Notas Científicas

Microsatellites for genetic studies and breeding programs in common bean

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Abstract – Twenty microsatelitte loci were identified and characterized in common bean. Microsatellites were tested in 14 genotypes. The allele number ranged from 1 to 3, and the polymorphism information content (PIC) was between 0.14 and 0.65. These polymorphic markers are available to be used for breeding programs.

Index terms: *Phaseolus vulgaris*, SSR, molecular markers.

Microssatélites para estudos genéticos e programas de melhoramento em feijoeiro

Resumo – Vinte locos de marcadores microssatélites foram identificados e caracterizados em feijoeiro. Os microssatélites foram testados em 14 genótipos. O número de alelos variou entre 1 e 3, e o conteúdo informativo de polimorfismo (PIC) entre 0,14 e 0,65. Esses marcadores polimórficos estão disponíveis para serem usados em programas de melhoramento.

Termos para indexação: *Phaseolus vulgaris*, SSR, marcadores moleculares.

Common bean (*Phaseolus vulgaris* L.) is the principal leguminosae used for human nourishment, mainly in South America and Africa, where it represents an important source of protein. Brazil detains the most important productivity and consumption of common bean in the world. However, this crop has not reached high productivity (Silva, 2000). Common bean occurred in two centers of origin in South and Central America, comprising two major gene pools, Andean or large-seeded bean type, and Mesoamerican or small-seeded type (Gepts & Debouck, 1991).

Microsatellites or simple sequence repeats (SSR) are polymerase chain reaction (PCR) based markers developed, for a wide range of plant species, around short segments of DNA, in which a specific motif of one to six nucleotides is repeated in tandem and distributed over the euchromatic part of the genome (Morgante & Olivieri, 1993; Powell et al., 1996). In common bean there are about 200 available SSR markers (Yu et al., 2000; Gaitán-Solís et al., 2002; Métais et al., 2002; Yaish & Pérez de la Vega, 2003; Buso et al., 2006), a small number when compared with other crops, like soybean (Cregan et al., 1999).

Microsatellite markers are powerful tools but their development is expensive and labour intensive. Consequently, many researchers have tried to use primer pairs developed for one species in another (Cipriani et al., 1999), attributing knowledge as transferability or cross-species amplification.

The variety IAC-UNA was used to construct a microsatellite enriched library for two dinucleotide repeat sequences (CT and GT). This enrichment was based on the procedure described by Billotte et al. (1999). IAC-UNA is a black-seeded variety, developed by Agronomic Institute (IAC, Campinas, SP, Brazil), resistant to anthracnose and susceptible to bean rust, *Fusarium* and angular leaf spot.

The genomic DNA was extracted from leaf tissue using a CTAB extraction method, as described by Hoisington et al. (1994). The extracted DNA was

digested with *Rsa*I restriction enzime, and the digested fragments were linked to *Rsa*I adapters. The library was enriched for dinucleotide sequences using $(CT)_8$ and $(GT)_8$ biotinylated microsatellite primers with labelled probes that were bound to Streptavidine MagneSphere Paramagnetic Particles as described by manufacturer.

Selected fragments were amplified by PCR using primer sequences complementary to the adapters, and then, attached to the vector pGEM-T (Promega). Plasmids were introduced into XL-1 Blue cells. Transformed cells were cultivated on agar plates containing 100 μg mL^{-1} of ampicilin and 50 μg mL^{-1} of X-galactosidase. Single white colonies were transferred onto microplates for long-term storage at 80ºC. Sequencing of the inserts was performed using the ABI 377 Big Dye Terminator and 20 sequences containing microsatellites were selected to have primer pairs designed.

Primer designing was performed using Primer Select with the following conditions: amplification DNA size from 150 pb to 350 pb; GC content between 40–60%; temperature annealing (*Ta*) between 45 and 60ºC; primer length between 18 and 22 pb; no hairpins or dimmers.

Twenty primers were designed for microsatellite loci, and were selected for characterization using 14 accessions from the IAC Germplasm Bank, including Andean (A) and Mesoamerican (M) gene pools. Total DNA was extracted from the following accessions: 'Sanilac' (M), 'Baetão' (M), 'Red Kidney' (A), 'Jamapa' (M), 'Flor de Mayo' (M), 'Tu' (M), 'Carioca Comum' (M), 'Jabola [CB]' (A), 'Fradinho Cruzeiro' (*Vigna* spp.), '87-JP-12' (A), 'BAT-93' (M), 'Jalo EEP-558' (A), 'IAC-UNA' (M), 'CAL-143' (A).

PCR reactions were carried out in a total volume of 25 μL containing 50 ng of template DNA, 0,8 μM of forward and reverse primer, 100 μM of each dNTP, 1,5 mM $MgCl_2$, 10 mM Tris-HCl, 50 mM KCl, and 0.5 U *Taq* DNA Polymerase (Invitrogen). Reactions were performed using the following conditions: 1 min at 94ºC; then, 30 cycles of [1 min 94ºC, 1 min at specific T*a*, 1 min at 72ºC], followed by 5 min at 72ºC. Amplification products were checked by electrophoresis on 3% agarose gels, and then loaded on 6% w/v denatured polyacrylamide gels using a 10 bp ladder (Invitrogen) as a size standard. After each run, gels were silver stained according to Creste et al. (2001).

The polymorphism information content (PIC) value was calculated by the following formula:

$$PIC = 1 - \sum_{i=1}^{n} f_i^{\,2} - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2 f_i^{\,2} f_j^{\,2}$$

in which n is the number of alleles; f_i and f_j are the frequencies of the i^{th} and j^{th} alleles, respectively (Botstein et al., 1980). Polymorphisms were observed among the amplified alelles.

Fifteen loci were polymorphic, four were monomorphic and one was unable to amplify among the twenty analyzed microsatellites (Table 1). Thirteen primer pairs were perfect dinucleotide motifs, one was imperfect (*FJ 20*), and six presented compound motifs. Two loci could separate Andean from Mesoamerican accessions; showing two different alleles, each one associated to a genetic pool (*FJ 05* and *FJ 17*). These SSRs would be tested in other genotypes, considering that they can be a tool to separate in domestication centers.

The number of alleles ranged from one to three, with an average of 2.07 alleles per locus. Yu et al. (2000) found an allele range that varied from 2 to 10, for a 24 polymorphic SSR evaluation in 12 genotypes. PIC values ranged from 0.14 to 0.65. The highest PIC (0.65) was found in *FJ 14*, that presented the greatest repetition motif, $(GA)_{10}$, and the most elevated number of alleles (3). Métais et al. (2002) published a range of 0.12 to 0.72 for PIC values, with an average of 0.44, when evaluating 15 polymorphic SSRs in 45 different bean lines of nine different quality types.

The primer pairs were also used to amplify one genotype of *Vigna* spp., accession 'Fradinho Cruzeiro', and one genotype of *Phaseolus lunatus*, accession 87-JP-12, using the same conditions optimized for *Phaseolus vulgaris* accessions. The efficiency of heterologous amplification was 100%. However, it would be necessary a resequencing of the amplification products to check if they were really related to the original sequence from which primer pairs were developed. Even so, this shows a considerable level of sequence, conservation within the primer regions flanking microsatellite loci. These results suggest that the new microsatellites reported in this paper could be used for synteny studies, establishing the conservation of genes between species such as *Phaseolus lunatus* and *Vigna* spp.

In common bean, most of the molecular markers used for genetic studies and breeding programs are RAPD

Table 1. Primer sequences and characteristics of 20 common bean (*Phaseolus vulgaris*) microsatellite markers, obtained from variety IAC-UNA, tested on 14 accessions, comprising Mesoamerican and Andean gene pools.

Locus/ GeneBank accession	Primers (5′→ 3′)	Repeat motif	Na[1]	Ta[2] (ºC)	Size of cloned alelle (size range) in bp	PIC[3]
FJ 01/ DQ469376	GTCGCCGCTACTTCTTTGTT TTTTAATGTTGTGGGAGTGATG	$(AC)_7$	2	60	270 (265-270)	0.51
FJ 02/ DQ469377	GGTCCACAATCAAGCAGTCA TATGGAACCTGATAGCAAGTG	$(AC)_{10}$ $(AT)_5$	2	51,4	251 (258-260)	0.52
FJ 03/ DQ469378	TTCGCGAGCAAGCAACTA TGAATGTTTTAAATGCGTTGAA	$(GT)_6$	1	45	178 (177)	0.00
FJ 04/ DQ469379	ATAGATGAAGGATTGGGAGAG GGGAAATTGAAGAGGAGATAC	$(AG)_8$	1	45	216 (218)	0.00
FJ 05/ DQ469380	AAGAAACAGAAACAATAAAAAC TTTCCATTTATTTTCAGTCACA	$(CT)_2$ $(CA)_6$	2	60	212 (220-222)	0.48
FJ 06/ DQ469381	TTGGAACACCGTGGAATGGA GAGGCTTTAGACGTTGGAGACA	$(GT)_7$	0	60	152 (-)[4]	0.00
FJ 07/ DQ469382	GAAAACGCGAAACAACCGA ATGTCTCCAAATCCCAAGTG	$(CA)_8$	2	60	290 (283-297)	0.52
FJ 08/ DQ469383	ATGGTCATGGTATCAGTTCA TCTTTTCCATAGTATTCTCTTG	$(CA)_6$ $(TA)_3$	1	60	195 (195)	0.00
FJ 09/ DQ469384	ACCTTAGATAGTGCTTGTTAGAG CATGACACCTAGGGCAAA	$(TG)_6$	1	45	155 (153)	0.00
FJ 10/ DQ469385	AGGGGAGTTGTGTTCTTAC ATACGTACGAGTGACTGGAGA	$(TG)_6$	2	45	209 (207-209)	0.14
FJ 11/ DQ469386	AAAAGGATCAAAGAGGAGAAAT GGGCAAGTAAAGCTAAACGAG	$(CA)_5$	2	60	297 (308-310)	0.25
FJ 12/ DQ469387	TATCAGCCTAGTTATTTTCAAG CATACTTTTCTTATTTTCTGGA	$(CA)_7$	2	60	256 (255-258)	0.25
FJ 13/ DQ469388	TCGATGCAGGATTGGATT CAGGTTGATTGTGATAGGTTAC	$(AC)_9$	2	60	266 (267-269)	0.48
FJ 14/ DQ469389	TTCATGGCAAGGTAAGTAAATA TGAATGAACACAACACAACAA	$(AG)_{10}$	3	60	148 (145-155)	0.65
FJ 15/ DQ469390	AGAATGGAGGGAAAAGCAAAAG CCGAAGTCCAAGATTAGAAGCC	$(ATGAG)_4$ $(GT)_3$	2	60	191 (200-205)	0.14
FJ 16/ DQ469391	TGGTGCTACAACAAAAGAGAAT TAGGCATGTGGGTAGGTCAG	$(TA)_6$ $(TG)_7$	2	60	284 (280-300)	0.20
FJ 17/ DQ469392	TCCCGATTTATAGTTCTCATTT AGGGACCTCCTTCATCTC	$(TG)_8$ $(TA)_3$	2	60	222 (220-230)	0.48
FJ 18/ DQ469393	CATTGAGATTTGAGGTTTCGTT AGGTATTTCCATCGTGCTTTTC	$(TG)_5$	2	60	224 (224-230)	0.48
FJ 19/ DQ469394	ATGTTAGTGCCTTATTTCTCT AAGGTAGGGTTGGGATTGT	$(CA)_7$	2	60	205 (210-222)	0.51
FJ 20/ DQ469395	TTGGAACACCGTGGAATGGA GAGGCTTTAGACGTTGGAGACA	$(AG)_3$ AA $(AG)_3$	2	60	251 (250-263)	0.51

[1]Number of alleles. [2]Temperature annealing. [3]Polymorphism information content. [4]No amplification.

markers, which are dominant, and not reproducible. SSR are codominant, more polymorphic and stable. These new informative microsatellites are an available tool in common bean research. They consist in important source of polymorphism which can be used in breeding programs or in genetic studies, as genetic and QTL mapping, marker-assisted selection and germplasm characterization in common bean.

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