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Analysis of Genetic Linkage in the Cowpea *Vigna unguiculata*

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ABSTRAK

Analisis genetik 12 loci yang mensyaratkan ciri-ciri morfologi menunjukkan beberapa rangkaian dalam kacang duduk (Vigna unguiculata *(L) Walp) Ddengan menggunakan kedua-dua kacuk balik dan data segregasi cantuman F2, 12 loci ini diletakkan pada lima kumpulan berangkai. Pg loci untuk pempigmeman nod, Pf untuk bunga ungu, Pc untuk salut biji benih licin, Na yang bermata kecil dan Br untuk salut biji benih coklat yang membentuk kumpulan berangkat 1 dengan susunan mungkin PgNaBrPc. Lokus Bpd untuk mencabangkan pedunkel dirangkaikan kepada Bp untuk pod kering coklat dan Dhp untuk pod yang terbuka bersam susunan mungkin Bpd Bp Dhp. Kumpulan berangkai yang ketiga mengandungi loci Crl untuk daun sesil. Bentuk daun hastate, Ha dan bilangan daun berseptrum, Spt, masing-masing bersekutu dengan kumpulan berangkai keempat dan kelima.*

ABSTRACT

Genetic analysis of 12 loci conditioning morphological characteristics indicated several linkages in cowpea Vigna unguiculata *(L.) Walp). Using both backcross and F2 joint segregation data, these 12 loci were assigned to five linkage groups. The loci Pg for nodal pigmentation, Pf for purple flower, Pc for smooth seed coat, Na for narrow eye, and Br for brown seed coat make up linkage group 1 with the probable order Pg-Na-BrP-cPf. The Bpd locus for branching peduncle was linked to Bp for brown dry pod and Dhp for pod dehiscence with the probable order Bpd-Bp-Dhp. The third linkage group consisted of loci Crl for crinkled leaf and Pt for sessile leaf. Hastate leaf shape, Ha, and septafoliolate leaf number, Spt, belong to the fourth and fifth linkage groups, respectively.*

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp) (Leguminosae) is an important grain legume crop in tropical and subtropical regions of the world. The genus *Vigna* contains about 170 species with growth habits ranging from erect, semi-upright to prostate and twining forms (Blackhurst and Miller 1980).

The International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria and the Genetics Unit of the Department of Agricultural Biology, University of Ibadan, Ibadan, Nigeria maintain more than 12,000 accessions of *Vigna unguiculata*; data are recorded for more than 50 characters, while some of the lines carry mutant traits.

Cowpeas are diploid species, 2n = 22 (Faris 1964; Mukherjee 1965; Pignone *et al.* 1990) with little genetic, and no chromosomal, divergence of the cultivated subspecies from their wild progenitors (Faris 1965). Several mutations have caused morphological and reproductive changes in the cowpea and these have led to the expression of such traits as leaf, branching, non-branching plants, etc. and an increase in useful genetic variability. A recessive mutant is of value to plant breeders as a genetic marker in isolating accidental selfs during hybridization and in identifying and evaluating marker-linked genes affecting specific quantitative traits. A marker may also be of value in surveying genetic variability in populations and interpopulation variability.

Reports of the inheritance of many morphological and disease resistance characteristics of cowpea were reviewed by Fery (1985). The

following mutant traits: branching peduncle, non-petiolate leaf, crinkled leaf and septafoliolate leaf number were described by Fawole (1988) and Fawole and Afolabi (1983). They reported each to be under single gene inheritance with the non-branching peduncle dominant to the branching peduncle, petiolate leaf dominant to non-petiolate leaf, non-crinkled leaf dominant to the crinkled leaf and trifoliolate leaf number dominant to the septafoliolate leaf number. Monogenic segregation pattern was also reported for nodal pigmentation (Harland 1919), hastate leaf shape (Ojomo 1977), flower colour (Kolhe 1970; (Hanchimal and Goud 1978), dehiscent pod (Aliboh *et al.* 1995), narrow eye pattern (Spillman 1913), dry pod colour (Saunder 1960), brown seed coat colour (Spillman 1913) and smooth seed coat (Rajendra *et al.* 1979).

According to these reports, nodal pigmentation was dominant to non-pigmentation, hastate leaf shape was dominant to subglobose leaf shape, purple flower was dominant to white flower, shattering of dry pod was dominant to non-shattering, and narrow eye pattern was recessive to the solid eye pattern. Also, the brown colour of the dry pod was dominant to the white seed coat colour, while the smooth seed coat was dominant to the rough seed coat texture. The symbols assigned to the genes controlling each of these characters are presented in Table 1.

However, there are few studies on gene linkage in cowpea and most links need further study and verification (Fery 1985). Calub (1968) reported that seed eye pattern and seed coat were inherited independently and Harland (1919) reported close linkage for seed characteristics such as colour and eye pattern. However, Saunders (1960) later reported that multiple effect of particular genes were responsible for the association between these traits.

Prior to the initiation of this study, no published work gave a precise linkage map of cowpea which could serve as a basis for further study. The lack of an encouraging foundation of good quality marker genes probably discouraged interest in mapping genes in cowpea. Also, many of the genes are involved in complex epistatic relationships, which makes them difficult to classify in segregating populations. However, the availability of a fairly detailed gene map in cowpea would be useful in genetic, cytogenetic and plant breeding studies.

The purpose of this study was to investigate the linkage relations of 12 easily scored, simply inherited traits to facilitate selection procedures and to initiate the construction of a genetic map for cowpea.

MATERIALS AND METHODS

The genetic materials were obtained from the cowpea genetics programme of the University of Ibadan, Nigeria and IITA. Preliminary screening of all genetic stocks was carried out for the 12 characters studied (Table 1). Each line was sown in 20 plastic pots with two plants per pot. The plants were maintained to maturity, and no segregation was observed for these traits. Their seeds collected for use in hybridization.

Ten plants from each parent were grown in the greenhouse and they were combined in the following six crosses: IBPC x IT82E - 9; IBPC x TVU1; IBPC x IBS876; IfeBPC x TVU4578; R10028 x G2497; IBPC x WCIBADAN - 10. A portion of the F1 seed was sown in the field in the first season of 1991 to produce F2 seeds. All mature pods that developed on the F1 plants were harvested and seeds were bulked after threshing and cleaning. Seeds of parents F1, BC1, BC2, and F2 progenies from the six crosses were planted in the dry season (December) of 1991 at the IITA experimental farm. The field was irrigated once every week for an 8-hour period throughout the growing period. The experimental design was a randomized complete block with four replications. For a cross, each parent had a total of 4 rows, F1 had 4 rows, backcross to parent 1 (BC1) had 8 rows, backcross to parent 2 (BC2), the recessive parent, had 16 rows, while F2 had a total of 60 rows. The length of each row was 10 m. Within rows, the spacing was 50 cm, and 75 cm between rows. Each plant was given a replication number, a row number and a within-row number. This enabled data collection from germination to weighing of seed after harvest for each plant without a mix-up.

During data collection, the characters were visually scored into alternate classes. The F2 and backcross data were analysed using the chi-square method to test the goodness-of-fit of observed ratios to expected ratios for each character.

Data were pooled when the chi-square test for heterogeneity indicated that families were homogeneous. For all categories of two-factor

TABLE 1
Gene designation and phenotypic description of cowpea

Trait	Classes		Line or Cultivar							
	Dominant (+)	Recessive (-)	IBPC	FE BPC	IT82E-9	TVU4578	IBS876	R10028	G2497	WCIB-10
Nodal pigmentation (Pg)	Pigmented	Non-pigmented	-	+	-	-	-	-	+	-
Branching peduncle (Bpd)	Normal	Branching	-	+	+	+	+	+	+	+
Crinkled leaf (Crl)	Normal	Crinkled	+	+	+	+	+	+	+	-
Non-petiolate leaf (Pt)	Petiolate	Non-petiolate	+	+	+	+	-	+	-	-
Hastate leaf shape (Ha)	Hastate	Normal	-	-	-	+	-	-	-	+
Flower colour (Ef)	Purple	White	-	+	+	-	-	+	+	
Narrow eye pattern (Na)	Solid	Narrow		+	+	+	+	+	-	+
Brown seed coat Colour (Br)	Brown	White	-	*	*	*	*	+	-	+
Brown dry pod (Bp)	Brown	Straw	-	*	+	-	-	*	+	
Seed coat texture (Pc)	Smooth	Rough	-	+	+	+		+	-	+
Dehiscent pod (Dhp)	Dehiscent	Non-dehiscent	-	-		+	-	—	+	
Leaf number (Spt)	Trifoliolate	Septafoliolate	+	+	+	+	+	-	+	+

* Not applicable

joint segregation ratios, contingency chi-square was used to detect linkage.

This was done by using Linkage-1, a computer program (Suiter *et al.* 1983). If the calculated chi-square is greater than the tabulated value, then linkage is indicated between the two traits. After detecting linkage by test of independence, linkage intensities were calculated using both F2 and backcross data. Linkage-1 was used to calculate recombination fractions and map distances, which were used to determine the gene order.

RESULTS

The 12 loci tested showed good agreement with expected 3:1 and 1:1 segregation ratios in the F2 and backcross generations respectively. Linkage tests indicated linkage between some of these loci and independence between others. Of the 45 F2 and backcross linkage tests between gene pairs in this study, 22 suggested independence while 23 indicated linkage (Table 2). The loci Pg, Br, Na, Pc and Pf make up linkage 1. Two of these loci, Br and Na, were linked with no recombination while they both showed tight linkage to Pc locus for smooth seed coat. Linkage group 2 is comprised of loci Bpd, Bp and Dhp. The loci Crl and Pt make up linkage group 3. The locus Ha showed independence from the six loci tested against it, hence it belongs to a separate linkage group. Also, locus Spt for septafoliolate leaf number was not linked to any of the loci tested against it and it thus forms linkage group 5. The respective gene orders and map distances of these linkage groups are presented in *Fig. 1.*

DISCUSSION

The results of inheritance studies were in agreement with the references, indicating single gene inheritance of these characters. From results of linkage tests, a relatively high incidence of linkage was observed. Five linkage groups, corresponding to five chromosomes, out of the 11 linkage groups possible in cowpea, were established from this study. Loci Na (narrow eye) and

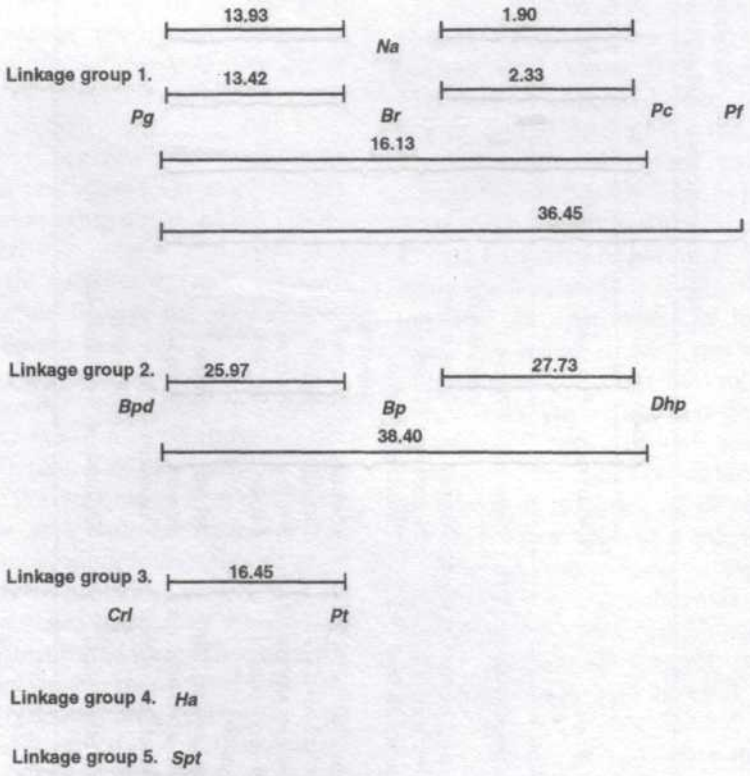

Fig 1. Cowpea gene order and map distances from F2 population

TABLE 2
Allelic constitution, F_2 and backcross progency distribution, calculated recombination values and map distances of linked traits in cowpea

Alleles	Generation	Number of Plants a	b	c	d	Total	X^2 linkage*	Recombination % and SE	Map Distance (MU) and SE	Linkage Phase
Pg pg Bpd bpd	F_2	558	1153	200	39	950	3.00	45.23 ± 2.56	74.85 ± 2.56	C
	BC	99	97	96	87	379	0.14	49.08 ± 2.57	116.98 ± 2.57	C
Pg pg Pf pf	F_2	601	203	0	68	872	163.55*	31.12 ± 3.02	36.45 ± 3.02	C
	BC	171	0	0	149	320	320.00*		≥50	C
Bpd bpd Pf pf	F_2	518	182	136	36	872	1.89	46.08 ± 2.64	79.98 ± 2.65	C
	BC	87	76	86	71	320	0.06	49.38 ± 2.79	126.92 ± 2.80	C
Bpd bpd Bp bp	F_2	650	126	79	120	975	163.02*	25.07 ± 2.96	27.56 ± 2.97	C
	BC	95	24	16	120	255	119.62*	15.69 ± 2.27	16.24 ± 2.28	C
Bpd bpd Dhp dhp	F_2	583	197	85	110	975	70.18*	34.38 ± 2.78	42.17 ± 2.79	C
	BC	99	46	14	96	255	78.21*	23.53 ± 2.65	25.54 ± 2.66	C
Bpd bpd Pc pc	F_2	612	151	133	63	259	13.73*	40.61 ± 2.66	56.67 ± 2.66	C
	BC	79	40	50	86	255	22.28*	35.29g± 2.99	43.94 ± 3.00	C
Bp bp Dhp dhp	F_2	618	112	86	159	975	224.46*	22.26 ± 3.01	23.93 ± 3.02	C
	BC	72	64	28	91	255	23.03*	36.08 ± 3.00	45.55 ± 3.01	C
Bp bp Pc pc	F_2	572	149	145	93	959	32.14*	37.77 ± 2.73	49.27 ± 2.73	C
	BC	78	33	51	93	255	30.46*	32.94 ± 2.94	39.53 ± 2.95	C
Dhp dhp Pc pc	F_2	561	149	151	98	959	30.45*	35.71 ± 2.56	37.13 ± 2.56	C
Ha ha Pt pt	F_2	348	144	147	39	678	4.72*	43.94 ± 3.07	68.52 ± 3.07	C
Ha ha Bpd bpd	F_2	341	143	135	49	668	0.55	48.31 ± 2.95	101.58 ± 2.9	C
	BC	75	81	72	82	310	0.05	49.35 ± 2.84	125.74 ± 2.84	C
Pt pt Bpd bpd	F_2	352	130	121	66	669	4.50*	44.22 ± 3.08	69.78 ± 3.08	C
Pg pg Pt pt	F_2	148	578	76	172	974	10.99*	42.51 ± 2.59	62.84 ± 2.60	C
Pg pg Crl crl	F_2	518	204	194	58	974	2.61	46.19 ± 2.50	80.72 ± 2.51	C
Pg pg Spt spt	F_2	548	176	186	64	974	0.17	49.04 ± 2.43	115.91 ± 2.43	C
Pg pg Br br	F_2	491	230	4	238	963	90.12*	13.11 ± 3.15	13.42 ± 3.16	C
Pg pg Pc pc	F_2	511	220	227	5	963	76.78*	15.59 ± 3.13	16.13 ± 3.13	C
Pg pg Na na	F_2	502	226	231	4	963	84.13*	13.58 ± 3.15	13.93 ± 3.15	C
Pt pt Crl crl	F_2	497	255	216	6	974	85.0*	15.88 ± 3.10	16.45 ± 3.11	C
Pt pt Br br	F_2	553	197	175	38	963	6.38*	43.15 ± 2.59	65.25 ± 2.60	C
Pt pt Pc pc	F_2	556	193	174	40	963	4.54*	44.32 ± 2.56	70.24 ± 2.57	C
	BC	30	32	31	31	124	0.03*	49.19 ± 4.49	120.19 ± 4.50	C
Pt pt Na na	F_2	557	188	179	39	963	5.05*	44.02 ± 2.57	68.88 ± 2.57	
	BC	31	31	37	25	124	1.17	45.16 ± 4.47	74.47 ± 4.48	

TABLE 2 (cont'd)

Alleles	Generation	Number of Plants a	b	c	d	Total	X^2 linkage*	Recombination % and SE	Map Distance (MU) and SE	Linkage Phase
Crl crl Spt spt	F_2	549	162	185	78	974	4.88*	45.07 ± 2.53	73.98 ± 2.53	C
Spt spt Na Na	F_2	547	182	164	70	963	2.24	46.55 ± 2.51	83.29 ± 2.51	R
Crl crl Na Na	F_2	551	166	188	58	963	0.02	49.71 ± 2.42	146.00 ± 2.45	R
Crl crl Br br	F_2	534	171	194	64	963	0.03	49.62 ± 2.43	139.22 ± 2.43	R
Spt spt Br br	F_2	544	183	180	56	963	0.20	48.90 ± 2.44	112.47 ± 2.44	R
Sp spt Pc pc	F_2	551	174	180	58	963	0.20	48.22 ± 2.43	110.78 ± 2.44	R
Br br Pc pc	F_2	716	9	13	225	963	847.87*	2.33 ± 3.22	2.33 ± 3.22	C
	BC	56	0	0	68	124	124.00*			C
Br br Na na	F_2	773	0	0	230	963	965.00*			C
	BC	56	0	0	68	124	124.00*			C
Pc pc Na na	F_2	719	12	6	226	963	868.15*	1.90 ± 3.82	1.90 ± .23	C
	BC	56	0	0	68	124	124.00*			C

*$P \leq 0.05$

a = dominant at both loci A-B-

b = dominant at the first locus, recessive at the second locus A-bb

c = recessive at the first locus, dominant at the second locus aaB-

d = recessive at both loci aabb.

Br (brown seed coat) were the only very closely linked loci. No recombinant was obtained from them. Nearness of these loci to the centromere may account for the difficulty in obtaining recombinants.

Gene order was consistent in different pairwise combinations to support the linkage map proposed. Calub (1968) reported that cowpea seed eye and seed coat colour were inherited independently. However, Harland (1919) had earlier reported tight linkage between seed characteristics such as colour and eye pattern, and flower colour, though such linkages in this study are not as tight as those reported by him.

Saunders (1960) observed that multiple effects of particular genes are responsible for the association between seed characteristics such as colour and eye pattern and flower colour, contrary to the findings of this study. This is because inheritance studies indicated different models for the characters and also because recombinants were obtained in linkage tests.

In cases where tight linkages occurred and or no recombinants were obtained for either or both recombinant classes, it was observed that this involved pigmentation genes. This could be because absence of pigments in vegetative parts usually leads to absence of pigmentation in other plant parts such as flower, pod and even seed. On the other hand, a plant with purple pigments in vegetative parts may or may not produce flowers or pods with pigments. Fery (1985) and Frahm-Levilveld (1965) have proposed that a general colour factor, C, must be present before any of several pigment genes can be expressed.

When it is lacking the plant lacks pigment in both vegetative and reproductive parts. The extent to which this gene causes anthocyanin pigment to be produced varies, and so is the location of the pigment.

CONCLUSION

This study has established five linkage groups corresponding to five chromosomes from the 11 linkage groups available in cowpea. These linkage groups comprise 12 loci. This is a significant contribution towards the development of a concise and comprehensive cowpea linkage map.

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