

A New Recessive Allele at the C Locus for Seedcoat Color in Common Bean

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Abstract. The inheritance of a new allele, *c'*, at the C locus for seedcoat color was studied in common bean (*Phaseolus vulgaris* L.) using plant introduction (PI) accession 527774 as the source of *c'*. The cross PI 527774 (yellow-brown seed) x v BC₂-5-593 (mineral-brown seed) genetic tester stock was studied in F₁ and F₂ progeny. An F₃ selection from the above cross, designated F₃*c'Gbv*, was crossed to 5-593 (a Florida breeding line with black seeds), and the F₁, and F₂ progeny were analyzed for color segregation. The second backcross [S-593 x F₁(F₃*c'Gbv* x 5-593)] was investigated in selfed progenies from 32 random BC₂-F₁ parents. Two of the BC₂-F₁ progenies were further tested in BC₂-F₂. A third backcross of *c'* to 5-593 was made and analyzed, and an allelism test of *c'BVBC₂-F₁5-593* with the cartridge huff *c'BC₂-F₁5-593* genetic tester stock confirmed that *c'* is an allele at C. The gene symbol, *c'*, is proposed for the new allele because it is only expressed with V and gives a grayish-brown seedcoat. Genotypes with *C/c'* do not show heterozygous mottling with *GBv* or *Gbv*, and there is no difference in seedcoat color between *CGBv* and *c'GBv*, or between *CGBv* and *c'GBv*.

The inheritance of seedcoat colors and patterns in common bean was reviewed by Prakken (1970). Eight genetic loci contribute to color inheritance in the synthesis developed by Prakken (1970, 1972), viz., P, C, D, J, G, B, V, and Rk. The C locus was described as a complex locus because it includes the R gene for dominant red color and other genetic factors affecting seedcoat color patterns, all tightly linked in the complex C locus (Prakken, 1974). An alternative view is that the C locus has a large multiple allele series, including C, C^r, C^{ma}, C^{cir}, C^{res}, Cst, C^{tho}, c, c^u, c^{ui}, and c^{cr} (Feenstra, 1960; Lamprecht, 1947; Nakayama, 1964, 1965). The experimental evidence cited above supports the hypothesis that the complex C locus consists of several tightly linked loci, one or more of which have a multiple-allelic series. There are multiple dominant alleles (C^{ma}, C^{cir}, C^{res}, Cst, C^{tho}) and multiple recessive alleles (c, c^u, c^{ui}, and c^{cr}) that control seedcoat color and pattern (Prakken, 1970). One of the goals of the bean genetics program at the Univ. of Florida is to develop demonstration genetic stocks with the seedcoat color genotypes in the yellow-black series (Prakken, 1970) in the common genetic background of Florida breeding line 5-593. The genotype of the black seedcoat color of 5-593 is *P[C r]DJGBVRk* (Bassett, 1994a). Parental lines can be found with various combinations of recessive genes for seedcoat color, and the desired set of recessive genes is then transferred into 5-593, usually with three backcrosses. One of the accessions in the seed collection of H. Lamprecht, V0722 [now U.S. Dept. of Agriculture plant introduction (PI) 527774], has a yellow-brown seedcoat color, which is expected to have the genotype *PCJGBvRk* (Prakken, 1970). The purpose of this study was to develop evidence for the existence of another recessive allele at C with a unique combination of genetic effects on seedcoat color and pattern.

Materials and Methods

PI 527774 was crossed with a mineral-brown genetic tester stock, v BC₂-5-593, and data were taken on segregation for seedcoat colors in the F₂. A selection was made in the F₂ for a plant with yellow-brown seedcoat color and the plant type of 5-593. The F₃ selection is designated F₃*c'Gbv*, where the *c'* allele is a hypothetical new allele at the C locus. The existence of the *c'* allele had no expression in the F₃ stock and its presence was unknown to me at that time. To make the paper easier to follow, the new allele will be used where genotypes are given. The proof of the existence of the new allele will be made by experimental evidence presented below.

The F₃*c'Gbv* stock was backcrossed to 5-593, and data were taken on the color of flowers and seedcoats of the F₁ and F₂ progeny. The second backcross to 5-593 was made as follows: 5-593 x F₁(F₃*c'Gbv* x 5-593) and progeny tests were made with 32 randomly selected BC₂-F₁ plants. Data were taken as above for the first backcross. Two of the BC₂-F₁ progenies were tested in BC₂-F₂ to confirm the genetic hypothesis made for the BC₂-F₂ parents. Data were taken on the BC₂-F₂ progenies as above.

A grayish-brown genetic stock was selected in the BC₂-F₂ with the genotype *Pc'JBV* and designated *c'BVBC₂-F₂5-593*. An allelism test was made by the cross *c'BVBC₂-F₂5-593* x *c'BC₂5-593*, the cartridge buff genetic tester stock (Bassett, 1994b). A third backcross to 5-593 was made with the same *c'BVBC₂-F₂5-593* plant used for the allelism test. Data were taken as above for both crosses.

Table 1. Segregation for seedcoat color (with genotypes) in the F₂ from the cross PI 527774 (*c'Gbv*, yellow brown) x v BC₂-5-593 (*CGBv*, mineral brown).

Mineral brown ^z <i>GB/-v</i>	Yellow brown ^z <i>Gbv</i>	χ^2 3:1	P
120	40	0.00	1.00

^zThere was no influence on seedcoat color from segregation for the hypothetical *c'* allele in PI 527774. The colors observed reflected only the genetic constitution at G, B, and V.

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Table 2. Segregation for flower and seedcoat color (with genotypes below) in the F₂ of the cross F₃c'G b v (plants 1-4) x 5-593 (C G B V).

Progeny no. ^x	Colored flowers ^z			White flowers		χ^2 (27:9:12:12:4)	P
	Seedcoat color						
	Black	Dark purple	Grayish brown ^y	Mineral brown	Yellow brown		
	C/-	C/-		B/-	b/b		
	B/-	b/b	c ^y /c ^y	B/-	b/b		
	V/-	V/-	V/-	v/v	v/v		
1	99	27	36	41	16	1.956	0.74
2	174	41	49	52	16	14.511	0.006

^yV/V gives bishops-violet flowers and V/v gives cobalt-violet flowers.

^zThe hypothetical new recessive allele, c', only produces a seedcoat color expression with V/-, i.e., it has no visible effect with v/v colors.

^xProgeny no. 1 was derived from crosses with F₃c'G b v plants no. 2 and no. 4, whereas progeny no. 2 was derived from crosses with F₃c'G b v plants 1 and 3. The segregation at V in progeny no. 2 was 264 V/- and 68 v/v, $\chi^2(3:1) = 3.615$, $P = 0.06$, and the segregation at C was 215 C/- and 49 c'/c', $\chi^2(3:1) = 5.838$, $P = 0.02$. The segregation at B in progeny no. 2, omitting the c'/c' segregation data, was 226 B/- and 57 b/b, $\chi^2(3:1) = 3.563$, $P = 0.06$.

Table 3. Segregation for seedcoat and flower color in the BC₂-F₂ from 5-593 (C G B V) x F₁(F₃c'G b v x 5-593), where 32 BC₂-F₂ progenies were separately classified.

Expected BC ₂ -F ₁ genotypes	No. of progenies ^y	Phenotypic classes with genotypes					Ratio tested	χ^2	P
		Colored flower, black seed	Colored flower, dark-purple seed	Colored flower, grayish-brown seed ^z	White flower, mineral-brown seed	White flower, yellow-brown seed			
		C/- B/- V/-	C/- b/b V/-	c ^y /c ^y V/-	B/- v/v	b/b v/v			
1. C/C B/B V/V	8	366							
2. C/C B/B V/v	8	287				84	3:1	1.101	
3. C/C B/b V/V	2	80	12				3:1	7.015	
4. C/C B/b V/v	3 ^x	112	5			24	9:3:3:1	29.64	
5. C/c ^y B/B V/V	5	176		56			3:1	0.092	
6. C/c ^y B/B V/v	6 ^w	160		63		68	9:3:4	1.698	
7. C/c ^y B/b V/V	0								
8. C/c ^y B/b V/v	0								

^zVariable color that is typical of c J colors, making color description difficult (Prakken, 1970).

^yThe number of BC₂-F₂ progenies (data combined) with the same segregation pattern, which corresponds to the expected segregation upon selfing the BC₂-F₁ parent (genotype). For 8, 8, 2, 3, 5, 6, 0, 0, the $\chi^2(1:1:1:1:1:1:1:1) = 18.5$, $P = 0.01$.

^xThe data for progeny 3-6, one of three progenies of BC₂-F₁ genotype 4, were 36, 4, 9, 3, with $\chi^2(9:3:3:1) = 5.026$, $P = 0.17$.

^wThe data for progeny 3-1, one of six progenies of BC₂-F₁ genotype 6, were 26, 11, 11, with $\chi^2(9:3:4) = 0.565$, $P = 0.75$.

Table 4. Segregation for seedcoat color in the BC₂-F₃ progeny of 27 randomly selected BC₂-F₂ parents grown from remnant seed of BC₂-F₂ progeny 3-1^w.

BC ₂ -F ₂ parent	No. of BC ₂ -F ₃ progenies ^y	Classification of BC ₂ -F ₃ progeny			Ratio tested	χ^2	P
		Black C/- V/-	Grayish brown c ^y /c ^y V/-	Mineral brown ^x -/- v/v			
C/C V/V	3	136					
C/C V/v	5	178		59	3:1	0.001	0.97
C/c ^y V/V	2	65	28		3:1	1.294	0.26
C/c ^y V/v	7	155	53	80	9:3:4	1.210	0.55
c ^y /c ^y V/V	2		91				
c ^y /c ^y V/v	4	144	39		3:1	1.328	0.25
-/- v/v	4			188			

^zThe data for progeny 3-1, one of six progenies of BC₂-F₁ parents with C/c^y B/B V/V, were 26, 11, 11, with $\chi^2(9:3:4) = 0.565$, $P = 0.75$ (Table 3).

^yThe number of BC₂-F₂ parents having the same genotype and, consequently, the same segregation pattern in BC₂-F₃ progeny tests, for which data were combined. For 3, 5, 2, 7, 2, 4, 4, the $\chi^2(1:2:2:4:1:2:4) = 3.667$, $P = 0.72$.

^xThe hypothesis is that the genotype at the C locus has no effect on seedcoat color when v/v is present, giving mineral brown color (G B understood to be present for all genotypes).

Results and Discussion

For the cross PI 527774 x v BC₂5-593, all F₁ and F₂ plants had white flowers due to v (data not shown). The F₂ segregated for only two seedcoat color classes, yellow brown and mineral brown, which were the parental classes (Table 1). The standard recessive allele at C produces mottled seedcoat colors when heterozygous (C/c) and with v (Prakken, 1970). The hypothetical c^v did not produce mottled seedcoat color when heterozygous in the F₂ of the above cross. The standard recessive allele at C also produces a paler color when homozygous; e.g., the yellow brown of C G b v becomes the maize yellow of c G b v and the mineral brown of C G B v becomes the buckthorn brown of c G B v (Prakken, 1970). No segregation for the paler colors produced by standard c were observed in the F₂ (Table 1). Thus, the hypothetical c^v lacks two important genetic effects of the standard recessive allele, viz., heterozygous mottling and (except with V) production of a paler color when homozygous.

The F₁ from the cross F₃ c^v G b v x 5-593 had cobalt-violet flower color due to V/v (Bassett et al., 1990) and shiny, unpatterned black seedcoats (data not shown). The F₂ from the same cross segregated for five seedcoat color classes (Table 2). The dark-purple and yellow-brown seedcoat classes are due to b/b and are well established in the literature (Prakken, 1970). The grayish-brown seedcoat class is attributed to segregation for a recessive allele at a third locus, C. The grayish-brown color name is not different from the brown tinged with greyish indigo hue that Prakken (1970) attributes to the genotype P c J G B V, i.e., to the standard recessive allele c. No mottled seedcoat colors or the lighter colors produced by c D J (Prakken, 1970) were observed in the F₂ progeny except for grayish brown (Table 2). Thus, the hypothetical c^v only ex-

presses a color effect with V; hence, the superscript v designation. The disturbed segregation observed for the progeny no. 2 (from plants no. 1 and no. 3 of Table 2) is a frequent occurrence in the extensive test crossing with the Lamprecht materials performed at this laboratory (Bassett, unpublished data).

The purpose of the original cross of PI 527774 x v BC₂5-593 was to construct a b v BC₂5-593 genetic tester stock. The presence of a unique recessive allele at C in PI 527774 was not suspected and delayed the construction of the intended tester stock. Thus, the discovery of a hypothetical c^v allele in PI 527774, after making the cross F₃ c^v G b v x 5-593, was purely fortuitous.

The hypothesis that three-factor segregation, involving a third locus besides B and V, produced the five seedcoat classes (Table 2) was tested by separately classifying 32 BC₂-F₂ progenies (field plots 3-1 through 3-32) from the cross 5-593 C G B V x F₁ (F₃ c^v G b v x 5-593) (Table 3). Of the eight patterns of segregation expected, two were not observed (no. 7 and no. 8) and two had significantly disturbed segregation (no. 3 and no. 4) (Table 3). Those results were attributed to a disturbed segregation at B, where there was a great shortage of b. The segregation at C and V (no. 5 and no. 6) was normal (Table 3).

Two of the BC₂-F₂ progenies (3-1 and 3-6) were selected for additional testing in BC₂-F₃ progenies (Table 3). Progeny 3-1 produced all expected segregation patterns, and the segregation within those patterns was normal (Table 4). Segregation in 3-1 was hypothesized to involve only the C and V loci. Progeny 3-6 produced all expected segregation patterns, but the segregation within all of the progenies segregating at B (B/v V/V, B/v V/v, and B/v v/v) was significantly disturbed (Table 5). Thus, the hypothesis of three-factor segregation is confirmed, in spite of disturbed segregation at B.

Table 5. Segregation for seedcoat color in the BC₂-F₃ progenies of 25 BC₂-F₂ randomly selected parents grown from remnant seed of BC₂-F₂ progeny 3 - 6^c.

BC ₂ -F ₂ parent	No. of BC ₂ -F ₃ progenies ^b	Classification of BC ₂ -F ₃ progeny ^a				Ratio tested	χ ²	P
		Black B/- V/-	Dark purple b/b V/-	Mineral brown B/- v/v	Yellow brown b/b v/v			
B/B V/V	5	215						
B/B V/v	6	195		64		3:1	0.012	0.91
B/B v/v	2			68				
B/b V/V	1	46	1			3:1	13.114	<0.001
B/b V/v	2	65	4	24		9:3:3:1	21.715	<0.001
B/b v/v	3			111	3	3:1	30.42	<0.001
b/b V/V	1		49					
b/b V/v	3		115		27	3:1	2.714	0.10
b/b v/v	2				62			

^aThe data from progeny 3-6, one of three progenies of BC₂-F₂ parents with C/C B/b V/v, were 36, 4, 9, 3, with x²(9:3:3:1) = 5.026, P = 0.17 (Table 3).

^bThe number of BC₂-F₂ parents having the same genotype and, consequently, the same segregation pattern in BC₂-F₃ progeny tests, for which data were combined. For 5, 6, 2, 1, 2, 3, 1, 3, 2, the x² (1:2:1:2:4:2:1:2:1) = 15, P = 0.06.

^cThe seedcoat colors are given above and the seedcoat color genotypes are given below.

Table 6. Segregation for seedcoat color in the F₂ from two crosses: no. 1, c^v B V B C₂-F₂5-593 (gray brown) x 5-593 (C B V, black) and no. 2, c^v B V B C₂-F₂5-593 x c^v B C₂5-593 (c^v B V, cartridge buff).

Cross no.	Classification of seedcoat color				χ ² 3:1	P
	Black C/-	Grayish brown c ^v /c ^v	Grayish brown c ^v /-	Cartridge buff c ^u /c ^u		
1	104	34			0.01	0.92
2			131	39	0.384	0.54

The hypothetical factor c^v was isolated in the genetic background of 5-593 and designated $c^v B V BC_2-F_3 5-593$. The allelism test at C was made with the cross $c^v B V BC_2-F_3 5-593 \times c^u BC_2 5-593$. The genetic tester stock $c^u BC_2 5-593$ was used because no $c^v BC_2 5-593$ stock has been constructed. The F_1 from the cross $c^v B V BC_2-F_3 5-593 \times c^u BC_2 5-593$ produced seed with grayish-brown coats (data not shown). The F_2 segregated for only the parental phenotypes, grayish brown and cartridge buff, in a 3:1 ratio, respectively (Table 6). No evidence of complementation was observed; hence, the hypothesis of allelism was confirmed. The third backcross to 5-593 segregated only for the parental phenotypic classes, black and grayish brown, in a 3:1 ratio, respectively (Table 6). Those results confirmed the hypothesis that only a single recessive gene substitution produced the grayish-brown seedcoat color class in the genetic background of 5-593. It is proposed that the new recessive allele at C be given the gene symbol c^v because it expresses only in the presence of V.

In summary, the c^v allele does not express mottled (dark pattern color/background pattern color) seedcoat color when heterozygous, C/c^v . In the presence of v/v , there is no color differential produced by C/C and c/c , as demonstrated with $G B v$ and $G b v$. With $V/-$ there is a color differential, e.g., black for $C G B V$ and grayish brown for $c^v G B V$. It is presumed that the colors produced by all other genotypes with c^v and V would be the same as with c and V as described by Prakken (1970). The basis for the presumption is that the grayish brown color name used in this paper is probably not different from the brown tinged with greyish indigo color name used by Prakken (1970) for $c J G B V$. Thus, I assume that $c^v J$ does not have an effect on pigment chemistry that is

different from $c J$ in the presence of $G B V$. The interactions of c^v with $B v$ and $b v$ are clearly different from the interactions of c with $B v$ and $b v$.

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