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Genetics of seed related attributes in cowpea [*Vigna unguiculata* (L.) Walp.]

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ABSTRACT

An investigation to study the genetics of seed related attributes was undertaken in cowpea. Three crosses (GC 2 x PGCP 5, GC 2 x PGCP 13 and GC 516 x PGCP 1) along with its parental seed, F_2, BCI and BCII made the complete set of six generations (P_1, P_2, F_1, F_2, BCI and BCII) for genetic analysis. The experiment was laid out in a Compact Family Block Design with three replications during *kharif* 2011. A single replication comprised of one row of parents and F_1, two rows of the backcrosses generations, BCI and BCII and four rows of the F_2. Genetics of seed related attributes, *viz.,* flower colour, seed colour, seed surface, pod beak colour, calyx pigmentation and pod colour at maturity, were studied following Chi-square test of statistical significance. The results evident that flower colour in all the three crosses studied appeared to be controlled by single gene inheritance with violet flower colour dominant over white flower colour. Similarly, seed surface, pod beak colour and calyx pigmentation also appeared to be controlled by single gene inheritance, in which smooth seed surface dominant over rough seed surface, green pod beak colour dominant over red pod beak colour and green calyx pigmentation dominant over red calyx pigmentation. The inheritance of seed colour and pod colour at maturity appeared to be controlled by two gene interactions with supplementary gene action, with dominance of black seed colour over brown and white seed colour, and black pod colour dominant to red and brown colour. Dominance expression of different characters could be utilized as a good marker for different breeding activities.

Key words: Chi-Square, Cowpea, Gene action, Inheritance.

INTRODUCTION

Cowpea is an important multi utility crop introduced in Indian sub-continent about 3500 years ago along with sorghum and millets. Among all the pulses, cowpea [*Vigna unguiculata* (L.) Walp.], locally known as *lobiya, chowla* (*chowli*), *southern pea* or *black eye pea*, is an annual legume that is adopted to warm condition and cultivated in the tropics and sub-tropics for dry grains, green edible pods for vegetable as well as fodder. Cowpea fits well in a variety of cropping system and is grown as cover crop, mixed crop, catch crop and green manure crop. It can be capable of restoring soil fertility and therefore, remains an integral part of subsistence and sustainable production system. Being a legume crop, cowpea fix substantial quantities of biological nitrogen by virtue of their symbiotic association with *Rhizobium* bacteria (Schultze and Kondorosi, 1998, Serraj, 2004) ranging from 73 to 80 kg / ha (Yamada, 1974). Besides it would supply up to 230 - 275 quintals of palatable hay per hectare as fodder (Haq, 1981). As such, cowpea has originated as heat loving crop (20^0 to 35^0C) with lower soil fertility requirements than many other crops that had given a misconception that the crop is suitable for less endowed areas. However, the crop has vast potential in varied agricultural situations in general and intensive agriculture in particular.

The crop has vast potential for yield enhancement through breeding interventions. The yield is dependent on yield components that have been immensely exploited in breeding programmes. Being a self pollinated crop, genetic improvement in cowpea has been made through conventional techniques such as selection from local materials, pedigree method of breeding *etc.* However, a high degree of heterosis has been reported in the crop, though its commercial exploitation has not been possible due to absence of male sterility and efficient pollinating system. Further, the seed attributes are important not only for yield but also for determination of quality of the produce and consumers' preference. For a sound breeding programme to be precisely effective, genetics of these attributes holds conspicuous pertinence. Therefore, the present investigation was planned to determine the genetics of seed related attributes.

MATERIALS AND METHODS

The present investigation to study the genetics of seed related attributes was undertaken in cowpea at the Centre of Excellence for Research on Pulses, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar during *kharif* 2011. Three crosses (GC 2 x PGCP 5, GC 2 x PGCP 13 and GC 516 x PGCP 1) were made at the centre during

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kharif 2010. These crosses (F_1) were grown along with its 5 parents (GC 2, GC 516, PGCP 5, PGCP 13 and PGCP 1) to make the F_2 (selfing of F_1), BCI (F_1 x Female Parent) and BCII (F_1 x Male Parent) generations during summer 2011 by hand emasculation and pollination. Therefore, the material for the present investigation consisting complete set of six generations (P_1, P_2, F_1, F_2, BCI and BCII) for inheritance studies. The experiment was laid out in a compact family block design with three replications. The three crosses formed the family block, whereas six generations of each cross represented individual plots within family. A single replication comprised of one row of parents and F_1, two rows of the backcrosses generations, BCI and BCII and four rows of the F_2. Inter and intra row spacing was kept 45 cm x 15 cm. Recommended agronomic practices and necessary plant protection measures were timely adopted for successful raising of the crop. Genetics of seed related attributes, *viz*., flower colour, seed colour, seed surface, pod beak colour, calyx pigmentation and pod colour at maturity, were studied to test the significance of difference between observed and expected frequencies or ratios. Chi-square test (commonly known χ^2 test) of statistical significance developed by Pearson (1900), which is commonly used in Mendelian and population genetics to test the significance of difference between observed and expected frequencies or ratios (Cochran, 1952), was used in the present study.

The general formula of x^2 test was as follows:

$$\chi^2 = \Sigma \frac{(O-E)^2}{E}$$

Where,

∑ = Summation,
O = Observed frequencies
E = Expected frequencies.

The expected frequencies can be calculated from the observed frequencies assuming a particular hypothetical segregation ratio. Each deviation (O – E) was squared and each squared deviation was then divided by the expected number in its class. The resulting quotients were then added together to give a single value of χ^2. This is tested against table value of Chi-Square at n-1 degree of freedom, where n is the number of classes.

RESULTS AND DISCUSSION

Flower colour: The inheritance pattern of flower colour was studied in cowpea using three cross combinations of violet and white colour flowered genotypes. All F_1 plants derived from cross, GC 2 (violet flower colour) × PGCP 5 (white flower colour) produced violet flowers. Segregation in the F_2 produced a 3 violet : 1 white flower colour ratio (Table 1). Backcross of F_1 plants involving violet colour flowered parent P_1 (GC 2) gave all violet colour flowered plants, whereas backcross of F_1 with white colour flowered parent P_2 (PGCP 1) gave a close fit to 1 violet : 1 white colour flowered plants. It indicated single gene inheritance with violet flower colour dominant over white flower colour. Similar results were observed in cross II (GC 2 x PGCP 13) and cross III (GC 516 x PGCP 1), in which GC 2 and GC 516 had violet flowers and PGCP 13 and PGCP 1 had white flowers. Sangwan and Lodhi (1998) reported that white flower colour is controlled by a single recessive gene.

TABLE 1: Genetic analysis of flower colour in segregating population of cowpea and test of goodness of fit to the expected ratio.

Cross	Generation	Observed number of plants			Expectedratio	x^2
		Purple colour	White colour	Total		
I	GC 2 (P_1)	16	0	16	-	-
	PGCP 5 (P_2)	0	18	18	-	-
	GC2 × PGCP 5 (F_1)	16	0	16	-	-
	F_2 (F_1 Selfing)	122	38	160	3:1	0.13
	F_1 × GC 2 (BCI)	64	0	0	-	-
	F_1 × PGCP 5 (BCII)	36	24	60	1:1	2.40
II	GC 2 (P_1)	18	0	18	-	-
	PGCP 13 (P_2)	0	16	16	-	-
	GC 2 × PGCP 13 (F_1)	20	0	20	-	-
	F_2 (F_1 Selfing)	115	41	156	3:1	0.13
	F_1 × GC 2 (BCI)	56	0	56	-	-
	F_1 × PGCP 13 (BCII)	32	26	58	1:1	2.76
III	GC 516 (P_1)	16	0	16	-	-
	PGCP 1 (P_2)	0	16	16	-	-
	GC 516 × PGCP 1 (F_1)	18	0	18	-	-
	F_2 (F_1 Selfing)	125	39	164	3:1	0.13
	F_1 × GC 516 (BCI)	56	0	56	-	-
	F_1 × PGCP 1 (BCII)	33	27	60	1:1	0.60

Non-significant at 5 per cent ($P_{0.05}$ = 3.841) level of significance

Seed colour: All the F_1 plants of the first cross, brown seed coloured female parent GC 2 (P_1) x white flower coloured male parent PGCP 5 (P_2) produced seeds, which had black colour, which does not resemble any of the parental lines indicated dominance of black seed colour over brown and white seed colour. Segregation in the F_2 produced a 9 black: 3 brown: 4 white seed colour ratio (Table 2.1), indicated two gene interactions with supplementary gene action in the inheritance of seed colour in cowpea. Backcross of F_1 plants with brown seed coloured parent P_1 (GC 2) gave a close fit to 1 black : 1 brown seed coloured plants, while Backcross of F_1 plants with white seed coloured parent P_2 (PGCP 1) gave a close fit to 1 black : 1 white seed coloured plants, which confirmed the dominance of black seed colour over brown and white seed colour and digenic inheritance of the trait. The similar results were observed in the third cross, which was made between brown seed coloured female parent GC 516 (P_1) and white seed coloured male parent PGCP 1 (P_2) (Table 2.3). The second cross was made between brown seed coloured female parent GC 2 (P_1) and green coloured male parent PGCP 13 (P_2). All the F_1 plants of this cross produced seeds, which possessed black colour, which does not resemble any of the parental lines indicated dominance of black seed colour over brown and green seed colour. Segregation in the F_2 produced a 9 black: 3 brown: 3 white : 1 green seed colour ratio (Table 2.2), indicated two gene interactions in the inheritance of seed colour in cowpea. Backcross of F_1 plants with brown seed coloured parent P_1 (GC 2) gave a close fit to 1 black : 1 brown seed coloured plants, while Backcross of F_1 plants with white seed coloured parent P_2 (PGCP 1) gave a close fit to 1 black : 1 white seed coloured plants, which confirmed the dominance of black seed colour over brown, white and green seed colour and digenic inheritance of the trait. The results of the present investigation are in agreement to the findings of Kehinde (2000). However, Drabo *et al.* (1988) reported

TABLE 2.1: Genetic analysis of seed colour in segregating population of cowpea and test of goodness of fit to the expected ratio in cross GC 2 x PGCP 5.

Generation	Observed number of plants				Expected ratio	x^2
	Blackcolour	White colour	Brown colour	Total		
GC 2 (P_1)	0	0	16	16	-	-
PGCP 5 (P_2)	0	18	0	18	-	-
GC2 × PGCP 5 (F_1)	16	0	0	16	-	-
F_2 (F_1 Selfing)	92	41	27	160	9:3:4	0.26
F_1 × GC 2 (BCI)	36	0	28	64	1:1	1.00
F_1 × PGCP 5 (BCII)	38	22	0	60	1:1	4.26

Non-significant at 5 per cent ($P_{0.05}$ = 5.991) level of significance

TABLE 2.2: Genetic analysis of seed colour in segregating population of cowpea and test of goodness of fit to the expected ratio in cross GC 2 x PGCP 13.

Generation	Observed number of plants					Expectedr atio	x^2
	Blackcolour	Browncolour	White colour	Green colour	Total		
GC 2 (P_1)	0	18	0	0	18	-	-
PGCP 13 (P_2)	0	0	0	16	16	-	-
GC 2 × PGCP 13 (F_1)	20	0	0	0	20	-	-
F_2 (F_1 Selfing)	78	32	34	12	156	9:3:3:1	2.63
F_1 × GC 2 (BCII)	29	27	0	0	56	1:1	-
F_1 × PGCP 13 (BCII)	32	0	0	26	58	1:1	2.76

Non-significant at 5 per cent ($P_{0.05}$ = 7.815) level of significance

TABLE 2.3: Genetic analysis of seed colour in segregating population of cowpea and test of goodness of fit to the expected ratio in cross GC 516 x PGCP 1.

Generation	Observed number of plants				Expectedratio	x^2
	BlackColour	White colour	Brown colour	Total		
GC 516 (P_1)	0	0	16	16	-	-
PGCP 1 (P_2)		0	16	0	16	- -
GC 516 × PGCP 1 (F_1)	18	0	0	18	-	-
F_2 (F_1 Selfing)	78	48	38	164	9:4:3	5.33
F_1 × GC 516 (BCI)	32	0	24	56	1:1	1.14
F_1 × PGCP 1 (BCII)	33	27	0	60	1:1	0.60

Non-significant at 5 per cent ($P_{0.05}$ = 5.991) level of significance five (R, P, B, M, N) major genes control seed coat colour in cowpea.

Seed surface: All F_1 plants derived from cross, GC 2 (smooth seed surface) × PGCP 5 (rough seed surface) had smooth seed surface. Segregation in the F_2 gave a 3 smooth : 1 rough seed surface ratio (Table 3). Backcross of F_1 plants involving smooth seed surfaced parent P_1 (GC 2) gave all smooth seed surfaced plants, whereas backcross of F_1 involving rough seed surfaced parent P_2 (PGCP 1) gave a close fit to 1 smooth : 1 rough seed surfaced plants. The above results indicated single gene inheritance with smooth seed surface dominant over rough seed surface. Similar results were observed in cross II (GC 2 x PGCP 13) and cross III (GC 516 x PGCP 1), in which GC 2 and GC 516 had smooth seed surface and PGCP 13 and PGCP 1 had rough seed surface. Singh and Ishiyaku (2000) reported the similar results for seed surface.

Pod beak colour: All F_1 plants derived from cross, GC 2 (green pod beak colour) × PGCP 5 (red pod beak colour) showed red pod beak colour. Segregation in the F_2 gave a 3 red : 1 green pod beak colour ratio (Table 4). Backcross of F_1 plants involving green pod beak coloured parent P_1 (GC 2) gave a close fit to 1 red : 1 green pod beak colour ratio, while backcross of F_1 involving red pod beak coloured parent P_2 (PGCP 1) gave all red pod beak coloured plants. These results indicated single gene inheritance with red pod beak colour dominant over green pod beak colour. Similar results were observed in cross II, which was also made between two parents, GC 2 with green pod beak colour and PGCP 13 with red pod beak colour. The third cross was made between GC 516 and PGCP 1, both had red pod beak colour showed all the plants with red pod beak colour in F_1, F_2, BCI and BCII.

Calyx pigmentation: The inheritance pattern of calyx pigmentation was studied in three cross combinations of red and green calyx pigmentation. Cross I was made between GC 2 (green pigmentation) × PGCP 5 (red pigmentation). All F_1 plants derived from this cross showed red calyx pigmentation. Segregation in the F_2 gave a 3 red : 1 green calyx pigmentation ratio (Table 5). Backcross of F_1 plants involving green color calyx pigmented parent P_1 (GC 2) gave a close fit to 1 red : 1 green calyx pigmentation ratio, while backcross of F_1 involving red calyx pigmented parent P_2 (PGCP 1) gave all red calyx pigmented plants. This indicated single gene inheritance with red calyx pigmentation dominant over green calyx pigmentation. Similar results were observed in cross II, which was involving GC 2 with green calyx pigmentation and PGCP 13 with red calyx pigmentation as parents. The third cross was made between GC 516 and PGCP 1, both having red calyx pigmentation showed all the plants with red calyx pigmentation in F_1, F_2, BCI and BCII.

Pod colour at maturity: The inheritance pattern of pod colour at maturity was studied with different cross combinations. In cross I, female parent, GC 2 recorded black pod color at maturity, whereas male parent PGCP 5 showed red pod colour. All F_1 plants derived from this cross had black pod color. Segregation in the F_2 gave 79 black, 48 brown, and 33 red pod coloured plants at maturity, which fitted to 9 black : 4 brown : 3 red pod coloured ratio at maturity (Table 6.1), indicated two gene interactions with supplementary gene action in the inheritance of pod colour

TABLE 3: Genetic analysis of seed surface in segregating population of cowpea and test of goodness of fit to the expected ratio.

Cross	Generation	Observed number of plants			Expectedratio	x^2
		Smooth surface	Rough surface	Total		
I	GC 2 (P_1)	16	0	16	-	-
	PGCP 5 (P_2)	0	18	18	-	-
	GC2 × PGCP 5 (F_1)	16	0	16	-	-
	F_2 (F_1 Selfing)	123	37	160	3:1	0.30
	F_1 × GC 2 (BCI)	64	0	64	-	-
	F_1 × PGCP 5 (BCII)	34	26	60	1:1	1.06
II	GC 2 (P_1)	18	0	18	-	-
	PGCP 13 (P_2)	0	16	16	-	-
	GC 2 × PGCP 13 (F_1)	20	0	20	-	-
	F_2 (F_1 Selfing)	120	36	156	3:1	0.31
	F_1 × GC 2 (BCI)	56	0	56	-	-
	F_1 × PGCP 13 (BCII)	27	31	58	1:1	0.28
III	GC 516 (P_1)	16	0	16	-	-
	PGCP 1 (P_2)	0	16	16	-	-
	GC 516 × PGCP 1 (F_1)	18	0	18	-	-
	F_2 (F_1 Selfing)	129	35	164	3:1	1.17
	F_1 × GC 516 (BCI)	56	0	56	-	-
	F_1 × PGCP 1 (BCII)	24	36	60	1:1	2.40

Non-significant at 5 per cent ($P_{0.05}$ = 3.841)level of significance

TABLE 4: Genetic analysis of pod beak colour in segregating population of cowpea and test of goodness of fit to the expected ratio.

Cross	Generation	Observed number of plants			Expectedratio	x^2
		Red pod beak colour	Green pod beak colour	Total		
I	GC 2 (P_1)	0	16	16	-	-
	PGCP 5 (P_2)	18	0	18	-	-
	GC2 × PGCP 5 (F_1)	16	0	16	-	-
	F_2 (F_1 Selfing)	129	31	160	3:1	2.70
	F_1 × GC 2 (BCI)	36	28	64	1:1	1.00
	F_1 × PGCP 5 (BCII)	60	0	60	-	-
II	GC 2 (P_1)	0	18	18	-	-
	PGCP 13 (P_2)	16	0	16	-	-
	GC 2 × PGCP 13 (F_1)	20	0	20	-	-
	F_2 (F_1 Selfing)	120	36	156	3:1	0.31
	F_1 × GC 2 (BCI)	35	21	56	1:1	3.50
	F_1 × PGCP 13 (BCII)	58	0	58	-	-
III	GC 516 (P_1)	16	0	16	-	-
	PGCP 1 (P_2)	16	0	16	-	-
	GC 516 × PGCP 1 (F_1)	18	0	18	-	-
	F_2 (F_1 Selfing)	164	0	164	-	-
	F_1 × GC 516 (BCI)	56	0	56	-	-
	F_1 × PGCP 1 (BCII)	60	0	60	-	-

Non-significant at 5 per cent ($P_{0.05}$ = 3.841)level of significance

TABLE 5: Genetic analysis of calyx pigmentation in segregating population of cowpea and test of goodness of fit to the expected ratio.

Cross	Generation	Observed number of plants			Expectedratio	x^2
		Red calyx pigmentation	Green calyx pigmentation	Total		
I	GC 2 (P_1)	0	16	16	-	-
	PGCP 5 (P_2)	18	0	18	-	-
	GC2 × PGCP 5 (F_1)	16	0	16	-	-
	F_2 (F_1 Selfing)	130	30	160	3:1	3.33
	F_1 × GC 2 (BCI)	39	25	64	1:1	3.06
	F_1 × PGCP 5 (BCII)	60	0	60	-	-
II	GC 2 (P_1)	0	18	18	-	-
	PGCP 13 (P_2)	16	0	16	-	-
	GC 2 × PGCP 13 (F_1)	20	0	20	-	-
	F_2 (F_1 Selfing)	126	30	156	3:1	2.76
	F_1 × GC 2 (BCI)	32	24	56	1:1	1.14
	F_1 × PGCP 13 (BCII)	58	0	58	-	-
III	GC 516 (P_1)	16	0	16	-	-
	PGCP 1 (P_2)	16	0	16	-	-
	GC 516 × PGCP 1 (F_1)	18	0	18	-	-
	F_2 (F_1 Selfing)	164	0	164	-	-
	F_1 × GC 516 (BCI)	56	0	56	-	-
	F_1 × PGCP 1 (BCII)	60	0	60	-	-

Non-significant at 5 per cent ($P_{0.05}$ = 3.841)level of significance

at maturity in cowpea. Backcross of F_1 plants with red pod coloured parent P_2 (PGCP 5) gave a close fit to 1 black : 1 red pod coloured plants, while backcross of F_1 plants with black seed coloured parent P_1 (GC 2) gave all black pod colored plants. These results indicated the dominance of black pod colour over brown and red colour and digenic inheritance of the trait. All F_1 plants derived from cross III, GC 516 (black pod color) x PGCP 1 (yellow pod color) had yellow pod color. Segregation in the F_2 gave 88 black, 68 brown and 8 yellow pod colored plants, which fitted to 9: 6 :1 ratio (Table 6.2). This indicated presence of epistatic gene interaction with polymeric gene action and digenic inheritance. Backcross F_1 plants involving brown pod color parent P_1 (GC 516) gave 34 black and 22 yellow pod coloured plants, which fitted to 1: 1 ratio of black and yellow pod colored plants, while backcross of F_1 with yellow pod

TABLE 6.1: Genetic analysis of pod colour at maturity in segregating population of cowpea and test of goodness of fit to the expected ratio in cross GC 2 x PGCP 5.

Generation	Observed number of plants				Expected ratio	x^2
	Blackcolour	Browncolour	Redcolour	Total		
GC 2 (P_1)	16	0	0	16	-	-
PGCP 5 (P_2)	0	0	18	18	-	-
GC2 × PGCP 5 (F_1)	16	0	0	16	-	-
F_2 (F_1 Selfing)	79	48	33	160	9:4:3	3.14
F_1 × GC 2 (BCI)	64	0	0	64	-	-
F_1 × PGCP 5 (BCII)	38	0	22	60	1:1	4.26

Non-significant at 5 per cent ($P_{0.05}$ = 5.991) level of significance

TABLE 6.2: Genetic analysis of pod colour at maturity in segregating population of cowpea and test of goodness of fit to the expected ratio in cross GC 516 x PGCP 1.

Generation	Observed number of plants				Expectedratio	x^2
	Black colour	Brown colour	Yellow colour	Total		
GC 2 (P_1)	16	0	0	16	-	-
PGCP 5 (P_2)	0	0	18	18	-	-
GC2 × PGCP 5 (F_1)	0	0	18	18	-	-
F_2 (F_1 Selfing)	88	68	8	164	9:6:1	1.38
F_1 × GC 2 (BCI)	34	0	22	56	1:1	2.56
F_1 × PGCP 5 (BCII)	0	0	60	60	-	-

Non-significant at 5 per cent ($P_{0.05}$ = 5.991) level of significance

colored parent P_2 (PGCP 1) gave all yellow coloured plants. These results indicated yellow pod colour is dominant over black and brown colour. Sangwan and Lodhi (1998) reported that black pod colour is partially dominant over white pod colour and is governed by one gene.

From the results, it can be concluded that the inheritance of various seed specific characters with dominance expression to different characters can be utilized as a good marker for execution of different breeding field operations. In the present study, flower colour in all the three crosses studied appeared to be controlled by single gene inheritance with violet flower colour dominant over white flower colour. Similarly, seed surface, pod beak colour and calyx pigmentation also appeared to be controlled by single gene inheritance, in which smooth seed surface dominant over rough seed surface, green pod beak colour dominant over red pod beak colour and green calyx pigmentation dominant over red calyx pigmentation. The inheritance of seed colour and pod colour at maturity appeared to be controlled by two gene interactions with supplementary gene action, with dominance of black seed colour over brown and white seed colour, and black pod colour dominant to red and brown colour.

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