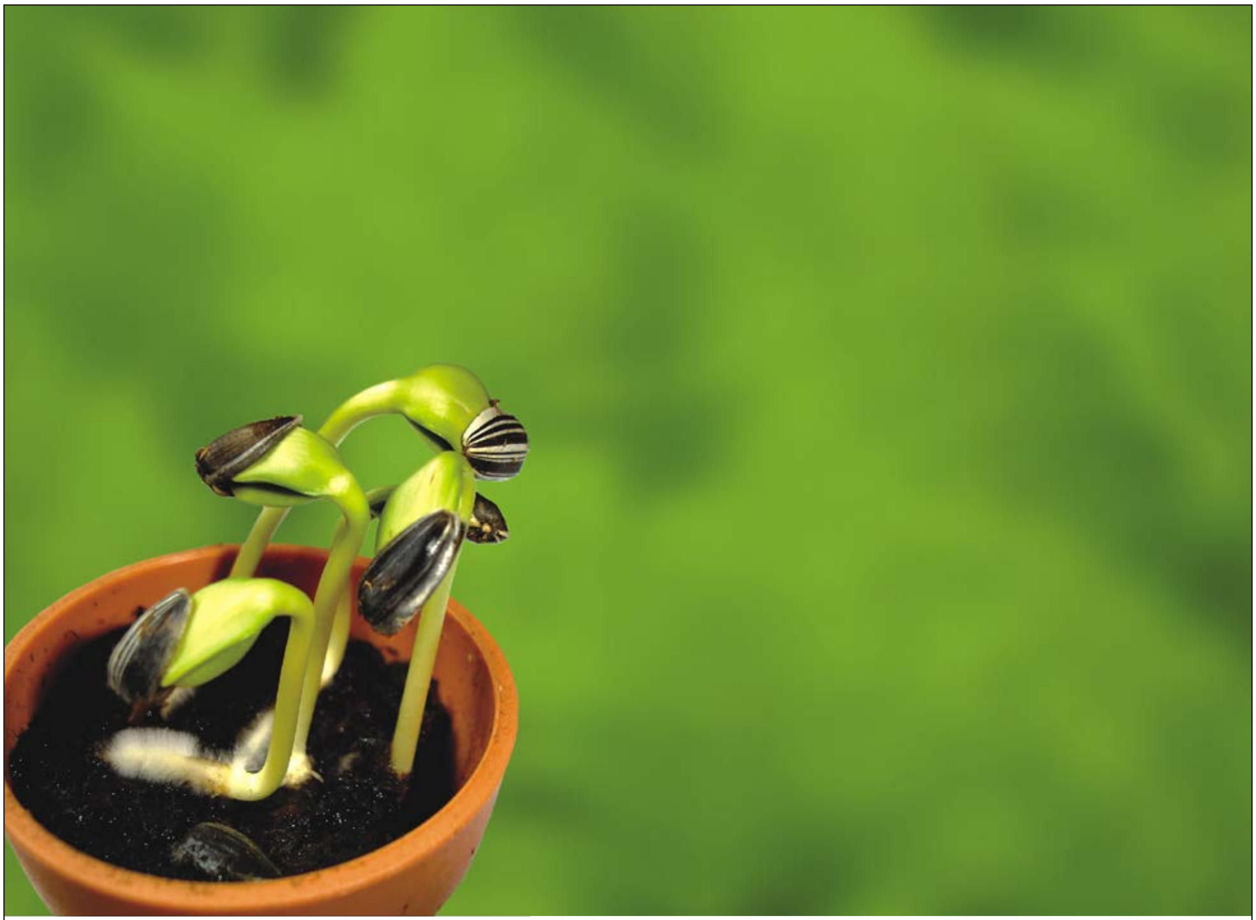

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Inheritance of Seed Coat Colour in Cowpea (*Vigna unguiculata* (L.) Walp)

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ABSTRACT

Cowpea seed coat colour is one of the important consumer traits of the crop in West Africa. The objective of the study was to find out the ratios of different seed coat colour types that would segregate from crosses involving cowpea genotypes of different colours. This would enable breeders to anticipate seed coat colours that would result in segregating populations and also help in estimating the number of genes controlling the trait. Six different bi-parental crosses were made and the resulting seeds grown to F_1 plants. Seeds were harvested from F_1 plants and grown on the field. An average of 250 plants was studied within each segregating population. The number of seed coat colour phenotypes produced varied from two for Bambey 21/Gbode to ten for Bambey 21/Tona. Individuals of some of the populations could not be classified based on seed coat colour as they exhibited continuous variation and therefore Chi-square goodness of fit test could not be conducted on them. It is suspected that many genes might be involved in seed coat colour inheritance in cowpea and as such the use of molecular and quantitative principles may be helpful in understanding the genetic control of the trait.

Key words: Cowpea, heterosis, qualitative, quantitative, segregation, seed coat colour

INTRODUCTION

Cowpea is very important crop in the tropics and subtropics (Stoilova and Pereira, 2013). In Ghana, cowpea is the second most consumed legume in human diet after groundnut. Its importance as feed and in soil improvement among others cannot be overemphasized. Cowpea is cultivated throughout many agro-ecologies of Ghana; however, the country imports the commodity to supplement its demand (Quaye *et al.*, 2011; Langyintuo *et al.*, 2003).

Grain yield of cowpea in most regions of Africa falls far below the potential of the crop due to many problems (Makoi *et al.*, 2010). High among production constraints of cowpea are insect pests attack, diseases infestation and weather failure (Dugje *et al.*, 2009). Development of new varieties addressing the various problems can help reduce the yield deficit. However, breeding against constraints without consumer acceptability considerations may result in the rejection of the improved varieties. This is because consumer preference is very important in cowpea utilization is Africa with Ghana as no exception.

Observation across markets in Ghana revealed that the imported types of cowpea are different from the Ghanaian varieties; imported ones having larger grains with cream or white coat colour

(Quaye *et al.*, 2011; Egbadzor *et al.*, 2013). Contact with farmers revealed that the imported types do not normally perform well under farmers' condition in the country. However, these are the types with high premium on the market.

Pigmentation of seeds and other parts of the plant is due to the synthesis of anthocyanins and other flavonoids. Many secondary plant metabolites belong to flavonoid class and among them anthocyanins are reported to be the most known, according to Holton and Cornish (1995). Anthocyanins are found in almost all plants and it is believed that they are present in all tissues (Chalker-Scott, 1999). Some anthocyanin genes are known to control the pigmentation of multiple parts of plants (Chandler *et al.*, 1989) while some such as pac 1 gene is known to control colour of only the seed coat in maize (Selinger and Chandler, 1999). Anthocyanins are known to be important to plants in response to abiotic such as drought (Chalker-Scott, 1999) as well as biotic (Makoi *et al.*, 2010; Sharma *et al.*, 2011) factors. Besides the roles of anthocyanin to the plant, it controls pigmentation of the seed coat which consequently has influence on consumer preference.

Seed coat colour has mainly been reported as a qualitative trait (Yilwa, 2012). However, crosses made over the years have pointed contrary in many cases. According to Oluwatosin (2000), two genes control the cross involving black or brown to cream seed coat; however, only one gene is responsible when red and cream are crossed. This research aimed at estimating the number of genes that might control seed coat colour in cowpea and thereby deciphering if the mode of inheritance is qualitative or quantitative. Six different bi-parental crosses were used. This would help in designing breeding programmes to produce consumer preferred seed coat colour type cowpea.

MATERIALS AND METHODS

Six bi-parental crosses were made between seven different true breeding cowpea genotypes namely; UCR779, CB27, Nhyira, Gbode, Bambey 21, Tona and Gh3710. The crosses were made in the West Africa Centre for Crop Improvement farms, University of Ghana in 2012. The F_1s were grown in one of the research fields of CSIR-Plant Genetic Resources Research Institute, Bunso, Ghana, in December, 2012. The F_1s harvested were grown in February, 2013 in Bunso and allowed to self. Dry pods of F_2 plants were harvested on plant by plant basis and the seed coat colour studied.

Selection of true hybrids: The seed harvested following artificial pollination might not be true hybrid. Cowpea is a natural inbreeder. The crop thus has tendency to self therefore, could easily have been pollinated before the emasculation process. To avoid selfing one needs to do emasculation as early as possible. However, emasculation done too early will also lead to abortion. To ensure that the putative hybrids are true hybrids, appropriate markers were used to identify true hybrids depending on the traits in which the parents were polymorphic.

Statistical analysis: It was planned to group seeds harvested on F_2 plants according to seed coat colour and use chi-square goodness of fit test for various genetic ratios. However, results made it difficult in grouping the seeds without ambiguity in many cases. Photographs of the parent phenotypes, their F_1s and F_2s were taken. Discussion was done based on the observation shown in the photographs.

RESULTS

The coat colours of most of the F_1 seeds were different from what were expected. The expected and observed seed coat colours of the six hybrids are presented in Table 1.

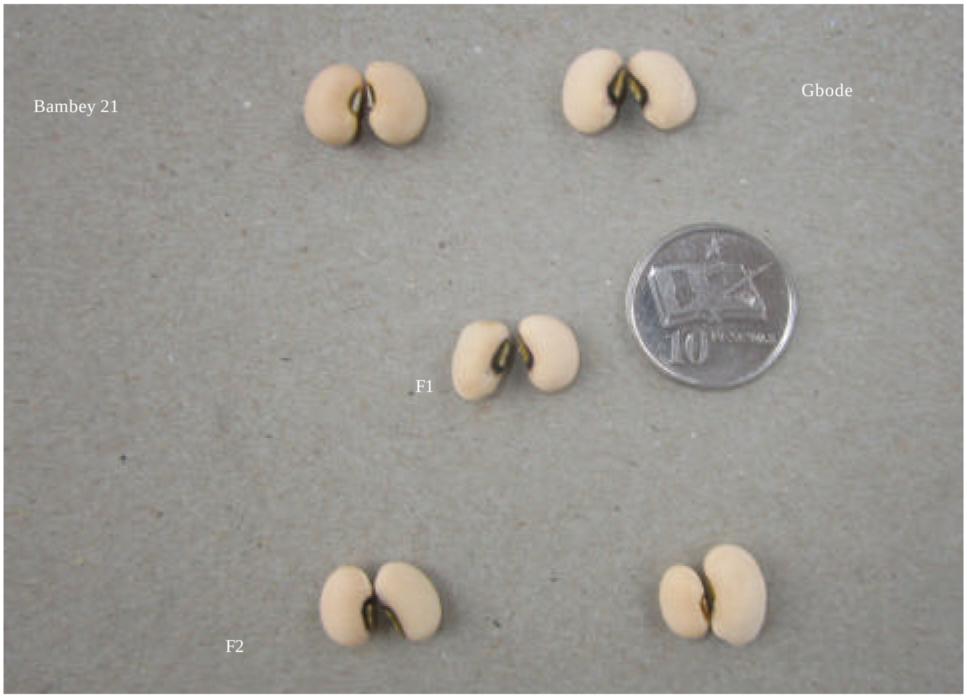

Fig. 1: Seeds of Bambey 21 and Gbode with their F_1 and F_2s

Table 1: Observed seed coat colours of cowpea hybrids

	F_1 seed testa pigmentation	
Cross	Expected	Observed
Bambey 21× Gbode	Black eye (Gbode)	Black eye
CB27×UCR779	Solid black	Solid black
Bambey 21×Tona	Solid brown	Solid black
Bambey 21×UCR779	Solid brown	Solid black
CB27×Gh3710	Solid black	Solid black
Bambey 21×Nhyira	Solid brown	Cream with black eye

Table 2: Segregation pattern for seed coat colour for the cross of Bambey 21/Gbode

Class	Observed (O)	Expected (E)	$(O-E)^2$	$(O-E)^2/E$
Gbode type	138	133	25	0.188
Bambey 21 type	39	44	25	0.568
χ^2				0.756

P {0.05, df 1 = 3.84}

Pigmentation of F_1 seeds of the cross of Bambey 21 and Gbode (Fig. 1) resembles that of Gbode indicating the dominance of the latter over the former. The F_2 although, had some variability within groups, they can be separated into the two parental types in terms of coat colour. The number of plants exibiting Gbode type were 138 to 39 Bambey 21 type fitting 3:1 ratio with Chi-square value of 0.756, suggesting monogenic control of the trait Table 2.

Nhyira has small brown eye while Bambey 21 has none. The F_1 of the cross between these genotypes had white seed coat with small black eye (Fig. 2). The F_2 had eye and eyeless seeds. The

Fig. 2: Seeds of Bambey 21 and Nhyira with their F_1 and F_2s

Table 3: Segregation pattern for seed coat colour for the cross of Bambey 21/Nhyira

Class	Observed (O)	Expected (E)	$(O-E)^2$	$(O - E)^2/E$
No eye	18	19.75	3.060	0.155
Black eye	45	39.50	30.250	0.766
Brown eye	16	19.75	14.063	0.712
χ^2				1.633

P {0.05, df 2 = 5.99}

eyes were either black or brown. Some of the black eyes were larger than others, however, distinction between the smalll and the larger types of eyes was difficult to make in some cases. There were 16 brown eye, 45 black eyes and 18 eyeless seeded plants fitting 1:2:1 ratio. The chi-square test for this is presented in Table 3. The calculated Chi-square 1.633 is smaller than table value of 5.99, meaning that the segregating ratio fits the 1:2:1.

The seed coat of the F_1 between brown seeded UCR779 and CB27 (white with black eye) was black (Fig. 3). The F_2 seeds had varied coat pigment from black to white. Some of the seeds had mottle coat. There were some solid seeds in that they did not have dinstinct eye colour. The eye types varied from normal eye to holstein type. Ten seed coat colour groups were identified.

Bambey 21 and UCR779 produced black seeded F_1 plants which segregate to seeds with black to white as shown in Fig. 4. There were both eye and eyeless seeds. A number of plants produced seeds inbetween the three main colours: Black, brown and white. It was difficult to classify some of the seeds into the black, brown or white as they were not clearly distinctive from each other in terms of seed coat colour. At least nine groups were identified in attempt to group the plants based on seed coat colour.

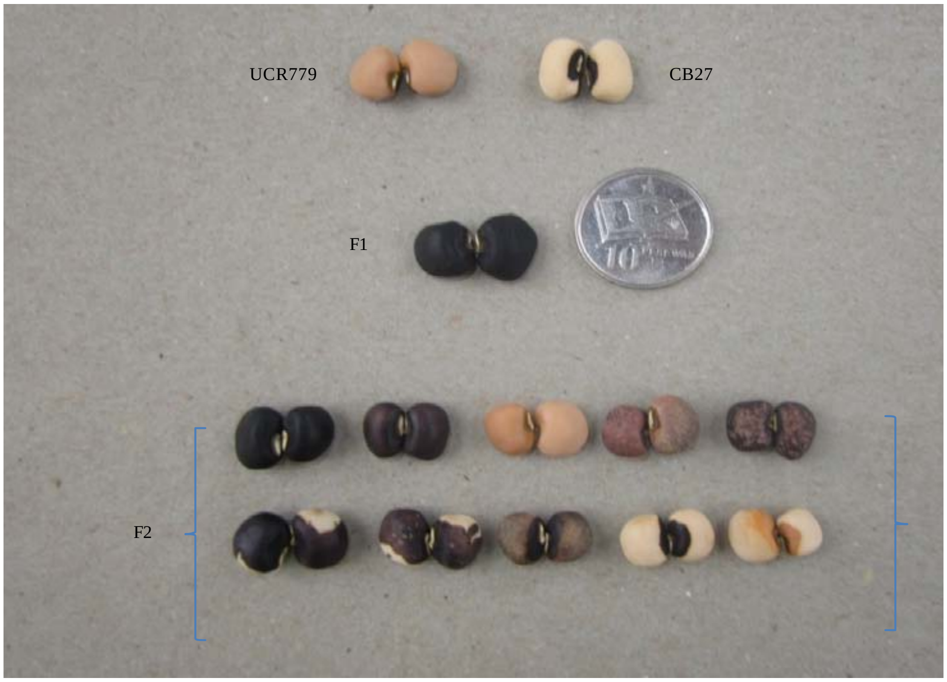

Fig. 3: Seeds of UCR779 and CB27 with their F_1 and F_2s

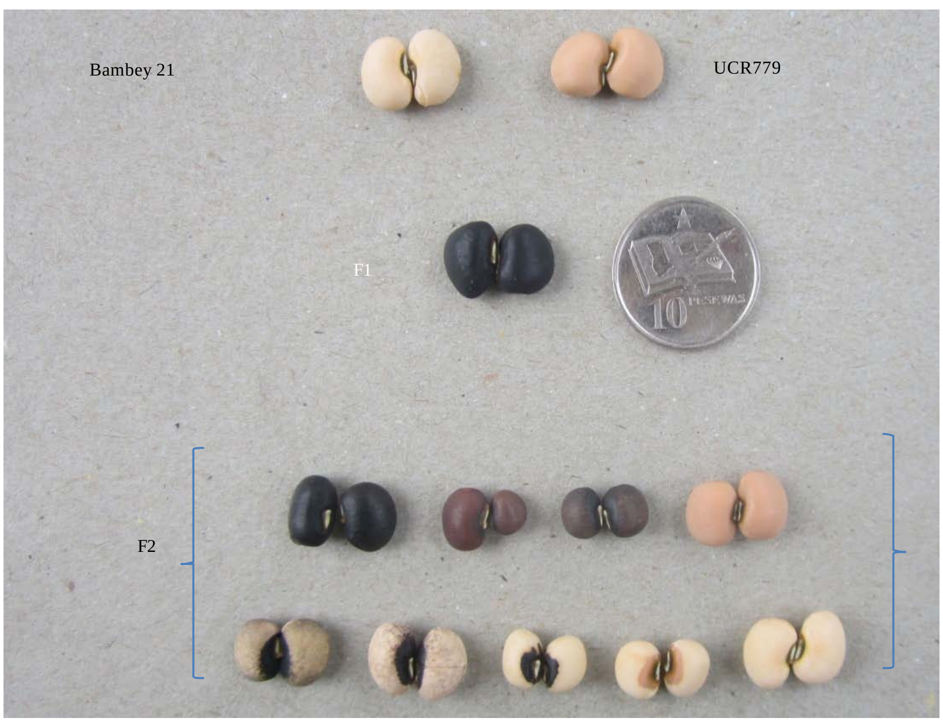

Fig. 4: Seeds of Bambey21 and UCR779 with their F_1 and F_2s

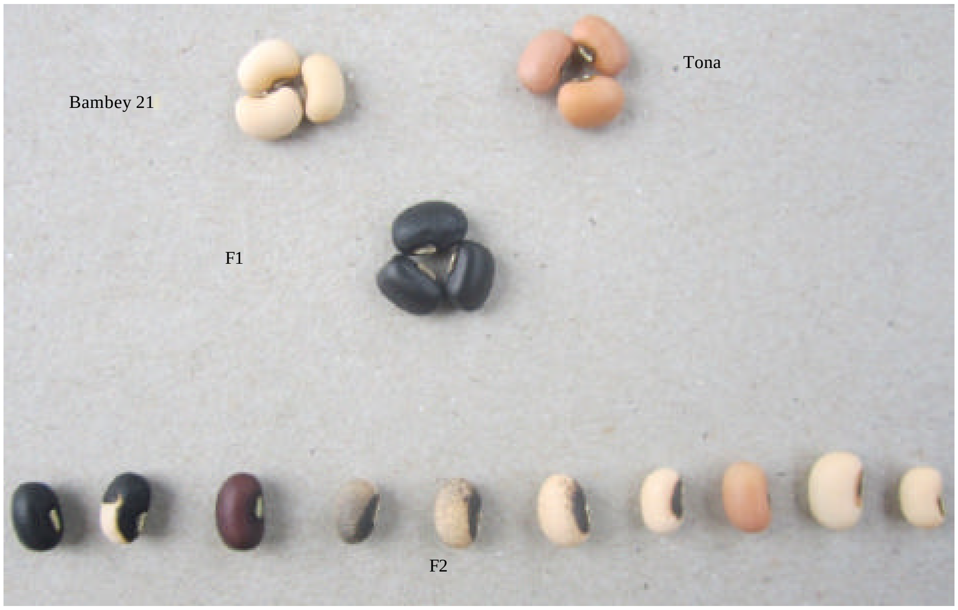

Fig. 5: Seeds of Bambey 21 and Tona with their F_1 and F_2s

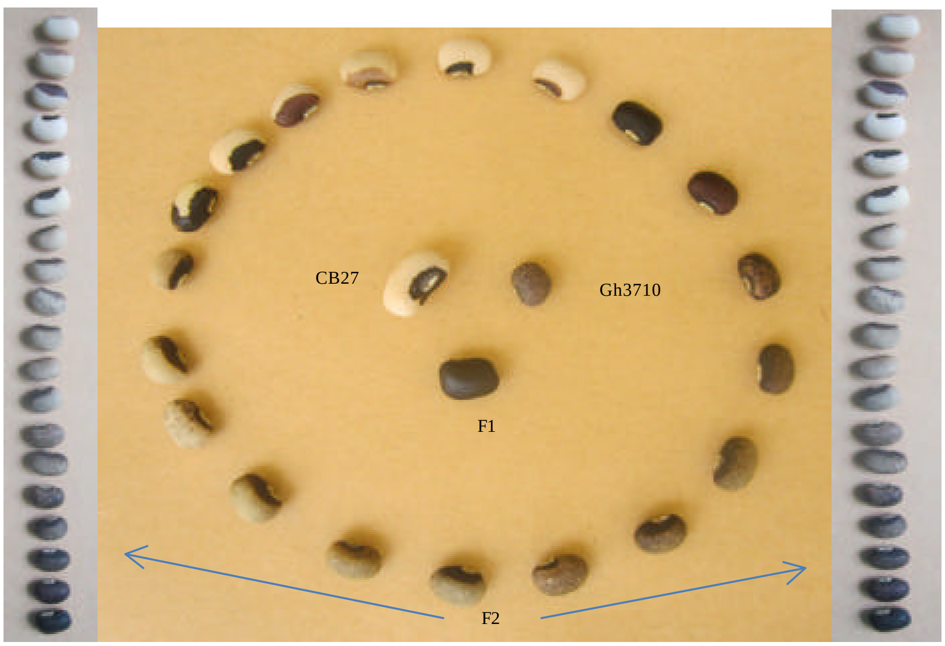

Fig. 6: Seeds of CB27 and Gh3710 with their F_1 and F_2s

The seed produced by F_1 plants of the cross of Bambey 21 (solid white) and Tona (solid brown) were solid black. F_2 plants produced black, brown and white seeds with a lot of internediates presented in Fig. 5. Some of the seeds were solid while some had eyes. Attempt to classify the seeds according to seed coat colour put them into ten different groups.

The hybrid of black eye CB27 and dark mottle GH3710 bore black seeds. The F_2 seed coat colours were much varied from black to white and very difficult to classify (Fig. 6). Most of the F_2 seeds had eye, however, eye could not be recognized on some.

DISCUSSION

Segregation for seed coat colour: Whilst individuals of two of the segregation populations could be grouped into definite seed coat colour groups, it could not be done for the other four. This suggests that no single cross may be able to give full insight into seed coat colour inheritance in cowpea, which also suggests that many genes might be involved.

The F_2 of Bambey 21 (all white) and Gbode (white with black eye) from F_1 that resembles Gbode could be grouped into two based on seed coat colour. This segregation approves the observation made on Bambey 21/CB27 by Ehlers *et al.* (2000) as reported in Fatokun *et al.* (2000). The Chi-square calculated was 0.76 for the 3:1 ratio. This result also agrees with Arshad *et al.* (2005) and Mustapha (2009) for blackgram and cowpea seed coat colour respectively. Similar report was made by Yilwa (2012).

F_2 of Bambey 21 and Nhyira had three groups of seeds based on coat colour; black eye, brown eye and solid white. Point of interest is the black eye observed at the F_1. Neither the male nor female parent had black seed coat. Production of black by white and brown parents suggests heterosis of seed coat colour and will be discussed later. This cross could also be likened to Bambey 21/Gbode, however in this case the heterozygous produced a distinct coat colour resulting in three different classes of coat colour at the $F_{2.}$

It became more difficulty and ambiguous in grouping plants according to seed coat colour as one moved progressively to cross number six. The cross between UCR779 (solid brown seed coat) and CB27 (white with black eye) produced F_1 with solid black seed coat. Black is reported to be dominant over brown (Mustapha, 2008). The black at the eye region of CB27 is dominant over brown UCR779 and because the latter lacks eye gene, solid black F_1 was produced. Different seed coat colours and patterns were observed in the F_2. At least ten seed coat colour groups were identified in the $F_{2,}$ indicating that many genes might be involved resembling what is known in maize (Chandler *et al.*, 1989).

Result of the cross and the segregating population of Bambey 21/Tona were unexpectedly very complex. Bambey 21 and Tona are all white and all brown respectively. However, the F_1 of this cross was solid black seeded. If dominance of black seed coat of cowpea over brown and other colours were universal, then white genotype crossed with brown could not produce black F_1. It is suggested that the result of this cross was due to epistasis or that the colours behaved in quantitative manner in which heterosis was observed from the cross with respect to seed coat colour. The cross of Bambey 21/Nhyira suggested that Bambey 21 has eye gene but not expressed because of lack of colour. The F_2 of Bambey 21/Tona therefore had some eyed genotypes because of the eye gene from Bambey 21. There was gradual decrease of colour from solid black to white in the segregating population. The varying seed coat colour might correlate with anthocyanin types and quantities.

Bambey 21/UCR779 followed Bambey 21/Tona in terms of seed coat colour inheritance as their F_1s were black and the segregating population had a number of groups with seed coat colour varying from black to white. Here also the expression of black at F_1 did not suggest its dominance over white and brown because if that were true, black could not have hidden under the white or brown of the parents. Epistasis may explain the expression of black at this F1 better than simple dominance and recessive. Yilwa (2012) reported four different crosses of white and brown in which she observed brown at the F1 and white and brown at the F2, with ratios suggesting two genes controlling the trait. The observation in this study is contrary to this as more than two coat colour types were observed in the crosses of brown to white. This brings suspicion of environment being affecting the seed coat colour expression, suggestion quantitative inheritance. In *Capsicum annum*, it is known that environmental factors greatly influence anthocyanin synthesis and therefore, pigmentation of various plant parts including the seed (Lightbourn *et al*., 2007).

The most complex segregation for seed coat colour was observed from the cross of CB27/Gh3710. The Gh3710 is dark mottling. F_1 of CB27/Gh3710 was black and could be attributed to the black eye of the CB27. The F_2 showed gradation from black to white with much difficulty in grouping them. This clearly showed quantitative nature of seed coat colour inheritance in cowpea.

Observations indicate continuous variation in the seed coat colour rather than classes. Attempt to identify nine classes in the segregation population of the cross of Bambey21/UCR779 means that at least four gene loci being involved in the seed coat colour inheritance. However, even the classification of seeds into coat colour groups in this research was arbitrary. The F_2 of the crosses of Bambey 21/Tona and CB27/Gh3710 on the other hand suggest more than four loci in each case. In this case, study of seed coat colour as quantitative trait may be appropriate. The segregation observed in the crosses of this experiment may not represent all the possible allele combination due to segregation distortion (Lambrides *et al*., 2004). The use of molecular tools in the study of seed coat colour segregation may give more accurate result.

Quantitative traits are known to be affected by environmental factors. Anthocyanin which is known to determine seed coat colour and pigmentation of other plant parts has the primary role of stress tolerance in plants especially when expressed in vegetative parts. Higher anthocyanin pigmentation of vegetative parts is caused by harsh environmental conditions (Chalker-Scott, 1999). Seed coat colour pigmentation may also be influenced by environmental factors such as solar radiation.

Overlap within genotypes and phenotype which is phenomenon of quantitative traits is very likely in attempt to classify seed coat colour in some of the crosses being discussed. The groupings are therefore, arbitrary. This is another proof of quantitative nature of seed coat pigmentation in cowpea. If white is taken as the lowest value for pigmentation and black as the highest, the cross of Bambey 21 (white) and Tona (brown) produced black offspring; an indication of heterosis and F_2 that spanned the whole spectrum of seed coat colour variability. Breeding for specific coat colour would thus be difficult as the F_2 showed much variability. Regarding seed coat colour of cowpea as qualitative trait will be misleading in breeding programmes as quantitative traits are more difficult to breed (Acquaah, 2007).

CONCLUSION

Seed testa pigmentation is as the result of presence of phenolic compounds including anthocyanins. Many anthocyanins are known to be present in the seed coat of cowpea. The type and amount of anthocyanins is related to the colour the coat might have and many genes have been implicated as reported in maize. In maize where the expression of anthocyanin is believe to be most understood in plants, many genes are reported to be involved. Anthocyanin content of the various parts of a plant can be quantified, implying that the pigmentation of the seed coat of cowpea can also be quantified.

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