J. Amer. Soc. Hort. Sci. 139(2):213–218. 2014.

Genetics and Mapping of the *Cl* Gene for Circumlineated Pattern in Common Bean Using AFLP-based Bulk Segregant Analysis and SNP-based Bidirectional Selective Genotyping

Luwbia Aranda
Department of Crop and Agro-Environmental Sciences, University of Puerto Rico, Mayaguez, Puerto Rico 00681-9000

Timothy G. Porch[1]
USDA-ARS, Tropical Agriculture Research Station, 2200 P.A. Campos Ave., Suite 201, Mayaguez, Puerto Rico 00680-5470

Mark J. Bassett
Horticultural Sciences Department, University of Florida, 1301 Fifield Hall, Gainesville, FL 32611

Laura Lara
USDA-ARS, Tropical Agriculture Research Station, 2200 P.A. Campos Ave., Suite 201, Mayaguez, Puerto Rico 00680-5470

Perry B. Cregan
Soybean Genomics and Improvement Lab (SGIL), USDA-ARS, 10300 Baltimore Blvd., Bldg 006, Beltsville Agricultural Research Center - West Beltsville, MD 20705-2350

Additional index words. *Phaseolus vulgaris*, seedcoat color, seedcoat pattern

Abstract. ***Circumlineatus* (*cl*) in common bean (*Phaseolus vulgaris* L.) is identified by a precipitation line in the seedcoat at the boundary of the white and colored zones. *Cl* is recessive and is expressed in partly colored seedcoats (*t*) with restricted patterns such as virgarcus. In this study, amplified fragment length polymorphism (AFLP) and single nucleotide polymorphism (SNP) markers, and the common bean genome sequence were used in combination with bulk segregant analysis and bidirectional selective genotyping to identify the genetic location of *Cl*. Markers were identified that cosegregated with *Cl* using *Cl/Cl* and *cl/cl* F_3 and F_5 progeny bulks from the cross *t z cl G b v* virgarcus BC_3 5-593 × *t* z^{sel} *Cl G b v* sellatus BC_3 5-593. Two bands from an AFLP primer combination, which yielded unambiguous polymorphisms between the bulks, were cloned and sequenced. The two sequences were used to interrogate the common bean whole genome sequence identifying a region also found through cosegregation analysis using bidirectional selective genotyping with SNPs. Thus, the *Cl* gene was localized on Pv09 using cosegregating AFLP and SNP markers, and the physical location was confirmed with the whole genome sequence.**

The seedcoat colors and patterns of common bean cultivars are major attributes enabling consumers to identify various dry bean market classes (Ernest et al., 2005). Some of the important market classes have seedcoat patterns that are partly colored; i.e., part of the seedcoat has a non-white color, whereas the remainder is white. The expression of partly colored patterns is primarily controlled at the *T* locus, and the genotype *t/t* is required for expression of the trait. Additional genes besides *t* are required to express various types of partly colored patterns, viz., *Cl*, *Z*, *Bip*, *J*, and *Fib* (Bassett, 2007). Seed coat colors are controlled by 10 genes: *P*, [*C R*], *Gy*, *Z*, *J*, *G*, *B*, *V*, and *Rk* (Bassett, 2007).

Prakken (1972) was the first author to report a new type of partly colored pattern from the cross 'White J' (genotype *T cl*) × 'Soldaat K' (genotype *t Cl*). This seedcoat trait was named *circumlineatus* because "each of the colour centres and even the smallest dots [were] bordered by a sharp precipitation-like line" (Prakken, 1972). Genotype *t cl* is required for expression of *cl*; hence, neither of Prakken's parents expressed the trait, which first appeared in the F_2. The data of Prakken (1972) supported the hypothesis that with *t/t*, the *cl* trait was controlled by a single additional gene that he gave the symbol *Cl*. There is a weak genetic linkage between the *T* and *Cl* loci of ≈36 cM (Prakken, 1972). Subsequently, gene *T* was located on linkage group B9 by McClean et al. (2002), and B9 was associated with chromosome Pv09 of common bean by Pedrosa-Harand et al. (2008). Thus, mapping of *Cl* has not been completed.

Received for publication 27 Sept. 2013. Accepted for publication 7 Jan. 2014.
This work is dedicated to an excellent scientist and exemplary person, Luwbia Aranda, a co-author in this study and a Master's student at the University of Puerto Rico from Bolivia who died in a car accident at the end of her graduate career.
These sequence data queried using the cloned AFLP products were produced by the U.S. Department of Energy Joint Genome Institute.
We thank Abraham Montes and Adolfo Quiles for assistance with the field and greenhouse experiments and Drs. Linda Beaver and Damaris Santana Morant for assistance with the statistical analyses.
Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.
[1]Corresponding author. E-mail: Timothy.Porch@ars.usda.gov.

Table 1. Levels of expression of the *circumlineatus* gene (*cl*) in three partly colored seedcoat patterns in four seedcoat colors of common bean; i.e., the interaction of *cl* with genes for pattern and color.

Seedcoat types		Seedcoat colors and genotypes			
Pattern	Genotypes	Black[z] *G B V*	Mineral brown[z] *G B v*	Yellow brown *G b v*	Chamois *g b v*
Anasazi	*t cl Z bip*^{ana}	Visible[y]	Visible	Visible	Visible
Virgarcus[z]	*t cl z Bip*	Not visible	Visible	Visible	Visible
Sellatus[z]	*t cl z*^{sel} *Bip*	Not visible	Variable	Visible	Visible

[z]Variable for groove expression, depending on background genotype for seedcoat color.
[y]Refer to *circumlineatus* phenotype in Figure 1. Visible is normal expression that is scorable by eye, whereas variable expression indicates that some seeds expressed *cl*, whereas others on the same plant did not.

Bassett (2004, 2007) summarized the interactions of three partly colored seedcoat patterns with four seedcoat colors in the presence of *cl* (with *t*) (Table 1). Bassett (2007) further clarified the anatomy of the precipitation line in partly colored seeds by describing a physical groove observed in the surface of the seed, an aspect of *cl* expression not reported by Prakken (1972). Expression of *cl* (with *t*) was suppressed by V in virgarcus and sellatus patterns but not with the Anasazi pattern (Table 1). Expression of *cl* (with *t*) was normal (obvious) for all combinations of seedcoat patterns and the non-black colors, except for variable expression (some seeds expressed *cl*, whereas others on the same plant did not) with a sellatus pattern in mineral brown color.

Tagging and mapping have been completed on a number of seedcoat pattern and color genes in common bean, viz., *T*, *Bip*, *C*, *Z*, *J*, *G*, *V*, and *Gy* (Bassett et al., 1999, 2002a, 2002b; Bassett and McClean, 2000; Brady et al., 1998; McClean et al., 2002). In these studies, the polymorphisms were discovered with random amplified polymorphic DNA markers and then converted to more reliable sequence tagged site markers. These markers were then used to determine the location of loci on the common bean molecular genetic map (McClean et al., 2002). With the advent of the common bean genome sequence, SNP markers, and tools such as mutagenesis populations and TILLING (Porch et al., 2009), novel methods are available for identifying genes of interest.

Although the genetics of *Cl* have been studied, the tagging of *Cl* and the determination of its genetic location have not been completed. The goal of this study was to confirm the genetics and identify the genetic map location of *Cl*.

Materials and Methods

Population development and *Cl* genetics. A population segregating for *cl* was created from the cross, *t z cl G b v* virgarcus BC_3 5-593 × *t z*^{sel} *Cl G b v* sellatus BC_3 5-593 at the University of Florida in Gainesville. A selection was made there in the BC_3-F_2 for a virgarcus (*z/z*) partly colored seedcoat type without the circumlineated border. In plot 2-44 in Gainesville, FL, the F_3 generation segregated for *cl* (Fig. 1) but was true breeding for virgarcus. A single seed of each F_3 was planted under greenhouse conditions at the U.S. Department of Agriculture, Agricultural Research Service (USDA-ARS) in Mayaguez, Puerto Rico, on 23 Feb. 2004 and all harvested seed of each plant were scored for *Cl*. Progeny testing was completed in the F_4 generation. A total of 49 F_4 progenies were planted on 3 Nov. 2004 in the field in Isabela, Puerto Rico, with an average of 10.3 plants per F_4 family for a grand total of 504 plants. Evaluation of *Cl* was performed on all seed of each plant harvested.

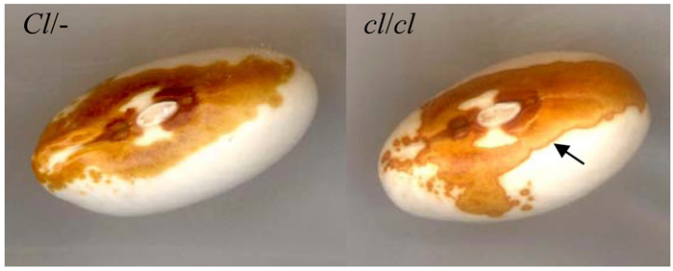

Fig. 1. *Circumlineatus* phenotype with precipitation line indicated by the arrow in representative lines from the *t z cl G b v* virgarcus BC_3 5-593 × *t z*^{sel} *Cl G b v* sellatus BC_3 5-593 population of common bean.

AFLP and bulk segregant analysis. DNA was extracted from leaf tissue of each of the F_3 lines using DNeasy extraction kits (Qiagen, Valencia, CA) at USDA-ARS in Mayaguez, Puerto Rico. AFLP analysis was performed using a AFLP pre-amplification kit (LI-COR, Lincoln, NE) and selective amplification was performed according to Vos et al. (1995) using labeled *Eco* RI primers (LI-COR) and non-labeled *Mse* I primers (Illumina, San Diego, CA). The AFLP products were separated on a sequencer (4300; LI-COR). Bulk segregant analysis (BSA) (Michelmore et al., 1991) was performed on bulks of four *cl/cl* or four *Cl/Cl* genotypes as determined by progeny testing. Two bulks of each genotype were prepared. *Eco* RI primers, one labeled with IR700 and the other with IR800, were amplified with a non-labeled *Mse* I primer. Both sets of primers contained three selective nucleotides. BSA was performed using 256 primer-pair combinations, eight *Eco* RI and 32 *Mse* I primers. Those primers revealing polymorphisms were then tested on the whole population. Cosegregating markers were identified and the genetic distance between the AFLP marker and the locus was calculated on the population derived from the 2-44 $F_{2:3}$ family using the Haldane mapping function.

Polymorphic AFLP band analysis and mapping. The AFLP analysis and electrophoresis were performed as described previously using labeled primers for the selected AFLP primers at the USDA-ARS in Mayaguez, Puerto Rico. The AFLP bands associated with the *cl* trait were located and isolated from polyacrylamide gels by interrupting electrophoresis of the sequencer (4300; LI-COR) as the bands entered the real-time gel display window. The bands were excised from their estimated position in the gels using a razor blade and the AFLP bands were extracted and then reamplified using the same AFLP protocol and primers, as described previously, and confirmed by electrophoresis of the reamplified bands

Table 2. Segregation for *circumlineatus* in the F_3 and F_4 generations of progeny from a single F2 plant selection of common bean from the cross *t z cl G b v* virgarcus BC_3 5-593 × *t z*^{sel} *Cl G b v* sellatus BC_3 5-593.[z]

F_3 segregation			F_4 segregation				
Plants (no.)[y]	*Cl* phenotype	Genetic hypothesis	Progenies (no.)[x]	*Cl*/-	*cl*/*cl*	χ^2 3:1	*P*
38	Not circumlineated	*Cl*/-	10	All			
			28	212	73	0.06	0.81
11	Circumlineated	*cl*/*cl*	11		All		

[z]The F2 selection had genotype *t z Cl*/- virgarcus.
[y]For the F_3 segregation data of 38:11, the $\chi^2_{3:1} = 0.017$, $P = 0.90$.
[x]For the F_3 segregation data of 10:28:11, the $\chi^2_{1:2:1} = 1.04$, $P = 0.59$.

alongside the original AFLP products. The bands were each purified from the polymerase chain reaction (PCR) products using a PCR purification kit (Qiagen) and then ligated into the pCR 2.1 TOPO cloning vector following the manufacturer's instructions (Invitrogen, Carlsbad, CA). Miniprepped plasmid DNA was tested for insert size, after blue/white selection, using plasmid specific M13 primers as well as a non-labeled version of the original AFLP primers. Those clones with PCR products matching the expected fragment sizes were sequenced. The two sequences were trimmed of vector sequence and blasted against the common bean whole genome sequence (genotype G19833) using PhaseolusGenes (Gepts and Dawei, 2013) and a cutoff e-value of 0.0001 was used for the determination of significant hits.

SNP analysis and bidirectional selective genotyping. DNA was extracted from F_5 plants, selected from different true breeding 49 F_4 progenies (above), using a DNeasy extraction kit (Qiagen) at USDA-ARS in Mayaguez, Puerto Rico. The DNAs were sent to the USDA-ARS Soybean Genomics and Improvement Laboratory (SGIL), in Beltsville, MD, for SNP analysis. In total, 10,914 SNPs were evaluated on 12 homozygous *Cl/Cl* or *cl/cl* F_5 lines. The SNPs were developed at USDA-ARS-SGIL through the BeanCAP project (manuscripts in preparation). The 12 lines were composed of six *cl/cl* lines and six *Cl/Cl* lines. The SNP data were analyzed at the USDA-ARS in Mayaguez, Puerto Rico, by grouping the lines by phenotype and then by visually evaluating them in an Excel spreadsheet (Microsoft, Redmond, WA) for SNPs with consistent polymorphism between the groups but homogenous within each group. Thus, SNPs were identified in which all of the six *cl/cl* lines carried one allele and all of the *Cl/Cl* lines carried the alternative allele. These were identified as putative markers for *cl*. The 'Stampede' × 'Redhawk' mapping population (manuscript in preparation) was used for anchoring the sequence scaffolds and for estimating genetic distance (cM).

A statistical test for marker-trait association was conducted using a binomial distribution to test the probability of cosegregation between the *cl* phenotype and the SNP marker genotype for the 12 genotypes selected.

Results

***Cl* genetics.** In the virgarcus pattern with yellow brown seedcoat color used in this study, the *cl* phenotype is obvious. We found a 3:1 ratio for *Cl*/- and *cl*/*cl* (Table 2) in the F_3 generation and a 1:2:1 ratio for *Cl/Cl*, *Cl*/*cl*, and *cl*/*cl* (Table 2) through progeny testing in the F_4 generation, thus confirming a single recessive gene. Scoring of the *cl* phenotype was completed by three people and the results were identical in all cases.

AFLP survey using BSA. In total, eight *Eco* RI and 32 *Mse* I primer combinations were surveyed for a total of 256 AFLP primer combinations. One primer combination was found to yield unambiguous polymorphisms between the bulks of *cl/cl* and *Cl/Cl* genotypes: *Eco* RI GACTGCGTACCAATTC**ACC** + *Mse* I GATGAGTCCTGAGTAA**CTC.** The *E*-**ACC**, *M*-**CTC** primer pair amplified two dominant markers, one linked to each allele from the contrasting bulks (Fig. 2). For the *Cl* allele, a 91-bp band was identified to be in coupling phase and was determined to be 12.5 cM from the locus. The 120-bp band linked to the *cl* allele was also in coupling phase and was determined to be 7.5 cM from the locus. Both calculations were made using the Haldane mapping function.

Polymorphic AFLP band evaluation and sequence analysis. The polymorphic 91- and 120-bp bands were cloned and sequenced. Use of the AFLP primers for mapping of *Cl* did not reveal any polymorphisms for the two identified markers in the available mapping populations. Subsequently, with the availability of the SNPs and the common bean genome sequence, the two markers were blasted on the genome sequence using the Phaseolusgenes database (Gepts and Dawei, 2013). Significant hits (cutoff e-value of 0.0001), based on sequence similarity, were found for each original AFLP band. There was one significant hit for the 91-bp band (*Cl* allele) that was localized on Pv09 in the region 30345818 to 30345883 bp (e value = 7.31795 e^{-30}). For the 120-bp band (*cl* allele), significant hits were localized on Pv09 in the region 30345841 to 30345883 bp (e value = 1.51997 e^{-13}) and 30345818 to 30345850 bp (5.78527 e^{-10}) and on Pv04 in the region 27696553 to 27696606 bp (e value = 8.70082 e^{-06}).

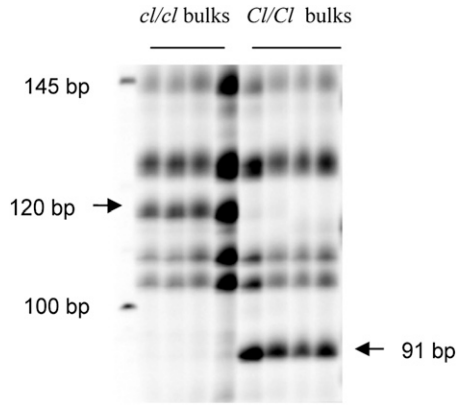

Fig. 2. Amplified fragment length polymorphism using the *E*-**ACC** and *M*-**CTC** primers on *cl/cl* or *Cl/Cl* bulks of common bean run on a polyacrylamide gel using a gel-based sequencer (4300; LI-COR, Lincoln, NE) and showing the polymorphic 91- and 120-bp bands and a 50 to 700-bp ladder in the first lane.

Table 3. Single nucleotide polymorphism (SNP) markers identified as cosegregating with the *circumlineated* locus through bidirectional selective genotyping in homozygous F_5 *cl/cl* and *Cl/Cl* lines of common bean from the cross *t z cl G b v* virgarcus BC3 5-593 × *t* z^{sel} *Cl G b v* sellatus BC3 5-593.

NCBI submitted SNP ID no.[z]	Chromosome no.	Position (cM)	Lines											
			6248-6	6248-9	6250-3	6250-10	6252-6	6328-1	6251-2	6251-4	6253-3	6253-6	6325-5	6325-6
			cl/cl F_5								*Cl/Cl* F_5			
ss715646359	7	26.2	BB[y]	BB	BB	BB	BB	BB	AA	AA	AA	AB	AA	AA
ss715646360	7	26.2	BB	BB	BB	BB	BB	BB	AA	AA	AA	AB	AA	AA
ss715646365	9	52.7	BB	BB	BB	BB	BB	BB	AA	AA	AA	AB	AA	AA
ss715646364	9	53.3	BB	BB	BB	BB	BB	BB	AA	AA	AA	AB	AA	AA
ss715650033	9	54.6	AA	AA	AA	AA	AA	AA	BB	BB	BB	AB	BB	BB
ss715646555	9	54.6	AA	AA	AA	AA	AA	AA	BB	BB	BB	AB	BB	BB
ss715646550	9	55.2	BB	BB	BB	BB	BB	BB	AA	AA	AA	AB	AA	AA
ss715646551	9	55.2	BB	BB	BB	BB	BB	BB	AA	AA	AA	AB	AA	AA
ss715646553	9	55.2	AA	AA	AA	AA	AA	AA	BB	BB	BB	AB	BB	BB
ss715646548	9	55.7	BB	BB	BB	BB	BB	BB	AA	AA	AA	AB	AA	AA
ss715646549	9	55.7	BB	BB	BB	BB	BB	BB	AA	AA	AA	AB	AA	AA
ss715646557	9	56.7	BB	BB	BB	BB	BB	BB	AA	AA	AA	AB	AA	AA
ss715648154	9	57.2	AA	AA	AA	AA	AA	AA	BB	BB	BB	BB	BB	BB
ss715648155	9	57.2	BB	BB	BB	BB	BB	BB	AA	AA	AA	AB	AA	AA
ss715648156	9	57.2	BB	BB	BB	BB	BB	BB	AA	AA	AA	AB	AA	AA
ss715648153	9	57.4	BB	BB	BB	BB	BB	BB	AA	AA	AA	AB	AA	AA
ss715639307	9	57.8	BB	BB	BB	BB	BB	BB	AA	AA	AA	AB	AA	AA
ss715646367	9	58.8	AA	AA	AA	AA	AA	AA	BB	BB	BB	AB	BB	BB
ss715639304	9	59.0	AA	AA	AA	AA	AA	AA	BB	BB	BB	AB	BB	BB
ss715639305	9	59.0	AA	AA	AA	AA	AA	AA	BB	BB	BB	AB	BB	BB
ss715639288	9	61.2	BB	BB	BB	BB	BB	BB	AA	AA	AA	AB	AA	AA
ss715639289	9	61.2	AA	AA	AA	AA	AA	AA	BB	BB	BB	AB	BB	BB
ss715639290	9	61.2	AA	AA	AA	AA	AA	AA	BB	BB	BB	AB	BB	BB
ss715639291	9	61.2	BB	BB	BB	BB	BB	BB	AA	AA	AA	AB	AA	AA
ss715646278	9	61.9	AA	AA	AA	AA	AA	AA	BB	BB	BB	AB	BB	BB
ss715646547	Unplaced		BB	BB	BB	BB	BB	BB	AA	AA	AA	AB	AA	AA
ss715646552	Unplaced		BB	BB	BB	BB	BB	BB	AA	AA	AA	AB	AA	AA

[z]National Center for Biotechnology Information (Bethesda, MD) SNP identification number.
[y]A and B refer to the two parental SNP alleles at each locus.
SNP = single nucleotide polymorphism.

Bidirectional selective genotyping with SNP markers. Of the 10,914 SNPs, 10,165 were scorable for the 12 genotypes evaluated. Analysis of the 12 lines in two groups based on *cl* and wild-type phenotype revealed 27 SNPs that showed consistent polymorphisms between the two groups, where all six genotypes in each group had the same genotype for the particular SNP (Table 3). The exception was genotype 6253-6, which showed a heterozygote genotype for all but one SNP and thus was largely uninformative. The polymorphic SNPs were located in two genomic regions: two were located on Pv07 at 26.2 cM and 23 were located on Pv09 between 52.7 and 61.9 cM, whereas two were not linked to any chromosome.

To determine if there was cosegregation of the SNP genotype and *cl* phenotype, the probability of the 27 SNPs cosegregating with the *cl* phenotype in the 12 lines evaluated, when independent assortment is assumed, was calculated. Under a null hypothesis of independent assortment of the *cl* phenotype and the homozygous SNP loci, the probability of the *cl* phenotype and a single SNP genotype cosegregating in all 12 lines evaluated is $\frac{2}{2^{12}} = \frac{1}{2048}$. To test ($\alpha < 0.01$) the alternative hypothesis of cosegregation of the *cl* phenotype and 27 or more SNPs in all 12 lines evaluated, X is defined as the number of SNP markers cosegregating with the *cl* phenotype. X is assumed to be binomially distributed with parameters n and P [$X \sim B(n, p)$], where n is the number of scorable SNPs (= 10,165) and P is the probability of *cl* phenotype and SNP genotype cosegregation in all 12 lines evaluated under the assumption of independent assortment $\left(= \frac{1}{2048}\right)$. In a binomial distribution of this type, the average number of cosegregating SNP markers would be 4.96 (np). The probability of 27 or more SNPs (out of 10,165) cosegregating with the *cl* phenotype in all 12 lines when independent assortment is assumed is 4.65×10^{-12}. Thus, we reject the null hypothesis and conclude that there is cosegregation between the *cl* phenotype and the 27 SNP genotypes in the 12 lines evaluated.

Discussion and Conclusions

This study has confirmed the genetics and identified the genetic map location of *Cl* in common bean. The linkage reported by Prakken (1972) between the *T* and *Cl* loci of ≈36 cM is a rate of almost 40% crossing over between these two loci. This weak level of linkage could equally indicate that *T* and *Cl* reside on the same or on different chromosomes. Therefore, molecular markers, more tightly linked to the locus, were needed to definitively map *Cl* to a chromosome and to more precisely locate its genetic position within that chromosome.

The *cl* gene was tagged using AFLP primers *E*-**ACC** and *M*-**CTC**. This mapping effort began before the development of SNP markers; thus, the initial tagging work was completed with AFLPs. Subsequent development of SNP markers and the genome sequence of common bean allowed for the mapping effort to be completed. Because of the close genetic similarity between the two parents of the population, *t z cl G b v* virgarcus BC_3 5-593 and *t* z^{sel} *Cl G b v* sellatus BC_3 5-593, a highly polymorphic marker system was needed to uncover polymorphisms. A single AFLP primer pair out of 256 was identified that showed polymorphisms between the bulks of *Cl/Cl* and *cl/cl* genotypes. Subsequently, a large set of 10,914 SNPs was evaluated on two phenotypically contrasting sets of genotypes within the population. These results indicate both the use of AFLP and SNP markers for uncovering polymorphisms in closely related populations and the importance of marker systems capable of detecting differences where low levels exist. The abundant and mapped SNP markers have added a powerful tool for genetic analysis in common bean, whereas the portable indel markers that are currently being developed will be an efficient and effective portable marker system for use in breeding programs.

To identify a map position for *cl*, two cosegregating and subsequently sequenced AFLP bands were found to localize on Pv09 for the 91-bp AFLP band and on Pv09 and Pv04 for the 120-bp AFLP band using the common bean genome sequence. In both cases, the most significant sequence similarity, based on e-value, was on Pv09. In addition, the physical location on Pv09 was within the same region, 30345818 to 30345883 bp, for both AFLP bands. The fact that both AFLP bands localized in the same region on Pv09 based on a high level of sequence similarity is ample evidence for the localization of *cl*. However, an additional region homologous with the 120-bp band on Pv04 suggests possible duplication.

Bidirectional selective genotyping of homozygous *Cl/Cl* or *cl/cl* lines using polymorphic SNP markers that cosegregated with the *Cl* locus identified 23 SNPs on Pv09 between 52.7 and 61.9 cM, whereas two SNPs were located on Pv07 at 26.2 cM, and two were unplaced on the genetic map. The statistical test, using a binomial distribution, showed the *cl* phenotype and SNP genotype cosegregated. Note that when there is random assortment between phenotype and genotype, the expected number of cosegregating markers, or false-positives, is 4.96 out of 10,165, the mean of the binomial distribution calculated. Of the 27 cosegregating bands observed, 23 were located on Pv09 and four were located on Pv07 or unlinked. Thus, these four SNPs, not located on Pv09, could represent the expected 4.96 false-positives.

Two independent approaches were implemented in this study for determination of the genetic map position of *Cl*. The Pv09 chromosome had the highest sequence similarity to the two cloned, cosegregating AFLP bands, and Pv09 also showed cosegregation of the phenotype with the SNP marker genotype for 23 of 27 markers. Therefore, both approaches, BSA with AFLP followed by physical mapping using the AFLP marker sequence, and bidirectional selective genotyping with a large set of SNP markers using the genetic map, identified a common region of the common bean genome. The genetic location of *cl* on Pv09 has thus been shown.

Literature Cited

Bassett, M.J. 2004. New observations on the expression of the gene *cl* for circumlineated patterns of partly colored seed coats—A gene carried by recurrent parent line 5-593. Annu. Rep. Bean Improv. Coop. 47:179–180.

Bassett, M.J. 2007. Genetics of seed coat color and pattern in common bean. Plant Breed. Rev. 28:239–315.

Bassett, M.J., R. Lee, C. Otto, and P.E. McClean. 2002a. Classical and molecular genetic studies of the strong greenish yellow seedcoat color in 'Wagenaar' and 'Enola' common bean. J. Amer. Soc. Hort. Sci. 127:50–55.

Bassett, M.J., R. Lee, T. Symanietz, and P.E. McClean. 2002b. Inheritance of reverse margo seedcoat pattern and allelism between the genes *J* and *L* for partly colored seedcoat pattern in common bean. J. Amer. Soc. Hort. Sci. 127:56–61.

Bassett, M.J. and P.E. McClean. 2000. A brief review of the genetics of partly colored seed coats in common bean. Annu. Rep. Bean Improv. Coop. 43:99–101.

Bassett, M.J., C. Shearon, and P.E. McClean. 1999. Allelism found between two common bean genes, hilum ring color (*D*) and partly colored seedcoat pattern (Z), formerly assumed to be independent. J. Amer. Soc. Hort. Sci. 124:649–653.

Brady, L., M.J. Bassett, and P.E. McClean. 1998. Molecular markers associated with *T* and *Z*, two genes controlling partly colored seed coat patterns in common bean. Crop Sci. 38:1073–1075.

Ernest, E.G., J.D. Kelly, and M.J. Bassett. 2005. A spontaneous mutation at a seedcoat pattern locus in the dark red kidney bean 'Red Hawk', which changes seed from self-colored to the partially colored virgarcus pattern. HortScience 40:57–59.

Gepts, P. and L. Dawei. 2013. PhaseolusGenes database. 4 Nov. 2013. <http://phaseolusgenes.bioinformatics.ucdavis.edu/>.

McClean, P.E., R.K. Lee, C. Otto, P. Gepts, and M.J. Bassett. 2002. Molecular and phenotypic mapping of genes controlling seed coat pattern and color in common bean (*Phaseolus vulgaris* L.). J. Hered. 93:148–152.

Michelmore, R.W., I. Paran, and R.V. Kesseli. 1991. Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. Proc. Natl. Acad. Sci. USA 88:9828–9832.

Pedrosa-Harand, A., T. Porch, and P. Gepts. 2008. Standard nomenclature for common bean chromosomes and linkage groups. Annu. Rpt. Bean Improv. Coop. 51:106–107.

Porch, T.G., M.W. Blair, P. Lariguet, C. Galeano, C.E. Pankhurst, and W.J. Broughton. 2009. Generation of a mutant population for TILLING common bean genotype BAT 93. J. Amer. Soc. Hort. Sci. 134:348–355.

Prakken, R. 1972. Inheritance of colours in *Phaseolus vulgaris* L. III. On genes for red seedcoat colour and a general synthesis. Mededelingen Landbouwhogeschool Wageningen 72-29: 1–82.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper, and M. Zabeau. 1995. AFLP: A new technique for DNA fingerprinting. Nucleic Acids Res. 23:4407–4414.