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ORIGINAL PAPER

Characterization of seed coat post harvest darkening in common bean (*Phaseolus vulgaris* L.)

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Abstract Seed coat post harvest darkening (PHD) represents a problem for producers and consumers of several market classes of dry bean. There are three PHD phenotypes: (1) non-darkening (ND), (2) slow darkening (SD) and (3) regular darkening (RD). The inheritance of PHD was elucidated by evaluating populations derived from crosses among multiple RD, SD and ND genotypes. Results indicate that at least two unlinked major genes control the PHD trait in common bean. Recessive epistasis with three phenotypic classes explains the segregation ratios of populations from crosses between SD and ND parents. One gene, *J*, is responsible for whether a bean will darken as seeds from plants that are *jj* do not darken at all. Another gene, *sd*, influences how quickly a seed coat will darken with *sdsd* individuals darkening more slowly than those with the dominant *Sd* allele.

Introduction

Seed coat post harvest darkening (PHD) causes a gradual change in the colour of the seed coat of some market classes of dry bean during storage. For example, the seed coat background colours of pinto, carioca, and cranberry or borlotti beans often change from cream to brown, a few months after the seed has reached physiological maturity. Both genotypic and environmental factors can influence the rate and extent of PHD and darkening tends to occur more rapidly in environments prone to elevated temperatures, humidity and exposure to light (Park and Maga 1999; Junk-Knievel et al. 2007).

There are at least three PHD phenotypes: (1) non-darkening (ND), (2) slow darkening (SD) and (3) regular darkening (RD). Post harvest darkening of the seed coat in common beans is an undesirable characteristic that results in lost value to producers, exporters and vendors. Common bean breeders have identified genotypes that darken slowly or not at all. There is interest among North American bean growers to produce slow darkening beans, particularly for Central American markets.

According to Prakken (1974) and Bassett (1996), the *J* locus is associated with PHD in common bean: the recessive *jj* produces seed coats that are far less subject to PHD compared to genotypes with the dominant *J* allele. However, the recessive genotype may have reduced levels of pigmentation in the coloured pattern of the seed coat. The limiter (*l*) locus is allelic with *J* (Bassett et al. 2002). Junk-Knievel et al. (2008) demonstrated that a single gene controlled whether a genotype was SD or RD; with RD being dominant. Initial observations of segregation among F_2 progeny of crosses between 1533-15, a SD pinto, and PI 608686 and PI 608688, both with the genotype *jj* (Genetic Markers 39 and 41, respectively), however, suggested a two-gene model rather than simply a new allele at *J*. The objective of this study was to demonstrate the existence of a second locus (*sd*) and investigate its interaction with *J*.

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Materials and methods

Parental genotypes

A total of eight genotypes of common bean and the progeny of crosses derived from them were intercrossed to determine the genetic relationships among the different PHD phenotypes (Fig. 1).

CDC Pintium is a RD pinto bean developed and released in 1999 by the Crop Development Centre (CDC) in Saskatoon, Saskatchewan. CDC Pintium has a seed coat that turns from creamy white to dark brown after approximately 6 months of storage at room temperature under normal light conditions.

1533-15 is a SD pinto bean developed by the CDC and registered as CDC WM-1 in 2009. 1533-15 has a seed coat that turns from creamy white to light brown, but does so at a much slower rate than CDC Pintium. After a complete year of storage at room temperature under normal light condition, 1533-15 seed coats remain very light in colour.

SDIP-1 is a SD pinto bean developed by Dr. S. P. Singh at the University of Idaho Kimberly Agricultural Research and Extension Centre (Singh et al. 2006). SDIP-1 has a seed coat that is almost identical to 1533-15 and turns from creamy white to light brown with age. As with 1533-15, SDIP seed coats stored for a complete year at room temperature under normal light condition remain very light in colour.

KVxUI-1 and KVxUI-6 are ND pinto beans that were developed by Dr. J. Myers at Oregon State University (OSU) to be homozygous for the *j* allele. Both of these lines have seed coats that are white with light brown spotting at harvest and retain their harvest colour after several years of storage at room temperature under normal light conditions.

Wit-rood boontje is a ND cranberry-like bean from the Netherlands. Wit-rood has a seed coat that is white at harvest and the cranberry mottled pattern is very faded and sometimes non-existent.

PI 608686 and PI 608688 are genetic testers for *j* (Genetic Markers 39 and 41, respectively) obtained from the United States Department of Agriculture (USDA) Plant Introduction Station at Pullman, Washington. PI 608686 also has the genotype *VV*, so has a black seed coat, while PI 608688 is *vv* and has a brownish-purple seed coat.

Populations

Crosses were made to generate a series of F_1–F_5 seed coat generations for phenotypic and genotypic evaluation of PHD. The crosses included RD × SD, RD × ND, SD × SD, SD × ND and ND × ND and in many cases, the reciprocals of these combinations were included. The populations were generated either through single seed decent (SSD) or random selection of seed from each filial generation. The progeny from the crosses was checked for variability in seed size and seed coat patterning and/or darkening to verify that each cross successfully produced hybrids.

Phenotyping

The UVC light protocol developed by Junk-Knievel et al. (2007) was used to darken the seed coats of all seeds used in this experiment. Only the exposed half of the seed coats darkened, while the other half maintained their original colour. In order to facilitate accurate phenotyping two visual comparisons were made: (1) between the exposed and unexposed sides of the same seed and (2) between exposed seeds and seeds that had not been exposed to UVC

Fig. 1 PHD phenotypes (from *left to right top row* CDC Pintium, 1533-15, SDIP-1, KVxUI-1, KVxUI-6, from *left to right bottom row* Wit-rood, PI 608686 and PI 608688). Seeds were exposed to UVC light for 36 h and three exposed and three unexposed sides are shown per genotype

light. Common bean seeds darkened using the UVC light protocol may darken further during storage; therefore, all seeds were scanned on a flatbed scanner within 2 weeks of UVC exposure to record the darkening phenotype for posterity. Six seeds of each parent and each progeny were scanned. A subset of checks, namely CDC Pintium (RD), 1533-15 (SD) and KVxUI-1 (ND), were used for comparison purposes. Each individual was classified as ND, SD, RD or bad seed (seed that was difficult to phenotype due to seed coat inconsistencies).

Statistical analysis

The statistical analyses of observed versus expected segregation ratios for two and three class goodness of fit tests were performed using Proc Freq of SAS version 9.2 (SAS Institute, Cary, NC). Pearson Chi-square *P* values (type 1 error of 0.05) were used to determine the significance of observed versus expected segregation ratios. A test of heterogeneity was computed in situations wherever pooling individual populations were possible. Tests of heterogeneity, H_0 the replicates are homogeneous, *P* values (type 1 error of 0.05) were used to determine if individual populations could be pooled; pooling populations were desirable as it increased the sample size and the degrees of freedom (*df*) of the populations under investigation, therefore, strengthening the statistical test. It was possible to combine all populations within each cross class ($p > 0.05$).

Results

Segregation ratios and Chi-square values of several F_1–F_5 populations from multiple crosses made between RD, SD and ND genotypes suggested a two-gene model for control of PHD in common bean.

Eight F_1 and 289 F_2 individuals from six populations made from crosses between ND × ND or ND × jj tester lines did not segregate for PHD. The seed coats of progeny from these crosses were all ND. Therefore, Wit-rood, KVxUI-1 and KVxUI-6 possessed the same alleles, *jj*, at the *J* locus, for ND.

Two F_1, 49 F_2, 43 F_3 and 104 F_4 individuals from four populations made from crosses between the two SD lines (1533-15 and SDIP-1) were phenotyped. The populations did not segregate for PHD; the seed coats of progeny from these crosses were all SD. Therefore, the genes controlling SD in 1533-15 and SDIP-1 are allelic at the SD locus.

Seven F_1, 364 F_2 and the resulting F_3 families from nine populations from crosses between CDC Pintium (RD) and the ND lines (Wit-rood, KVxUI-1 and KVxUI-6) were phenotyped. The F_1 individuals from these populations had RD seed coats. All F_2 progeny had segregation ratios that were not significantly different from 3RD:1ND (Table 1) suggesting a single gene model. Crosses between CDC Pintium and the *jj* testers PI 608686 and PI 608688 had F_1 seed coats that were too dark to phenotype for PHD. The subset of the F_2 progeny from these F_1s that had seed coat colours that could be phenotyped (pinto-like) all segregated in a 3RD:1ND manner, suggesting CDC Pintium carries the dominant *J* allele. F_3 individuals from one PI 608688 × CDC Pintium cross, however, showed segregation for all PHD phenotypes (RD, SD and ND, Table 2), suggesting a two-gene model in this cross. The discrepancy between the F_2 and F_3 genotypes is believed to be a function of differences in the alleles present at a second locus in the different parental genotypes; one allele being dominant to the other. It is likely that seed from the PI 608688 genotype was heterogeneous, accounting for the segregation of the additional phenotype in the F_3.

Six F_1, 432 F_2 and F_3 families from 14 populations from crosses between 1533-15 (SD) and the ND lines (Wit-rood, KVxUI-1, KVxUI-6) and 1533-15 and the *jj* testers were phenotyped. The F_1 individuals from these populations had RD seed coats with the exception of those from the crosses with the *jj* testers, which were too dark to phenotype. The F_2 progeny from the crosses with Wit-rood, KVxUI-1 and KVxUI-6 had segregation ratios that were not significantly

Table 1 Observed and tested PHD segregations ratios and Chi-squared, *P* values and putative genotypes for the F_2 seed coats from crosses between CDC Pintium (RD) and *jj* genotypes

Female parent	Male parent	Observed RD:SD:ND	Tested RD:ND	χ^2	*P* value
KVxUI-1	Pintium	71:0:21	3:1	1.0582	0.63
Pintium	KVxUI-1	68:0:22	3:1	0.148	0.93
Pintium	KVxUI-1	69:0:18	3:1	0.8621	0.3532
Pintium	KVxUI-1	59:0:20	3:1	0.0042	0.98
PI 608688	Pintium	10:0:2[a]	3:1	0.4444	0.505
Pintium	PI 608686	2:0:2[a]	3:1	1.3333	0.2482
Pooled		279:0:85	3:1	0.5275	0.4677

[a] Only the seeds with pinto-like colouring could be phenotyped in crosses with the two PI lines

Table 2 Observed and tested PHD segregations ratios and Chi-squared, *P* values and putative genotypes for the seed coats of F_3 families of PI 608688 (*jj*) × CDC Pintium (RD)

F2 individual	Observed RD:SD:ND	Tested RD:SD:ND	χ^2	*P* value	Putative F_2 genotype
3254S-1-1	26:6:0	3:1:–	0.6667	0.4142	*JJSdsd*
3254S-1-2	28:4:9	9:3:4	2.9783	0.2256	*JjSdsd*
3254S-1-3	12:1:3	9:3:4	2.5833	0.2748	*JjSdsd*
3254S-1-4	12:5:3	9:3:4	1.2667	0.5308	*JjSdsd*
3254S-1-5	26:0:0	–	–	–	*JJSdSd*
3254S-1-6	29:0:0	–	–	–	*JJSdSd*
3254S-1-7	17:0:6	3:–:1	0.0145	0.9402	*JjSdSd*
3254S-1-8	19:5:9	9:3:4	0.3064	0.858	*JjSdsd*
3254S-1-9	29:0:0	–	–	–	*JJSdSd*
3254S-1-10	25:0:6	3:–:1	0.5269	0.4679	*JjSdSd*
3254S-1-11	0:0:7	–	–	–	*jj–*
3254S-1-12	0:0:21	–	–	–	*jj–*
3254S-1-13	0:0:10	–	–	–	*jj–*
3254S-1-14	17:0:0	–	–	–	*JJSdSd*
3254S-1-15	12:0:5	3:–:1	0.1765	0.6744	*JjSdSd*
3254S-1-16	0:0:3	–	–	–	*jj–*
3254S-1-17	8:0:2	3:–:1	0.1333	0.7150	*JjSdSd*
3254S-1-18	7:0:1	3:–:1	0.6667	0.4142	*JjSdSd*
3254S-1-19	5:0:1	3:–:1	0.2222	0.6374	*JjSdSd*
3254S-1-20	8:0:4	3:–:1	0.4444	0.5050	*JjSdSd*
3254S-1-21	9:0:4	3:–:1	0.2308	0.6310	*JjSdSd*
3254S-1-22	8:1:0	3:1:–	0.9259	0.3359	*JJSdsd*
3254S-1-23	6:0:0	–	–	–	*JJSdSd*
3254S-1-24	9:0:3	3:–:1	0	1.0	*JjSdSd*
3254S-1-25	7:0:0	–	–	–	*JJSdSd*
3254S-1-26	6:0:1	3:–:1	0.4286	0.5127	*JjSdSd*

Table 3 Observed and tested PHD segregations ratios and Chi-squared, *P* values and putative genotypes for the F_2 seed coats from crosses between 1533-15 (SD) and *jj* genotypes

Female parent	Male parent	Observed RD:SD:ND	Tested RD:SD:ND	χ^2	*P* value
1533-15	P1 608686	14:3:11	9:3:4	3.4444	0.1787
1533-15	P1 608686	19:7:6	9:3:4	0.7222	0.6969
1533-15	P1 608686	13:8:9	9:3:4	2.1926	0.3341
1533-15	P1 608688	23:6:13	9:3:4	0.5214	0.5891
1533-15	P1 608688	14:5:14	9:3:4	5.3569	0.0687
P1 608688	1533-15	14:3:11	9:3:4	3.4444	0.1787
P1 608688	1533-15	23:6:14	9:3:4	1.5685	0.4565
1533-15	KVxUI-6	16:4:8	9:3:4	0.2757	0.8007
1533-15	KVxUI-1	16:6:7	9:3:4	0.0728	0.9643
1533-15	KVxUI-1	11:3:2	9:3:4	1.4444	0.4857
PI 608688	1533-15	5:1:2	9:3:4	0.2222	0.8948
Wit-rood	1533-15	17:1:10	9:3:4	4.8254	0.0896
Wit-rood	1533-15	44:9:16	9:3:4	1.9823	0.3712
Wit-rood	1533-15	9:5:1	9:3:4	3.7556	0.2457
Pooled		238:67:124	9:3:4	4.9062	0.0860

different from 9RD:3SD:4ND (Table 3). Segregation in the F_3 families confirmed the F_2 genotypes. The F_2 progeny of crosses between 1533-15 and *jj* tester lines that had seed coats that could be phenotyped had phenotypic ratios of 9RD:3SD:4ND suggesting a two-gene model (Table 3). Segregation of F_3 families confirmed this two-gene model.

Discussion

Results from this inheritance study suggest that at least two unlinked genes control the PHD trait in common bean. One gene, *J*, is responsible for whether a bean will darken and is epistatic to the second gene. The presence of the dominant allele *J* results in a tendency to darken, while the homozygous recessive *jj* condition results in a ND phenotype. The second gene, *Sd*, is responsible for how quickly a seed coat will darken. Individuals carrying the dominant allele, *Sd*, will have a RD phenotype, while those that are homozygous for the recessive allele (*sdsd*) will be SD (Junk-Knievel et al. 2008). Any plant with the genotype *jj*, regardless of the genotype at *Sd* will be ND; variability at the *Sd* locus only occurs when the dominant *J* allele is present. The gene symbol *sd* for slow darkening was accepted by the Genetics Committee of the Bean Improvement Cooperative (BIC) in February 2011.

The segregation ratios for the PHD classes were used to generate putative genotypes for each of the parents used in this inheritance analysis (Table 4). The results suggest that all parental RD and SD genotypes used in the inheritance analysis were homozygous dominant at the *J* locus. Parental RD genotypes were homozygous dominant, and parental SD genotypes were homozygous recessive, at the *sd* locus. Parental ND genotypes were always homozygous recessive at the *j* locus and in all cases but one, parental ND genotypes were homozygous dominant at the *Sd* locus. The seed used from the genotype PI 608688 was likely heterogeneous at the *Sd* locus and homozygous recessive at the *j* locus.

Table 4 Genotypes of parental lines from the inheritance study

Parental line	Market class	PHD phenotype	Putative genotype
CDC Pintium	Pinto	RD	*JJSdSd*
1533-15	Pinto	SD	*JJsdsd*
SDIP	Pinto	SD	*JJsdsd*
Wit-rood	Cranberry-like	ND	*jjSdSd*
KVxUI-1	Pinto	ND	*jjSdSd*
KVxUI-6	Pinto	ND	*jjSdSd*
PI 608686	Black	ND	*jjSdSd*
PI 608688	Dark grey	ND	*jjSdsd* or *jjSdSd*

The parents used in this inheritance study were of great value in identifying the underlying genetic control of PHD in common bean; however, some of the parents were more useful than the others. The *jj* tester genotypes PI 608686 and PI 608688 have black and brownish-purple seed coat colours, respectively, which cannot be evaluated for darkening under UVC light. Therefore, phenotypic evaluation of PHD was more difficult for these genotypes because progeny from crosses with these lines segregated for black or brownish-purple seed coat colours. Crosses between these tester lines and bean lines with light backgrounds resulted in only subsets of F_2 progeny with seed coat colours that could be phenotypically evaluated for PHD; some progeny were too dark to phenotype, while others had white or cream coloured seed coats, which were easily phenotyped.

In contrast, the genotypes CDC Pintium, 1533-15, KVxUI-1, KVxUI-6 and Wit-rood had white or cream background colours prior to exposure to UVC light. These light background colours made these parental lines much more useful in genetic studies of PHD as it helped reduce experimental error in phenotyping filial generations. PI 608686 and PI 608688 were used to confirm that Wit-rood and the KVxUI lines were *jj* and could be useful as *jj* testers in the future. 1533-15 could also be used as a *sdsd* tester and will be deposited to the gene bank at the USDA Plant Introduction Station in Pullman, Washington.

Variability was apparent in the seed coats prior to UVC exposure, suggesting that there is more dynamic seed coat chemistry at work, causing an underlying seed coat background and obscuring phenotypic evaluation from a visible perspective. Seeds from the same plant or from different plants may have varying degrees of biochemical and environmental interactions that alter the resulting background colour. In some cases, this variability can make phenotyping the PHD trait a quality judgment in distinguishing between ND and SD or SD and RD seeds. The potential for not correctly identifying a phenotype can result in distorted segregation ratios, however, the results obtained in this study showed statistically strong positive correlations between observed and expected phenotypic ratios when subjected to goodness of fit tests. Phenotypic observations of the F_3 families also made it possible to confirm the phenotype of F_2 individuals. Clearly this is a trait for which molecular markers would be beneficial. There is a dominant SCAR marker for *J*, $OL4_{525}$, which is linked at 1.2 cM (Bassett et al. 2002) but it is dominant and cannot be used to distinguish between *JJ* and *Jj* individuals. The linkage is also limited to the Mesoamerican gene pool (Bassett et al. 2002), precluding use of the marker in breeding programs for Andean beans. Currently, there are no molecular markers for *sd*.

Conclusion

There are at least two genes controlling the extent of darkening that occurs in dry bean during storage. One, *J*, has been known for decades (Prakken 1974; Bassett 1996) and has been mapped to B10 (McClean et al. 2002). The second, *sd,* is new and does not appear to be linked to *J*.

Knowledge of the parental PHD genotypes identified in this inheritance study may prove to be an important tool for future inheritance studies, biochemical profiling and breeding associated with seed coat genetics in common bean as well as other crops that experience this phenomenon.

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