



ISSN (E): 2277-7695

ISSN (P): 2349-8242

NAAS Rating: 5.23

TPI 2022; 11(5): 39-45

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www.thepharmajournal.com

Received: 02-02-2022

Accepted: 16-04-2022

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Genetic variability studies in early segregating population of cowpea (*Vigna unguiculata* L. Walp)

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Abstract

The present investigation was carried out in F₁, F₂ and F₃ generations of four inter sub-specific crosses (RC101×Vyjayanthi, RC101× Vellayani jyothica, ACM 05-02×Ettumanoor local and ACM 05-07× Vyjayanthi) of cowpea to identify the promising segregants for further use in future cowpea breeding programmes. The F₂ and F₃ progenies were evaluated along with their parents. The breeding nature of the segregants were investigated by their mean performance, phenotypic and genotypic variability for the traits viz., days to first flowering, plant height, number of clusters per plant, number of pods per plant, pod length, number of seeds per pod, hundred seed weight and single plant yield. Among the four crosses, the variances due to phenotypic and genotypic causes were high in F₂ compared to F₃ generation for all characters except pod length which had higher values in F₃ than F₂ in crosses RC101×Vyjayanthi and RC101× Vellayani jyothica. The increase in the pod length in F₃ might be due to persistence of heterozygosity and expression of hidden genes in F₂. The estimates of heritability and genetic advance were high in both F₂ and F₃ generations of all the crosses for number of pods per plant and single plant yield. Selection for these traits might be very effective in crosses ACM 05 07 × Vyjayanthi and RC101 × Vyjayanthi.

Keywords: Cowpea, (*Vigna unguiculata* L. Walp), Segregating population, Variability, Heritability

Introduction

Cowpea (*Vigna unguiculata* L. Walp) is a multi-purpose, underutilized legume crop mostly grown in dry tropical areas. It is predominantly cultivated by resource limited small holder farmers usually women with average farm size of 0.5–1 ha (Wiggins and Sharda, 2013) [19]. It is one of the most important food legume crops, exhibits considerable morphological variability in its wild as well as cultivated forms. (Belay and Fisseha, 2021) [2]. Cowpea can derive up to 99% of its N nutrition from symbiotic fixation and fix substantial amounts of symbiotic N. In fact, cowpea has been shown to contribute about 240 kg N/ha, with N benefit of 60–70 kg/ha to succeeding crops in rotation in unfertile soils (Owusu *et al.*, 2018) [12]. The poor average yields of cowpea speak of the need for adopting appropriate method of handling segregating populations to ensure quick progress in the plant breeding programmes.

The techniques employed to estimate and assess the variability also varied among the different investigators. Improvement in this crop, though attempted through many methods, substantial progress in yield has been achieved only through recombination breeding. It generates high variability particularly in the early segregating generation and selection is exercised in this generation since genotypes possessing desirable genes occur either in homozygous or heterozygous conditions with their frequency declining in subsequent generations. Hence, the F₂ population which has high variability serves as an ideal source for exercising selection. It is achieved through adopting suitable selection procedures involving the basis of selection, stage of selection and method of selection. The basic aim of the breeder is to perpetuate the best progeny of the best family. The success of any crop improvement program depends on the efficiency of selection methods, because of differences in the population size, per cent is considered instead of actual number of segregants, which ultimately lies in the isolation of superior segregants from segregating generations. The focus of the study is to identify the promising segregants for further use in future breeding programmes. Also to combine the desirable characters into the progenies from the different subspecies and to select a desirable plant type with high yield form the segregating progenies.

Materials and Methods

Pure selfed seeds of the following six varieties, three in dwarf, early flowering with short pod and grain (*V. unguiculata subspecies sinensis*) types and three in climber, late flowering with long pod and vegetable (*V. unguiculata subspecies sesquipedalis*) types were collected from Kerala, Tamil Nadu and Rajasthan. Parents seeds used for hybridization were illustrated in figure 1. The cross combinations were effected, the hybrids (F_1) was studied in the Department of Plant Breeding and Genetics, Agricultural College and Research Institute Madurai during main rainy season. The F_1 seeds from four highly promising heterotic crosses were utilized as the experimental material for the present study of F_2 and F_3 generations. Morphological characteristics features of parents and their four crosses given table 1.

Evaluation of F_2 generations: The F_2 generation of four cross combinations was raised along with their six parents in a Randomized Block Design replicated twice. A total of 300 plants were raised per cross combinations in 30 rows adopting a spacing of 45 cm between rows and 30 cm between plants in a row. The recommended agronomic practices were followed throughout the crop growth period. Observations were recorded in all the plants in each cross of the F_2 and ten plants in parents. Based on single plant wise F_2 plants are harvested and dried then kept under optimum moisture storage condition. Based on single plant yield wise 30 superior plants were selected and these seed materials were used for source of F_3 generation. Flower colour variation were observed in F_2 generation were given in Figure 2 & 3.

Evaluation of F_3 generation: The F_3 generation consisted of plants chosen based on the single plant yield from four crosses which were raised in F_2 . In F_3 a total of 120 families (30 families from each cross) of four crosses were raised with their six parents in a Randomized Block Design replicated twice. The F_3 generation was raised at the rate of two rows per progenies (F_2), each rows consisting of ten plants. Each cross had two rows of the respective parents per replication. Observations were recorded in randomly selected five single plants per family and ten plants for each parent per replication. The data recorded on eight quantitative traits in F_2 and F_3 generations were statistically analyzed.

Coefficients of variations: The genotypic and phenotypic coefficient of variability were computed according to the formula given by Burton and Devane, (1953) [3].

$$PCV \% = \frac{\sqrt{\sigma_p^2}}{\text{Grand mean}} \times 100$$

$$GCV \% = \frac{\sqrt{\sigma_g^2}}{\text{Grand mean}} \times 100$$

Where,

σ_g^2 = Genotypic variance

σ_p^2 = Phenotypic variance

Heritability: Heritability, in its broad-sense, was estimated, according to Warner (1952) [18], as follows:

$$h^2 = \frac{VG}{VP} \times 100$$

Where h^2 = broad sense heritability; V_p = phenotypic variance of F_2 individuals (V_{F_2}) and V_G = Genotypic variance of F_2 individuals [$V_{F_2} - 1/3(V_{F_1} + V_{p_1} + V_{p_2})$]

Genetic advance: It was derived according to the method of Johnson *et al.*, (1955) [8] for each character.

$$\text{Genetic advance} = h^2 \times K \times P$$

Where,

h^2 = Heritability

K = Selection differential, the value of which is 2.06 at 5 intensity (Falconer, 1981)

P = Phenotypic standard deviations of F_2

Genetic advance as per cent mean (GAM): Genetic advance was expressed as percentage of mean by using the formula suggested by Johnson *et al.* (1955) [8].

$$\text{GA expressed as percentage of mean} = \frac{\text{Genetic advance}}{\text{Grand mean}} \times 100$$

Results and Discussion

Simple measures of variability like range, mean and coefficients of variation reveal the extent of variability but not the heritable portion of total variation. To have the knowledge of heritable portion of variability, it is necessary to estimate the heritability of each character. The broad sense heritability gives an idea about the portion of observed variability attributable to genetic differences. Heritability indicates the accuracy with which a genotype can be identified by its phenotypic performance. Burton (1952) [4] suggested that heritability estimate coupled with genotypic coefficient of variation would provide an accurate picture about the extent of genetic advance to be expected through the selection.

The expression of any trait in advanced generations after selection from early segregating generation depends upon the mean and genetic variation in the base population (Grafius, 1956) [6]. Effective selection of superior plant is successful only when the segregating progenies had high mean coupled with high variability, because such population may serve as an ideal source for exercising selection. Variability parameters are presented in table 2&3. Flower colour variation was observed in F_2 generation given in figure 4. When the high mean was considered in the present study, the F_2 of cross RC101 x Vyjayanthi recorded higher value for number of clusters per plant and number of pods per plant. Cross RC101 x Vellayani jyothica registered high mean value for pod length, while high mean for number of seeds per pod was observed in cross ACM 05 07 x Vyjayanthi. Besides, higher mean values were observed in cross RC101 x Vyjayanthi, for number seeds per pod and in cross ACM 05-02 x Ettumanoor local for pod length higher mean values were observed in F_3 generation. Also cross ACM 05-07 x Vyjayanthi recorded high mean for number of clusters per plant and number of pods per plant. In both generations, crosses RC101 x Vellayani jyothica and ACM 05-07 x Vyjayanthi showed higher mean values for days to first flowering and plant height respectively. The mean values were high for hundred seed weight and single plant yield in both generation of cross RC101 x Vyjayanthi. Such high

variability was observed for single plant yield by Mafakheri *et al.* (2017)^[9] and Owusu *et al.* (2020)^[12] in cowpea. These results have clearly indicated that cross RC101×Vyjayanthi would be the best combination for yield components while cross ACM 05-07× Vyjayanthi might be suitable for improvement of number of clusters per plant and number of pods per plant. Segregation pattern of seed colour in F₂ generation of four crosses were illustrated in Figure 5,6,7&8.

Estimates of genotypic and phenotypic coefficients of variation in different crosses: From the study, the variances due to phenotypic and genotypic causes were high in F₂ compared to F₃ generation for all characters except pod length which had higher values in F₃ than F₂ in crosses RC101×Vyjayanthi and RC101× Vellayani jyothica. The increase in the pod length in F₃ might be due to persistence of heterozygosity and expression of hidden genes in F₂. The analysis of variance for seed yield and its components indicated significant difference among the crosses for all the characters. Results revealed that, the magnitude of phenotypic coefficient of variation was higher than corresponding genotypic coefficient of variation for all the characters under study as illustrated in table 2. It revealed that apparent variation was not only due to genotypes but also due to the influence of environment. However, narrow difference between genotypic and phenotypic coefficients of variation estimates indicated little influence of environment on the expression of these traits and variability was mainly due to genetic constitution. This implies that phenotypic variability has been a reliable measure of genotypic variability.

When high mean associated with high variability in F₂ is considered as an

index for selection, the following crosses could be identified as promising. Cross ACM 05-07× Vyjayanthi recorded desirable mean along with high heritability for number of clusters per plant, number of pods per plant and number of seeds per pod. Such high variability was observed for number of clusters per plant (Singhal *et al.*, 2010)^[16]. High variability for number of pods per plant was observed by Dinesh *et al.* (2017)^[4]. For number of seeds per pod high variability was observed by Gomes *et al.* (2020)^[7].

Cross RC101×Vyjayanthi had high mean with high variability for single plant yield when compared to other crosses such high variability for single plant yield was also noticed by and Belay and Fisseha (2021)^[3]. In the present study, the variability was the highest for pod length in cross ACM 05-02×Ettumanoor local as it had the highest PCV and GCV values in F₂ and F₃ generation. In both F₂ and F₃ all the characters except days to first flowering showed moderately high GCV, thereby suggesting that these characters could be improved genetically. Days to first flowering had low value of GCV in both F₂ and F₃ indicating the limited scope for further improvement. The relative magnitude of PCV and GCV indicated the presence of environmental influences in the expression of the characters studied by Gerrano *et al.* (2015)^[5]; Pandian *et al.* (2020). In general the Environmental Coefficient of Variation (ECV) was high in F₂ generation than an F₃ except for days to first flowering which indicated that F₂ generation is more liable for environmental influence.

From the aforesaid facts, desirable mean with high variability in both F₂ and F₃ were recorded at genotypic and phenotypic

levels by cross ACM 05-07× Vyjayanthi for number of clusters per plant, number of pods per plant and number of seeds per pod and cross RC101×Vyjayanthi for single plant yield. High mean with high variability was recorded by cross ACM 05 02×Ettumanoor local for days to first flowering and pod length in both generations. Hence, among the crosses studied, crosses ACM 05 07 x Vyjayanthi and RC101 x Vyjayanthi were found to be promising populations for exercising selection.

Estimates of heritability in broad sense and expected genetic advance:

In the present study, the heritability in broad sense of higher magnitude was observed in F₂ for days to first flowering, plant height, number of clusters per plant, number of pods per plant and number of seeds per pod than in F₃ generation (Nehru *et al.* 2009)^[11]. The characters hundred seed weight and single plant yield showed high heritability values in both the F₂ and F₃ generations. Also high heritability was observed in F₃ for pod length than F₂. High heritability was also reported for single plant yield by Owusu *et al.* (2021)^[13]; Ajayi *et al.* (2020)^[1].

The crosses which showed high heritability combined with high genetic advance in F₂ were not necessarily being with such high estimates in F₃ generation. The crosses RC101×Vyjayanthi, RC101× Vellayani jyothica and ACM 05-02×Ettumanoor local for days to first flowering and hundred seed weight had high estimates of both heritability and genetic advance in F₃. In the present study, estimates of high heritability and genetic advance were observed in both the F₂ and F₃ generation for plant height, number of clusters per plant, number of pods per plant, number of seeds per pod and single plant yield in all the crosses.

High heritability with moderate genetic advance was observed in both the generations for days to first flowering in cross ACM 05-07× Vyjayanthi in F₂, for pod length in cross RC101×Vyjayanthi and for hundred seed weight in cross ACM 05-07× Vyjayanthi of F₃. According to Panse (1957)^[15] the traits under the influence of high heritability with low genetic advance were under the control of non additive (dominance and epistasis) gene action. Hence the parents involved in these cross combinations are mostly suitable for heterosis breeding for aforesaid traits.

In the present study, the estimates of heritability and genetic advance were high in both F₂ and F₃ generations of all the crosses for number of pods per plant and single plant yield and crosses RC101×Vyjayanthi, RC101× Vellayani jyothica and ACM 05-02×Ettumanoor local for days to first flowering. Selection for these traits might be very effective in crosses RC101×Vyjayanthi and ACM 05-07× Vyjayanthi. High heritability coupled with high genetic advance as per cent of mean was observed for all characters. This indicated that most likely the high heritability might be due to additive gene effects and hence, it could be improved by simple selection methods like pureline selection, progeny selection or family selection. High heritability coupled with medium genetic advance as per cent of mean indicated the presence of both additive and non-additive gene actions for the inheritance of these characters and high genotype x environment interaction. These characters could be improved suitably by modified selection procedure for mixed effects of additive and non-additive gene actions like cyclic hybridization followed by selection.

Table 1: Morphological characteristic of parents and their crosses in F₂ generation

Crosses/Varieties	Plant type	Flower colour (Standard petal)	Pod colour	Seed colour and size
RC 101	Dwarf, early flowering	White	Short, green	Dull white and round, small
ACM 05 02	Medium, early flowering	Cream	Short and green with purple tip	Pure white and round, medium
ACM 05 07	Medium, early flowering	Light purple	Medium, green	Cream and round, small
Vyjayanthi	Climber, late flowering	purple	Long, Purple	Purple and oval, long
Vellayani Jyothica	Climber, late flowering	Pale yellow	Long, green	Purple with white mottled and oval long
Ettumanoor local	Climber, late flowering	Cream with purple	Long, green	Dark purple and long, bold
RC101 × Vyjayanthi	Dwarf late, dwarf early, medium early, medium late, tall early and tall late,	White, white purple shade, light purple and purple	Short green, short purple, medium green, medium purple, long green and long purple	White oval medium, white oval long, black round small, black round medium, black oval long and purple with white oval long
RC101 × Vellayani Jyothica	Short early, short late, tall early and tall late	White, pale yellow with purple shade, pale yellow	Short green, medium green and long green	White oval small, white oval medium, white mottled black, white mottled black oval small, white mottled black oval medium and brown oval medium
ACM 05 02 × Ettumanoor local	Medium early and short early	Cream, cream with light purple shade and cream with dark purple shade.	Medium green with purple tip and long green with purple tip	Black mottled cream oval small, black mottled cream oval medium, black mottled cream oval long, brown oval medium and brown mottled cream oval medium
ACM 05 07 × Vyjayanthi	Medium early, medium late, tall early, tall late	Light purple, dark purple, white, white with light purple shade and white with more purple shade	Small purple, medium purple, long purple, small green, medium green and long green	Cream oval medium, cream oval long, brown oval small, brown oval medium, dark black oval medium, dark black round medium and purple oval medium.

Table 2: Genetic variability parameters for quantitative characters in F₂ and F₃ generation of RC101 × Vyjayanthi & ACM 05 07 × Vyjayanthi

Crosses Characters	Generation	RC101 × Vyjayanthi							ACM 05 07 × Vyjayanthi						
		Mean	PCV	GCV	ECV	h ² (bs)	GA	GAM	Mean	PCV	GCV	ECV	h ² (bs)	GA	GAM
Days to first flowering (days)	F ₂	47.55	13.32	12.91	0.41	93.90	11.77	25.77	48.82	9.40	8.66	0.74	84.86	7.43	16.44
	F ₃	45.68	12.56	12.16	0.40	93.67	11.53	24.24	45.19	7.95	7.19	0.76	81.83	6.54	13.40
Plant height (cm)	F ₂	27.38	31.69	29.96	1.73	89.40	15.98	58.36	43.60	26.32	24.74	1.58	88.32	20.88	47.89
	F ₃	23.95	34.50	32.42	2.08	88.31	15.03	62.76	40.52	21.04	18.68	2.36	78.83	13.84	34.16
No. of clusters/ plant	F ₂	9.98	33.31	32.64	0.68	95.98	6.57	65.87	9.13	46.77	46.43	0.34	98.53	8.67	94.94
	F ₃	8.65	30.85	29.87	0.98	93.76	5.15	59.58	9.87	40.68	40.34	0.34	98.34	8.13	82.41
No. of pods/ plant	F ₂	19.97	34.36	33.64	0.72	95.85	13.55	67.84	18.25	46.77	46.48	0.29	98.76	17.37	95.15
	F ₃	17.42	31.13	30.07	1.05	93.34	10.43	59.86	19.76	40.67	40.38	0.29	98.60	16.32	82.60
Pod length (cm)	F ₂	18.54	12.20	9.90	2.31	65.75	3.06	16.53	19.29	25.97	25.38	0.59	95.54	9.86	51.11
	F ₃	10.47	24.99	21.56	3.43	74.42	4.01	38.32	18.94	20.39	19.61	0.78	92.50	7.36	38.86
Seeds/ pod	F ₂	8.99	23.13	20.79	2.34	80.77	3.46	38.49	10.43	33.96	32.93	1.03	94.00	6.86	65.77
	F ₃	10.87	18.86	16.89	1.97	80.22	3.39	31.16	9.24	34.31	33.00	1.31	92.51	6.04	65.38
100 seed weight (g)	F ₂	12.97	30.08	30.07	0.01	99.97	8.03	61.94	9.09	14.53	14.52	0.01	99.83	2.72	29.88
	F ₃	13.60	16.58	16.58	0.01	99.90	4.64	34.13	11.81	8.68	8.66	0.01	99.71	2.11	17.82
Single plant yield (g)	F ₂	15.15	40.12	40.06	0.07	99.68	12.48	82.39	10.59	31.71	31.57	0.14	99.11	6.86	64.74
	F ₃	13.60	27.52	27.40	0.12	99.14	7.64	56.21	13.18	23.87	23.75	0.12	98.99	6.42	48.68

Table 3: Genetic variability parameters for quantitative characters in F₂ and F₃ generation of RC101 × Vellayani Jyothica & ACM 05 02 × Ettumanoor local

Crosses Characters	Generation	RC101 × Vellayani Jyothica							ACM 05 02 × Ettumanoor local						
		Mean	PCV	GCV	ECV	h ² (bs)	GA	GAM	Mean	PCV	GCV	ECV	h ² (bs)	GA	GAM
Days to first flowering (days)	F ₂	51.20	11.23	10.83	0.40	93.04	11.02	21.52	43.74	16.65	16.43	0.23	97.31	14.60	33.38
	F ₃	47.44	11.39	10.93	0.46	92.11	10.25	21.61	42.72	15.14	14.88	0.26	96.59	12.87	30.13
Plant height (cm)	F ₂	25.65	37.62	35.62	2.00	89.65	17.82	69.48	20.39	23.76	19.90	3.86	70.14	7.00	34.33
	F ₃	27.53	30.69	28.54	2.15	86.49	15.05	54.68	20.52	21.99	17.81	4.18	65.59	6.10	29.71
No. of clusters/ plant	F ₂	7.79	28.41	26.49	1.92	86.94	3.97	50.88	6.28	42.96	41.55	1.40	93.58	5.20	82.81
	F ₃	8.55	22.65	20.63	2.02	82.93	3.31	38.69	6.83	35.86	34.44	1.42	92.22	4.65	68.13
No. of pods/ plant	F ₂	15.59	28.41	26.92	1.48	89.82	8.19	52.56	12.49	43.10	41.47	1.63	92.57	10.27	82.18
	F ₃	16.70	24.20	22.67	1.53	87.78	7.31	43.75	13.67	34.34	32.62	1.72	90.23	8.73	63.83
Pod length (cm)	F ₂	19.43	16.89	15.66	1.24	85.91	5.81	29.89	16.97	31.81	30.78	1.03	93.61	10.41	61.35
	F ₃	18.27	20.33	19.18	1.15	89.00	6.81	37.28	20.35	22.09	21.05	1.04	90.79	8.41	41.32
Seeds/ pod	F ₂	9.44	31.53	30.39	1.14	92.90	5.70	60.33	9.09	37.61	36.41	1.20	93.73	6.60	72.62
	F ₃	8.94	28.08	26.64	1.44	90.02	4.65	52.06	10.26	27.05	25.73	1.32	90.48	5.17	50.42
100 seed weight (g)	F ₂	9.88	41.88	41.84	0.04	99.79	8.51	86.09	9.27	25.85	25.83	0.02	99.83	4.93	53.17
	F ₃	10.72	34.53	34.48	0.05	99.74	7.60	70.94	10.03	20.05	20.02	0.02	99.75	4.13	41.20
Single plant yield (g)	F ₂	9.77	37.06	36.89	0.17	99.06	7.39	75.63	11.47	35.44	35.41	0.03	99.84	8.36	72.88
	F ₃	12.91	23.13	22.97	0.16	98.62	6.07	47.00	11.45	33.21	33.18	0.03	99.81	7.82	68.29



Fig 1: Parents used for Hybridization



Fig 4: Pod colour variation in F2 generation of cross RC 101 X Vyjayanthi



Fig 2: Flower colour variation in F2 generation of cross RC 101 x Vyjayanthi



Fig 5: Seed colour variation in F2 generation of cross RC 101 X Vyjayanthi



Fig 3: Flower colour variation in F2 generation of cross ACM 05 07 x Vyjayanthi



Fig 6: Seed colour variation in F2 generation of cross RC101 X Vellayani Jyothica



Fig 7: Seed colour variation in F₂ generation of cross ACM 05 02 × Ettumanoor local



Fig 8: Seed colour variation in F₂ generation of cross ACM 05 07 x Vyjayanthi



Fig 9: Superior single plant selection in F₃ generation of cross RC 101 X Vyjayanthi.



Fig 10: Superior single plant selection in F₃ generation of cross ACM 05 07 X Vyjayanthi

Conclusion

From the aforesaid facts, desirable mean with high variability in both F₂ and F₃ were recorded at genotypic and phenotypic levels by cross ACM 05 07 × Vyjayanthi for number of cluster per plant, number of pods per plant and number of seeds per pod and cross RC101 × Vyjayanthi for single plant yield. Hence, among the crosses studied, ACM 05 07 × Vyjayanthi and RC101 × Vyjayanthi were found to be promising in providing better source population for exercising selection. Also some of the F₃ segregants in all the crosses had besides their parental type, colour, size and shapes (Fig 9&10). It will be much useful for the plant breeders to develop different coloured and various sized new cowpea genotypes in future breeding programmes.

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