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Effect of Chemical Mutagen on Expression of Characters in Arid Legume Pulse -Cowpea (*Vigna unguiculata* (L.) Walp.)

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Abstract: Studies on chemical induced mutagenesis, Ethyl Methane Sulphonate (EMS) were performed by exposing the healthy and dry seeds of cowpea variety Co 4 to E MS at 10, 20, 30, 40 and 50mM. The study was economically important to evolve mutants with varied seed coat colour as against dark grey colored seed coat of Co 4. The LD_{50} value was found at 30mM for EMS. Under field conditions germination, seedling survival, plant height on 30th day, pollen fertility, seed fertility, pods per plant, pod length, seeds per pod, 100 seed weight and single plant yield was reduced as compared to the control. In M_2 generation, viable macro mutants like dwarf mutant, spreading type, late mutant, early mutant, semi sterile type, single and tri cotyledonary leaf mutant, basal branching, multiple leaf mutant, white flower mutant, and chimeric mutant were observed. Lower concentrations resulted in single type and higher concentration produced multiple type mutations. Economically important macro mutants such as white seed coat colour mutants were observed in M_2 generation.

Key words: cowpea, EMS, genetic advance, heritability, mutagen, seed colour, yield characters

INTRODUCTION

Arid legumes are the pulse crops cultivated with less water requirement or in dry land conditions. Cowpea is one among them, being a self pollinated crop creation of variability is important for the crop improvement programmes. Pulses are important source of protein and are essential adjunct to a predominantly cereal based diet. Because of its high protein content (20-25%), cowpea is referred as poor man 's meat. Its young leaves, pods and seeds contain vitamins and minerals which have fuelled its usage for human consumption and animal feeding. It gives a heavy vegetative growth and covers the ground, that it checks the soil erosion in problem areas. Most of the crop improvement programmes attempted through conventional breeding methods have exploited only the natural variability available in the germplasm. Adequate variability is not available in the gene pool to change the plant ideotype. Under such circumstances, induced mutagenesis can be efficiently employed as an alternative to induce the variability in morphological and physiological characters. Among the cowpea varieties, Co 4 has duration of 85 days with the yield potential of 961 kg per hectare under irrigated condition. In spite of this, it lacks consumer preference, because of its unacceptable seed coat colour. Therefore altering the seed coat colour without affecting the other desirable characteristics can pave the way for more market preference. Keeping the above consideration in view, the present investigation was undertaken using the potent mutagen Ethyl Methane Sulphonate (EMS) in the variety Co 4 in order to change the testa colour of seed and to study the genetic variability in M_2 generations induced and to select the economic mutants in M_2 generation.

MATERIALS AND METHODS

Selfed seeds of parental line Co 4, were treated with the chemical mutagen Ethyl Methane Sulphonate (mono functional alkylating agent). Seven different concentrations of EMS ranging from 10 to 70mM with an interval of 10mM were used initially to fix the LD_{50} value. A total of 50 seeds were sown in germination paper, replicated twice for each treatment. Well filled 200 seeds were pre-soaked for six hours for each treatment in distilled water and there after, the soaked seeds were placed between the folds of blotting paper to remove the excess water adhering to the seed surface. Seeds were treated with different doses of EMS in double distilled water and pH of the mutagenic solution was adjusted to seven by phosphate buffer. The seeds were immersed for six hours in the required concentration of mutagen with intermittent shaking. After that, the seeds were thoroughly washed in tap

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water for ten times to eliminate the residual effect of the chemical and immediately sown in the field. A total of 60 treated seeds per treatment per replication were sown with single seeds per hill in the field along with control in randomized block design with two replications at the spacing of 30cm between rows and 15cm between plants. The recommended agronomic practices and plant protection measures were followed uniformly for all treatments. Non-irradiated dry seeds and pre-soaked seeds in distilled water for the six hours were used as control. Observations were recorded on shoot length, root length germination on 5^{th} and 10^{th} day, survival of plants on 30^{th} day, plant height on 30^{th} day, days to 50 per cent flowering, plant height at maturity, number of pods per plant, length of the pods, number of seeds per pods, 100 seed weight, seed yield per plant, pollen fertility, seed fertility/seed set, seed protein content and single plant yield in M_1 and M_2 generation.

Mutagenic Effectiveness and Efficiency: Mutagenic effectiveness pertains to the rate of mutation induction as related to mutagenic dose. Mutagenic efficiency is referred to as the mutation rate in relation to M_1 damage like lethality, injury and sterility. The effectiveness and efficiency of EMS were worked out by using the formulae suggested by Konzak *et al*.[11].

$$\text{Mutagenic effectiveness} = \frac{\text{Mx100}}{\text{Conc. (mM)}}$$

Where,

M = mutation frequency for 100 M_2 plants, Conc. = concentration of chemical mutagen in mM or per cent

Mutagenic efficiency = M x 100/L
= M x 100/I
= M x 100/S where,

M = mutation frequency for 100 M_2 plants, L = percentage of lethality or survival reduction,

I = percentage of injury or reduction in seedling size, S = percentage of sterility i.e., reduction in seed fertility.

The mean and variance of M_2 generation of the different treatments were subjected to appropriate statistical analysis. The over all sum of square due to treatments was partitioned among different sources following the method of Allard [2]. Heritability in broad sense was computed for each character using the following formula[14]. Genetic advance for a particular trait was estimated by adopting the method as suggested by Johnson *et al*.[9]. Analysis of skewness and kurtosis was estimated by adopting the following formula suggested by Fisher[6]. The seed protein content was estimated by microkjeldhal method[8].

RESULTS AND DISCUSSION

Analysis of variance for different characters under in M_1 generation was given in Table 1. Estimates of mean for different characters in M_2 generation were given in Table 2. The effect of EMS on germination, shoot length and root length was studied and results were presented in Table 3. Under laboratory conditions, the germination percentage ranged from 29.00 (70mM) to 84.00 (10mM) in EMS treatments. Since 50 per cent reduction was obtained at 30mM, the LD_{50} value was fixed as 30mM for EMS treatments.

The mean pod length decreased from 16.71 cm to 15.64cm Single plant yield decreased from 23.79g (10mM) to 18.90g (40mM) (Table 2). The per cent reduction over control varied from 21.46 to 58.11.Reduction in pod number may be due to a probable inhibiting action of enzymes, changes in the enzymes activity and the toxicity of the mutagen, on these attributes[3]. The marked reduction caused by mutagens in seed yield per plant can be attributed to high seed sterility and reduced pod number as caused by physiological and biochemical disturbances in the development of plants[16]. The decline in yield could also be probably due to indirect influence of altered yield contributing components. The seed protein content ranged from 22.62 (50mM) to 23.91 (20mM) per cent. Except 20mM all the other mutagenic treatments recorded lesser protein content than their respective control. The hampered protein synthesis in the embryonic cells could also prevent passage of cells in different stages of mitosis thereby retarding the emergence of root and shoot. Reduction in seedling height was noticed to be proportionate to the increase in dosage of mutagen. Cherry and Lessman[4] reported that the reduction in plant height can be attributed to the inhibition of growth due to low rate of cell division, decreased amylase activity and increased peroxide activity.

Physical and chemical mutagens induce physiological damages (injury), gene mutations (point mutations) and chromosomal mutations (chromosomal aberrations) in the biological material in M_1 generation [7]. The biological damage caused by the mutagens in M_1 generation could be measured based on seed germination, survival reduction (lethality), plant height reduction (injury) and seed fertility reduction (sterility) (Table 7).

The decrease in germination due to mutagenic treatments observed was also in conformity with the earlier reports of Deepalakshmi[5] and Thanga Hemavathi[21] in black gram. The seed germination was reduced more under chemical mutagen Gaul[7] reported that the damage to the biological material as reflected in the above parameters might be considered as an

indication of the mutagenic effects. In the present study considerable reduction in shoot and root development was noted. A linear relationship was exhibited between the mutagenic dosage and development of shoot and root. The influence on shoot and root growth has been related to many factors which include chromosomal abnormality with height reduction, reduction in auxin levels, inhibition of auxin synthesis, failure of assimilation mechanisms and chromosomal damage-cum-mitotic inhibition[17]. The per cent reduction for pollen fertility ranged from 9.93 (10mM) to 48.54 (50mM). An increase in dose/concentration of the mutagen led to an increase in per cent reduction in pollen fertility. Larik[13] reported that the pollen fertility reduction may be due to cumulative effects of various aberrant meiotic stages as well as physiological and genetic damages that induced probably by the breakage of chromosome through formation of an anti metabolic agent in the cell or may be due to irregular disjunction of chromosomes at anaphase. The disjunction of chromosome may result from the formation of interchanges and multivalent or orientation of chromosome at metaphase I[12].

The spectrum of chlorophyll mutants and the relative frequencies of different types of chlorophyll mutants are given in percentage and presented in Table 4 & 5. Four types of chlorophyll mutants *viz.,* albino, xantha, chlorina and viridis were observed in M_2 seedlings. Xantha found in higher proportion in all the treatments followed by chlorina. Viridis occurred only in 20mM and 30mM concentrations. Chlorina occurred 100 per cent in 50mM concentrations. The pooled segregation showed inconsistent trend with dosage of treatments. Viable mutants were recorded from early seedling stage to complete maturity stage. The pooled segregation ratio of chlorophyll mutants on M_1 plant basis showed an inconsistent trend with dosage of gamma rays and EMS. These findings are in conformity with Ahmed John[1] in black gram. In the present study, maximum segregation was less than 25 per cent in gamma rays and less than 40 per cent in EMS.

The data on frequency of viable mutations computed on M_1 plant and M_2 seedling basis (Table 8). In chemical mutagen, the minimum and maximum frequency was observed in 40mM and 20mM with the value of 20.00 and 33.33 per cent respectively on M_1 plant basis. The efficiency was higher mostly at lower doses both for chlorophyll and viable mutants than at higher doses on M_1 plant and M_2 seedling basis. This was in confirmation with the findings of Khan[10] in black gram. This may be due to the fact that the biological damages increased with the increase in dose at a rate greater than the frequency of mutation[11]. Thus, the mutagenic effectiveness and efficiency will also depend upon the nature of induced mutation or aberrations. On M_2 seedling basis, the range was from 1.25 (20mM) to 2.05 (30mM) for EMS treatments. The mutants were scored in M_2 generation. The viable mutants were grouped in to plant height, leaf modifications, variation in branching habit, floral mutants, pod and seed mutants and others. In the present investigation viable macro mutations with changes in attributes like stature, duration, cotyledon, stem, leaf, pod, flower and seed mutants were recorded. Stature mutants namely dwarf, spreading and duration mutants like early and late mutant were observed. Vanniarajan[22] observed semi spreading mutants in gamma ray treatment alone in black gram.

Multiple leaf mutants and other type of leaf abnormalities were noticed (Table 6). This includes leaf let with varied shapes and textures. The leaf shape mutants showed leaflet which were ovate, broad, narrower, crinkled and smaller than normal leaflets. Isolation of more than one type of mutation from single M_1 plant progeny is termed as multiple mutations or multi mutations.. The mutagenic effectiveness of chlorophyll mutations on M_1 plant basis and M_2 seedlings basis are furnished in Table 7. The effectiveness varied from 13.34 (50mM) to 133.30 (10mM) on M_1 plant basis and 1.33 (40mM) to 3.90 (20mM) on M_2 plant basis. It can be assumed that multi mutational events affect several genes and thus several enzymes or proteins, resulting in pleiotropic effect. Most of the mutants bearing multi mutational events thus may be lethal in the first generation, affecting the frequency of occurrence of multi mutations in M_2 and future generations[23]. In the present study the mutants exhibiting brownish white seed coat colour were identified. Similar findings were obtained by Singh and Yadav[19] in green gram. Chimeric mutants were identified in gamma ray treatments. Similar type of mutants was recorded by Thakur[20] in cowpea. Total mutation frequency was arrived at by adding up frequency of chlorophyll, non-viable and viable mutations. The total mutation frequency rate was 3.05 in 30mM and 1.83 at 10mM (Table 8). Assessment of variance has been the most dependable statistical measure to find the mutagenic effect on the polygene. The genotypic coefficient of variation provides a mean to study the genetic variability generated in quantitative characters[9]. The response of mutagens as measured by the magnitude and the nature of variability varied from character to character. Maximum GCV 8.57 and maximum PCV 9.48 per cent was obtained at 10mM. The maximum heritability of 95.33 recorded at 20mM and maximum GA as per cent of mean (15.98) was obtained at 10mM. Maximum GCV and PCV for plant height at maturity were noticed at 10mM. At 30mM recorded maximum GCV, PCV for days to 50 per cent flowering, the maximum GCV, PCV was observed at 50mM for number of pods per plant, pod length, 10mM registered the maximum PCV for pod length, 30mM and 40mM recorded maximum GCV for number of seeds per pod, 20mM recorded maximum GCV,

PCV for 100 seed weight, 40mM for single plant yield. This result was consonance with Sheeba[18], the maximum GCV was in plant height and number of seeds per capsule in sesame. Mathew *et al.*[15] reported highest estimates of GCV for plant height, seed yield per plant, pods per plant and 100 seed weight.

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Table 1: Analysis of variance for different characters under in M_1 generation

Characters	EMS		
	Replication	Treatment	Error
Germination	0.25	1389.10**	1.96
Shoot length	0.02	253.34**	0.79
Root length	0.525	32.21**	0.57
Germination on 5 th day	0.33	834.00**	1.13
Germination on 10 th day	5.33	733.28**	1.53
Survival on 30 th day	3.00	850.93**	0.60
Plant height on 30 th day	1.92	135.58**	0.51
Days to 50 % flowering	4.08	24.55**	0.68
Pollen fertility	0.62	625.37**	0.19
Seed fertility	0.35	439.85**	1.20
Pod length	0.06	10.02**	0.47
Number of pod / plant	6.31	19.69**	0.80
Number of seeds / pod	2.47	21.15**	0.30
Plant height at maturity	0.16	145.49**	2.15
100 seed weight	0.43	9.83**	0.52
Single plant yield	0.08	60.78**	0.69

** Significance at 1 % level
*Significance at 5 % level

Table 2: Estimates of mean for different characters in M 2 generation

EMS (mM)	Days to 50% flowering	Plant height	No. of pods/pl.	Pod length	No. of seeds/pod	100 seed weight	Single plant yield
Control	49.03 ± 1.25	64.01 ± 1.41	19.46 ± 0.93	16.71 ± 0.72	16.56 ± 0.80	12.01 ± 0.67	21.68 ± 0.25
10	50.13 ± 0.58	53.84 ± 1.77	19.11 ± 0.96	16.38 ± 0.92	15.40 ± 1.01	11.24 ± 0.75	23.79 ± 1.00
20	49.20 ± 0.95	55.17 ± 0.68	18.20 ± 0.80	15.64 ± 0.88	14.97 ± 0.97	10.31 ± 0.71	19.84 ± 0.72
30	52.37 ± 1.10	50.48 ± 1.14	20.33 ± 0.90	16.52 ± 0.82	15.86 ± 1.13	11.71 ± 0.60	22.58 ± 0.43
40	53.17 ± 1.05	47.29 ± 1.01	16.34 ± 0.77	15.92 ± 0.53	13.28 ± 1.12	10.29 ± 0.82	18.90 ± 0.00
50	56.10 ± 0.90	48.07 ± 1..52	19.04 ± 1.03	16.19 ± 0.85	15.13 ± 0.92	11.36 ± 1.12	23.02 ± 1.07

Table 3. Effect of treatments on seedling growth under laboratory condition in M_1 generation

EMS (Conc.)	Germination (%)				Shoot length (cm)			Root length (cm)		
	Mean ± SE (per cent)	Transformed mean	Per cent on control	Per cent reduction	Mean ± SE(cm)	Transformed mean	Per cent on control	Mean ± SE (per cent)	Transformed mean	Per cent on control
Control	98.50	83.33	100.00	0.00	33.20	100.00	0.00	14.29	100.00	0.00
10	84.00	71.42	85.28	14.72	32.25	97.13	2.87	14.01	98.04	1.96
20	72.50	58.82	73.60	26.40	30.79	92.74	7.26	11.74	82.15	17.85

Table 3: Continue

30	49.50	45.45	50.25	49.75	24.72	74.45	25.55	9.02	63.12	36.88
40	39.50	40.00	40.10	59.90	20.51	61.77	38.23	6.23	43.59	56.41
50	37.00	38.46	37.56	62.44	13.59	40.93	59.07	7.83	54.79	45.21
60	34.50	37.03	35.02	64.98	7.65	23.04	76.96	3.89	27.22	72.78
70	29.00	33.33	29.44	70.56	4.62	13.91	86.09	4.88	34.14	65.86
	SE = 1.40 CD (0.05) = 3.31			SE = 0.89 CD (0.05) = 2.11			SE = 1.35 CD (0.05) = 3.31			

Table 4: Frequency of chlorophyll mutants in M_2 generation

EMS (mM)	Number of M_1 plant progenies		Number of M_2 seedlings		Mutation frequency	
	Scored	Segregated	Scored	Segregated	Per 100 M_1 plants	Per 100 M_2 seedlings
10	15	2	750	2	13.33	0.27
20	15	2	645	5	13.33	0.78
30	15	4	600	6	26.67	1.00
40	15	3	570	3	20.00	0.53
50	15	1	525	4	6.67	0.76

Table 5: Spectrum of chlorophyll mutants in M_2 generation

EMS (mM)	Total number of mutants in M_2	Spectrum of (Relative percentage) chlorophyll mutants			
		Albina	Xantha	Chlorina	Viridis
10	2	-	50.00	50.00	-
20	5	-	40.00	20.00	40.00
30	6	16.67	66.67	-	16.67
40	3	-	33.33	66.67	-
50	4		-	100.00	-

Table 6: Frequency and percentage of M_1 plant progenies segregating for single and multiple chlorophyll mutants in M_2 generation.

EMS (mM)	Number of M_1 plant progenies segregating	M_1 plant progenies segregating for chlorophyll mutants					
		Frequency			Relative percentage		
		One type	Two type	Three type	One type	Two type	Three type
Control	-	-	-	-	-	-	-
10	2	1	1	-	50.00	50.00	-
20	2	1	1	-	50.00	50.00	-
30	4	1	3	-	25.00	75.00	-
40	3	1	1	1	33.33	33.33	33.34
50	1	1	-	-	100.00	-	-

Table 7: Mutagenic effectiveness and efficiency based on chlorophyll mutants -M_1 plant basis

EMS (mM)	Percent survival reduction Lethality(L)	Percent height reduction Injury (I)	Percent seed fertility reduction Sterility (S)	Mutants per 100 M_1 plants	Effectiveness	Efficiency		
					M x 100 Conc.mM	M x 100 L.	M x 100I.	M x 100S
10	10.41	6.54	8.42	13.33	133.30	128.04	203.82	158.31
20	14.46	22.29	12.28	13.33	66.65	92.19	59.80	108.55
30	22.55	22.90	21.83	26.67	44.43	118.27	116.49	122.17
40	37.00	39.47	25.13	20.00	50.00	54.05	50.67	79.59
50	56.67	41.72	44.98	6.67	13.34	11.77	15.99	14.83

Table 8: Mutagenic effectiveness and efficiency based on viable mutants – M_1 plant basis

EMS (mM)	Per cent survival reduction Lethality (L)	Per cent height reduction Injury (I)	Per cent seed fertility reductionSterility (S)	Mutants per 100 M_1 plants	Effectiveness	Efficiency		
					M x 100 Conc.mM	M x 100 L.	M x 100 I.	M x 100 S
10	20.00	6.54	8.42	26.67	266.7	133.35	407.80	316.75
20	21.54	22.29	12.28	33.33	166.65	154.74	149.53	271.42
30	31.29	22.90	21.83	26.67	88.9	85.23	116.46	122.17
40	43.08	39.47	25.13	20.00	50.00	46.43	50.67	79.59
50	61.54	41.72	44.98	20.00	40.00	32.50	47.94	44.46

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