Extension of the core map of common bean with EST-SSR, RGA, AFLP, and putative functional markers

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Abstract Microsatellites and gene-derived markers are still underrepresented in the core molecular linkage map of common bean compared to other types of markers. In order to increase the density of the core map, a set of new markers were developed and mapped onto the RIL population derived from the 'BAT93' × 'Jalo EEP558' cross. The EST-SSR markers were first characterized using a set of 24 bean inbred lines. On average, the polymorphism information content was 0.40 and the mean number of alleles per locus was 2.7. In addition, AFLP and RGA markers based on the NBS-profiling method were developed and a subset of the mapped RGA was sequenced. With the integration of 282 new markers into the common bean core map, we were able to place markers with putative known function in some existing gaps including regions with QTL for resistance to anthracnose and rust. The distribution of the markers over 11 linkage groups is discussed and a newer version of the common bean core linkage map is proposed.

Keywords *Phaseolus vulgaris* · Core linkage map · EST-SSR · AFLP · NBS-profiling method · Resistance gene analogs

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Introduction

Common bean (*Phaseolus vulgaris* L.) is the most important legume for direct human consumption in the developing world (Broughton et al. 2003). Production is especially important in Eastern and Southern Africa and Latin America where, in particular, rice and beans are an inseparable pair of staple foods for millions of people, representing a major source of dietary protein. Therefore, an increased effort has been devoted to improving bean varieties and expanding the area under bean cultivation. However, the productivity of most bean varieties grown in developing countries is still low.

Molecular markers have been used to assist common bean breeding programs in various ways (Jarne and Lagoda 1996), including studies on the

origin and diversity of current cultivars (Gepts 1998), the domestication of *P. vulgaris* (Koinange et al. 1996), and the genetic control of resistance to important diseases (Miklas et al. 2003; Miklas et al. 2006a; Nodari et al. 1993b; Yu et al. 1998). The first molecular linkage maps of common bean were developed by means of restriction fragment length polymorphism (RFLP) markers (Adam-Blondon et al. 1994; Nodari et al. 1993a; Vallejos et al. 1992). Subsequently, common markers were used to integrate these maps by establishing the correspondence of their linkage groups (Freyre et al. 1998). For instance, a population of RILs derived from a cross between 'BAT93' and 'Jalo EEP558' was used as a core mapping population to construct an integrated linkage map. Although RFLP markers have been important to establish a genome-wide framework for anchoring and cross-referencing plant linkage maps, their widespread use in common bean was restricted by the lack of probes and the low-throughput nature of RFLP markers. Different types of markers, mainly random amplified polymorphic DNA (RAPD) were then used to increase the density of the common bean molecular linkage map (Freyre et al. 1998). More recently, single sequence repeats (SSR), also called microsatellites, have been developed and used to increase the density of existing maps, especially the core 'BAT93' × 'Jalo EEP558' linkage map (Blair et al. 2003; Grisi et al. 2007; Yu et al. 2000).

Currently, high-throughput markers, such as AFLP, are preferred for map saturation purposes. Putative functional markers are still underrepresented in the common bean molecular linkage map compared to other types of markers (López et al. 2003; Miklas et al. 2006b; Mutlu et al. 2006; Rivkin et al. 1999). Among these are resistance gene analogs (RGA), which are associated with a number of known *R*-genes (Brugmans et al. 2008; Ferrier-Cana et al. 2003; Kanazin et al. 1996; Mutlu et al. 2006) and quantitative resistance loci (Flandez-Galvez et al. 2003; Mutlu et al. 2006; Timmerman-Vaughan et al. 2002). In addition, microsatellites represent a limited proportion of the markers on the bean core map (Blair et al. 2003; Grisi et al. 2007; Yu et al. 2000), in spite of their many desirable attributes, including their presence in both non-genic and genic regions of plant genomes (reviewed by Li et al. 2004; Oliveira et al. 2006). A large number of unmapped microsatellite markers have been reported in the literature (Benchimol et al.

2007; Buso et al. 2006; Caixeta et al. 2005; Campos et al. 2007; Cardoso et al. 2008; Gaitán-Solís et al. 2002; Guerra-Sanz 2004; Hanai et al. 2007; Métais et al. 2002; Murray et al. 2002; Yaish and Pérez de la Vega 2003). In addition, the density of the common bean core map is still low for purposes of marker-assisted selection and map-based cloning.

The objectives of the present work were to test new SSR markers developed from common bean expressed sequence tags (EST) in a set of *P. vulgaris* genotypes, assess their capacity to render SSR polymorphisms and to map them and other markers such as RGA and AFLP onto the common bean core map.

Materials and methods

Plant material and DNA extraction

A set of 23 common bean inbred lines and one accession of *P. acutifolius* were used to evaluate allelic variation at EST-SSR loci. Among *P. vulgaris* accessions, 17 genotypes belonged to the Mesoamerican gene pool ('Apetito Blanco', 'Baetão', 'Barbunya', 'BAT93', 'Brasil2', 'Carioca Comum', 'Flor de Mayo', 'Garbancillo', 'Great Northern', 'IAC-UNA', 'Jamapa', 'Mulatinho', 'Porrillo', 'Puebla152', 'Rio Tibagi', 'Sanilac' and 'Tu'), and six to the Andean gene pool ('Antioquia 8', 'CAL 143', 'Jabola', 'Jalo EEP558', 'Pompadour' and 'Red Kidney'). For map construction the inter-gene pool 'BAT93' × 'Jalo EEP558" population (Freyre et al. 1998; Nodari et al. 1993a) consisting of 74 F$_8$ RILs was used.

Total genomic DNA was extracted from young leaf tissue using the CTAB extraction method as described by Doyle and Doyle (1987). DNA concentrations were estimated by electrophoresis on ethidium bromide-stained agarose gels using appropriate molecular weight standards. From this quantification, aliquots at 10 and 50 ng/μl were prepared for EST-SSR amplifications and enzymatic digestions, respectively.

Development and amplification of EST-SSR markers

As reported previously (Hanai et al. 2007), a total of 3,126 bean EST sequences were obtained from the bean EST project homepage (http://lgm.esalq.usp.br/BEST/). EST-containing SSR exhibiting at least five

repeat units of di-, tri-, and tetranucleotide motifs were analyzed and 156 primer pairs were designed. PCR reactions were performed in a final volume of 16 μl in 96-well plates using a Gen Amp® PCR System 9700 thermocycler (Applied Biosystems). Approximately 20 ng of template DNA were mixed in a solution containing 0.2 μM of both forward and reverse primers, 0.2 mM of each dNTP, 1.5 mM MgCl$_2$, 10 mM Tris–HCl (pH 8.3), 50 mM KCl and 0.4 units *Taq* DNA polymerase (Promega). Four PCR program profiles were used to amplify EST-SSR loci. All four profiles started with a preliminary denaturation step at 94°C for 2 min followed by two stages. In both stages, denaturing and elongation conditions were held constant at 94°C for 30 s and 72°C for 60 s, respectively. The duration of primer annealing was 45 s, but the temperature varied in stage 1, decreasing 1°C per cycle during 12 cycles (65–53°C), 0.5°C per cycle during 10 cycles (55–50°C) or 1°C per cycle during 10 cycles (55–45°C) in programs 1, 2 and 3, respectively. In program 4, the initial annealing temperature was 45°C and increased 1°C per cycle during 10 cycles (45–55°C). Stage 2 comprised 25 cycles of amplification. In all programs, the annealing temperature of stage 2 was the one reached at the end of the first PCR stage. Final elongation was performed at 72°C for 7 min.

Development and sequencing of RGA markers

RGA markers were amplified based on the NBS-profiling methodology (van der Linden et al. 2004). This methodology is based on the digestion of DNA, ligation with adapters, and a PCR reaction where one primer targets the adapter while the other, which is degenerate, targets the conserved nucleotide binding site (NBS) domain of plant disease resistance genes. This amplification results in several fragments per reaction due to the abundance of resistance gene-like sequences in plant genomes.

About 200 ng of genomic DNA from the parental line 'BAT93' and 'Jalo EEP558' were digested with *Rsa*I (New England BioLabs). Digestions were performed in a final volume of 20 μl using 6 U of the enzyme according to the manufacturer's recommendations, during 4 h at 37°C. Reactions were terminated by heat inactivation (20 min at 65°C). An adapter previously prepared from an equimolar mixture of LA (long arm) oligonucleotide (5′-ACTCGATTCTCAA CCCGAAAGTATAGATCCCA-3′) and SA (short arm) oligonucleotide (5′-TGGGATCTATACTT*-3′) was ligated to the restriction fragment ends. The 3′ end of the SA oligonucleotide was blocked for *Taq* DNA polymerase extension by the addition of an amino group (*). Ligation was performed by adding 20 μl of a mixture containing 1.25 μM adapter, 1× ligation buffer (New England BioLabs), 80 units T4 DNA ligase (400 units/μl; New England BioLabs) to 20 μl of digested DNA. The reaction was incubated at 16°C overnight and terminated by heat inactivation (65°C for 10 min). As template DNA 3 μl of ligation products were used for the amplification of selected fragments anchored to the NBS-domain. For this, an AP (adapter primer) complementary to the adaptor (5′-ACTCGATTCTCAACCCGAAAG-3′) and one of four NBS-primer were used in distinct reactions (Table 1). The amplification was performed in a final volume of 20 μl containing 0.25 μM of both primers, 0.2 mM of each dNTP, 1.5 mM MgCl$_2$, 10 mM Tris–HCl (pH 8.3), 50 mM KCl and 1.0 unit *Taq* DNA polymerase (Promega). The amplification was carried out in a Gen Amp® PCR System 9700 thermocycler (Applied Biosystems). A two-stage PCR was used after an initial denaturing step at 94°C for 5 min. The first

Table 1 Designation and sequence of primers used to generate RGA markers based on the NBS-profiling method

Primer designation[a]	Sequence 5′–3′	Protein domain/motif
AP	ACTCGATTCTCAACCCGAAAG	Adapter[b]
NBSa-F	GGAATGGGKGGACTYGGYAARAC	NBS/kinase
Kdc-F	ATGGGAAGGAAGTATTCCAA	NBS/kinase
Kdc-R	ARGTTCCACAGGACATCACC	NBS/kinase
RGP-F	GGNATGGGYGGBRTHGGYAARAC	NBS/hydrophobic

[a] F for forward and R for reverse

[b] Primer specific to the adapter sequence used in combination with RGA specific primers

stage consisted of 8 cycles at 94°C for 45 s, at 58°C (−1°C per cycle) for 1 min, and at 72°C for 1 min, whereas the second stage consisted of 25 cycles at 94°C for 45 s, 50°C for 1 min and 72°C for 1 min. A final extension of 7 min at 72°C was conducted. The amplification of fragments anchored to NBS-domain was assured by designing an adapter that does not have an AP-primer annealing site in the SA oligonucleotide. This annealing site is constructed after elongation of the NBS-primer, when the end of LA oligonucleotide is used as template for AP-primer annealing (for details see van der Linden et al. 2004).

RGA markers of interest were excised from polyacrylamide gels and reamplified following the same PCR conditions initially used. Fragments were resolved on agarose gels, purified (PCR purification kit, Qiagen), and cloned into pGEM®-T Easy vector (Promega) for sequencing. Inserts were sequenced in the forward and reverse directions. The sequencing reaction was performed as described by Sanger et al. (1977) using DYEnamic™ ET dye Terminator Cycle Sequencing Kit (Amersham Pharmacia Biotech, Inc.) on MegaBACE 1000 (Amersham Biosciences). Sequences were assembled using the Phred/Phrap/Consed package using a phred score ≥20 as the threshold for base quality. Nucleotide sequences were compared to sequences deposited in the Genbank database (http://ncbi.nlm.nih.gov/BLAST) using the BLAST tool (Altschul et al. 1990).

Generation of AFLP markers

AFLP markers were amplified based on the protocol described by Vos et al. (1995) with modifications. Restriction and ligation enzymes required for AFLP analysis were obtained from the New England Biolabs Company. Briefly, 200 ng of genomic DNA from each of the parents and the 74 F_8 RILs were double digested with EcoRI and MseI or PstI and MseI enzymes (5 U of each) in a 25 μl reaction mixture (10 mM Tris–acetate, pH 7.5; 10 mM Mg-acetate, 50 mM K-acetate) during 4 h at 37°C. All resulting fragments were ligated to adapter sequences by adding an equal volume of a ligation solution i.e. 30 mM of Tris–HCl (pH 7.5), 10 mM of MgCl2, 10 mM of DTT, 1.0 mM of ATP, 0.25 μM of EcoRI or PstI adapter, 2.5 μM of MseI adapter, and 80 units of T4 DNA ligase (400 units/μl). These reactions were incubated at 16°C overnight and terminated by heat

inactivation (65°C for 10 min). The adapter-ligated DNA (3 μl) was used for the pre-selective amplification using AFLP primers based on the sequence of the EcoRI or PstI and MseI adapters, with one selective nucleotide at the 3′ end. The pre-selective reaction was amplified using the following conditions: 94°C for 2 min; 26 cycles of 94°C for 60 s, 56°C for 60 s, 72°C for 60 s; and a final elongation at 72°C for 5 min. Five microliters of a 10-fold diluted pre-selected PCR product were used as DNA template for the selective amplification using AFLP primers with three additional selective nucleotides at the 3′ end. The following cycling parameters were used for selective amplification: 94°C for 2 min; 12 cycles of 94°C for 30 s, 65°C for 30 s, 72°C for 60 s; 23 cycles of 94°C for 30 s, 65°C for 30 s, 72°C for 60 s; and a final elongation at 72°C for 5 min.

Genotyping

All markers were resolved in 6% w/v denaturing polyacrylamide gels (19:1; acrylamide:bis-acrylamide) using the electrophoresis apparatus Sequi-Gen® GT (Bio-Rad). Samples (3 μl) of amplified products were denatured with 1× loading buffer (0.2% each bromophenol blue and xylene cyanol, 10 mM EDTA, pH 8.0, and 95% formamide) at 95°C for 5 min before they were loaded onto the gels. The EST-SSR markers were electrophoresed for 2 h 30 min at 70 W, and AFLP and RGA markers for 4 h 30 min at 80 W. The loci were visualized by silver staining according to Creste et al. (2001). AFLP loci were coded using the name of the primer pair followed by the fragment size (bp). RGA loci were coded using the initials of the enzyme and NBS primer names followed by the fragment size (bp). The codes for EST-SSR markers are shown in Table 2.

Map construction

The segregation of each AFLP and RGA locus was scored by the presence or absence of the fragment. EST-SSR as well as rare codominant AFLP loci were scored by identifying the parental fragments in the segregating population. Goodness-of-fit tests for 1:1 segregation ratio were performed for all makers segregating in the 74 F_8 RILs.

For the construction of the map, we adopted the following approach using the software MAPMAKER

Table 2 PvM (*Phaseolus vulgaris* Microsatellites) loci derived from bean expressed sequence tags (EST)

Marker code (Bean unigene)	Repeats motif[a]	Sequence of forward and reverse primers (5′–3′)	Predicted/observed allele (bp)[b]	PCR program[c]	Na/PIC[d]	Linkage group	Amplification in *Phaseolus acutifolius*
PvM010 (PVEPSE3030I07)	ACT5	GAAGGGCAATGGCTGAAG TCCCGTGGAAACACTTGG	256/256	1	Nd	1	
PvM023 (PVEPSE2004G02)	AG5	CCTGTTCCCTCGTGTGAGA AATCAACCCCATCAAACCAA	219/700	2	Mono		N
PvM031 (PVEPSE3030F06)	ATC5	GAGAAGCCAGGAACTTA GCATCCCTTTGTGTC	159/159	3	Nd		
PvM043 (PVEPSE2026A06)	AG10	CGAGCAAAGACATCGGTTT CATTGTAAGCCAGGGAAGG	112/112	4	Mono		N
PvM047 (PVEPSE2022E03)	CA5	AGCACTATGATTTTTGAAT CCTGGGTTGAAGGAAT	205/700	3	Nd		
PvM050 (PVEPSE2025C02)	TC5	GCGTGGGGTCTTTTCTCTCT AACGCATGGAAGTGCAAGTT	206/206	4	Mono		N
PvM051 (PVEPSE2019C07)	CA5	CCAACCACCCTTCTTCTCTCT ATCCGTGCCAACCATCTTT	199/199	4	2/0.22		Y
PvM052 (PVEPSE2018H03)	CA7	TCCTCTGCAAACAAAACCCTA TTCGCCAAATTCGACAAATCT	204/500	4	3/0.62	5	Y
PvM053 (PVEPSE2016A02)	TC6	TGGGGGAATTGAGTGAACAT TCTTTCTTGCGCTTTTCTCTTC	200/200	3	4/0.51	9	Y
PvM054 (PVEPSE2015G11)	TC5	GCATCGTTGTTTCCTTCACA GATTGGAGAGCGGATTGGTA	193/193	3	2/0.09		Y
PvM055 (PVEPSE2014C04)	AT6	TGGTCTTCCTGGAGATGAAAG GCACAAAGTGCCCAAATG	209/209	1	Mono		N
PvM056 (PVEPSE2013B09)	TA6	AGCTGCAGAAAACTTGGAAA TTGAAACCCAGGTCTATTCTCTTC	214/214	3	4/0.62	1	Y
PvM057 (PVEPSE2030D11)	CT5	TCCTCAAATGCTTGGTTTCA CCGGTTCTCACCAGATTGTT	199/199	1	Mono		N
PvM058 (PVEPSE2031B01)	TC5	ATTGGGACGGGGATTG ACTTTTCCTTCTCTCCTCT	151/151	3	3/0.38		Y
PvM059 (PVEPSE2031C12)	CT5	TGAGGCACACTTCGACTACAA ATGGCAAGAACGTGAAAAGG	158/158	2	2/0.38	8	Y

Table 2 continued

Marker code (Bean unigene)	Repeats motif[a]	Sequence of forward and reverse primers (5'–3')	Predicted/ observed allele (bp)[b]	PCR program[c]	Na/ PIC[d]	Linkage group	Amplification in Phaseolus acutifolius
PvM060 (PVEPSE2032B08)	AC5	TTCACATTCTTCACTCACA ATCCCTTGGACGATGAC	161/161	3	2/0.08		Y
PvM061 (PVEPSE2033A05)	AC6	GAAAAAGCTATGGCCACAC CGGTAGAGAATCCAGCGAGA	231/231	2	2/0.39	9	N
PvM062 (PVEPSE2033F05)	TC5	GCACCACTTTCTCAGATTTGG CTGTCATGGCGATGGTGTTA	172/172	3	3/0.48	5	Y
PvM064 (PVEPSE2035G05)	GA6	AGTACCCTACACGTATCACTG GCCACTAGCCCATCTTCT	163/163	1	Nd		
PvM065 (PVEPSE2129G03)	TA5	GAGCGCACAAATGCAATAAT GGGAGGATAAACGACAAATAGG	241/241	1	2/0.09		Y
PvM066 (PVEPSE3001H04)	AT5	CCTCAGCAAGCTCCGTAAAC CATGCAACCTGAAACACTCC	196/196	1	2/0.51	6	Y
PvM067 (PVEPSE3002D09)	GT5	CTGGTGCAGCAAGGTTTTTC TGCGCCAGAATTTACAATACAC	216/216	1	2/0.09		Y
PvM068 (PVEPSE3007B09)	AT6	CCATTACGTGTCCAAGGTCTC CAAAAAGGAATGGACTCTAGG	269/269	3	3/0.51		Y
PvM069 (PVEPSE3008F01)	TC7	CAATAAAAACCGCAGAGAATCC GCGTAAGCAGGTGGGTAAGT	217/217	1	2/0.09		N
PvM070 (PVEPSE3010C09)	AT6	GAAGGGTCATTGTGGAC TCTTTTGTCACTCCTAATCC	161/161	1	Nd	11	
PvM072 (TC144)	TC9	GAAACACAATGGCTCAAGCA CAAACCAGCAGCAACAAGAG	200/200	3	Mono		Y
PvM073 (TC154)	AAG6	CTTCACCGATCTGACAGCAG TTCTCCAGGAACCCCTTCTTT	194/600	4	3/0.44	9	Y
PvM074 (TC241)	GCG5	TGAACACCCTTTCGTCAACTC CCTTCTCCTCCCCTGATTTT	200/450	4	2/0.08		Y
PvM075 (PVEPLE1001E04)	GAT5	ATTGGAAGGGGATGAACCT TAGGAGAGTGCCCAGTGCTT	222/222	4	3/0.44	9	Y
PvM078 (PVEPSE2003D01)	TCT6	CATTACTCCCTTCCCTTCCA ACTTCACTGGCACTCACAGG	174/174	1	Nd	3	

Table 2 continued

Marker code (Bean unigene)	Repeats motif[a]	Sequence of forward and reverse primers (5'–3')	Predicted/observed allele (bp)[b]	PCR program[c]	Na/PIC[d]	Linkage group	Amplification in *Phaseolus acutifolius*
PvM079 (PVEPSE2003F03)	GAA5	TCCGAAACTAACAGAACAAGGA TTATGGACAGTGGACGAACG	191/191	1	2/0.5		Y
PvM080 (PVEPSE2004B12)	CAC5	ACTATCCCAACAACCACCA TCATAGCCTCCACCAGAAGG	204/204	4	Mono		Y
PvM081 (PVEPSE2004F01)	ATG5	TCCCAGTGAAATCTGCTACAT CCAACAATAAAATGGCACAAA	176/176	4	2/0.08		Y
PvM082 (PVEPSE2004G10)	TTA5	TTTCATCTTCAAAACTCTCATCAA ATGGCAGACCGAACTCTCTA	183/183	4	2/0.16		Y
PvM083 (PVEPSE2005C02)	TTC5	AGAAATGAGTGAGCGTGTCG ACGGCTGATAGAGCGGGTA	152/152	1	Mono		Y
PvM084 (PVEPSE3013B07)	TG5	AGAAGGTGAAGTATGATTTA TACATTGATTTTGGGTCA	244/244	1	Mono		N
PvM085 (PVEPSE3015B05)	TA6	ATTCACGGTTAGGATAGAT TGGCTGTCTTGTGTTTCT	111/111	1	Mono		N
PvM086 (PVEPSE3018D04)	AT5	ACCGGGAGAAAAGGAAGAAC GCCAAAAAGTAAGAAGCAGCA	210/250	1	2/0.09		Y
PvM087 (PVEPSE3021G12)	TA5	CATCACGACAGTTTTCC CATTACATTACACCTATTCA	278/278	1	Nd	3	
PvM088 (PVEPSE3022A06)	AG5	AGGTGCAAAAGCAAGAATGC TCCCCAGCAGTATGATGACA	208/208	1	Nd	2	Y
PvM089 (PVEPSE3023B04)	TC5	CGAAGTAGCACGCAGAGAGA GCAAGACGGGAGTGATGG	185/290	1	2/0.08		Y
PvM090 (PVEPSE3023H10)	TC6	CCCTTCACCGCTCCATACT CGGAAACAGTGGCTGGTCTA	250/250	1	2/0.08		Y
PvM092 (PVEPSE3027F14)	TA6	CGTGGGCTACACTGGAAGAC CATCTGCAAATGCTTGCTGT	240/240	1	3/0.35		Y
PvM093 (PVEPSE3029J06)	GA5	TTATATGCGGAGCAAAGGATG GACCGACAAAGCCTTCATTC	216/216	1	2/0.38	2	Y
PvM094 (PVEPSE3030F08)	CT6	AGTCACCGATGCTCTGAAG GGGGTTTAGGGAAAGCAAAA	185/185	1	Mono		Y

Table 2 continued

Marker code (Bean unigene)	Repeats motif[a]	Sequence of forward and reverse primers (5'–3')	Predicted/observed allele (bp)[b]	PCR program[c]	Na/PIC[d]	Linkage group	Amplification in *Phaseolus acutifolius*
PvM095 (TC17)	AC9	GGGCGAGTTCATCTTCACAA / AGGATCAACAGCCTCCAGTG	243/400	1	4/0.69	3	Y
PvM096 (TC18)	CT5	GCCAGTTGTTGCTTGGAAT / GAAGAATGGTTGCTCCCTCA	190/190	1	Mono		Y
PvM097 (TC19)	TA5	CAAGAGTGAAGGGGCAGTTT / CGGCCAACCACTACTTTTAG	155/155	1	3/0.66	1	N
PvM098 (TC70)	TC5	CTCCCTCCTTCACACTCTTTG / GGGGTAGTCCTTTGGAACG	114/114	1	3/0.46	11	Y
PvM099 (TC70)	TC5	CTCTTTGTGTGGCTCCCTGT / GGTGGCATCTTTTTCTACCTCA	203/2000	1	Mono		Y
PvM100 (TC147)	AT7	GCCAATAACATCACCTCAT / GGGTAGTCAGTTTCTAAGCA	290/290	1	4/0.36	2	Y
PvM101 (TC158)	TC6	TCACATACAAGCACATCAACCA / CCAGCAACTCCAGACACAGT	176/660	1	Mono		Y
PvM102 (TC161)	CA5	CGGAAATAGAAACTCAAACTT / AATGGAGAGAGAGCAAGG	259/259	1	Mono		Y
PvM103 (TC168)	AG8	CGAGAAAGAGAGAGAGAAGAGTTT / GTGTTGGTGTGATGCTGAG	145/145	1	3/0.58	8	Y
PvM104 (TC187)	AC5	CCAATCGAAAGACCCAAATC / GATCGCAGAGCCTTCAAATC	191/191	1	2/0.08		Y
PvM105 (TC188)	AT5	CCGTATGAACATCAACCATT / CACAAGAGGATTTCCAACTCT	232/500	1	2/0.29		N
PvM106 (TC195)	AT5	GCTGGTGTGGAGATGATACT / TGTTGATGTTGAAAGGAGTG	219/219	1	Mono		Y
PvM107 (TC198)	AT5	GGAGCCCCTCACCAATAGAC / CGCCCTGGATCGTTAGATAG	347/347	1	Mono		Y
PvM108 (TC202)	CT5	CATCTTGAGTTTATCCCTGCTC / AATTCCAAGGCCATTGACAC	238/238	1	Nd		
PvM110 (TC325)	CT5	CGCACACGCTCTCTCT / CTTCTGCTGCTCCTCAATCTT	202/	2	Nd		

Table 2 continued

Marker code (Bean unigene)	Repeats motif [a]	Sequence of forward and reverse primers (5'–3')	Predicted/observed allele (bp) [b]	PCR program [c]	Na/PIC [d]	Linkage group	Amplification in *Phaseolus acutifolius*
PvM111 (TC427)	TA5	TGGCTGGTAACAACAACTTCA TACGACATGGACCAAAACGA	200/200	1	Mono		N
PvM112 (TC471)	AG5	GGTTCCTGCCCCAGAATC TTGCTGCTTCCGTTTCAGTA	251/251	1	Mono		Y
PvM113 (TC514)	CT5	CTGGTGGAGTTGTGGCTGTA GGATTCTGCTCAAAGGGAGA	180/180	1	Mono		Y
PvM114 (TC529)	CT5	TCTTCTTCTTCCAAGCCTTCC TATGGCAGCAAACGGGTAT	201/1000	1	Mono		Y
PvM115 (PVEPLE1003G09)	CT25	AAATGTAAAGTGCTCCAT CTGAGAGAAAGAAAGAGACA	143/143	2	13/0.91	2	Y
PvM116 (PVEPSE2002D05)	GA5	TATTGCCCAAAGATGCTTCC ATACCCAAAATGCACCCACT	179/179	1	Mono		Y
PvM117 (PVEPSE2004H03)	TC7	CACACATCTCACCTTGTCTCTCTC GCGGCATTCAACACTGCT	165/700	1	Mono		Y
PvM118 (PVEPSE2006F05)	TC8	GCGAACACATTCACACAACA AATCCCGACCAAGTCTCCTT	215/215	1	5/0.54	8	Y
PvM119 (PVEPSE2006H01)	TC9	AGAAGAAGAAGTAGAACAGAATC GCTGGAGTTGGGGTCAGT	226/1100	1	Nd		
PvM120 (PVEPSE2009C06)	TC6	CCCATCCTCACTCACAAAC TCATTCTTCTGGTGCTGCTTT	211/211	1	2/0.39	1	Y
PvM123 (PVEPSE2013B07)	CT9	CCCACTACCACTCCTTTG AGCGTCTGAACCCATTG	194/194	2	4/0.47	1	Y
PvM124 (PVEPSE2013E06)	TA5	TCCCCTACTGATGTGTTC AAATGTCTGGTTTTGC	193/193	2	2/0.39	3	N
PvM125 (PVEPSE2014B09)	AC5	CAAACTCAACACCCTCTCA GGAAACAGCAGCACCAC	198/198	1	Mono		N
PvM126 (PVEPSE2014F08)	TC7	AAATCCTCTTCCACCTTTG AACACGCACACACAGACA	132/132	1	3/0.49	3	N
PvM127 (PVEPSE2015H07)	TC5	AACTTTCTTTGACCCTCTC GCTTTGTCTTGTTCTTCCA	155/155	2	6/0.72	10	Y

Table 2 continued

Marker code (Bean unigene)	Repeats motif[a]	Sequence of forward and reverse primers (5'–3')	Predicted/observed allele (bp)[b]	PCR program[c]	Na/PIC[d]	Linkage group	Amplification in *Phaseolus acutifolius*
PvM128 (PVEPSE2016C01)	GA11	GTTTCTGCGGTGAGAGTG AGCCTTCGTCGTTCTTCT	130/130	1	3/0.43	9	N
PvM129 (PVEPSE2017D09)	GA5	GGTAGGACAACAAGGTAACG AAAACAACGCAGGAAATG	167/167	2	Mono		N
PvM130 (PVEPSE2018H02)	TA5	TGGACAACAACTACACA AAGACATTCACATAATAAGG	265/265	2	2/0.18		N
PvM132 (PVEPSE2018H10)	AG5	GCCGAAGCAGATAAAAGG TGTTCTCGTCTCCAATGC	191/191	2	4/0.51	3	Y
PvM133 (PVEPSE2019C09)	AT5	GAAGTCTCTTGGGTTTCAGA CCCTATTTTTCACTCTCACC	193/193	2	Mono		N
PvM134 (PVEPSE2020C04)	CA5	TCATACTACCTTGTTCTTACATTCA CACGCTGTTCCACCTG	182/182	2	Mono		Y
PvM135 (PVEPSE2005C06)	TTG5	TCTCTCTGTTCTTTTCTGGGTTT CGTTGTCTTTTCTCCGCTTT	151/225	1	Mono		N
PvM136 (PVEPSE2006E08)	TGG5	GCAACTATGCTGGGAAAGGA GGGCATTCTTCAGATTCTCG	217/217	1	2/0.08		Y
PvM137 (PVEPSE2007B06)	TTC6	TTCTCCGTCCCTCCTTGTG GCCTTCCCACCAGGTTTAG	197/197	1	2/0.16		Y
PvM139 (PVEPSE2011H08)	AGC5	AGAGGACGACGATGGATTGGT TCAGAACAAGGGTTTTCG	288/288	2	Mono		N
PvM141 (PVEPSE2013G03)	CCA5	CTTCGTCATCTCCCACAA CCATCATCTCCTTTGGAA	214/214	2	Mono		N
PvM142 (PVEPSE2014H11)	CAG5	CAGATAAATAGCCCTGTGC GCATTCAAACGATGACTCTT	188/188	1	2/0.16		N
PvM143 (PVEPSE2016B08)	ATG5	TCTTCCACTCCTCCACCT CCTAAGCACCCCAAAAAT	183/170	1	Mono		N
PvM145 (PVEPSE2019C10)	TCC5	TTTCAGTTCGGGATTGTTCC ATTGGTGGAGGTGGGAGAG	203/203	1	3/0.16	5	Y
PvM146 (PVEPSE2020D07)	CGC5	ACCATTGACTCCGATGT ACGATAAACCCAATCCACCA	187/400	1	Mono		Y

Table 2 continued

Marker code (Bean unigene)	Repeats motif[a]	Sequence of forward and reverse primers (5'–3')	Predicted/observed allele (bp)[b]	PCR program[c]	Na/PIC[d]	Linkage group	Amplification in *Phaseolus acutifolius*
PvM147 (PVEPSE2020F11)	TCA5	ATTTGCTGTGTTTCTGCTG / GAAGTTGAGGTGGGAGGT	151/151	1	Mono		N
PvM148 (PVEPSE2023B04)	CCA7	ACCTCAAAACCCACCACAAA / GAAGTGCTCCCAGATGAAGG	191/191	1	3/0.48	3	Y
PvM149 (PVEPSE2025B02)	ACG5	ATGACGAGGAACCAGACACC / GTCGCTTCAACGAGGATGTT	213/213	1	2/0.08		Y
PvM150 (PVEPSE2030D10)	TCT5	CTCCCAAGTTCCCCTTTTTC / ATGCGTCCAACCCTTATGTC	199/199	1	2/0.43	11	Y
PvM151 (PVEPSE2031D01)	TTA6	GGGCTGCAAAGGTGACATTA / ACCGAAAACCCATGCTACTC	204/215	1	3/0.29		Y
PvM152 (PVEPSE2032F06)	TTG6	ATTTTGGAGCGAAACAGCAT / GAGAACCTCGTCGTCGTCTT	200/200	1	4/0.36		Y
PvM153 (PVEPSE3002B08)	AGA5	ATGGGCGATCTGGACTATGT / TTGAAGACCAAGCCAAGAGAA	183/270	1	2/0.41	2	Y
PvM154 (PVEPSE3021F08)	TCA5	ATTCACCTCCTTCTGCTTCG / GCTCCTTCTTCTCCAGTCTCG	216/490	1	Mono		Y
PvM155 (PVEPSE3029G07)	AAC5	AAGTATGGAAACTGGGGCTGT / GTGCGTGGAATAATGTGGTG	206/206	2	2/0.08		Y
PvM156 (PVEPSE2021C05)	TTC6	CACACTTCAACTCCAAAGG / CCAACCCTCGCAAAAT	219/1100	2	Nd		Y

[a] Following the repeats motif, the number of repeat units is shown

[b] At least one genotype amplified the observed allele

[c] Detailed in Material and methods

[d] Na number of alleles observed considering all of the bean genotypes (24), PIC polymorphic information content calculated as described in Hanai et al. (2007), Nd not determined as there was no amplification or weak amplification, Mono monomorphic locus in all of the studied bean genotypes

v. 3.0 (Lander et al. 1987). Segregation data here obtained (EST-SSR, RGA and AFLP markers) were merged with available data of markers placed on the framework of the core map (Freyre et al. 1998). Initially, three RFLP markers equally distant were anchored to their respective linkage group using the 'anchor' command. The 'assign' command (LOD > 3) was used to assign all other markers to the linkage groups. In each linkage group, the order of 10 highly informative markers was defined using the 'order' command. Then, the other markers were added to the map using the 'build' command (LOD > 2.0). Loci that could not be ordered with the default log-likelihood threshold value were referred to as accessory markers and placed in the most likely interval. The Kosambi mapping function was used for the calculation of map distances (Kosambi 1944). Map drawings were generated using MapChart (Voorrips 2002). The nomenclature and orientation of linkage groups followed the recent changes adopted by the Genetics Committee of the Bean Improvement Committee (Pedrosa-Harand et al. 2008).

Results and discussion

Allelic variation at EST-SSR loci

Out of 156 primer pairs designed initially, 139 amplified 140 loci of which 40 were analyzed previously (Hanai et al. 2007). The remaining 100 loci were evaluated in the present study using a set of bean genotypes (Table 2). About 13 amplicons were larger than expected, probably due to the presence of introns between primer sites. Nevertheless, only 11 EST-SSR loci presented a non-optimal amplification pattern and were not analyzed. A total of 89 pairs of primers amplified fragments that were easy to score, 54 of which revealed polymorphisms among the 24 bean genotypes. The number of alleles per polymorphic locus varied from 2 to 13 with an average of 2.7. The polymorphic information content (PIC) of the 54 loci ranged between 0.08 and 0.89, with a mean PIC of 0.40.

Our data agree with previous findings that reported an average of 2.9 (Yu et al. 1999) and 3.1 alleles per locus (Guerra-Sanz 2004) as both studies analyzed genic SSR in bean accessions. Generally, SSRs from anonymous sequences are regarded as more informative than SSRs of genic origin. For example, average values of six and seven alleles per locus were described by Gaitán-Solís et al. (2002) and Buso et al. (2006), respectively. The fact that expressed sequences are associated with biological processes and therefore are more conserved than anonymous ones could explain these differences; however, when the same set of bean genotypes were analyzed using SSR loci developed from genic and genomic sources, the results showed similar average values for PIC and number of alleles per locus between genic and genomic SSRs (Hanai et al. 2007).

A considerable number of polymorphic SSR loci is generally required for variety identification or even for pedigree analysis when polymorphisms are used for tracing parentage (Guerra-Sanz 2004). The locus PvM115 containing a CT motif repeated 25 times showed both higher PIC (0.89) and number of alleles (13) in the 24 genotypes analyzed (Fig. 1). In our previous study, 12 alleles were detected at the PvM21 locus (Hanai et al. 2007). Thus, these two EST-SSR loci are very informative and may be useful in both types of analyses.
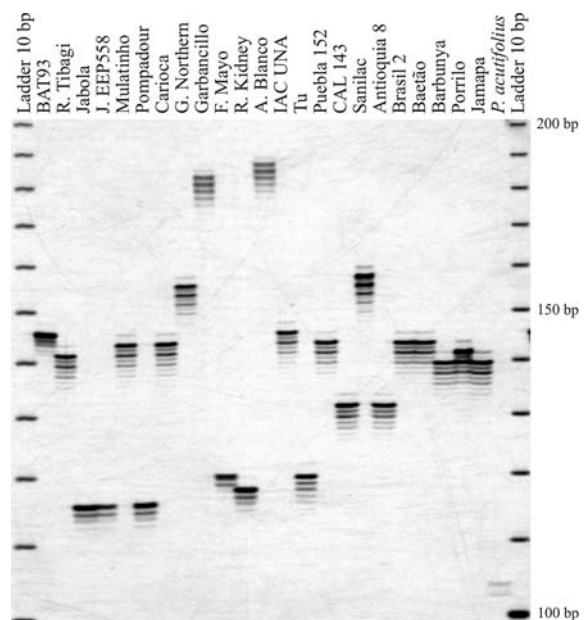

Fig. 1 Electrophoretic pattern at PvM115 locus (EST-SSR) in 24 *Phaseolus* genotypes detected in 6% silver-stained polyacrylamide gel. 10-bp DNA ladder (Invitrogen) are in first and last lane

An updated common bean core map

We screened 'BAT93' and 'Jalo EEP558' for polymorphisms using 156 EST-SSR primer pairs of the PvM series, 16 combinations of restriction enzymes (*Rsa*I, *Hind*III, *Alu*I and *Dra*I) and NBS primers (Table 1) through the NBS-profiling methodology and 28 combinations of AFLP selective primers. A total of 140 EST-SSR primer pairs amplified scorable loci, of which 50 were polymorphic. Regarding the NBS-profiling method, the restriction enzyme *Rsa*I was chosen because it generated better amplification profiles and more polymorphic RGA loci (data not shown). The four NBS primers in combination with *Rsa*I produced 194 loci, of which 32 were polymorphic (Fig. 2). In addition, the AFLP selective primer combinations (12 from *Eco*RI/*Mse*I and 16 from *Pst*I/*Mse*I digestions) amplified 1,252 loci, 29% of them being polymorphic. From these, 15 combinations producing 203 polymorphic loci were selected for genotyping the mapping population (Table 3).

The merging of the data of the 285 loci developed in this study (50 EST-SSR, 32 RGA and 203 AFLP) with the data of 143 markers previously mapped into the core common bean linkage map (Freyre et al. 1998) resulted in a map comprised of 413 DNA markers (Table 4) distributed in 11 linkage groups (LG). About 15 markers remained unlinked. The length of the new linkage map was estimated at 1,259 cM, corresponding to an average of one marker per 3.0 cM. The number of markers per LG ranged from 32 (10) to 50 (8) while the LG lengths varied from 75 (5) to 147 cM (2).

The levels of efficiency of both QTL mapping and selection based on DNA markers is enhanced when regularly spaced markers are available throughout the linkage groups (Lander and Botstein 1989). If the number of markers allocated to map positions is high, existing gaps are more likely to be saturated. With the integration of 282 new markers (50 EST-SSR, 32 RGA and 200 AFLP) into the common bean core map, we were able to place new markers in some

Fig. 2 Amplification pattern of RGA markers in 6% silver-stained polyacrylamide gel. The DNA was digested with *Rsa*I and amplified using the primers AP and NBSa-F. The parental lines are represented in duplicate ('BAT 93', lanes 1 and 2, and 'Jalo EEP558', lanes 3 and 4); lanes 6 through 35 correspond to F$_8$ RILs. *Arrows* indicate polymorphic loci. Lane 5 shows the 25-pb DNA ladder (Invitrogen)
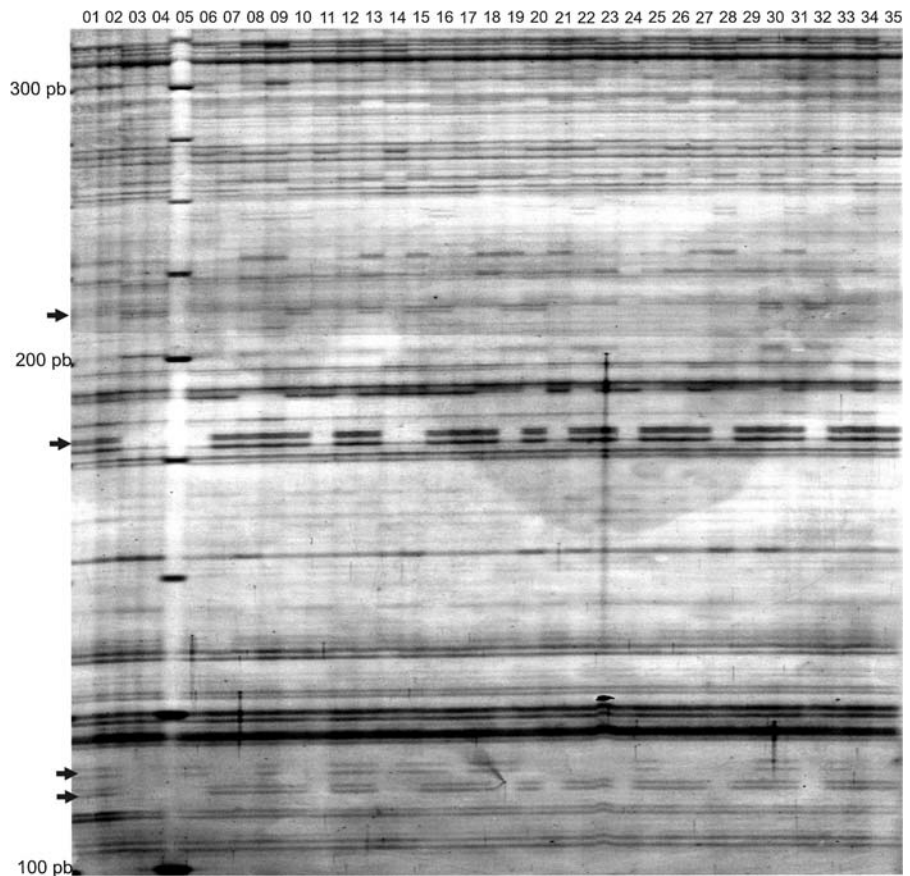
Table 3 Number of polymorphic loci per combination of AFLP primers

Primer combination	Selective nucleotides	Number of polymorphic loci
P31M64	P + AAA, M + GCT	18
P32M63	P + AAC, M + GCA	19
P37M64	P + ACG, M + GCT	5
P39M65	P + AGA, M + GGA	8
P39M63	P + AGA, M + GCA	11
P38M65	P + ACT, M + GGA	4
P32M64	P + AAC, M + GCT	6
E31M48	E + AAA, M + CAC	20
E39M49	E + AGA, M + CAG	10
E40M56	E + AGC, M + CGC	23
E37M60	E + ACG, M + CTC	23
E31M60	E + AAA, M + CTC	18
E40M49	E + AGC, M + CAG	8
E31M49	E + AAA, M + CAG	18
E39M48	E + AGA, M + CAC	12
Total		203

E EcoRI complementary primer, P PstI complementary primer, M MseI complementary primer

existing gaps. In LG 3, for instance, five AFLP, one RGA, and one EST-SSR markers were positioned between markers *Bng12* and *D1151* and in LG 6 three AFLP and one EST-SSR markers were positioned between markers *ROF7a* and *D1086*. Similarly, additional markers were placed in two linkage groups within regions where QTL for resistance to

anthracnose were mapped before (Miklas et al. 2006b): the QTL interval (40–75 cM) in LG 1 was saturated with seven AFLP and two EST-SSR markers whereas the QTL interval in LG 2 (65–95 cM) was saturated with 14 new markers, including 4 RGA (Fig. 3).

Slight discrepancies were observed between the present map and the previous one (Freyre et al. 1998). Linkage groups 2, 6 and 11 became shorter while LG 1, 3, 8, 9 and 10 became longer. These discrepancies are probably due to the size of the mapping population and the kind of markers used. In order to develop a core linkage map and align the existing RFLP maps, Freyre et al. (1998) used a number of markers with more missing data and a smaller population. For instance, the LG 2 of the Freyre's map had 175 cM while the corresponding groups in the Florida and Davis' maps had 128 and 107 cM, respectively (Nodari et al. 1993a; Vallejos et al. 1992). In addition, the present map is about 30 cM longer than the one reported by Freyre et al. (1998), because new markers were positioned at the end of the groups, e.g., LG 10 had eight new loci mapped as terminal markers, expanding its size to more than 36 cM (Fig. 3).

Segregation features

Most of the markers (82.5%) showed a 1:1 segregation ratio of the parental alleles ($P < 0.05$), as expected in a RIL population. Among the newly

Table 4 Distribution of different types of marker over the linkage groups (LG) of the novel common bean core map, and their lengths in centimorgans (cM)

LG	No. of markers previously mapped[a]	No. of novel markers			Total	Length (cM)
		EST-SSR	RGA	AFLP		
1	14	6	3	21	44	129.0
2	15	6	4	14	39	146.7
3	12	7	2	21	42	143.0
4	8	1	3	23	35	99.6
5	15	5	1	18	39	75.2
6	15	2	0	16	33	97.1
7	14	2	4	14	34	105.4
8	11	7	4	28	50	146.1
9	7	7	1	11	26	123.1
10	9	4	5	14	32	108.4
11	11	3	5	20	39	85.2
Total	131	50	32	200	413	1258.8

[a] Markers previously assigned to the framework of the common bean integrated map (Freyre et al. 1998). *EST-SSR* expressed sequence tag derived single sequence repeats, *RGA* resistance gene analog markers based on NBS-profiling method
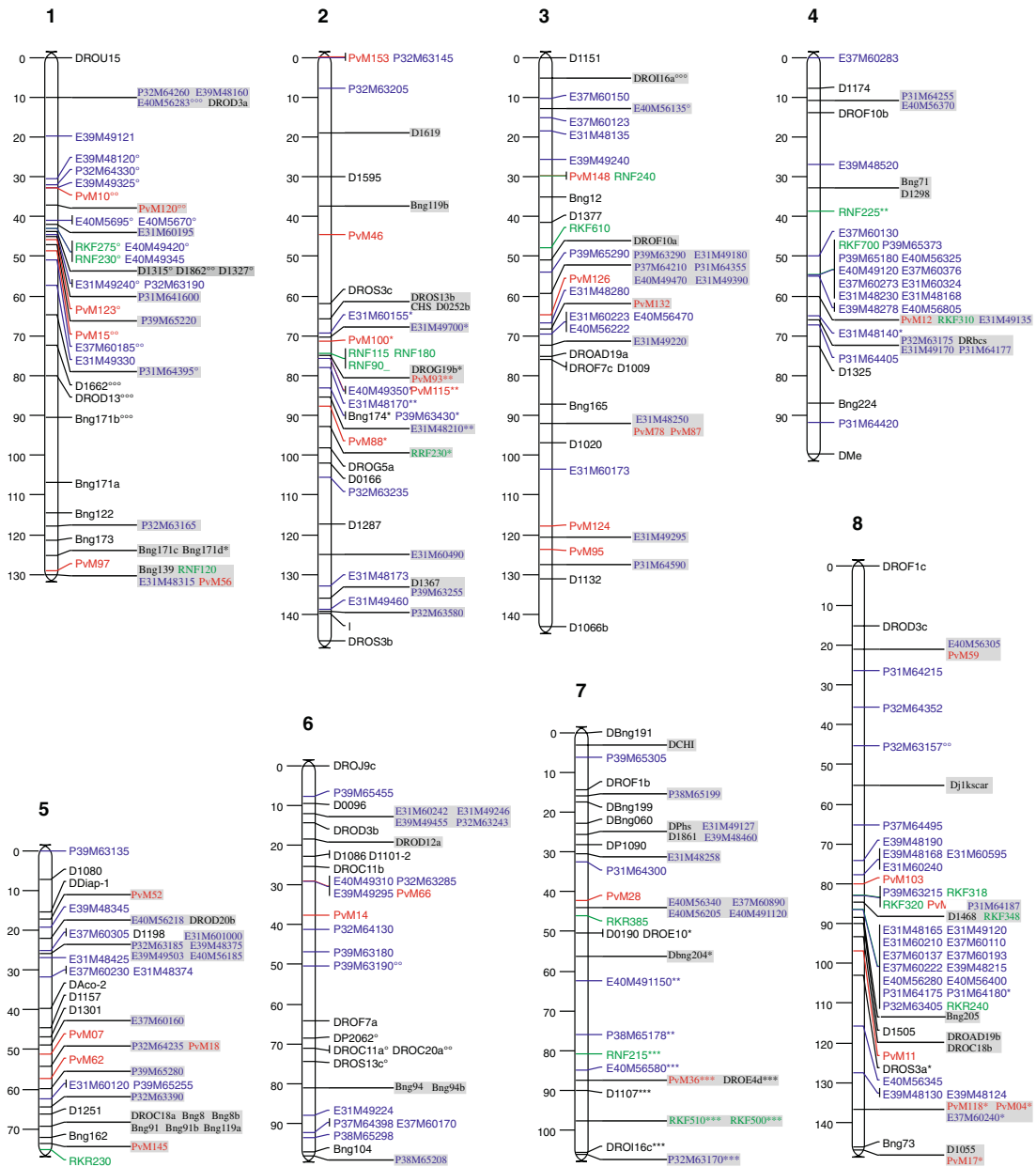
Fig. 3 An updated common bean molecular linkage map based on the 'BAT93' × 'Jalo EEP558' RIL population. The revised orientation and nomenclature of linkage groups follows that of the Bean Improvement Cooperative (http://www.css.msu.edu/bic/Genetics.cfm). About 261 markers were ordered with LOD > 2.0 and their distances (cM) from top to bottom are indicated to the left of linkage groups (*LG*). About 152 markers were assigned to linkage groups but not ordered in

multipoint analyses (LOD > 2.0) and were placed in the most likely interval (*gray boxes*) without changing the map distances. Loci with distorted segregation ratios are indicated by asterisks (*P < 0.05; **P < 0.01; ***P < 0.001 for markers skewed toward 'BAT93') or degree symbols (°P < 0.05; °°P < 0.01; °°°P < 0.001 for markers skewed toward 'Jalo EEE558')

positioned markers, 13 EST-SSR (26%), 7 RGA (22%) and 28 AFLP (14%) showed segregation distortion. Gametes in direct competition, meiotic

drive, and genes controlling pollen lethality are factors that affect allele segregation during meiosis and can produce distorted ratios (Moyle and Graham
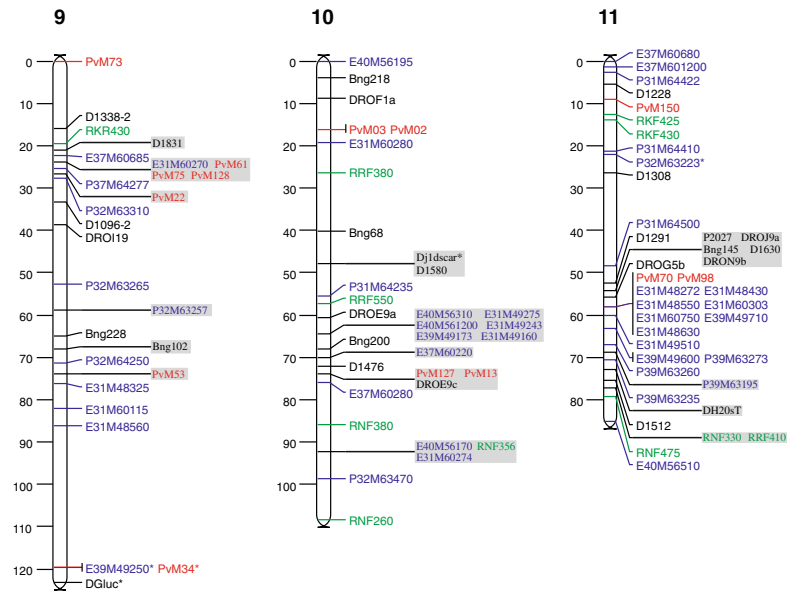
Fig. 3 continued

2006). Among the 28 AFLP loci with distorted segregation, similar numbers were found skewed towards 'BAT93' alleles (15) and 'Jalo EEP558' alleles (13). In contrast, segregation distortion affecting EST-SSR and RGA loci favored 'BAT93' alleles. Nine EST-SSR and five RGA loci showed an excess of 'BAT93' alleles, while four EST-SSR and two RGA loci showed an excess of 'Jalo EEP558' alleles. This could be explained by the higher adaptability of lines carrying 'BAT93' alleles. Since we used SSR markers that are within genes it is possible that their products were affected by natural selection leading to the observed bias in the segregation ratios. Following allelic frequencies of SSR loci in 24 generations, Rodrigues and dos Santos (2006) reported that all loci were affected by natural selection, which favored one of the parental common bean accessions ('Carioca MG') at 29 out of 30 loci.

Interestingly, markers showing distorted segregation were mainly located in six linkage groups. An excess of 'Jalo EEP558' alleles were positioned in two regions located on LG 1 and 6, while an excess of 'BAT93' alleles were mapped in four regions located in LG 2, 7, 8 and 9. The clustering of markers with segregation distortion possibly reflects the location of genes that were favored by either meiotic selection or inadvertent selection in BJ population.

Marker distribution

Table 4 shows the distribution of marker types in the common bean linkage groups together with group lengths (in cM). Non-anonymous EST-SSR and RGA markers were mapped to all linkage groups, excluding LG 6. About 50 EST-SSR markers were ordered with LOD > 2.0 into 40 distinct positions of the 11 linkage groups of the BJ map. Although EST-SSR were positioned on all LGs, their distribution was not uniform and varied from one in LG 4 to seven markers in LG 3, LG 8 and LG 9 (Table 4). Interestingly, LG 4 is the group in which most of the microsatellites studied by Yu and coauthors were mapped (Yu et al. 2000). This illustrates the complementariness of both studies regarding the saturation of the core map with SSR markers.

In general, we did not observe a direct relation between number of markers and length of the LGs. The shortest linkage group (75 cM) had as many markers (39) as the largest one (123 cM), which contained 26 markers (Table 4). This could be explained by the clustering pattern in some regions of the bean genome. Using absolute co-segregation of three or more markers as a criterion for defining a cluster we inferred clustering in seven LGs: 1, 2, 3, 4, 6, 8 and 11, with large clusters (>8 markers) in

groups 4, 8 and 11 (Fig. 3). AFLP markers formed the majority of the clusters. The uneven distribution of AFLP markers derived from double digestion with *Eco*RI and *Mse*I was previously reported in other legume species such as soybean (Young et al. 1999) and adzuki bean (*Vigna angularis*; Han et al. 2005) but also in the yellow passion fruit (Lopes et al. 2006). The preferential localization of AFLP loci in DNA regions of recombination suppression may be associated with the restriction enzymes used, which may favor the finding of these markers in centromeric regions, for example (Troggio et al. 2007), as they may require methylation to maintain their specialized functions. Here, we used the combinations *Pst*I with *Mse*I and *Eco*RI with *Mse*I, as suggested by Young et al. (1999). These authors proposed that *Pst*I, which is blocked by methylation would be more efficient than *Eco*RI in producing AFLP markers placed at random on linkage maps. In fact, out of 51 *Pst*I/*Mse*I-derived markers, only five were found completely linked, two being located in the cluster of LG 4 and three in the cluster of LG 8.

Mapping markers with putative known function

Several EST-SSR showing similarities with known and unknown proteins were mapped in several linkage groups. These include markers with highly significant e-values ($<e^{-30}$) such as PvM61 (LG 9) from a sequence similar to members of the stress-related NAC transcription factor family (Fang et al. 2008); PvM18 (LG 5) from a sequence similar to the leucine-rich repeat (LRR) domain of plant disease resistance genes (Ryan et al. 2007); and PvM87 (LG 3) from an EST similar to subtilisin-like proteases, a class of enzymes involved in recognition of pathogens and activation of defense responses (van der Hoorn and Jones 2004). A complete list of the putative function of ESTs from which SSR markers were developed in this work is presented in Table 5.

Mapping of RGA markers

About 32 RGA loci generated with the NBS-profiling method using four degenerated primers that target the NBS domain were mapped at 26 positions on 10 linkage groups (Fig. 3). The remaining six mapped at exactly the same loci (RKF275 and RNF230 on LG 1; RNF90, RNF115 and RNF180 on LG 2; RKF500 and

RKF510 on LG 7; RKF318 and RKF320 on LG 8; and RNF330 and RNF475 on LG 11). This was expected, as many disease resistance genes occur in clusters as complex loci in many plant species, including common bean. The anthracnose resistance locus Co-4 is such an example as it contains five copies of the *COK-4* gene (Melotto et al. 2004). Vallejos et al. (2006) showed that multiple copies of a gene member of the TIR–NBS–LRR family were present within the *I* locus, which confers resistance to *Bean common mosaic virus*.

At least five RGA markers mapped to regions containing resistance genes for anthracnose and rust diseases. In LG 1 the marker RNF120 co-located with *Co-1*, *Co-x*, *Co-w* and *Ur-9* genes; in LG 7 the marker RKR385 was mapped near to *Co-6* gene; and in LG 11 three markers (RNF330, RRF410 and RNF475) were positioned in the region containing the *Co-2* gene against anthracnose and *Ur-3*, *Ur-11* and *Ur-Dorado* genes against rust (Miklas et al. 2006a). The RGA markers were also positioned in the vicinity of QTLs located on the core map (Miklas et al. 2006a). For instance, markers RKF275 and RNF230 (LG 1) were mapped near to QTLs for resistance to common bacterial blight and white mold. In LG 2, the marker RRF230 was positioned in a region containing QTLs for resistance to common bacterial blight and anthracnose as well as RNF115, RNF180 and RNF90 co-located with a QTL for resistance to white mold. The RGA RNF240 mapped to a region that a QTL for resistance to *Fusarium* root rot was located in LG 3. The marker RKR230 (LG 5) was mapped together a QTL for resistance to white mold, and markers RKF500 and RKF510 (LG 7) co-positioned with a QTL conditioning common bacterial blight. The marker RRF550 (LG 10) was mapped near a QTL for resistance to angular leaf spot, and the markers RNF330, RRF410 and RNF475 (LG 11) were mapped near a QTL region for resistance to anthracnose (Miklas et al. 2006a).

About 16 RGA fragments were sequenced, and five sequences showed high similarity with plant resistance proteins (Table 5). The BLASTx analyses showed that RNF356 and RRF380 sequences are similar to resistance proteins from *Glycine max*. Similarly, sequences of RNF475 and RRF410 were highly similar to resistance proteins from *P. vulgaris* as well as the RNF330 sequence has similarity with a resistance protein from *Medicago truncatula*. Several

Table 5 EST-SSR and RGA clones with similarity (e-value $< e^{-5}$) to protein sequences deposited in GenBank

Marker code (Sequence name)	Linkage group	GenBank access number	Homology	E-value	Organism matched
EST-SSR					
PvM001 (TC34)	8	P25699	Ferritin	4e-67	*Phaseolus vulgaris*
PvM002 (TC116)	10	ABE77731	Conserved hypothetical protein	4e-16	*Medicago truncatula*
PvM007 (TC127)	5	ABA40448	Fructokinase 2-like protein	5e-71	*Solanum tuberosum*
PvM010 (PVEPSE3030I07)	1	NP_194345	Cytokinin-responsive GATA factor 1	1e-9	*Arabidopsis thaliana*
PvM012 (PVEPSE3030L20)	4	ABE81465	C2 domain-containing protein	5e-34	*Medicago truncatula*
PvM013 (PVEPSE3030M09)	10	NP_565320	Proteína ribosomal 40S S17 (RPS17B)	3e-46	*Arabidopsis thaliana*
PvM017 (PVEPSE2030E02)	8	CAA42617	Ribulose bisphosphate carboxylase	8e-41	*Phaseolus vulgaris*
PvM018 (PVEPSE3003E05)	5	NP_188718	Leucine-rich repeat family protein	2e-29	*Arabidopsis thaliana*
PvM022 (PVEPSE2004H10)	9	BAG09558	Chloroplast RNA binding protein	9e-28	*Mesembryanthemum crystallinum*
PvM034 (PVEPSE3027C10)	9	BAA19769	SRC2 cold induced protein	1e-37	*Glycine max*
PvM036 (PVEPSE3011F10)	7	NP_194937	Galactosyltransferase	8e-17	*Arabidopsis thaliana*
PvM046 (PVEPSE2024B07)	2	Q94A43	BES1/BZR1 homolog protein 2	4e-21	*Arabidopsis thaliana*
PvM052 (PVEPSE2018H03)	5	NP_196885.1	Glutaredoxin family protein	7e-05	*Arabidopsis thaliana*
PvM059 (PVEPSE2031C12)	8	CAN61408	Hypothetical protein	1e-44	*Vitis vinifera*
PvM061 (PVEPSE2033A05)	9	ACD39379	NAC domain protein	1e-50	*Glycine max*
PvM066 (PVEPSE3001H04)	6	NP_001077728	Dormancy/auxin associated family protein	3e-12	*Arabidopsis thaliana*
PvM073 (TC154)	9	AAB00554	Dehydrin	3e-21	*Phaseolus vulgaris*
PvM075 (PVEPLE1001E04)	9	CAN73178	Hypothetical protein	7e-25	*Vitis vinifera*
PvM078 (PVEPSE2003D01)	3	CAO64383	Unnamed protein product	2e-28	*Vitis vinifera*
PvM087 (PVEPSE3021G12)	3	AAY54007.1	Subtilisin-like protease	3e-76	*Arachis hypogaea*
PvM093 (PVEPSE3029J06)	2	Q40287	Anthocyanidin 3-O-glucosyltransferase 5	9e-18	*Manihot esculenta*
PvM095 (TC17)	3	P49357	Serine hydroxymethyltransferase 1	2e-66	*Flaveria pringlei*
PvM097 (TC19)	1	AAO91809	Drought induced protein	1e-38	*G. latifolia*
PvM098 (TC70)	11	NP_200264	Hypothetical protein	5e-35	*A. thaliana*
PvM103 (TC168)	8	AAN03469	Ubiquitin-conjugation enzyme	6e-71	*G. max*
PvM118 (PVEPSE2006F05)	8	ABF70090.1	Putative protein kinase	3e-60	*Musa balbisiana*
PvM120 (PVEPSE2009C06)	1	ABO78563	Glycine cleavage system P-protein	7e-06	*M. truncatula*
PvM124 (PVEPSE2013E06)	3	AAM63014.1	Unknown	5e-27	*A. thaliana*
PvM127 (PVEPSE2015H07)	10	CAN64365	Hypothetical protein	2e-07	*V. vinifera*
PvM148 (PVEPSE2023B04)	3	AAW28572.1	Putative oxygen evolving enhancer protein 3, identical	1e-38	*Solanum demissum*

Table 5 continued

Marker code (Sequence name)	Linkage group	GenBank access number	Homology	E-value	Organism matched
PvM150 (PVEPSE2030D10)	11	NP_191557.1	tRNA-binding region domain-containing protein	3e-47	*A. thaliana*
PvM153 (PVEPSE3002B08)	2	AAL50980	Bax inhibitor-like protein	5e-13	*Brassica oleracea*
RGA					
RNF330 (RNBSaF330)	11	ABE86051.1	Disease resistance protein	9e-25	*Medicago truncatula*
RNF356 (RNBSaF356)	10	AAC49509	Disease resistance protein homolog	2e-16	*Glycine max*
RNF475 (RNBSaF475)	11	AAC33296.1	NBS type putative resistance protein	3e-74	*Phaseolus vulgaris*
RRF380 (RRGPF380)	10	AAF44094.1	Disease resistance-like protein	9e-45	*Glycine max*
RRF410 (RRGPF410)	11	ABN42886.1	Resistance protein	1e-42	*Phaseolus vulgaris*
RKF310 (RKDCF310)	4	ABD28710	Polynucleotidyl transferase, Ribonuclease H fold	2e-7	*Medicago truncatula*
RKR240 (RKDCR240)	8	AAR13317	gag-pol polyprotein	2e-7	*Phaseolus vulgaris*

tagging approaches in candidate genes have been used for analyzing resistance genes in crop species, including common bean (López et al. 2003; Miklas et al. 2006b; Rivkin et al. 1999). Here we demonstrate the feasibility of NBS-profiling methodology for generating RGA loci as well as a complementary tool for tagging RGA that were not yet mapped in the common bean genome.

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