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Microsatellite marker diversity in common bean (*Phaseolus vulgaris* L.)

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Abstract A diversity survey was used to estimate allelic diversity and heterozygosity of 129 microsatellite markers in a panel of 44 common bean (*Phaseolus vulgaris* L.) genotypes that have been used as parents of mapping populations. Two types of microsatellites were evaluated, based respectively on gene coding and genomic sequences. Genetic diversity was evaluated by estimating the polymorphism information content (PIC), as well as the distribution and range of alleles sizes. Gene-based microsatellites proved to be less polymorphic than genomic microsatellites in terms of both number of alleles (6.0 vs. 9.2) and PIC values (0.446 vs. 0.594) while greater size differences between the largest and the smallest allele were observed for the genomic microsatellites than for the gene-based microsatellites (31.4 vs. 19.1 bp). Markers that showed a high number of alleles were identified with a maximum of 28 alleles for the marker BMd1. The microsatellites were useful for distinguishing Andean and Mesoamerican genotypes, for uncovering the races within each genepool and for separating wild accessions from cultivars. Greater polymorphism and race structure was found within the Andean gene pool than within the Mesoamerican gene pool and polymorphism rate between genotypes was consistent with genepool and race identity. Comparisons between Andean genotypes had higher polymorphism (53.0%) on average than comparisons among Mesoamerican genotypes (33.4%). Within the Mesoamerican parental combinations, the intra-racial combinations between Mesoamerica and Durango or Jalisco race genotypes showed higher average rates of polymorphism (37.5%) than the within-race combinations between Mesoamerica race genotypes (31.7%). In multiple correspondance analysis we found two principal clusters of genotypes corresponding to the Mesoamerican and Andean gene pools and subgroups representing specific races especially for the Nueva Granada and Peru races of the Andean gene pool. Intra population diversity was higher within the Andean genepool than within the Mesoamerican genepool and this pattern was observed for both gene-based and genomic microsatellites. Furthermore, intra-population diversity within the Andean races (0.356 on average) was higher than within the Mesoamerican races (0.302). Within the Andean gene pool, race Peru had higher diversity compared to race Nueva Granada, while within the Mesoamerican gene pool, the races Durango, Guatemala and Jalisco had comparable levels of diversity which were below that of race Mesoamerica.

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Introduction

Common beans (*Phaseolus vulgaris* L.) are a diverse food legume important to the diet of many people around the world, especially in Latin America and Eastern and Southern Africa (Broughton et al. 2003). Common bean originated and was domesticated in the New World and has two major gene pools, the Andean and the Mesoamerican, based on their centers of origin in South and Central America, respectively (Gepts and Debouck 1991). Within these gene pools are a total of six races including three Mesoamerican (Mesoamerica, Durango, and Jalisco) and three Andean (Peru, Nueva Granada, and Chile) races, which can be distinguished by morphological and biochemical characteristics (Singh et al. 1991a, b). An additional Mesoamerican race has been designated Guatemala that includes certain climbing beans from Central America (Beebe et al. 2000). These races have been subjected to analysis using a range of

molecular markers especially isozymes (Debouck et al. 1993) and RAPDs (Beebe et al. 2000, 2001) but to date have not been characterized for microsatellites.

Microsatellites or simple sequence repeats (SSR) are PCR-based molecular markers developed around short segments of DNA in which a specific motif of one to six nucleotides is repeated in tandem, multiple times (Morgante and Olivieri 1993; Powell et al. 1996). Due to the high mutation rates and resulting variability at SSR loci, these markers have been ideal for genetic mapping and characterizing genetic diversity in crop species at the inter-specific, inter-subspecific, inter-varietal and even intra-varietal levels (Lee 1995; Mitchell et al. 1997; Matus and Hayes 2002). SSR markers have been developed for common bean (Yu et al. 2000; Gaitán-Solís et al. 2002; Blair et al. 2003; Yaish and Perez de la Vega 2003) but their number lags behind the number in other cereals and legumes such as rice and soybeans (Temnykh et al. 2000; Cregan et al. 1999).

An essential characteristic of microsatellites is their ability to detect polymorphisms on panels of diverse germplasm. Microsatellites have been found to vary in the polymorphism they detect depending sometimes on the length and sequence of the repeat motif they contain and their location in gene-coding or non-coding segments of the genome (Thoquet et al. 2002; Temnykh et al. 2000, 2001; Eujay et al. 2002). Polymorphism information content (PIC) analysis can be used to evaluate markers so that the most appropriate can be selected for genetic mapping, phylogenetic analysis or association genetics (Anderson et al. 1993). In common bean, microsatellite markers have been used to construct a PCR-based genetic map (Yu et al. 2000; Blair et al. 2003), to evaluate intra-specific diversity within the genus (Gaitán-Solís et al. 2002) and to fingerprint genetic diversity in commercial varieties of common beans from Europe (Métais et al. 2002; Masi et al. 2003) and from Nicaragua (Gomez et al. 2004) but have not been extensively tested in other regional collections or used for association mapping. Initial sets of fluorescent microsatellite panels have been constructed for common beans (Masi et al. 2003) although extensive panels such as those that exist for rice (Blair et al. 1999; Coburn et al. 2002) and soybean (Narvel et al. 2000) have not been developed.

The objectives of this work, therefore, were (1) to evaluate the mapped common bean microsatellites used in Blair et al. (2003) for their ability to detect genetic diversity within a representative set of 43 common bean cultivars and wild accession spanning both the Andean and Mesoamerican gene pools; and (2) to describe the structure of diversity within this group of genotypes. The study provides a baseline for allele size determination which is useful for the design of fluorescent microsatellite fingerprinting panels and also serves as a parental survey for common bean genotypes that are parents of recombinant inbred line and advanced backcross populations among wild and cultivated germplasm, and among and within the gene pools.

Materials and methods

Plant material

A total of 43 common bean (*P. vulgaris*) and one tepary bean (*P. acutifolius*) genotypes (Table 1) were used in this study. The genotypes represented parents of genetic mapping populations being studied at Centro Internacional de Agricultura Tropical (CIAT) for the inheritance of disease resistance (common bacterial blight caused by *Xanthomonas anopodis* pv. *phaseoli*, angular leaf spot caused by *Phaeoisariopsis griseola*, anthracnose caused by *Colletotrichum lindemuthianum* and bean golden yellow mosaic virus), insect resistance (*Apion godmani* and *Thrips palmi*), abiotic stress tolerance (aluminum, drought and low phosphorous), grain quality (micronutrient content) as well as yield and its components. The genotypes were grouped in three parental surveys with common control genotypes run in each gel, namely the Mesoamerican DOR364 and the Andean G19833. Among the 43 common bean genotypes were a total of 12 Andean (10 cultivated and 2 wild) and 31 Mesoamerican (30 cultivated and 1 wild) genotypes. The three wild accessions represented accessions from Argentina, Colombia, and Mexico. Among the cultivated genotypes, 16 were advanced breeding lines from CIAT and the remainder were landraces or locally bred varieties. CIAT lines included 3 from the BAT series, 1 from the BRB series, 4 from the DOR series, 2 from the MAM series, 1 from the MAR series, 3 from the SEA series, 1 from the SEL series, 1 from the SEQ series and 1 from the VAX series. BAT, DOR, MAM, MAR, SEA, and VAX lines are predominantly Mesoamerican while BRB and SEQ lines are predominantly Andean. MAM lines have mixed Durango and Mesoamerica race pedigrees while the SEL and VAX lines have some tepary bean ancestry. Germplasm accessions included representatives of the Nueva Granada and Peru races within the Andean gene pool and representatives of the Guatemala, Durango, Jalisco, and Mesoamerica races within the Mesoamerican gene pool according to previous classifications by Beebe et al. (2000, 2001). Local varieties included one variety each from Brazil and Mexico, and two varieties each from Colombia and Honduras (EAP). A single tepary bean genotype, G40001 was included as an outgroup and because it is a parent with the Mesoamerican cultivar ICA Pijao of a congruity backross population (Muñoz et al. 2004). The growth habit of each genotype was classified from I (determinate bush) to IV (indeterminate climber) according to Singh (1982).

Microsatellite analysis

DNA was extracted by standard mini prep methods (Afanador and Hadley 1993; Mahuku 2004) and quantified with a Hoefer DyNA Quant 2000 fluorometer for dilution to a standard concentration of 10 ng/μl. Microsatellite markers included the 150 microsatellites analyzed by Blair et al. (2003) with a total of 65 gene-based and 85

Table 1 Common bean genotypes used for assessment of microsatellite diversity and their accession number, phaseolin status, race and gene pool identity, origin and growth habit

Genotype	Ph	Genepool	Race	Status	Origin	GH
G 11360	S	Mesoamerican	J	Cultiv	Mexico	IV
G 11350	S	Mesoamerican	M	Cultiv	Mexico	III
G 21657	C	Andean	P	Cultiv	Bulgaria	III
G 21078	T	Andean	P	Cultiv	Argentina	IV
G 21242	C	Andean	na	Cultiv	Colombia	IV
G 14519	S	Mesoamerican	M	Cultiv	USA	IV
G 4825	B	Mesoamerican	M	Cultiv	Brazil	III
G 19833	C	Andean	P	Cultiv	Peru	III
DOR 364	S	Mesoamerican	M	Cultiv	CIAT	II
BAT 477	S	Mesoamerican	M	Cultiv	CIAT	II
G 3513	S	Mesoamerican	M	Cultiv	Mexico	II
BAT 881	S	Mesoamerican	M	Cultiv	CIAT	II
G 21212	B	Mesoamerican	M	Cultiv	Colombia	II
G 24404	C	Andean	na	Wild	Colombia	IV
Radical Cerinza	T	Andean	P	Cultiv	Colombia	I
G 24390	M	Mesoamerican	na	Wild	Mexico	IV
DOR 390	S	Mesoamerican	M	Cultiv	CIAT	II
G19892	T	Andean	na	Wild	Argentina	IV
DOR 476	S	Mesoamerican	M	Cultiv	CIAT	II
SEL 1309	S	Mesoamerican	M	Cultiv	CIAT	II
BAT 93	S	Mesoamerican	M	Cultiv	CIAT	II
Jalo EEP558	T	Andean	NG	Cultiv	Brazil	III
ICA Pijao	B	Mesoamerican	M	Cultiv	Colombia	II
G 40001	na	Tepary Bean	na	Cultiv	Mexico	III
VAX 6	S	Mesoamerican	M	Cultiv	CIAT	II
MAR 1	S	Mesoamerican	M	Cultiv	CIAT	II
J 117	S	Mesoamerican	J	Cultiv	Mexico	III
JAMAPA	S	Mesoamerican	M	Cultiv	Mexico	II
G 2333	S	Mesoamerican	G	Cultiv	Mexico	IV
G 19839	T	Andean	P	Cultiv	Peru	III
G 855	Sb	Mesoamerican	J	Cultiv	Mexico	IV
BRB 191	T	Andean	NG	Cultiv	CIAT	II
MAM 49	S	Mesoamerican	D	Cultiv	CIAT	III
G 5273	T	Andean	NG	Cultiv	Mexico	II
MAM 38	S	Mesoamerican	D	Cultiv	CIAT	III
SEQ 1027	T	Andean	NG	Cultiv	CIAT	III
G 4090	Sd	Mesoamerican	M	Cultiv	El Salvador	II
Tio Canela	S	Mesoamerican	M	Cultiv	EAP	II
DOR 714	S	Mesoamerican	M	Cultiv	CIAT	II
SEA 5	S	Mesoamerican	D	Cultiv	CIAT	II
MD 23–24	S	Mesoamerican	M	Cultiv	EAP	II
SEA 15	S	Mesoamerican	D	Cultiv	CIAT	II
G 685	Sb	Mesoamerican	G	Cultiv	Guatemala	IV
SEA 21	S	Mesoamerican	M	Cultiv	CIAT	II

Races (*D* Durango, *G* Guatemala, *J* Jalisco, *M* Mesoamerica, *NG* Nueva Granada) as determined by morphology and multiple correspondance analysis this article, *GH* growth habits as described in materials and methods of text, *na* not applicable

genomic derived markers. Amplification conditions were as recommended previously (Blair et al. 2003; Gaitán-Solís et al. 2002; Yu et al. 2000). PCR reactions were carried out in 12 μl reaction volumes containing 20 ng of genomic DNA, 0.15 μM each of forward and reverse primers, 200 μM of total dNTP and 1 unit of *Taq* polymerase in 1× PCR buffer [10 mM of Tris–HCl (pH 7.2), 50 mM of KCl]. Final $MgCl_2$ concentration ranged from 1.5 to 2.5 mM depending on the microsatellite and was controlled by adding this component separately to the PCR reaction mix. Amplification products were mixed with a loading buffer (5 μl formamide, 0.4% bromophenol blue and 0.25% w/v xylene cyanol FF) and denatured at 96°C for 4 min. A 2.5 μl sample of this mixture was loaded onto 4% denaturating polyacrylamide gels (29:1 acrylamide:bis-acrylamide) which were run in Sequi-Gen GT electrophoresis units (Bio-Rad, Hercules, CA, USA) at 100 constant Watts for approximately 1.5 h depending on the expected size of the microsatellites. Gels were silver stained according to manufacturers instructions for the Silver Sequence® DNA Sequencing System kit (Promega, Madison, WI, USA). Allele sizes were estimated for clear single-copy banding patterns based on comparison of microsatellite bands to a 10-pb molecular-weight ladder (Invitrogen, Carlsbad, CA, USA) that was placed every twentieth lane in one hundred lane gels. For the comparison between the three parental surveys, any gels that did not show the same allele size for the control genotypes, DOR364 and G19833, which were loaded adjacent to the molecular weight size standard, were not used for the analysis. Of the 150 microsatellites, a total of 21 could not be compared in this manner due to differences in amplification pattern or multiple banding patterns and were not used for data analysis.

Data analysis

Comparable allele sizes could be ascertained across the three parental surveys for a total of 129 of the microsatellites and these were used for all subsequent analysis. Null alleles were uncommon (4.8 and 6.7% for genomic and gene-based microsatellites, respectively) and were not used in diversity assessment. Allele assignments for the 23 common bean and one tepary bean accessions were used to calculate the PIC of each microsatellite according to Anderson et al. (1993). PIC was calculated using the formula: $\mathrm{PIC} = 1 - \Sigma p_{ij}^2$ where p_{ij} is the frequency of the patterns (j) for each marker (i). The microsatellite dataset was also used for a multiple correspondence analysis (MCA) of the genotypes where the MCA defines a matrix of distances between each pair of individuals calculated using a chi-squared metric involving a weighting factor that considers the quantity of information for each individual and each band (Hair et al. 1992). Subsequently the MCA data were used for UPGMA clustering in SAS program software v. 9.1.3 (SAS Institute, Cary NC, USA). Principal inertia adjustments to calculate the variance explained by each dimension in the MCA were made according to Benzecri (1992) in this version of SAS. Values for total diversity (H_t), intra population diversity (H_s) and inter population diversity (H_{si}) as well as population differentiation (G_{st}) coefficients were also calculated (Nei 1987).

Results

Characterization of microsatellite markers

Across all the microsatellites tested there was an average of 7.8 alleles and an average polymorphism information

content of 0.534, however genomic microsatellites had a higher average number of alleles (9.2) than gene-based microsatellites (6.0) (Table 2). Similarly, the average polymorphism information content was higher for the genomic microsatellites (PIC = 0.594) compared to the gene-based microsatellites (PIC = 0.446 on average). Correlations between the number of alleles and the PIC values were high for both gene-based ($r = 0.772$, $P < 0.0001$) and genomic ($r = 0.820$, $P < 0.0001$) microsatellites. The allele size range was generally a good predictor of the number of alleles present for a locus and vice versa, however there were several microsatellites such as BMd18 and PC-CTT002 with large size ranges (above 40 bp) but few alleles (below 5). Conversely there were several gene-based (PV-AG003 and PV-AG004b) and genomic (BM151, BM165, BM181, BM189, and BMd33) with a small size range (below 15) and more than 5 alleles. Overall the number of alleles was correlated with the size range for both the gene-based ($r = 0.494$, $P = 0.0001$) and genomic ($r = 0.848$, $P < 0.0001$) microsatellites. The size differences between the largest and the smallest alleles or allele range was wider on average for the genomic microsatellites (31.4 bp) compared to gene-derived microsatellites (19.1 bp). Several gene-based (BMd18, PV-AG004, PV-AT001, PV-TTTC001) and genomic microsatellites (BM53, BM137, BM187, BM154, BM156, BM160) which tended to be highly polymorphic had large size differences between smallest and largest alleles. The observed allele sizes for the 129 microsatellite agreed well with the expected sizes from Blair et al. (2003), Gaitán-Solís et al. (2002) and Yu et al. (2000) and in all but 6 gene-based (BMd26, BMd31, BMd32, BMd46, PV-GGC001, and PV-TTC001) and 2 genomic microsatellites (BM195 and GATsIIB) the expected allele size from these previous reports was within the range of observed allele sizes (Table 2). The highest number of alleles (23) among the genomic microsatellites was found for BM137 and BM154 with the next highest being for BM153 (21 alleles). These three markers had the highest PIC values ranging between 0.927 and 0.937. The highest number of alleles (28) among the gene-based microsatellites was found for BMd1 with the next highest being PV-AAT001 (24 alleles) while all the remaining microsatellites in this class had 20 alleles or fewer. Monomorphic markers across the 44 individuals tested included the gene based microsatellites BMd35, BMd48, PV-AAAT001, PV-ATCC001, and the genomic microsatellite BMd39. The trends both in allele number and polymorphism information content for genomic versus gene-based microsatellites are visualized by plotting these two variables against each other for both types of markers (Fig. 1).

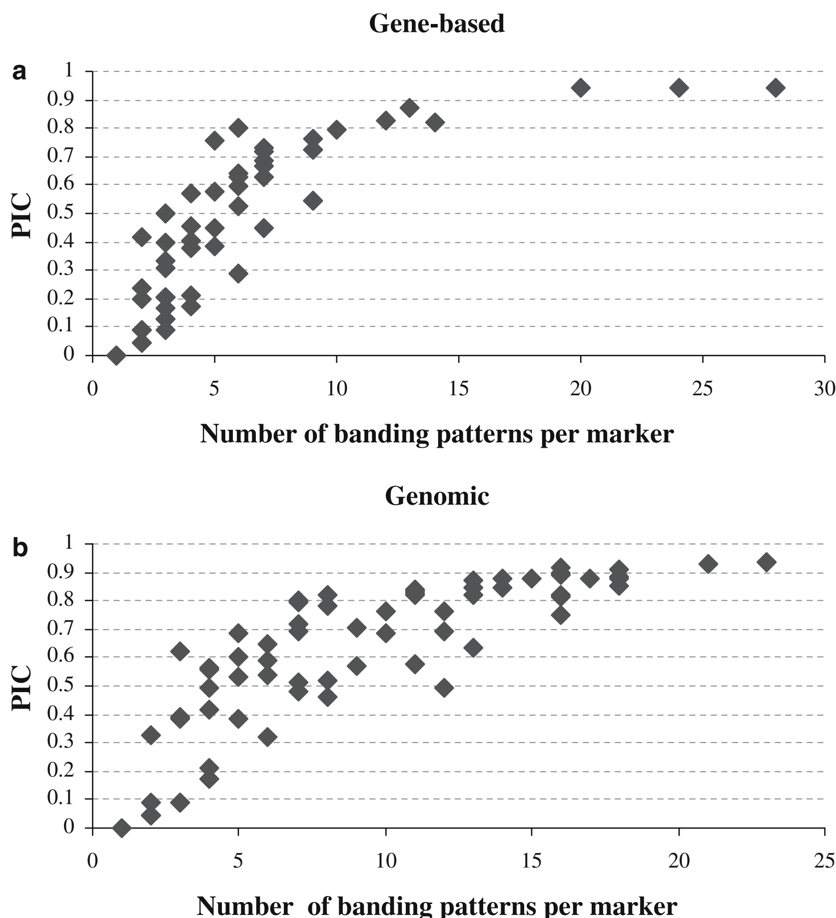

Fig. 1 Relationship between polymorphism information content (*PIC*) of each microsatellite and the number of allele banding patterns detected by 57 gene-based (**a**) and 72 genomic (**b**) microsatellites

Table 2 Number of alleles and polymorphism information content (PIC) of 129 common bean microsatellites (57 gene-based and 72 genomic) markers

Marker	No. of alleles	PIC	Expected Allele	Min.	Max.	Marker	No. of alleles	PIC	Expected Allele	Min.	Max.
(A) Gene-based						(A) Gene-based					
BMd1	28	0.942	165	172	200	BMd37	6	0.799	134	123	134
BMd2	6	0.629	106	100	110	BMd45	2	0.416	129	92	130
BMd3	7	0.626	223	187	228	BMd46	4	0.379	158	320	330
BMd4	3	0.129	146	142	146	BMd47	4	0.573	150	128	154
BMd5	3	0.129	122	117	118	BMd48	1	0.000	131	131	131
BMd6	2	0.087	122	121	122	BMd49	2	0.201	95	94	94
BMd7	7	0.730	166	168	171	BMd50	3	0.334	124	122	126
BMd8	9	0.724	176	174	190	BMd51	4	0.407	116	107	118
BMd9	6	0.287	135	136	180	BMd53	5	0.574	105	108	112
BMd10	9	0.761	139	138	144	BMd55	3	0.088	188	185	185
BMd13	3	0.168	194	193	194	PV-CTT001	14	0.822	152	152	172
BMd14	3	0.206	186	188	190	PV-AG001	9	0.546	157	130	160
BMd15	9	0.722	166	163	202	PV-AG003	7	0.721	164	157	168
BMd16	6	0.526	136	126	150	PV-GAAT002	5	0.448	156	156	166
BMd17	7	0.666	116	100	118	PV-TTTC001	7	0.448	161	135	210
BMd18	5	0.757	156	154	242	PV-AT007	20	0.941	192	190	216
BMd19	4	0.209	154	155	162	PV-AT001	24	0.943	170	215	295
BMd20	10	0.793	123	116	132	PV-CTT002	3	0.305	218	168	218
BMd21	5	0.387	190	146	196	PV-AG004	9	0.546	201	202	276
BMd22	6	0.596	121	118	123	PV-AG004b	12	0.829	202	202	216
BMd23	2	0.087	127	127	128	PV-AAAT001	1	0.000	205	205	205
BMd25	3	0.400	118	116	118	PV-ATCC001	1	0.000	172	172	172
BMd26	4	0.458	141	133	140	PV-ATCC002	2	0.044	192	192	200
BMd27	2	0.236	109	157	157	PV-ATCC003	3	0.501	178	174	178
BMd28	13	0.874	151	130	157	PV-ATCT001	3	0.206	196	200	200
BMd30	4	0.170	134	134	136	PV-CCCT001	3	0.501	150	142	150
BMd31	2	0.044	161	146	146	PV-CCT001	7	0.684	137	137	158
BMd32	3	0.206	150	100	112	PV-TTTC001	6	0.638	143	162	258
BMd35	1	0.000	128	128	128						
(B) Genomic						(B) Genomic					
AG1	7	0.483	132	126	142	BM181	9	0.571	192	182	193
BM003	2	0.325	193	195	195	BM183	11	0.839	149	134	160
BM006	2	0.044	153	154	154	BM184	10	0.684	160	150	168
BM048	2	0.087	232	232	232	BM185	11	0.833	105	100	117
BM053	21	0.927	287	278	360	BM187	18	0.854	191	150	270
BM068	4	0.493	170	129	173	BM188	18	0.880	177	142	190
BM098	5	0.601	247	242	252	BM189	6	0.647	114	107	116
BM114	13	0.818	234	225	275	BM195	2	0.044	138	150	150
BM137	23	0.937	155	122	238	BM197	4	0.557	201	195	203
BM138	8	0.779	203	195	205	BM199	18	0.913	304	290	330
BM139	13	0.632	107	84	118	BM200	16	0.893	221	227	295
BM140	8	0.459	190	160	210	BM201	11	0.820	102	94	114
BM141	16	0.813	218	160	229	BM202	7	0.796	156	138	158
BM142	6	0.537	157	155	159	BM205a	9	0.707	137	135	154
BM143	18	0.887	143	118	176	BM209	14	0.848	129	95	146
BM146	4	0.170	281	278	288	BM210	17	0.880	166	147	200
BM147	3	0.088	178	178	180	BM211	13	0.848	186	180	237
BM148	2	0.044	295	290	290	BM212	8	0.517	214	196	214
BM149	6	0.320	273	242	258	BM213	3	0.394	154	156	160
BM151	8	0.819	153	139	154	BMd11	3	0.088	161	158	163
BM152	16	0.897	127	92	138	BMd12	3	0.623	167	164	170
BM153	15	0.876	226	188	255	BMd33	7	0.690	110	97	108
BM154	23	0.936	218	210	360	BMd36	12	0.762	164	160	180
BM155	5	0.387	114	114	126	BMd38	2	0.044	178	170	172
BM156	16	0.818	267	210	315	BMd39	1	0.000	126	126	126
BM157	7	0.510	113	100	130	BMd40	7	0.801	197	190	213
BM159	6	0.590	198	193	203	BMd41	7	0.716	245	232	255
BM160	11	0.580	211	183	265	BMd42	10	0.764	149	128	160
BM161	13	0.873	185	148	190	BMd43	4	0.414	176	138	178
BM164	12	0.495	182	139	186	BMd44	4	0.210	135	134	136
BM165	11	0.826	177	178	192	BMd56	5	0.534	193	186	192
BM166	2	0.044	151	148	150	BMd57	2	0.044	140	140	140
BM167	12	0.693	165	115	165	GATS11	5	0.683	306	210	236
BM170	14	0.877	179	155	182	GATS11B	3	0.385	160	100	105
BM172	11	0.580	107	82	110	GATS54	4	0.563	114	98	117
BM175	16	0.749	170	145	215	GATS91	16	0.914	229	210	275

Comparison of within and inter-gene pool parental combinations

Among the parental combinations represented in this survey were crosses between species, between cultivars, between gene pools and between wild accessions and cultivated genotypes (Table 3). The average level of polymorphism was higher in the comparison between ICA Pijao (*P. vulgaris*) and G40001 (*P. acutifolius*) than in the comparisons among common bean parents. The inter-genepool (Andean × Mesoamerican) combinations had higher polymorphism (59.7%) than within-genepool (37.9%) combinations. The most polymorphic of these was G855 × BRB191 which had an average level of polymorphism of 66.7%, however, many of the other combinations between cultivated Mesoamerican and Andean beans had similar levels of polymorphism (from 58 to 65% on average). The combinations between the wild and cultivated parents of different genepools, Radical Cerinza × G24390 and DOR390 × G19832 were similar to the averages of Andean × Mesoamerican combination within the cultivated genotypes. Among the inter-genepool combinations, DOR364 × G19833 was included because it was used to create the microsatellite map in Blair et al. (2003), while BAT93 × Jalo EEP558 was included because it was the basis for the integrated genetic map of Freyre et al. (1998). Both of these inter-genepool comparisons had similar levels of polymorphism although the combination DOR364 × G19833 was slightly higher in overall polymorphism.

Among the within-gene pool parental combinations, comparisons between Andean genotypes had higher polymorphism (53.0%) on average than comparisons between Mesoamerican genotypes (33.4%). This was especially notable with the parental combination of the cultivated Radical Cerinza and the wild accession G24404 followed by the parental combination of the Peru race genotype G21078 with G21242 compared to the parental combination of the same parent G21078 with the Nueva Granada race parent G21657. Within the Mesoamerican gene pool combinations, the intra-racial combinations between Durango or Jalisco and Mesoamerica race genotypes showed higher average polymorphism (37.5%) than the within-race combinations between Mesoamerica race genotypes (31.7%). Examples of inter-racial parental combinations with high polymorphism were G11360 × G11350, J117 × Jamapa and SEA5 × MD23-24. By comparison, polymorphism was low in the intra-racial combinations VAX6 × MAR1 and DOR476 × SEL1309, both derived from CIAT breeding lines that are predominantly of the Mesoamerica race. Polymorphism was even lower for the parental comparison between two parents of the same grain color class such as Tio Canela and DOR714, both small red seeded genotypes of the same subrace of the Mesoamerica race.

Table 3 Level of polymorphism in parental combinations across or within Mesoamerican (M) and Andean (A) gene pools and between common and tepary beans for gene-based and genomic microsatellite markers

Parental combination		Type of cross[a]	Gene-based (57)		Genomic (72)		Total (129)	
Female parent	Male parent		No. Poly	% Poly	No. Poly	% Poly	No. Poly	% Poly
G 11360	G 11350	M(j) × M (m)	14	24.6	32	43.8	46	35.7
G 21657	G 21078	A(p) × A(p)	21	36.8	37	50.7	58	45.0
G 21078	G 21242	A(p) × A(na)	24	42.1	44	60.3	68	52.7
G 14519	G 4825	M(m) × M(m)	14	24.6	29	39.7	43	33.3
DOR 364	G 19833	M(m) × A(p)	32	56.1	53	72.6	85	65.9
DOR 364	BAT 477	M(m) × M(m)	17	29.8	27	37.0	44	34.1
DOR 364	G 3513	M(m) × M(m)	10	17.5	28	38.4	38	29.5
BAT 881	G 21212	M(m) × M(m)	15	26.3	30	41.1	45	34.9
Cerinza	G 24404	A(p) × A (w)	28	49.1	51	69.9	79	61.2
Cerinza	G 24390	A(p) × M (w)	30	52.6	45	61.6	75	58.1
DOR 390	G 19892	M(m) × A (w)	30	52.6	49	67.1	79	61.2
DOR 476	SEL 1309	M(m) × M(m)	11	19.3	30	41.1	41	31.8
BAT 93	Jalo EEP558	M(m) × A(ng)	29	50.9	51	69.9	80	62.0
ICA Pijao	G 40001	Inter-specific	49	86.0	60	82.2	109	84.5
VAX 6	MAR 1	M(m) × M(m)	12	21.1	29	39.7	41	31.8
J 117	Jamapa	M(j) × M(m)	15	26.3	42	57.5	57	44.2
G 2333	G 19839	M(g) × A(p)	29	50.9	53	72.6	82	63.6
G 855	BRB 191	M(j) × A(ng)	31	54.4	55	75.3	86	66.7
BRB 191	MAM 38	A(ng) × M(d)	28	49.1	49	67.1	77	59.7
G 5273	MAM 38	A(ng) × M(d)	30	52.6	54	74.0	84	65.1
BRB 191	MAM49	A(ng) × M(d)	28	49.1	54	74.0	82	63.6
MAM 49	G 5273	M(d) × A(ng)	30	52.6	55	75.3	85	65.9
SEQ 1027	G 4090	A(ng) × M(m)	29	50.9	55	75.3	84	65.1
Tio Canela	DOR 714	M(m) × M(m)	8	14.0	24	32.9	32	24.8
SEA 5	MD 23–24	M(d) × M(m)	12	21.0	32	43.8	44	34.1

[a] Inter genepool and inter race combinations indicate by abbreviations where A = Andean and M = Mesoamerican genepools followed by an additional letter in parenthesis where (d) = Durango race, (g) = Guatemala race, (j) = Jalisco, (m) = Mesoamerica race, (ng) = Nueva Granada race, (p) = Peru race and (w) = wild accession as determined by morphology and multiple correspondance analysis in this article

Relationships between genotypes

In the overall multiple correspondence analysis (Fig. 2a), the tepary bean accession that had been used as an outgroup was differentiated from all common bean accessions in a first dimension which explained 24.3% of the variance. The wild accessions G24404 (Andean genotype from Colombia) and G24390 (Mesoamerican genotype from Mexico) were also distinguished from the domesticated common bean genotypes of each gene pool in a second dimension within the multiple correspondence analysis which explained 19.3% of the variance. Within the cultivated genotypes of common beans there were two principal clusters in the multiple correspondence analysis (Fig. 2b) corresponding to the Andean and Mesoamerican gene pools as expected. These were predominantly separated by the third dimension of the multiple correspondence analysis which explained 7.0% of the variance.

Within the Andean group there was evidence for two subgroups one clustering around the Argentinean wild accession G19892 and one separate from this group. These same groups could be identified more clearly in Fig. 2b which represents only the cultivated accessions within the multiple correspondence analysis. The first subgroup of Andean genotypes corresponded to the Peru race and the second to the Nueva Granada race, where the Peru race contained type IV (G21078), type III (G19833, G19839, and G21657) and type I (Radical Cerinza) growth habit beans; while the Nueva Granada race (BRB191, G5273, Jalo EEP558, and SEQ1027) did not include any climbing beans. Meanwhile, one of the cultivated Andean genotypes, G21242, showed signs of introgression from the Mesoamerican gene pool and was found half way between these two gene pools.

Within the Mesoamerican group there was less distinction or race structure although the Guatemala race genotypes (G685 and G2333) were associated and were separate from both Jalisco and Mesoamerica race genotypes. Jalisco race genotypes (G855, G11360, and J117) were intermediate between Guatemala and Mesoamerica race genotypes. Durango genotypes (MAM38, MAM49, SEA5, and SEA15) were not well separated from the Mesoamerica genotypes although they were clustered together.

Heterogeneity was calculated for each of the groups and subgroups described above using both the gene-based and genomic microsatellites as well as the overall dataset (Table 4). Inter-population diversity (H_s) was highest when considering the two species, *P. vulgaris* and *P. acutifolius* or when comparing the Andean and Mesoamerican gene pools within common bean, but was lower when comparing the races. Intra population diversity (H_{si}) was high and similar for cultivated and wild common beans whether the analysis was with gene-based or genomic microsatellites or both. Population differentiation did not distinguish the cultivated and wild common beans as they had overlapping patterns of diversity ($G_{st} = 0.035$, 0.029, and 0.031 for gene-based, genomic, and total microsatellites, respectively). On the other hand, population differentiation was evident when comparing the Andean and Mesoamerican gene pools ($G_{st} = 0.170$, 0.147, and 0.155, respectively). Intra-population diversity within the Andean gene pool was higher than within the Mesoamerican gene pool and this pattern was observed for both gene-based and genomic microsatellites. Greater population differentiation was

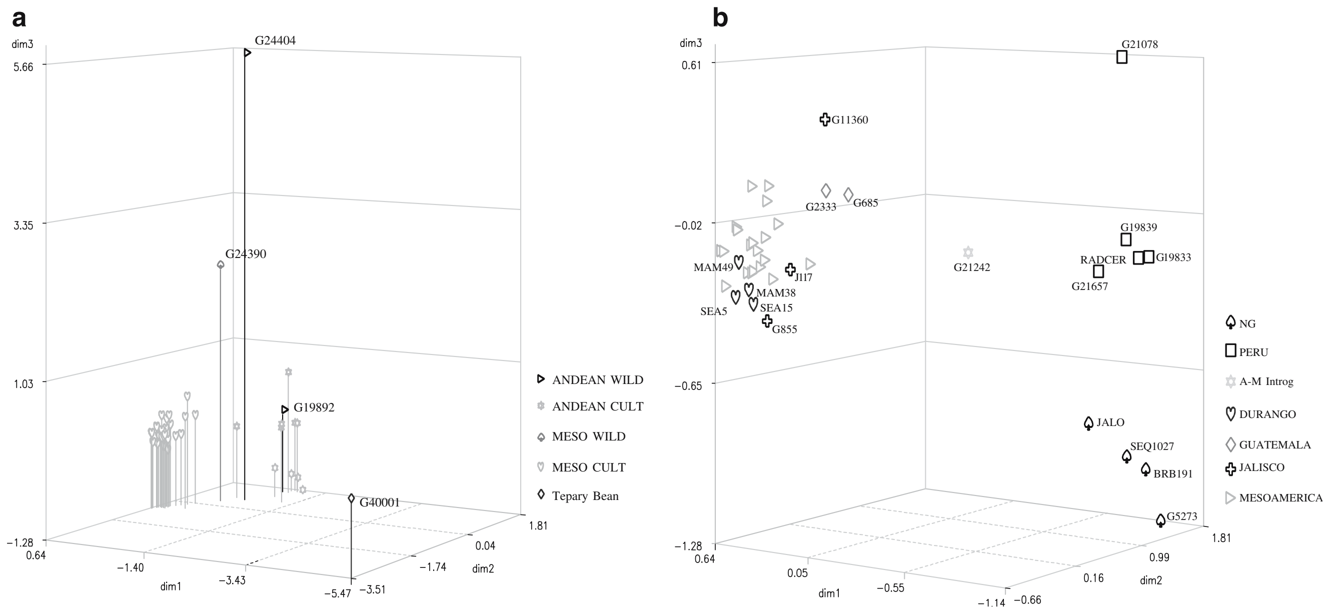

Fig. 2 Multiple correspondance analysis using UPGMA clustering for 44 common bean genotypes based on 129 microsatellite markers where **a** includes wild common bean (*Phaseolus vulgaris* L.) and tepary bean (*P. acutifolius Gray*) outgroups and **b** includes only cultivated common bean classified according to races within Andean and Mesoamerican genepools. Each subfigure is derived from the same overall multiple correspondence analysis and has the same dimensions represented

Table 4 Observed intra (H_s) and inter population (H_{si}) diversity for genotypes belonging to wild and cultivated common beans, to Andean and Mesoamerican gene pools and to races within each gene pool

Category	*N*	Observed heterogeneity			Value
		cDNA based (57)	Genomic (72)	Total	
Total	44	0.444	0.593	0.527	H_t
Species/status[a]	44	0.429	0.575	0.511	H_s
Cultivated *P. vulgaris*	40	0.432	0.583	0.516	H_{si}
Wild *P. vulgaris*	3	0.388	0.477	0.437	H_{si}
Tepary bean *P. acutifolius*	1	0.000	0.000	0.000	H_{si}
Gene pools	40	0.343	0.486	0.422	H_s
Mesoamerican	30	0.319	0.481	0.410	H_{si}
Andean	10	0.412	0.500	0.461	H_{si}
Races	40	0.253	0.363	0.314	H_s
Nueva Granada	4	0.215	0.352	0.292	H_{si}
Peru	5	0.397	0.436	0.419	H_{si}
Introgressed	1	0.000	0.000	0.000	H_{si}
Durango	4	0.154	0.325	0.249	H_{si}
Guatemala	2	0.246	0.292	0.271	H_{si}
Jalisco	3	0.257	0.367	0.319	H_{si}
Mesoamerica	21	0.289	0.430	0.368	H_{si}

[a] Status distinguishes wild versus cultivated *Phaseolus vulgaris*

observed with the races (G_{st} = 0.361, 0.323, and 0.337, respectively) compared to the gene pools. Intra-population diversity within each of the races was lower than within the corresponding gene pool as a whole. Furthermore, intra-population diversity within the Andean races (0.356 on average) was higher than within the Mesoamerican races (0.302). Within the Andean gene pool, race Peru had higher diversity compared to race Nueva Granada, while within the Mesoamerican gene pool, the races Durango, Guatemala, and Jalisco had comparable levels of diversity which were below that of race Mesoamerica.

Discussion

Significantly lower average number of alleles per locus and polymorphism information content were found for microsatellites from gene sequences than for microsatellites from non-coding sequences ($t = 2.72$, $P < 0.001$, and $t = 3.06$, $P < 0.005$, unpaired t-test with 72 and 57 *df*, respectively). The correlation between number of alleles per locus and polymorphism information content means that either estimator is useful for determining the value of a marker for diversity studies. The gene-based microsatellites were frequently bi-allelic or tri-allelic and distinguished the difference between Andean and Mesoamerican genepools. Meanwhile most of the genomic microsatellites detected more than three alleles and were able to resolve within-genepool variation. These trends have been observed in other crop species (Brown et al. 1996; Cho et al. 2000; Temnykh et al. 2001; Schloss et al. 2002). Differences in allelic variability at specific microsatellite loci may be due to differences in the mutation rate and selection pressure inherent for each locus (Métais et al. 2002; Chen et al. 2002). Microsatellite variability is thought to be influenced by the structure, motif, length and genomic context of the simple sequence repeat loci (Cho et al. 2000; Temnykh et al. 2001). The level of polymorphic information content found with the gene-based and genomic microsatellites was not associated with their map position or in the case of the gene-based microsatellites with their position within the translated or untranslated regions of the gene as described in Blair et al. (2003).

The polymorphism detected between parental combinations evaluated in this study agreed well with whether the comparison was across species, gene pools or races. Microsatellite polymorphism was low in parental comparisons from the same race within the Mesoamerican gene pool, slightly higher for parents from different races within the Mesoamerican gene pool or for races within the Andean gene pool, higher still for crosses between gene pools, and highest of all for the cross between common and tepary beans. The combinations between germplasm accessions were more polymorphic than the combinations between advanced breeding lines or varieties. This difference in polymorphism rate was equally evident when using cDNA derived and genomic microsatellites although the average polymorphism rate for genomic microsatellites was higher (57.9%) than for gene derived microsatellites (40.0%) over all the intra-specific comparison but not in the interspecific comparison where both rates were very high (above 80%). The parental comparisons made in this study were representative of the types of parental combinations used in common bean research and show the value of recently-developed microsatellites for efficient genetic analysis of *Phaseolus*, especially for populations derived from within gene pool parental combinations which hitherto have been difficult to analyze.

In terms of the diversity assessment, several observations are pertinent. First, microsatellite polymorphism in common beans used in this study appeared to be higher than within other cultivated legumes including peanuts (*Arachis* spp.), chickpea (*Cicer arietinum*), soybean (*Glycine max*) or cowpea (*Vigna unguiculata*) (Cheng et al. 2001; Ferguson et al. 2004; Hopkins et al. 1999; Huttel et al. 1999; Narvel et al. 2000). The higher intra-specific diversity of microsatellites within common bean may reflect the dual domestication events of the Andean and Mesoamerican gene pools and a greater level of inter gene pool hybridization (Gepts and Debouck 1991).

A second observation was that the microsatellite results agree with previous analysis of the origins of cultivated common bean conducted with isozymes (Santalla et al. 2002; Singh et al. 1991b), restriction fragment length polymorphism (Becerra-Velazquez and Gepts 1994; Sonnante et al. 1994) randomly amplified polymorphic DNA markers (Beebe et al. 2000: Johns et al. 1997), and amplified fragment length polymorphism markers

(Tohme et al. 1996; Beebe et al. 2001). However in this study, unlike previous evaluations of Andean beans (Tohme et al. 1996; Beebe et al. 2001; Chacón et al. 2005), two groups were identified in the Andean cluster corresponding to the Nueva Granada and Peru races while groups were not clearly identified within the Mesoamerican cluster. The Guatemala race genotypes (G685 and G2333) and one Jalisco genotype (G11360) were distant however from the Mesoamerica, Durango and other Jalisco race genotypes. Microsatellites were useful previously for the analysis of diversity in common bean breeding lines from Canada (Yu et al. 1999) in wild accessions and related species (Gaitán-Solís et al. 2002), in snap beans (Métais et al. 2002) and in dry bean landraces from Europe (Masi et al. 2003) and Nicaragua (Gomez et al. 2004), however this study is the first to evaluate a broad set of tropically adapted wild and cultivated dry bean genotypes of various growth habits, as well as improved and unimproved germplasm from various seed classes ranging from carioca, small red, black, large red, red mottled, cream mottled to yellow mottled, with microsatellite markers.

A third observation was that within gene-pool diversity was higher in the Andean gene pool than within the Mesoamerican gene pool and as a result microsatellites may be more useful for genetic mapping in Andean × Andean parental combinations than for Mesoamerican × Mesoamerican parental combinations. The greater diversity within Andean beans may reflect their multiple growth habits and origin in different agroecologies, while the similarity of some of the Mesoamerican genotypes may be a result of their being derived from an inter-racial mix of parents, this despite the fact that the Mesoamerican gene pool was represented by a greater number of races (Durango, Guatemala, Jalisco, and Mesoamerica) than the Andean gene pool (Nueva Granada and Peru). Alternatively, the higher diversity of the Andean genotypes could be due to the selection of genotypes from a greater range of agroecologies typical of the regions where Nueva Granada and Peru race cultivars are grown (Singh et al. 1991a) compared to the Mesoamerican genotypes many of which were from the CIAT breeding program or from Central America. Higher diversity within the Andean gene pool may have also been due to introgression of Mesoamerican or wild accession alleles into this gene pool (Beebe et al. 2001). The accession G21242 was notable because it appeared to be intermediate between Andean and Mesoamerican gene pools and therefore could be a possible hybrid from Mesoamerican–Andean gene pool introgression.

A final observation was that the results of this microsatellite study show some differences with previous morphology based classification of common bean races (Singh et al. 1991a). Notably, growth habit was not important for distinguishing the Andean genotypes into different races, as type I bush as well as type IV climbing beans were found amongst the Peru race individuals defined in this study and type II and III growth habit beans were found in the Nueva Granada race individuals likewise defined here. Finally the microsatellite survey found a close association of the type II Mesoamerica race individuals and type III Durango race individuals however it would be useful to confirm this result with additional landraces from the Durango race as some of the genotypes used in this study were from mixed ancestry (Terán and Singh 2002).

In closing, the present survey represent a baseline for the choice of microsatelite markers for future genetic fingerprinting and marker assisted selection. The more polymorphic genomic microsatellites may well become the mainstay of mapping studies since they will be useful even in narrow intra-genepool crosses. They will also be very useful for analyzing recent changes in population structure and the history of selection in closely-related germplasm from a given area or from a specific commercial class. Meanwhile the more conserved and stable cDNA-derived microsatellites may find their greatest utility in mapping in wide inter-genepool or inter-specific crosses and in the phylogenetic analysis of the genus.

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