

Full Length Research Paper

Studies on the inheritance pattern of joints, pod and flower pigmentation in cowpea [*Vigna unguiculata* (L) walp.]

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Genetic studies were carried out to elucidate the inheritance pattern of plant, flower and pod-tip pigmentation in cowpea. The parental varieties were selected based on their contrasting plant and flower pigmentation. These varieties were grown in the screen house between May, 2002 and October, 2003, and sufficient number of parental F₁, F₂, backcross and F₃ seeds were obtained. The F₁, F₂, and backcross along with parent seed were planted in the field during the cropping season covering July-October, 2003. Observations were taken on joint pigmentation, flower colour and pod traits on individual plant basis in each population. The results were analyzed for genetic segregation, to determine the number of genes controlling these traits and interactions between them. The joint pigmentation was controlled by one dominant gene. The coloured margins on the standard and wings of flower were controlled by one dominant gene; the purple standard and wings was also controlled by a single dominant gene and it was allelic to the gene controlling the coloured margins. The purple wing with white standard was controlled by another dominant gene which was different from the ones controlling pigments on the standard petal. The pigmented (purple) calyx was controlled by two genes and it was pleiotropic (tightly linked) to pod-tip as well as purple backline on standard petal. Of the two genes controlling the calyx colour, one controls joint pigmentation such that in the absence of joint pigmentation is prerequisite for calyx, pod-tip and backline pigmentation.

Key words: Inheritance, segregation, genes, pleiotropic, association.

INTRODUCTION

Cowpea [*Vigna unguiculata* (L) Walp] is an important food legume of the tropics and a versatile crop primarily cultivated in the semi-arid regions of tropical Africa, Asia and South America. It is used; as seed in processed foods, as vegetables (fresh leaves, peas and pods) and as a dry haulms and fodder (Rachie, 1985). Cowpea is an inexpensive source of vegetable protein, and a hardy crop well adapted to relatively dry environments. The seed contains about 23 - 25% protein and 50 – 67%

starch, and is a major source of minerals and vitamins (Quin, 1997). By 1981, cowpea was traditionally considered as food legume of the poor in Nigeria, but recent survey (Nnanyelugo et al., 1997) showed that this perception has changed. Cowpea is now considered to be food for the rich, the informed, the salaried worker and those who can afford it.

The cowpeas grown in different parts of the tropics vary widely in seed colour and type, and preferences changes from region to region (IITA, 1983). In West Africa, the preferred types are white and brown seeds with rough coat. In East Africa and parts of Latin America, red and brown seeds with smooth coat are preferred. But in some Latin American countries, particularly Cuba and

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parts of the Caribbean, the black colour is preferred (IITA, 1983). These varying preferences show the need to develop varieties with different characteristics, as no single variety can be suitable for all regions.

Preference for various cowpea varieties makes the study of different traits of cowpea, such as flower colour, pod traits and seed traits of immense importance. Having basic knowledge of the genetics of plant characteristics is particularly useful in generating genetic maps that would help breeders in their selection work. It also enables the geneticists to study existing association within/and between qualitative traits and desired quantitative traits.

The genus *Vigna* has great potentials for improvement since gene exchange has proved successful between all wild and cultivated forms (Maréchal et al., 1978). Cowpea is ideal for genetic research and plant breeding. It is a diploid, with a relatively short cycle.

Pigmentation on cowpea is present on the cotyledons, joints, flower petals, seeds pods, peduncles, stems and leaves. In 1914, Mann cited by Fery (1985) showed that anthocyanin and melanin like substances are responsible for colour in cowpea and the expression of any pigment on the plant is the result of the interaction between several pigment genes and a general colour factor. A number of recent studies have added further information on the genetics of important traits in cowpea such as plant pigmentation, flower colour, seed colour and seed coat texture. A few studies on linkage and mapping have been conducted (Singh, 2000). The author, however, indicated the need for further studies due to confusions and overlaps in assigning gene symbols for different plant parts.

Genetic research in cowpea has been subject of immense interest since the beginning of the 1900's (Rachie, 1985). Genetic information is needed to devise or select efficient breeding procedures which could lead to development of improved and high yielding cowpea varieties suitable for different ecological zones and cropping systems. This would not only add to the knowledge but can also be used as the tool/marker in breeding programmes. However, the early reports did not specify the joints pigmentation rather they refer to it as pigmented plant. None of the earlier reports has endeavored to study plant pigmentation on a holistic basis, explaining the relationship between pigmentation in different plant parts (Singh, 2000). This study therefore makes it specific to joints pigmentation.

MATERIALS AND METHODS

The work was carried out at the International Institute of Tropical Agriculture (IITA), Kano station in the screen house between May - October, 2002. Progeny testing was carried out at IITA Research Farm, Minjibir between July - October, 2003 during the rainy season. Six cowpea varieties were obtained from IITA breeding programme facility and used for hybridization. Preference was given to lines with contrasting characters for pigmentation, flower trait and pod traits. For the hybridization studies, planting was done in the

screen house in plastic pots that were filled with fresh sandy loam topsoil. The seeds were carefully selected and directly sown in well labeled prepared pots. They were watered regularly, and hand weeding was carried out at regular intervals.

After germination the plants were thinned to three or four plants per pot for effective observation. Hybridization studies were carried out in the screen house. Hybridization of the cowpea flowers was done using hand emasculation and pollination. The materials used for hybridization were: sharply pointed forceps, small tags and 70% alcohol. The forceps used were periodically immersed in alcohol between crosses to prevent contamination by unwanted pollens. Pollination was performed immediately after emasculation for best result. After pollination an appropriately labeled tag was fixed to the peduncle directly below the pollinated flower to identify the cross. Crosses were made between varieties with contrasting traits such as: Purple flower crossed with pale (purple wing) tinge (purple on margin standard) or white flower (completely devoid of colour), pigmented plant with non pigmented plant.

Fortnightly planting was carried out to ensure continuous hybridization process. This enabled crossing between late maturing lines of first planting date with early maturing lines of second planting date and so on. Observations were made on several cowpea parameters such as flower colour, plant pigmentation and pod traits.

Data collected were subjected to statistical analyses. Chi-square test was carried out to determine the goodness of fit to various genetic ratios.

RESULTS AND DISCUSSIONS

Joint pigmentation

This refers to a purple pigmentation present on the joints, between stem and peduncles, stem and branches, stem and leaflets. In the crosses between pigmented and pigmented plants, all the parental lines as well as all the F_1 , F_2 , F_3 and backcross plants were pigmented. Thus, there was no segregation indicating that the gene for joint pigmentation on these parents was the same. The results of all the crosses involving parents with pigmented joints and non pigmented joints (Table 1) indicate a monogenic inheritance of this trait with pigmentation being dominant.

Cowpea has pigmentation on vegetative parts of the plant, such as joints (between stem and peduncles, stem and branches and stem and leaflets); flowers; comprising calyx, standard and wings as well as on peduncles and pods. In all, these pigmentation appeared to interact in a way to suggest the presence of a factor responsible for their expression. This factor was referred to as the colour factor (Fery, 1985). Most of the traits were simply inherited involving one or two major genes with some levels of interactions and pleiotropism. Birader et al. (1997) reported three genes for calyx colour, three genes for seed coat colour, four genes for pod tip pigmentation and four genes for flower colour with some genes showing pleiotropic effects. Fery (1985) suggests that anthocyanins were responsible for all colours in the flower petals, seed pods, peduncles, petioles, stem and leaves. The colour factor appears to be present in all the plants studied.

Table 1. Segregation Pattern for joints pigmentation in different populations of Cowpea involving the cross pigmented x non-pigmented.

Variety/Cross	Generation	Number of Plants		Expected Ratio	χ^2	Probability
		PP	NP			
ITK-568-18	Parent	0	20			
IT95K-238-3	Parent	20	0			
IT93K-452-1	Parent	20	0			
Aloka Local	Parent	20	0			
238-3 x 568-18	F ₁	16	0			
238-3 x 568-18	F ₂	98	33	3 : 1	0.003	0.025 – 0.05
F ₁ x 568-18	BC1	5	4	1 : 1	0.11	0.25 – 0.150
F ₁ x 238-3	BC2	5	0			
452-1 x 568-18	F ₁	13	0			
452-1 x 568-18	F ₂	71	28	3 : 1	0.57	0.50 – 0.75
F ₁ x 568-18	BC1	3	2	1 : 1	1.00	0.5 – 0.75
F ₁ x 452-1	BC2	4	0			
Aloka x 568-18	F ₁	14	0			
Aloka x 568-18	F ₂	78	25	3 : 1	0.03	0.50 – 0.10

PP = Pigmented plants; NP = Non pigmented plants

The results obtained are in agreement with previous study of Karkannavar et al. (1991) who reported a single dominant gene conditioning stipule colour and that red colour is dominant over green.

Flower colour

Inheritance of flower colour was also found to be simply inherited when different types of pigmentation were considered separately. Two gene pairs were observed to control the trait. Multiple alleles was observed in the cross between purple and margin (tinged) (Table 2). Both colour genes were present in the purple and tinged flowers. The tinged colour gene is dominant in the tinged flower but recessive in the purple flower. The 3 purple: 1 ratio obtained suggests that segregation in the F₂ and F₃ occurred only in gene controlling the tinged colour. One gene appears to segregate in the cross involving purple standard and wings and purple wings (Table 2) and purple standard and wings and white standard and wings (Table 2) as such dominance was observed with purple being dominant. A 3:1 ratio was obtained from the F₂ and F₃ segregating populations.

The purple wing was controlled by another dominant gene which was different from the ones controlling pigments on the standard petal. When both genes are present, the purple colour is expressed. Presence of the purple wing gene alone gives the flower its purple wings with white standard colour. Absence of the allelic gene of the purple standard and wings results in white flower, since the effect of the purple wing gene cannot be expressed in the absence of the former.

A similar report was given by Fery (1985), suggesting that white flowers may result whenever one general

colour factor, C, or the complementary genes are absent. He suggested that anthocynin factor, L, is responsible for purple wings (pale) colour and a factor, D, is responsible for increasing colour intensity in the presence of L, and an allelic gene appears to control margin colour on standard and wings. It appears that the basic colour gene in purple standard and margins is different from the one which has only purple wings.

Presence of backline on standard petal

This refers to the presence of purple dash in the back of standard petal. This was observed to be in dominant over its absence. In all the crosses studied (Table 3), the F₂ and F₃ populations segregate in 3 backline positive: 1 backline negative indicating the dominance of backline and that segregation occurred in one gene only. These observations agree with Kolhe (1970) who reported that stripes on the dorsal surface of the standard petal are dominant over yellow.

Calyx and pod-tip pigmentation

The Inheritance pattern of pigmentation on both pod tip and calyx was observed to be similar; therefore, discussions presented on pod-tip pigmentation apply to calyx pigmentation as well. Two patterns of inheritance, which appeared to be correlated with joint pigmentation were observed for this trait. These segregation ratios differed based on the presence of or absence of pigmentation on the joints of the plant. Inheritance pattern of pigmentation on both pod tip and calyx was studied and was observed to be similar. Consequently, discussions on pod-tip pigmentation apply to calyx pigmentation

Table 2. Segregation pattern for flower colour involving various colour combinations PSWxMSW, PSWxPW, WSWxMSW, MSWxPSW and MSWxPW in different population of cowpea crosses.

Variety/Cross	Generation	Number of Plants				Expected Ratio	X ²	Probability
		PSW	MSW	WSW	PW			
Aloka Local	Parent	20	0	0	0			
IT93K-452-1	Parent	0	20	0	0			
IT97K-568-18	Parent	0	0	0	20			
IT90K-277-2	Parent	0	0	20	0			
Alokax 568-18	F ₁	14	-	-	0			
Alokax 568-18	F ₂	78	-	-	25	3:1	0.03	0.10-0.75
277-2xAloka	F ₁	6	-	0	-			
277-2xAloka	F ₂	41	-	13	-	3:1	0-025	0.80-0.90
F ₁ x Aloka	BC	5	-	2	-	1:1	2-78	0.75-0.90
452-1X Aloka	F ₁	5	0	-	-			
452-1X Aloka	F ₂	25	8	-	-	3:1	0.01	0.10-0.95
F ₁ x 452-1	BC	3	1	-	-	1:1	1.00	0.5-0.75
277-2x452-1	F ₁	-	16	0	-			
277-2x452-1	F ₂	-	97	32	-	3:1	0.003	0.05-0.10
F ₁ x 452-1	BC1	-	6	2	-	1:1	2.78	0.75-0.90

PSW = Purple standard and wings; MSW = margin standard and wings; WSW = white standard and wings; PW = purple wings.

Table 3. Segregation pattern for presence of backline on standard petal involving the combination white standard and wings without backline x white standard and wings with backline in different populations of cowpea crosses.

Variety/cross	Generation	Number of Plants		Expected Ratio	X ²	Probability
		WSWb+	WSWb-			
		0	20			
Komboinse local	Parent	20	0			
277-2 x Kamboinse	F ₁	6	0			
277-2 x Komboinse	F ₂	28	11	3 : 1	0.21	0.25 – 0.5
F ₁ x Kamboinse	BC1	3	4	1 : 1	0.14	0.1 – 0.25

WSWb+ = White standard and wings with backline on standard petals.

WSWb- = White standard and Wings without backline on standard petals.

as well. In the crosses involving pigmented and pigmented pod-tip all the parental lines F₁, F₂ and F₃ progenies plants had pigmented pod-tips showing no segregation thereby indicating that the genes controlling the traits are the same in all the varieties used.

In the cross between cowpea plants with pigmented pod-tip and non pigmented pod tip, the results indicate pigmentation to be dominant over non pigmentation and the trait was observed to be controlled by two genes. These two genes appear to be complementary, 9 pigmented : 7 non pigmented ratio. However, on close examination of the parent and segregating population, it was evident that the joint pigmentation gene is a prerequisite for pod-tip colours gene to express. When one or both genes are absent the pod-tip is not pigmented. This is presented in the cross involving plants with pigmented joints and pod-tip and plants lacking pigmentation on both the joints and pod-tips (Table 4). The F₂ and F₃ progenies

segregated into 9:7 ratio of pigmented and non pigmented pod-tip respectively indicating that in the pigmented plants the two gene pairs were dominant, while in the non pigmented plants the two gene pairs were recessive. In the cross involving plants having pigmentation on both the joints as well as pod-tip and plants having pigmentation on the joints but not on the pod-tip gave a 3:1 ratio of pigmented and non pigmented pod-tips respectively in the F₂ and F₃ progenies (Table 4). Here the two genes are present together, therefore the pod-tip appear pigmented and this suggests that segregation occurred in only one of the two genes controlling the trait, while the gene was the same in both the parental lines. This is in agreement with Premsekar and Raman (1972) and Kolhe (1970) who reported that dark green pod is dominant over pale green and segregation pattern observed in the F₂ population suggests monogenic form of inheritance.

Table 4. Segregation pattern for pod-tip pigmentation in different populations of cowpea involving the cross combination pigmented x non-pigmented.

Variety/cross	Generation	No. of Plants		Expected Ratio	X ²	Probability
		PP	NPP			
Kamboinse Local	Parent	20	0			
IT93K-452-1	Parent	20	0			
IT90K-277-2	Parent	0	20			
IT97K-568-18	Parent	0	20			
277-2 x Kamboinse	F ₁	6	0			
277-2 x Kamboinse	F ₂	28	11	3 : 1	0.213	0.25 – 0.5
F ₁ x 277-2	BC1	4	3	1 : 1	0.14	0.1 – 0.25
F ₁ x Kamboinse	BC2	5	0			
452-1 x 568-18	F ₁	13	0			
452-1 x 568-18	F ₂	56	43	9 : 7	0.004	0.05 – 0.1
F ₁ x 568-18	BC1	3	6	1 : 3	0.33	0.25 – 0.5
F ₁ x 452-1	BC2	3	0			

PP = Pigmented pod; NPP = Non-pigmented pod.

Table 5a. Segregation Pattern for F₂ populations of cowpea cross, showing association between joints pigmentation flower colour and backline.

Cross	PP/PSWb+	NP/PWb-	Ratio	X ²	Probability
238-3 x 568-18	98	33	3 : 1	0.003	0.25 – 0.50

NP/PWb- = Non-pigmented plant/purple wings flower without backline.

PP/PSW b+ = Pigmented plant/purple standard and wings flowers with backline.

Table 5b. Segregation pattern for F₂ populations of various cowpea crosses, showing association between joints pigmentation, pod tip and calyx pigmentation.

Cross	Number of plants			Ratio	X ²	Probability
	PP/PPT/PC	PP/NPT/NPC	NP/NPT/NPC			
452-1 x 568-18	56	NA	43	9 : 7	0.004	0.05 – 0.1
238-3 x 568-18	74	NA	57	9 : 7	0.004	0.105 – 0.1
277-2 x kamboinse	28	11	NA	3 : 1	0.213	0.25 – 0.5
277-2x452-1	99	30	NA	3 : 1	0.209	0.25 – 0.5

PP = Pigmented plant; PPT = pigmented pod-tip; PC = pigmented calyx; NPT = non pigmented pod-tip; NPC = non pigmented calyx; NP = non-pigmented plant; NA = not applicable.

Linkage /associations

Association was observed among the coloured traits of cowpea. These traits included Joints pigmentation, flower colour, pod-tip pigmentation, calyx pigmentation and backline on standards petals (Tables 5a and 5b). Some of the associations are attributed to being tightly linked and some to pleiotropy. This suggests that the gene controlling joints pigmentation is a prerequisite for the expression of calyx colour, pod-tip colour and backline. Joshi et al. (1994) reported a pleiotropic gene P₁ for pigmentation in calyx, corolla and pod-tip with localized genes conditioning colouration on individual parts. It was

observed that pigmented calyx, pod-tip and backline were expression of the same gene as they went together as one trait and needed joints pigmentation gene as a prerequisite. Therefore, they are tightly linked or pleiotropic.

CONCLUSION

The study revealed that inheritance pattern of qualitative traits (plant pigmentation, flower colour and pod traits) varied from trait to trait. Some traits were monogenic and simply inherited, including plant pigmentation. Some of the traits appeared to be controlled by two gene pairs,

with various gene interactions, such as dominance, epistasis and complementarities.

Pigmentation was observed to be dominant over non-pigmentation. The pigmented (purple) calyx was controlled by two genes and it was pleiotropic (tightly linked) to pod-tip, they go together. Purple standard flower was dominant over white standard, purple wings (pale), margin standard (tinged) flowers and the gene controlling purple standard and wings was allelic to the gene controlling the coloured margins. The interaction between genes controlling purple wings (pale) and margin standard (tinged) exhibit modifying gene effect by giving purple flower in both F_1 and F_2 generations.

Backline presence on standard petal was found to be dominant over its absence. The results obtained from this study strongly indicated that the coloured traits of cowpea were associated. Some of the associations are attributed to being tightly linked and some to pleiotropy.

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