

<http://www.pjbs.org>

PJBS

ISSN 1028-8880

**Pakistan
Journal of Biological Sciences**

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Genetic Analyses of Pigmentation in Cowpea

Francis Kwame Padi

CSIR, Savanna Agricultural Research Institute, P.O. Box 52, Tamale, Ghana

Abstract: Genetic control of pigmentation in different parts of cowpea (*Vigna unguiculata* (L.) Walp.) was studied in six F₂ segregating populations and their corresponding F₃ families derived from eight parents. Monogenic control for colour expression was found for leaf node pigmentation, flower (petal) colour, immature pod colour, seed coat colour, seed eye colour and seed eye colour pattern. Presence of pigment was dominant over absence of pigment and the black seed eye was dominant over brown eye. For seed eye pattern however, partial dominance of the very small eye type over the Holstein eye type was observed. Different flower colour genes induce similar white flower colours in the parents and non-allelic interactions between these white flower colour loci produced novel flower colour variants in the F₂ population. In the parental lines, flower and seed pigmentation are due to the same single locus effects, although flower colour may be influenced by epistatic gene interactions that have no effects on seed coat pigmentation. Pleiotropic effects of the recessive locus that causes loss of pigmentation of the leaf node include loss of pigment in petals and at least, in a genetic background of lines with a white seed coat, induces intense pigmentation around the hilum. By contrast, there are no apparent pleiotropic effects of the pod colour locus and the seed eye colour locus on pigmentation in other tissues.

Key words: Cowpea, epistasis, pleiotropy, pigmentation

INTRODUCTION

Colour production is an integral part of the development of various plant parts; the type of colour may adapt the plant part for a specific function, such as the brightly coloured petals of insect-pollinated plant species. The formation of colour in plant parts has therefore been the subject of a number of studies. It is known that the various shades of purple, red and orange colour of plant organs result from deposition of anthocyanins in the cell vacuole and pigmentation patterns result from cell-specific expression of combinations of regulatory proteins (Mol *et al.*, 1998). The final colour tonality of a plant organ is however affected by the amount of pigment and type of tissues pigmented, co-pigmentation, the presence of metal ions and vacuolar pH (Freitas *et al.*, 1991 and Martin and Gerats, 1993). Experiments have shown that anthocyanin pigmentation is developmentally regulated (Jaakola *et al.*, 2002 and Honda *et al.*, 2002) and may be affected also by a number of abiotic stress factors (Kumar and Sharma, 1999; Edreva *et al.*, 2002). Colour production is however primarily vested in the transcriptional control of expression of the anthocyanin bio-synthetic genes, with distinct regulators controlling specific steps of the bio-synthetic pathway (Martin and Gerats, 1993). The number of interacting loci and mode of action of these colour loci are of interest to breeders because their manipulation leads to the development of novel plant types with

specific colour characteristics. Commonly, monogenic control of pigmentation have been found with absence of colour recessive to presence of colour, as in petal colour in *Brassica rapa* (Rahman, 2001) and petal colour in *Leucaena* (Simioni *et al.*, 1998) and the green cotyledon trait in cowpea (Fery and Dukes, 1994). Quattrocchio *et al.* (1999) noted that the recessive alleles that condition non-pigmentation result from loss of function mutation of anthocyanin bio-synthetic genes or regulatory loci.

Cowpea (*Vigna unguiculata* (L.) Walp.) is a self-pollinating plant and an important grain and fodder legume crop in the semi-arid tropics that lie 30°N to 30°S of the equator, where it provides an important part of the dietary protein requirements. For both vegetative and reproductive parts, a number of pigmentation types are commonly observed in cultivated cowpea. Spillman (1912) noted that interaction of pairs of five dominant anthocyanin bio-synthetic genes is responsible for the range of seed coat and flower pigmentation types in cowpea. To gain further insight into the genetic control of pigmentation of various plant parts of cowpea, the segregation of colour in F₂ populations and their corresponding F₃ families were studied.

MATERIALS AND METHODS

The study was carried out at the experimental fields of the Savanna Agricultural Research Institute (SARI) located in the northern region of Ghana, at an altitude of

183 m, 09° 25' and 0° 580' longitude. The cultivars and breeding lines used as parents and their relevant characteristics are listed in Table 1. The crossings were done at the University of California, Riverside (UCR) and the seeds were kind donations from Dr. Jeffrey Ehlers of the UCR.

Experimental procedures: Parents, F₂ and their corresponding F₃ segregating populations were planted in rows with 60 cm spacing between and within rows. Plantings of the F₂ segregating populations were done on July 23, 2002 and the F₃ populations were planted on January 14, 2003. The size of each F₂ population used is indicated in Table 2. Typically, 25 to 40 F₃ families for a pigmentation pattern, each originating from a single F₂ plant were evaluated. Fifty individuals per F₃ family were observed.

Plants were monitored for pigmentation patterns for the various plant parts. Leaf node colour was assessed on plants six weeks after sowing. Flower (petal) colour was recorded on the first flush of opened flowers whereas the pod colour was taken on one-week old pods. Seed coat colour, seed eye colour and seed eye pigmentation pattern were recorded on dried seeds after harvesting dried pods.

A chi-square test for the distribution of plant frequencies versus the particular pigmentation character was used to check the genetic hypotheses of control of pigment inheritance (Snedecor and Cochran, 1989).

RESULTS AND DISCUSSION

The observed and expected segregation ratios for pigmentation in the various plant parts studied are presented in Table 2. For each trait, the phenotypic ratios fitted the hypothesised genetic control for pigment expression as indicated by the probability values greater than 5 % ($p > 0.05$) using χ^2 analyses.

For all crosses observed involving parents that differed for pigment or non-pigment expression in a particular plant part, the F₁ plants showed pigmentation. In all individuals possessing leaf node pigmentation, purple colouration of the leaf node and petioles became evident 10 days after planting. Purple pigmentation in cowpea has been attributed to the accumulation of anthocyanins (Fery, 1985).

The Melakh X KV61-1-1 cross: The F₂ individuals obtained from this cross segregated for leaf node colour. Segregation for leaf node colour was consistent with that expected for a single gene, with purple pigmentation dominant to non-pigmentation, (Table 2). This segregation ratio was confirmed in the F₃ generation by

testing 40 families obtained from F₂ plants that had pigmented leaf nodes. Fifteen families were homozygous for the pigmented leaf node locus and 25 segregated for purple and green leaf node. Chi-square analysis showed that this ratio fits the expected 1:2 for non-segregating: segregating families for the F₃ generation ($X^2_{[1:2]} = 0.33$; $p = 0.57$). Within the F₃ segregating families, the phenotypic ratios fitted monogenic inheritance (data not shown).

The KVx61-1-1 X Mouride cross: The F₂ population segregated for leaf node pigmentation, flower colour and seed coat colour. Monogenic inheritance for leaf node pigmentation was obtained in this cross (Table 2). Plants with pigmented leaf nodes were grown in the F₃ generation and families either produced plants with pigmented leaf nodes only, or segregated for leaf node pigmentation.

Four distinct flower colour types were uncovered in the F₂ population. These were the parental types, a deep purple type and a white flower type with a tinge of purple in the wing petals. Two seed coat colour types of brown and white were recovered. Node pigmentation, flower colour and seed coat colour showed unique associations as indicated in table 3. The appearance of deep purple flower type in the F₁ and four flower colour variants in the F₂ generation indicated that the genes for flower colour in the parents are non-allelic and some sort of epistatic interaction resulted in the various colour types observed. The observed F₂ ratios however did not fit that expected for disgenic epistatic interaction. In the F₂ generation, plants with white-purple tinged flowers had non-pigmented nodes and suggested recessive epistasis between the leaf node colour locus and the mutant purple flower type operating in addition to the epistatic interaction between the major flower colour loci. These epistatic interactions resulted in a shift from the ratio of 3:4:9 expected for disgenic recessive epistasis to 9:12:16:27 expected for two sets of recessive epistasis involving three loci. A good fit to this model was obtained using chi-square analysis (Table 2). When the purple flower colour and white-purple tinge groups were combined (i.e. neglecting the effect of the leaf node locus on flower colour), a significant fit was obtained for disgenic recessive epistasis ($X^2_{[3:4:9]} = 0.43$; $p = 0.81$). In the F₃ generation, 25 families that had the Mouride flower type were tested, 7 families produced only the Mouride type of flowers and 18 families segregated for the Mouride type and white flowers. This ratio fits the expected 1:2 for non-segregating: segregating families ($X^2_{[1:2]} = 0.31$; $p = 0.58$). All F₃ individuals had white seed coats. Chi-square analysis showed that in the F₂ generation, segregation for white and brown seed coat types fit a 7:9 ratio (Table 2).

Table 1: Pigmentation characteristics of parents used

Parent	Node colour	Flower colour	Pod colour	Seed coat colour	Eye colour	Eye pattern ²
ITP-148	Purple	White	-	White	Light brown	Very small
Sul-518	¹	-	-	White	Brown	-
Mouride	Purple	White, slight purple pigment in standard petal	Deep green	White	-	-
Melakh	Purple	-	-	White	-	-
KVx61-1-1	Green	White	-	White	-	Holstein group
IT82E-18	-	Purple	Light green	Brown	-	-
01-15-52	-	-	-	White	Black	-
01-11-52	-	-	-	White	Black	-

¹Colour type not relevant to this study, ²Classification as suggested by IBPGR, 1983;

Table 2: Segregation for pigmentation of various plant parts in cowpea F₂ populations

Populations	Trait	Observed	Expected	² n	Ratio	X ²	Prob.
KVx61-1-1 X Mouride	Leaf node colour	Green (39): Purple (111)	Green (37.5): Purple (112.5)	150	1:3	0.08	0.78
KVx61-1-1 X Mouride	Flower colour	Tinged (24): ¹ Mouride (25): White (38): Purple (63)	Tinged (21.1): Mouride (28.1): White (37.5): Purple (63.3)	150	9:12:16:27	0.75	0.86
Kvx61-1-1 X Mouride	Seed coat colour	White (63): Brown (87)	White (65.6): Brown (84.4)	150	7:9	0.18	0.67
Melakh X KVx61-1-1	Leaf node colour	Green (42): Purple (141)	Green (45.75): Purple (137.25)	183	1:3	0.41	0.52
ITP-148 X KVx61-1-1	Leaf node colour	Green (47): Purple (147)	Green (48.5): Purple (145.5)	194	1:3	0.06	0.81
ITP-148 X KVx61-1-1	Seed eye pattern	Very small (48): Narrow eye (99): Holstein (47)	Very small (48.5): Narrow eye (97): Holstein (48.5)	194	1:2:1	0.47	0.79
Mouride X IT82E-18	Flower colour	Mouride (34): Purple (110)	Mouride (36): Purple (108)	144	1:3	0.15	0.70
Mouride X IT82E-18	Pod colour	Light green (39): Green (105)	Light green (36): Green (108)	144	1:3	0.33	0.57
Mouride X IT82E-18	Seed coat colour	White (34): Brown (110)	White (36): Brown (108)	144	1:3	0.15	0.70
01-15-52 X Sul-518	Seed eye colour	Brown (40): Black (110)	Brown (37.5): Black (112.5)	150	1:3	0.14	0.71
01-11-52 X ITP-148	Seed eye colour	Brown (41): Black (117)	Brown (39.5): Black (118.5)	158	1:3	0.08	0.78

¹Mouride refers to the flower colour of the variety Mouride described in Table 1, ²n is the number of F₂ individuals observed

Table 3: Colour association between plant parts in the KVx61-1-1 X Mouride cross

Flower colour	Leaf node colour	Seed coat colour
Mouride type	Pigmented	White
Purple	Pigmented	Brown
White, purple tinge	No pigment (green)	Brown
White	Pigmented or green	White

In cowpea, flower colour is generally associated with seed coat colour; purple flowers are associated with self-coloured or pigmented seed coats and white flowers with cream or white seed coats (Saunders, 1960). It is evident from this study however that this relationship may be affected depending on the presence of modifying genes. The leaf node colour locus has no effect on seed coat colour, but its masking effect on the purple flower trait resulted in ratios different from that expected between flower colour and seed coat colour.

The Mouride X IT82E-18 cross: The F₂ progeny segregated for flower colour, immature pod colour and seed coat colour. For each of these traits, monogenic inheritance was found (Table 2) with dominance of purple flower over white flower, deep green pod colour dominant over light green pod colour and brown seed coat was dominant over white seed coat. Association between purple flower and brown seed coat suggested pleiotropic effect of the flower colour locus. This observation considered together with the segregation pattern in the KVx61-1-1 X Mouride cross provide evidence that pigmentation of the flower and seed coat in cowpea are controlled by the same locus. Depending on the choice of

parents in a hybridisation programme, non-allelic gene interactions may occur to produce novel flower and seed coat colour types. Where allelic variants are crossed, control of flower and seed coat colour has been found to follow a monogenic Mendelian inheritance pattern (Sangwan and Lodhi, 1998). In addition, depending on the presence of genes with modifying effects on the flower colour phenotype (such as the epistatic effects of the recessive non-pigmented leaf node locus) flower colour variants may be produced that will not be indicative of the segregation ratios expected for seed coat colour. This has important implications where selection for seed coat colour (an important economic trait) is based on flower colour variants.

The ITP-148 X KVx61-1-1 cross: The F₂ generation segregated for leaf node colour and seed eye colour pattern. Segregation for leaf node colour was consistent with single locus control for pigmentation (Table 2). Partial dominance for very small eye pattern over the Holstein eye pattern was detected with the F₁ generation having a narrow eye pattern. In the F₂ generation, three seed eye pattern types; the very small eye, the narrow eye and the Holstein eye types were observed. The phenotypic ratios observed indicated monogenic inheritance (Table 2). This was further confirmed in the F₃ generation by testing 40 families each derived from F₂ plants that had the very small eye pattern and 40 families that had the narrow eye pattern. In the families derived from the very small eye type, no segregation for eye

pattern was evident. Segregation for eye pattern in the F₃ families derived from the narrow eye F₂ individuals was consistent with the expectation that all narrow eye types were heterozygous for the seed eye pattern locus, (data not shown).

Observation of a direct association between leaf node colour and the seed eye colour pattern suggested a pleiotropic effect of the leaf node locus. As such all the F₃ families derived from the very small seed eye type did not segregate for leaf node colour whereas those derived from the narrow seed eye type segregated for leaf node colour. The recessive mutation that causes non-pigmentation of the leaf node also induces intense pigmentation around the hilum resulting in the Holstein eye pattern. Within cowpea producing regions where preference is for white seed coat types with minimum amount of pigmentation around the hilum (such as within West Africa, Hall *et al.*, 1997), variability studies have indicated predominance of pigmented leaf node types compared with non-pigment types (Bennett-Lartey and Ofori, 1999 and Ezueh and Nwoffiah, 1984) and suggests an indirect selection against the green leaf node type by farmers through selection for reduced pigmentation around the hilum.

The 01-15-52 X Sul-518 and 01-11-52 X ITP-148 crosses: The F₁ individuals had black eye seeds indicating dominance of black eye over the brown eye. In each of the crosses, a good fit of the observed phenotypic ratios was established for single locus control of pigmentation (Table 2). This was further confirmed in the F₃ generation by growing 40 families obtained from F₂ plants with black eye for each cross. The proportions of segregating to non-segregating families obtained fitted the theoretical 2:1 ratio as confirmed by chi-square test, with probability values of p= 0.37 and p= 0.44 for the 01-15-52 X Sul-518 and 01-11-52 X ITP-148 crosses, respectively.

ACKNOWLEDGMENT

The author gratefully acknowledges the funding support of the Agricultural Services Sub-sector Investment Program (AgSSIP) of the Government of Ghana in carrying out this research.

REFERENCES

Bennett-Lartey, S.O. and I. Ofori, 1999. Variability studies in some qualitative characters of cowpea (*Vigna unguiculata*) (L.) Walp) accessions from four cowpea-growing regions of Ghana. Ghana J. Agric. Sci., 32: 3-9.

- Edreva, A., A. Gürel, E. Gesheva and H. Hakerlerler, 2002. Reddening of cotton (*Gossypium hirsutum* L.) leaves. *Biologia Plantarum*, 45: 303-306.
- Ezueh, M.I. and G.N. Nwoffiah, 1984. Botanical observations on a local collection of vegetable cowpea cultivars in south-eastern Nigeria. *Trop. Grain Legume Bull.*, 29: 2-6.
- Fery, R.L., 1985. The genetics of cowpea: a review of the world literature. In: S.R. Singh and K.O. Rachie (Eds), *Cowpea research, production and utilisation*, John Wiley and Sons, Chichester, pp: 25-62.
- Fery, R.L. and Dukes, 1994. Genetic analysis of the green cotyledon trait in southernpea (*Vigna unguiculata* [L.] Walp.). *J. Amer. Soc. Hort. Sci.*, 119: 105-1056.
- Freitas, L.H.C., M.T. Schifino-Wittmann and N.R. Paim, 1991. Floral characteristics, chromosome number and meiotic behaviour of hybrids between *Leucaena leucocephala* (2n = 104) and tetraploid *L. diversifolia* (2n = 104) (Leguminosae). *Braz. J. Genet.*, 14: 781-789.
- Hall, A.E., B.B. Singh and J.D. Ehlers, 1997. Cowpea breeding. *Plant Breeding Reviews*. 15: 215-274.
- Honda, C., N. Kotoda, M. Wada, S. Kondo, S. Kobayashi, J. Soejima, Z. Zhang, T. Tsuda and T. Moriguchi, 2002. Anthocyanin bio-synthetic genes are coordinately expressed during red coloration in apple skin. *Plant Physiol. Biochem.*, 40: 955-962.
- IBPGR (International Board for Plant Genetic Resources), 1983. Descriptors for cowpea. Rome, IBPGR. pp: 29.
- Jaakola, L., K. Määttä, A.M. Pirttilä, R. Törrönen, S. Kärenlampi and A. Hohtola, 2002. Expression of genes involved in anthocyanin biosynthesis in relation to anthocyanin, proanthocyanidin and flavonol levels during bilberry fruit development. *Plant Physiol.*, 130: 729-739.
- Kumar, V. and S.S. Sharma, 1999. Nutrient deficiency-dependent anthocyanin development in *Spirodela polyrhiza* (L.) Schleid. *Biologia Plantarum*, 42: 621-624.
- Martin, C. and T. Gerats, 1993. Control of pigment biosynthesis genes during petal development. *Plant Cell.*, 5: 1253-1264.
- Mol, J., E. Grotewold and R. Koes, 1998. How genes paint flowers and seeds. *Trends Plant Sci.*, 3: 212-217.
- Quattrocchio, F., J. Wing, K. van der Woude, E. Souer, N. de Vetten, J. Mol and R. Koes, 1999. Molecular analysis of the *anthocyanin2* gene of petunia and its role in the evolution of flower color. *Plant Cell.*, 11: 1433-1444.
- Rahman, M.H., 2001. Inheritance of petal colour and its independent segregation from seed colour in *Brassica rapa*. *Plant Breeding*, 120: 197.

- Sangwan, R.S. and G.P. Lodhi, 1998. Inheritance of flower and pod colour in cowpea (*Vigna unguiculata* L. Walp.). *Euphytica*, 102: 191-193.
- Saunders, A.R., 1960. Inheritance in the cowpea (*Vigna sinensis* Endb.). 2: Seed coat colour pattern; flower, plant and pod colour. *South African J. Agric. Sci.*, 3: 141-162.
- Simioni, C., M.T. Schifano-Wittmann and N.R. Paim, 1998. A model for flower colour inheritance in *Leucaena* (Leguminosae). *Genet. Mol. Biol.*, 21: No.3
- Snedecor, G.W.W. and W.G. Cochran, 1989. *Statistical Methods*. Iowa State University, Ames.
- Spillman, W.J., 1912. The present status of the genetics problem. *Sci.*, 35: 757-767.