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Induced chemical mutagenesis in cowpea [*Vigna unguiculata* (L.) Walp.]

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Abstract

A mutagenesis programme was carried out using three chemical mutagens *viz.*, EMS, MMS and SA on two varieties of cowpea [*Vigna unguiculata* (L.) Walp.] namely, RC 19 and RC101. The M_1 generation was raised only from higher doses of the mutagens which adversely affected their survival. In M_2 generation, a wide spectrum of macromutations were observed in the progenies of both the varieties including few seed color mutants. The MMS treatment was found most effective and efficient as well. Several M_2 progenies of the two cowpea varieties were significantly superior to their respective parents for seed yield per plant. A considerable number of M_2 progenies were consistently superior to their parents in M_3 generation also.

Key words : Cowpea, chemical mutagenesis, polygenic variation

Introduction

Cowpea [*Vigna unguiculata* (L.) Walp] is an important arid legume crop of the country and consumed as grain, vegetable and cattle feed. Although there exists adequate genetic variability in the species for yield and yield attributes, but efforts made to breed a desirable plant type with higher yield potential is generally not successful either due to lack of suitable parent or failure of seed setting on emasculated buds [1]. A mutation breeding programme is thus advocated [2]. However, for an efficient mutation breeding programme, the knowledge on efficiency of mutagens being used is of basic importance. Further, information on application of chemical mutagens on cowpea is scanty particularly with reference to its cultivation in arid regions [3]. Keeping these points in view, a study was undertaken with three chemical mutagens on two varieties of cowpea with an objective to induce both macro and micro mutational changes.

Materials and methods

Two morphologically distinct cultivars of cowpea namely, RC 19 (tendrilar, viny, purple flowered with small and fawn colored seeds) and RC 101 (semi determinate, non-viny, white flowered with white bold seeds) were treated with graded doses of ethylmethane sulphonate (EMS), methylmethane sulphonate (MMS) and Sodium Azide (SA). The LD_{50} were determined for the three chemical mutagens *viz.*, 0.25% for EMS, 0.025% for MMS and 1 mM for SA. However, for the two cowpea varieties, the M_1's were raised only from treatment doses higher than LD_{50} of the three chemical mutagens. For chemical treatment, the seeds of the two varieties were presoaked for 6h in distilled water (3 samples each containing 250 seeds of a variety) and then treated with EMS (0.5%, pH 7.0), MMS (0.05%, pH 7.0) and SA (3mM, pH 3.0) freshly prepared in 0.1 M phosphate buffer for 4 h at room temperature and then washed thoroughly in running tap water for 6h to remove the traces of chemicals. The parent seeds treated in similar way but without mutagen served as control. The treated seed samples (including control) of both the varieties were immediately sown in the field in RBD with two replications at the spacing of 30 × 10 cm during *kharif* 2002 to raise M_1 generation. Pollen fertility was determined by staining with 1% acetocarmine (only stained pollens were considered fertile). The M_1 plants from each treatment were harvested separately and their M_2 progenies (70 progenies of RC 19 and 69 progenies of RC 101) were raised during *kharif* 2003. The size of M_2 population was small because high doses of MMS and EMS treatments adversely affected the plant growth and seed setting in both the varieties in M_1 generation. The M_2 progenies were raised in single row (3m) plots. On the basis of significantly higher yield over respective parent, selection of superior progenies and superior plants from these superior progenies was done in M_2 generation. Thus, 100 plant progenies of RC 19 and 70 progenies of RC101 were randomly selected for raising M_3 generation. The M_2 or M_3 progenies of the two varieties were sown in separate experiments in single row plots of 3 m length during *kharif* 2004 with similar agronomical practices. In both M_2 and M_3 generations, respective control rows were repeated once after every 10 rows of the progenies. The observations were recorded on yield and various yield traits on 10 normal looking plants selected randomly in each progeny both in M_2 and M_3 generations. In

M_2 generation, the chlorophyll and other morphological mutations were examined and classified as per Blixt [4]. Mutation frequency, mutagenic effectiveness and efficiency were calculated on M_2 family basis as per Konzak *et al.* [5]. The statistical analysis were carried out using the standard procedure as outlined by Snedecor and Cochran [6].

Results and discussion

Chlorophyll and morphological mutations : In the present study 3 types of chlorophyll deficient mutations *viz.*, chlorina, virescent and others were identified in the M_2 progenies of the two varieties (Table 1). Except for EMS on RC 19, only one type of chlorophyll deficient mutants was observed for other treatments. The spectrum of morphological mutants mainly comprised of four types *viz.*, short and erect, spreading and giant, altered leaf and non-viny in RC 19 whereas, spreading, altered leaf, tendrilar and dwarf were observed in RC 101. The M_2 population used in the present study was relatively small being derived only from the higher dose of mutagens employed in the study. Mahna *et al.* [7] also observed several chlorophyll and morphological mutants in cowpea, upon treatment with SA. Generally in any mutation breeding experiment the convention is to use both, higher and lower doses than LD_{50} (including LD_{50}) in order to have a reasonable chance of recovery of mutations. However, in the present study we concentrated only on higher dose of mutagens with an objective to recover rare desirable mutations, if any. Relatively higher mutation frequency both for chlorophyll and morphological mutations were observed for EMS and MMS. It may be noted that the population in which the macro-mutants were observed was too small i.e. 723 M_2 individuals for RC19 and 606 M_2 individuals for RC 101. For morphological mutations SA on RC 101 appeared potent, indicating the influence of genotype. Few seed color mutants were also observed in M_2 progenies of both the varieties *viz.*, black, green, rajma type, roan chocolate and castor type. Out of these, black, rajma and castor type were found stable in M_3 and M_4 generations also. Black, dull black and maroon seed color mutants have been reported earlier also [8].

Mutagenic effectiveness and efficiency : On the basis of effectiveness, the mutagenic potency of these mutagens was more or less same on the two varieties. The MMS treatment was most effective on RC 19 followed by SA and EMS while on RC 101 SA was most effective indicating differential response of the genotypes (Table 1). In terms of mutagenic efficiency also MMS was most potent on both the varieties followed by EMS (RC 19) or SA (RC 101).

Polygenic variability in M_2 generation : Analysis of variance revealed that between progeny and within progeny mean sum of squares were significant for all the characters studied in M_2 generation indicating existence of induced variability in progenies of both the varieties. Perusal of data revealed that the mean of the traits were shifted to both the negative and positive directions to varying magnitudes depending upon the trait and genotype (Table 2). Murugan and Subramanian [8] observed similar shifts in the mean values in cowpea varieties upon radiation with gamma rays. The progenies with positive shifts in the mean values were more in RC 19 for plant height, pods/plant and seed yield/plant while RC 101 showed relatively

Table 1. Spectrum and frequency of macro-mutations and estimates of mutagenic effectiveness/efficiency of chemical mutagens in cowpea

Treatments	Dose	Total plants observed	Macromutations							Total freq.	Total no. of M_2 families	% of M_2 family segregating for mutations (Mf)	% of pollen sterility in M_1 (S)*	Mutagenic effectiveness (Mf/tc)	Mutagenic efficiency (Mf/s)
			Per cent chlorophyll mutations			Morphological mutation									
			Chlorina	Virscent	Other	Short erect	Spreading giant	Altered leaf	Non viny						
Variety RC-19															
MMS	0.05%	23	-	-	-	4.34	-	-	-	4.34	3	33.33	51.53	166.65	0.65
EMS	0.5%	149	1.34	-	-	1.34	0.67	2.01	-	5.36	15	20.00	47.14	10.00	0.42
SA	3mM	551	-	0.36	-	0.36	0.36	0.36		1.44	50	8.00	40.50	100.00	0.20
Control	-	142	-	-	-	-	-	-	-	-	7	-	-	-	-
Variety RC-101						Spreading	Altered leaf	Tendrillar	Dwarf						
MMS	0.05%	81	-	1.23	-	1.23	3.70	1.23	-	7.39	5	40.00	51.10	200.00	0.78
EMS	0.5%	243	0.82	-	0.82	0.82	1.23	0.41	-	4.10	29	17.24	46.87	8.62	0.37
SA	3mM	282	0.35	-	-	1.41	1.77	1.06	0.70	5.29	36	16.16	39.37	208.25	0.42
Control	-	95	-	-	-	-	-	-	-	-	7	-	-	-	-

Table 2. Distribution of number of M_2 progenies of cowpea varieties showing significant increase or decrease in the mean performance than their respective parent for various characters

Varieties	Progenies derived from mutagenic doses	Total number of progenies	Plant height			Branches/plant			Pods/plant			Seed yield/plant		
			a	b	c	a	b	c	a	b	c	a	b	c
RC 19	MMS (0.05%)	3	1	1	2	3	-	-	1	-	2	-	1	2
	EMS (0.5%)	15	3	8	4	8	2	5	7	1	7	5	2	8
	SA (3 mM)	51	14	31	6	14	20	17	19	9	23	30	5	16
	Total	69	18	39	12	25	22	22	27	10	32	35	8	26
RC 101	MMS (0.05%)	5	1	2	2	5	-	-	2	-	3	3	-	1
	EMS (0.5%)	29	6	7	16	13	5	11	11	6	12	7	15	7
	SA (3 mM)	36	9	4	23	10	11	8	11	16	9	14	15	7
	Total	70	16	13	41	38	16	19	24	22	24	24	31	15

a = Significantly higher than the control; b = Significantly lower than the control; c = At par with the control

more number of superior progenies only for branches per plant. Superior progenies identified on the basis of seed yield/plant of both the varieties also showed higher magnitude of CV indicating that these progenies are segregating and can give rise to desirable mutations in further generations and thus formed a basis for their M_3 generation evaluation. Furthermore, these selected progenies also showed high mean values for 1 or 2 more yield attributes. Plant progenies from 13 superior M_2 progenies from RC 19 (46, 54, 48, 47, 49, 20 and 34) and 10 from RC 101 (27, 103, 119, 120, 77 and 117) were selected for further evaluation (Table 3).

Polygenic variability in M_3 generation : The analysis of variance in both the varieties revealed that for all the characters studied. The between progeny and within progeny mean sum of squares were highly significant. This is in agreement with earlier reports on cowpea [9]. Since only high yielding M_2 progenies were advanced to M_3 generation, apparently it would seem that the variation would converge, however, the results of the present study indicated to the possibility of exercising even further selection in M_3 progenies to identify relatively more superior individuals. A comparison of mean of M_3 progenies with the mean of their control; progenies revealed that for yield/plant, the progenies 46-1, 34-7, 46-2, 48-6, 49-6, 47-4, 20-1, 54-3 and 46-8 of RC 19 and progenies 77-2, 127-5, 117-8, 117-9, 103-5, 120-3 and 129-6 of RC 101 were significantly superior (Table 4). These progenies besides their significantly higher seed yield also showed significantly higher values for 4-6 yield attributes out of the seven studied and hence can be advanced for further selection and yield trials. It has earlier been shown that selection is most effective in M_2 generation [10, 11]. This inference is substantiated in the present study because the superiority of several M_2 progenies for yield/plant was consistent in M_3 generation also.

Table 3. Superior M_2 progenies of cowpea varieties identified on the basis of seed yield/plant (g)

Variety					
RC 19			RC 101		
Progeny	Mean	C.V.	Progeny	Mean	C.V.
46	16.59**	35.61**	127	17.70**	32.20**
54	16.53**	25.84**	103	15.21**	25.84**
48	16.08**	34.41**	129	15.01**	11.45**
47	15.35**	15.37**	120	14.56**	27.25**
49	13.26**	39.88**	77	12.77**	30.09**
20	12.79**	38.49**	117	09.84**	14.22**
34	11.81**	22.33**	112	17.31**	51.84**
5	16.31**	18.94**	73	16.22**	63.54**
55	16.05**	35.78**	107	14.36**	26.54**
52	15.57**	42.85**	75	12.46**	21.22**
58	15.14**	38.17**	-	-	-
62	13.90**	43.25**	-	-	-
65	13.34**	17.06**	-	-	-
Control	8.50	14.78	Control	8.72	6.76

**Significant at p = 0.01

Genetic parameters of variation were estimated for all the characters studied in order to asses the possibility of improvement and also to determine selection criteria for improvement of seed yield per plant (Table 5). High heritability alongwith relatively higher genetic advance as percentage of mean were recorded for plant height, branches/plant in RC 101 whereas in RC 19 it was pods/plant followed by plant height which is highly desirable. This indicated that selection for these quantitative characters in further generations will be highly responsive. These findings are in close conformity with the results obtained by Choulwar and Boriker [12] in cowpea.

From the results of present study it may be inferred that relatively higher doses of mutagens are effective in inducing not only macro mutations but also micromutations in desirable direction, at least in cowpea.

References

1. **Singh B. B., Chambhss O. L. and Sharma B.** 1997. Recent advances in cowpea breeding. *In*: B.B. Singh, Mohan Raj, K Dashiel and Jackai, L.E.M. (eds.). Advances in cowpea research. UTA/JIRCAS, ITTA, Ibadan, Nigeria pp. 30-49.

Table 4. Mean and C.V. in respect of various yield attributes of high yielding M_3 progenies for different characters in cowpea varieties RC-19 and RC-101

Progenies	Seed yield/plant (g)		Plant height (cm)		Branches/ plant		Pods/ plant		Clusters/ plant		Seeds/ pod		Pod length (cm)		100-seed weight (g)	
	Mean	cv	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV
RC-19																
46-1	6.00**	17.11**	20.00	16.83**	2.40**	40.25**	11.10**	13.06**	3.20**	13.18**	6.40**	19.76**	8.30**	15.08**	8.10	5.12**
34-7	5.91**	23.93**	23.80**	13.84**	2.50**	38.87**	10.40**	9.29**	2.80**	22.59**	6.70**	23.39**	8.60**	17.51**	8.12	6.75**
46-2	5.88**	29.09**	24.00**	11.62**	2.30**	21.00**	9.80**	16.52**	3.10**	32.08**	7.60	12.71**	9.90**	10.04**	7.45**	10.06**
48-6	5.79**	21.34**	30.90**	6.90**	2.40**	52.70**	10.00**	8.16**	3.20**	19.76**	9.60**	10.06**	11.10**	6.98**	8.12	7.63**
49-6	5.79**	27.26**	20.50	5.27	2.40**	21.52**	6.80	16.70**	2.20	41.77**	9.70**	12.90**	11.20**	10.35**	8.08	5.95**
47-4	5.69**	22.80**	22.90**	6.33*	2.60**	41.34**	10.80**	19.91**	2.50**	50.77**	8.30**	8.13**	9.96**	6.81**	8.13	4.99**
20-1	5.62**	24.65**	19.50	7.74*	2.30**	35.79**	11.10**	10.79**	2.20	51.60**	8.30**	16.11**	9.40	15.21**	8.52**	3.39
54-3	5.37**	24.00**	20.80	11.73**	2.30**	41.25**	9.20**	16.84**	2.80**	32.82**	10.50**	8.09**	12.02**	7.66**	8.04	4.96**
46-8	5.32**	22.11**	22.40**	7.35*	2.70**	46.36**	9.40**	16.02**	3.10**	32.08**	7.70	29.39**	8.85**	20.98**	8.08	5.99**
22-1	5.31**	21.75**	19.60	16.3.1**	2.70**	25.00**	9.10**	9.62	2.70**	17.89**	7.60	18.81**	9.60	30.35**	8.15	8.32**
Control	4.03	6.39	20.12	5.06	1.94	10.87	6.61	5.55	2.06	6.25	7.50	4.64	9.40	2.70	7.96	3.09
RC-101																
102-6	6.70**	14.95**	19.00	9.28**	2.20**	28.75**	8.40**	17.92**	2.50**	21.08**	8.40**	13.97**	10.70"	14.48**	8.52	2.79
77-2	6.05**	21.25**	17.35**	15.25**	2.10**	47.35**	8.70**	19.57**	2.20	28.75**	8.00	13.18**	10.00	18.56**	8.55	2.63
127-5	5.61**	15.01**	21.10**	4.71	2.30**	29.35**	7.50**	11.33**	2.40*	21.52**	8.00	13.18**	9.54*	12.67**	8.54	2.31
117-8	5.38**	23.25**	20.50*	13.26**	2.00**	33.33**	7.90**	11.08**	1.90**	29.88**	8.60**	18.34**	10.47**	13.93**	8.71**	8.29**
139-4	5.35**	45.41**	13.60**	21.42**	2.10**	27.03**	8.10**	32.11**	2.90**	61.79**	6.10**	26.15**	8.60**	18.34**	8.47	3.94
117-9	5.32**	16.38**	20.60*	15.21**	2.30**	21.00**	9.20**	12.34**	2.40*	21.52**	7.80	17.93**	9.82	13.36**	8.51	6.69**
103-5	5.30**	26.01**	16.30**	10.04**	2.10**	35.14**	7.50**	19.12**	1.80**	23.42**	8.40**	12.80**	10.35**	10.45**	8.42	4.87*
120-3	5.30**	18.28**	18.20**	8.90**	2.60**	19.86**	8.20**	14.99**	2.20	41.77**	8.90**	12.37**	11.40**	9.43**	8.53	2.88
91-1	5.29**	25.31**	22.70**	27.48**	1.20**	65.73**	8.90**	27.75**	2.80**	40.55**	8.30*	17.09**	11.90**	12.18**	8.52	2.18
129-6	5.27**	28.36**	20.50**	6.60*	2.30**	41.25**	9.20**	19.03**	2.30	35.79**	9.30**	16.07**	11.24**	12.07**	8.33	2.25
Control	4.11	10.73	19.43	4.67	1.71	13.11	6.86	5.92	2.20	10.67	7.84	4.22	9.91	2.39	8.37	3.17

*,**Significant at p = 0.01 and 0.05 respectively

Table 5. Habitability in broad sense (h^2_{bs}) and genetic advance as percentage of mean (GA) in M_3 progenies of two cowpea varieties

Characters	Varieties			
	RC 19		RC 101	
	h^2_{bs}	GA	h^2_{bs}	GA
Plant height (cm)	45.00	16.08	59.78	29.40
Branches/plant	10.58	09.30	30.18	38.29
Pods/plant	46.08	25.14	18.36	08.42
Clusters/plant	18.89	1483	14.93	13.14
Seeds/pod	30.47	13.85	27.46	12.94
Pod length (cm)	27.82	10.11	35.78	13.47
100 seed weight (g)	11.78	01.68	18.20	02.11
Seed yield/plant (g)	21.90	10.58	21.05	13.31

2. **Kharkwal M. C., Pandey R. N. and Pawar S. E.** 2004. Mutation breeding for crop improvement. *In*: Plant Breeding — Mendelian to Molecular Approaches (H.K. Jain and M. C. Kharkwal eds.). Narosa Publishing House Pvt. Ltd., New Delhi: 601-646.

3. **Kumar D. and Narayan P.** 2005. Production Technology for Cowpea. AICRP on And Legumes, CAZRI, Jodhpur, Rajasthan, India.

4. **Blixt S.** 1972. Mutation Genetics in *Pisum*. Agn. Hort. Genet., **30**: 1-293.

5. **Konzak C. P., Nilan R. A., Wagnor J. and Foster R. J.** 1965. Efficient chemical mutagensis. Radiat. Bot. (Suppl.), **5**: 49-70.

6. **Snedecor G. W. and Cochran W. C.** 1967. Statistical Methods. Oxford and IBH Publishing Company, New Delhi pp. 100-113.

7. **Mahana S. K., Bhargava A. and Mohan L.** 1990. Alkaline azide mutagenecity in cowpea. Mut. Breed. News, **36**: 6-7.

8. **Murugan S. and Subramanian M.** 1993. Variability studies for polygenic traits in M_3 and M_4 generations of cowpea (*Vigna unguiculata*). Crop Res. Hissar, **6**: 264-269.

9. **Lawhale A. D.** 1982. Note on the genetic variability in quantitative characters of cowpea in M_3 generation. Indian J. Agric. Sci., **52**: 22-23.

10. **Kharkwal M. C.** 2001. Induced mutations in chickpea (*Cicer arietinum* L.) V. Evaluation of micro-mutations. Indian J. Genet., **61**: 115-124.

11. **Sharma B.** 1995. Mutation breeding through polygenic variability. *In*: Genetic research and education: Current trends and next fifty years (B. Sharma eds.). Indian Society of Genetics and Plant Breeding . New Delhi : 1210-1219.

12. **Choulwar S. B. and Borikar S. T.** 1985. Variability studies for polygenic traits in M_3 generation of cowpea. J. Maharashtra Agric. Univ., **10**: 198-199.