Seed Coat Color in *Phaseolus vulgaris* L. , Its Chemistry and Associated Health Related Benefits

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Seeds of common bean (*Phaseolus vulgaris* L.) show a very large variation in size, shape, and color. This fact is quite obvious to anyone who has grown beans in the garden, used them as a research model, or visited produce markets in Africa, and Central and South America. The variation in seed phenotype of bean is as important to commerce as it is interesting to the scientist and naturalist. Consumers associate variations in seed color and color patterns to intrinsic differences in processibility, flavor, and texture (Adams and Bedford, 1973). The industrialists of dry bean commerce use the same seed variations to formalize commercial (market) classes, which separate unique characteristics into specific end uses. The scientist views color variation in bean as an opportunity to codify the responsible genes and determine their (the genes) biochemical functions.

Since the beginning years of the Twentieth Century, extensive research has been carried out on the inheritance of seed coat color in *P. vulgaris*. However, many results of the early investigations could not be readily related to each other and often led to disagreements among researchers. For example, there was a life-long dispute between Prakken (1970) and Lamprecht (1951) based on whether certain genes should be regarded as color genes or color modifiers (Leakey, 1988). Lack of agreement among investigators on the interpretation of the genetic data and descriptions of the color types were other factors that contributed to the early confusion surrounding the inheritance of seed coat colors in *P. vulgaris*. Because of the complexity of the genetic synthesis of seed coat color, especially for seeds with patterned colors, I will limit my remarks to the currently accepted model (Prakken, 1970, 1972) for the genes in *P. vulgaris* that give entirely colored (non-patterned) seed coats. For comprehensive reviews of seed coat colors in common bean, the reader is referred to articles by Kooiman (1931), Lamprecht (1941), Prakken (1970, 1972), and Leakey (1988).

The gene symbols, descriptions of the color types, and classification of the genes controlling seed coat color in *P. vulgaris* is due mainly to the research of Kooiman (1920, 1931), Lamprecht (1932), and Prakken (1970, 1972). Mark Bassett (1994 a, b; 1997; 1998 a, b, c) is also to be commended for the outstanding contributions he has made over the past decade to the understanding of seed coat color inheritance, especially of patterned seed coats, reconciliation of genes and gene symbols, determinations of interactions between color genes, and developing tester stocks for further genetic and chemical analyses of the genes. Prakken (1970, 1972) reviewed the literature accumulated over the first six decades of the Twentieth Century and developed a synthesis for the inheritance of seed coat color in *P. vulgaris* that is accepted today. In his synthesis, Prakken (1970, 1972) indicated a "ground factor" gene, *P* (described by Kooiman, 1931), which does not produce any color by itself but which must be present for pigment formation in the seed coat to be possible. Lamprecht (1936) mentioned, besides *P*, a

second gene, *Gri*, is necessary for bean seed coats to have color. Bassett (1994 a) reconciled the *Gri* gene ("ground factor" gene of Lamprecht) with *P* by showing that Gri is an allele at the P locus. The dominance series is $P > p^{gri} > p$. According to Prakken's interpretation (1970, 1972) of seed coat color in *P. vulgaris* , *C*, *D*, and *J* are **color genes** and can only be expressed in the presence of *P*. The genes, *G*, *B*, *V*, and *Rk* are **modifying genes** (have an intensifying effect or darkening influence upon pale colors formed by the action of the color genes) but do not impart color in themselves. The gene, *V*, is also called the violet factor and the dominant allele causes bluish, or violet to black, colors to develop in the seed coat.

Concomitant with the elucidation of the genetics of seed coat color variation was an interest in the relationship between genes and pigment synthesis. Prakken (1940) expressed the desirability that thorough chemical investigations should proceed along with the genetical studies (cited by Feenstra, 1960). Feenstra's (1960) work was the first systematic attempt to reconcile the relationship between genes and pigment synthesis. Feenstra (1960) studied the pigment chemistry of 12 genotypes, each with a different combination of alleles at *C*, *Sh* [same as *J* of Prakken, (1970)], and *V* . One of the 12 genotypes was 'Canadian Wonder Improved'. The remaining 11 genotypes were derived by inbreeding to homozygosity from eight parental cross combinations, which involved five named varieties and eight numbered breeding lines. Thus, the genetic backgrounds of his (Feenstra,1960) 12 seed coat genotypes were considerably diverse. However, the diversity of the genetic backgrounds of the materials had no effect on the results of the chemical analyses (Feenstra, 1960). This finding meant that the determination of the biochemical action of the color and color modifying genes could proceed without the tedious task of developing a series of isogenic lines differing from each other only at each particular locus. Feenstra (1960) isolated 18 different compounds from his 12 experimental lines. The compounds were anthocyanins, flavonol glycosides, and leuco-anthocyanidins, all pigments belonging to the class of secondary plant metabolites known as flavonoids. Today, the research community accepts the conclusion that the pigments responsible for the wide variations in color of bean seed coats are flavonoids (Beninger et al., 1998). Since the **modifying genes** interact with the **color genes** to form various pigments in bean seed coats, (Beninger and Hosfield, 1999; Beninger et al., 1999), I will refer to the eight genes–*P*, *C*, *D*, *J*, *G*, *B*, *V*, and *Rk*–throughout this paper as color determining genes.

Although Feenstra (1960) found associations between *C* , *J* , and *V*, genes and flavonoid compounds, he was unable to determine the biochemical effects of single genes. There was a need to develop additional genetic stocks to those of Feenstra (1960) that would be useful for studies associating pigments produced by all the color determining genes-those with gene substitutions at the various loci which have visible effects on seed coat color. Bassett (1994a, 1998a) developed a set of genetic stocks, each of which was homozygous recessive for different genes affecting seed coat color substituted into a common genetic background [Florida dry bean breeding line, 5-593 (FLA 5-593)] that was homozygous dominant at all other color determining loci. These stocks proved useful for a new series of investigations to study the biochemical action of the genes controlling seed coat color (Beninger et al., 1998, 1999). The analysis of flavonoids present in 'Prim', a manteca type dry bean, was the beginning of the renewed efforts to establish the relationship between the Mendelian genes controlling seed coat color in *P. vulgaris* and the pigments present (Beninger et al., 1998). 'Prim' was useful for relating genes to

pigment chemistry because it had a light yellow seedcoat and had recessive alleles at several color determining loci [genotype, $P P$, $[C r]$ $[C r]$, $d d$, $j j$, $G G$, $b b$, v^{lae} v^{lae} (Bassett et al., 1999)]. Two flavonol glycosides, kaempferol-monoglucoside (astragalin) and kaempferol 3-glucoxyloside were isolated from the methanol extracts of 'Prim' and were responsible for imparting the yellow color to the seed coat. No proanthocyanidins (condensed tannins) were found in the 'Prim' seed coat. Although this work did not establish the biochemical actions of any of the genes responsible for seed coat color, the work showed that when the dominant alleles, C and G were present and the remaining loci were recessive, only flavonol monomers were produced in the seed coats.

The metabolic pathway for flavonoid biosynthesis begins with the isomerization of chalcone to form a flavanone, which is then converted successively into the flavonols, dihydrokaempferol, and kaempferol (Heller and Forkman, 1988). To date, our knowledge of the flavonoids resulting from the action of the seed coat color determining genes has been inferred from other plant systems. The general pathways, and the genes that control them have been elucidated from such plant species as maize (*Zea mays* L.) and petunia (*Petunia hybrida* Hort. Vilm.-Andr.) (Koes, 1988). However, the pathways and products resulting from the seed coat color genes in *P. vulgaris* have not been elucidated and are speculative.

Feenstra (1960) concluded from his trallelic model of alleles at the C, J, and V loci, that C promotes the formation of anthocyanins and flavonol glycosides. Leakey (1988) reviewed Feenstra's (1960) work and the chemistry of various flavonoid interconversions and speculated that the genes, G and B control the production of a 3,5 diglycoside on the quinoid ring of the flavonol and the hydroxylation of the B-ring of the flavonoid nucleus, respectively. Both of these theories (Leakey, 1988) have been refuted by Beninger et al. (1998, 2000). In fairness to Leakey (1988), he had no direct experimental evidence for the biochemical action of G and B but drew inferences from the flavonoid pathways of other species.

Beninger et al. (1999, 2000) studied genotypes that were dominant at the V locus and others that were homozygous $v v$. When V was present, anthocyanins were produced, but the $v v$ genotypes were devoid of anthocyanins. However, when $V V$ genotypes were in the presence of $b b$ instead of B, the production of anthocyanins was greatly reduced. Dark brown violet (genotype, $P P$, $[C r]$ $[C r]$, $D D$, $J J$, $G G$, $b b$, $V V$, $Rk Rk$) had 19% of the total anthocyanin content as FLA 5-593 (genotype, $P P$, $[C r]$ $[C r]$, $D D$, $J J$, $G G$, $B B$, $V V$, $Rk Rk$). From this work, Beninger and Hosfield (1999) and Beninger et al. (1999, 2000) concluded that anthocyanin production is dependent on V and, since B acts in a quantitative rather than qualitative fashion regulating the amount of anthocyanin in seed coats, the B gene may act to regulate a common precursor to all anthocyanins. Accordingly, the dominant allele B controls the production of more of the precursor than $b b$. The precursor is then converted to anthocyanins (Beninger et al., 2000). In addition, Beninger et al. (1999) showed that the substitution of allele b for B, changing bean genotype $P P$, $[C r]$ $[C r]$, $J J$, $G G$, $B B$, $v v$, (dark brown seed coat) to $P P$, $[C r]$ $[C r]$, $J J$, $G G$, $b b$, $v v$ (yellow-brown seed coat), caused a decrease in the amount of the main flavonoid monomer, astragalin (kaempferol 3-*O*-glucoside). Since dihydrokaempferol is needed for synthesis of both anthocyanins and flavonol glycosides, B may act to promote synthesis of a common precursor, either at or before conversion of the flavanone, naringenin, to

dihydrokaempferol (chalcone synthase or chalcone isomerase steps) in the flavonoid biosynthetic pathway. Feenstra (1960) inferred that dominant J in $P.$ $vulgaris$ probably promotes the production of proanthocyanidins (condensed tannins). Experimental evidence supporting Feenstra's (1960) hypothesis was provided by Beninger and Hosfield (1999a, b) and Beninger et al. (1999, 2000) who found that only genotypes with a dominant allele at the J locus had proanthocyanidins.

Dark red kidney beans have garnet red seed coats and have the genotype P P, [c^u r] [c^u r], $J J$, g g, B B, v v, rk^d rk^d (Bassett, 1998c). Beninger and Hosfield (1999) found three flavonol glycosides in 'Montcalm' (dark red kidney)–astragalin and two quercetins. No anthocyanins were found but proanthocyanidins were present. The conclusions drawn from this work (Beninger and Hosfield, 1999) was that with P and c^u, three flavonol monomers are produced, but the alleles, rk and rk^d interact with c^u to restrict the production of the flavonol glycosides to one kaempferol (astragalin) compound, the other two flavonols being quercetins. The flavonol monomers had little effect on the color of the 'Montcalm' seed coat, but the proanthocyanidins did.

Polyphenolic compounds found in seed coats of colored seeded dry beans are known to adversely affect the nutrition of humans who eat this crop (Salunkhe et al., 1990). While there is copious literature regarding the antinutritional effects of seed coat phenolics in $P.$ $vulgaris$ (Aw and Swanson, 1995; Elias et al., 1979; Salunkhe et al., 1990) nothing has been reported on what, if any, beneficial effects are associated with these compounds. Flavonoids obtained commercially (Husain et al., 1987; Robak and Gryglewski, 1998; Sichel et al., 1991) and isolated from plant species (Gamez et al., 1998; Wang et al., 1999) are known to be effective free radical scavengers, i.e., show antioxidant activity. Recently, condensed and hyrolyzable tannins of high molecular weight also have been shown to be effective antioxidants with even greater activity than simple phenolics, e.g., flavonoid monomers (Hagerman et al., 1998).

Recently Beninger (unpublished data, 2000) in a liposome antioxidant assay (Wang et al., 1999; Arora and Strasburg, 1997) tested kaempferol glucoside and kaempferol-glucoxyloside, quercetins, anthocyanins, and condensed tannin extracts from $P.$ $vulgaris$. The anthocyanins, delphinidin 3-O-glucoside, petunidin 3-O-glucoside and the flavonol quercetin, 3-O-glucoside were the most active of the pure compounds tested (Beninger, unpublished data, 2000). Although the antioxidant activity of these compounds was significantly less than butylated hydroxy toluene (BHT), they still inhibited lipid destruction by over 50% relative to the iron control. The third anthocyanin, malvidin 3-O-glucoside was also active but had significantly less activity than BHT, delphinidin 3-O-glucoside, petunidin 3-O-glucoside, and quercetin 3-O-glucoside. Kaempferol 3-O-glucoside had the least amount of antioxidant activity of the pure compounds tested. This flavonol inhibited liposome breakdown by less than 20% relative to the control; its activity was not significantly different from the control. The tannin extracts were generally found to be as active, or slightly more active, than the pure flavonoid compounds (Beninger, unpublished data, 2000).

Recent work has shown that the most important structural feature of flavonoids for antioxidant activity is the B-ring ortho 3' 4' dihydroxy orientation (Cao et al., 1997; Dziedzic and Hudson, 1983, 1984; Husain et al., 1987) Letan, 1966; Sichel et al., 1991). The most active flavonoids in

our study were delphinidin 3-*O*-glucoside and quercetin 3-*O*-glucoside, which have OH groups at 3' and 4', and petunidin 3-*O*-glucoside, which has a dihydroxy group at 4' and 5'. However, malvidin 3-*O*-glucoside with both the 3' and 5' hydroxy groups methylated had significantly lower activity than the above compounds, all of which have an ortho dihydroxy substitution on the B-ring. Wang et al. (1999) tested three anthocyanins found in tart cherries, all of which had a B-ring 3', 4' substitution, and these were all found to have good antioxidant activity. Finally, kaempferol 3-*O*-glucoside, which only has a single B-ring 4' hydroxyl had no significant activity compared to the iron control. This finding is consistent with the results of the antioxidant assay of the methanol extract from 'Prim', in which no significant activity was observed. 'Prim' has no tannins, and only two kaempferol compounds are present in the seed coat (Beninger et al., 1998).

There is increasing evidence that flavonoids consumed in natural foods convey health benefits to humans by virtue of their antioxidant activity (Laughton et al., 1991; Hertog et al., 1993; Frankel et al., 1993). Dry beans are an integral part of diets in a significant portion of the world population, but the potential benefits of consuming beans from a nutriceutical standpoint have largely been overlooked. Beninger (unpublished data, 2000) showed that pure flavonoid compounds such as anthocyanins, quercetin glucoside, and proanthocyanins (condensed tannins) that are the seed coat pigments of colored seeded dry beans had significant antioxidant activity in a fluorescence-based liposome assay. Resolution of the genes responsible for flavonoids and tannin formation, along with the antioxidant activity of these compounds may enable breeders to select for varieties that have a range of antioxidant activities and also, perhaps, balance antioxidant activity with antinutritional effects.

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