') were treated with gamma rays (10, 20, 30 and 40 kR) and ethyl methane sulphonate (0.1, 0.2, 0.3 and 0.4%). A steady reduction in germination and subsequent survival of the treated population, seedling height and pollen fertility were observed with the increasing doses/concentrations of mutagens in both the cultivars regardless of the mutagens used. Both mutagenic effectiveness and efficiency generally decreased with increasing doses/concentrations of mutagens. The efficiency of gamma rays was more in all the 3 parameters of biological damage compared to ethyl methane sulphonate in both the cultivars. The coefficients of variation for all the quantitative characters were of higher magnitude compared to control in both the cultivars. Linear increase in variability in the treated population was recorded with the increasing doses/concentrations of mutagens in both the cultivars.

Key words: Clusterbean, Effectiveness, Efficiency, Mutagens, Variability

The usefulness of a mutagen in crop improvement depend both on its effectiveness and efficiency. A highly effective mutagen may not necessarily show high efficiency and *vice versa*, therefore, estimation of effectiveness and efficiency of the mutagens have practical utility for induction of desirable mutants in higher frequency. Mutagenesis is rewarding in situations where naturally occurring variability is low. This applies both to overall variability as well as variability for specific traits and second where a simply inherited defects needs to be rectified in an otherwise agronomically superior cultivar (Chopra and Sharma 1985). Mutation breeding is a powerful tool for inducing variability in crops like clusterbean, where exploitable and useful genetic variation is meager. Moreover creation of variability in this crop through hybridization is very difficult and cumbersome owing to very small and delicate flower structures resulting in very poor seed setting in the manually hybridized buds and higher frequency of flower drop during and after crossing (Arora and Pahuja 2008). There are limited variability for characters of economic importance in existing cultivars, therefore, mutagenic treatments were employed for creation of variability in 2 cultivars of clusterbean ('RGC 936' and 'HG 365') having better adaptability over the locations.

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MATERIALS AND METHODS

The present study was conducted during 2003–06 at central farm of CAZRI, Jodhpur for creation of variability and to workout effectiveness and efficiency of mutagens in clusterbean. Healthy and pure seeds of 2 clusterbean cultivars ('RGC 936' and 'HG 365') were treated with gamma rays [(10, 20, 30 and 40 kR) Source Co^{60}, IARI, New Delhi] and ethyl methane sulphonate (0.1, 0.2, 0.3 and 0.4%). The ethyl methane sulphonate treatments were performed in freshly prepared phosphate buffer (*p*H 7.0). In M_1 generation the data on reduction in germination and subsequent survival (lethality), seedling height reduction (injury) and reduction in pollen fertility (sterility) were recorded to estimate the damage caused by the mutagens. M_1 plants were individually harvested to grow the M_2 generation following the plantto row method. The M_2 generation was critically screened for chlorophyll mutations and mutation frequency was determined as per the method suggested by Gaul (1964). Proper agronomic practices were followed for raising a healthy crop in both the generations. For estimation of induced variability 5 plants were randomly selected from each treatment in M_2 generation and mean values and coefficient of variation were calculated using standard statistical procedures. Both mutagenic effectiveness and efficiency were determined using the formulae and procedures of Konzak *et. al.* (1965).

RESULTS AND DISCUSSION

Damage in M_1 generation

In the M_1 generation the initial damage caused by the mutagenic treatments was judged by the reduction in germination and subsequent survival of the treated plants, seedling height reduction (injury) and reduction in pollen fertility (sterility). Linear reduction in germination and subsequent survival of the treated population, seedling height and pollen fertility were observed with the increasing doses/ concentrations of mutagens in both the cultivars regardless of the mutagens used. The perusal of data in Table 1 clearly shows that among the mutagens ethyl methane sulphonate caused more damage than gamma rays. Differential behaviour of the 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 in close agreement with the earlier findings of Badami and Bhalla (1992) in clusterbean and Kharkwal (1998) in chickpea.

Mutation frequency and effectiveness and efficiency in M_2 generation

The M_2 generation was screened critically as it is the most variable population and all the variants are expected to express themselves. Since chlorophyll deficient mutants could not survive long and observed in treated population for variable length of their life-cycle depending on the deficiency of chlorophyll. Therefore, these mutants are of no agronomic value but their frequency reflects the effectiveness of the mutagens and chlorophyll mutants are used as tests for evaluation of genetic action of mutagenic factors. They are the most frequently observed and can be easily identified factorial mutations in M_2-generation. Different mutation frequencies and width of mutation spectra were induced under the action of applied mutagens. There were positive trend with the increasing doses of the mutagens and mutation frequency (Table 2). In this regard gamma rays were found more effective (4.15 and 5.88%) compared to ethyl methane sulphonate (3.04 and 5.70%) and cultivar 'HG 365' better responded (5.88 and 5.70%) than 'RGC 936' (4.15 and 3.04%). These findings are in concurrence with the earlier findings of Badami and Bhalla (1992) in clusterbean.

The data on mutagenic efficiency and effectiveness are presented in Table 2. The mutagenic efficiency was worked out using 3 criteria of biological damage (lethality, injury and sterility) induced by the mutagenic treatments. In all the 3 cases the efficiency of lower doses/concentrations were more compared to higher doses in producing desirable mutants. However in 'HG 365' increase in concentration of ethyl methane sulphonate from 0.1 to 0.2% increased the

Table 1 Effect of mutagens on germination, survival, seedling height and pollen fertility on clusterbean cultivars in M_1 generation

Treatment	Germination (%)	Survival (%)	Seedling ht.(cm)	Pollen fertility (%)
		'RGC 936'		
Control	75.33 (0.0)	50.44 (0.0)	6.24 (0.0)	96.64 (0.0)
10 kR γ rays	64.66 (14.16)	48.45 (3.94)	6.01 (3.68)	87.17 (9.80)
20 kR γ rays	62.66 (16.81)	45.74 (9.31)	5.74 (8.01)	80.38 (16.82)
30 kR γ rays	59.33 (21.23)	40.44 (19.82)	5.15 (17.46)	71.53 (25.98)
40 kR γ rays	48.00 (36.28)	34.72 (31.16)	4.66 (25.00)	62.16 (35.68)
Mean	61.99 (22.12)	42.34 (16.06)	5. 39 (13.62)	75.31 (22.07)
0.1% EMS	58.66 (22.12)	48.86 (3.13)	5.82 (6.73)	83.26 (13.84)
0.2% EMS	54.00 (28.31)	46.91 (6.99)	5.60 (10.25)	77.42 (19.88)
0.3% EMS	52.66 (30.09)	36.70 (27.24)	5.15 (17.47)	70.76 (26.78)
0.4% EMS	42.00 (44.24)	30.15 (40.22)	4.44 (28.85)	56.67 (41.36)
Mean	51.83 (31.19)	40.65 (19.40)	5.25 (15.86)	72.03 (25.46)
		'HG 365'		
Control	68.13 (0.0)	60.66 (0.0)	6.42(0.0)	95.75 (0.0)
10 kR γ rays	65.67 (3.61)	58.66 (3.29)	6.06 (5.61)	88.29 (7.80)
20 kR γ rays	58.06 (14.78)	53.33 (12.08)	5.87 (8.56)	82.47 (13.87)
30 kR γ rays	53.44 (21.56)	46.57 (31.64)	5.50 (14.33)	73.24 (23.51)
40 kR γ rays	46.93 (31.11)	36.00 (40.65)	4.90 (23.67)	60.55 (36.76)
Mean	56.02 (17.77)	48.64 (19.81)	5.58 (13.08)	76.14 (20.48)
0.1% EMS	61.36 (9.93)	54.66 (9.89)	5.85 (8.88)	81.64 (14.74)
0.2% EMS	57.50 (15.60)	51.33 (15.38)	5.65 (12.00)	73.84 (22.88)
0.3% EMS	48.66 (19.78)	45.66 (24.73)	5.12 (20.25)	64.50 (32.64)
0.4% EMS	42.59 (37.48)	38.14 (37.12)	4.55 (29.13)	56.43 (41.10)
Mean	52.53 (22.90)	47.45 (21.78)	5.30 (17.56)	69.10 (27.83)

Figures in parentheses show per cent reduction over respective control values

Table 2 Effectiveness and efficiency of mutagenic treatments in clusterbean

Treatment	Mutation frequency (Mf)	% Lethality (L)	Mutagen efficiency (Mf/L)	Seedling injury (I)	Mutagen efficiency (Mf/I)	Pollen sterility (S)	Mutagen efficiency (Mf/S)	Mutagenic effectiveness (Mf/kR or txc)
				'RGC 936'				
Control								
10 kR γ rays	2.56	3.94	0.650	3.68	0.696	9.80	0.261	0.256
20 kR γ rays	4.44	9.31	0.477	8.01	0.554	16.82	0.264	0.222
30 kR γ rays	4.52	19.82	0.228	17.46	0.259	25.98	0.174	0.151
40 kR γ rays	5.08	31.16	0.163	25.00	0.203	35.68	0.142	0.127
Mean	4.15	16.06	0.380	13.62	0.428	22.07	0.210	0.189
0.1% EMS	1.67	3.13	0.533	6.73	0.248	13.84	0.121	2.783
0.2% EMS	2.36	6.99	0.337	10.25	0.230	19.88	0.118	1.966
0.3% EMS	4.00	27.24	0.145	17.47	0.229	26.78	0.149	2.222
0.4% EMS	4.13	40.22	0.103	28.85	0.143	41.36	0.100	1.720
Mean	3.04	19.40	0.280	15.86	0.212	25.46	0.122	2.173
				'HG 365'				
Control								
10 kR γ rays	3.35	3.29	1.018	5.61	0.597	7.80	0.429	0.245
20 kR γ rays	4.86	12.08	0.402	8.56	0.568	13.87	0.350	0.300
30 kR γ rays	7.07	31.64	0.223	14.33	0.493	23.51	0.301	0.222
40 kR γ rays	8.23	40.65	0.202	23.67	0.348	36.76	0.224	0.192
Mean	5.88	19.81	0.461	13.08	0.502	20.48	0.326	0.240
0.1% EMS	2.45	9.89	0.248	8.88	0.276	14.74	0.166	4.083
0.2% EMS	6.00	15.38	0.390	12.00	0.500	22.88	0.262	5.000
0.3% EMS	6.66	24.73	0.269	20.25	0.329	32.64	0.204	3.700
0.4% EMS	7.69	37.12	0.207	29.13	0.264	41.10	0.187	3.204
Mean	5.70	21.78	0.278	17.56	0.342	27.83	0.205	3.996

EMS, Ethyl methane sulphonate

efficiency considerably irrespective of the parameters of biological damage. The perusal of data clearly shows that the efficiency of gamma rays was more in all the 3 parameters of biological damage compared to ethyl methane sulphonate in both the cultivars. Badami and Bhalla (1992) also observed the similar trend in clusterbean cultivars treated with gamma rays, magnetic field and sodium azide. This might be due to the detrimental effect of higher doses/concentrations of the mutagens in causing more biological damage to cells and production of more undesirable effects at higher doses/ concentrations. The low efficiency of certain mutagens may be attributed to the use of low doses corresponding to their mutation induction. The higher efficiency of a mutagen indicates relatively less biological damage in relation to mutations induced (Jain and Khandelwal 2009). The estimation of efficiency can be influenced by the criteria used for defining the biological damage. However, on comparative grounds mutagenic efficiency is a better yard stick for comparing the usefulness of the mutagens than mutagenic effectiveness.

The degree of effectiveness of mutagens and the response of cultivars was observed to be varying. Both the mutagens were less effective in 'RGC 936' and linear reduction in effectiveness were observed with the increasing doses/ concentration of mutagens with the mutation rate of 0.189 and 2.173 for gamma rays and ethyl methane sulphonate, respectively. While cultivar 'HG 365' showed increased effectiveness at lower doses/concentrations of both gamma rays and ethyl methane sulphonate and then gradual reduction in effectiveness was observed with higher mutation rates (0.240 and 3.996). The results of the present study on efficiency and effectiveness are in line with earlier findings of Badami and Bhalla (1992) in clusterbean, Kharkwal (1998) in chickpea (*Cicer arietinum* L.) and Gaikwad and Kothekar (2004) in lentil (*Lens culinaris* Medik) and Jain and Khandelwal (2009) in blackgram (*Vigna mungo* Hepper). Kharkwal (1998) observed that both mutagenic effectiveness and efficiency generally decreased with increasing doses/ concentrations of mutagens as in the case of present study, nevertheless, the highest total mutation rates were in general obtained with higher doses/concentrations both in case of physical as well as chemical mutagens. It would thus be seen that higher mutagenic effectiveness and efficiency does not reflect the *per se* mutation frequency and they cannot be used an index for maximization of mutation rates.

Induced variability

In the M_2 generation for estimation of induced variability

Table 3 Mean and coefficient of variation of yield and yield components in M_2 generation in clusterbean cultivars treated with mutagens

Treatment	Plant height (cm)		Branches/plant		Clusters/plant		Pods/plant		Pod length		Seeds/pod		Yield/plant	
	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)
'RGC 936'														
Control	58.60	6.78	8.33	15.49	26.53	13.18	68.15	13.08	5.56	5.99	8.42	6.85	20.95	12.21
10 kR γ rays	59.93	18.07	8.53	16.01	30.80	15.98	81.23	18.20	6.20	6.67	9.13	8.13	18.90	49.92
20 kR γ rays	56.73	20.01	8.13	27.37	26.00	17.74	70.42	23.94	5.95	7.92	8.55	12.29	17.40	53.00
30 kR γ rays	58.00	21.27	8.66	35.87	29.85	21.53	65.38	27.95	5.83	11.97	7.60	13.56	24.65	59.48
40 kR γ rays	53.66	23.63	6.33	37.68	23.06	29.65	67.05	33.39	5.70	13.98	7.15	16.95	22.70	62.25
Mean	57.08	17.95	7.91	26.48	27.43	19.61	71.02	23.31	5.92	9.30	8.11	11.55	20.91	47.37
0.1% EMS	51.33	18.46	7.20	10.78	25.33	12.10	70.45	17.31	5.93	9.48	8.86	13.33	18.90	51.79
0.2% EMS	52.86	19.38	7.53	17.22	28.06	17.43	72.86	20.83	5.80	11.65	8.40	18.35	21.80	57.84
0.3% EMS	53.80	20.04	7.00	25.07	23.53	23.97	71.20	25.64	5.78	11.86	8.75	19.26	16.50	62.67
0.4% EMS	51.53	20.89	6.40	26.92	21.83	28.88	65.75	29.62	5.92	12.07	7.95	22.94	18.20	64.08
Mean	52.38	17.11	7.03	19.09	24.69	19.11	70.06	21.29	5.86	10.21	8.49	16.14	18.85	49.73
'HG 365'														
Control	72.86	10.50	10.65	11.16	33.40	8.81	76.85	11.63	5.75	3.90	8.20	8.23	23.65	15.95
10 kR γ rays	67.20	13.07	12.46	12.81	40.40	14.51	88.60	15.51	6.01	5.73	8.75	9.79	22.90	50.38
20 kR γ rays	73.86	13.18	12.19	14.52	46.86	23.50	86.30	19.14	6.23	7.69	8.60	11.17	26.85	52.40
30 kR γ rays	69.20	14.56	11.80	17.00	37.56	26.28	74.15	25.94	5.62	9.04	8.55	13.18	25.75	56.71
40 kR γ rays	77.06	15.86	10.00	20.56	32.35	30.75	76.33	30.72	5.83	9.74	8.30	13.35	20.40	62.69
Mean	70.63	13.43	11.61	15.21	39.29	20.77	81.34	20.58	5.92	7.22	8.55	11.14	23.97	47.62
0.1% EMS	65.46	12.83	11.33	12.77	37.60	13.21	80.45	18.83	5.98	4.35	8.66	10.64	27.60	39.42
0.2% EMS	59.00	18.02	10.00	18.30	29.26	22.04	78.67	23.39	5.65	11.29	8.40	12.30	25.00	45.80
0.3% EMS	71.20	19.23	11.06	24.26	36.65	27.65	88.20	24.75	5.33	14.20	8.10	13.00	32.40	48.68
0.4% EMS	74.80	20.04	12.13	26.66	34.30	31.76	70.17	33.10	6.00	15.49	8.25	14.06	27.15	53.95
Mean	67.60	16.12	11.13	18.63	34.45	20.69	79.37	22.3	5.74	9.84	8.35	11.64	28.04	40.76

through mutagenesis, the mean values and coefficient of variation were calculated for yield and yield components (Table 3). The coefficient of variation for all the quantitative characters were of higher magnitude compared to control (parent varieties) in both the cultivars treated with either gamma rays or ethyl methane sulphonate which shows induction of variability for these traits. In general there was linear increase in variability in the treated population with the increasing doses/concentrations of mutagens in both the cultivars. Yield/plant is considered most variable and dependent trait showing polygenic inheritance showed high coefficient of variation over the control. This clearly shows induction of variability for this important trait through the contribution of component traits. Amrita *et al.* (2003) also created variability through gamma rays and identified some promising mutants in clusterbean. Yadav *et al.* (2004) exposed seeds of clusterbean cv. 'RGC 197' to gamma rays (10–80 kR) and isolated superior progenies in M_2 which showed higher mean values with lower coefficient of variation in M_3 generation. An increase in genetic variability might be due to the occurrence of extreme types, both on the positive and negative side of the control and the means of the treated progenies were almost comparable with the control means. This is due to the fact that the effect of mutagens on the quantitative traits has been interpreted as that owing to detrimental mutations which are supposed to occur more frequently than favourable ones. Brock (1965) reported that in the species which had previously been selected to breeding and selection, random mutations resulted in an increase in variance and a shift in the mean away from the direction of previous selection. He further suggested that the increased genetic variation permitted effective selection response in each direction even in characters approaching the limits within the species.

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