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Research Article

# Genetic diversity in cultivated carioca common beans based on molecular marker analysis

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## Abstract

A wide array of molecular markers has been used to investigate the genetic diversity among common bean species. However, the best combination of markers for studying such diversity among common bean cultivars has yet to be determined. Few reports have examined the genetic diversity of the carioca bean, commercially one of the most important common beans in Brazil. In this study, we examined the usefulness of two molecular marker systems (simple sequence repeats – SSRs and amplified fragment length polymorphisms – AFLPs) for assessing the genetic diversity of carioca beans. The amount of information provided by Roger's modified genetic distance was used to analyze SSR data and Jaccards similarity coefficient was used for AFLP data. Seventy SSRs were polymorphic and 20 AFLP primer combinations produced 635 polymorphic bands. Molecular analysis showed that carioca genotypes were quite diverse. AFLPs revealed greater genetic differentiation and variation within the carioca genotypes (Gst = 98% and Fst = 0.83, respectively) than SSRs and provided better resolution for clustering the carioca genotypes. SSRs and AFLPs were both suitable for assessing the genetic diversity of Brazilian carioca genotypes since the number of markers used in each system provided a low coefficient of variation. However, fingerprint profiles were generated faster with AFLPs, making them a better choice for assessing genetic diversity in the carioca germplasm.

*Key words:* AFLPs, genetic structure, genetic variability, *Phaseolus vulgaris* L., SSRs.

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## Introduction

The genus *Phaseolus* originated in the Americas and contains 55 species, five of which are widely cultivated: *P. vulgaris* L., *P. lunatus* L., *P. coccineus* L., *P. acutifolius* A., Gray var. *latifolius* Freeman and *P. polyanthus* Greenman (Debouck, 1993). Of these five species, the common bean (*Phaseolus vulgaris* L.) is the most important because its high protein content has made it the major staple food crop in Africa and Latin America (Yu and Bliss, 1978).

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The cultivated common bean has two major gene pools and several races within these pools (Beebe *et al.*, 2000). The carioca cream-striped grain type belongs to the Mesoamerican gene pool and shows marked genetic variability that accounts for many traits such as disease resistance. The common bean is essentially an autogamous species with breeding based on self-crossings and the identification of segregant populations from which superior lines are selected. For this reason, it is important to know the extent of parental genetic dissimilarity in order to direct future crosses and enhance the chances of recovering superior genotypes in segregating generations (Gepts and Debouk, 1991).

In 1967, the Agronomic Institute (IAC, Campinas, SP, Brazil) released a new carioca variety named the 'origi-

nal carioca'. This new cream-striped grain type cultivar quickly became popular and was widely cultivated in Brazil and abroad, especially in Africa, because of its excellent grain quality and high grain yield. Recombination events moved the genetic structure of the original carioca forward and, with time, introduced new variability into the new carioca cultivars that were named after the original carioca because of their similar cream-striped grain type.

Molecular techniques have altered the way plant breeding is being done. Molecular markers have great potential to help breeders develop new improved varieties since they may be used to estimate the genetic diversity and level of heterozygosity among plants and animals (Dani *et al.*, 2008; Kumar *et al.*, 2008). Molecular markers have been used for genetic mapping (Grisi *et al.*, 2007), marker-assisted selection (Ender *et al.*, 2008), and to measure spatial and temporal gene flow within and among populations (Papa and Gepts, 2003).

A range of molecular data can be used to assess crop genetic diversity and has been applied to study the population structure of Mesoamerican and Andean bean gene pools (Dìaz and Blair, 2006; Blair *et al.*, 2007, 2009; Kwak and Gepts, 2009). Talukder *et al.* (2010) analyzed the genetic diversity and seed mineral content of a set of common beans in order to enhance seed Zn and Fe content. Santalla *et al.* (2010) used simple sequence repeats (SSRs) to elucidate how adaptation to environmental conditions has sculpted the common bean genomes in southern Europe. In addition, molecular markers have been used to evaluate genetic diversity in snap bean varieties from Europe (Métais *et al.*, 2002; Masi *et al.*, 2003), wild populations from Mexico (Payró de la Cruz *et al.*, 2005) and dry bean genotypes from Italy (Marotti *et al.*, 2007), Bulgaria (Svetleva *et al.*, 2006), Nicaragua (Gomez *et al.*, 2004), Slovenia (Maras *et al.*, 2006) and East Africa (Asfaw *et al.*, 2009).

Among molecular markers, microsatellites or SSRs deserve special attention as tools for analyzing diversity. These codominant, multiallelic markers are widely distributed throughout genomes and can be highly polymorphic (Chin *et al.*, 1996). SSRs have been used to distinguish among Mesoamerican and Andean accessions (Blair *et al.*, 2006; Benchimol *et al.*, 2007; Campos *et al.*, 2007) and have identified greater genetic variability among Andean than among Mesoamerican genotypes (Gepts *et al.*, 2008). SSRs have been successfully used to evaluate genetics, pedigree, phylogeny and/or identify various traits and/or germplasm accessions (McCouch *et al.*, 2001); they have been especially important in assessing the genetic diversity and genetic maps of common beans (Yu *et al.*, 1999; Guo *et al.*, 2000; Métais *et al.*, 2002; Gaitán-Solís *et al.*, 2002; Masi *et al.*, 2003; Blair *et al.*, 2003, 2006; Benchimol *et al.*, 2007; Grisi *et al.*, 2007; Hanai *et al.*, 2007; Zhang *et al.*, 2008).

Amplified fragment length polymorphism (AFLP) is a polymerase chain reaction (PCR)-based molecular marker assay (Vos *et al.*, 1995) that can detect a higher number of polymorphic *loci* in a single assay than restriction fragment length polymorphism (RFLP) or random amplification of polymorphic DNA (RAPD) (Powell *et al.*, 1996). AFLP is highly polymorphic and shows considerable reproducibility within a laboratory. The effectiveness and reliability of AFLP has led to its increasing use in diversity studies, phylogeny, genomic linkage mapping and identification of varieties (Tohme *et al.*, 1996; Papa and Gepts, 2003; Rosales-Serna *et al.*, 2005). AFLPs can detect a large number of polymorphic bands in a single lane rather than high levels of polymorphism at each *locus* such as occurs with SSRs. AFLP has been used to distinguish very closely related genotypes belonging to the same commercial class, such as the yellow bean class (Pallottini *et al.*, 2004). AFLPs are also reliable for distinguishing closely related cocoa varieties (Saunders *et al.*, 2001).

Single nucleotide polymorphisms (SNPs), including insertion/deletions (Indels), are informative genetic markers. Although at any given site SNPs could, in principle, involve four different nucleotide variants, in practice they are generally biallelic. When compared to multiallelic markers such as SSRs, biallelic SNPs are less polymorphic (the expected heterozygosity is lower), but this disadvantage is compensated for by the relative abundance of SNPs (Oraguzie *et al.*, 2007). SNP markers are useful in a variety of applications, including the construction of high resolution genetic maps, mapping traits, genetic diagnostics, analysis of the genetic structure of populations and phylogenetic analysis (Rafalski, 2002). Efforts to develop SNP- and Indel-based markers for the common bean (*Phaseolus vulgaris* L.) have been reported (Galeano *et al.*, 2009).

A comparison of the different marker systems used to estimate crop genetic diversity is important in order to assess their usefulness in germplasm conservation and as plant breeding tools. Garcia *et al.* (2004) used different marker systems (AFLP, RAPD, RFLP and SSR) to examine the diversity of inbred tropical maize lines and concluded that AFLP was the best molecular assay for fingerprinting and assessing genetic relationships because of its high accuracy. Geleta *et al.* (2005) reported that both AFLP and SSR markers were efficient tools in assessing the genetic variability among sorghum genotypes.

In this study, we sought to determine the most suitable molecular marker system (SSRs or AFLPs) for assessing the genetic diversity in commercial carioca common beans and examined the genetic variability among various genotypes.

## Materials and Methods

### Plant material and DNA extraction

Sixty carioca genotypes from the Agronomic Institute Germplasm Bank (IAC, Campinas, SP, Brazil) were used in this study (Table 1). Total genomic DNA was extracted

**Table 1** - Common bean (*Phaseolus vulgaris* L.) accessions evaluated by SSRs and AFLPs.

| Number of genotypes | Genotypes | Genealogy | Origin* |
|---|---|---|---|
| 01 | A-449 | G2910 / A19 | CIAT |
| 02 | Aporé | Carioca / México 168 /4/ Carioca /// Porrillo No. 1 / Gentry 21439 // 51052 / Cornell 49-242 | EMBRAPA |
| 03 | Branquinho | Unknown | Creole variety |
| 04 | BRS – Cometa | A 769 / 4 / EMP 250 /// A 429 / XAN 252 // C 8025 / G 4449 /// WAF 2 / A 55 // GN 31 / XAN 170 | EMBRAPA |
| 05 | BRS – Horizonte | EMP 250 / 4 / A 769 /// A 429 / XAN 252 // Pinto VI 114 | EMBRAPA |
| 06 | BRS – Pontal | BZ3836 // FEB 166 / AN910523 | EMBRAPA |
| 07 | BRS – Requinte | Carioca MG // POT 94 / AN910523 | EMBRAPA |
| 08 | BRSMG-Talismã | Selection involving the following parents: BAT 477, IAPAR 14, FT 84-29, Jalo EEP, A 252, A 77, Ojo de Liebre, ESAL 645, Pintado, Carioca, ESAL 645, P 85, P 103, H-4, AN910522, ESAL 624, Carioca MG | EMBRAPA |
| 09 | Campeão II | Aporé / Carioca comum | Creole variety |
| 10 | Caneludo | Unknown | Creole variety |
| 11 | Carioca | Mass selection in local material (Palmital, SP, Brazil) | IAC |
| 12 | Carioca Lustroso | Unknown | Creole variety |
| 13 | Carioca MG | Carioca / Cornell 49242 // Rio Tibagi | UFLA |
| 14 | Carioca Precoce | Not found | EMBRAPA |
| 15 | CV-48 | Recurrent selection involving the following parents: BAT 477, IAPAR 14, FT 84-29, Jalo EEP, A 252, A 77, Ojo de Liebre, ESAL 645, Pintado, Carioca, ESAL 645, P 85, P 103, H-4, AN910522, ESAL 624, Carioca MG | UFLA |
| 16 | FEB-186 | A525 // A767 // G2500C / A445 // G12727 / XAN11 | CIAT |
| 17 | FEB-200 | A767 // G4495 / PVA 1111 // G4449 / XAN112 | CIAT |
| 18 | FT-Bonito | IAPAR-14 / IAC-Carioca 80 | FT-Seeds |
| 19 | FT-Paulistinha | Carioca / México 168 // Carioca 1070 | FT-Seeds |
| 20 | FT-Porto Real | FT 85-75 | FT-Seeds |
| 21 | Goytacazes | A 106 / A 63 | Creole variety |
| 22 | Guará | Not found | EPAGRI |
| 23 | H96A28 - P4-1 - 1-1 - 1 | Vax! / Aruã // Akytã / IAPAR14 // A686 | IAC |
| 24 | H96A102-1-1-152 | Aruã/G5686 // Xan251 / Akytã // Pyatã / Mar1 // Pérola | IAC |
| 25 | H96A31-P2-1-1-1-1 | Vax1 / Aruã // Aruã / Mar1 // Maravilha / Cal143 | IAC |
| 26 | IAC – Alvorada | Pyatã / A686 // Maravilha / G2338 // Maravilha / And277 // L317-1 | IAC |
| 27 | IAC-Apuã | Emp81 / H853-50-2 | IAC |
| 28 | IAC-Aysó | Carioca / Cornell 49-242 | IAC |
| 29 | IAC-Carioca | Carioca / Cornell 49-242 | IAC |
| 30 | IAC-Carioca Akytã | DOR 41 // 10-3-1 / TU1B1-2 / 10-9-1 | IAC |
| 31 | IAC-Carioca Aruã | 10771.122 // H5380-41 / A156 // H5380-41 / AB136 | IAC |
| 32 | IAC-Carioca Pyatã | DOR 41 // 10-3-1 / TU1B1-2 / 10-9-1 | IAC |
| 33 | IAC-Carioca Tybatã | L933 / LM30630 | IAC |
| 34 | IAC-Votuporanga | Emp81 / H853-50-2 // H853-50-2 / *Phaseolus aborigineus* | IAC |
| 35 | IAC-Ybaté | G4000 / H858-50-2 | IAC |
| 36 | IAPAR - 14 | Carioca 99 / G / N / Nebraska 1 Sel / 27 // BAT 614 | IAPAR |
| 37 | IAPAR - 57 | Porrillo Sintético / Aeté 1-38 // CENA 83-1 / IAPAR BAC32 // CENA 83-2 / CENA 83-1 | IAPAR |
| 38 | IAPAR - 80 | A 2488 / EMP 117 /5/ Veranic 2 / Tlalnepantla 64 // Jamapa / Tara /// Carioca 99 / G.N.Nebraska1#27 /4/ Sel.Aroana | IAPAR |
| 39 | IAPAR - 81 | Veranic 2 / Tlalnepantla 64 // Jamapa / Tara /// [(Carioca 99 / G.N.Nebraska 1#Sel 27) // Sel.Aroana]} /5/ Aroana /// Veranic 2 / Tlalnepantla 64 // Jamapa / Tara /4/ A 259 | IAPAR |

**Table 1 (cont.)**

| Number of genotypes | Genotypes | Genealogy | Origin* |
|---|---|---|---|
| 40 | IAPAR -72 | Carioca / *Phaseolus coccineus* | IAPAR |
| 41 | IAPAR - 31 | IAPAR BAC 4 / RAI 46//IAPAR BAC2 / IGUAÇÚ /3/ BAT 93/ IAPAR BAC 4 | IAPAR |
| 42 | IPR- Aurora | RM8454-21-1/ IAPAR-14 | IAPAR |
| 43 | Juriti | BAT93 / 2 / Carioca Sel.99 / Great Northern Nebraska 1 sel#27 / 3 / sel. Aroana / 4 / A176 / A259 / 5 / II 133 / XAN87 | IAPAR |
| 44 | L 507-1 | Not found | IAC |
| 45 | L-476-2 | Not found | IAC |
| 46 | LH-II | Carioca MG / Carioca / EMGOPA 201 Ouro // Carioca / EMGOPA 201 Ouro | UFLA |
| 47 | LP 01-38 | Not found | IAPAR |
| 48 | LP 9979 | Not found | IAPAR |
| 49 | LP88-175 | Not found | IAPAR |
| 50 | Mar 2 | A252 / G5653 | CIAT |
| 51 | MD-806 | Not found | CIAT |
| 52 | Mex 279 | Not found | CIAT |
| 53 | OPNS-331 | Ouro Negro / Pérola | UFLA |
| 54 | OPS-16 | Ouro Negro / Pérola | UFLA |
| 55 | Pérola | Carioca / México 168 / 4 / Carioca /// Porrillo No. 1 / Gentry 21439 // 51052 / Cornell 49-242 | EMBRAPA |
| 56 | Rubi | Carioca / México 168 / 4 / Carioca /// Porrillo No. 1 / Gentry 21439 // 51052 / Cornell 49-242 | EMBRAPA |
| 57 | Rudá | Carioca / Rio Tibagi | CIAT |
| 58 | Taquarí | Unknown | CATI |
| 59 | TO | Not found | CIAT |
| 60 | Z-28 | IAPAR 81 / AN9022180 // PF 9029975 / A-805 | UFLA |

CATI – Coordination of Integral Technical Assistance; CIAT- International Center for Tropical Agriculture; EMBRAPA – Brazilian Company of Agricultural Research; EPAGRI – Brazilian Company of Agricultural Research and Rural Extension of Santa Catarina; IAC – Agronomic Institute of Campinas; IAPAR – Agronomic Institute of Paraná; UFLA – Federal University of Lavras.

from powdered lyophilized young leaves using the CTAB method (Hoisington *et al.*, 1994).

## SSR analysis

A total of 85 SSRs (Table 2) were developed from two microsatellite-enriched libraries, one for the 'CAL-143' line and another for the 'IAC-UNA' variety (Benchimol *et al.*, 2007; Campos *et al.*, 2007; Cardoso *et al.*, 2008; Oblessuc *et al.*, 2009). Sixty-five of the 85 SSRs were genotyped using 6% polyacrylamide silver stained gels (Creste *et al.*, 2001) whereas the remaining 20 SSRs were genotyped using a fluorescent labeling method that allowed high-throughput genotyping (Schuelke, 2000).

To explore the potential nature of the SSR *loci* assessed in this study, the Gene Ontology functional annotation tool Blast2GO (Conesa *et al.*, 2005) was used to assign GO IDs, enzyme commission (EC) numbers and INTERPRO codes. Sequence similarities were researched using BLASTX (Altschul *et al.*, 1990) against a databank of non-redundant protein sequences (NR, E-value cutoff = $1e^{-05}$) and BLASTN against a databank of expressed sequenced tags (dbEST; E-value cutoff = $1e^{-05}$). Further functional manual annotation was done using AmiGO and PFAM.

For polyacrylamide assays, amplifications were done in a final volume of 25 μL containing 50 ng of DNA, 1X buffer, 0.2 μM of each forward and reverse primer, 100 μM of each dNTP, 2.0 mM $MgCl_2$, 10 mM Tris-HCl (pH 8,0), 50 mM KCl, and 0.5 U of *Taq* DNA polymerase. The reactions were run using the following conditions: 1 min at 94 °C, then 30 cycles of 1 min at 94 °C, 1 min at the specific annealing temperature for each SSR and 1 min at 72 °C, with a final cycle of 5 min at 72 °C.

Amplifications using fluorescent SSRs were done with the M13 universal primer. The reactions were amplified in a final volume of 15 μL containing 30 ng of DNA, 1 U of *Taq* DNA polymerase, 1.5 mM $MgCl_2$, 0.15 mM of each dNTP, 1X buffer, 0.8 pmol/μL of reverse primer and labeled M13 and 0.2 pmol of forward primer/μL. The reactions were run using the following conditions: 1 min at 94 °C, then 30 cycles of 1 min at 94 °C, 1 min at the specific annealing temperature (56 °C or 60 °C) for each SSR and

1 min at 72 °C, followed by 8 cycles of 1 min at 94 °C, 1 min at 53 °C and 1 min at 72 °C, with a final cycle of 10 min at 72 °C. The amplicons sequenced with a 3730 DNA Analyzer (Applied Biosystems) and analyzed with GeneMapper® v 3.7 software.

## AFLP analysis

AFLP analysis was done as described by Vos *et al.* (1995), with some modifications. DNA (100 ng) was digested with *Eco*RI and *Mse*I restriction enzymes, linked to specific adapters, and fragments were amplified. The pre-amplification reactions were done with primers containing one selective nucleotide. The pre-amplification PCR consisted of 3 µL of digested DNA and with the adapters already linked, 0.2 µM of the primer combinations, 100 µM of each dNTP, 2.0 mM $MgCl_2$, 10 mM Tris-HCl (pH 8,0), 50 mM KCl, and 0.5 U of *Taq* DNA polymerase in a final volume of 25 µL. The reactions were run using the following conditions: 1 min at 94 °C, then 26 cycles of 1 min at 94 °C, 1 min at 56 °C, 1 min at 72 °C, with a final cycle of 5 min at 72 °C. The pre-amplification products were diluted 1:9 in water.

Selective amplification was done with primers that had three selective nucleotides (for *Eco*RI and *Mse*I primers). Twenty primer combinations were used in this step (Table 3). PCR for selective amplification was done as described for the pre-amplification reaction, using 3 µL of the pre-amplification dilution. The reactions were done using the following conditions: 2 min at 94 °C, then 12 cycles of 0.30 s at 94 °C, 0.30 s at 65 °C and 1 min at 72 °C, followed by 23 cycles of 0.30 s at 94 °C, 0.30 s at 56 °C and 1 min at 72 °C, with a final cycle of 5 min at 72 °C. The PCR amplification products were separated on 7% denaturing polyacrylamide silver stained gels. The AFLP bands were scored manually.

## Data analysis

The AFLP data were scored for presence (1) or absence (0) of bands, whereas for SSRs the results were transformed into genotypic data in order to identify *locus* and allele frequencies. Pair-wise comparisons were used to estimate Jaccard's similarity coefficient (Jaccard, 1908) for AFLP data using NTSYS software, version 2.02E (Rohlf, 1993). Genetic distances (GDs) were calculated from the SSR data for all possible inbred pairs using Rogers modified genetic distance (RMD; Goodman and Stuber, 1983) version 1.3 (Miller, 1997). Cluster analyses for SSRs and AFLPs were done using UPGMA (Unweighted Pair-Group Method with Arithmetic Averages).

Polymorphism information content (PIC) values were calculated using the formula:

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

where fi is the frequency of the $i^{th}$ allele (marker) for the $i^{th}$ SSR *locus* (Lynch and Walsh, 1998). Discrimination power (DP) analysis values for the $k^{th}$ primer were calculated using the formula:

$$\mathrm{DP}_k = 1 - \sum_{j=1}^{J} p_j \frac{N p_j - 1}{N - 1},$$

where $N$ is the number of individuals and $p_j$ is the frequency of the $j^{th}$ pattern (Tessier *et al.*, 1999). PIC was used to measure the information of a given marker *locus* for the pool of genotypes, while DP was used to measure the efficiency of SSRs and AFLPs in order to identify varieties by taking into account the probability of two randomly chosen individuals having different patterns.

The bootstrap procedure (Efron and Tibshirani, 1993) was used to verify whether the number of polymorphic SSRs and AFLPs used to estimate genetic similarity was large enough to supply a precise estimation of molecular markers among the genotypes (Tivang *et al.*, 1994). The polymorphic markers were submitted to sampling with the replacement of markers to create new samples from the original data. Genetic similarities for each of these subsets were calculated by obtaining 1000 bootstraps estimates of SSRs and AFLPs for each of these combinations. The coefficients of variation (CV) were used to construct box plots for each sample size. These analyses were done using the R software, which is a language and environmental asset for statistical computing. For each marker system (SSRs and AFLPs), the exponential function was adjusted to estimate the number of *loci* needed to obtain a CV of 10%. The median and maximum CV values were used to evaluate the accuracy of the genetic distance estimates because although the mean CV is often used in the literature, caution is needed when dealing with molecular marker data for which there is no assurance that the CV values have a symmetrical distribution.

Principal coordinate analysis (PCO; Gower, 1966) was done with the SSR MRD distance matrix and the first three principal coordinates were used to describe the dispersion of the 60 accessions according to their allele data.

Wrights F statistics for SSRs were estimated using the GDA program (Lewis and Zaykin, 2000). This set of statistics was used to test the structure of genetic diversity of the carioca genotypes evaluated. AMOVA (Analysis of Molecular Variance) was used to test the structure of the genetic diversity of the genotypes based on the AFLP data, and the analyses were done using Arlequin 2.0 software (Schneider and Excoffier, 1999). The significance of the fixation indices was tested by a permutation procedure with 10,000 permutations. Arlequin 2.0 software was also used to estimate the diversity fraction ($F_{ST}$) generated by AFLP analysis. The genetic differentiation coefficient, $F_{ST}$, measures the relative degree of gene differentiation among subpopulations such that each genotype can be considered

**Table 2** - Data from 85 microsatellites used to genotype the 60 carioca accessions (cream-striped grain type). Of the 85 SSRs screened, 20 were genotyped using a fluorescence technique (*).

| N° | SSRs | Motif | Ta** | Allele range (bp) | Number of alleles | PIC** | DP** |
|---|---|---|---|---|---|---|---|
| 01 | SSR-IAC01* | (CT)8 | 56 | 240-262 | 2 | 0.43 | 0.38 |
| 02 | SSR-IAC05 | (TG)6(GA)5 (AG)10 (ACA)5 | 50 | 164-166 | 2 | 0.10 | 0.10 |
| 03 | SSR-IAC09 | (CA)9C (CA)2(TA)6 | 56 | 160-168 | 2 | monomorphic | - |
| 04 | SSR-IAC10 | (GA)12(AG)6 (AG)6 | 56 | 176-188 | 4 | 0.68 | 0.47 |
| 05 | SSR-IAC11 | (GA)24 | 56 | 186-204 | 4 | 0.60 | 0.58 |
| 06 | SSR-IAC13 | (GA)10A (GA)4GG (GA)9 | 56 | 180 | 1 | monomorphic | - |
| 07 | SSR-IAC14* | (GT)7 | 56 | 226-256 | 5 | 0.30 | 0.32 |
| 08 | SSR-IAC16 | (GA)8 | 56 | 220-224 | 3 | 0.29 | 0.32 |
| 09 | SSR-IAC18* | (GT)8 | 56 | 270-300 | 3 | 0.59 | 0.61 |
| 10 | SSR-IAC20 | (GA)7AA (GA)2 | 56 | 182 | 1 | monomorphic | - |
| 11 | SSR-IAC21 | (AC)6 | 56 | 138-140 | 2 | 0.40 | 0.20 |
| 12 | SSR-IAC22 | (TA)8(GA)9 | 56 | 146-148 | 2 | 0.09 | 0.11 |
| 13 | SSR-IAC24 | (AC)7(AT)6 | 56 | 166-168 | 2 | 0.06 | 0.06 |
| 14 | SSR-IAC25 | (CA)6CAA (CA)2 CAA(CA) 3CG (CA)5 | 56 | 260-300 | 3 | 0.49 | 0.12 |
| 15 | SSR-IAC27 | (GT)5 | 56 | 260-278 | 2 | 0.11 | 0.16 |
| 16 | SSR-IAC28 | (GT)5(TC)10(TA)14 | 56 | 280 | 1 | monomorphic | - |
| 17 | SSR-IAC29 | (GA)23 | 56 | 58-158 | 2 | 0.10 | 0.23 |
| 18 | SSR-IAC32* | (TG)7 (TA)6 | 56 | 62-80 | 3 | 0.48 | 0.35 |
| 19 | SSR-IAC34 | (GA)12 | 56 | 180-182 | 2 | 0.49 | 0.52 |
| 20 | SSR-IAC35 | (CT)5 | 56 | 240-242 | 2 | 0.50 | 0.56 |
| 21 | SSR-IAC45 | (TG)5 | 56 | 202 | 1 | monomorphic | - |
| 22 | SSR-IAC46 | (CA)7 | 56 | 220-260 | 4 | 0.63 | 0.31 |
| 23 | SSR-IAC47* | (GA)20 | 56 | 300-330 | 4 | 0.56 | 0.52 |
| 24 | SSR-IAC49 | (AG)9 | 56 | 228-230 | 2 | 0.13 | 0.21 |
| 25 | SSR-IAC51 | (GA)5 CA (GA)9 CA (GA)2 | 56 | 150-160 | 2 | 0.25 | 0.38 |
| 26 | SSR-IAC52 | (GA)11 | 56 | 221-225 | 3 | 0.56 | 0.56 |
| 27 | SSR-IAC53 | (GA)9 | 56 | 164-168 | 3 | 0.52 | 0.12 |
| 28 | SSR-IAC54 | (AC)6 CAAA (TA)3 C (AT)5 | 56 | 110-112 | 2 | 0.09 | 0.09 |
| 29 | SSR-IAC55 | (GA)13 | 56 | 194-202 | 3 | 0.52 | 0.53 |
| 30 | SSR-IAC56* | (AC)8 | 56 | 270-300 | 3 | 0.37 | 0.36 |
| 31 | SSR-IAC57 | (GT)5 | 56 | 280 | 1 | monomorphic | - |
| 32 | SSR-IAC58 | (TG)10 | 56 | 184 | 1 | monomorphic | - |
| 33 | SSR-IAC59* | (AC)7 | 61 | 35-170 | 3 | 0.55 | - |
| 34 | SSR-IAC62 | (AG)14 | 45.3 | 198-210 | 4 | 0.67 | 0.67 |
| 35 | SSR-IAC63 | (AC)6 | 59.8 | 210 | 1 | monomorphic | - |
| 36 | SSR-IAC64* | (AC)6 | 56 | 270-290 | 4 | 0.53 | 0.57 |
| 37 | SSR-IAC65 | (TG)5 | 60 | 270-272 | 2 | 0.10 | 0.10 |
| 38 | SSR-IAC66 | (GA)10 | 56 | 136-144 | 3 | 0.49 | 0.73 |
| 39 | SSR-IAC67 | (GT)7 | 56 | 110 | 1 | monomorphic | - |
| 40 | SSR-IAC68 | (CT)8 | 56 | 260-272 | 4 | 0.53 | 0.82 |
| 41 | SSR-IAC70 | (AC)8 | 60 | 186-188 | 2 | 0.48 | 0.48 |
| 42 | SSR-IAC73 | (AT)6(GT)6 | 60 | 198-230 | 3 | 0.53 | 0.34 |
| 43 | SSR-IAC77 | (CA)6(CT)4 | 60 | 188-190 | 2 | 0.44 | 0.45 |
| 44 | SSR-IAC83 | (TC)11 | 45 | 250-260 | 3 | 0.61 | 0.63 |
| 45 | SSR-IAC87 | (AC)9 | 63.5 | 220-240 | 3 | 0.41 | 0.29 |
| 46 | SSR-IAC88 | (CA)7(AT)7 | 60 | 210-220 | 3 | 0.52 | 0.53 |
| 47 | SSR-IAC91 | (AC)3(TC)2 | 60 | 200-210 | 2 | 0.06 | 0.06 |
| 48 | SSR-IAC96 | (CA)5(TA)2 | 60 | 254-258 | 2 | 0.47 | 0.48 |
| 49 | SSR-IAC97 | (AC)3(TC)2 | 60 | 240 | 1 | monomorphic | - |

**Table 2 (cont.)**

| Nº | SSRs | Motif | Ta** | Allele range (bp) | Number of alleles | PIC** | DP** |
|---|---|---|---|---|---|---|---|
| 50 | SSR-IAC98 | (CT)8(TA)3(TG)8 | 60 | 230-290 | 3 | 0.60 | 0.65 |
| 51 | SSR-IAC100 | (AT)4(GT)8 | 60 | 206-210 | 2 | 0.09 | 0.17 |
| 52 | SSR-IAC101 | (AC)7 | 60 | 186-190 | 2 | 0.29 | - |
| 53 | SSR-IAC102 | (CT)7 GTCA (CT)8 | 60 | 176-178 | 2 | 0.39 | 0.42 |
| 54 | SSR-IAC127 | (TA)3 T (TGA)3 G (TA)3 | 63.3 | 168-170 | 2 | 0.50 | 0.50 |
| 55 | SSR-IAC128 | (AC)7 GGA (TC)2 | 56.7 | 168-190 | 2 | 0.31 | 0.35 |
| 56 | SSR-IAC129 | (TG)2 G (CT)2 TCT (GA)2 | 56.7 | 250-258 | 2 | 0.47 | 0.53 |
| 57 | SSR-IAC134 | (AC)6 | 56.7 | 218-250 | 2 | 0.41 | 0.39 |
| 58 | SSR-IAC136 | (CA)7 (AT)5 | 56.7 | 240-270 | 2 | 0.43 | 0.17 |
| 59 | SSR-IAC141 | (TCT)3 A (CT)13 | 59.4 | 214-218 | 2 | 0.40 | 0.46 |
| 60 | SSR-IAC143* | (TC)2 T (TC)2 T (TC)2 | 63.3 | 170-200 | 4 | 0.51 | 0.49 |
| 61 | SSR-IAC144* | (CT)10 | 56.7 | 170-220 | 4 | 0.70 | 0.49 |
| 62 | SSR-IAC147 | (CA)5 | 56.7 | 230-240 | 2 | 0.46 | 0.42 |
| 63 | SSR-IAC155 | (AG)9 | 56.7 | 196-200 | 2 | 0.04 | 0.01 |
| 64 | SSR-IAC156 | (TC)3 TG (GC)2 | 56.7 | 230 | 1 | monomorphic | - |
| 65 | SSR-IAC159 | (AC)6/(AC)4 C (CT)2 | 56.7 | 284-296 | 2 | 0.29 | 0.42 |
| 66 | SSR-IAC160 | (TG)2 (TA)2 (TG)5 | 56.7 | 170-174 | 2 | 0.44 | 0.49 |
| 67 | SSR-IAC166 | (CA)2 AA (AC)3/(TA)2 GAC (TG)3 | 56.7 | 186-190 | 2 | 0.05 | 0.36 |
| 68 | SSR-IAC167 | (TG)7 (CG)3 | 56.7 | 138-168 | 2 | 0.34 | 0.31 |
| 69 | SSR-IAC174 | (AT)3 A (AT)2 (AC)7 TTT (CA)3 | 53.2 | 140 | 1 | monomorphic | - |
| 70 | SSR-IAC179 | (AC)2 CTTT (AC)2 CTA (TC)5 | 63.3 | 180-186 | 2 | 0.48 | 0.53 |
| 71 | SSR-IAC180 | (AC)3 T (CA)3 TAA/ (AC)3(AC)3 G (CA)2 | 63.3 | 206 | 1 | monomorphic | - |
| 72 | SSR-IAC181 | (AT)2 AC (AT)3/(AG)5 TAA (AG)2 C (AG)2 | 58.4 | 120 | 1 | monomorphic | - |
| 73 | SSR-IAC183 | (AG)18 A (AC)4 | 56 | 190-196 | 2 | 0.27 | 0.34 |
| 74 | SSR-IAC209 | (AC)2 (TG)3 | 56.7 | 198-200 | 2 | 0.48 | |
| 75 | SSR-IAC211 | (CA)10 (TA)8 | 43.8 | 176 | 1 | monomorphic | - |
| 76 | SSR-IAC226* | (TG)8 | 60 | 240-260 | 4 | 0.56 | 0.65 |
| 77 | SSR-IAC239* | (AG)15 | 60 | 260-300 | 6 | 0.61 | 0.62 |
| 78 | SSR-IAC240* | (CT)10 | 60 | 196-210 | 4 | 0.64 | 0.62 |
| 79 | SSR-IAC242* | (AT)2 (GT)3 | 60 | 256-300 | 2 | 0.36 | 0.38 |
| 80 | SSR-IAC244* | (TC)9 | 60 | 200-226 | 5 | 0.27 | 0.27 |
| 81 | SSR-IAC251* | (AC)11 (AT)12 | 45 | 144-296 | 5 | 0.69 | 0.53 |
| 82 | SSR-IAC272* | (CA)6 | 60 | 200-236 | 4 | 0.42 | 0.45 |
| 83 | SSR-IAC390* | (GT)4 AT (GT)3 | 60 | 190-250 | 5 | 0.64 | 0.62 |
| 84 | FJUNA 167* | (AT)4 AG (GT)6/(AT)4 (GT)6 | 60 | 290-310 | 4 | 0.41 | 0.41 |
| 85 | FJUNA 384* | (CA)5 | 60 | 160-206 | 3 | 0.67 | 0.38 |

**Ta- annealing temperature; PIC – polymorphism information content; DP – Discrimination power.

a subpopulation. Wright's $F_{ST}$ is considered to be identical to $G_{ST}$ (Nei, 1978) for fully homozygous diploids in AFLP analysis and was calculated as: $F_{ST} = G_{ST} = D_{ST}/h_T$, where $H_T = H_S + D_{ST}$, and $D_{ST} = (H_T - H_S)$.

## Results and Discussion

### Survey of molecular marker polymorphism

Seventy (82.4%) of the SSRs (Table 2) were polymorphic and produced 196 polymorphic alleles. The allele number ranged from 2 to 6 with a mean of 2.8 alleles per SSR. The highest numbers of alleles observed were found for SSR-IAC10, SSR-IAC62, SSR-IAC144 and SSR-IAC251. AFLP markers produced 725 bands of which 635 (87.6%) were polymorphic. The number of fragments amplified per primer pair varied from 20 (E-ACC/M-CGG) to 71 (E-TAA/M-GAA) (Table 3).

The highest polymorphic information content (PIC) found for SSRs was 0.70 (SSR-IAC144) and the lowest was 0.03 (SSR-IAC155), with a mean value of 0.47. The high-

**Table 3** - AFLP primer combinations and their characteristics.

| Primer combination | Number of bands | Polymorphic bands | Polymorphism rate (%)* | DP values |
|---|---|---|---|---|
| E-TAA/M-GAA | 71 | 65 | 91.5% | 0.89 |
| E-TCA/M-GAA | 23 | 21 | 91.3% | 0.82 |
| E-TCA/M-GAC | 24 | 22 | 91.6% | 0.83 |
| E-TCA/M-GAC | 33 | 29 | 87.9% | 0.70 |
| E-TAT/M-GTA | 33 | 29 | 87.9% | 0.79 |
| E-TAT/M-GTG | 59 | 52 | 88.1% | 0.75 |
| E-TTA/M-GAT | 34 | 30 | 88.2% | 0.78 |
| E-TTG/M-GAA | 24 | 20 | 83.4% | 0.72 |
| E-TTG/M-GAT | 32 | 29 | 90.6% | 0.78 |
| E-TCT/M-GAA | 27 | 24 | 88.9% | 0.80 |
| E-TCT/M-GAT | 37 | 34 | 91.9% | 0.83 |
| E-TCT/M-GTA | 41 | 33 | 80.5% | 0.86 |
| E-TCT/M-GTT | 42 | 37 | 88.0% | 0.93 |
| E-TTT/M-GTA | 54 | 47 | 87.0% | 0.92 |
| E-TTT/M-GTC | 26 | 20 | 83.4% | 0.91 |
| E-AAG/M-CGG | 47 | 45 | 95.7% | 0.88 |
| E-AAG/M-CCT | 33 | 29 | 87.9% | 0.91 |
| E-AAG/M-CTC | 39 | 35 | 89.7% | 0.90 |
| E-ACC/M-CGG | 20 | 16 | 80.0% | 0.85 |
| E-ACC/M-CCC | 26 | 18 | 69.2% | 0.94 |
| Totals | 725 | 635 | 87.6% | - |

*Percentage of polymorphic bands.

est PIC value for AFLP was 0.37 (E-AAG/M-CGG) and the lowest was 0.03 (E-TAT/M-GTG), with a mean value of 0.29. Benchimol *et al.* (2007) analyzed genotypes from the Andean and Mesoamerican gene pools (*P. vulgaris* L.) with 123 polymorphic microsatellites and found PIC values from 0.04 to 0.83. These genotypes were expected to be more diverse than those of the carioca commercial type (Mesoamerican). Fifty-three SSRs used by Benchimol *et al.* (2007) were also used here with the carioca genotypes, and six of these SSRs showed similar PIC values (SSR-IAC-01, SSR-IAC32, SSR-IAC55, SSR-IAC64, SSR-IAC70 and SSR-IAC-83). Thus, most of the SSRs differed in their PIC, a situation that probably reflected the type and number of genotypes that were evaluated. Moreover, the higher PIC values found for the SSR markers were related to the size of the SSR motifs; perfect SSRs with a higher number of repetitions and compound SSRs showed higher PIC (Table 2). The PIC values were compatible with those obtained for SSRs used in genotyping carioca cultivars.

Discrimination power (DP) analyses for SSRs (Table 2) yielded values from 0.01 (SSR-IAC155) to 0.82 (SSR-IAC68) with most being around 0.40. Based on parameters such as the number of alleles, DP and PIC, the polymorphism estimated for the carioca genotypes with a subset of 85 SSRs showed that these *loci* could generate sufficient polymorphism to allow their use as molecular markers to establish genetic relationships among very closely related genotypes. These SSRs could be also useful for a wide range of genetic investigations such as linkage map construction and association mapping studies. The DP values for AFLPs were higher than for SSRs, ranging from 0.70 (E-TCA/M-GAC) to 0.94 (E-ACC/M-CCC), with most of the values being around 0.92 (Table 3).

To understand the genomic nature (functional or non-functional regions) of the SSR markers used, the 85 contigs were compared with non-redundant GenBank databases using BLASTX and BLASTN (dbEST). Of the sequences analyzed, 22% were found in the BLASTX – NR DB and 49% in the BLASTN dbEST database. GO analyses yielded a hierarchy of terms that varied at many levels and were divided into functional groups identified as "molecular function", "biological process" and "cellular component". Nineteen functional SSRs were identified (Table S1), and the SSRs that shared similarity with ESTs deposited in dbEST are shown in Table S2.

## Genetic diversity of the carioca genotypes

Various studies have shown that the accuracy of genetic distance measurements is enhanced by using a mean CV of 10% (dos Santos *et al.*, 1994; Halldén *et al.*, 1994; Thormann *et al.*, 1994; Tivang *et al.*, 1994). However, according to Garcia *et al.* (2004), the use of an average value is not a good indicator of central tendency for skewed data. Hence, in this work, the minimum number of SSR and AFLP *loci* necessary for an accurate representation of genetic distances was calculated in order to construct an exponential function based on the average, median and maximum CV values of genetic distances obtained by bootstrap sampling data for each SSR and AFLP. The CV values used to calculate the median were used as follows: the sample size (number of *loci*) required for 50% of the genetic distances to have CV values < 10% ($n_{median}$), the sample size required for no genetic distance to have a CV > 10% ($n_{maximum}$), and the sample size required for all genetic distances to have an average CV of 10% ($n_{mean}$) (Figure 1). The results obtained based on these criteria are shown in Table 4.

The choice of the appropriate number of polymorphic *loci* required for a reliable estimate of genetic distance is influenced by the criteria used. According to Garcia *et al.* (2004), the median CV value is the best choice for evaluating the precision of genetic distance estimates based on different molecular systems. The extremely high (almost 100%) coefficients of determination for the adjusted equations for SSRs and AFLPs indicated that extrapolation to outlying points could be done. The results of the 10% CV analysis ($n_{median}$) showed that 44 SSRs were necessary to assess the genetic variability among genotypes, whereas 100 AFLPs were required for the same analysis (Table 4). Thus, AFLPs were more suitable for analyzing genetic diversity in the set of common bean genotypes evaluated here since they provided information more rapidly and more accu-

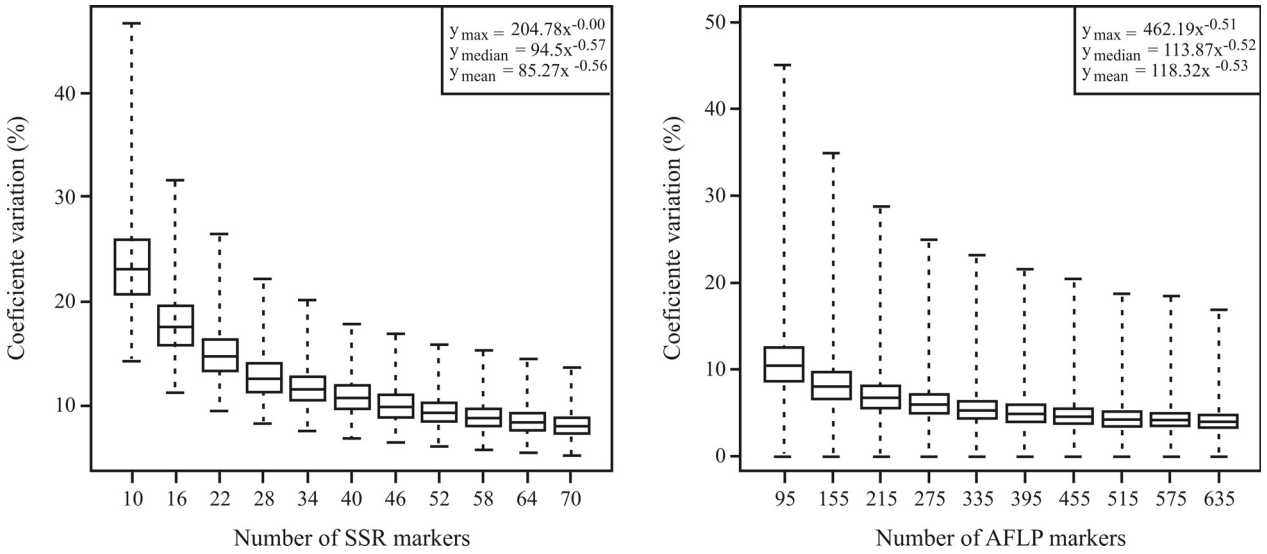


**Figure 1** - Box plots of the coefficients of variation for the genetic similarity among all genotypes estimated by bootstrap analysis for subsamples with different number of SSRs and AFLPs.

**Table 4** - Sample size (number of loci sampled) required for genetic distances to have the specified coefficient of variation (CV%) in carioca common beans.

| Coefficient of variation (CV%) | Sample size (number of *loci* sampled) | |
|---|---|---|
| | SSRs | AFLPs |
| Average of 10% ($n_{mean}$) | 45.66 | 109.6 |
| 50% less than 10% ($n_{median}$) | 44.39 | 100.56 |
| 100%less than 10% ($n_{max}$) | 100.34 | 1738.13 |

rately. Similar results were reported by Pallottini *et al.* (2004) for AFLPs used to assess the yellow bean class.

Maluf *et al.* (2005) characterized the genetic diversity of *Coffea arabica* genotypes using various molecular markers (AFLP, RAPD and SSR). RAPD and SSR were more efficient in kinship analysis; however, despite the high discriminatory power of SSRs, these markers were not efficient in separating the genotypes into well-defined groups. The authors concluded that AFLP markers were more appropriate for assessing the genetic diversity of kin groups. More recently, Kumar *et al.* (2008) concluded that AFLP markers were very useful for assessing the genetic diversity among narrow-based common bean accessions.

Of the 70 SSRs used here, 37 were mapped in the 'IAC-UNA' x 'CAL-143' genetic map (UC map; Campos *et al.*, 2010). These SSRs were anchored in eight out of the 11 linkage groups (GLs) generated in the UC map (B1 – SSR-IAC01, SSR-IAC21 and SSR-IAC226; B6 – SSR-IAC47, SSR-IAC128 and SSR-IAC183; B9 – SSR-IAC55, SSR-IAC62 and SSR-IAC242). The linkage group B2 had 10 SSRs (SSR-IAC18a, SSR-IAC24, SSR-IAC46, SSR-IAC51, SSR-IAC57, SSR-IAC70, SSR-IAC134, SSR-IAC141, SSR-IAC166 and SSR-IAC251), the B3 linkage group had one SSR (SSR-IAC77), with one SSR in the B8 linkage group (SSR-IAC22). The B4 linkage group had four SSRs (SSR-IAC25, SSR-IAC66, SSR-IAC67 and SSR-IAC179), with the same number of SSRs being observed in the B5 linkage group (SSR-IAC10, SSR-IAC88, SSR-IAC96 and SSR-IAC159). The B7 linkage group had six SSRs (SSR-IAC18b, SSR-IAC64, SSR-IAC101, SSR-IAC143, SSR-IAC272 and SSR-IAC239) and the B10 linkage group had two SSRs (SSR-IAC155 and SSR-IAC244). These results show that the number of SSRs used was not sufficient to scan the entire bean genome since not all of the 11 chromosomal pairs were assessed and some linkage groups were irregularly scanned. Indeed, the SSR map positions in the UC map were not known at the time these SSRs were chosen for this study.

The SSR dendrogram (Figure 2) showed high genetic variability, with genetic distances varying from 0.37 to 0.63. However, a low genetic structure was observed as some groups were not well-defined. This could be explained by the fact that the SSRs used to assess the genetic variability of common genotypes in 'carioca' beans did not cover the entire genome of the common bean since the SSRs used in the analysis were not distributed in all linkage groups of the common bean.

The AFLP dendrogram provided a better clustering pattern for the carioca genotypes, which formed two major groups (Figure 3); five accesses ('Campeão II', 'LP9979', 'IAC-Aruã', 'Aporé' and 'CariocaMG') could not be classified with either of the two major groups. Genetic distances varied from 0.09 to 0.63 and indicated marked genetic variability. The AFLP average genetic distance ($GD_{AFLP}$ = 0.88) was higher than the average genetic distance for SSRs ($GD_{SSR}$ = 0.58). Some of the genotypes clustered according to the institutional breeding program from which they were derived. This was observed for the IAPAR (The Agronomic Institute of the State of Paraná, in southern

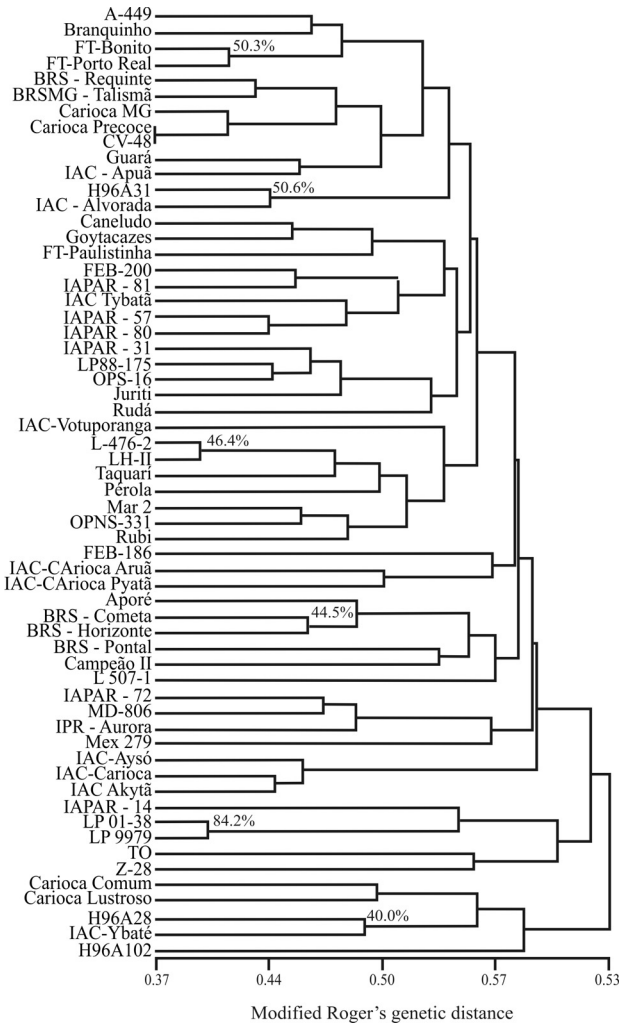


**Figure 2** - UPGMA dendrogram for the 60 carioca common beans based on SSR markers. Genetic distances were calculated using Rogers modified genetic distance.

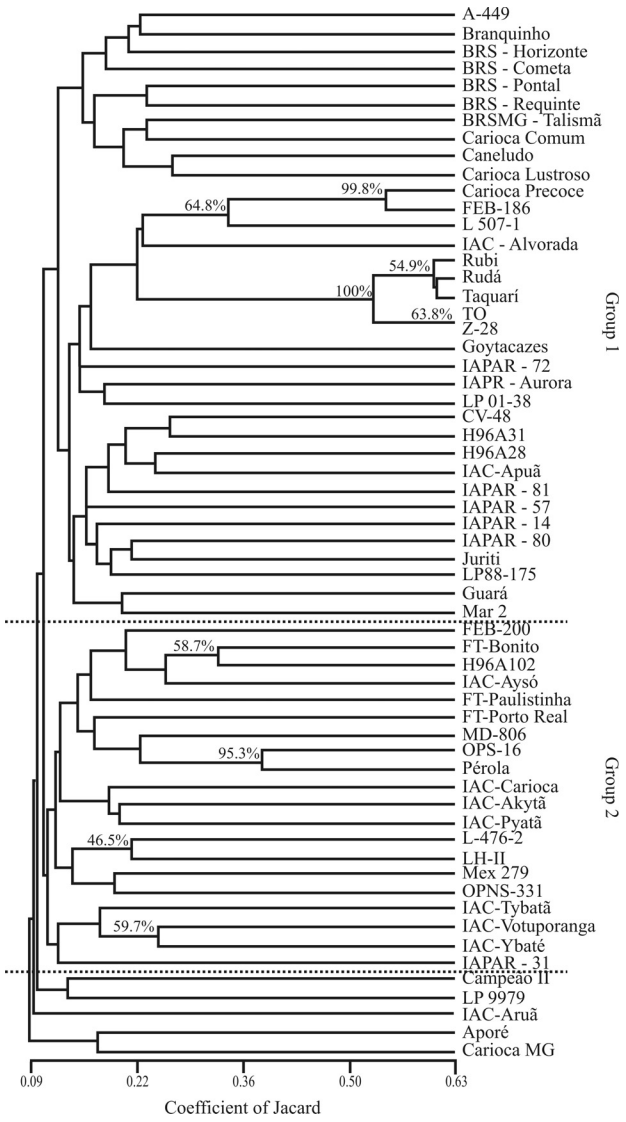


**Figure 3** - UPGMA dendrogram for the 60 carioca common beans based on AFLP data. Genetic similarity values were calculated using Jaccards coefficient.

Brazil) genotypes ('IAPAR 81', 'IAPAR57', 'IAPAR 14', 'IAPAR 80', 'Juriti' and 'LP88-175'), for the IAC (The Agronomic Institute, Campinas, SP, Brazil) genotypes ('IAC-Carioca', 'IAC-Akytã' and 'IAC-Pyatã'; 'IAC-Tybatã', 'IAC-Votuporanga' and 'IAC-Ybaté') and for those from EMBRAPA ('A-449', 'Branquinho', 'BRS-Horizonte', 'BRS-Cometa', 'BRS-Pontal', 'BRS-Requinte' and 'BRSMG-Talismã'). This clustering pattern could be explained by the fact that each breeding Institution usually displays and uses a different genetic core collection and each breeding program has its own goals.

The Pearson correlation coefficient for SSR and AFLP genetic distances was negative (r = -0.08) indicating lack of correlation between these marker systems. The differences between SSRs and AFLPs reflect the extent of genome coverage and different evolutionary properties (Tautz and Schlötterer, 1994). The genomic distribution of SSRs is non-random (Li *et al.*, 2004), whereas it is reasonable to expect that AFLPs should have complete genome coverage (Nowosielski *et al.*, 2002). Saini *et al.* (2004) reported a low correlation (0.50) between these two classes of markers in rice and assumed that this reflected different genomic fractions that involved repeat and/or unique sequences; these sequences may have had different patterns of involvement or preservation during natural or human selection.

Menezes *et al.* (2004) and Palomino *et al.* (2005) studied the genetic diversity within carioca cultivars using RAPD markers and verified high genetic variability within carioca cultivars that differed from the 'original carioca'. Although RAPD markers have been extensively used for common bean fingerprinting and genetic analysis, AFLP analysis provides a higher level of polymorphism than RAPD (Pejic *et al.*, 1998). AFLP markers are advantageous because they reveal a larger number of reproducible markers, thereby increasing the probability of identifying polymorphic markers, even among closely related genotypes

such as in common beans (Tohme *et al.*, 1996; Beebe *et al.*, 2001).

Principal coordinate analysis (PCO) failed to provide a clear division of the genotypes in the first three axes for both markers. For SSRs, 20.95% of the total variance was explained in three axes (Figure 4). According to SSR PCO, the 'original carioca', which is the ancestor of most of the carioca genotypes that are currently being used, was detached from the other cariocas. This finding supports the hypothesis that carioca genotypes have diverged from the 'original carioca' ancestor and shows that there is still considerable genetic variability to be exploited among carioca common bean accessions. Indeed, carioca common bean accessions have derived from many recombination events and from crosses with different genotypes, with much of this variability being preserved. Other factors, such as retrotransposons, may also be involved in generating and maintaining carioca genetic diversity.

GDA analysis of the SSR data attributed 83% of the total molecular variance to $F_{ST}$, indicating a high level of genetic differentiation in the carioca samples. Based on the AFLP data, AMOVA showed 98% $G_{ST}$. Meanwhile, the observed heterozygosity was very low for all genotypes (0.16) and reflected the inbreeding index characteristic of an autogamous species (Beebe *et al.*, 2000). Both groups of markers revealed high genetic variability among the carioca genotypes.

Common bean breeding programs usually explore only a low proportion of the available genetic diversity. Since most breeding programs use only a limited number of cultivars, the genetic base of elite cultivars tends to be narrow (Cooper *et al.*, 2001). However, our findings suggest that genetic diversity is still preserved in the carioca collection. The degree of variability detected here supports the idea that the carioca group has a broader genetic base than

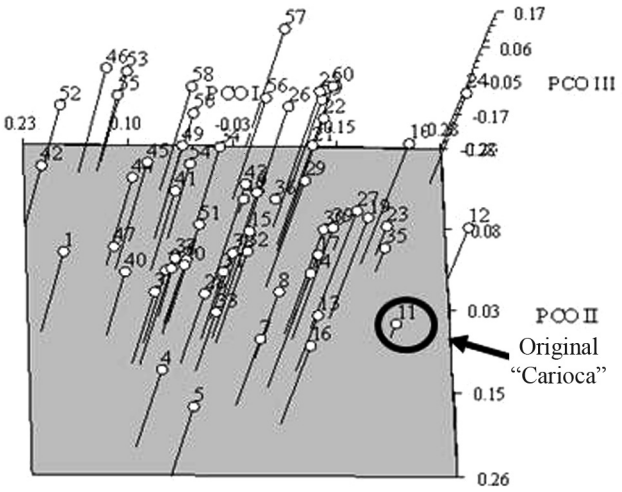


**Figure 4** - Principal coordinate analysis (PCO) for the SSR data of the 60 carioca common bean accessions. The position of original 'carioca' is indicated.

expected, with a large number of unique alleles and low gene flow among them.

The success of common bean breeding programs is intimately related to the appropriate choice of divergent parents. To make this choice researchers need to know the genetic diversity contained in the available germplasm since the best parental combination can be obtained by combining two parents with a high degree of divergence; this will allow the exploitation of heterosis to improve breeding programs. The 'carioca' genotypes of common beans are currently the most sold in Brazil, largely because of their agronomic and commercial characteristics. Most of the genotypes have a high yield and resistance to various bean diseases, such as rust (examples: 'Aporé', 'Carioca MG', 'Rudá'), golden mosaic virus (examples: 'IAPAR 72', 'MD-806' and 'IPR-Aurora'), to bacterial diseases (examples: 'IAC-Tybatã', 'LP88-175', 'IAC-Carioca Pyatã', 'IAC-Carioca Akytã', 'IAC-Ybaté', 'BRS-Pontal'), anthracnose (examples: 'IAC-Carioca Tybatã', 'IAC-Carioca Pyatã', 'IAC-Carioca Akytã', 'IAC-Ybaté', 'H96A28', 'H96A102', 'IAPAR-31', 'BRS-Requinte', 'BRS-Pontal', 'IAC-Alvorada', 'H96A31', 'LP 9979') and angular leaf spot (examples: 'BRS-Pontal', 'IAC-Carioca Tybatã', 'Pérola', 'IAPAR-31', 'IAC-Carioca Aruã', 'BRS-Requinte'). Knowledge of the appropriate agronomic information and genetic distances among a set of 'carioca' genotypes can be used by bean breeding programs to assist in selecting superior parents and allow the exploitation of heterosis.

Information on possible crosses based on genetic diversity can also be used to assist breeding programs. Thus, for example, based on the AFLP genetic distances and the agronomic traits associated with each genotype, the following crosses could be suggested: 'IAC-Alvorada x H96A102' ($GD_{AFLP}$ = 0.89), 'IAC-Alvorada' x 'LP 0181' ($GD_{AFLP}$ = 0.87), 'IAC-Alvorada' x 'LP 9979' ($GD_{AFLP}$ = 0.89), 'IAC-Alvorada' x 'H96A28' ($GD_{AFLP}$ = 0.87), 'IAC-Alvorada' x 'IPR-Aurora' ($GD_{AFLP}$ = 0.90), 'BRS-Requinte' x 'H96A102' ($GD_{AFLP}$ = 0.90), 'BRS-Pontal' x 'H96A102' ($GD_{AFLP}$ = 0.87), 'Branquinho' x 'H96A102' ($GD_{AFLP}$ = 0.85), 'BRS-Talismã' x 'H96A102' ($GD_{AFLP}$ = 0.83) and 'H96A31' x 'H96A102'($GD_{AFLP}$ = 0.80).

A large number of markers have been used to assess genetic diversity in plants. For wide-scale use in germplasm characterization and breeding, it is important that these marker technologies be exchanged between laboratories and be standardized to yield reproducible results that will allow the direct comparison of data among laboratories and studies, thereby reducing project expenses. Jones *et al.* (1997) described a network experiment involving several European laboratories in which the reproducibility of three popular molecular marker techniques (AFLP, RAPD and SSR) was examined. The authors concluded that the costs involved in developing SSR markers meant that AFLP was more useful for studying genetic diversity – the major limi-

tation inherent with SSRs resides in the logistic difficulty of increasing the number of useful *loci* for assignment tests. Indeed, developing and applying large numbers of SSRs may be technically challenging, expensive and time-consuming (Goldstein and Pollock, 1997).

AFLP provides a practical alternative for studying genetic diversity since the technique generates a large number of *loci*, and the cost and time required are lower than for SSRs (Vos *et al.*, 1995; Rieseberg, 1998; Mueller and Wolfenbarger, 1999). Furthermore, the high reproducibility of AFLP means that multiple bands can be generated in a single assay (Oliveira *et al.*, 2004). AFLP has been successfully used to estimate genetic diversity in the common bean (Tohme *et al.*, 1996; Caicedo *et al.*, 1999; Maciel *et al.*, 2003; Pallottini *et al.*, 2004; Rosales-Serna *et al.*, 2005).

Comparisons of the efficiency of SSRs and AFLPs in assessing the genetic diversity of plants have generally shown that AFLP is the preferred technique. Barbosa *et al.* (2003) investigated the genetic diversity of tropical maize using SSRs and AFLPs and concluded that the former was less efficient than the latter for assigning lines to heterotic groups in tropical maize, and for predicting single cross performance in this culture. Similarly, Garcia *et al.* (2004), in a comparison of AFLP, RAPD, RFLP and SSR markers to evaluate genetic diversity in tropical maize, found that AFLP was the best-suited molecular assay for accurate fingerprinting and assessment of genetic relationships among tropical maize inbred lines.

The advantages and disadvantages of different marker systems for analyzing diversity in breeding populations or germplasm bank accessions, such as the carioca commercial type, must be considered in genotyping data for crop improvement and for the *ex-situ* conservation of plant genetic resources. Other studies that have compared the efficiencies of AFLPs and SSRs have concluded that both marker systems are useful for assessing the genetic structure and diversity of common bean accessions (Maras *et al.*, 2008; Masi *et al.*, 2009).

In conclusion, this is the first study to use powerful molecular markers such as AFLP and SSRs to assess the genetic variability of carioca commercial beans. Other studies of cream-striped carioca genotypes have used RAPD markers, which are less powerful in revealing the extent of genetic diversity (Menezes *et al.*, 2004; Palomino *et al.*, 2005). AFLP and SSRs and the number of markers they generated were appropriate for assessing the genetic diversity among carioca genotypes. In practice, AFLP markers were easier to use when screening the whole genome for genetic diversity and were more suitable in distinguishing among very closely related genotypes belonging to the same commercial class, such as the carioca genotypes.

## Acknowledgments

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## Supplementary Material

The following online material is available for this article:

Table S1 - Functional annotation off SSRs sharing similarity with proteins in GenBank.

Table S2 - SSRs with similarity to ESTs deposited in the dbEST databank.

This material is available as part of the online article from http://www.scielo.br/gmb

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