A Test Cross Protocol for Determining the Seedcoat Genotype at the C Locus in Common Bean

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Abstract. Studying the genetics of seedcoat color in common bean (Phaseolus vulgaris L.) in F1 progeny is very difficult because of complex epistatic interactions, and the analysis is complicated further by multiple allelism, especially at the C locus. An alternative approach is to study seedcoat genetics by analyzing the F1 progeny of test crosses between a variety with unknown seedcoat genotype and genetic tester stocks with known genotypes. Twenty varieties, 18 with known genotype at C, were test crossed with the genetic tester stock c+ BC3, 5-593, where 5-593 is a recurrent parent with seedcoat genotype P [cR] D J G B V Rk. The resulting F1 progenies were classified into seven phenotypic classes and discussed. The crosses g B v BC3, 5-593 x c+ BC3, 5-593 and c+ BC3, 5-593 x v BC3, 5-593 were made and the F1 progeny classified for flower color and seedcoat color and pattern. No tiny cartridge buff flecks were observed in the segregants with Cle+Vr, whereas Cle+Vf always showed such flecks. The contrasting seedcoat color expression at C in different environmental conditions is discussed.

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Materials and Methods

Table 1 presents the list of varieties studied, sources of the seed, and seedcoat colors. The varieties were chosen for one of two reasons: 1) because the genotypes for seedcoat color (or at least the genotype at C) were previously determined; or 2) the variety contributed to the representation of the complete range of possible interactions encountered. For 18 of the varieties tested, the genotype at the ‘complex’ C locus is known, and the sources of that information are cited (Table 2). The variety ‘Wax Digoin’ has particular importance because it carries the “standard” recessive allele at c, which produces mottled seedcoat colors when heterozygous, Cle (Lamprecht, 1932). In Prakken’s (1970) summary of seedcoat color genetics, the Group VI color series gives the “shiny dark pattern colors” produced by Cj, whereas substitution of the standard c for C produces the Cj Group V color series, which Prakken calls the “shiny background colors.” Each seedcoat color with C is changed to a slightly paler color with c, hence, the Cle mottling is subtle. To my knowledge, no commercial dry bean varieties grown in the United States are known to have a Cle genotype.

The genetic tester stock used in the test cross program described below is C+ BC3, 5-593. The tester carries only the recessive alleles for the seedcoat color genes indicated in the name, with the exception of r, which is always present unless otherwise stated. Those recessive alleles are in backcross three (BC3) to the recurrent parent 5-593, a Florida dry bean breeding line with the seedcoat color genotype P [cR] D J G B V Rk (Bassett, 1994b, 1996a). The c+ allele in c+ BC3, 5-593 was derived from Prakken’s “all recessive” tester (Bassett, 1994b, 1996a). In the 5-593 genetic background, the C+ allele produces cartridge buff seedcoat color, and C+ allele produces nearly black seedcoat with widely dispersed tiny flecks of cartridge buff.

The 20 varieties listed in Table 1 were crossed to c+ BC3, 5-593 in the greenhouse over a period of several years, and the F1 progeny were either grown (most commonly) in the greenhouse or in the field in various years. Data were taken on the seedcoat color and pattern in the F1 progeny. The genotypes at seedcoat color loci other than C are not given (Table 2) because they do not contribute any useful information for interpreting the F1 phenotypes of the test crosses. Data were taken on attributes of the seed from F1 test cross plants immediately after harvest.

The genetic tester stock g B v BC3, 5-593 was developed from the cross ‘Calima’ [C+ R] j g b v Rk x b v BC3, 5-593 by selection in the field (Spring 1994) for the segregants with
Table 1. Seedcoat phenotypes of parental bean varieties of common bean studied in test crosses and the sources of the seed.

| Parental varieties | Seedcoat phenotype | Reference |
|--------------------|--------------------|-----------|
| Akasando*, Commodore*, G23623* | Oxblood red |          |
| Jacobs Cattle* | Oxblood red/white; partly colored |          |
| Montcalm*, Garnet*, Huetar*, UI-37*, Rufus* | Garnet brown (Dark Red Kidney or Small Red classes) |          |
| Redkloud* | Testaceous (Light Red Kidney class) |          |
| Sutter Pink* | Pink (Pink class) |          |
| S-593* | Black |          |
| Opal* | Silvery gray |          |
| Wax Digoin (PI 226936)*, Masterpiece*, Prim*, Citroen* | Pale greenish yellow or creamish pale buff |          |
| Wagenaar* | Greenish yellow |          |
| PI 527735 (V0400)*, PI 527808 (V0874)* | White |          |

*The red color names (oxblood, garnet brown, testaceous, and pink) are from Smith (1939), and the remaining names follow Prakken (1970, 1972).

Table 2. Genotypes at the C locus for the seedcoats of common bean varieties (all pure lines) tested and references to research supporting the genotype.

| Parental varieties | C locus genotype | Reference |
|--------------------|------------------|-----------|
| Montcalm, Redcloud, Sutter Pink | c* | Bassett, 1998a |
| Wax Digoin | c | Lamprecht, 1932 |
| PI 527734, PI 527808 | c | Stig Blixt |
| Garnet, Rufus, Huetar, UI-37 | C | Bassett, 1998a |
| S-593 | Bassett, 1994b |
| Prim | C | Bassett, 1999 |
| Masterpiece | C | Nakayama, 1961 |
| Wagenaar, Citroen | C | Prakken, 1972 |
| Akasando | R | Nakayama, 1961 |
| Commodore | R | Smith and Madsen, 1948 |
| Jacobs Cattle | [7 R] | Bassett, 1998a |
| G 23623, Opal | ? | (Genotype not known) |

From genetic notes transcribed by Blixt from Lamprecht’s (1932) seed packet data after Lamprecht’s death; information now in the common bean genetic stock collection at Pullman, Wash. See the Genetic Resources Information Network (GRIN) to access the data.

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| Garnet, Rufus, Huetar, UI-37 | C | Bassett, 1998a |
| S-593 | Bassett, 1994b |
| Prim | C | Bassett, 1999 |
| Masterpiece | C | Nakayama, 1961 |
| Wagenaar, Citroen | C | Prakken, 1972 |
| Akasando | R | Nakayama, 1961 |
| Commodore | R | Smith and Madsen, 1948 |
| Jacobs Cattle | [7 R] | Bassett, 1998a |
| G 23623, Opal | ? | (Genotype not known) |

Results and Discussion

In extensive test crosses of common bean varieties with the c BC, 5-593 genetic tester, seven different phenotypic classes were observed among the test cross F1 progenies (Table 3). The first phenotype is the result of simple allelism, giving the cartridge buff of the tester, i.e., the variety tested carries c (Bassett, 1998a). The second phenotype (Table 3) is due to a recessive c allele, but not the same allele as the tester. When the test cross F1 progeny express seedcoats with brown tinged with grayish indigo, the c allele present in the variety tested must be either the standard c or some other recessive c not c (Table 3).

The third phenotypic class (Table 3) is the result of nonallelism. The unpatterned black F1 progenies from crosses with the Small Red market class parents, ‘Garnet,’ ‘Rufus,’ ‘Huetar,’ and ‘UI-37,’ are nonallelic. A fourth phenotypic class (Table 3) resulted from the crosses with 5-593 and G23623, which produced F2 progeny with black seedcoats having a highly restricted pattern, viz., widely distributed tiny flecks of cartridge buff. Thus, the C allele in 5-593 and G23623 produces a F1 heterozygote with very limited patterning. The flecks are so small that they can only be verified under 15X magnification. The flecks otherwise may be mistaken for dust, tiny particles of debris, or minute seedcoat damage.

The question remains whether the Cc heterozygote having tiny cartridge buff flecks with Vl- will produce a typical marbling pattern with vLv. Illustration of the marbling pattern was given in Bassett (1996b). Conclusive evidence was obtained from the data from two crosses: g B v BC, 5-593 x c- BC, 5-593 and c- BC, 5-593 x v BC, 5-593, where g B v BC, 5-593 and v BC, 5-593 carry the C allele from 5-593. The tiny cartridge buff flecks observed with vLl- were not observed with vLv (Table 4).

The fifth test cross phenotype (Table 3) is the result of a nonallelic interaction, giving black/cartridge buff marbling. This type of interaction was previously reported by Nakayama (1960, 1961, 1964) for C x c* crosses. The marbling pattern was expressed with all possible genotypic combinations at G, B, and V.

The pattern descriptors, matte and marble, are terms of art. The Cc interaction produces mottling, which is a subtle patterning between two colors that are close in hue and also varies greatly from seed to seed in the relative proportions of “dark pattern” to “light pattern” color (Prakken, 1970). On the other hand, the Cc interaction produces marbling, which can be a high or low contrast patterning depending on the hue of the “dark pattern” color controlled by the genotype at G, B, and V. Also, the marbling pattern has relatively constant proportions (from seed to seed) of “dark pattern” to “light pattern” color. Illustration of mottling and marbling patterns was given by Bassett (1996b).

The marbling pattern produced by the Cc interaction is hardly distinguishable from the same pattern produced by a dominant “allele” at the C locus that produces a true-breeding marbling (Prakken, 1970). Various symbols have been used for the dominant marbling allele by various authors over many decades: C*, M, or some tightly linked combination of C and M indicated by a curving line above the two letters and joining them. The same ‘complex C locus’ also controls anthocyanin color patterning in all plant parts, e.g., hypocotyl, cotyledon, seedcoat, stem, petiole, leaf lamina, flower bud, and banner petal (see Prakken, 1970, for a review).
Table 3. Parental common bean varieties tested crossed with the $c^u$ BC, 5–593 tester, the seedcoat phenotypes of the F$_1$ progeny, and the genetic interpretation of the test cross results, i.e., the putative allele at the $C$ locus.

| Parental varieties tested | Class no. | Test cross F$_1$ seedcoat | C locus genotype |
|---------------------------|-----------|---------------------------|------------------|
| Montcalf, Redklood, Sutter Pink | 1 | Cartridge buff | $c^u$ |
| Wax Digoin, PI 527735, PI 527808 | 2 | Brown tinged with grayish indigo | $c$ |
| Garnet, Rufen, Huetar, UI-37 | 3 | Unpatterned black | $C$ |
| 5–593, G23623 | 4 | Black with widely distributed tiny cartridge buff flecks | $C$ |
| Masterpiece, Wagenaar, Prim, Citroen | 5* | Black/cartridge buff; marbled pattern | $C$ |
| Akasando, Commodore, Jacobs Cattle | 6* | Black, dark purple, or navy blue/cartridge buff (sometimes tinted various colors); marble pattern | $c^u$ |
| Opal (C* $j$) | 7* | Black/cartridge/buff stripe pattern | $C^u$ |

Note: $c^u$ is the recessive allele.

Table 4. Segregation (with genetic hypothesis) for flower color and seedcoat color and pattern in the F$_2$ from two common bean crosses: 1) $g$ B v BC, 5–593 x $c^u$ B v, 5–593; and 2) $c^u$ BC, 5–593 x v BC, 5–593.

| VI: purple flowers | v/v white flowers |
|--------------------|--------------------|
| Cross no. | Cl- Black seeds | $c^u$C- Cartridge buff seeds | Cl- G B | G B | Cl- $c^u$ G B | $c^u$C- Cartridge seeds | $c^u$C- Cartridge buff seeds | $\chi^2$ ratio tested | $\chi^2$ | $P$ |
| 1 | 177 | 54 | 32 | [32, both classes combined] | 36 | 12.9:7.9 | 2.939 | 0.40 |
| 2 | ND$^a$ | ND$^a$ | 30 | 8 | 3:1 | 0.316 | 0.57 |

$^a$ND = data not shown.

Nakayama (1965), working with the cross ‘Sanpaku’ (P C j g b v $c^u$) x ‘Ever Green’ (P C j g b V), demonstrated that the $c^u$ allele in ‘Ever Green’ had mutated into a new, completely recessive allele $c^v$, i.e., does not produce marbling with $C^c$.

The results in Table 3 demonstrate that dominant $C$ alleles have also acquired mutations, allowing expression of either $C^c$-marbling or $C^u$-unpatterned color. Thus, a geneticist must be very careful to test any putative $c^u$ allele with appropriate testers, such as ‘Masterpiece’ or ‘Akasando’ (Table 3).

The sixth test cross phenotype (Table 3) is the result of a cross between a variety carrying unpatterned dominant red (or partly colored dominant red/white in the case of ‘Jacobs Cattle’) and the $c^v$ BC, 5–593 tester (Bassett, 1996b, 1998a). The coloring of the marbled pattern can be quite variable among varieties, and the presence of $[ ? R]$ needs to be confirmed with a test cross with either $b$ v BC, 5–593 or $g$ b v BC, 5–593 (Bassett, 1996b, 1998a).

The seventh test cross phenotype (Table 3) is the result of a cross between a variety with a seedcoat pattern controlled at $C$ and the $c^v$ BC, 5–593 tester, where the seedcoat pattern of test cross F$_1$ progeny is identical with that of the variety tested (data not shown). Great variation is possible for seedcoat patterns controlled at $C$, with almost continuous variation between such types as marbled, stripes, pinto, etc. The possible variations in the types of stripes is so great and finely graded in differences that no descriptive catalog of the differences seems possible.

Any $C$ locus pattern may be carried cryptically due to epistasis by $p$, $t$, or $j$ (Prakken, 1970, 1972). The only variety tested that carries a dominant $C$ locus pattern was ‘Opal’ (Table 3). The seedcoat pattern controlled by $C^u$ in ‘Opal’ is obscured by the action of $j$ (Bassett, unpublished data). In marbled seedcoats the action of $j$ cannot completely suppress the pattern expression, but does weaken it to a faint expression level (Bassett, unpublished data). For seedcoat patterns with less extensive “dark pattern color” zones the pattern is completely obliterated, as in ‘Opal’.

In general, the test cross protocol for determining seedcoat color in common bean (Bassett, 1992), as it is applied to any given locus, results in the conclusion that the variety being tested carries either the dominant or the recessive allele at that locus. For the $C$ locus the results of the test cross have more than two allelic possibilities. Two distinct recessive allelic classes (Table 3, classes 1 and 2) can be distinguished, viz., the $c^v$ allele, which includes the $c^v$ allele, and the $c^u$ allele, which includes the standard $c$ and possibly others. The $C$ locus results also included five dominant allelic classes (Table 3, classes 3 through 7). Class 3 is completely unpatterned, class 4 is only slightly patterned, and class 5 is marbled. Class 6 carries the dominant gene $R$ for red (oxblood), which results in marbling pattern, but both the “dark pattern color” and the “light pattern color” are not typical for class 5 $C$ alleles. The most reliable indicator of class 6 is the tinting of the cartridge buff zone with various colors.

Class 7 is a special case where a dominant pattern allele at $C$ has been entirely or nearly obscured by the interaction of the recessive allele at the $J$ locus (Table 3). The result of the test cross is to make the underlying (unexpressed) pattern visible by removing the $j$ effect. Varieties that carry visible (with J dominant) $C$ patterns do not need the test cross to reveal the genotype at $C$ for those who know how to identify those patterns. There are also recessive seedcoat pattern genes in common bean: partly colored patterns dependent on $t$ for expression (Prakken, 1970), and three patterns, each determined by a different recessive allele at the stippled locus (Bassett, 1996c, 1999b). Thus, when a variety with a seedcoat pattern is test crossed with $c^u$ BC, 5–593 and the $F_1$ progeny show the same pattern observed in the patterned parent, then the pattern must be controlled at the $C$ locus.

The $F_2$ classes 3–6 are interpreted as being allelic with $C$ because this is the simplest hypothesis consistent with the data. Various loci closely linked together within the ‘complex $C$ locus’ of Prakken (1970) are probably playing a role in the seedcoat pattern expression, but the data produced by this protocol are not adequate to determine those genotypes. Genes not linked to $C$ are probably not influencing the observed results because such interactions have not been reported during decades of genetic investigations, many of which were capable of exposing them (Prakken, 1970).

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