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Research Article

Effect of physical mutagen on expression of characters in arid legume pulse cowpea *(Vigna unguiculata (L.) Walp.)*

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Abstract

Studies on physical induced mutagenesis, gamma rays were performed by exposing the healthy and dry seeds of cowpea variety CO 4 to gamma rays 20, 30, 40, 50, 60 and 70 kR. The study was to evolve economically important mutants with varied seed coat colour as against dark grey coloured seed coat of CO 4. The LD_{50} value was found at 50kR for ^{60} Co gamma rays. 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, semi sterile type, single and tri cotyledonary leaf mutant, basal branching, multiple leaf mutant, white flower mutant, chimeric mutant and seed coat colour mutant were observed. Gamma rays induced higher proportion of chlorophyll mutations. Single type and multiple type mutations occurred more frequently. Economically important macro mutants such as brownish white seed coat colour mutants were observed in M_2 generation.

Key words: mutagen, gamma rays, seed colour, yield characters, macromutation.

Introduction

Pulses form an important part of Indian dietry. They are an important source of protein and are essential adjunct to a predominantly cereal based diet and enhance the biological value of the protein consumed. Cowpea is tropical grain legume which plays an important role in developing countries of the tropics and sub tropics, especially in sub Saharan Africa, Asia, Central and South America, and is cultivated over area of 12.5 million hectare, with an annual production of over 3 million tonnes world wide (Singh *et al*., 1991). 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. Cowpea is a most versatile kharif pulse crop because of its smothering nature, drought tolerant characters, soil restoring properties and multipurpose uses.

Most of the crop improvement programmes attempted through conventional breeding methods have exploited only the natural variability available in the germplasm. Selection within local types exercised for a long time, exhausted the natural genetic variability of the crop. Adequate variability is not available in the gene pool to change the plant ideotype or to correct specific deficiency of otherwise outstanding genotypes. 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. Inspite 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 potent mutagens gamma rays in the variety Co 4 in order to change the testa colour of seed through mutagenesis, 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 physical mutagen gamma rays (ionizing radiation). Six different dosages starting from 20 to 70 kR with an interval of 10kR were administered for fixing the LD_{50} value. A total of 50 seeds were sown in germination paper, replicated twice for each

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treatment. Seed germination was counted in all the treatments. Well filled, 200 seeds were sealed in polythene bags and placed in a 5000 curie ^{60}Co gamma chamber with capacity to release 576kR/hr. 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 5th and 10th day, survival of plants on 30th day, plant height on 30th day, days to 50 per cent flowering, plant height at maturity, number of pods per plant, length of 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 gamma rays were worked out by using the formulae suggested by Konzak *et al*. (1965).

$$\text{Mutagenic effectiveness} = \frac{M \times 100}{\text{Krad}}$$

where,

M = mutation frequency for 100 M_2 plants
Krad= dose of physical mutagen in kilorad

$$\text{Mutagenic efficiency} = M \times 100/L = M \times 100/I = M \times 100/S$$

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 values of M_2 generation of the different treatments were subjected to appropriate statistical analysis. The over all sum of square due to treatments was partioned among different sources following the method of Allard (1960). Heritability in broad sense was computed for each character using the following formula (Lush, 1940). Genetic advance for a particular trait was estimated by adopting the method as suggested by Johnson *et al*. (1955). Analysis of skewness and kurtosis was estimated by adopting the following formula suggested by Fisher (1950). The seed protein content was estimated by microkjeldhal method (Jackson, 1973).

Results and Discussion

Analysis of variance for different characters under gamma ray treatments 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 gamma ray on germination, shoot length and root length was studied and results were presented in Table 3. Under laboratory conditions, the germination percentage ranged from 24.00 (70kR) to 86.00 (20kR) in gamma ray treatments. Since 50 per cent reduction was obtained at 50kR, the LD_{50} value for germination was fixed as 50kR for gamma ray treatments.

The mean pod length decresed from 18.73 cm to 14.73 cm. Single plant yield decreased from 24.40g to 17.31g (Table 2). The per cent reduction in single plant yield varied from 16.30 (20kR) to 51.90 (60kR). 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 (Blinks, 1952).

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 (Ramaswamy, 1973; Prabhakaran, 1992). The decline in yield could also be probably due to indirect influence of altered yield contributing components.

The seed protein content ranged from 21.89 (70kR) to 23.84 (40kR) per cent in gamma ray treatment. Except 40kR 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. In the present investigation, there was proportionate reduction in germination under field condition with increase in dosage of gamma rays. A reduction in seedling height was noticed to be proportionate to the increase in dosage of the mutagen. Cherry and Lessman (1967) 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 (Gaul, 1970). 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).

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 (Riley, 1954).

The per cent reduction for pollen fertility ranged from 16.01 (20kR) to 64.02 (70kR). An increase in dose of the mutagen led to an increase in per cent reduction in pollen fertility. Larik (1975) 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 antimetabolic 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 (Kumar and Das, 1973).

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.,* albina, xantha, chlorina and viridis were observed in M_2 seedlings. Albina occurred at 60kR, while xantha and chlorina occurred in all the treatments. Viridis found in 50kR and 60kR only. The pooled segregation showed an inconsistent trend with dosage of treatments. Viable mutants were recorded from early seedling stage to complete maturity stage. The highest chlorophyll mutation frequency was noticed in gamma ray treatment. This was in confirmation with the findings of Raveendran and Jayabalan (1997) in cowpea. In the present study, maximum segregation was less than 25 per cent in gamma rays.

The data on frequency of viable mutations computed on M_1 plant and M_2 seedling basis are furnished in Table 8. The viable mutation frequency was observed to be 13.00 (60kR) to 33.33 (50kR) on M_1 plant basis and 1.43 (40kR) to 1.93 (50kR) on M_2 seedling basis with maximum frequency of 33.33 per cent in 50kR and 1.93 in 50kR for M_1 plant basis and M_2 seedling basis respectively. 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 (1999) in blackgram. This may be due to the fact that the biological damages increased with the increased with the increase in dose at a rate greater than the frequency of mutation (Konzak *et al*., 1965). Thus, the mutagenic effectiveness and efficiency will also depend upon the nature of induced mutation or aberrations. The viable mutants were grouped into 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 early and late duration mutants were observed. Vanniarajan (1989) observed semi spreading mutants in gamma ray treatment alone in blackgram.

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 multimutations. . Sharma and Orav (1965) reported the occurrence of multimutations to be non-random in peas. The mutagenic effectiveness of chlorophyll mutations on M_1 plant basis and M_2 seedlings basis are furnished in Table 7. The effectiveness of chlorophyll mutations for gamma rays ranged from 11.12 (60kR) to 66.67 (30kR) on M_1 plant basis and 1.27 (70kR) to 3.53 (30kR) on M_2 plant basis

It can be assumed that multimutational events affect several genes and thus several enzymes or proteins, resulting in pleiotropic effect. Most of the mutants bearing multimutational events thus may be lethal in the first generation, affecting the frequency of occurrence of multimutations in M_2 and future generations (Waghmare and Mehra, 2001).

In the present study the mutants exhibiting brownish white seed coat colour were identified. Similar findings were obtained by Singh and Yadav (1991) in greengram. Chimeric mutants were identified in gamma ray treatments. Similar type of mutants was recorded by Thakur (2004) 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 observed in the study was 3.96 in 60kR and 1.96 in

40kR (Table 8). Assessment of variance has been the most dependable statistical measure to find the mutagenic effect on the polygenes. The genotypic coefficient of variation provides a mean to study the genetic variability generated in quantitative characters (Johnson *et al*., 1955). The response of mutagens as measured by the magnitude and the nature of variability varied from character to character.

The maximum GCV was observed at 60kR for plant height, days to 50 per cent flowering, single plant yield, 70kR for number of pods per plant, pod length, number of seeds per pod, 40kR for 100 seed weight. The maximum PCV was recorded at 60kR for plant height, days to 50 per cent flowering, 70kR for number pods per plant, pod length, number of seeds per pod, 40kR for 100 seed weight and 60kR for single plant yield. This result was consonance with Sheeba (2002), the maximum GCV was in plant height and number of seeds per capsule in sesamum. Mathew *et al.* (2005) 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 gamma ray treatments in M_1 generation

Characters	Gamma rays		
	Replication	Treatment	Error
Germination	1.14	1416.00**	6.80
Shoot length	7.26	253.14**	0.57
Root length	5.99	38.33**	1.83
Germination on 5 th day	0.07	1027.74**	1.71
Germination on 10 th day	5.78	1055.40**	2.12
Survival on 30 th day	1.14	1052.9**	1.81
Plant height on 30 th day	0.75	22.57**	1.51
Days to 50 % flowering	2.57	39.48**	1.57
Pollen fertility	3.43	1227.68**	0.25
Seed fertility	0.46	173.16**	0.91
Pod length	0.11	4.51**	0.43
Number of pod / plant	0.05	17.26**	0.67
Number of seeds / pod	0.10	8.40**	0.32
Plant height at maturity	4.57	160.05**	1.78
100 seed weight	0.29	11.53**	0.27
Single plant yield	0.56	42.21**	0.36

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

0

Table 2. Estimates of mean for different characters in M_2 generation

Gamma rays(kR)	Days to 50% flowering	Plant height (cm)	No. of pods/ plant	Pod Length (cm)	No.of seeds/pod	100 seed weight (g)	Single plant yield
Control	48.43 ± 0.65	62.89 ± 0.90	19.26 ± 0.90	18.62 ± 0.67	16.66 ± 0.75	12.32 ± 0.89	24.24 ± 1.16
20	49.40 ± 1.15	52.17 ± 1.09	18.60 ± 0.79	16.68 ± 0.92	16.03 ± 1.21	11.27 ± 0.96	23.54 ± 0.20
30	48.33 ± 1.06	49.03 ± 1.15	21.01 ± 0.86	18.73 ± 0.82	17.14 ± 0.88	12.37 ± 1.04	24.40 ± 1.12
40	50.27 ± 0.78	46.69 ± 1.58	17.26 ± 0.64	16.34 ± 0.78	14.20 ± 0.88	10.69 ± 1.18	22.80 ± 0.92
50	53.10 ± 1.05	48.25 ± 0.94	17.72 ± 0.86	16.50 ± 0.82	14.77 ± 0.58	11.43 ± 0.86	22.86 ± 0.75
60	51.83 ± 0.95	45.10 ± 1.38	15.01 ± 0.53	14.73 ± 0.66	12.63 ± 0.99	9.59 ± 0.69	17.31 ± 0.85
70	55.07 ± 0.94	46.81 ± 1.40	18.04 ± 0.99	15.32 ± 0.92	14.26 ± 0.98	10.04 ± 0.82	19.56 ± 1.39

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

Germination (%)				Shoot Length (cm)			Root Length(cm)		
Mean ± SE (per cent)	Transformed mean	Per cent on control	Per cent reduction	Mean ± SE(per cent)	Transformed mean	Per cent on control	Mean ± SE (per cent)	Per cent on control	Per cent reduction
98.00	83.33	100.00	0.00	34.12	100.00	0.00	18.86	100.00	0.00
86.00	71.43	87.75	12.25	36.64	107.38	-7.38	18.97	100.58	-0.58
83.50	66.67	85.20	14.80	26.91	78.86	21.14	12.48	66.17	33.83
69.50	58.82	70.92	29.08	27.79	81.44	18.56	14.50	76.88	23.12
51.50	47.62	52.55	47.45	17.69	51.84	48.16	13.77	73.01	26.99
39.50	40.00	40.31	59.69	11.77	34.49	65.51	9.75	51.69	48.31
24.00	29.41	24.48	75.72	6.96	20.39	79.61	7.15	37.91	62.09
SE = 2.56 **CD (0.05) = 6.27** **CD (0.01) = 9.50**				**SE = 0.75** **CD (0.05) = 1.85** **CD (0.01) = 2.81**			**SE = 0.75** **CD (0.05) = 1.78** **CD (0.01) = 2.63**		

Table 4. Frequency of chlorophyll mutants in M_2 generation

Gamma rays(kR)	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
20	15	2	750	5	13.33	0.70
30	15	3	750	8	20.00	1.06
40	15	3	750	4	20.00	0.53
50	15	5	675	9	33.33	1.33
60	15	1	600	8	6.67	1.33
70	15	2	450	4	13.33	0.89

Table 5. Spectrum of chlorophyll mutants in M_2 generation

Gamma rays(kR)	Total number of mutants in M_2	Spectrum of (Relative percentage) chlorophyll mutants			
		Albina	Xantha	Chlorina	Viridis
20	5	-	40.00	60.00	-
30	8	-	37.50	62.50	
40	4	-	50.00	50.00	-
50	9	-	33.33	44.45	22.22
60	8	12.50	37.50	25.00	25.00
70	4	-	75.00	25.00	-

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

Gamma rays (kR)	Number of M_1 plant progenies segregating	M_1 plant progenies segregating for chlorophyll mutants					
		Frequency			Relative percentage		
		One type	Two types	Three types	One type	Two types	Three types
Control	-	-	-	-	-	-	-
20	2	1	1	-	50.00	50.00	
30	3	1	2	-	33.33	66.67	
40	3	1	1	1	33.33	33.33	33.34
50	5	1	3	1	20.00	60.00	20.00
60	1	1	-	-	100.00	-	-
70	2	-	1	-	50.00	50.00	-

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

Gamma rays (kR)	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 / kR or Conc.	M x 100 / L.	M x 100 / I.	M x 100 / S
20	8.65	3.23	12.27	13.33	66.65	154.1	412.6	108.6
30	16.76	6.77	18.99	20.00	66.67	119.3	295.4	105.3
40	28.11	7.80	17.99	20.00	50.00	71.1	256.4	111.1
50	35.68	10.09	13.47	33.33	66.67	93.4	330.3	247.4
60	61.09	12.71	27.13	6.67	11.12	10.9	52.4	24.5
70	64.32	20.77	30.91	13.33	19.04	10.3	32.1	21.5

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

Gamma rays (kR)	Per cent survival reduction Lethality (L)	Per cent height reduction Injury (I)	Per cent seed fertility reduction Sterility (S)	Mutants per 100 M_1 plants	Effectiveness	Efficiency		
					M x 100 / kR or conc.	M x 100 / L.	M x 100 / I.	M x 100 / S
20	8.65	3.23	1.21	26.67	133.35	308.32	825.70	217.36
30	16.76	6.77	18.99	26.67	88.9	159.13	393.94	140.44
40	28.11	7.80	17.99	20.00	83.33	71.15	256.41	111.17
50	35.68	10.09	13.47	33.33	40.00	93.41	330.33	247.43
60	61.09	12.71	27.13	13.00	21.67	21.28	102.28	47.92
70	64.32	20.77	30.91	20.00	28.57	31.09	96.29	64.70