

INDUCED GENETIC VARIABILITY IN COWPEA [*VIGNA* UNGUICULATA (L.)WALP] VAR. PUSA KOMAL

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INTRODUCTION

Cowpea is widely cultivated throughout the world but especially in Africa, Asia and South American continent where plant protein comprises 3% of the total dietery protein (Mahe et al., 1994). Cowpea represents one of the best hopes for combating shortage in food supply. Dry seeds of cowpea are known as "Vegetable meat" due to high amount of quality protein (23.4%), carbohydrate (60.3%), fat (1.8%), iron (57 mg/100g) and vitamins such as riboflavin (0.18 mg/100 g), nicotinic acid (1.9 mg/100 g) and thiamin (0.92 mg/100 g).

ABSTRACT

Genetic variability is the most essential prerequisite for any successful crop improvement programme as it provides spectrum of variants for the effective selection, which can be achieved through the processes of hybridization, recombination, mutation and selection. Genetic variability has been exhausted in legumes due to natural selection and hence conventional breeding methods are not fruitful. They generally loose different alleles for high productivity, seed quality, pest and disease resistance during the processes of adaptation to environmental stress. Exploitation of recombination variations through hybridization is less in cowpea since it is self-fertilized, due to dehiscence of anthers taking place before the opening of the flower. In addition, high rate of flower abortion nearly 70 to 75 per cent shed before anthesis and half of the remaining abort prematurely (Ojehomon, 1968). Hence, alternate methods of generating variability have gained greater importance in breeding of crop plants. In this context, artificial

assess the genetic variability in M₂ and M₃ generation. Analysis of variance in M₂ and M₃ population showed significant differences among the treatments for all the twelve yield and its attributing characters under study except protein per cent. For all the characters under study in both the generations, phenotypic coefficient of variation was higher in magnitude than genotypic coefficient of variation. High GCV as well as PCV was obtained in M₂ and M₃ generation for traits number of primary branches per plant, protein percent, yield per plant and hundred seed weight. High heritability estimates in coupled with high genetic advance expressed as percent of mean was recorded in protein percent in M₂ as well as M₃ populations.

In the present investigation, cowpea var. Pusa Komal was treated with physical mutagen gamma rays (100, 200,

300, 400, 500 Gy) and chemical mutagen EMS (0.25%, 0.30%, 0.35%, 0.40% and 0.45%) with an objective to

induction of genetic variation has been proposed as one of the quick methods of enlarging genetic variability in crop plants. Radiations are an important source of rearrangement of chromosomes and transmutation of genes. Gamma rays are known to influence plant growth and development by inducing cytological, genetical, biochemical, physiological and morphogenetic changes in cells and tissue (Gunckel and Sparrow, 1961). Chemical mutagens on the other hand, are known to produce high rate of gene mutation. Nevertheless, they create problem such as uncertain penetration to relevant target cell, poor reproducibility, persistence of the mutagen or its metabolites in the treated material and rick of safe handling. Among the chemical mutagens, EMS induces a vastly higher proportion of point mutations. Extensive studies over the past 70 years have been undertaken on mutagenesis for the induction of genetic variability and improvement of economic traits in several crop plants. It has also enhanced the genetic diversity in the germplasm of food and commercial crops. Radiations and chemical mutagens are the potential mutagenic agent for induction of mutations. During this period, around 2672 mutant varieties have been officially released in the world for commercial cultivation (http://www.mvgs.iaea.org/). The major contribution is from cereals followed by ornamental, legumes and oilseeds. Most of the mutant varieties were released in China (27.70%), India (12.70%), Russia (9.80%), Japan (8.70%), Germany (6.50%), USA (4.70%) and others (22.9%). Seven cowpea mutant varieties of radiation and chemical origin viz., V 16 (Amba), V-37 (Shreshtha), V-38

(Swarna), V-240, Co-5 (forage cowpea), Cowpea-88 (forage cowpea) and Khalleswari (TRC-77-4) have been released in India [Reddy and Dhanashekar (2007) and Maluszynski et *al.* (2000)]. Thus, proving its role in the creation of genetic variations resulting in new varieties with better characteristics (Arulbalachandran et *al.*, 2010).

Genetic improvement of any crop largely depending on the magni-tude of several genetic parameters like analysis of variance of each mean value, phenotypic and genotypic variance, phenotypic and genotypic coefficient of variation (PCV and GCV), broad sense heritability (h²) and genetic ad-vance (GA) on which the breeding methods are formulated for its further improvement. Cowpea being a potential leguminous vegetable crop of India has immense scope for genetic improvement for various qualitative and quantitative characters besides widening the genetic base through induced mutagenesis. Keeping this in view, the present study was planned, to ascertain the magnitude of induced genetic variability through the use of gamma rays and EMS in M₂ and M, generations for various yield and its attributing traits in cowpea var. Pusa Komal. It is a widely adaptable, indeterminate, dwarf, bushy and photo insensitive variety of cowpea. It has synchronous bearing habit with good quality less fibrous pods.

MATERIALS AND METHODS

The experimental material consisted of healthy well matured, non-dormant seeds of cv. Pusa Komal which were irradiated with physical mutagen gamma rays (100, 200, 300, 400, 500 Gy) and treated with chemical mutagen Ethyl Methane Sulphonate (CH₂SO₂OC₂H₅) of concentration 0.25%, 0.30%, 0.35%, 0.40% and 0.45% for 6h. Untreated seeds were used as control. For EMS treatment different concentration. The LD_{50} is of great importance to know the sensitivity of genotypes to the critical dose of mutagens causing 50% mortality. Criteria such as LD_{50} (50% viability) or GR_{50} (50% growth reduction) are used to choose the dose range. Ethyl Methane Sulphonate (EMS) is highly toxic therefore, it is used in low concentration. Dry seeds were irradiated in the gamma rays chamber at Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur with a source strength of 2000 Curie Co60 at a dose of 3000 rads/ minute. Dry seeds should be used for irradiation because the proportion of water is low hence the damage to cells is reduced. For treatment with chemical mutagen seeds were soaked in double distilled water for six hours at room temperature prior to treatment with mutagen solution to ensure complete saturation of seeds with water and initiate physiological activities in their embryo. The aqueous solution of different concentrations of EMS was prepared using double distilled water. Seeds were soaked in the freshly prepared mutagenic solutions for six hours and kept at room temperature with intermittent shaking after prior soaking in double distilled water for 6 hours. The seeds at the end of the treatment were washed in running tap water to make them free from the residues of mutagen sticking to the seed coat, dried with filter paper and sown immediately.

A set of 300 seeds were chosen for each dose/treatment including the control and control were sown in the field to raise the M_1 generation in Randomized Block Design with

three replications at a spacing of 30×10 cm. Based on M_1 attributes, plants are selected for growing in the M_2 generation. Seeds are harvested from the main branches and first fruit cluster of M_1 plants to ensure high percentage of M_2 mutation. Seeds harvested from M_1 were sown in M_2 to identify the mutants in single seed descents or plant to row method in 6 m long rows 60 cm apart in Randomized Complete Block Design with three replications along with untreated control. Plants selected from the M_2 population were grown in M_3 to test the uniformity of the plants. All the recommended cultural measures namely, irrigation, weeding and plant protection methods were carried out during the growth period of the crop.

Data collected for plant height, number of branches per plant, days to first flowering, days to first picking, days to last picking, number of pods per cluster, number of pods per plant, pod length, number of seeds per pod, hundred seed weight, yield per plant and protein % in the M₂ and M₃ generations were subjected to statistical analysis. In order to assess the extent of induced variation, significant differences were identified using the Least Significance Difference estimated from the error mean square and tabulated 't'values at 1% and 5% level of significance. Mean values were subjected to analysis of variance to test the significance for each traits as per Panse and Sukhatme (1967). For statistical analysis of genetic parameters, genotypic, environmental and phenotypic coefficients of variation (expressed in percentage) were calculated by using the formula given by Burton (1952). Heritability in broad sense was determined according to the methodology given by Hanson et al., (1956). The estimate of the expected genetic advance (GA) expressed as a percentage of the mean value with an assumed 5% (2.06 after Kang et al., 1983) intensity of selection pressure was computed by the formula given by Singh and Chaudhary (1985) as:

$GA = h^2 (bs) X \sigma \rho X k$

Where, k = 2.06, constant for 5% selection intensity (i.e. the highest-performing 5% are selected), $h^2 =$ broad-sense heritability, σ_{σ} = genotypic variance of the treated population.

RESULTS AND DISCUSSION

The magnitude of genetic variability in particular decides the effectiveness of selection. The phenotypic variance measures the magnitude of variance arising out of phenotypic or genotypic values. It is an established fact that greater the variability among the genotypes better is the chance for further improvement in the crop. 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. The response of mutagens as measured by the magnitude and the nature of variability varied from character to character. However, mutagenic treatments induced both chromosomal and non-heritable physiological changes that contribute to total induced variation and assessment of the heritable component of variation, which will be useful in the mutation breeding programme. Genotypic coefficient of variation provides a mean to study the genetic variability generated in quantitative characters (Johnson et al.,

	otein	99 70 24		% ui	
	er Pro	4* 2.5 0.2		Protei	13.16 47.41 12.69
	Yield pe plant (g)	567.97 7929.24 574.05		ield per ant (g)	44 58.59* 66
	100 seed weight (g)	2.44 28.91 * 2.60		t (g) pl.	* 75 6.
	N umber of seeds per pod	6.28 32.93* 6.51		r 100 se weigh	0.32 17.00 1.03
	od length n)	37 7.89* 54		Numbe of seeds per pod	0.01 3.22* 0.04
	of Pc (cr	9.47. 9.		Pod length (cm)	0.03 2.61* 0.18
	Number pods per plant	0.20 505.12* 1.18		Number of pods per plant	2.97 104.14* 1.65
	Number of pods per cluster	4.61 33.87* 4.34		Number of pods per cluster	0.11 0.28* 0.01
a Komal	Days to last picking	31.11 151.47* 6.36	sa Komal	Days to last picking	5.39 167.22* 3.14
of variety Pus	Days to first picking	6.57 142.77* 4.72	of variety Pu	Days to first picking	69.37 131.90* 4.96
² population o	Days to first flowering	4.46 243.42* 5.50	l ₃ population e	Days to first flowering	2.75 220.47* 1.70
aracters in M	squares Number of primary branches per plant	11.27 284.81* 6.83	aracters in M	of squares Number of primary branches per plant	0.05 5.68* 0.16
of different ch	Mean sum of Plant height (cm)	1.68 49.13* 2.74	of different ch	Mean sum c Plant height (cm)	0.08 51.66* 1.77
ariance c	d.f.	2 10 20	ariance (d.f.	2 10 20
: Analysis of ve	Source of variation	Replication Treatments Error	: Analysis of v	Source of variation	Replication Treatments Error
Table 1:	S.No	1. 3.	Table 2:	s.No	1. 3.

1955). It is an indirect measure of the environmental influence on the inheritance pattern of the yield attributes, whereas heritability gives the picture of heritable portion of phenotypic variance. The genetic advance shows the extent of genetic gain that could be expected through selections in the character to be improved (Burton, 1952 and Johnson et al., 1955). Heritability is also influenced by en-vironmental factors, the information on heritability alone may not help in pin pointing characters enforcing selec-tion. Simply, heritability gives the information on the magnitude of in-heritance of metrical attributes, i.e. polygenic inheritance, while genetic advance will be helpful in formulating suit-able selection procedures.

In order to assess the nature and magnitude of induced polygenic variability or micro mutations in gamma rays and EMS treatment population in M₂ and M₃ generation of var. Pusa Komal were evaluated for twelve yield and its attributing traits through statistical parameters such as mean and variance. Analysis of variance in M₂ and M₂ population means and variances for the traits showed significant differences among the treatments for all the characters under study except for protein % as depicted in Table 1 and 2 for M, and M, generations respectively. Conversely, Jain and Khandelwal (2009) in black gram observed a significant difference among protein % and EMS treatment. Nevertheless, results observed by Lawhale (1982) in cowpea were similar to the present findings.

Coefficient of variation

The phenotypic coefficient of variation was higher in magnitude than genotypic coefficient of variation, for all the characters under study in both the generations of Pusa Komal, indicating that all the characters to some degree interacted with environment. Contrary, to that obtained by Singh et al., 2013 Since the coefficient of variation measures the magnitude of variability present in a population, selection from a population with such coefficient of variation values is very likely to be effective in improvement of the traits studied. Genotypic and phenotypic coefficient of variation were classified as low (0-20) and high (30 and above).

High genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PVC) in M, generation (Table-3) was recorded for traits number of primary branches per plant followed by number of pods per cluster, number of pods per plant, protein percent, yield per plant, number of seeds per pods, hundred seed weight and pod length. reported similar results in for branches per plant, yield per plant, plant height and number of pods per plant in chickpea.

However, in the M₃ generation (Table 4) number of primary branches per plant, protein percent, yield per plant and hundred seed weight recorded high GCV and PCV revealing that the expression of these characters were less influenced by the growing environent and offers an opportunity to improve further by selection. Khan (1984) for traits number of branches per plant, number of pods per plant and hundred seed weight also obtained high GCV in population treated with EMS. High GCV value for yield per plant indicates that simple selection for yield may be advantageous as compared to its components under study. However, high GCV as well as PCV was obtained in M₂ and M₃ generation for traits number of primary branches per plant, protein percent, yield per plant

Table 3: Genotypic Coefficient of Variation, Phenotypic Coefficient of Variation, Heritability and Genetic advance (GA) in percent of mean for twelve characters in M, generation of cowpea var. Pusa Komal

S.N.	Characters	Coefficient of Variation		Heritability	GA in percent of mean
		Genotypic $\sigma^2 g$	Phenotypic $\sigma^2 p$	h² (bs)%	
1.	Plant height (cm)	8.25	8.95	59.69	13.14
2.	Number of primary branches per plant	74.27	76.96	93.13	14.80
3.	Days to first flowering	17.43	18.03	60.51	34.72
4.	Days to first picking	8.76	9.20	47.70	17.19
5.	Days to last picking	7.13	7.58	58.38	13.80
6.	Number of pods per cluster	65.27	78.39	69.32	11.19
7.	Number of pods per plant	44.40	45.56	60.82	72.04
8.	Pod length (cm)	21.11	27.89	57.26	32.88
9.	Number of seeds per pod	34.75	45.83	22.50	54.33
10.	100 seed weight (g)	26.11	29.73	77.13	47.27
11.	Yield per plant (g)	37.13	37.45	38.29	47.79
12.	Protein percent (%)	37.47	38.53	89.39	75.06

Table 4: Genotypic Coefficient of Variation, Phenotypic Coefficient of Variation, Heritability and Genetic advance in percent of mean for twelve characters in M₂ generation of cowpea var. Pusa Komal

S.N.	Characters	Coefficient of Variation		Heritability	GA in percent of mean
		Genotypic $\sigma^2 g$	Phenotypic $\sigma^2 p$	h² (bs)%	
1.	Plant height (cm)	9.08	9.50	61.34	14.79
2.	Number of primary branches per plant	62.97	63.50	98.33	20.73
3.	Days to first flowering	16.55	16.74	67.72	33.71
4.	Days to first picking	8.26	8.73	59.51	16.09
5.	Days to last picking	7.48	7.69	64.57	14.97
6.	Number of pods per cluster	18.12	18.93	71.66	28.95
7.	Number of pods per plant	19.49	19.96	45.39	27.31
8.	Pod length (cm)	7.11	7.54	49.39	27.67
9.	Number of seeds per pod	16.04	16.21	44.93	18.33
10.	100 seed weight (g)	23.42	23.47	79.58	38.44
11.	Yield per plant (g)	33.60	33.78	28.93	37.59
12.	Protein percent (%)	44.14	48.09	85.86	80.23

and hundred seed weight. These results were in consonance to that obtained by Meshram *et al.* (2014) in black gram irradiated with gamma rays for yield per plant; Arulbalachandran and Mullainathan (2009) in black gram treated with EMS for number of branches per plant, protein percent and Deepalakshmi and Anandakumar (2004) for number of primary branches per plant, yield per plant in black gram treated with gamma rays and EMS. In view of the fact, that coefficient of variation measures the magnitude of variability present in the population, selection from a population with such high values of GCV and PCV is very likely to be effective in improvement of these traits.

Heritability and Genetic Advance in percent of mean

Heritability determines the relative amount of heritable proportion of variability and is an important biometrical tool for adopting appropriate breeding procedure. It also acts as a predictive instrument in expressing the reliability of phenotypic value. Therefore, it helps the plant breeder to select a particular character when heritability is high. Wide range of induced variability was exhibited in the treated population according to estimates of heritability and genetic advance in percent of mean. The estimates of heritability in broad sense genetic advance in percent of mean for the twelve yield and its attributing characters have been described in Table 2 and 3 for M_2 and M_3 generation respectively. Heritability was classified as low (0-50), moderate (50-70) and high (70 and above) and genetic advance in percent of mean was also classified as low (0-30), moderate (30-70) and high (70 and above)

Broad sense heritability in M₂ as well as M₃ generation of Pusa Komal was high for characters number of primary branches per plant, protein percent and hundred seed weight. Similarly, Kumar (2008) also observed high heritability in hundred seed weight. Whereas, it was moderate for number of pods per plant, days to first flowering, plant height, days to last picking and days to first picking. This indicates that these characters are less influenced by environmental changes and selection based on phenotypic performances would be reliable and useful.

Genetic advance is indicative of the expected genetic progress for a particular trait under selection procedure and consequently carries much significance in self pollinated crops. Genetic advance in percent of mean was estimated to be high for characters (Table 3 and 4) yield per plant, protein percent and number of pods per plant, in the M_2 and only protein percent in M_3 generation of Pusa Komal. This indicates that selection of these traits will be gratifying because they are governed by additive gene action. Number of pods per plant, hundred seed weight, number of primary branches per plant, yield per plant, days to first flowering and plant height had moderate value of genetic advance in percent of mean in both the generations. High heritability estimates in conjunction with high genetic advance expressed in percent of mean was recorded in protein percent in M₂ as well as M₂ populations of Pusa Komal. This indicates the lesser influence of environment in expression of these characters and prevalence of additive gene action in their inheritance Johnson et al. (1955). Hence, simple selection of these characters may be acquiescent to develop further through induced mutagenesis. Correspondingly, high heritability along with moderate genetic advance was recorded for hundred seed weight in both the generations, which conclude that this trait might be assigned to additive gene action, which controls its expression, and phenotypic selection for their amelioration can be done. The increase in heritability and genetic advance as percent of mean in the mutagenic treatments is largely genetic, besides being additive and fixable. It is perhaps due to mutations at additive gene loci. The success of selection however, will be greater in subsequent generations when there will be increased recombination and elimination of cytological variants (Kumar, 1972).

High heritability and low advance expressed as percent of mean was recorded for number of primary branches per plant in M_2 with M_3 population while number of pods per cluster in M_3 generation only. Combination of high heritability with low genetic advance is indicative of non-additive gene action (Manimaran and Raveendran, 2004) i.e. the expression of these traits might be due to mutations at non-additive loci. Here the high heritability is due to favourable influence of environment rather than the genotype and hence simple selection may not be rewarding.

Low heritability and moderate genetic advance was recorded for number of seeds per pod in M₂ while number of seeds per pod and pod length in M₃ generation. This was contrary to the results obtained by Khan (1988) and Ignacimuthu and Babu (1992) in mung bean who recorded high heritability coupled with high genetic advance as percent of mean for number of seeds per pods. Low heritability coupled with low genetic advance was obtained in the M₃ generation of var. Pusa Komal for traits pod length and number of seeds per pods indicating that these traits are highly subjected to environment, consequently their selection would be ineffective.

An overview of this investigation indicates that the variety Pusa Komal have proved to be highly responsive to physical and chemical mutagen. Analysis of variance revealed prevalence of highly significant differences among the treatments for all the twelve characters studied except for protein per cent. Hence, physical mutagen (gamma rays) and chemical mutagen (EMS) both exhibited greater variability than control which can be utilized in the future crop improvement programme.

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