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Linkage of genes for anthocyanin and chlorophyll pigmentation in cowpea [*Vigna unguiculata* (L.) Walp.]

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Abstract

The inheritance of anthocyanin and chlorophyll pigmentation of various vegetative and pod characters were studied in cowpea [*Vigna unguiculata* (L.) Walp] cross P-737-2 × V-23. One to three pairs of genes were responsible for inheritance of these characters. Monogenic ratio of 3 : 1 was observed for both pigmentation of pod tip and surface and pod streak. Duplicate ratio of 15 : 1 and trigenic duplicate ratio of 63 : 1 were obtained for pigmentation on peduncle tip and unripe pod colour respectively, while pigmentation on stipules and peduncle surface were controlled by any two of the three complementary genes and two threshold dominant genes giving F_2 ratios of 54 : 10 and 11 : 5 respectively. Pleiotropy and differential gene action were established among pigmentation on peduncle surface, stipules and unripe pod colour. Pigmentation on peduncle surface (Pgpls) was linked with peduncle tip (Pgpit), surface (Pgplts) and pod streak (Pst) with recombination value of 33.10, 43.71 and 38.41 per cent respectively. Pgplti was proved to be linked to four gene linkage group and Pgplt2 was linked to Gnp which belonged to separate two genes linkage group.

Key words : Cowpea, linkage, anthocyanin and chlorophyll pigmentation, *Vigna unguiculata*

Introduction

Some of the qualitative characters are economically important. In cowpea [*Vigna unguiculata* (L.) Walp] light pod colour is preferred over green types for vegetable. There are a number of pigmented characters scattered on various parts of the plant. Information on the inheritance and inter-relationships of these characters is very scanty in cowpea. This paper deals with inheritance and inter relationships of various pigmentation and pod characters in cowpea.

Material and methods

Three pigmented vegetative characters and three pod characters in a cross P-737-2 × V-23 were studied in the present investigation. F_1 in comparison with parents, F_2 and F_3 generations were subjected to x^2 test to work out the nature of inheritance. F_2 ratios were confirmed by the F_3 breeding behaviour of 40 families ranging in number from 62 to 102 plants. Family segregation was not assessed as the test of heterogeneity was non-significant. Joint segregation was worked out as per the standard procedure to assess the association of characters. Linkage value was estimated by the product ratio method of Fisher and Balmukund [1]. Pleitropy was also estimated as per the joint segregation procedure up to 2 genes common between any two characters. Gene symbols were adopted as per the recommendations of both the International committee on genetic symbols and Nomenclature as per Tanaka, *et al.*, [2] and Tomato genetics co-operative by Bortan, *et al.*, [3] and Clayberg, *et al.*, [4-5], Fery [6] proposed the rules adopted in Cucurbitaceae to be adopted in Vigna.

Results and discussion

Individual characters: The phenotypes of parents, F_1 and segregating F_2 plants are given in Table 1. Pigmentation and green unripe pod colour were found to be dominant over absence of pigmentation and white pods respectively. F_2 phenotypic ratios of 11 : 5, 15 : 1, 54 : 10, 3 : 1, 63 : 1 and 3 : 1 for pigmentation of peduncle surface, pigmentation on peduncle tip, pigmentation on stipules, pigmentation of pod tip and surface, unripe pod colour green *vs* white and pod streak respectively. All the F_2 phenotypic ratios were confirmed through F_3 breeding behaviour (Table 2).

Information on the genetics of pigmentation in cowpea is scanty [8]. In this study two threshold dominant genes, two duplicate genes and combinations of two of the three complementary genes were found to be responsible for pigmentation on peduncle surface, peduncle tip and stipules respectively. Inheritance for the latter two characters were studied by Viswanath [7] and Karkannavar *et al.*, [8] who reported two complementary genes and a single dominant gene respectively. A ratio of 11 : 5 for pigmentation on peduncle surface is reported for the first time. Harland, *et al.*, [9-10] noted that the factor P, B_1 and E caused

Table 1. Behaviour of parents, F_1 and F_2 generation for six characters in cowpea cross P-737-2 × V-23

Sl. No.	Character	P_1	P_2	F_1	Obs exp.	F_2 Dominance Present	F_2 Recessive Absent	Ratio	X_2	P (probability)
1.	Pigmentation on peduncle surface	Absent	Present	Present	Obs	186.00	98.00	11:5	1.40	0.2-0.3
					exp.	195.25	88.75			
2.	Pigmentation on peduncle tip	Absent	Present	Present	Obs	271.00	13.00	15:1	1.36	0.2-0.3
					exp.	266.25	17.75			
3.	Pigmentation on stipules	Absent	Present	Present	Obs	238.00	55.00	54:10	2.20	0.1-0.2
					exp.	247.00	45.78			
4.	Pigmentation on pod tip and surface	Absent	Present	Present	Obs	213.00	67.00	3:1	0.17	0.5-0.7
					exp.	210.00	70.00			
5.	Unripe pod colour	Green	Green	Green	Obs	305.00	3.00	63:1	0.36	0.5-0.7
					exp.	303.88	4.81			
6.	Pod streak	Streak	Non-streak	Streak	Obs	180.00	58.00	3:1	0.05	0.8-0.9
					exp.	178.50	59.50			

Table 2. Segregation behaviour for six characters of cowpea cross P-737-2 × V-23 in F_3 generation

a)	Pigmentation on peduncle surface	BTD (PP)			Segregating into		BTR (AP)	Probability
	(Presence Vs. Absence) (11 : 5)			1 : 3	11 : 5			
	Expected ratio	7		4	4		1	
	Observed number of families	20.00		6.00	13.00		1.00	
	Expected number of families	17.50		10.00	10.00		2.50	0.2-0.3
b)	Pigmentation on peduncle tip	BTD (PP)			Segregating into		PTR (AP)	
	(Presence Vs. Absence) (15:1)			15 : 1	3 : 1			
	Expected ratio	7		4	4		1	
	Observed number of families	21.00		7.00	10.00		0.00	
	Expected number of families	17.50		10.00	10.00		2.50	0.2-0.3
c)	Pigmentation on stipules	BTD (PP)			Segregating into		BTR (AP)	
	(Presence Vs. Absence) (54 : 10)		54 : 10	15 : 1	9 : 7	3 : 1		
	Expected ratio	10	8	12	12	12	10	
	Observed number of families	11.00	3.00	5.00	5.00	7.00	9.00	
	Expected number of families	6.25	5.00	7.50	7.50	7.50	6.25	0.1-0.2
d)	Pigmentation on pod tip and surface	BTD (PP)			Segregating into		BTR (AP)	
	(Presence Vs. Absence) (3:1)				3 : 1			
	Expected ratio	1			2		1	
	Observed number of families	10.00			15.00		15.00	
	Expected number of families	10.00			20.00		10.00	0.1-0.2
e)	Unripe pod colour	BTD (GPC)			Segregating into		BTR (WPC)	
	(Green Vs. White) (63 : 1)		63 : 1		15 : 1	3: 1		
	Expected ratio	37	8		12	6	1	
	Observed number of families	24.00	1.00		8.00	6.00	1.00	
	Expected number of families	23.13	5.00		7.59	7.59	0.625	0.2-0.3
f)	Pod streak	BTD (PS)			Segregating into		BTR (AS)	
	(Presence Vs. Absence) (3:1)				3 : 1			
	Expected ratio	1			2		1	
	Observed number of famHies	12.00			17.00		11.00	
	Expected number of families	10.00			20.00		10.00	0.5-0.7

BTD = Breeding true for dominant character; BTR = Breeding true for recessive character, PP = presence of pigmentation, AP = Absence of Pigmentation, GPC = Green pod colour, WPC = White pod colour, PS = Presence of streak and AS = Absence of streak

the development of anthocyanin in the peduncle tip. There is no information as to how these factors interact to produce the pigmentation. Pigmentation on pod tip and surface and streaked pods were controlled by a single gene dominant over their absence. Harland, *et al.*, [9-10] proposed several factors; B, E, P and R to be responsible for pigmentation of pod and envisaged triple series of multiple allelomorphs to explain his

Table 3. Joint segregation of pigmentation on peduncle surface (11:5) with other characters in cowpea cross P-373 × V-23

Sl. No.	Character with ratio	Joint ratio	Obs exp.	Remnarks	F_2 phenotypic joint segregation					Probability
					AB	Ab	aB	ab	X^2	
1.	Pigmentation on peduncle tip (15 : 1)	165 : 11	Obs:		273.00	16.00	19.00	2.00		<0.001
		75 : 5	Exp :	Independence	199.80	13.32	90.82	6.05	86.85	0.7-0.8
			Exp :	Linkage	269.39	14.78	22.95	2.89	1.1036	
			Cov :	33.10%						
2.	Pigmentation on stipules (54 : 10)	594:110	Obs :		253.00	35.00	17.00	5.00		<0.01
		270 : 50	Exp :	Independence	179.82	33.30	81.74	15.14	87.94	0.1-0.2
			Exp :	One gene Common (pleiotropy)	256.72	27.45	14.53	11.30	6.068	
3.	Pigmentation on pod tip and surface (3:1)	33 : 11	Obs :		218.00	71.00	15.00	6.00		<0.0001
		15 : 5	Exp :	Independence	159.84	53.28	72.66	24.22	86.52	0.7-0.08
			Exp :	Linkage	217.14	17.66	67.01	8.19	1.23	
			Cov :	43.71%						
4.	Unripe pod colour (63 :1)	693 : 11	Obs :		287.00	0.00	23.00	0.00		<0.0001
		325:5	Exp :	Independence	211.15	5.35	95.98	1.52	84.57	0.3-0.5
			Exp :	One gene	282.55	1.62	24.22	1.62	3.370	0.05-0.1
				common	284.17	0.00	19.37	6.46	7.17	
				(Pleiotropy) Two genes common (Pleiotropy)						
5.	Pod streak (3:1)	33 : 11	Obs :		214.00	13.00	76.00	7.00		<0.0001
		15 : 5	Exp :	Independence	159.84	53.28	72.65	24.22	61.19	0.2-0.3
			Exp :	Linkage	220.89	16.05	63.25	'9.81	4.17	
			Cov :	38.41%						

Obs, Exp and Cov observed, expecte and cross over (%) values respectively

Table 4. Joint segregation of pigmentation on peduncle tip (15 :1) with pigmentation on stipules (54:10), pigmentation on pod tip and surface (3:1), unripe pod colour (63:1) and pod streak (3:1)

Sl. No.	Character with ratio	Joint ratio	Obs. exp.	Remarks	F_2 phenotypic joint segregation					Probabi lity
					AB	Ab	aB	ab	X^2	
1.	Pigmentation on stiputes (54 : 10)	810 : 150	Obs :	Independence One	259.00	33.00	15.00	8.00		
		54: 10	Exp :	gene common	249.17	46.14	16.61	3.08	12.07	<0.0001
			Exp :	(Pleiotropy)	254.71	40.61	11.07	8.61	2.24	0.3-0.5
2.	Pigmentation on pod tip and surface (3:1)	45 : 15	Obs :		224.00	68.00	10.00	8.00		
		3 : 1	Exp :	Independence	217.97	72.65	14.53	4.84	3.94	0.2-0.3
3.	Unripe pod colour (63 : 1)	945 : 15	Obs :		290.00	4.00	15.00	2.00		<0.0001
		63 : 1	Exp :	Independence	287.00	4.56	19.13	0.30	10.47	0.7-0.8
			Exp :	One gene common (Pleiotropy)	287.00	3.64	18.22	1.22	1.15	
			Exp :	Linkage	286.92	3.71	18.24	1.13	1.28	0.7-0.8
			Cov :	3.04%						
4.	Pod streak (3 : 1)	45 : 15	Obs :	Independence	219.00	76.00	9.00	6.00		
		3 : 1	Exp :		217.97	72.66	14.53	4.84	2.54	0.3-0.5

Table 5. Joint segregation of unripe pod colour (63 : 1) with pigmentation of pod tip and surface pod streak

Sl. No.	Character with ratio	Joint ratio	Obs exp.	Remnarks	F_2 phenotypic joint segregation					Probability
					AB	Ab	aB	ab	X^2	
1.	Pigmentation of pod tip and surface (3:1)	189 : 63	Obs :		234.00	0.00	76.00	0.00		0.1-0.2
		3 : 1	Exp :	Independence	228.87	3.63	76.29	2.21	4.96	
2.	Pod streak (3 : 1)	169 : 63	Obs :		227.00	80.00	2.00 3.63	1.00		0.8-0.9
		3 : 1	Exp :	Independence	228.87	76.29		1.29	0.96	

AB = Both characters showing dominant phenotype; Ab = First character showing dominance and second character showing recessive phenotype; aB = First character showing recessive and second character showing dominant phenotype; ab = Both characters showing recessive phenotype.

results. A multiple allelic series of P locus P^t, P^y and P^g and two independent factors P^s and P^u were contemplated-by Sen and Bhowal [11] in explaining pigmentation on the pod.

Mortenson and Brittingham [12] considered a single gene, S that conditioned speckled pod and there is no information on its mode of inheritance. Green unripe pod colour was found to be dominant over white pods and three duplicate genes are known to influence this character. Several studies reporting genes that control different degrees of green pod colour have been published by Krishnaswamy, *et al.*, [13] and Kolhe [14]. All these clearly indicate that there is enormous variation created for these characters. Spontaneous mutation appears to have occurred widely in this crop to create variability. The role of wide range of mutator genes (transposable elements) to create enormous variability in cowpea crop was postulated by Sharma [15-16].

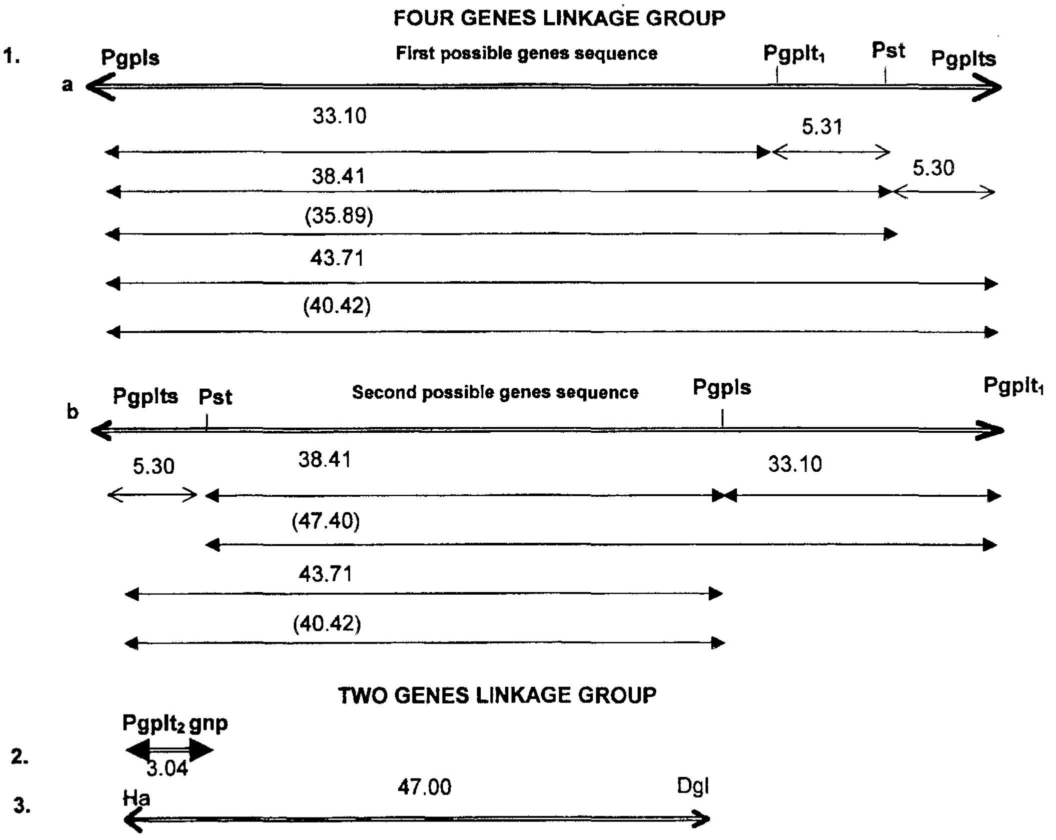

Fig. 1. Linkage map between the linked genes in cowpea cross P-737-2 × V-23 figure in the parenthesis indicate corrected map distance

(a) Inter-relationships of genes: Joint segregation data presented in (Table 3,4,5) revealed the activity of one pleiotropic gene in developing pigmentation on peduncle surface, peduncle tip, stipules and unripe pod colour. Linkage was detected among the genes controlling accumulation of pigment in plant organs like peduncle surface, peduncle tip and surface, pod streak and unripe pod colour. All other characters showed independent inheritance.

(b) Linkage relationship: Though the studies of inheritance in cowpea was started as early as 1911 by Spilman, the first linkage relationship was established by Saunders [17] Later on twenty one linkage groups were established by various workers but with only two genes each by Saunders [18-19], Harland [10], Spillman and Sando [20], Harland [10] and Kolhe [14]. In the present study, four gene linkage group was established for the first time. Pigmentation on peduncle surface (pgpls) was linked with pigmentation peduncle tip (Pgplt1), pigmentation on peduncle tip and surface (Pgplts) and pod streaks (Pst) with recombination values of 33.10., 43.17 and 38.41 per cent respectively (Table 3). Pgplt2 is linked to unripe pod colour (Gnp) with a distance of 3.04 per cent (Table 4). Gnp assorted independently of pgplts and Pst (Table 5). The gene Pgplt1 was located along with others three markers on the same chromosome and Pgplt2 was linked to Gnp forming a separate linkage group. There are two possible gene sequences in four markers linkage group (Fig. 1a&b). Kosambi [22] formula was used for getting the corrected map distance which are given in parenthesis.

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References

1. **Fisher R. A. and Balmukund.** 1928. The estimation of linkage by offspring of selfed heterozygotes. J. Genetic., **20**: 79-92.
2. **Tanaka Y., Ephurssi B., Hadarn E., Hagberg A., Kemp T., Love A., Nachtsheim H., Pontecarvo G. and Rhoades M. M.** 1957. Report of the International committee on genetic symbols and Nomenclature Inter. Uni. Biol. Sci. Colleagues, B, **30**: 1-6.
3. **Barton D. W., Butler L., Jenkins J. A., Rick C. M. and Young P. A.** 1955. Rules for nomenclature in Tomato genetics. J. Hered., **46**: 22-26.
4. **Clayberg C. D., Butler L., Kerr E. A., Rick C. M. and Young P. A.** 1960. Second list of known genes in Tomato including supplementary rules for nomenclature. J. Hered., **51**: 167-174.
5. **Clayberg C. D., Butler L., Kerr E. A., Rick C. M. and Robinson R. W.** 1966. Third list of known genes in Tomato with revised linkage map and additional rules. J. Hered., **57**: 189-196.
6. **Fery R. L.** 1980. Genetics of Vigna. Hort. Rev., **2**: 311-394.
7. **Viswanath K. P.** 1979. Inheritance studies of few qualitative characters in cowpea unpublished M.Sc. (Agri.) Thesis, Univ. Agri. ScL, Bangalore.
8. **Karkannavar J. C., Venugopal R. and Goud J. V.** 1981. Inheritance and linkage studies in cowpea [*Vigna unguiculta* (L) Walp]. Ind. J. Genet., **51**: 203-207.
9. **Harland S. C.** 1919b. Notes of inheritance in cowpea. Agr. New Barbados, **18**: 20.
10. **Harland S. C.** 1920. Inheritance of certain characters in the cowpea (*Vigna sinensis*). J. Genet., **10**: 193-205.
11. **Sen N. K. and Bhowal J. G.** 1961. Genetics of *Vigna sinensis* (L.) Savi. Genetics, **32**: 247-266.
12. **Mortensen J. A. and Brittingham W. H.** 1952. The inheritance of pod colour in Southern pea. [*Vigna sinensis* (L.) Savi] and [*Vigna sesquipedalis* (L.) Frunw], Madras Agri. J., **159**: 449-456
13. **Krishnaswamy N., Nambiar K. K. and Mariakulandai A.** 1945. Studies in cowpea [*Vigna unguiculata* (L) walp]. Madras Agri. J., **33**: 145-160, 193-200.
14. **Kolhe A. K.** 1970. Genetic studies in *Vigna* sp. Poona Agr. Coll. Mag., **59**: 120-137.
15. **Sharma B.** 1969a. Chemically induced mutations in cowpea [*Vigna sinensis* (L.) Savi] Curr. Sci., **38**: 520-521.
16. **Sharma B.** 1969b. Induction of a wide range mutator in *Vigna sinensis*. Proc. Sym. on radiation and radiomimetic substances in mutation breeding. Sept. 26-29, 1969, Bombay, Bhabha Atomic Research centre, Bombay, p. 13-22.
17. **Saunder A. R.** 1960b. Inheritance in the cowpea [*Vigna sinensis* (L) Savi.] (Endb) III. Mutations and linkages. S. Afr. J. Agr. Sci., **3**: 327-348.
18. **Saunders A. R.** 1959. Inheritance in the cowpea *Vigna sinensis* (Endb). I. Colour of the seed Coat. S. Afr. J. Agr. Sci., **2**: 285-307.
19. **Saunders A. R.** 1960a. Inheritance in the cowpea *Vigna sinensis* (Endb) II. Seed coat colour pattern, flower, plant and pod colour S. Afr. J. Agr. Sci., **3**: 141-162.
20. **Spillman W. J. and Sando B. S.** 1930. Mendelian factors in cowpea (*Vigna* sp). Michigan Acad. Sci., Arts and letters. Papers **11**: 249-283.
21. **Kosambi D. D.** 1944. The estimation of map distances recombination values. Ann. Eugen., **12**: 172-175.