Inheritance of Seedcoat Colour in Wild and Landrace Genotypes of Cowpea Grown in Bauchi, Nigeria

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ABSTRACT

For increased value addition and preference of the cowpea grain, the need to determine the basic sources of genes conditioning, seed coat colour and colour patterns in natural population is desirable. Screen house and field evaluation studies involving landrace cowpeas and their wild relative (Dekindtiana *var. pubescens*) were carried out in Tafawa Balewa and Bar Arewa in Bauchi Northern Guinea Agroecology of Nigeria, to study the inheritance of seed coat colour in cowpea. Thirty genotypes of cowpea, comprising 10 parents, nine F_1, two backcrosses and nine F_2 were advanced from collection and hybridization trial. These were laid out in a randomized complete block design with three replications in the wet season (July – October) of 2018. The parental genotypes bred true to type and all the F_1 plants of the nine crosses had 1Black: 1Black spotted with brown within locules of a single pod irrespective of the parental seed coat colour. The backcrosses and the F_2 population in all the crosses had plants with seed coat colour and colour patterns in the range of five to twelve. The results unveils the genetic bases of seed coat colour in cowpea, where, early introgression of genes between landrace cowpeas and their wild relative (Dekindtiana *var. pubescens*) accounted for the varying cowpea seed coat colour and colour patterns observed in natural populations. These can deliberately be developed as options for researchers, growers and consumers preference.

Keywords: Inheritance, Cowpea genotype, Landrace, Wild, Seedcoat colour.

INTRODUCTION

There is a great deal of diversity in preference for seed coat colour of cowpea in some regions of the world especially, central and southern America, particularly in the drier regions of Brazil, Venezuela, Panama,Elsalvador, Haiti, Ecuador, Guyana and Suriname. Therefore, varieties with different seed colours such as white, brown, cream, black and speckled are found in the region (Watt *et al*. 1985). In Nigeria, the white seeded cowpea is preferred over the brown in the northern region and vise- versa in the western region.

The first attempt to explain the inheritance of cowpea seed colour was reported by Spillman in 1912. He noted that a general colour factor, C, was responsible for colour and that its absence resulted in white seeds. The C factor in combination with R, U, N and B conditions red, buff, brown and blue seed coats, respectively. Harland (1919) proposed a model with R as a general colour factor conditioning red seed coat. He suggested that the R factor in combination with B, N, M as well as N and M conditions black, buff, maroon and brown, respectively.

Spillmann and Sander (1930) designated the general colour factor as R and described N as an anthocyanin pigment factor. They used symbols B, F, P and U for brown, fine and dense speckling, purple and buffs, respectively, and showed how these genes interacted to produce ten different seed coat colours. Saunders (1960) reported that most of the common colour patterns of the cowpea seed resulted from interactions between two or more genes. He stated that the gene responsible for black colour was dominant to all but the purple seed coat colour. Calub (1968) suggested that black was epistatic to all colours regardless of the presence of other colour genes.

Seed coat patterns are inherited independently of seed coat colour, but the appearance of any pattern is dependent upon the presence of the general colour factor C (Calub, 1968; Fery, 1980). Drabo *et al.,* (1988) observed that incomplete dominance of several seed coat pattern genes made classification difficult in progeny segregation for the Holstein, Watson, small eye and hilum ring traits. Although several works have reported on the genetics of cowpea seed coat colour, there are interactions and modifier genes that are not yet understood (Fery, 1980).

Individuals of some of the cowpea populations could not be classified based on seed coat colours, as they exhibited continuous variation (Egbadzor *et al*., 2014) and the F_2 plants have seed coat colours outside the phenotypic limits of the two parents (Joshua, 2009). Therefore, chi-square goodness of fit test could not be conducted on them (Egbadzor *et al.,* 2014). It was suspected that many genes might be involved in seed coat colour inheritance in cowpea (Joshua 2009; Egbadzor *et al.,* 2014) and as such the use of molecular and quantitative principles may be helpful in understanding the genetic control of the trait ((Egbadzor *et al.,* 2014).

Joshua (2009) reported a segregation ratio of 13 Black: 3 White and 9 Black: 4 Black/White: 3 White in crosses between improved varieties and the wild (*var. pubescens*) cowpeas namely: IT93K-452-1 x Wild (*var. pubescens*) and IT97K-499-38 x Wild (*var. pubescens*). However, F_2 populations in crosses between the wild and the local varieties namely: Kannando x Wild (*var. pubescens*) and Achishiru x Wild (*var. pubescens*) could not be classified on the basis of Mendelian genetics as the seed coats had colours and colour patterns range of 4 – 10. Cowpea varieties, especially those with pigmented seed coats, are believed to be loaded with antioxidant agents, i.e vitamins A and C, which help get rid of free radicals, and stop the growth of cancerous cells. Also, these antioxidants are found to be of benefit to the skin, thereby helping in delaying signs of ageing, they help repair the skin and rejuvenate it accordingly (Chowdhury, 2019).

This study therefore, attempted to discover the genetic bases of seed coat colour in cowpea and the sources of the genes involved, as this will allow for the production at ease of cowpeas with variable seed coat colour and colour patterns for preference options to researchers, growers and consumers of the grain.

MATERIALS AND METHODS

Site and Genetic material

The germplasm evaluation study was conducted on a field in Bar Arewa village which is situated seven kilometres south of Tafawa Balewa town and five kilometres north of Bogoro town in Bauchi State during the wet season of 2018. Bauchi is located at 10^0 22N and 9^0 46"E and at 609 metres above sea level. Bauchi lies in the Northern Guinea Savannah zone of Nigeria.

Sources and Description of Materials: Materials used in this study consisted of 10 parental genotypes, nine F_1 genotypes, two backcross genotypes and nine F_2 genotypes of cowpea; making a total of 30 genotypes. The parental genotypes were advanced from the collection and agronomic evaluation study in 2015. The F_1, back crossses and the F_2 genotypes were developed from the hybridization trials in 2016 and 2017, respectively, in the screen house in Tafawa Balewa town of Bauchi State, Nigeria.

Cultural Practices

The land used for the field evaluation was planted with sorghum the previous year. The vegetation in the field were burnt using a non- selective chemical herbicide called Vinash as the trade name with the active ingredient: Glyphosate IPA 41% WW(in the form of 480 g/l Glyphosate isopropylanine salt) SL at the rate of 1litre: 60 litres of water. The shrubs were cleared using cutlass and the left- over grasses and trashes were removed from the experimental field. Thereafter, the land was ploughed using cattle- drawn ridger.

The entire experimental field measured 100 m x 50 m which was then marked out with a measuring tape into three blocks of 25 m x 50 m each. The Parental and the F_1 genotypes plots measured 0.8 m x 25 m each, while the Back crosses and the F_2 genotype plots measured 1.6 m x 25 m, respectively, to obtain large population. Relays between adjacent blocks measuring 2.0 m and 0.8 m served as discards between adjacent plots. These were clearly separated using pegs. The 30 genotypes were laid out in a randomized complete block design with three replications

Sowing of the seeds was done on July 24, 2018, following a heavy rainfall the previous day. Seeds free of any physical defect were used. Seed scarification was used for the wild parent (*var. pubescens*) only to break dormancy and to allow for prompt germination. Inter- and intra-row spacing was 0.8 m and 1.0 m, respectively, giving an estimated plant population of 12,500/ha. Two seeds were sown about 2.0 cm per hole for all the genotypes except the wild parent in which three seeds were sown in 1.0 cm depth because of the small seed size. The seedlings were later thinned down to one plant per stand two weeks after emergence.

Hand-weeding was employed using small hoe at four weeks after sowing. This was repeated at eight weeks after sowing. Pre-and post-flowering insecticides were sprayed to control and manage damage by insect pests on leaves, flowers and pods.

The broad-spectrum insecticide called Sharp shooter (Profenofos 40%+Cypermethrin 4%) at the rate of 1 litre: 120 litres of water was used.

Pods were harvested at full maturity, 12-14 weeks after sowing. Maturity was observed when pods changed from green colour to the characteristic pod colour of the genotype as yellow, pink or grey. Pod shattering was observed in the wild parent, F_1, Back crosses and F_2 genotypes. Pods from five randomly sampled plants in each plot of the three replications in the parents and the F_1 genotypes and all plants in the back crosses and F_2 plots were harvested into well-labeled polyethylene bags. Harvesting operation commenced on September 10, 2018 and was completed on November 8, 2018.

Observation and Data Collection

The following parameters were observed and data collected:

Plant height: This was measured using a long wooden metric rule from the base of the plant to the apical bud of the central branch.

Leaf number: Leaf number per plant was determined by counting.

Number of branches per plant: This was done by counting the number of branches on each of the sampled plants eight weeks after sowing.

Number of peduncles per plant: This was determined by counting at harvest.

Days to first flower per plant: This was observed and recorded each morning between 6.00 am-12.00 noon of each day for each of the sampled plants that started flowering.

Days to first pod maturity: This was recorded from each of the sampled plants, whenever the first pod attained maturity.

Number of pods per plant: This was done by counting the number of pods in each of the sampled plants for all the genotypes.

Length of pod: Five pods randomly picked from each sampled plant were measured using ruler and the values added and averaged to obtain the mean length per pod for each plant after harvest.

Number of seeds per pod: This was determined on five randomly picked pods of each sampled plant. The number of seeds for each of the five pods were added up and averaged to obtain the mean number of seeds per pod.

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Number of seeds per plant: This was obtained by multiplying the number of pods per plant by the number of seeds per pod for each plant.

100-seed weight: This was obtained by counting 100 seeds from each sampled plant and then weighing using a digital top-balance.

Seed yield per plant: Seeds obtained for each plant were weighted as described in above.

Evaluation of qualitative characters

The following qualitative characters were recorded by counting:

i. Number of plants with purple, pale-purple and white with purple margin flowers
ii. Number of plants with grey, pink and yellow pods was noted
iii. Number of plants with or without hairs on their stems
iv. Number of plants with or without hairs on their pods. Number of plants with or without hairs on their stems and pods were assessed manually by hand feeling on all F_2 plants.
v. Number of plants with shattering and non- shattering pods.
vi. Number of plant with a particular seed coat colour or colour pattern was noted and
vii. Heterosis was tested for each quantitative character recorded. In the case of F_1 performance poorer than the worst parent, negative heterosis was declared.

Data Analysis

Data collected during this study were subjected to one or more of the various forms of analytical tools available in System Analytical Statistics (SAS) Software: Analysis of Variance (ANOVA) and Statistical Analysis for Agricultural Research (STAR) (2014). Means were separated using Duncan's Multiple-Range Test (DMRT) Duncan's, 1955). Test for goodness of fit on the qualitative characters, Broad-sense heritability (h^2 *b %*) and Genetic Advance (*GA %*) estimates were carried out using the Plant Breeding Tools, Version 1.3 (2014).

RESULTS

Table 1. Agronomic Characteristics of the Cowpea Genotypes Evaluated

Character	Yar Ja	Nafyali	Yarwuri	Ngb 00769	Kanannado	Ngb 00765	Yaro da kokari	Yar dunga	Achi shiru	Var. pubescens
Growth Habit	Spreading	Spreading	Semi-erect	Spreading	Spreading	Erect	Spreading	Spreading	Semi-erect	Spreading
Flower colour (Standard)	Pale purple	Pale purple	White with purple margin	Pale purple	Pale purple	Purple	White with purple margin	Pale purple	Purple	Purple
Seedcoat colour	Brown	White	White	White	White	Red	White	White	Light brown	Black
Hilum colour	White	Narrow dark	Black	Narrow dark brown	Light brown	Narrow White	Black	Conspicuous black	White	Dull White
Seedcoat texture	Rough	Rough	Rough	Rough	Rough	Smooth	Rough	Rough	Smooth	Smooth

Cowpea Germplasm Field Evaluation Results.

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Plate 1. Inner Part of the Screen House Containing the Experimental Plants.

Table 2. Seed Coat Colour in F_1Crosses Involving the Wild (*var. pubescens*) and some Landraces of Cowpea Grown in Tafawa Balewa in 2017

S/N	Crosses	Parental phenotype	F_1 Phenotype
1	Achi shiru x Wild	♀Brown x ♂Black	Black/Black with dotted brown
2	Kanannado x Wild	♀White x ♂Black	Black/Black with dotted brown
3	Nafyali x Wild	♀White x ♂Black	Black/Black with dotted brown
4	NGB 00765 x Wild	♀Red x ♂Black	Black/Black with dotted brown
5	NGB 00769 x Wild	♀White x ♂Black	Black/Black with dotted brown
6	Yaro da kokari x Wild	♀White x ♂Black	Black/Black with dotted brown
7	Yar dunga x Wild	♀White x ♂Black	Black/Black with dotted brown
8	Yar ja x Wild	♀Brown x ♂Black	Black/Black with dotted brown
9	Yar wuri x Wild	♀White x ♂Black	Black/Black with dotted brown

Plate 2. Seed coat Colour Segregation in locules of a single pod in F_1 Plants. Source: Author (2018).

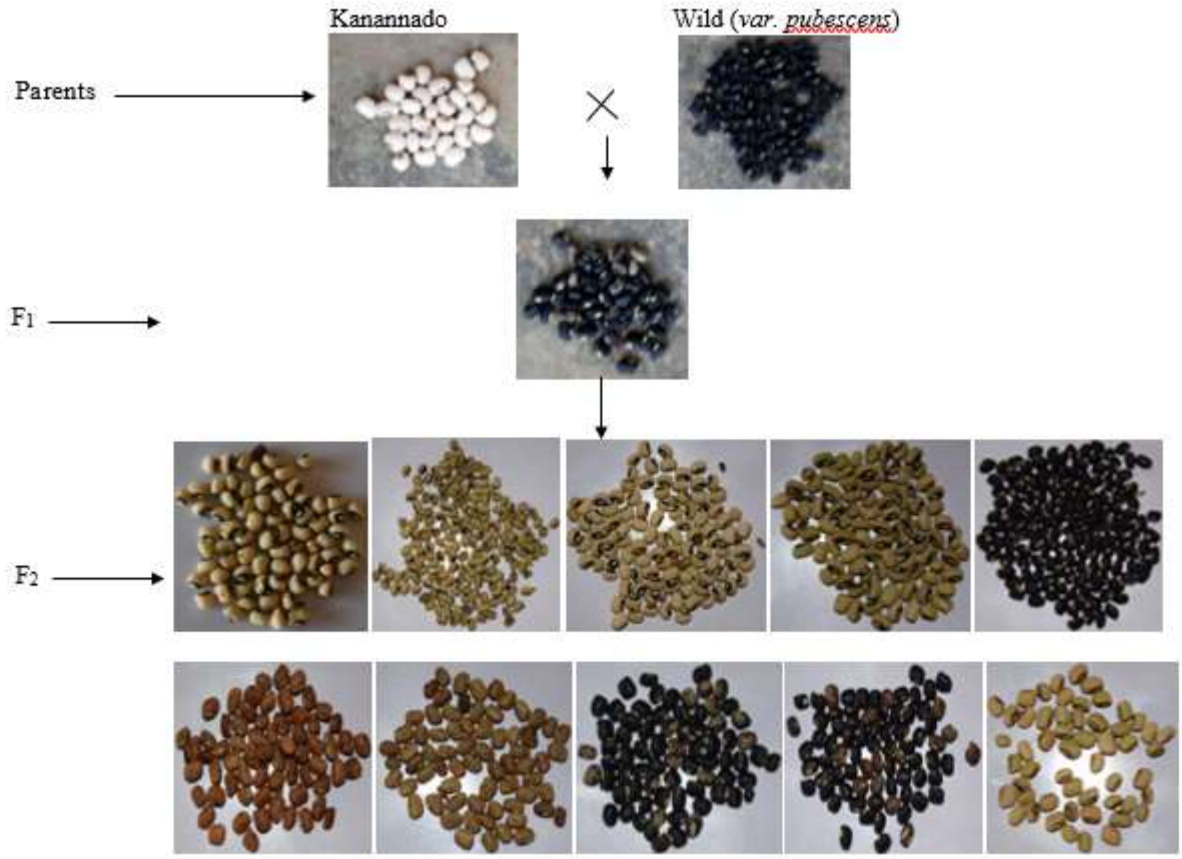

Figure 1. Parents, F_1 and F_2 Crosses between Kanannado and Wild (*var. pubescens*). Source: Author (2018).

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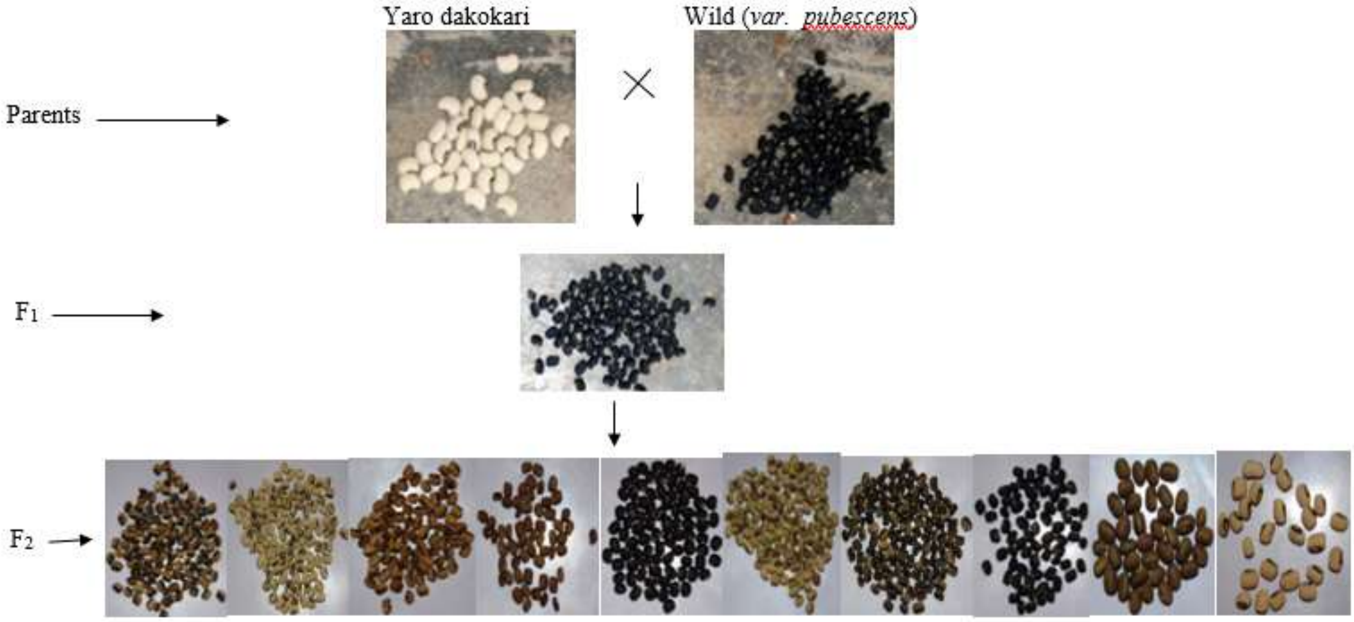

Figure 2. Parents, F_1 and F_2Crosses between Yaro Dakokari and Wild (*var. pubescens*). Source: Author (2018).

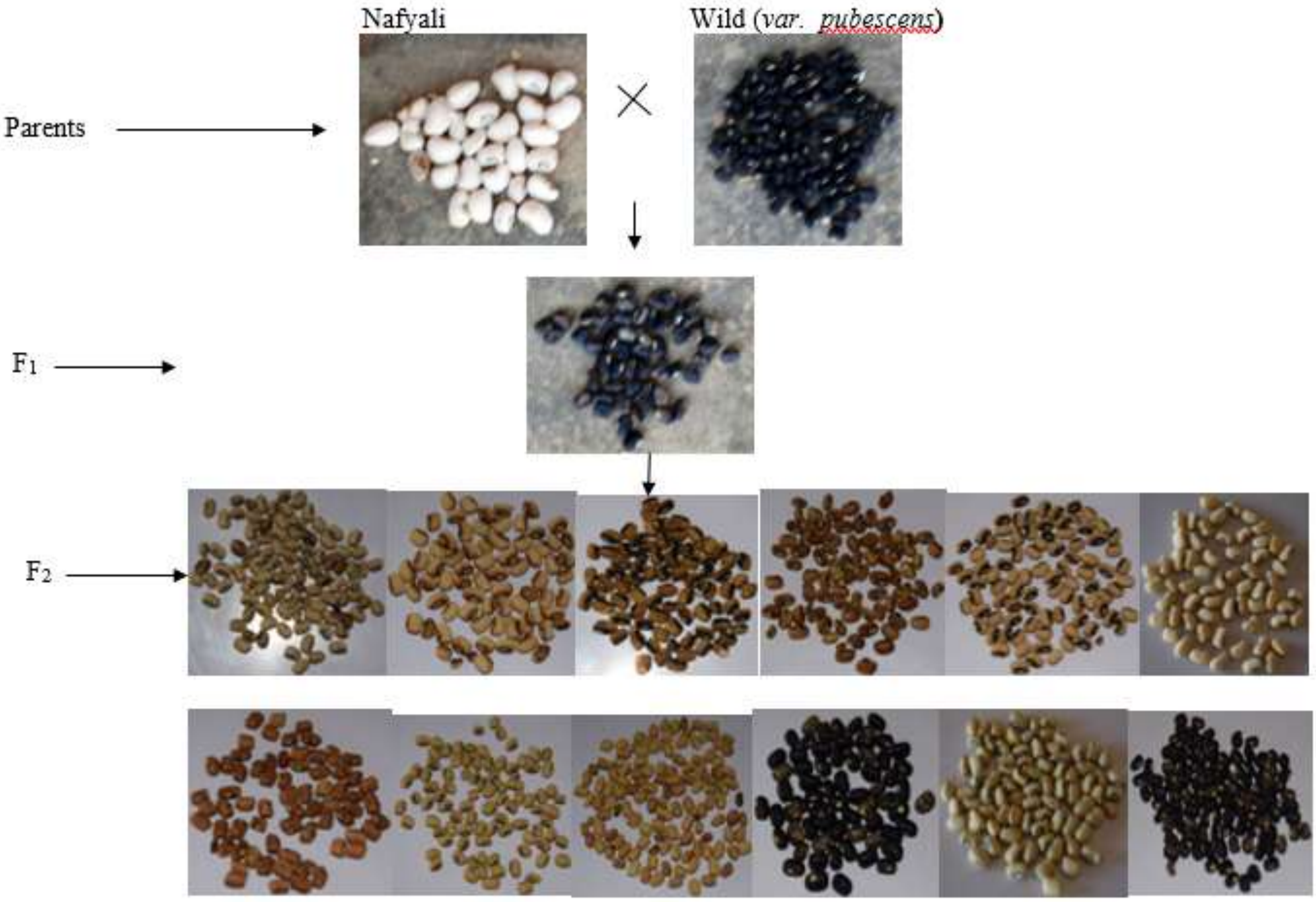

Figure 3. Parents, F_1 and F_2 Crosses between Kanannado and Wild (*var. pubescens*). Source: Author (2018).

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Table 3. Seed Coat Colour of the Parents and Segregation Pattern for Seed Coat Colour in the F_1 and F_2 Crosses Involving Land Race Cowpeas and their Wild Relative (Dekindtiana *var. pubescens)*Grown in Bar-Arewa in 2018.

	Genotype	Seed Coat Colour																		
	Parents	Wht	Blk	Brw	Red	BBb	BBc	BrC	CrW	WBr	Bcr	Buf	LBr	DGr	LRe	LGr	BWh	YBl	CrB	Total
1	Achishiru			22																22
2	Kanannado	24																		24
3	Nafyali	23																		23
4	NGB 00765				24															24
5	NGB 00769	25																		25
6	Yarodakokari	24																		24
7	Yardunga	23																		23
8	Yarja			22																22
9	Yarwuri	24																		24
10	Wild (*Var. Pubescens*)		25																	25
	F_1 crosses																			
11	Achi shiru x Wild					33														33
12	Kanannado X Wild					26														26
13	Nafyali xwild					15														15
14	NGB 00765 x Wild					42														42
15	NGB 00769 xWild					49														49
16	Yaro da kokari x Wild					11														11

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17	Yar dunga x Wild					20														20
18	Yarja x Wild					12														12
19	Yar wuri x Wild					27														27
	Backcross																			
20	Achishiru x Wild x Achishiru		5	3	0	9	6	0	0	0	0	0	0	0	0	0	0	0	4	27
21	Yarwuri x Wild xYarwuri	9	6	7	0	10	1	3	3	3	3	1	0	0	0	0	0	0	0	46
	F_2 crosses																			
22	Achishiru x Wild	0	20	14	0	48	40	0	0	0	0	0	0	0	0	0	0	0	14	136
23	Kanannado x Wild	14	17	9	0	48	12	20	9	3	6	6	0	0	0	0	0	0	0	144
24	Nafyalix wild	20	13	13	0	33	20	0	11	0	0	7	0	0	0	11	0	0	0	128
25	NGB 00765x Wild	0	30	6	7	22	5	0	0	0	0	0	9	2	4	4	2	0	0	91
26	NGB 00769xWild	42	10	6	4	53	0	0	0	0	0	12	0	9	0	0	9	0	0	145
27	Yaro da kokari x Wild	12	16	0	0	16	0	8	0	12	0	12	8	5	16	0	0	5	0	110
28	Yardunga x Wild	18	43	7	0	22	0	0	18	0	0	0	12	9	0	0	0	0	0	129
29	Yarja x Wild	0	14	9	0	38	0	19	0	19	0	0	0	0	16	0	0	8	0	123
30	Yarwuri x Wild	31	18	13	0	35	4	13	11	9	11	4	0	0	0	0	0	0	0	149

S/No	Abbreviation	Seed Coat Colour/Colour Pattern Distribution
1	Wht	White
2	Blk	Black
3	Brw	Brown
4	Red	Red
5	BBb	Black and black dotted with brown
6	BBc	Black and black dotted with cream
7	BrC	Brown dotted with cream
8	CrW	Cream white
9	WBr	White dotted with brown
10	BCr	Black dotted with cream
11	Buf	Buff
12	LBr	Light brown
13	DGr	Dark green
14	LRe	Light red
15	LGr	Light green
16	BWh	Brown with dull white
17	YBl	Yellow with black
18	CrB	Cream dotted with black

The results of seed coat colour distribution in the parents and segregation pattern in the F_1, Backcrosses and F_2 are presented in Table 3. All the parents bred true to type, as expected in self fertilising plants Table 1. The results show that there was seed coat colour segregation in all the F_1crosses of 1 black: 1black spotted with brown within locules of a single pod Table 2. The segregation pattern in the Backcrosses and F_2 crosses falls outside the Mendelian segregation pattern; therefore, seed coat colour and colour patterns were only counted and recorded accordingly.

DISCUSSION

The results of seed coat colour distribution in the parents and segregation pattern in the F_1, Backcrosses and F_2 are presented in Table 3. All the parents bred true to type. The results show that there was seed coat colour segregation in all the F_1crosses of 1 black: 1black spotted with brown within locules of a single pod and not from plant to plant as expected, this indicates a rare phenomenon. The segregation pattern in the Backcrosses and F_2 crosses falls outside the Mendelian segregation pattern; therefore, seed coat colour and colour patterns were only counted and recorded accordingly in the range of five to 10. Joshua (2009), reported F_2 populations in crosses between the wild (*var. pubescens*) and local genotypes namely: Kanannado and Achishru which had seed coat colours outside the phenotypic limits of the two parents, therefore, chi- square goodness of fit test could not be conducted on them. Whereas, crosses between the wild and improved genotypes: IT93K-452-1 and IT97K-499-38, segregated into 13: 3 and 9: 4: 3 respectively. This explained that landraces or local varieties of cowpea carries colour genes that can condition various seed coat colour and colour patterns when they are crossed to specifically the wild (*var. pubescens*). Many genes with smaller effects are involved in the inheritance of seed coat colour and colour patterns in cowpea.

CONCLUSION AND RECOMMENDATION

The results of this study unveils the genetic bases of seed coat colour in cowpea, where, early introgression of genes between landrace cowpeas and their wild relative (dekindtiana *var. pubescens*) accounted for the varying cowpea seed coat colour and colour patterns observed in natural populations. The findings also implied that seed coat colour and colour patterns can deliberately be developed as options for researchers, growers and consumers preference. Hence, it is suggested that the use of molecular and quantitative principles be employed to help further in understanding the genetic control of seed coat colour in landrace cowpeas and their wild relative (*var. pubescens*).

Conflict of Interests

The authors have not declared any conflict of interests.

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