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ORIGINAL PAPER

Genetic diversity of Chinese common bean (*Phaseolus vulgaris* L.) landraces assessed with simple sequence repeat markers

Xiaoyan Zhang · Matthew W. Blair · Shumin Wang

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Abstract Common beans were introduced from the Americas to China over 400 years ago and presently constitute an important export crop in many areas of the country. Evaluation of the genetic diversity present in Chinese accessions of common beans is essential for conservation, management and utilization of these genetic resources. The objective of this research was to evaluate a collection of 229 Chinese landraces with 30 microsatellite markers to evaluate the genetic variability, genepool identity and relationships within and between the groups identified among the genotypes. A total of 166 alleles were detected with an average of 5.5 alleles per locus for all microsatellites. The landraces were clustered into two genepools with two subgroups each. The level of diversity for Chinese landraces of Andean origin was higher than for the Chinese landraces of Mesoamerican origin due to the presence of more infrequent alleles in this first group. The range of marker prevalence indices was from 0.288 to 0.676 within the Andean group and from 0.426 to 0.754 within the Mesoamerican group. Two subgroups were identified in each genepool group with one of the Mesoamerican subgroups arising from introgression. Gene flow (N_m) was 0.86 or below between subgroups from different gene pools and 2.6 or above between subgroups within the genepools. We discuss the existence of a secondary center of diversity for common beans in China and the importance of inter genepool introgression.

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X. Zhang · S. Wang (✉)
ICS, CAAS-Institute of Crop Science,
Chinese Academy of Agricultural Sciences,
Beijing 100081, China
e-mail: smwang@mailcaas.net.cn

M. W. Blair (✉)
CIAT-International Center for Tropical Agriculture,
Cali AA6713, Colombia
e-mail: m.blair@cgiar.org

S. Wang
NFCRI-The National Key Facility for Crop Gene Resources
and Genetic Improvement, Beijing 100081, China

Introduction

Common bean (*Phaseolus vulgaris* L.) is the most important edible food legume in the world, representing 50% of grain legumes for direct human consumption (McClean et al. 2004). The crop originated and was domesticated in the New World in two centers of origin (Andes and Mesoamerica), which gave rise to two major gene pools (Andean and Mesoamerican) distinguished by seed size and other differences (Broughton et al. 2003). Genotypes from the cultivated Andean gene pool generally are large seeded (>40 g 100-seed weight^{-1}) while genotypes from the cultivated Mesoamerican gene pool are small-seeded (<25 g 100-seed weight^{-1}) or medium seeded (25–40 g 100-seed weight^{-1}) (Evans 1973, 1980). Evidence based on phaseolin seed proteins (Gepts et al. 1986), allozymes (Singh et al. 1991c; Santalla et al. 2002), morphological traits (Singh et al. 1991b), and DNA markers (Beebe et al. 2000, 2001; Blair et al. 2006) have confirmed the existence of the two gene pools. Singh et al. (1991a, b) further divided the two gene pools into six races, three Andean (Nueva Granada, Peru and Chile) and three Mesoamerican (Mesoamerica, Durango and Jalisco), with an additional race reported for Guatemalan climbing beans (Beebe et al. 2000). Common bean is widely distributed around the world and secondary centers of diversity exist in the Caribbean (Castiñeiras et al.

1991; Durán et al. 2005), South America outside the Andean primary center (Maciel et al. 2003), Europe (Rodiño et al. 2001, 2003, 2006; Santalla et al. 2002), Africa (Khairallah et al. 1990; Martin and Adams 1987a, b) and potentially in Asia (Singh 1999). Within Asia, collections exist in China (Wang et al. 1999), India (Tiwari et al. 2005) and Iran (Pribalouti et al. 2006), however common bean diversity has been less well-studied in Asia and Africa than in Europe or the Americas.

China is a major producer of common beans (fifth worldwide in dry beans and first in snap beans) with production distributed in many agricultural areas of the country, including primary bean growing areas in the provinces of Guizhou, Heilongjiang, Neimenggu, Sichuan and Yunnan (Wang et al. 1999). A total of 1,204,000 ha of dry beans and 213,000 ha of snap beans are grown in China (FAO 2006). The crop is thought to have a history of over 400 years in China according to sources reviewed by Zheng (1997) and was suspected of having been introduced directly from Latin America. Given this history, common bean is considered a traditional crop in China. Common beans in China are mainly produced under rain-fed conditions in traditional farming systems that often include rotation with vegetables or intercropping with maize (Zheng 1997). Some commercial classes have become an important export crop and are favorites of international trade reaching 799,690 ton exported (FAO 2006) making China one of the largest exporters of the crop.

Chinese grain types are characterized by being mainly small to medium seeded, some large, with predominance of white, cream, red, brown or black seed colors although cream mottled or red mottled seeds and some striped or bicolor patterns are also observed (Wang et al. 1996, 1997). Among the growth habits found in Chinese landraces, type IV climbing beans are the most common but type I, II and III types are represented. More than 4,900 accessions of common bean are conserved in the National Gene Bank of China located in the Chinese Academy of Agricultural Sciences in Beijing. Morphological characteristics, disease resistance, and quality traits of some of these common bean accessions have been catalogued, but little information is available regarding the genetic relationship of Chinese common bean landraces to each other and to international germplasm both within and between gene pools. Landraces are thought to have valuable traits in terms of agro-ecological adaptation, cooking quality or consumer preference, and resistance to diseases or abiotic stresses (Wang 2006). It is also noteworthy that few collections of Chinese beans are found outside of China and those that are have small numbers of accessions, with only 186 in the International Center for Tropical Agriculture and 131 in the United States Department of Agriculture plant genetic resource units. Chinese germplasm is presumed to include genotypes from the two centers of origin given the range in seed size but this has not been studied with molecular markers before. Marker studies are also needed to validate the designation of China as a secondary center of diversity for the crop.

A range of molecular techniques can assess crop genetic diversity, however among the most ideal for distinguishing closely related germplasm are microsatellites, which are highly informative markers that detect length polymorphisms at loci with simple sequence repeats (Powell et al. 1996). Their advantages for diversity studies include uniform genome coverage, high levels of polymorphism, codominance, and an easy-to-implement, specific PCR-based assay (Pejic et al. 1998). Although relatively few diversity studies in common bean have used microsatellite markers, a basic polymorphism survey exists for a range of loci (Blair et al. 2006) and two population structure studies have been carried out to analyze Mesoamerican and Andean gene pools (Díaz and Blair 2006; Blair et al. 2007). In addition, microsatellites and a related marker system called Inter Simple Sequence Repeat 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) and Slovenia (Maras et al. 2006).

The objective of this study was to apply microsatellite markers for the evaluation of genetic diversity of common bean germplasm from China in order to confirm the country's status as a secondary center of diversity for the crop and to obtain a baseline of information for the preservation and utilization of this important food species for Chinese and world agriculture. We were especially interested in (1) identifying the prevalence of the Andean and Mesoamerican gene pools in China, (2) detecting the presence of subgroups within the gene pools in China along with their possible relationships to common bean races and (3) comparing the polymorphism and allele frequencies in each of these gene pools and the geneflow or introgression between the identified groups and subgroups.

Materials and methods

Plant material

A total of 229 common bean genotypes from China were used in this study, of which 131 were supplied by the Genetic Resource Unit of CIAT (the International Center for Tropical Agriculture), and 98 were provided by the National Gene Bank of CAAS (Chinese Academy of Agricultural Sciences) http://www.ciat.cgiar.org/urg/beans.htm; http://www.caas.net.cn/engforcaas/index.htm). In addition

to these accessions, two check cultivars were included for comparisons: G19833, with large, yellow and red mottled seed, originally collected in Peru representing the Andean gene pool; and DOR364 with small red seed representing the Mesoamerica gene pool (Blair et al. 2006). A total of 164 genotypes had collection site data and these landrace accessions represented the following provinces: Sichuan (total 32 genotypes), Guizhou (23), Neimenggu (21), Heilongjiang (20), Hebei (17), Hubei (16), Shanxi (9), Shannxi (6), Yunnan (4), Beijing (4), Shandong (3), Jilin (3), Zhejiang (2), and Gansu (1), Hunan (1), Henan (1), Jiangxi (1). A total of 65 accessions from CIAT did not have detailed geographic information but were collected in China and had gene bank entry numbers. In general terms, the accessions from CIAT were mostly from the northeast and southeast of China while the genotypes supplied by CAAS were mostly from the northwest and southwest of China. Given this complementarity the two sets of genotypes were analyzed together to represent all of Chinese germplasm. Since the accessions obtained from both gene banks were not segregating for seed color we decided to evaluate a bulk of tissue from four plants per accession for DNA polymorphisms. Plants were grown for three weeks in a greenhouse and total genomic DNA was extracted from young trifoliate leaves using a CTAB extraction method described in Afanador et al. (1993). DNA quality was checked on a 1% agarose gel, quantified with a DyNA Quant 2000 fluorometer (Hoefer Pharmacia Inc.) and diluted to a concentration of 5 ng μl^{-1} for PCR reactions.

Microsatellite analysis

Thirty microsatellite markers were selected according to polymorphism and stability of amplification as per Blair et al. (2006). The PCR reactions were carried out in 15 µl final volumes containing 25 ng of genomic DNA, 0.1 µM each of the forward and reverse primers, 10 mM Tris–HCl (pH 7.2), 50 mM KCl, 1.5–2.5 mM $MgCl_2$ depending on the primer, 250 µM total dNTP and 1 unit *Taq* polymerase. The temperature cycling profile involved a hot start of 92°C for 3 min; then 34 cycles of denaturation at 92°C for 30 s, annealing at 50–65°C depending on the melting temperature for the given primer pair for 30 s and extension at 72°C for 45 s, followed by final extension at 72°C for 5 min. After amplification, each PCR reaction was ixed with 5 µl of formamide containing 0.4% bromophenol blue and 0.25% w/v xylene cyanol FF and denatured at 95°C for 3 min. A total of 4 µl of each mixture was then loaded with a Hamilton multi-pipetter onto 4% denaturing polyacrylamide (29:1 acrylamide:bis-acrylamide) gels that contained 5 M urea and 0.5× TBE. Gels were run in Sequi-Gen GT electrophoresis units (Biorad, Hercules, CF, USA) at a constant power of 100 W for 1–2 h and PCR amplification products were detected via silver staining according to manufacturer's instructions (Promega Inc., Madison, WI, USA) using a re-circulating tank system developed at CIAT.

Data analysis

Microsatellite allele sizes for the 30 loci were scored for all genotypes on the basis of comparison to a 10-bp molecular-weight ladder and an allele matrix was prepared from this dataset. Multiple correspondence analysis was performed with NTSYS-pc 2.1 (Rohlf 2002) based on calculations of Euclidean distance between genotypes. The inertia of each axis was calculated using the principal components procedure of the SAS software v. 9.1.3 (SAS Institute, Cary NC, USA). Subsequently the genetic distance matrix was used for UPGMA clustering also in NTSYS. The polymorphism information content (PIC) was calculated using the formula: $PIC = 1 - \Sigma p_{ij}^2$, where p_{ij} is the frequency of the patterns (j) for each marker (i), for each microsatellite across the entire sample of genotypes and for the Andean and Mesoamerican groups that were identified. To examine the relationship between the prevalence and rarity of markers, we computed the frequency of each of 30 markers within each gene pool. We then computed a 'marker prevalence index' for each cultivar (Beer et al. 1997) based on the average of the frequency values of the markers present in that cultivar as was applied for the study of introgression in common bean by Islam et al. (2004). Genetic variation within and among the groups and subgroups detected was analyzed with POPGENE software (Yeh et al. 1997) using parameters such as percentage of polymorphism (P), observed heterozygosity (H_o), Nei's (1978) coefficient of genetic diversity, coefficient of gene differentiation (G_{ST}), gene flow (N_m), genetic distance (GD) and genetic identity (I). The relationship between populations (K) was evaluated with the software Structure (Pritchard et al. 2000) based on populations of $K = 2$ to $K = 4$. Data was collected on seed color, seed pattern, growth habit and seed weight for each of the groups and subgroups and a non-random distribution of these traits was evaluated with contingency tests using SAS software. Monte Carlo random simulations were used for probability calculation when observation values were below 5.

Results

Characterization of microsatellite loci

The level of polymorphism among the Chinese common bean accessions in terms of allele size, total and predominant alleles and PIC values for each of the 30 microsatellites

evaluated is reported in Table 1. A total of 166 alleles were found across the full set of genotypes. The average number of alleles per microsatellite was 5.5, and ranged from 2 alleles for BM142, BMd26, BMd45, BMd46, BMd53 and PVgaa001 to 19 alleles for BM200. Other microsatellites showing a high number of alleles were BM160 with 15 alleles, and BM143 and BM210 each with 10 alleles. The PIC values, a reflection of allele diversity and frequency among these, were 0.541 for all the microsatellites, and ranged from a low of 0.235 for AG1 to a high of 0.878 for BM200. The size range between smallest and largest allele observed for a given microsatellite (minimum and maximum as listed in Table 1) varied from 2 to 80 bp. Among the 30 microsatellites used in this study, 20 were genomic and 10 were gene-based. The average allele number, average PIC value and size ranges were higher and broader for the genomic microsatellites compared to the gene-based microsatellites but were similar to the values obtained by Blair et al. (2006). These differences were significant comparing genomic and gene-based microsatellites for the average number of alleles per locus in unpaired *t*-tests ($t = 2.89$, $p = 0.00734$). Correlations between number of alleles and the PIC values were high for both genomic ($r = 0.7137$, $p = 0.0004$) and gene-based ($r = 0.7380$, $p = 0.0148$) microsatellites. Meanwhile, correlations between number of alleles and size range were significant for genomic microsatellites ($r = 0.7814$, $p < 0.0001$) but not for gene-based microsatellites.

Genetic structure of the germplasm collection

Microsatellite analysis uncovered two major groups of Chinese germplasm that corresponded to Andean and Mesoamerican gene pools based on the position of the control genotypes, DOR364 and G19833, in the multiple correspondence analysis (Fig. 1). The division between the Andean and Mesoamerican groups (dimension 1) explained the greatest proportion of the variance (43.3%) while the differentiation within the Andean group (dimensions 2 and 3) explained less variance (6.7 and 5.6%, respectively). Introgression between the two genepools was suggested by the existence of intermediates between the Andean and Mesoamerican groups.

Fewer genotypes were found in the Andean group than in the Mesoamerican group. The Andean group was composed of 58 accessions with average Euclidean distance of 1.00. The most common seed colors in this group were cream (24.14%), red (24.14%) and white (17.24%) with average 100 seed weight of 38.9 g and a mix of seed patterns including mottled (29.3% of total), bi-colored (12.1%) and striped (3.4%) with the remainder (55.2%) un-patterned). It is interesting to note that bi-color seed pattern was unique for the Andean group and mottling was more prevalent in this group than in the Mesoamerican group. Meanwhile, the Mesoamerican group consisted of 171 genotypes with a lower average Euclidean distance compared to the Andean group of 0.27. Mesoamerican accessions had small to medium sized seed with average 100 seed weight of 31.2 g. The dominant seed colors were brown (38.01%), cream (19.3%) and black (17.54%), however some white, yellow, pink or red seeded beans were also found in this group. It was noteworthy that yellow beans were less common in the Mesoamerican group than in the Andean group and that pink beans were found only in the Mesoamerican group. An off-gray color was also exclusive to the Mesoamerican group. The results also showed that striped seed pattern was common in the Mesoamerican group (21.1% of the total) but that seed mottling was infrequent (6.4%) and the majority of the genotypes were unpatterned (72.5%). All four growth habit types were present in both Andean and Mesoamerican groups, although type I growth habit was more prevalent in the first while type IV and type III growth habits were more common in the second. In contingency tests, the distributions of seed color, seed pattern and the four growth habits were found to be non-random between Andean and Mesoamerican groups.

Allele number, predominant allele and PIC value varied between the Andean and Mesoamerican groups for both types of microsatellites (Table 1). It was notable that the allele number was higher among Mesoamericans than among Andeans for both genomic (5.7 vs. 4.9, respectively) and gene-based (2.7 vs. 2.3) microsatellites, however the differences were not significant (paired *t*-test, $p > 0.05$). The lowest PIC values observed in the Andean group was with gene-based microsatellites such as BMd15 and BMd46 which only produced single alleles each, at the same time the highest average PIC value was also present in the Andean group for the genomic microsatellite BM200. The average number of alleles for the study was 6.07 with 2.64 effective number of alleles and 0.535 as the average Nei's heterozygosity (Table 2).

The marker prevalence indices were calculated for all the accessions together and for the Andean and Mesoamerican groups separately (Fig. 2). The accessions with large marker prevalence indices contain markers that are widespread in the germplasm pool, while accessions with small indices contain relatively rare markers (Islam et al. 2004). In the overall genotypes, the indices evaluated in this study ranged from 0.226 to 0.616 with an average index of 0.481. Andean accessions had lower marker prevalence indices (0.311) compared to Mesoamerican accessions (0.539) due to the greater frequency of Mesoamerican genotypes over Andean genotypes in the Chinese germplasm set. We were able to divide all the accessions into four classes based on prevalence indices. The first class consisted of 143 accessions (62.45%) all from Mesoamerican genepool with the

Table 1 Allele size and size range, allele number and predominant allele found across all genotypes and for each group as defined in the text as well as polymorphism information content (PIC) based on the evaluation of 229 common bean landraces with 20 genomic and 10 gene-based microsatellites

Locus	Allele size (bp)			Total alleles			Predominant allele			PIC		
	Minimum	Maximum	Range	All	Andean	Meso	All	Andean	Meso	All	Andean	Meso
Genomic												
AG1	123	155	32	4	3	2	137	137	137	0.235	0.586	0.034
BM139	82	118	36	8	8	4	84	102	84	0.534	0.702	0.289
BM140	160	202	42	8	6	5	160	200	160	0.622	0.778	0.512
BM142	153	155	2	2	2	2	153	155	153	0.347	0.289	0.034
BM143	115	173	58	10	8	8	131	155	131	0.576	0.824	0.335
BM151	146	154	8	5	4	5	150	148	150	0.665	0.554	0.555
BM152	92	132	40	9	7	8	96	132	96	0.732	0.702	0.651
BM155	116	120	4	3	2	3	120	118	120	0.519	0.180	0.448
BM157	100	118	18	3	3	3	100	114	100	0.496	0.553	0.230
BM160	180	260	80	15	10	13	184	232	184	0.859	0.788	0.812
BM170	150	180	30	8	5	5	158	168	158	0.593	0.395	0.417
BM175	158	190	32	9	6	8	158	170	158	0.513	0.737	0.265
BM181	180	202	22	6	6	3	180	184	180	0.470	0.638	0.109
BM197	198	202	4	3	2	3	200	202	200	0.554	0.033	0.369
BM200	220	262	42	19	10	17	244	232	244	0.878	0.851	0.801
BM210	164	184	20	10	4	10	170	170	182	0.835	0.512	0.854
BM211	180	190	10	3	3	3	180	190	180	0.369	0.656	0.064
GATS11	226	230	4	3	3	3	226	230	226	0.651	0.096	0.562
BMd33	96	110	14	4	4	3	100	108	100	0.666	0.171	0.562
BMd36	164	188	24	6	3	6	176	164	176	0.496	0.155	0.269
Average	150	176	26.1	6.9	5.0	5.7	155	166	156	0.580	0.510	0.408
Gene-based												
BMd15	164	204	40	3	1	3	168	168	168	0.390	0.000	0.469
BMd20	120	128	8	3	3	3	120	124	120	0.436	0.212	0.150
BMd26	136	142	6	2	2	2	142	136	142	0.425	0.330	0.241
BMd45	92	128	36	2	2	2	92	128	92	0.428	0.110	0.171
BMd46	318	321	3	2	1	2	318	318	321	0.499	0.000	0.458
BMd53	106	109	3	2	2	2	106	109	106	0.333	0.367	0.045
PV-CTT001	151	164	13	5	4	5	161	161	159	0.765	0.475	0.753
PV-AG001	148	156	8	4	4	3	148	156	148	0.488	0.478	0.218
PV-AG003	164	168	4	3	2	3	166	164	166	0.399	0.153	0.098
PV-GAA001	135	139	4	2	2	2	135	139	135	0.455	0.222	0.288
Average	153	166	12.5	2.8	2.3	2.7	156	160	156	0.462	0.235	0.289

highest indices ranging from 0.5 to 0.7; the second class included 32 accessions (13.97%) with indices from 0.4 to 0.5, of which 28 were from the Mesoamerican genepool while only four (G19286A, G19286B, G20408, G24541) were from the Andean genepool. The last two classes were composed of 24 (10.48%) and 30 accessions (13.1%) with lower indices from 0.3 to 0.4 and 0.2 to 0.3, respectively. Most Andean accessions (93.1%) belonged to these two classes. When prevalence indices were calculated within each genepool rather than across the entire dataset we found that the indices increased dramatically both in Andean and Mesoamerican groups although the value for the Andean group as a whole (0.584) was still lower than for the Mesoamerican group (0.649) as a whole.

Differentiation between subgroups

Among the two major Andean and Mesoamerican groups separating at a Euclidean distance value of 1.83, we found a total of four subgroups according to the UPGMA dendrogram

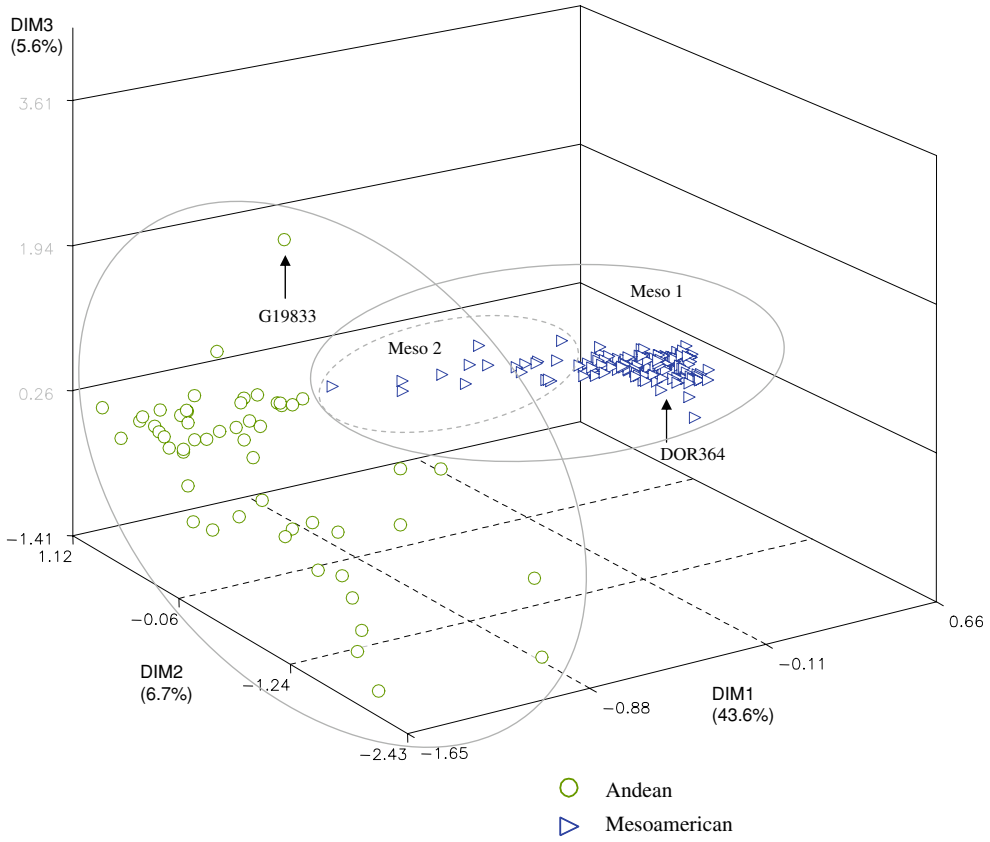

Fig. 1 Multiple correspondence analysis for 229 common bean genotypes based on 30 microsatellite markers showing accessions falling in the Andean (*circles*) or Mesoamerican (*banners*) groups. Positions of control genotypes indicated by *arrows* and *names*

Table 2 Genetic diversity for Chinese common bean accessions classified by subgroups within the Andean and Mesoamerican gene pools

Groups	na	ne	H_0	Nei's	P	%
Andean 1	2.5667	1.7875	0.0333	0.3043	22	73.33
Andean 2	3.7333	2.2797	0.0273	0.4420	30	100.00
Andean total	3.9667	2.2963	0.0294	0.4233	30	100.00
Meso 1	5.0000	1.9674	0.0500	0.3434	30	100.00
Meso 2	3.1333	2.1174	0.2533	0.4455	29	96.67
Meso total	5.3000	2.0465	0.0682	0.3665	30	100.00
Overall total	6.0667	2.6420	0.0581	0.5351	30	100.00

na observed number of alleles, *ne* effective number of alleles, H_0 observed heterozygosity, genetic diversity according to Nei (1978), *P* number of polymorphic loci, % percentage polymorphic loci

produced from the distance matrix (Fig. 3). Within the Andean group there were two subgroups separating at a Euclidean distance of 1.5 which were validated by STRUCTURE analysis, while in the Mesoamerican group there were two subgroups separating at a lower distance of 0.65 which in STRUCTURE analysis were distinguished by the amount of Andean introgression. Further subdivision of the first Mesoamerican group was possible with STRUCTURE analysis at a $K = 4$ but the subgroups were closely related in the dendrogram and were not studied further. Average seed size was higher in the Andean group on average compared to the Mesoamerican group (Table 3) and unpaired *t*-test showed significant difference ($p < 0.0000$) in seed weight between the genepools and between the subgroups.

Among the Andean subgroups, Andean 2 comprised 22 accessions and was more polymorphic (Nei's = 0.442) than Andean 1 comprised of 36 accessions (Nei's = 0.304). This subgroup also had the highest observed and effective number of alleles (3.73 and 2.28, respectively) and was diverse in seed pattern and growth habits (Tables 2, 3). Average seed size was larger in this subgroup (40.70 g) compared to the other subgroup although a few small-seeded types were observed (eg. G24541, 19.22 g). The control genotype for the Andean genepool, G19833 was most closely related to this subgroup but was clustered apart. Similar kinds of seed colors, more seed patterns but fewer growth habits and smaller seed size were observed in the Andean 1 subgroup compared to the Andean 2 subgroup. Among the Mesoamerican subgroups, the majority (91.2%) was clustered with the control genotype DOR364 in the Mesoamerican 1 subgroup, which presented a range of seed colors and seed sizes but had the lowest average seed weight of any subgroup. The Mesoamerican 2 subgroup had slightly higher

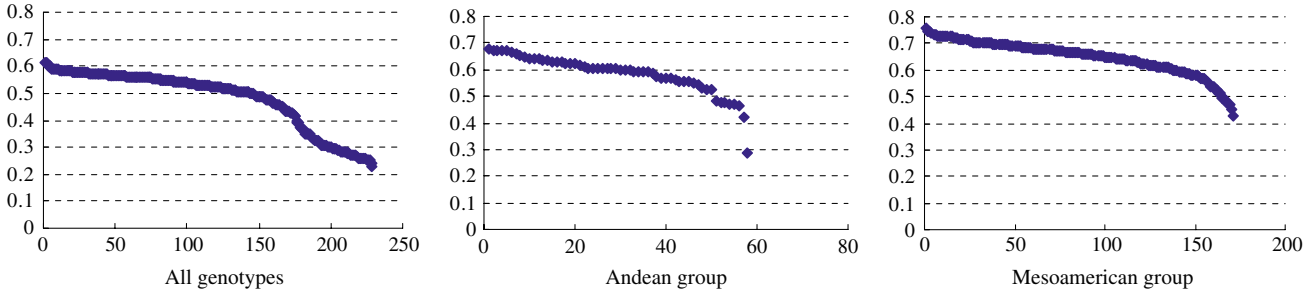

Fig. 2 Marker prevalence indices of 229 Chinese common bean accessions based on alleles identified at 30 microsatellite loci analyzed together or separately by genepool (Andean and Mesoamerican)

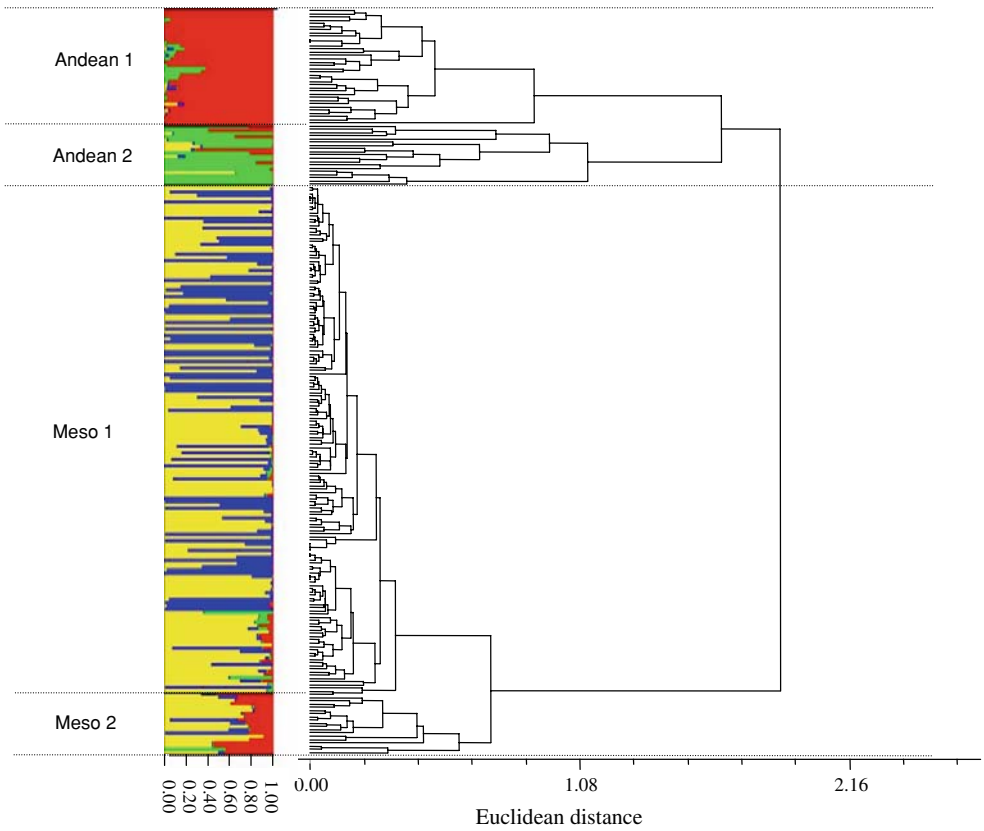

Fig. 3 UPGMA dendrogram and STRUCTURE analysis (with $K = 4$) for 229 Chinese common bean genotypes showing Andean and Mesoamerican subgroups as described in the text

average seed weight and a more limited range of seed colors some with mottling, which was uncommon in the previous subgroup. Mesoamerican 1 subgroup was predominantly type IV growth habit while Mesoamerican 2 subgroup was predominantly of type III growth habit.

Observed heterozygosity was generally low (average 0.058); however, for the Mesoamerican (0.068) subgroups the average was higher than for the Andean subgroups (0.029). The highest H_0 (0.253) was observed in the second Mesoamerican subgroup with the next highest (0.050) found in the first Mesoamerican subgroup while overall observed heterozygosity was 0.058. The higher observed heterozygosity in the second Mesoamerican group could reflect within accession diversity or high levels of outcrossing. Genetic differentiation, gene flow, genetic distance and genetic identity between the four subgroups are shown in Table 4. Similar gene flow (N_m higher than 2.6) was found within the Andean and Mesoamerican groups, which was higher than in a previous evaluation of Mesoamerican races by Díaz and Blair (2006); but comparable to that found in Andean races by Blair et al. (2007). Genetic differentiation was highest between genepool groups and lowest within them ranging from 0.08 and 0.09 within Andean and Mesoamerican group, respectively to 0.41 and 0.32 between

Table 3 Seed color, seed pattern, growth habit and 100-seed weight distribution in the subgroups of 229 Chinese common bean accessions with total genotypes in each category for Andean and Mesoamerican gene pools and overall indicated

Groups	Number	Seed color[a]									Seed pattern				Growth habit[b]				100-seed weight (g)		
		1	2	3	4	5	6	7	8	9	None	Bi-color	Mottle	Stripe	1	2	3	4	Average[c]	Minimum	Maximum
Andean 1	36	6	8	5	3	–	9	2	3	–	21	5	10	–	24	8	4	–	37.85	25.80	55.00
Andean 2	22	4	6	2	–	–	5	2	3	–	11	2	7	2	4	1	8	9	40.70	19.22	66.40
Andean group	58	10	14	7	3	–	14	4	6	–	32	7	17	2	28	9	12	9	38.91	19.22	66.40
Meso 1	156	25	31	1	63	2	6	1	24	3	118	–	4	34	2	7	28	119	30.86	15.47	52.00
Meso 2	15	–	2	1	2	–	3	1	6	–	6	–	7	2	–	1	10	4	32.70	24.00	44.00
Meso group	171	25	33	2	65	2	9	2	30	3	124	–	11	36	2	8	38	123	31.22	15.47	52.00
Overall total	229	35	47	9	68	2	23	6	36	3	156	7	28	38	30	17	50	132	35.07	15.47	66.40

[a] Seed color: *1* white, *2* cream, *3* yellow, *4* tan, *5* pink, *6* red, *8* black, 9 other color

[b] Growth habit: *1* determinate bush, *2* indeterminate bush, *3* indeterminate prostrate, *4* indeterminate climbing beans

[c] Average for each gene pool and overall indicated in last three rows of this column

Table 4 Genetic differentiation, gene flow, genetic distance and genetic identity among and between subgroups of Chinese accessions analyzed with microsatellite markers

Groups	G_{ST}				I			
	Andean 1	Andean 2	Meso 1	Meso 2	Andean 1	Andean 2	Meso 1	Meso 2
Andean 1	–	0.08	0.41	0.29	–	0.9039	0.2387	0.5179
Andean 2	2.87	–	0.32	0.23	0.1010	–	0.3346	0.5218
Meso 1	0.37	0.53	–	0.09	1.4327	1.0948	–	0.8589
Meso 2	0.62	0.86	2.61	–	0.6580	0.6505	0.1521	–
	N_m				GD			

Genetic differentiation (G_{ST}) and genetic identity (I) in upper diagonals in left and right panels of the table, respectively. Gene flow (N_m) estimated as $G_{ST} = 0.25(1 - G_{ST})/G_{ST}$ (N_m); genetic distance (GD) in lower diagonal of left and right panels, respectively

Mesoamerican 1 subgroup and the two Andean subgroups. Correspondingly, the lowest genetic distance was found for the subgroups within each group. Genetic identity was 0.904 within the Andean group and 0.859 within the Mesoamerican group and ranged from 0.239 to 0.522 between the genepool groups. Gene flow across genepools was 0.86 or below, while genetic distances ranged from 0.65 to 1.43 being widest between the first Andean and first Mesoamerican subgroups. The second Mesoamerican subgroup was closer to both Andean subgroups in genetic identity (average 0.520) than the first Mesoamerican group (average 0.287) suggesting it was partly introgressed with the Andean genepool also supporting results from STRUCTURE analysis shown in Fig. 3.

Discussion

Several observations about Chinese bean germplasm can be made from the results of this study. First, Chinese common bean accessions could be classified into the two gene pools of common bean clustering into Andean and Mesoamerican groups based on the microsatellites analysis. The predominant alleles in each group were for the most part different except for AG1 and BMd15, which possessed the same predominant allele between the two groups. Genetic differentiation (G_{ST}) and genetic distance (GD) between Andean and Mesoamerican group were 0.331 and 1.112, respectively, which were similar but lower than the values reported by Blair et al. (2006) for a cross genepool germplasm set. We can conclude that Chinese common bean germplasm includes genotypes from both gene pools and that inter genepool introgression is likely to be important, a topic to which we will return. This is not surprising given that in most studies of secondary centers of diversity, both Andean and Mesoamerican genotypes have been found, constituting further evidence that both genepools were taken from their center of origin and were disseminated as part of the "Post-Colombian exchange" of genetic resources between 'Old' and 'New' worlds. Co-existence of Andean and Mesoamerican genotypes has been observed both in regions within the Western hemisphere that were outside of the centers of origin such as Brazil and the Caribbean (Castiñeiras et al. 1991; Duarte et al. 1999; Maciel et al. 2003; Durán et al.

2005) and in other parts of the world to which common beans were transported during the colonial period such as Europe and Africa (Martin and Adams 1987a, b; Khairallah et al. 1990; Skroch and Nienhuis 1995; Rodiño et al. 2001; Santalla et al. 2002; Svetleva et al. 2006; Marotti et al. 2007). We can conclude from our study that the same process occurred with common beans brought to China.

A second observation was that the polymorphism level between landraces within the Mesoamerican group was lower than between accessions within the Andean group (Nei's indices of 0.367 and 0.423, respectively) even though the sample evaluated contained more of the former genepool than the latter genepool. Greater than expected diversity within the Andean genepool detected seems to be a common feature of microsatellite analyses and agrees with previous results from Blair et al. (2006) who used microsatellite markers to evaluate a mini-core set of common bean genotypes. Lower diversity within the Mesoamerican genepool compared to the Andean genepool contrasts with results from Becerra-Velásquez and Gepts (1994); Beebe et al. (2000, 2001) and Islam et al. (2004), but these studies evaluated broader, multi-country samples of germplasm. The high diversity in the Andean group of Chinese accessions might be explained given their multiple growth habits, wide seed color range and unique seed patterns. The higher level of diversity within the Andean group may also reflect the introduction of a greater amount of germplasm from this genepool to China compared to a more limited selection of germplasm introduced for the Mesoamerican genepool.

The third observation was that the level of diversity in Chinese common bean accessions was potentially higher than in other secondary centers of diversity (Maciel et al. 2003; Skroch and Nienhuis 1995; Tiwari et al. 2005) although less than in the primary centers of diversity (Díaz and Blair 2006) based on comparable estimates of diversity, with the caveats that genetic diversity could be influenced by the selection of genetic markers and germplasm and that diversity was not equal between the genepools in our study. Recombination between the gene pools seems to have contributed to overall diversity of each genepool within the Chinese germplasm. Accessions that had unique microsatellite alleles but possessed other gene pool traits were found. For example in the Andean group, 100 seed weights of accessions below 30 g were about 24% of all genotypes, even though Andean genotypes had higher average seed weight than Mesoamerican beans. Similarly, a few genotypes in the Mesoamerican group possessed type I growth habit (1.2%) and seed size larger than 40 g (9.9%) or red mottled seed pattern (6.4%), all characteristics more typical of Andean beans. As a result of this recombination the average 100-seed weight within Andean group detected for Chinese accessions was 38.9 g, which was lower in comparison with typical Andean beans for which seed weight usually exceeds 40 g and the average seed weight in the Mesoamerican group of Chinese accessions (31.2 g) was higher than typical for the genepool which usually ranges from 20 to 30 g (Singh et al. 1991a). In terms of seed characteristics, black seeded genotypes were found in the Andean group although this seed type is more typical of Mesoamerican beans (Voysest et al. 1994). Common bean is considered a predominantly self-pollinated species with outcrossing rate below 5% but crossing does occur in nature (Debouck et al. 1993; Freyre et al. 1996; Beebe et al. 1997) creating inter gene pool recombinants (Beebe et al. 2001; Islam et al. 2004).

The fourth observation was that diversity within the genepools was divided unevenly between the subgroups. Within the Mesoamerican genepool, higher diversity was observed for Mesoamerican 2 subgroup, which had higher gene flow ($N_m = 0.68$) with the Andean subgroups than the Mesoamerican 1 subgroup. This was consistent with the position of Mesoamerican 2 subgroup genotypes between the genepools in the multiple correspondence analysis suggesting that this subgroup arose from inter genepool introgression, which seems most likely for 15 accessions, mostly from South China, including six from Sichuan (F2641, F27351, F2774, F2780, F2789 and F2822), four from Guizhou (F2972, F2980, F3020 and F3145), two from Hubei (F3624 and F3643), one from Hunan (F2305) and two additional accession (G5 and G14193). Mottled seed pattern was also present in this subgroup even though mottling is usually absent in the Mesoamerican gene pool and could have been derived from the Andean race Nueva Granada where it is common (Singh et al. 1991a). South China was found to be a region that was sympatric for both Andean and Mesoamerican genotypes and therefore a likely site for introgression between the genepools.

Introgression of seed traits across genepool boundaries, formation of genepool subgroups and novel recombinants have been described in common bean germplasm from the Iberian Peninsula, which is another secondary center of diversity where some very large seeded Andean type landraces had Mesoamerican isozyme alleles and vice versa some medium seeded Mesoamerican type landraces had Andean isozyme alleles (Rodiño et al. 2003; Rodiño et al. 2006). Introgression between races has also been observed (Rosales-Serna et al. 2005; Díaz and Blair 2006; Blair et al. 2007) in primary centers of diversity and introgression may be even more common in secondary centers of diversity (Maciel et al. 2003; Durán et al. 2005; Maras et al. 2006). At present, only a limited number of introductions of common bean into China are suspected (Zheng 1997), however the wide range of climates and soil types in China even within the same province could have encouraged selection by farmers of novel recombinants adapted to different environment,

field management regimes and market preferences. Several additional studies have found that environmental or human selective pressure or the production and preservation of seed mixtures in secondary centers of diversity can result in introgression between gene pools and may create new genetic variation in common bean (Islam et al. 2002; Negri and Tosti. 2002; Santalla et al. 2002).

A fifth observation was that Chinese accessions we found to be in the Mesoamerican group were likely to be from race Mesoamerica given their close association with the control genotype DOR364, which is from this race. Within the Mesoamerican gene pool, race Mesoamerica is characterized by small black, small red and small white seeded genotypes and these were precisely the seed colors common in the Chinese accessions belonging to the Mesoamerican group we identified along with genotypes having brown and cream-colored seed. It seems less likely that races Durango or Jalisco arrived in China since overall diversity within the Mesoamerican genepool was poor and diversity that was present was due to introgression between the genepools. We had hoped to uncover diversity within Chinese accession of Mesoamerican origin by using many of the same microsatellite markers as in Díaz and Blair (2006), where a dichotomous race structure was distinguished for Mesoamerica race versus Durango–Jalisco race genotypes. Further arguing against the role of Durango–Jalisco races in China was the fact that the yellow, pink, stippled or pinto seed coat colors typical of these races (Singh et al. 1991a) were for the most part absent or infrequent in the Mesoamerican genotypes we evaluated. Therefore, we can hypothesize that only one race contributed to Mesoamerican bean diversity in China, explaining why diversity in this genepool is so much lower than in the corresponding Andean gene pool accessions.

In contrast, the Chinese accessions in the Andean group probably represented both race Nueva Granada and race Peru germplasm, although mostly race Nueva Granada given that the majority of Andean genotypes from China were fairly distant from the control genotype G19833, which is a race Peru accession. Race Nueva Granada is known to include medium-to-large seeded accessions with bush bean growth habits, mostly with temperate climate adaptation, and represents the majority of the commercial large seeded cultivars in use today (Blair et al. 2007). Chinese accessions are less likely to represent race Peru, which consists mainly of climbing beans, adapted to highland environments above 2,000 masl, given the growing conditions in China. However, there was some evidence for race Peru seed patterns in the Chinese collection given the prevalence of bi-color patterns, the fact that there were many type IV growth habit beans which could have originated from race Peru and the observation that the Andean genotypes from China split into two subgroups. However, since many of the climbing types had medium sized seed they could also have been a result of introgression from the Mesoamerican genepool where climbing beans were also found. Finally, it may be possible that some race Chile accessions, which are characterized by prostrate type III growth habit, medium sized, rounded to oval seed, and usually pale colors (Singh et al. 1991a), could have influenced germplasm in China since we find some of these traits in Andean beans from China. If this had occurred, it might have contributed to the higher level of diversity in the Andean genepool compared to the Mesoamerican genepool. Beebe et al. (2001) lists race Chile as occurring at higher latitudes in some parts of Asia including in China. Further study on which races of common bean are present in China and Asia should be conducted.

In conclusion, our results suggest that China is an important secondary center of diversity for common bean equivalent to other 'Old World' secondary centers of diversity such as Southwestern and Southeastern Europe (Maras et al. 2006; Martins et al. 2006; Negri and Tosti 2002; Rodiño et al. 2001; 2003; 2006; Santalla et al. 2002) or Eastern and Southern Africa (Martin and Adams 1987a, b). Our designation of China as a secondary center of diversity, is based on the similar or higher levels of diversity found in our study (Nei's = 0.535) as has been found in these regions that are already reported as secondary centers of diversity (eg. H_t of 0.293 and 0.317 for genotypes from Martins et al. (2006) in Portugal and Santalla et al. (2002) in Spain, respectively). Like in these centers, we believe that the co-existence of the two genepools in the region and evidence of hybridization between them qualifies China as a secondary center of diversity for the crop. The discovery of large amounts of diversity in China is not surprising given that China is a large producer of dry beans and currently produces the most snap beans of any country on earth as well as having intensive horticultural systems based on family farms. In addition, China probably has a history with the crop that is comparable in length to that of Europe and Africa through Spanish trade with South East Asia or early Chinese navigation around the Pacific and Indian oceans. The discovery of inter genepool introgression, which has been observed before in other secondary centers of diversity, is particularly interesting given that it seems to be a characteristic of the regions where the two gene pools overlap and might also be related to adaptation of common bean to the diverse agro-ecosystems found in China which are potentially among the most diverse on earth ranging from subtropical to temperate and sea level to 3,000 masl. In summary, we believe our study provides a baseline for common bean germplasm studies in Asia and that this research represents the first report of China as a secondary center of diversity for common bean.

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