Molecular Ecology Notes (2006) **6**, 252–254 doi: 10.1111/j.1471-8286.2006.01210.x

PRIMER NOTE

Microsatellite markers for the common bean *Phaseolus vulgaris*

G. S. C. BUSO,* Z. P. S. AMARAL,* R. P. V. BRONDANI† and M. E. FERREIRA*‡
*Embrapa Recursos Genéticos e Biotecnologia — Laboratório de Genética Vegetal, C.P. 02372 Brasília — DF 70770-900, Brazil, †Embrapa Arroz e Feijão — Laboratório de Biotecnologia, C.P. 179 Goiânia — GO 74001-970, Brazil, ‡Universidade Católica de Brasília — SGAN 916 Módulo B Brasília DF, Brazil

Abstract

Efforts to develop molecular tools for genetic analysis and breeding of common bean in the tropics are still limited. The number of microsatellite markers available for the crop is small compared to other crops of similar social and economic importance. As part of a project to broaden the use of molecular tools in bean breeding, a genomic library enriched for AG/TC repeat sequences was constructed for *Phaseolus vulgaris*. Twenty microsatellite markers were initially developed and 10 were characterized using a panel of 85 representative accessions of the bean gene bank. The number of alleles per marker ranged from three to 10. The polymorphism information content (PIC) varied from 0.23 to 0.80. The results indicate that the new markers can be readily used in genetically analysis of common bean.

Keywords: common bean, conservation, *Phaseolus vulgaris*, SSR

Received 6 July 2005; revision received 7 September 2005; accepted 17 October 2005

Common bean is a staple food in a great number of developing countries, where it is used daily as a primary source of proteins. The average yield of bean varieties cropped in developing countries is still very low. The variables limiting yield include diverse cultivation systems, restricted improved cultivar adoption and susceptibility to biotic and abiotic stresses. The narrow genetic base of breeding programs has been limiting the rate of expected genetic improvement of several crops (Tanksley & McCouch 1997), and this is probably true for common bean as well. The rational use of germplasm stored in gene banks as a source of genetic variability for breeding programs can certainly impact the development of higher yielding cultivars. A prerequisite for the use of the genetic resources is the detailed knowledge of the extension and distribution of genetic variability of the cultivated species and their wild relatives. This can be accomplished by several means, including the employment of technology based on DNA sequence polymorphism. However, efforts to develop molecular tools for the genetic analysis and breeding of common bean are still limited when compared with human and economic resources allocated to the research of other commercial crops. There is therefore a great need to develop molecular tools for genetic studies of beans. Among them, molecular markers such as simple sequence repeats (SSRs), are highly desirable, since they can be used for in-depth genetic analysis. Here we report the development and the characterization of new microsatellite markers for this economically valuable species.

Correspondence: G. S. C. Buso, Fax: (61) 3340-3624; E-mail: buso@cenargen.embrapa.br

Microsatellites are short tandem repeats of DNA, generally distributed throughout the genome of an organism. The polymorphism is based on the number of repetitions of a short DNA motif at a given locus. Microsatellite markers are becoming the markers of choice in many plant breeding programs. They are polymerase chain reaction (PCR)-based, codominants, easily reproducible, highly multiallelic and can be genotyped on semi-automated DNA sequencers using multiplex assays, allowing for high throughput DNA typing. Today, around 180 SSR markers are available for common bean, half of them obtained from gene sequences deposited in GenBank (Yu *et al*. 2000). These markers are usually less informative than SSR markers obtained from enriched genomic libraries (Gaitán-Solís *et al*. 2002). Although the cost involved in library construction is relatively high, the polymorphism observed with markers obtained by this procedure is usually worthwhile (Rafalski *et al*. 1996; Brondani *et al*. 1998; Ritschel *et al*. 2004). Given the simplicity and specificity of SSR-based assays and the ease with which the widely reproducible typing of an

Table 1 A battery of 20 new microsatellite markers for genetic analysis of *Phaseolus vulgaris* developed from a genomic library enriched for AG/TC repeat sequences. A sample of 85 accessions of beans were used to estimate annealing temperature (T_a), the total number of alleles per locus (A), observed heterozygosity (H_O), expected heterozygosity (H_E), and Hardy–Weinberg equilibrium (HWE)

Locus/GenBank no	Primer sequences (5′–3′)	Repeat array	Allele size (bp)	T_a	A	H_O	H_E	HWE	PIC
PVBR1/ DQ185872	gCAgAggTTCTACCTgATCg TggTTATCAACTggCTCTCC	$(AG)_{10}$	404	56	—	—	—	—	—
PVBR2/ DQ185873	CTAACCgCCATCAACATAgC CgCACTgAAgTCAATggTCC	$(CT)_{10}T(CT)_4$	236	56	—	—	—	—	—
PVBR4/ DQ185874	CAATTCTAGCAAGGACGAACG AAACATTGTGACCGCATCCT	$(CTTTG)_3$	159	56	—	—	—	—	—
PVBR5/ DQ185875	ATTAgACgCTgATgACAgAg AgCAgAATCCTTTgAgTgTg	$(GA)_{22}$	195	56	6	0.00	0.77	**	0.73
PVBR 6/ DQ185876	ggCATAAAgAggAATCAAAg CgCAgCTgTgTTCTCTACg	$(CA)_5$	247	56	—	—	—	—	—
PVBR 7/ DQ191409	AATggCAggTCAgTgAAAC ATgACCACgCAgTgACAgAg	$(AG)_{12}$	306	56	—	—	—	—	—
PVBR 9/ DQ185877	CAACCCATACAAATCATCC gCTAATCCATTCTCgAAg	$(CT)_{19}$	356	43	—	—	—	—	—
PVBR10/ DQ185878	ACAACAACAgAgAATCTCTC AAggATgAgAAggAggAC	$(AG)_{23}$	88	54	6	0.00	0.24	NS	0.23
PVBR11/ DQ185879	AAACTCAAAgTCgTTgTTCC CCACTgACTCTAgCTCCTCC	$(TC)_8(GT)_4$	142	56	3	0.02	0.49	**	0.44
PVBR12/ DQ185880	AAgTCTCCACCTTTCAAATgC ACgCCATCCACCATCCTT	$(CTTT)_4(CT)_{28}$	193	60	—	—	—	—	—
PVBR14/ DQ185881	TgAgAAAgTTgATgggATTg ACgCTgTTgAAggCTCTAC	$(AG)_{23}$	196	56	7	0.01	0.83	**	0.80
PVBR15/ DQ185882	CCTACgCgACAAgATTggCA CAgAAAggAAACATAAAC	$(CT)_{23}$	191	50	—	—	—	—	—
PVBR16/ DQ185883	AAgCAACAgAAAAgCAgAAgAC TTgTggTgTTgAgTgTggTg	$(GA)_{28}$	198	56	5	0.00	0.59	**	0.51
PVBR18/ DQ185884	gTTCTgCTTgCAgCATACC AgAAACACAATCggAAgAg	$(CT)_{29}$	198	46	10	0.00	0.52	**	0.50
PVBR19/ DQ185885	gAgAgggAggCATAAAgAgg TgCACTCCgACgATCAAgTT	$(AC)_6$	159	45	—	—	—	—	—
PVBR20/ DQ185886	TgAgAAAgTTgATgggATTg TACgCTgTTgAAggCTCTAC	$(AG)_{22}$	197	56	10	0.00	0.81	**	0.78
PVBR21/ DQ185887	gAAgAACCgCAAgTAgAgAAgT TAACATCAgACgCCgACgA	$(AG)_{18}$	229	56	7	0.05	0.59	**	0.55
PVBR23/ DQ185888	gAACggAgACATAACAgCCA gAggACCAACCAAACCAAgC	$(AG)_{19}$	345	56	6	0.00	0.60	**	0.57
PVBR24/ DQ185889	ggTAACCAAgCTAAAgAC CTCTACCTCAACAAATAgTg	$(AG)_{25}$	250	55	—	—	—	—	—
PVBR25/ DQ185890	gAgCTTCTCCgTCCTgTgT CgAACTgAATCAgAAAggAA	$(CT)_{23}$	158	56	9	0.00	0.78	**	0.74

The empty cells for genetic diversity parameters means that the loci were amplified but were not polymorphic for the sample analysed. NS, not significant; **$P < 0.01$, and polymorphic information content (PIC) of 10 molecular markers.

allele at a locus can be carried out, a collection of microsatellite primers were obtained in this study based on the development of microsatellite-enriched libraries.

Initially, an enriched genomic library from a single individual plant of *Phaseolus vulgaris*, Perola cultivar, was constructed with *Tsp 509*-digested DNA, following protocols described by Brondani *et al*. (1998). Recombinant clones in plasmid vectors were transformed into *Escherichia coli* cells, and colonies having SSR were identified by poly AG/TC probe hybridization and anchor PCR. Positive clones were picked and sequenced on an ABI 377 DNA sequencer (Applied Biosystems) using dye terminator fluorescent chemistry. Primers annealing at the SSR flanking regions were designed using the software PRIMER (Lincoln *et al*. 1991).

A sample of 286 SSR positive recombinant colonies screened by hybridization were considered for this study, of which 145 were selected and 100 were found informative after anchor PCR analysis (Rafalski *et al*. 1996). A subsample of 91 clones were sequenced and 28 showed sufficient adjacent DNA sequence for SSR primer design. A total of 20 primer pairs were finally synthesized and their amplification conditions tested (Table 1).

Microsatellite amplification was performed in a 13-μL reaction mix containing 0.9 μM of each primer, 1 U *Taq* DNA polymerase, 200 μM of each dNTP, 1% reaction buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$), DMSO 50% and 10.0 ng of template DNA. Amplifications were performed using a PT-100 thermal cycler (MJ Research) with the following conditions: 96 °C for 2 min (1 cycle), 94 °C for 1 min, 54 or 56 °C for 1 min (according to each primer pair annealing temperature), 72 °C for 1 min (30 cycles), and 72 °C for 7 min (1 cycle). The amplified products were separated in 4% denaturing polyacrylamide gels stained with silver nitrate (Bassam *et al*. 1991) and sized by comparison to a 10-bp DNA ladder. Following microsatellite marker screening by silver nitrate detection in polyacrylamide gels, 10 loci were selected for further characterization based on the level of allelic variation, low stuttering and band quality using a sample of the 85 bean accessions from the germplasm collection. The data allowed for the estimation of the polymorphism information content (PIC) of the markers, calculated as the probability that an individual is informative with respect to the segregation of its inherited alleles (Botstein *et al*. 1980). All but two of the microsatellite markers consisted of nucleotide motifs of the type used for enrichment. Eighty five per cent of the microsatellites found were perfect dinucleotide repeats, 5% imperfect and 10% compound (Table 1). The number of alleles per locus ranged from three to 10, with mean of seven alleles per locus. The PIC values ranged from 0.23 to 0.80 (Table 1). Most of these markers should be useful for common bean germplasm characterization and construction of linkage maps. The development of molecular tools will greatly contribute to the genetic analysis and marker-aided selection of the common bean.

Acknowledgements

The authors would like to thank the Ministry of Education CAPES master and doctoral fellowship to RPVB.

References

Bassam BJ, Caetano-Anolles G, Gresshoff PM (1991) Fast and sensitive silver staining of DNA in polyacrylamide gels. *Analytical Biochemistry*, **196**, 80–83.

Botstein D, White RL, Skolnick M, Davis RW (1980) Construction of a genetic linkage map in man using restriction fragment polymorphism. *American Journal of Human Genetics*, **32**, 4–33.

Brondani RPV, Brondani C, Tarchini R, Grattapaglia D (1998) Development, characterization and mapping of microsatellite markers in *Eucalyptus grandis* and *E. urophylla*. *Theoretical Applied Genetics*, **97**, 816–827.

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

Lincoln SE, Daly MJ, Lander ES (1991) *PRIMER Software*. Available from E. Lander, Whitehead Institute, Cambridge, Massachusetts.

Rafalski JA, Vogel JM, Morgante M, Powell W, Andre C, Tingey SV (1996) Generating and using DNA markers in plants. In: *Analysis of Non-Mamalian Genomes: A Practical Guide* (eds Birren B Lai E), pp. 75–134. Academic Press, New York.

Ritschel PS, de Lins TCL, Tristan RL, Buso GSC, Buso JA, Ferreira ME (2004) Development of microsatellite markers from an enriched genomic library for genetic analysis of melon (*Cucumis melo* L.). *BMC Plant Biology*, **4**, 9–23.

Tanksley SD, McCouch S (1997) Seed banks and molecular maps: unlocking genetic potential from the wild. *Science*, **277**, 1063–1066.

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.). *Journal of Heredity*, **91**, 429–434.