Inheritance of growth habit detected by genetic linkage analysis using microsatellites in the common bean (*Phaseolus vulgaris* L.)

Tatiana de Campos · Paula Rodrigues Oblessuc · Danilo Augusto Sforça ·
Juliana Morini Kupper Cardoso · Renata Moro Baroni · Adna Cristina Barbosa de Sousa ·
Sérgio Augusto Morais Carbonell · Alisson Fernando Chioratto ·
Antonio Augusto Franco Garcia · Luciana Benchimol Rubiano ·
Anete Pereira de Souza

Abstract The genetic linkage map for the common bean (*Phaseolus vulgaris* L.) is a valuable tool for breeding programs. Breeders provide new cultivars that meet the requirements of farmers and consumers, such as seed color, seed size, maturity, and growth habit. A genetic study was conducted to examine the genetics behind certain qualitative traits. Growth habit is usually described as a recessive trait inherited by a single gene, and there is no consensus about the position of the locus. The aim of this study was to develop a new genetic linkage map using genic and genomic microsatellite markers and three morphological traits: growth habit, flower color, and pod tip shape. A mapping population consisting of 380 recombinant F10 lines was generated from IAC-UNA × CAL143. A total of 871 microsatellites were screened for polymorphisms among the parents, and a linkage map was obtained with 198 mapped microsatellites. The total map length was 1865.9 cM, and the average distance between markers was 9.4 cM. Flower color and pod tip shape were mapped and segregated at Mendelian ratios, as expected. The segregation ratio and linkage data analyses indicated that the determinacy growth habit was inherited as two independent and dominant genes, and a genetic model is proposed for this trait.

T. de Campos · D. A. Sforça · A. C. B. de Sousa ·
A. P. de Souza (✉)
Centro de Biologia Molecular e Engenharia Genética
(CBMEG), Universidade Estadual de Campinas
(UNICAMP), Cidade Universitária Zeferino Vaz,
CP 6010, Campinas, SP CEP 13083-970, Brazil
e-mail: anete@unicamp.br

P. R. Oblessuc · J. M. K. Cardoso · R. M. Baroni ·
S. A. M. Carbonell · A. F. Chioratto · L. B. Rubiano
Centro de Pesquisa e Desenvolvimento de Recursos
Genéticos Vegetais, Fazenda Santa Elisa, IAC,
Campinas, SP, Brazil

A. A. F. Garcia
Departamento de Genética, Escola Superior de
Agricultura Luiz de Queiroz (ESALQ), Universidade de
São Paulo (USP), CP 83, Piracicaba,
SP CEP 13400-970, Brazil

A. P. de Souza
Departamento de Biologia Vegetal—Instituto de Biologia
(IB), UNICAMP, Cidade Universitária Zeferino Vaz,
CP 6010, Campinas, SP CEP 13083-970, Brazil

Abbreviations

AFLP	Amplified fragment length polymorphism
CTAB	Cetyltrimethylammonium bromide
CNL	Coiled-coil nucleotide-binding site leucine-rich repeat
EST	Expressed sequence tag
IAC	Agronomic Institute of Campinas
PCR	Polymerase chain reaction

RAPD Random amplified polymorphic DNA
RIL   Recombinant inbred line
RFLP Restriction fragment length polymorphism
SSR   Single sequence repeat
UC   IAC-UNA × CAL143

Introduction

The common bean (*Phaseolus vulgaris* L.) is a major staple food crop in Africa and Latin America, and is valued as one of the primary protein sources in the diet of low-income populations in developing countries. Moreover, it is a legume that is favorable to genetic studies because it has certain model plant characteristics, such as a small genome (637–675 Mb per haploid genome; Arumuganathan and Earle 1991; Guo et al. 2007), diploidy ($2n = 2x = 22$), and a highly self-pollinated breeding system.

The cultivated bean is morphologically diverse and has variations in many traits, such as growth habit, phenology, pod pigmentation, and seed pigmentation (Singh et al. 1991). Determinacy is an important agronomic trait in the common bean. Indeterminate growth habit was first reported to be controlled by a single dominant gene by Lamprecht (1935). It is considered a simple trait, and there is no consensus about its genomic location or the number of loci involved (McClean et al. 2002).

Genetic maps provide useful information for many studies, such as the localization of genomic regions that control phenotypic traits, synteny studies, and gene cloning. Moreover, they can be used to study the genetic architecture of quantitative traits. Morphological traits can be used as markers when qualitative variation and discrete phenotypic segregation classes are present.

Microsatellites or simple sequence repeats (SSRs) (Tautz and Renz 1984) are one of the most popular markers used to study polymorphisms between DNA sequences. Variations in these repetitive sequences within loci are detected using polymerase chain reaction (PCR). Microsatellites present a high level of polymorphism, co-dominance, multi-allelism, and a Mendelian pattern. They have been used in genetic analyses in mammals (Moore et al. 1998), insects (Harper et al. 2003), birds (Primmer et al. 1997), fishes (Rico et al. 1993) and plants (Condit and Hubbell 1991; Yang et al. 1994; Yu et al. 2000).

As a result of research efforts from many groups aiming to increase knowledge of the common bean, microsatellites have recently been described (Yu et al. 1999; Gaitán-Solís et al. 2002; Métais et al. 2002; Blair et al. 2003, 2008; Buso et al. 2006; Hanai et al. 2007; Benchimol et al. 2007; Campos et al. 2007; Cardoso et al. 2008). When microsatellites are derived from ESTs (expressed sequence tags), they can be associated with genes of known function and can be used as functional markers, tagging genes of interest in a more efficient manner (Hackuf and Wehling 2002; Oliveira et al. 2009).

Genetic mapping of the common bean began with the use of morphological markers and isozymes (Basset 1991). The maps developed by Vallejos et al. (1992), Gepts et al. (1993), Nodari et al. (1993) and Adam-Blondom et al. (1994) were first created using restriction fragment length polymorphism (RFLP)-based markers. A core map was established in the common bean that was based on a recombinant inbred population BAT 93 × Jalo EEP558 (Freyre et al. 1998). Random amplified polymorphic DNA (RAPD) (Adam-Blondom et al. 1994; Bai et al. 1997; Rodríguez-Suárez et al. 2006) and amplified fragment length polymorphism (AFLP) (Tar'an et al. 2002) were also used to construct linkage maps for the crop.

Microsatellites have been used in diversity analyses (Blair et al. 2006a, 2009; Zhang et al. 2008) and genetic maps (Yu et al. 2000; Blair et al. 2003, 2006b; Grisi et al. 2007), but some linkage groups that exist have low saturation and gaps. A central effort of the research on the common bean genome is to integrate physical and genetic maps. Schlueter et al. (2008) recently published a draft physical map for the common bean genome. Our primary objective was to characterize new microsatellites and develop a linkage map based on genic and genomic microsatellite markers. Another major objective was to map phenotypic markers and to study the genetic control of the growth habit trait.

Materials and methods

Mapping population

A recombinant inbred line (RIL) population was obtained from a cross between the CAL143 and IAC-UNA inbred lines. CAL143 is an Andean line with

cream coat color, red stringed seeds, and calima type. IAC-UNA is a black seeded variety developed by the Agronomic Institute of Campinas (IAC) and is from the Mesoamerican gene pool. The two parents are divergent for many phenotypic traits and have contrasting responses to many common bean pathogens. This mapping population was advanced by inbreeding to the F10 generation and 380 lines were obtained.

Microsatellite amplification and analysis

Total genomic DNA for all recombinant inbred lines was isolated from bulked young leaves of ten plants per line, using the CTAB extraction method as described in Hoisington et al. (1994).

A total of 871 microsatellites were tested for polymorphisms among the CAL143 and IAC-UNA lines. Of these, 567 have been published previously. There were 44 markers from the PV series (Yu et al. 2000; Caixeta et al. 2005); 46 markers from the BM, AG, and GAT series (Gaitán-Solís et al. 2002); 2 markers from the PVbng series (Murray et al. 2002); 49 markers from the Bmd series (Blair et al. 2003); 15 markers from the M series (Yaish and Pérez de la Vega 2003); 18 markers from Guerra-Sanz (2004); 71 markers from the PVBR series (Buso et al. 2006; Grisi et al. 2007); 80 markers from the PvM and FJ series (Hanai et al. 2007); 222 markers from SSR-IAC series (Benchimol et al. 2007; Cardoso et al. 2008); and 20 markers from the FJ series (Campos et al. 2007). The gene-based microsatellites used were from the PvM series that was developed from ESTs, Bmd, and PV from Yu et al. (2000) and the markers from Guerra-Sanz (2004) that were developed from GenBank gene searches. The remaining 304 tested markers were developed in this work according to Campos et al. (2007). The polymorphic markers developed are further described in Table 1.

Microsatellite fragments were amplified using PCR, with each reaction containing 40 ng of template DNA, 0.8 μM each of forward and reverse primer, 150 μM of each dNTP, 1.5 mM MgCl$_2$, 10 mM Tris–HCl, 50 mM KCl, and 0.5 U Taq DNA polymerase. Reactions were performed using the following cycling conditions: 1 min at 94°C; followed by 30 cycles of 94°C for 1 min, the specific annealing temperature for 1 min and 72°C for 1 min; and a final step of 72°C for 5 min. Some microsatellites were

mapped using cycles of touchdown (TD) PCR (Table 1), as described by Hanai et al. (2007). PCR products were separated using electrophoresis in denaturing polyacrylamide gels (6% w/v) and visualized using silver staining (Creste et al. 2001). The molecular size of the fragments was estimated using a 10-bp ladder (Invitrogen). Fluorescent primers were used for 39 loci as described by Schuelke (2000), and these loci were genotyped using an ABI 3730 automatic sequencer.

Morphological markers

Three morphological markers were evaluated in the mapping population: flower color, growth habit, and pod tip shape. Flower color is controlled by the *V* locus (Lamprecht 1939) and segregates as purple (IAC-UNA) and pink (CAL143) in the mapping population. Growth habit segregates as determinate type I, present in the Andean parent, and indeterminate type III, present in the Mesoamerican parent. Determinacy has been described as a qualitative trait controlled by the *fin* locus (Lamprecht 1935; Rudorf 1958). The pod tip shape can be curved (*Ct*; Al-Mukhtar and Coyne 1981), present in IAC-UNA, or straight (*ct*), present in CAL143.

Segregation and linkage analyses

The chi-square (χ^2) test for 1:1 segregation ratios was performed for all polymorphic markers. The expected segregation ratios were tested based on *P*-values after performing Bonferroni corrections (Lynch and Walsh 1998). The linkage map was constructed using the software MAPMAKER 3.0b (Lander et al. 1987). The threshold for considering markers to be linked was a LOD score of 3.0 and a maximum genetic distance of 37.5 cM using the Kosambi (1944) map function.

To order the markers within groups, markers with known positions in other published maps were anchored in the corresponding group. Markers whose position could not be anchored with high precision were verified based on their LOD score.

Ordering analyses of markers within groups were performed following the guidelines suggested by Mollinari et al. (2009). First, a subset of nine informative markers was chosen based on missing data and segregation distortion. For each group, all possible

Table 1 Description of the polymorphic microsatellites for CAL143 and IAC-UNA that were developed, including primer sequences, repeat motifs, allele sizes and annealing temperatures (T_a)

	Locus/GenBank accession no.	Primer sequence (5′–3′)	Motif	Size (bp)	T_a (°C)
1	SSR-IAC223/FJ529449	GTGGCACCTGGAATAAGACAAC ACAAATGCTCACTCACCAAATC	(AG)15	214–220	TD*
2	SSR-IAC224/FJ529450	ATTATGGATTTAGGGTAGGTGA GCTTGACAATTGGAAGTGG	(TC)13	116–120	TD
3	SSR-IAC226/FJ529452	TTTTTCTCGTTTTACTTTTATCTG TACTGATTTATTTCTTCCACTTC	(TG)8	150–155	60
4	SSR-IAC227/FJ529453	GGATCTGTGCCTTCTCTGTG TTCCATATCCCCAAAACTT	(AG)18	135–148	45
5	SSR-IAC228/FJ529454	CAAATTTTTGCTTGAACTGAT TTCTCTCCTCAAATGTAACTAAC	(CA)6	203	60
6	SSR-IAC229/FJ529455	CCTAAGGATGAACCACTCTAATA ACCCTTGCATGTGTTGTTT	(CA)7	198	60
7	SSR-IAC230/FJ529456	GGATTCGGCATTTGATAGAC AGAGAAGAAATTAGGGAGAACAG	(CT)6	188–190	60
8	SSR-IAC231/FJ529457	TGCATTTTGAAAGGACAGAT ACCAGGCATAGGTAGGACA	(TA)7 A (AG)16	246	60
9	SSR-IAC232/FJ529458	GGTTTGCATTCTTCATTTATTT ACCCATTCCCAGACTTCC	(TG)6(TA)5	241–245	60
10	SSR-IAC233/FJ529459	GGCAACTCTAAGGCAATCC CGAAGAATGTCAGAGAAATAAAC	(GT)9(TA)7	271	60
11	SSR-IAC234/FJ529460	GACCGTTAAAATGGAATCAGTT TGCCTATGTTTATGTGCTTGTT	(TC)5	258	60
12	SSR-IAC235/FJ529461	TTCAGCATGCATATTCAAGTGT GGCTCCCAGGCATAGTTCT	(TA)10	253	60
13	SSR-IAC236/FJ529462	TCTTCCTCTTCCTCTTCCTCTA TCTGATGTGGCAACGATAAAAT	(CT)12	170	60
14	SSR-IAC237/FJ529463	TGAAAGGCCACAACGACAAGT GCACCCAATCTCCCACAAA	(GT)2 AT (TG)5	155–160	60
15	SSR-IAC238/FJ529464	GAGATAGATCATAGACCCCAA CTTTCAATTTTAACTCATCCTC	(AG)18	233	60
16	SSR-IAC239/FJ529465	AAAGAAAAAGTGGGGAAAGA GCTCATGCAAAAATAGACTCA	(AG)15	247	60
17	SSR-IAC240/FJ529466	TGAATGTATGAGATTTCGTAGTTA ATTTTATTAGTGGCATTGTCCT	(CT)10	213–228	60
18	SSR-IAC241/FJ529467	AATGTGGCTATGATGGAAGAGG AAGAGAATGGGGGAAAAGGAG	(TC)8	151	60
19	SSR-IAC242/FJ529468	ATGAGATGCGAGGATTTTTTAT GGATGGGTAAGAAGGCTGAA	(CA)5	242–280	60
20	SSR-IAC243/FJ529469	GCTGGTGAGGCTTGTTGA AATTGACCTTGGATCTTCTTAG	(AT)2 (GT)3	145	60
21	SSR-IAC244/FJ529470	CGCGTTGTATTGCAGTAAAGAA AACTCCGTGGAACCCTGTGAT	(TC)9	192	60

Table 1 continued

	Locus/GenBank accession no.	Primer sequence (5′–3′)	Motif	Size (bp)	T_a (°C)
22	SSR-IAC245/FJ5294471	TAGGCCATCATCATTCAGTTTG GGTTGCCCTCATAGTCATCAC	(AT)2 (GT)3	185–192	60
23	SSR-IAC246/FJ529472	TTCCCTTCATGGTCAACA CAGTAACTACAGATCAGCACAC	(AG)10	225–228	60
24	SSR-IAC247/FJ529473	TGGATGAGACGGCTTTT CTAGATACATTCTTGTCTTCAGT	(AT)3 (GT)3	210–214	TD
25	SSR-IAC248/FJ529474	TTATAAGTTTACATCAGTTTGGTT AGAATCAGGCCTTGCTTGT	(GT)2 GC (GT)4	286–288	TD
26	SSR-IAC249/FJ529475	TTTATGAGTGGTCGAAGTGAAC AAGGGGAGGGTATTAGACAGT	(AG)9	227–231	60
27	SSR-IAC250/FJ529476	TATCTAGATCTTTGCCCTTGAA CATTGCCTGTATTGTTTGAA	(GT)4 AT (GT)3	238	60
28	SSR-IAC251/FJ529477	AAGTCTTGCCCTTTTTGTTTAT TTTTTGGCTAAGTTGGTTCC	(AC)11 (AT)12	278	45
29	SSR-IAC252/FJ529478	AATGAATTCGCAACTGTC GTGGCTAAGATGAAGAACAT	(GA)5	303–305	60
30	SSR-IAC253/FJ529479	GTTCGGTCGGGGATGGA TTAGACGGCTACTGGCAAAGAG	(GA)10	218–220	60
31	SSR-IAC254/FJ529480	TTGGGTTTTATGTTTTCTGTGA CCATATCTTGTTGTGCCTTGAC	(TG)6	245–249	60
32	SSR-IAC255/FJ529481	TTACAATTCACAACAACAGAGA TTAAGAGAAAATGAAGGATGAG	(AG)23	148–168	60
33	SSR-IAC256/FJ529482	TATTTTATTAGTGGCATTGTCC TGAGAGATCCTGTAGAAGTAGTAGT	(GA)9	156–160	60
34	SSR-IAC257/FJ529483	GCAACTGAAAGGCTAAGATT TATTGGAAAATATGGGAGAA	(GT)8	300–302	60
35	SSR-IAC258/FJ529484	CATTGTCGGTGTCGGAGAAGTC CCCACGCTCTTGTTGCTGTC	(GT)7	175–178	60
36	SSR-IAC259/FJ529485	TATATGCCTGCACCACTGTAAC GAAAATCCGGAAACTCAAGAA	(TC)11	241–247	45
37	SSR-IAC260/FJ529486	TGAACAGTGCAGCAGTAACAA CACCAGACACCAATCATCAA	(AG)9	119–129	60
38	SSR-IAC261/FJ529487	TTCCCAAACACCACACCTAAGT TCACCGCGCACGAGATAA	(AC)8	260–270	60
39	SSR-IAC262/FJ529488	ATATCGTTTGATATCCTTACACA CAAACACTGGTTCACATCTCAC	(GT)9	243–245	60
40	SSR-IAC263/FJ529489	TGCAATTGACAAAAAGTTCGTA TGTATGATAGGCCTCCACCA	(AC)9	156–160	60
41	SSR-IAC264/FJ529490	TGGGATCTGTGCCTTCTC TTCCATATCCCCAAAACTT	(GA)15	125–135	45
42	SSR-IAC265/FJ529491	GTAGGTTTGTGTGCGTGC GGAAAGAAAGTTAAGATTGAGT	(TG)5	245–247	60
43	SSR-IAC266/FJ529492	TTGAGGATGTAGATTATTTTGTT CATCATTTGTGCAGTTACCAG	(TA)5 (TG)8	269–273	60

Table 1 continued

	Locus/GenBank accession no.	Primer sequence (5′–3′)	Motif	Size (bp)	T_a (°C)
44	SSR-IAC267/FJ529493	TGAGTGAACCAGCATAATCTAA CACCCGGTTGAAAATACA	(CT)15	139–162	51.4
45	SSR-IAC268/FJ529494	ATGTAGATCTGGTAAGGAGTGAA AATTAGATTAGTCAAGAAACAAAAC	(TC)9	190–193	60
46	SSR-IAC269/FJ529495	TGCGCCACTGTTTCGTATT ACCCAACCCAGGACTTCAC	(AC)6	145–147	60
47	SSR-IAC270/FJ529496	ATGTGTTTAGCAAGGATGGTCT GCTGATTTGCTGTAACGAAC	(TG)6	214–216	60
48	SSR-IAC271/FJ529497	GATTTTCTTTTCTTCCCTCAA TGCTTCCATTCCATCAA	(CA)7	227–228	49
49	SSR-IAC272/FJ529498	TAACTGAACAGGCTAAAAGAAC TGATGAGACTAAAAATGACACTT	(CA)6	210–215	60
50	SSR-IAC274/FJ529500	TCTACACATAACTGCCCTGAAC AACACTGCCACAACTAAAGAAA	(TG)7	200–205	60
51	SSR-IAC275/FJ809021	AGCAACTCATAGCACTTCTG TGAGTAATTCATTGTCTGTCCT	(TA)3G(AC)6	255	48.6
52	SSR-IAC276/FJ809022	AAGCCCAAGCCCAAACCAG CACCACCAAGACCCGATTCAG	(GAA)4(GT)7	249	52.8
53	SSR-IAC277/FJ809023	ATGGAAGGCTGCAATACATCAG CAGCGACGGTGCTTACTGG	(AC)6	286	52.1
54	SSR-IAC278/FJ809024	TCTGTCAGTTTAGCTTCGTCAC CCGTTGGAGGGTCGTTAC	(CT)10	191	51.9
55	SSR-IAC279/FJ809025	CAATACCACAAAACGCAA CATTATGATTAAATTCTGGTGT	(TG)3GG(TG)2	220	45.4
56	SSR-IAC280/FJ809026	ACTCCTGGCAAAAATCTCG GCAAAACCTCCATGAAGACAG	(TTGA)3	141	49.0
57	SSR-IAC281/FJ809027	AGTCCAGGCAGAGCAAGCAAG CGCATTCATCTCATCCATTATCC	(GT)9	189	52.7
58	SSR-IAC282/FJ809028	CTCTTGGTTACGCGTGGACTAC CGCCTATGTTGGGTTGAGATG	(CAA)2T(CA)3	234	54.8
59	SSR-IAC283/FJ809029	GAACTCATTCCCCTCTCC GCAGCTCCTCCATTCTAC	(TTA)7	208	47.9
60	SSR-IAC284/FJ809030	AAGGAGAAAATAAAATCACAGTC GTTTCGAGGTTATTGGGGA	(CT)13	240	50.7
61	SSR-IAC285/FJ809031	ACCTAGATGGATTGTGACC GTCTAGTTTTACGCATTCATTC	(AT)11(TG)11(TA)6(AG)7	204	48.1
62	SSR-IAC286/FJ809032	TTGTTCTGAGGACTGGATGTAT TCGGTTGATTTTTCGTTTTA	(TC)18	181	49.1
63	SSR-IAC287/FJ809033	ACAACGTTAGAAAGGGAAGG GAAAAAAGCGAGAAAAAGAGT	(GA)13	179	51.4
64	SSR-IAC288/FJ809034	TCCCGTGAGTTACAAATAG ATCACCACTCCTCTCCAG	(ATTT)4	182	46.6

TD: Touchdown PCR described in Hanai et al. 2007

orders of the nine markers were compared based on their log-likelihoods. Additional markers from each group were added using the 'TRY' command, starting with more informative markers. The final order was verified using the 'RIPPLE' command, with a window with six markers. Finally, multipoint estimates of distance were obtained using the 'MAP' command.

Results

Microsatellite polymorphisms tests

The conditions for amplification of the 871 microsatellites were optimized and the polymorphisms were tested for the parents of the IAC-UNA × CAL143 population. A total of 248 (28.5%) microsatellites were polymorphic and presented adequate visualization patterns with no stutter or non-specific bands.

There were eight (38.1%) polymorphic markers from the PV series described by Caixeta et al. (2005), 19 (41.3%) from the BM, AG, and GAT series, one (50.0%) from the PVbng series, 38 (53.5%) from the PVBR series, 11 (26.3%) from the FJ series described by Hanai et al. (2007), 69 (31.1%) from the SSR-IAC series, and five (25.0%) from the FJ series described by Campos et al. (2007). All the 15 M series markers were monomorphic. Among the gene-based microsatellites, there were five (21.8%) described by Yu et al. (2000), 15 (32.7%) from the Bmd series, three (16.7%) described by Guerra-Sanz (2004), and 10 (25.0%) from the PvM series that were polymorphic. In total, there were 33 (25.4%) polymorphic markers from 130 gene-based markers tested and 232 (31.3%) polymorphic markers from 741 genomic-based markers. The 64 new IAC series microsatellites, described in Table 1, were also polymorphic.

Genetic map

A total of 198 microsatellites and three phenotypic markers were mapped, with distributions in all 11 linkage groups (Fig. 1). The total map length was 1865.9 cM with an average linkage group length of 170.5 cM. The average distance between markers was 9.4 cM, with an average of 18 markers in each group. The number of markers in each group varied from five (B11 group) to 37 (B2 group).

The B2, B3, B4, B5, B7, B8, and B9 linkage groups had gaps that require more saturation. The B2, B4, B8, and B9 groups have markers with genetic distances greater than 37.5 cM; in these cases, sub-groups were discriminated by segmented lines (Fig. 1). The B8 group was split into A and B sub-groups, as it was not possible to determine the correct sense order between them. The 8A subgroup was formed by ten markers with previous mapping position unknown in the literature, not allowing further comparison. While there was statistical evidence for the PVBR251 marker being linked to the B1 group, its distance from the adjacent marker (IAC235) was greater than 37.5 cM. The same phenomenon occurred in the B9 group with marker IAC242.

The Bonferroni correction was used, but even markers with segregation distortion were included in the map. Distortion was observed for 92 (37.3%) microsatellite loci, and 76 of these were positioned on the map (Fig. 1). As expected, these markers clustered in certain chromosomal regions.

Phenotypic markers

All three phenotypic markers were mapped. The *V* locus was detected in the B6 group and was closely linked to the microsatellites IAC268 (0.2 cM) and IAC183 (0.6 cM) at the edge of the B6 group. The *fin* and *Ct* loci were mapped, but their position in the linkage groups could not be estimated with sufficient accuracy; therefore they are indicated by arrows in Fig. 1. The pod tip shape trait, determined by the *Ct* locus, was linked to the B9 group and was close to the molecular markers FJ17 and PVBR60.

Phenotypic segregation (Table 2) was tested. The expected Mendelian segregation ratio (1:1) was observed for flower color and pod tip shape. However, the growth habit trait showed significant deviation from this value ($P < 10^{-5}$), which could be the result of two loci controlling this phenotype. A ratio of 259:106, which is a good fit to the ratio 3:1 ($\chi^2 = 3.62$, $P = 0.06$), was observed for growth habit, confirming this hypothesis. In addition, the F1 phenotype was 100% determinate growth habit. We propose a novel genetic model wherein two recessive loci control the phenotype of the indeterminate growth habit. For example, consider the cross *finfinfin'fin'* (indeterminate growth) × *FinFinFin'Fin'*

Fig. 1 Genetic map for IAC-UNA × CAL143 (UC). B1 to B11 refer to linkage groups, and I to XI are the corresponding chromosomes (Pedrosa et al. 2008). The underlined microsatellites were anchored to form the linkage groups. (*) Markers with segregation distortion to CAL143 and to (**) IAC-UNA. (***) Phenotypic marker *V* linked to group B6. *Arrows* indicate possible position of the *fin* and *Ct* loci, and dotted lines indicate distances larger than 37.5 cM
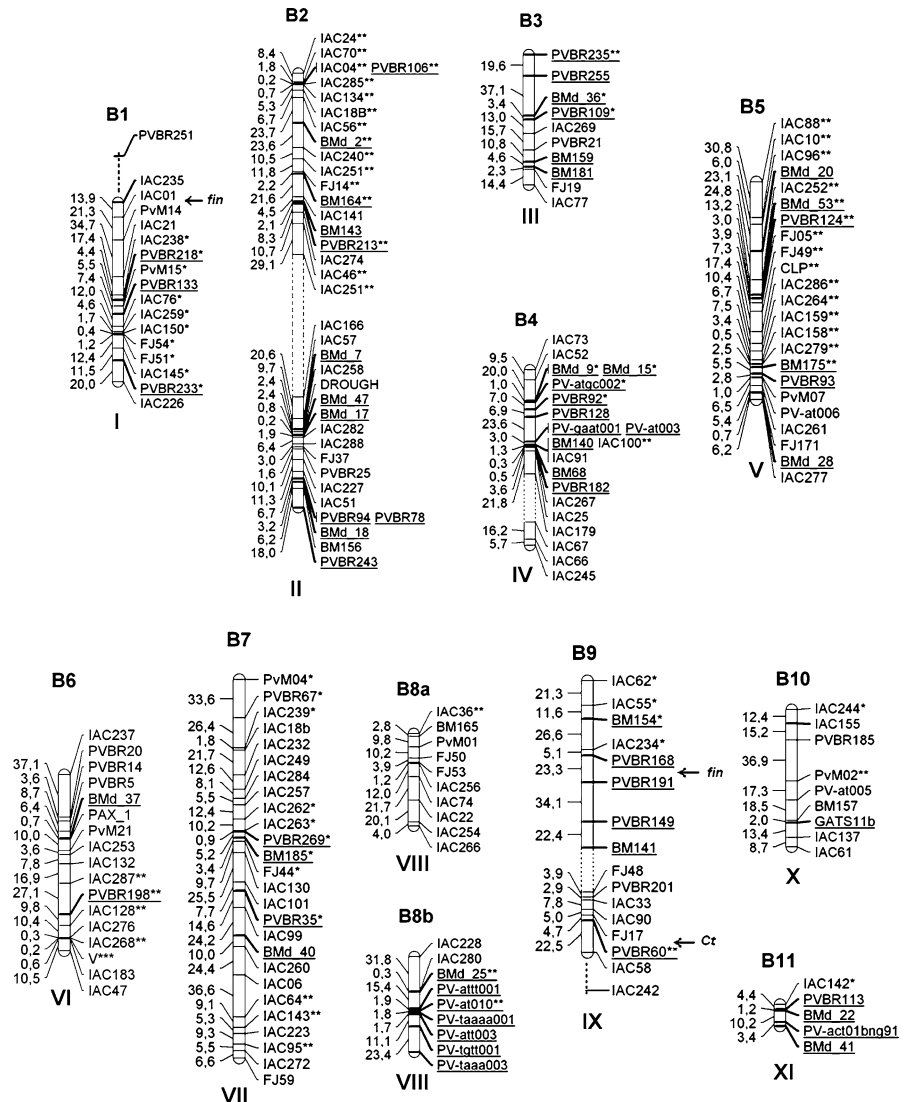
(determinate growth). Four homozygous genotypes with equal proportions are expected in a RIL population: *FinFinFin′Fin′*, *FinFinfin′fin′*, *finfinFin′Fin′* and *finfinfin′fin′*. If only the homozygous genotype with two recessive alleles (*finfinfin′fin′*) expressed indeterminate growth, a segregation of 3:1 would be expected. The growth habit trait (*fin*) was mapped in the B1 (LOD = 3.76) and B9 (LOD = 3.95) groups, supporting the hypothesis that two loci could be involved in the genetic control of this trait.

Table 2 Segregation of three qualitative traits from an F10 population of the common bean cross IAC-UNA × CAL143

Morphological marker	Segregation ratio 1:1 (CAL143:IAC-UNA)	χ^2	P	Map location
Pod tip shape	165:195	2.50	0.11	B9
Flower color	161:197	3.17	0.07	B6
Growth habit	259:106	64.14	$<10^{-5}$	B1 and B9

Discussion

Mapping population and polymorphism tests

The new mapping population IAC-UNA × CAL143 (UC) established in this study represents the largest population currently developed with regard to the number of recombinant inbred lines ($n = 380$) and generations of self-breeding. The UC population is able to detect recombinant events because of the high number of recombinant lines. For this reason, a well-saturated map with precise estimates of distance and marker order is expected.

The parents were chosen from different gene pools (Mesoamerican and Andean) and have contrasting features for several agronomical traits. Segregation for disease resistance, such as resistance against anthracnose (caused by *Colletotrichum lindemuthianum*), angular leaf spot (caused by *Pseudocercospora griseola*) and rust (caused by *Uromyces phaseoli*), was observed. These parents define an interesting mapping population, as several traits can be mapped.

A moderate polymorphism rate (28.5%) was found between microsatellite markers. Low diversity is expected for a cross between two cultivated genotypes. The observed polymorphism rate is similar to results reported in other studies, such as 45.7% (Blair et al. 2006b) and 42.8% (Grisi et al. 2007). If the parents were from the same gene pool, they would most likely have a narrower genetic base and less genetic variability.

One probable reason for the moderate polymorphism rate found is the extensive number of microsatellites developed with small motif repeats, considered from five units in enriched library screening (Benchimol et al. 2007; Campos et al. 2007; Hanai et al. 2007). For example, the PVBR series contains larger repeated motifs and was the most polymorphic microsatellite series (53.5%) in polymorphism tests. In this work, we described 64 new microsatellite markers that presented parental UC polymorphisms, which will be useful for other genetic studies in the common bean.

Genetic map

The final UC genetic map covered all 11 linkage groups of the common bean. The total length (1865.9 cM) was consistent with other genetic mapping studies (Blair et al. 2003; Grisi et al. 2007). Some linkage groups presented a highly dense coverage distribution of markers (e.g., B5 and B7), while others contained gaps (e.g., B2 and B8) or presented few markers (e.g., B11, with only five markers). The microsatellite-based genetic map presented by Grisi et al. (2007) also found a small number of markers in the B11 group (only four markers were detected). The Co-2 cluster, containing one of the R genes involved in anthracnose disease resistance in the common bean, was found by molecular analysis in the subtelomeric region of the B11 group (Creusot et al. 1999). R genes contain leucine-rich regions called coiled-coil nucleotide-binding site leucine-rich repeat (CNL), and can contain heterochromatic knobs. There is evidence that the Co-2 cluster, through an ectopic recombination, gives rise to the Pv B4-CNL sequences in the B4 group (David et al. 2009). The difficulty in mapping the B4 linkage group could be due to the presence of two knobs detected in the CNL subtelomeric region (Geffroy et al. 2009).

A total of 201 markers have been positioned in the UC map to date, including 131 microsatellite markers that were not mapped previously. The use of anchor markers was efficient to form the linkage groups and to order the markers within them. Among the gene-based microsatellites, in the B4 linkage group the loci *BMd9*, *Bmd15* and *PVatgc002* were mapped very close together and were derived from different phytohemagglutinin gene sequences, suggesting a single location of a gene family cluster in this region. It confirms the results of Blair et al. (2003), who found other markers (*Bmd16* and *PVatgc004*) related to phytohemagglutinin gene sequences also in the B4 group.

In the UC map, a concentration of markers with distortion to Andean alleles was detected in the B1 group, and a concentration of markers with distortion to Mesoamerican alleles in the B2 and B5 groups. In the B2 linkage group only a small region presented markers with segregation distortion. For the B5 group, the region with distortion is larger, and in the B1 group, Andean alleles predominated (Fig. 1). Blair et al. (2003) found the same segregation distortion to Mesoamerican alleles in regions of the B5 group. Freyre et al. (1998) also found the same distortion for the B1 and B2 groups for the same parental gene pools.

The phenomenon of markers with segregation distortion in the common bean has been extensively described (Freyre et al. 1998; Blair et al. 2003; Grisi et al. 2007; Hougaard et al. 2008). This phenomenon was observed not only for microsatellite markers, but also for legume anchor markers (Hougaard et al. 2008) and RFLP markers (Freyre et al. 1998). The reasons for this segregation distortion could be associated with genomic regions related to adaptive advantages that were selected during each self-breeding cycle of the population mapping development. These distortion genomic regions did not present mixture between the Andean and Mesoamerican pools, supporting an incompatible genomic region hypothesis. The distortion of Mendelian ratios associated with these markers clustering inside the linkage groups represents a trend towards retaining the ancestral pool origin.

The segregation distortion observed for some markers did not exclude them from being potentially informative for recombinant detection. For this reason, they were incorporated into the linkage analysis. The selection effect cannot be ignored, however, even in a controlled cross in a population mapping experiment. According to Shizhong (2008), the use of markers with segregation distortion could actually increase the mapping saturation and statistical power of quantitative trait locus mapping.

The phenotypic mapping of the V locus in the B6 group is consistent with other genetic maps, as it was also obtained by Nodari et al. (1993) and McClean et al. (2002). The Ct locus was previously identified by Al-Mukhtar and Coyne (1981), but it was never mapped. Flower color is related to seed color, an important trait selected by the consumer market. The markers that are tightly linked to the V and Ct loci can be used for future cloning experiments. The use of markers for simply inherited traits in marker-assisted backcrossing and introgression across Andean and Mesoamerican gene pools is also suggested.

The fin and fin' loci were mapped in two distinct groups (B1 and B9), with almost the same LOD score for each. It is necessary to obtain more markers for these groups to saturate the regions in order to estimate the positions of these loci. Koinange et al. (1996) and Blair et al. (2006b) also mapped the fin locus to the B1 group. Kwak et al. (2008) found a candidate gene for the fin locus, $PvTFL1y$, and they also mapped it to the B1 linkage group. Ta'an et al.

(2001) and Tar'an et al. (2002) mapped the growth habit gene to the B9 linkage group.

The existence of only one locus to define growth habit in the common bean has been previously questioned, and it is probable that multiple genes controlling growth habit exist in the common bean (McClean et al. 2002). We propose a genetic model based on two loci (fin and fin'), according to Mendelian segregation ratios and linkage mapping results. This genetic model approach related the phenotypic and genotypic analyses of segregating progeny. The single cross UC confirmed that the two growth habit loci probably resulted from the huge effective size of the mapping population, with the segregation and linkage data analyzed together.

The source of determinacy of CAL143 may be an example of a dominant determinate genotype derived from the Andean region that has been domesticated without maize as a physical support for the climbing bean in traditional agriculture, as proposed by Koinange et al. (1996). The F1 cross UC also revealed the dominant inheritance of the determinate growth habit trait, which was usually inherited recessively. Determinant genotypes must represent different sources of genetic control of determinacy, and the frequent recessive state cannot be an exclusive state for the common bean growth habit. This is in agreement with Kornegay et al. (1992), whose results also suggested a genotype (G13624) with two dominant determinate growth habit genes.

The use of qualitative traits such as morphological markers allows the study of genetic models and represents basic genetic knowledge about any species. We propose a new genetic model regarding growth habit, a characteristic of simple inheritance that is important in an agronomic context. Further studies, such as fine-structure mapping in these regions, other crosses and segregating progeny could be performed to study both loci. It is evident that advances can be achieved in genetic breeding of the common bean with the development of genetic mapping. The presented map gives an overview of the genomic organization and provides anchor points for future maps.

References

Adam-Blondom AM, Sévignac M, Dron M (1994) A genetic map of common bean to localize specific resistance genes against anthracnose. Genome 37:915–924

Al-Mukhtar FA, Coyne DP (1981) Inheritance and association of flower, ovule, seed, pod and maturity characters in dry edible beans (Phaseolus vulgaris L.). J Am Soc Hort Sci 106:713–719

Arumuganathan K, Earle ED (1991) Nuclear DNA content of some important plant species. Plant Mol Biol 9:208–218

Bai Y, Michaels TE, Pauls KP (1997) Identification of RAPD markers linked to common bacterial blight resistance genes in Phaseolus vulgaris L. Genome 40:544–551

Basset MJ (1991) A revised linkage map of common bean. Hort Sci 26:834–836

Benchimol LL, Campos T, Carbonell SAM, Colombo CA, Chioratto AF, Formighieri EF, Gouvea LRL, Souza AP (2007) Structure of genetic diversity among common bean (Phaseolus vulgaris L.) varieties of Mesoamerican and Andean origins using new developed microsatellite markers. Genet Res Crop Evol 54:1747–1762

Blair MW, Pedraza F, Buedia HF, Gaitán-Solís E, Beebe SE, Gepts P, Tohme J (2003) Development of a genome-wide anchored microsatellite map for common bean (Phaseolus vulgaris L.). Theor Appl Genet 107:1362–1374

Blair MW, Giraldo MC, Buendia HF, Tovar E, Duque MC, Beebe SE (2006a) Microsatellite marker diversity in common bean (Phaseolus vulgaris L.). Theor Appl Genet 113:100–109

Blair MW, Iriarte G, Beebe S (2006b) QTL analysis of yield traits in an advanced backcross population derived from a cultivated Andean × wild common bean (Phaseolus vulgaris L.) cross. Theor Appl Genet 112:1149–1163

Blair MW, Buendía HF, Giraldo MC, Métais I, Peltier D (2008) Characterization of AT-rich microsatellites in common bean (Phaseolus vulgaris L.). Theor Appl Genet 118:91–103

Blair MW, Díaz LM, Buendía HF, Duque MC (2009) Genetic diversity, seed size associations and population structure of a core collection in common beans (Phaseolus vulgaris L.). Theor Appl Genet 119:955–972

Buso GSC, Amaral ZPS, Brondani RPV, Ferreira ME (2006) Microsatellite markers for the common bean Phaseolus vulgaris. Mol Ecol Notes 6:252–254

Caixeta ET, Borém A, Kelly JD (2005) Development of microsatellite markers based on BAC common bean clones. Crop Breed Appl Biotechnol 5:125–133

Campos T, Benchimol LL, Carbonell SAM, Chioratto AF, Formighieri EF, Souza AP (2007) Microsatellites for genetic studies and breeding programs in common bean. Pesq Agropec Bras 42:589–592

Cardoso JMK, Oblessuc PR, Campos T, Sforça DA, Carbonell SAM, Chioratto AF, Formighieri EE, Souza AP, Benchimol LL (2008) New microsatellite markers developed from an enriched microsatellite common bean library. Pesq Agropec Bras 43:929–936

Condit R, Hubbell SP (1991) Abundance and DNA sequence of two-base regions in tropical tree genomes. Genome 34:66–71

Creste S, Tulmann A, Figueira A (2001) Detection of single sequence repeat polymorphism in denaturing polyacrylamide sequencing gels by silver staining. Plant Mol Biol 19:299–306

Creusot F, Macadré C, Ferrier Cana E, Riou C, Geffroy V, Sévignac M, Dron M, Langin T (1999) Cloning and molecular characterization of three members of the NBS-LRR subfamily located in the vicinity of the Co-2 locus for anthracnose resistance in Phaseolus vulgaris. Genome 42:254–264

David P, Chen NWG, Pedrosa-Harand A, Thareau V, Sévignac M, Cannon SB, Debouck D, Langin T, Geffroy V (2009) A nomadic subtelomeric disease resistance gene cluster in common bean. Plant Physiol 151:1048–1065

Freyre CI, Skroch P, Geffroy V, Adam-Blondon AF, Shirmohamadali A, Johnson W, Llaca V, Nodari R, Pereira P, Tsai SM, Tohme J, Dron M, Nienhuis J, Vallejos C, Gepts P (1998) Towards an integrated linkage map of common bean. 4. Development of a core map and alignment of RFLP maps. Theor Appl Genet 97:847–856

Gaitán-Solís E, Duque MC, Edwards KJ, Tohme J (2002) Microsatellite in common bean (Phaseolus vulgaris): isolation, characterization, and cross-species amplification in—Phaseolus ssp. Crop Sci 42:2128–2136

Geffroy V, Macadré C, David P, Pedrosa-Harand A, Sevignac M, Dauga C, Langin T (2009) Molecular analysis of a large subtelomeric nucleotide binding-site-leucine-rich-repeat family in two representative genotypes of the major gene pools of Phaseolus vulgaris. Genetics 181:405–419

Gepts PR, Nodari R, Tsai R, Koinange EMK, Llaca V, Gilbertson R, Guzman P (1993) Linkage mapping in common bean. Annu Rep Bean Improve Coop 36:24–38

Grisi MCM, Blair MW, Gepts P, Brondani C, Pereira PAA, Brondani RPV (2007) Genetic mapping of a new set of microsatellite markers in a reference common bean (Phaseolus vulgaris) population BAT93 × Jalo EEP558. Genet Mol Res 6:691–706

Guerra-Sanz JM (2004) New SSR markers of Phaseolus vulgaris from sequence databases. Plant Breed 123:87–89

Guo X, Castill-Ramirez S, Gonzalez V, Bustos P, Fernandez-Vazgueq JL, Santamaria RI, Arellano J, Cevallos MA, Davila G (2007) Rapid evolutionary change of common bean (Phaseolus vulgaris L.) plastome, and the genomic diversification of legume chloroplasts. BMC Genomics 8:228

Hackuf B, Wehling P (2002) Identification of microsatellite polymorphism in an expressed portion of rye genome. Plant Breed 121:17–25

Hanai LR, Campos T, Camargo LEA, Benchimol LL, Souza AP, Melloto M, Carbonell SAM, Chioratto AF, Consoli L, Formighieri EF, Siqueira M, Tsai SM, Vieira MLC (2007) Development, characterization, and comparative analysis of polymorphism at common bean SSR loci isolated from genic and genomic sources. Genome 50:266–277

Harper GL, Maclean N, Goulson D (2003) Microsatellite markers to assess the influence of population size, isolation and demographic change on the genetic structure of the UK butterfly Polyommatus bellargus. Mol Ecol 12:3349–3357

Hoisington D, Khairallah M, González-de-León D (1994) Laboratory protocols: CIMMYT applied molecular genetics laboratory, 2nd edn. CIMMYT, Mexico, DF

Hougaard BK, Madsen LH, Sandal N, Moretzsohn MC, Fredslund J, Schauser L, Nielsen AM, Rohde T, Sato S, Tabata S, Bertioli DJ, Stougaard J (2008) Legume anchor markers link syntenic regions between *Phaseolus vulgaris*, *Lotus japonicus*, *Medicago truncatula* and *Arachis*. Genetics 179:2299–2312

Koinange EMK, Singh SP, Gepts P (1996) Genetic control of the domestication syndrome in common bean. Crop Sci 36:1037–1045

Kornegay J, White JW, Cruz OO (1992) Growth habit and gene pool effects on inheritance of yield in common bean. Euphytica 62:171–180

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Kwak M, Velasco D, Gepts P (2008) Mapping homologous sequences for determinacy and photoperiod sensitivity in common bean (*Phaseolus vulgaris*). J Hered 99:283–291

Lamprecht H (1935) Zur Genetik von *Phaseolus vulgaris*. X. Über Infloreszenztypen und ihre Vererbung. Hereditas 20:71–93

Lamprecht H (1939) Zur Genetik von *Phaseolus vulgaris*. XIV. Über die Wirkung der Gene P, C, J, Ins, Can, G, B, V, Vir, Och und Flav. Hereditas 25:255–288

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) Mapmaker: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Lynch M, Walsh B (1998) Genetics and analysis of quantitative traits. Sinauer Associates, Sunderland 980p

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

Métais I, Hamon B, Jalouzot R, Peltier D (2002) Structure and level of genetic diversity in various bean types evidenced with microsatellite markers isolated from a genomic enriched library. Theor Appl Genet 104:1346–1352

Mollinari M, Margarido GRA, Vencovsky R, Garcia AAF (2009) Evaluation of algorithms used to order markers on genetic maps. Heredity 103:494–502

Moore SS, Hale P, Byrne K (1998) NCAM: a polymorphic microsatellite locus conserved across eutherian mammal species. Anim Genet 29:33–36

Murray J, Larsen J, Michaels TE, Schaafsma A, Vallejos CE, Pauls KP (2002) Identification of putative genes in bean (*Phaseolus vulgaris*) genomic (Bng) RFLP clones and their conversion to STSs. Genome 45:1013–1024

Nodari RO, Tsai SM, Gilbertson RL, Gepts P (1993) Towards an integrated map of common bean-2: development of a RFLP-based linkage map. Theor Appl Genet 85:513–520

Oliveira KM, Pinto LR, Marconi TG, Molinari M, Ulian EC, Chabregas SM, Falco MC, Burnquist W, Garcia AAF, Souza AP (2009) Characterization of new polymorphic functional markers for sugarcane. Genome 52:191–209

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

Primmer CR, Raudsepp T, Chowdhary BP, Møller AP, Ellegren H (1997) Low frequency of microsatellites in the avian genome. Genome Res 7:471–482

Rico C, Zadworny D, Kuhnlein U, Fitzgerald GJ (1993) Characterization of hypervariable microsatellite loci in the threespine stickleback *Gasterosteus aculeatus*. Mol Ecol 2:271–272

Rodríguez-Suárez C, Méndez-Vigo B, Pañeda A, Ferreira JJ, Giraldez R (2006) A genetic linkage map of *Phaseolus vulgaris* L. and localization of genes for specific resistance to six races of anthracnose (Colletotrichum lindemuthianum). Theor Appl Genet 114:713–722

Rudorf W (1958) Genetics of *Phaseolus aborigineus* Burkart. Proc X Intl Congr Genet 2:243 (Abstr.)

Schlueter JA, Goicoechea JL, Collura K, Gill N, Lin J, Yu Y, Kudrna D, Zuccolo A, Vallejos CE, Muñoz-Torres M, Blair MW, Tohme J, Tomkins J, McClean P, Wing RA, Jackson SA (2008) BAC-end sequence analysis and a draft physical map of the common bean (*Phaseolus vulgaris* L.) genome. Trop Plant Biol 1:40–48

Schuelke M (2000) An economic method for the fluorescent labeling of PCR fragments. Nat Biotechnol 18:233–234

Shizhong X (2008) Quantitative trait locus mapping can benefit from segregation distortion. Genetics 180:2201–2208

Singh S, Nodari R, Gepts P (1991) Genetic diversity in cultivated common bean. II. Marker-based analysis of morphological and agronomic traits. Crop Sci 31:23–29

Ta'an B, Michaels TE, Pauls KP (2001) Identification of markers associated with quantitative traits in beans. Annu Rep Bean Improve Coop 44:9–10

Tar'an B, Michaels TE, Pauls KP (2002) Genetic mapping of agronomic traits in common bean. Crop Sci 42:544–556

Tautz D, Renz M (1984) Simple sequence repeats are ubiquitous repetitive components of eukaryotic genomes. Nucleic Acids Res 12:4127–4137

Vallejos CE, Sakiyama NS, Chase CD (1992) A molecular marker-based linkage map of *Phaseolus vulgaris* L. Genetics 131:733–740

Yaish MWF, Pérez de la Vega M (2003) Isolation of (GA)n microsatellite sequences and description of a predicted MADS-box sequence isolated from common bean (*Phaseolus vulgaris* L.). Genet Mol Biol 26:337–342

Yang GP, Shagai Maroof MA, Xu CG, Zhang Q, Biyashev RM (1994) Comparative analysis of microsatellite DNA polymorphism in landraces and cultivars of rice. Mol Gen Genet 245:187–194

Yu K, Park SJ, Poysa V (1999) Abundance and variation of microsatellite DNA sequences in beans (*Phaseolus vulgaris* and *Vigna*). Genome 42:27–34

Yu K, Park SJ, Poysa V, Gepts P (2000) Integration of simple sequence repeat (SSR) markers into a molecular linkage map of common bean (*Phaseolus vulgaris* L.). J Hered 91:429–434

Zhang X, Blair MW, Wang S (2008) Genetic diversity of Chinese common bean (*Phaseolus vulgaris* L.) landraces assessed with simple sequence repeat markers. Theor Appl Genet 117:629–664