New Alleles, rk^{cd} and rk^p , at the Red Kidney Locus for Seedcoat Color in Common Bean

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ABSTRACT. Among light red and dark red kidney common bean (*Phaseolus vulgaris* L.) varieties, pink seedcoat color (light red kidney) is dominant to dark red, but when Red Mexican varieties (with dark red seedcoats) are crossed with dark red kidney varieties, dark red seedcoat is dominant to the pink segregants observed in an F_2 population. A genetic investigation of this reversal of dominance was performed by making crosses in all combinations among standard varieties of the four recessive-red market classes—Light Red Kidney ‘California Early Light Red Kidney’, Pink ‘Sutter Pink’, Red Mexican ‘NW 63’, and Dark Red Kidney ‘Montcalm’—and observing segregation for seedcoat colors in F_2 and F_3 progenies. The data were consistent with the hypothesis that ‘NW 63’ carries a new allele at Rk , viz., rk^{cd} , where cd stands for *convertible dark red kidney*. Thus, $C rk^{cd}$ expresses dark red kidney seedcoats and $c^u rk^{cd}$ expresses pink seedcoats. Also, $C B rk^{cd}$ expresses garnet brown seedcoats, whereas $C B rk^d$ expresses liver brown seedcoat color. Thus, we propose the gene symbol rk^{cd} for the Rk locus gene in ‘NW 63’. The rk gene from Light Red Kidney ‘Redkloud’ and ‘Sutter Pink’ was backcrossed (with $c^u b v$) into the recurrent parent 5-593, a Florida dry bean breeding line with seedcoat genotype $P [C r] J G B V Rk$. In the F_2 progenies of BC_2 to 5-593, the $c^u b v rk$ segregants from ‘Redkloud’ gave true pink seedcoats, whereas those derived from ‘Sutter Pink’ gave consistently very weak pink color under humid Florida growing conditions. We propose the gene symbol rk^p , where p stands for *pale pink*, for the distinctive rk allele in ‘Sutter Pink’. The more general implications of the above findings were discussed.

Prakken (1972) wrote a comprehensive review of the genetics of red seedcoat color and a general synthesis of all previous research on seedcoat color. Dominant red seedcoat color was given the gene symbol R by Tjebbes in 1921 (as reported by Lamprecht, 1947). The current view of the R gene is that it is very tightly linked to the complex C gene, which is really a series of tightly linked genes (Bassett, 1991; Prakken, 1974). A bracket convention for gene symbols involving the complex C gene has been adopted, e.g., $[C^{ma} R]$ for dominant red (marbled) seedcoats (Bassett, 1991) and $[c^u Prp^i]$ for the whole-plant syndrome of *intensified* (i) anthocyanin expression in the flower buds, corolla, (purple) pods, stems, petioles, and leaves (Bassett, 1994a).

Recessive red seedcoat color was first reported by Gloyer (1928), and Smith (1939) proposed the gene symbol Rk for the locus in the ‘Red Kidney’ variety. The new recessive gene rk produced testaceous (light red kidney color) with m (now given the c^u gene symbol), and $m Rk$ gives buff (Smith, 1939). Smith (1939, 1947) also demonstrated that bean seedcoats with dominant patterns (pintos, marbled, or striped) controlled at C could be changed from [cartridge] buff to pink (same as testaceous) in the light pattern area of the seed by the gene substitution Rk to rk . The Dark Red Kidney market class has a recessive red color controlled by a different allele at Rk , viz., rk^d , and the heterozygote rk/rk^d shows complete, or nearly complete, dominance of rk (Smith and Madsen, 1948). Thus, the dominance order of the alleles is $Rk > rk > rk^d$, where c^u is present with rk and rk^d .

Lamprecht (1947) rejected the hypothesis of Smith (1939) that

a recessive gene rk for the red kidney color really exists, and did so by speculating on possible alternative genotypes that Smith’s (1939) experiments had not ruled out. Circa 1960, Lamprecht sent Smith his Line 214 P -white all-recessive tester, and Smith sent him a (Light) Red Kidney selection that Lamprecht designated Line 687. Lamprecht (1961) crossed Line 159 ($P C j Rk$) x Line 687 ($P c J rk$) and found that rk exists and that the color gene j prevents its expression. Similarly, Smith (1961), using Line 214, found that the color gene J is necessary for the expression of red colors by rk and rk^d . In summary, the current view is that rk and rk^d are modifying genes that change the cartridge buff of $P c^u J$ to their respective recessive red colors (Prakken, 1972).

Prakken (1972) reported the interactions of the rk^d gene (in ‘Soldaat K’) with all gene combinations at the G and B color modifying genes. Bassett (unpublished data) found that the rk^d interactions reported by Prakken (1972) were the same he observed for rk interactions with G and B color modifying genes, whereas the color expression of rk^d in Red Mexican varieties was not altered by the genotype at G and B (Bassett, 1998). Ten commercial varieties of the Red Mexican market class were found to have the dominant allele C (Bassett, 1998), whereas all light or dark red kidney varieties reported have the recessive allele c^u (Bassett, 1998; Smith, 1939, 1947, 1961).

Feenstra (1960) reported that the c^u gene in the seedcoat genotype $P c^u d J g b V Rk$ blocks the production of flavonol glycosides and anthocyanins, but permits production of three leuco-anthocyanidins. Beninger and Hosfield (1999) reported that the rk^d gene in the dark red kidney bean ‘Montcalm’ with seedcoat genotype $P c^u J g B v rk^d$ produced three flavonol glycosides, but attributed the garnet brown (dark red) seedcoat color to proanthocyanidins.

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Excellent quality photographic illustrations of common bean seedcoat colors are available (Voysest, 2000) for the four most important color classes (listed below) described in this paper. For cartridge buff color, see the light pattern colors in Pinto and Carioca market class beans. For the synonymous color names, pink, testaceous, and light red kidney, see the Light Red Kidney class beans. For the synonymous color names, garnet brown and dark red kidney, see the Dark Red Kidney class beans. For the oxblood (dominant red color) expressed by *R*, see the Sangretroro class beans.

The observation that pink color was not dominant over dark red kidney color and light red (redder than pink) color segregants in F_2 progenies from the cross 'Sutter Pink' x 'NW 63' (dark red kidney color seed) was contrary to the findings of Smith and Madsen (1948) and prompted the research presented below. The observed reversal of dominance expresses as though the *rk^d* allele somehow became dominant over *rk*, but a more plausible hypothesis might be developed from appropriate experimental work. The objective of this research was to test the interactions of various alleles at the *C* and *Rk* loci as found in a representative variety of each of the four principal market classes of the United States that have recessive red seedcoat colors, viz., Light Red Kidney, Pink, Red Mexican, and Dark Red Kidney.

Materials and Methods

In 1985 a program was initiated to develop genetic tester stocks for common bean seedcoat colors and patterns by backcrossing selected recessive alleles, singly and in combination, into a recurrent parent 5-593, a Florida dry bean breeding line with the seedcoat genotype *TP [C r] Z J G B V Rk* (Bassett, 1994b; Bassett and Blom, 1991; Bassett et al., 1999). Details of the backcross procedure for developing genetic tester stocks were previously described (Bassett, 1994c). The seedcoats of 5-593 are shiny, unpatterned black.

The seedcoat genetic study used four varieties, one each from the four market classes: Light Red Kidney 'California Early Light Red Kidney' (CELRK), Pink 'Sutter Pink', Red Mexican 'NW 63', and Dark Red Kidney 'Montcalm' (Table 1). Following the procedures of Bassett (1999), three genetic tester stocks - *c^u* BC₃ 5-593, *z j* (margo *z*) BC₃ 5-593, and *g b v* BC₃ 5-593 - were crossed to each of the four test varieties to determine the genotype at the loci *C*, *J*, *G*, *B*, and *V*. The test crosses and the genetic interpretation of seedcoat colors of F_1 progenies were performed in greenhouse facilities at Gainesville, Fla., during Fall 1999 and Winter 2000, respectively. The genotype for the four test varieties (Table 1) at the *Rk* locus was considered adequately established (Bassett, 1998; Smith, 1939, 1961).

The four test varieties were crossed in all possible combinations, including reciprocal crosses, in Summer 1999 (June to August) in greenhouse facilities at Prosser, Wash. Subsequently, the F_2 populations were generated from self-pollinated F_1 plants grown in the greenhouse during Fall 1999 (September to November) at Prosser. The seedcoat color of the F_2 seed (F_1 phenotype) lots was recorded, and the F_2 seed lots were sent to Dr. James Beaver, University of Puerto Rico at Mayaguez, Puerto Rico, for field planting in Winter 1999/2000 (December to February). Seedcoat color of the F_3 seed lots from individual F_2 plants (self-pollinated) was recorded. The F_3 progenies were planted in the field at Prosser in Summer 2000 (June to August) in 3 m rows spaced 0.56 m apart. Depending on seed availability, 25 to 50 seeds were planted for each F_3 plot. At harvest maturity, a single seed was obtained

from each F_3 plant within the progeny row, and the seedcoat color was classified and recorded. The observations for the degree of seedcoat vein expression (high, intermediate, or low) was also recorded. Due primarily to a lack of enough seed (<25) and limited field space, not all F_3 progenies (from F_2 parents classified for seedcoat color) could be sown. Seedcoat color phenotypes recorded for reciprocal crosses had similar classifications and patterns of segregation (data not shown), but the reciprocal cross data were not included in the overall analysis because 1) of missing reciprocal crosses for 'Sutter Pink' x 'CELRK' and 'Sutter Pink' x 'Montcalm', and 2) F_3 progeny rows for some reciprocal crosses were not planted due to limited field space.

At the University of Florida, Gainesville, a program was initiated in 1994 to develop genetic stocks in BC₃ to 5-593 (used as the recurrent parent) with the red kidney gene from 'Redcloud' (for the Light Red Kidney market class) and 'Sutter Pink' (for the Pink market class). To facilitate the study of genotypes of the color modifying genes, *G*, *B*, and *V*, parallel development (for Light Red Kidney and Pink classes) was made in the *C*/*-* and *c^u*/*c^u* genetic backgrounds because the *c^u*/*c^u* genotype masks the genotype at the color modifying genes. The final goal was to develop genetic stocks for both recessive red market classes in the background genotype *c^u J G b v*, which was accomplished by 1997.

Results and Discussion

SEEDCOAT COLOR GENOTYPES OF PARENTS. Test crosses of *c^u* BC₃ 5-593 with the four test varieties produced two phenotypic classes in F_1 progeny: 1) unpatterned black seedcoats for 'NW 63', a nonallelic reaction interpreted as a *C* allele reaction (Bassett, 2000) and 2) cartridge buff seedcoats for 'CELRK', 'Sutter Pink', and 'Montcalm', an allelic reaction with the *c^u* allele present in the test varieties (Bassett, 2000). Test crosses of *z j* BC₃ 5-593 with the four test varieties produced in F_1 progeny shiny, unpatterned black seedcoats, which is a nonallelic reaction interpreted as a *J* allele present in the four test varieties (Bassett, 1999). Test crosses of *g b v* BC₃ 5-593 with the four test varieties produced two phenotypic classes in F_1 progeny: 1) buffy citrine seedcoats for 'CELRK' and 'Montcalm', which is a nonallelic reaction interpreted as a *g B v* genotype (Prakken, 1972) and 2) shamois seedcoats for 'Sutter Pink' and 'NW 63', which is an allelic reaction interpreted as genotype *g b v* in the test varieties. All the above test cross F_1 results are summarized in Table 1. We have proposed the tentative gene symbol *rk^{cd}* for a new allele at *Rk* in 'NW 63', where *cd* indicates *convertible dark* red kidney color. With *c^u rk^{cd}*, the seedcoat color is pink, whereas with *C rk^{cd}* the color is garnet brown, a synonym for dark red kidney. Also, we have proposed the tentative gene symbol *rk^p* for another new

Table 1. Genetic hypothesis for the seedcoat color and market class of four red bean parental varieties.

Cultivar (no.)	Seedcoat genotype ^z	Market class
CELRK (P ₁)	<i>c^u J g B v^{lae} rk</i>	Light Red Kidney
Sutter Pink (P ₂)	<i>c^u J g b v rk^p</i>	Pink
NW63 (P ₃)	<i>C J g b v rk^{cd}</i>	Red Mexican
Montcalm (P ₄)	<i>c^u J g B v rk^d</i>	Dark Red Kidney

^zGenotype *c^u*/*c^u* (with *P J*) expresses cartridge buff seedcoat color that is *unchangeable* (indicated by superscript *u*) by the genotype at *G*, *B*, and *V*. The superscript *lae* in gene symbol *v^{lae}* stands for laelia (pink or Rose purple) flower color. The superscripts used with gene symbol *rk* are *p* for *pale* pink seedcoat color, *d* for *dark* red kidney seedcoat color, and *cd* for *convertible dark* red kidney seedcoat color.

allele at *Rk*, where *p* indicates *pale* pink seedcoat color when the beans are grown in humid conditions.

RESULTS OF DIALLEL CROSSES AMONG FOUR PARENTS. The cross 'CELRK' x 'Sutter Pink' produced F_1 and F_2 plants with light red kidney (LRK, same as pink) color (Table 2). Progeny tests of 72 pink F_2 parents gave true breeding pink F_3 progenies (Table 3). The action of c^u/c^u prevents the expression of *B* from 'CELRK', which would otherwise (with *C/-*) produce various brown colors (Prakken, 1970). These results agree with those of Smith (1939, 1961) and Smith and Madsen (1948), who called the color produced by genotype (using the symbols of Prakken, 1970) $c^u J rk v$ (or v^{lae}) testaceous. The apparent differences in the purity and brightness of pink colors in various varieties of the Pink and LRK market classes are mostly due to the degree of absence of pale grayish or beige vein patterns in the seedcoat color background.

The cross 'CELRK' x 'Montcalm' produced pink seedcoats in the F_1 generation, and the F_2 generation segregated 3:1 for the two parental phenotypes, viz., pink to dark red kidney (DRK, same as garnet brown) seedcoat colors, respectively (Table 2). The segregation observed in the F_3 generation supports the hypothesis that a single factor segregation is occurring in F_2 that involves only the alleles at the *Rk* locus that are known to control the parental seedcoat colors, viz., *rk* in 'CELRK' and *rk^d* in 'Montcalm' (Table 3). The *rk* allele is fully dominant to *rk^d*, as evidenced by the pink F_2 class being either true breeding in F_3 or having 15 of the F_3 progenies from F_2 LRK parents segregating 3:1 for pink to DRK, respectively (Table 3). The chi-square test fit a 1:2:1 ratio for the three expected F_3 classes: true breeding DRK, segregating 3 pink:1 DRK, and true breeding pink, respectively (Table 3).

The cross 'Sutter Pink' x 'Montcalm' produced pink seedcoats in the F_1 generation and segregated 3:1 in F_2 for pink to DRK seedcoat colors, respectively. The segregation observed in F_3 confirmed the hypothesis that segregation is occurring at a single locus (*Rk*) where the *rk^p* allele from 'Sutter Pink' is dominant to the *rk^d* allele from 'Montcalm'. The chi-square test fit a 1:2:1 ratio for the three expected classes: true breeding DRK, segregating 3 pink:1 DRK, and true breeding pink (Table 3).

The results from the cross of either 'CELRK' or 'Sutter Pink' with 'Montcalm' agree with Smith and Madsen (1948) except that

the *rk* gene of 'CELRK' or 'Sutter Pink' is completely dominant to the *rk^d* of 'Montcalm' rather than partially dominant as in the 'Michigan Dark Red Kidney' x 'Red Kidney' cross of Smith and Madsen (1948). In previous work with Red Mexican market class materials, Bassett (1998) found that the DRK seedcoat color of Red Mexican beans was due to a gene for recessive red color, which was assumed to be *rk^d*. In retrospect, Bassett (1998) observed that all Red Mexican varieties (≈ 35 g/100 seeds) tested carried *C*, whereas all recessive red LRK and DRK (≈ 55 g/100 seeds) varieties tested had c^u . This may be due to coincidence, founder effects, or some genetic necessity not yet discovered.

For the cross 'Sutter Pink' x 'NW 63', the F_2 and F_3 seedcoat color classes observed were dark red ('NW 63' parental type), medium red, light red (redder than pink), and pink ('Sutter Pink' parental type). Complete analysis of all the data demonstrated that only the pink class was always reliably reproduced in derived progenies in all seasons and growing facilities. Consequently, we combined the observed seedcoat color classes to only two: pink and all other red colors for further analysis and presentation.

The cross 'Sutter Pink' x 'NW 63' produced garnet brown seedcoats in the F_1 generation, indicating full dominance of the *rk^{cd}* allele from 'NW 63' over the *rk^p* from 'Sutter Pink'. The F_2 segregated 3:1 for red to pink seedcoats, respectively (Table 2), and the F_3 segregation confirmed that a single factor (*C* locus) was responsible for the segregation in F_2 (Table 3). The chi-square test fit a 1:2:1 ratio for the three expected classes: true breeding red, segregating 3 red:1 pink, and true breeding pink (Table 3). Our hypothesis is that a two-factor interaction is involved, viz., $C/c^u rk^{cd}/rk^p$, where *rk^{cd}* is a new allele carried by 'NW 63' that has convertible seedcoat color expression depending on the genotype at *C* (Table 4). With *C/-* *rk^{cd}* the seedcoat will be red, and with $c^u/c^u rk^{cd}$ it will be pink. The genotype *C/-* *rk* gives a red color (hue dependent on other genes) or red haze over some background color, whereas only $c^u/c^u rk/rk$ gives pink seedcoat color (Bassett, 1998; Prakken, 1972; Smith, 1961). Thus, the only gene segregating that can shift the color expression of *rk^{cd}* in F_2 and F_3 is the *C/c^u* genotype of the *C* locus (Tables 1 and 2).

For the cross 'CELRK' x 'NW 63', the F_2 and F_3 seedcoat color classes observed were: dark brownish red, dark red ('NW 63' parental type), light red, pink ('CELRK' parental type), light

Table 2. Segregation for seedcoat color (with genotypes) in F_1 and F_2 progenies of six crosses among four red seeded bean varieties².

Cross	F_1 seedcoat genotype and color ^y	Brown and red	Red	Pink	Ratio tested	χ^2	<i>P</i>
$P_1 \times P_2$	$c^u B/b rk/rk^p$ Pink			$c^u rk/-$ (or rk^p) All			
$P_1 \times P_3$	$C/c^u B/b rk^{cd}/rk$ Dark brown	<i>C/-</i> 91		$c^u rk^{cd}/-$ (or <i>rk</i>) 21	3:1	2.333	0.13
$P_1 \times P_4$	$c^u B rk/rk^d$ Pink		$c^u rk^d/rk^d$ 21	$c^u rk/-$ 53	1:3	0.451	0.50
$P_2 \times P_3$	$C/c^u b rk^{cd}/rk^p$ Garnet brown		<i>C/-</i> 58	$c^u rk^{cd}/-$ (or $rk^?$) 15	3:1	0.772	0.38
$P_2 \times P_4$	$c^u B/b rk^p/rk^d$ Pink		$c^u rk^d/rk^d$ 31	$c^u rk^p/-$ 88	1:3	0.070	0.79
$P_3 \times P_4$	$C/c^u B/b rk^{cd}/rk^d$ Dark Brown	<i>C/-</i> <i>-/-</i> or $c^u rk^d$ 133		$c^u rk^{cd}/-$ 31	13:3 3:1	0.003 3.252	0.96 0.07

²The variety names of the parents and their complete seedcoat genotypes are given in Table 1.

³Garnet brown is the color of the dark red kidney (DRK) market class, and pink is the color of the light red kidney market class. Red colors include DRK, other nonpink red colors, but none of the brown colors produced by interactions with *B*. DRK color genotypes include *C/-* *rk^{cd}/rk*, *C/-* *rk^{cd}/rk^{cd}*, *C/-* *rk^d/rk^d* and $c^u/c^u rk^d/rk^d$. Pink color genotypes include $c^u/c^u rk/rk^d$, $c^u/c^u rk^{cd}/rk$, $c^u/c^u rk^{cd}/rk^{cd}$, and $c^u/c^u rk/rk$, where *rk^p* does not affect color expression in freshly harvested materials grown in arid conditions.

pink, and three brown classes (with or without a red hue), viz., dark brown, medium brown, and light brown. Because we were unable to model the brown color genetics (a difficult trigenic interaction with variable expression over environments), the analysis that follows will combine the data into only two classes: pink (includes light pink class) and all other red or brown seedcoat colors. However, only the data from F₃ progeny tests of red seedcoat class F₂ parents are presented, i.e., no F₃ data from brown or dark brownish red seedcoat color F₂ parents are presented for reasons discussed below.

The cross 'CELRK' x 'NW 63' produced dark brown seedcoats in the F₁ generation, an expression of the interaction of *B/-* with *C/- J rk^{cd}/-* (Tables 1 and 2). The F₂ and F₃ generations segregated for many different red and brown colors, which are listed above. Careful analysis of that data showed that no genetic model could be found for all the classes in the observed frequencies corresponding to existing knowledge of seedcoat genetics. The analysis did show that some genotypes were misclassified

because they had different color expressions in different environments (seasons) (data not shown). Cumulative errors of this sort, and perhaps other types, made Mendelian analysis of so many color classes impossible. The only seedcoat color classification (within crosses) that could be made with minimal errors was pink versus not pink (brown and red, or other). The F₂ segregated 3:1 for brown and red to pink, respectively (Table 2). Sixty-one F₂ parents with brown or red seedcoat color were progeny tested in F₃, and all segregated in a 3:1 ratio for brown or red seedcoat to pink seedcoats, respectively (Table 3). All 12 pink F₂ parents tested in F₃ were true breeding. Our hypothesis is that the single factor segregation observed in F₂ and F₃ was at the *C* locus for *C/-* versus *c^u/c^u*. Those results support the hypothesis that both *c^u/c^u rk^{cd}/-* and *c^u/c^u rk/rk* express pink seedcoat color.

For the cross 'NW 63' x 'Montcalm', the F₂ and F₃ seedcoat color classes observed were very dark red (nearly black), dark brownish red, dark red (nonparental), dark red ('NW 63' parental type), dark red ('Montcalm' parental type), medium red, light red,

Table 3. Segregation for seedcoat color (with genotypes) in F₃ progenies from randomly selected F₂ plants derived from six crosses among four red bean varieties.^z

Cross	F ₂ parent seedcoat color ^y	F ₃ segregation			Ratio tested	χ ²	P
		Seedcoat color class ^y					
		No. progenies	Brown and red	Red			
P ₁ × P ₂							
	Pink	72			<i>c^u rk</i> (or <i>rk^p</i>)		
P ₁ × P ₃	Brown and red	61	<i>C/- -/-</i> 685		<i>c^u/c^u rk</i> (or <i>rk^{cd}</i>)	3:1	0.062
	Pink	12			224		0.80
					All		
^x P ₁ × P ₄				<i>c^u rk^d/rk^d</i>	<i>c^u rk/-</i>		
	DRK	7 ^x		All			
	Pink	15 ^x		34	88	1:3	0.536
	Pink	4 ^x			All		0.46
^w P ₂ × P ₃				<i>C rk^{cd}</i>			
	Red	9 ^w		All			
				<i>C/- -/-</i>	<i>c^u/c^u -/-</i>		
	Red	20 ^w		429	144	3:1	0.005
					<i>c^u rk^p or c^u rk^{cd}</i>		0.98
	Pink	12 ^w			All		
^v P ₂ × P ₄				<i>c^u rk^d/rk^d</i>	<i>c^u rk^p/-</i>		
	DRK	16 ^v		All			
	Pink	31 ^v		123	373	1:3	0.011
	Pink	26 ^v			All		0.92
^u P ₃ × P ₄				<i>c^u rk^d</i>	<i>c^u rk^{cd}/-</i>		
	Pink	9 ^u			All		
	Pink	16 ^u		62	224	1:3	1.683
	DRK	8 ^u		All			0.20
P ₃ × P ₄			<i>C/-</i> or <i>c^u rk^d</i>		<i>c^u rk^{cd}/-</i>		
	Red ^t	16	170		38	13:3	0.032
						3:1	5.026
							0.03

^zThe variety names of the parents and their complete seedcoat genotypes are given in Table 1. For the cross P₁ × P₃, F₃ data were omitted for brown and red F₂ parents that did not segregate for pink seed.

^yGarnet brown is the color of the dark red kidney (DRK) market class, and pink is the color of the light red kidney market class. Red colors include DRK, other nonpink red colors, but none of the brown colors produced by interactions with *B*. DRK color genotypes include *C/- rk^{cd}/rk*, *C/- rk^{cd}/rk^{cd}*, *C/- rk^d/rk^d* and *c^u/c^u rk^d/rk^d*. Pink color genotypes include *c^u/c^u rk/rk^d*, *c^u/c^u rk^{cd}/rk*, *c^u/c^u rk^{cd}/rk^{cd}*, and *c^u/c^u rk/rk*, where *rk^p* does not affect color expression in freshly harvested material grown in arid conditions.

^xFor the data 7, 15, and 4, the χ² (1:2:1) = 1.308, *P* = 0.52.

^wFor the data 9, 20, and 12, the χ² (1:2:1) = 0.463, *P* = 0.79.

^vFor the data 26, 31, and 16, the χ² (1:2:1) = 4.397, *P* = 0.11.

^uFor the data 9, 16, 8, the χ² (1:2:1) = 0.091, *P* = 0.96.

^tFor P₃ × P₄ there were 150 F₃ plots, and 86 did not segregate for pinks and 64 did; for 86, 64 the χ² (1:1) = 3.227, *P* = 0.07. Among red F₂ parents, 30 segregated for pink seedcoats and 55 did not.

very light red (redder than pink), bright red, very dark brown, dark brown, medium brown, light brown with or without a red hue, pink, and pale (light) pink. The analytical treatment of the data followed the same rules as given above for the cross 'CELRK' x 'NW 63', and for the same reasons.

For the cross 'NW 63' x 'Montcalm' the F_1 generation produced dark brown seedcoats, expressing the interaction of $B/-$ with $C/-rk^{cd}/rk^{cd}$ (Tables 1 and 2). The F_2 and F_3 generations segregated for similar groups of different red and brown seedcoat colors as the cross 'CELRK' x 'NW 63' and led to the same failure of Mendelian analysis of those color classes for the same reasons (data not shown). Our genetic model for this cross has the hypothesis that the F_1 carries the genotype $C/c^u rk^{cd}/rk^{cd}$ (Table 5). The 164 F_2 plants segregated for a much better fit to a 13:3 ratio between brown and red to pink seedcoat color, respectively, than to a 3:1 ratio for the same classes although the test was not quite significant at the 5% level, which is predicted by our model (Table 2). In contrast to the 'CELRK' x 'NW 63' cross where all pink F_2 were true breeding in F_3 (Table 4), the model for this cross predicts that 2/3 of the pink F_2 parents will segregate 3:1 for pink to DRK, respectively, in F_3 and the remaining 1/3 will be true breeding (Table 5). The model also predicts that DRK F_2 parents will be true breeding (Table 5). Those segregation results were observed in F_3 , and the expected 1:2:1 ratio of the expected three classes—true breeding pink, segregating 3 pink: 1 DRK, and true breeding DRK, respectively, was also observed (Table 3). A 1/3 fraction of the other brown and red seedcoat F_2

parents are expected to segregate 13:3 for brown and red to pink seedcoat color classes, respectively, and this was observed with significance (Table 3).

AN ALTERNATIVE HYPOTHESIS WITH C RATHER THAN Rk . An alternative hypothesis was examined to account for the reversal of dominance observed in crosses of Red Mexican market class parents with LRK or DRK market class parents. There is no doubt that recessive red seedcoat colors are expressed as an interaction of the C and Rk loci, but the new allele may be at C rather than Rk . The hypothesis is that there is only one recessive allele at Rk , viz., rk^d (all four parents of Table 1). Thus, a true rk allele does not exist. Instead, there are two allelic forms of c^u ; one (c^u) does not convert rk^d to pink seedcoat expression and another (c^{ulr} , 'CELRK' and 'Sutter Pink') that does convert rk^d to pink. Application of this alternative model was compatible with all the F_2 and F_3 data (Tables 2 and 3) except for the data from cross $P_3 \times P_4$. The alternative genetic model does not predict (is inconsistent with) the observed segregation for plants with pink seedcoats in the cross 'NW 63' x 'Montcalm'. Therefore, the hypothesis of two alleles at C was rejected.

AN ALTERNATIVE HYPOTHESIS FOR CONVERTIBILITY AT Rk . A second alternative hypothesis may be given for the new allele, rk^{cd} , hypothesis. Perhaps the rk allele, believed by Smith (1961) and Smith and Madsen (1948) to be common to both LRK and Pink market classes, gives pink with c^u and DRK color with $C/-$. Thus, the rk^{cd} allele designation would be just a synonym for rk in a noncustomary genetic background with respect to the C locus. This alternative hypothesis was found to be unsupported

Table 4. The genetic model for segregation in F_2 and F_3 progenies from the common bean cross 'CELRK' ($c^u rk$) x 'NW 63' ($C rk^{cd}$), where rk^{cd} is a hypothetical new allele at Rk that expresses dark red kidney seedcoat color with C and pink with c^u , and where rk^{cd} is dominant to rk with C .

Genotype	F ₂ segregation		F ₃ segregation
	Frequency (1/16)	Seedcoat color ^z	Seedcoat colors ^z (with expected ratios)
$C/C rk^{cd}/rk^{cd}$	1	Other	All other
$C/C rk^{cd}/rk$	2	Other	All other
$C/C rk/rk$	1	Other	All other
$C/c^u rk^{cd}/rk^{cd}$	2	Other	3 other : 1 pink
$C/c^u rk^{cd}/rk$	4	Other	3 other : 1 pink
$C/c^u rk/rk$	2	Other	3 other : 1 pink
$c^u/c^u rk^{cd}/rk^{cd}$	1	Pink	All pink
$c^u/c^u rk^{cd}/rk$	2	Pink	All pink
$c^u/c^u rk/rk$	1	Pink	All pink

^zOther = all brown and red seedcoat colors except for pink. Pink = light red kidney color.

Table 5. The genetic model for segregation in F_2 and F_3 progenies from the common bean cross 'NW 63' ($C rk^{cd}$) x 'Montcalm' ($c^u rk^d$), where rk^{cd} is a hypothetical new allele at Rk that expresses dark red kidney seedcoat color with C and pink with c^u , and where rk^{cd} is dominant to rk^d with c^u .

Genotype	F ₂ segregation		F ₃ segregation
	Frequency (1/16)	Seedcoat color ^z	Seedcoat colors ^z (with expected ratios)
$C/C rk^{cd}/rk^{cd}$	1	Other	All other
$C/C rk^{cd}/rk^d$	2	Other	All other
$C/C rk^d/rk^d$	1	Other	All other
$C/c^u rk^{cd}/rk^{cd}$	2	Other	3 other : 1 pink
$C/c^u rk^{cd}/rk^d$	4	Other	13 other : 3 pink
$C/c^u rk^d/rk^d$	2	Other	All other
$c^u/c^u rk^{cd}/rk^{cd}$	1	Pink	All pink
$c^u/c^u rk^{cd}/rk^d$	2	Pink	3 pink : 1 DRK
$c^u/c^u rk^d/rk^d$	1	DRK	All DRK

^zOther = all brown and red seedcoat colors except for pink. Red = all red seedcoat colors except pink. Pink = light red kidney color. DRK = dark red kidney.

Table 6. Segregation for pink bean seedcoat colors in the F₂ from crosses of pink breeding lines (in 5-593 genetic background) derived from ‘Redkloud’ (RK) or ‘Sutter Pink’ (SP) with *b v BC*₃ 5-593 (yellow brown seedcoat tester line).

Cross	<i>C/- Rk/-</i> Y.B. ^z	<i>C/- rk/rk</i> Y.B. ^z with reddish haze	<i>c^u/c^u Rk/-</i> C.B. ^y	<i>c^u/c^u rk/rk</i> Pink	χ ² 9:3:3:1	<i>p</i>
RK <i>c^u b v rk BC</i> ₁ 5-593						
<i>x b v BC</i> ₃ 5-593	183	53	51	16	2.232	0.53
RK <i>c^u b v rk BC</i> ₂ 5-593						
<i>x b v BC</i> ₃ 5-593	91	34	31	5	3.052	0.38
SP <i>c^u b v rk BC</i> ₁ 5-593						
<i>x b v BC</i> ₃ 5-593	170	56	46	16 ^x	1.8765	0.60
SP <i>c^u b v rk BC</i> ₂ 5-593						
<i>x b v BC</i> ₃ 5-593	99	40	36	8 ^x	2.210	0.53

^zY.B. = yellow brown seedcoats.

^yC.B. = cartridge buff seedcoats.

^xThe pink seed segregants from crosses with lines derived from ‘Sutter Pink’ had low expressivity and were barely discernible under humid Florida growing conditions.

by results from the parallel development for genetic stocks with their recessive red genes derived from ‘Redkloud’ and ‘Sutter Pink’ in both *C/-* and *c^u/c^u* backgrounds (Table 6). The genotype *C/- rk/rk* (as derived from ‘Redkloud’ and ‘Sutter Pink’) gave yellow brown seedcoats with a reddish haze and not the garnet brown of the DRK class predicted by the alternative hypothesis. Thus, the distinction between the interaction characteristics with the *C* locus of *rk^d* and *rk^{cd}* is due to a unique allele at *Rk* in the Red Mexican market class.

DISCUSSION AND CONCLUSIONS FOR THE *rk^{cd}* HYPOTHESIS. On the basis of the above results for the diallel crosses among the four recessive red varieties tested and their F₂ and F₃ data, we formally propose the gene symbol *rk^{cd}* for a third recessive allele at the *Rk* locus. The dominance order is *Rk* > *rk* or *rk^{cd}* > *rk^d*, where *c^u* is present with the three recessive alleles. We were not able to explore the interactions of the three recessive alleles with *C* because of the interactions of *B* and the environmental sensitivity (variable expressivity) of red colors with *C*.

In spite of our inability to model the interactions of *B* with *C* and *Rk* for three of our crosses (Tables 2 and 3), there is one difference in interaction between *B* and the two alleles, *rk^d* and *rk^{cd}* that is beyond doubt. Bassett (1998) reported that PI 173763 with seedcoat genotype [*C r*] *J B v rk^d* expressed garnet brown seedcoat color, which differs from the results reported by Smith (1961) where *C J B v rk^d* (Prakken’s gene symbols) expresses liver brown. This conclusion is based on an interpretation of the results of Smith (1961) by the senior author that is different from that of Smith (1961). In his cross ‘Michigan Dark Red Kidney’ *x* Line 214 (the all recessive tester), Smith (1961) mistakenly attributed the F₂ segregation of the drab and liver brown vs. buff and garnet brown seedcoat color classes to segregation at *Br* (now *B*), whereas the observed color segregation is due to genetic segregation for *C* vs. *c^u*, respectively. Line 214 must carry *C* because all red kidney varieties carry *c^u*. Also, the *B* gene is carried by ‘Michigan Dark Red Kidney’, not by Line 214 as Smith (1961) assumed. Smith (1961) correctly attributed liver brown seedcoat color to genotype *C J B v rk^d*, rather than the garnet brown of PI 173763 with the same genotype. Therefore, the *rk^d* gene in PI 173763 (and all other Red Mexican varieties) must be a different allele from the *rk^d* in ‘Michigan Dark Red Kidney’ (and probably all other DRK varieties). Our hypothesis is that PI 173673 carries *rk^{cd}*, which (with *C*) is not modified by segregation at *B*, whereas the standard *rk^d* is modified by segregation at *B* (with *C*).

THE *rk^p* HYPOTHESIS FOR A HUMIDITY-SENSITIVE ALLELE. The

results presented in Table 6 support the hypothesis that the *rk* gene in ‘Sutter Pink’ is different from the one in ‘Redkloud’. With genotype *C/-*, the *rk* gene from both parents gave the same seedcoat color, viz., yellow brown with a reddish haze. With genotype *c^u/c^u*, the *rk* gene from ‘Redkloud’ gave a typical pink seedcoat color, whereas the *rk* gene from ‘Sutter Pink’ gave a weakly expressed pink color that was barely discernible from the cartridge buff of *c^u* (Table 6). Our hypothesis is that ‘Sutter Pink’, and probably all other indeterminate, small-seeded Pink market class varieties, carry a recessive red allele at *Rk* that is different from the determinate, large-seeded LRK class. We propose the gene symbol *rk^p*, where *p* stands for *pale* pink seedcoats, for the Pink class allele as represented by ‘Sutter Pink’. We speculate that the Pink class requires an environment with low relative humidity for the genotype *c^u/c^u rk^p/rk^p* to express a true pink color.

Conclusions

We propose that the specific results found for the four recessive red varieties tested may be generalized over their respective market classes. For example, probably all Red Mexican market class varieties carry the *rk^{cd}* allele of ‘NW 63’, which is why all Red Mexican varieties carry *C* (and not *c^u*) to avoid the pink seedcoat expression of *c^u rk^{cd}*. The current hypothesis for the chemistry of red color controlled by the *Rk* locus is that proanthocyanidins (polyphenols, tannins) and their oxidation/polymerization products are in some way, not yet understood, able to express the red color of the LRK and DRK market classes of beans (Beninger and Hosfield, 1999). Thus, the presumption is that all LRK varieties carry *c^u* with *rk* because the concentration of proanthocyanidins (tannins) produced by *rk* is too low to adequately cover the background color effects of the color modifying genes *G* and *B*. Similarly, all DRK varieties carry *c^u* with *rk^d* because the higher concentration of tannins produced by *rk^d* (Hosfield, Michigan State Univ., personal communication) is not adequate to cover the color effects of *B* (Smith, 1961). The gene *c^u* is able to protect the expression of *rk* and *rk^d* by blocking the production of the anthocyanins and flavonol glycosides required for the color modifying expression of the genes *G*, *B*, and *V* (Feenstra, 1960). The new gene *rk^{cd}* must either 1) produce enough of the proanthocyanidins and their oxidation/polymerization products to cover up colors controlled by *G* and *B* or 2) be able to block their expression in a way analogous to *c^u*. More research of pigment chemistry in seedcoats is needed to resolve such questions.

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