Genetic diversity and selection of genotypes to enhance Zn and Fe content in common bean

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Abstract

Common bean (*Phaseolus vulgaris* L.) is an important source of dietary protein and minerals worldwide. Genes conditioning variability for mineral contents are not clearly understood. Our ultimate goal is to identify genes conditioning genetic variation for Zn and Fe content. To establish mapping populations for this objective, we tested mineral content of 29 common bean genotypes. Chemical analyses revealed significant genetic variability for seed Zn and Fe contents among the genotypes. Genetic diversity was evaluated with 49 primer pairs, of which 23 were simple sequence repeats (SSR), 16 were developed from tentative consensus (TC) sequences, and 10 were generated from common bean NBS-LRR gene sequences. The discriminatory ability of molecular markers for identifying allelic variation among genotypes was estimated by polymorphism information content (PIC) and the genetic diversity was measured from genetic similarities between genotypes. Primers developed from NBS-LRR gene sequences were highly polymorphic in both PIC values and number of alleles (0.82 and 5.3), followed by SSRs (0.56 and 3.0), and markers developed from TC (0.39 and 2.0). genetic similarity values between genotypes ranged from 14.0 (JaloEEP558 and DOR364) to 91.4 (MIB152 and MIB465). Cluster analysis clearly discriminated the genotypes into Mesoamerican and Andean gene pools. Common bean genotypes were selected to include in crossing to enhance seed Zn and Fe content based on genetic diversity and seed mineral contents of the genotypes.

Keywords

Common bean; genetic diversity; mineral nutrients; breeding

Common bean (*Phaseolus vulgaris* L.) is consumed worldwide and is a staple food in many countries (Broughton et al. 2003). Beans are a rich source of protein, energy (approx. 380 kcal 100 g^{-1} of seeds), vitamins (thiamine, riboflavin, niacin, vitamin B6, folic acid), dietary fiber (especially soluble fraction), and minerals (calcium, iron, zinc, phosphorus, potassium,

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magnesium) (Reyes-Moreno and Paredes-Lopez 1993). The role of dry beans as functional foods in chronic disease risk reduction has been given increasing attention. Several recent literature reviews have highlighted the positive effects of dry bean on improving serum lipid profiles in patients with coronary heart disease or Type 2 diabetes (Anderson and Major 2002; Anderson et al. 2004; Winham et al. 2007).

Common bean ($2n=2x=22$) represents 50% of the grain legumes consumed worldwide. Common bean landraces exhibit wide genetic variability for seed color, shape, shininess, size, and have demonstrated adaptability to several environmental conditions (Rodiño et al. 2003). The genome size for common bean is estimated to be about 450 to 650 million base pairs (Mb)/haploid (Bennett and Leitch 1995) and is considered to be one of the smallest genomes among major crop species. Based on phaseolin seed storage protein variation (Gepts and Bliss 1986; Gepts 1990), marker diversity (Becerra-Velasquez and Gepts 1994), and morphology (Gepts and Debouck 1991), two major gene pools of wild common bean were identified. The Middle American gene pool extends from Mexico through Central America and into Colombia and Venezuela, meanwhile the Andean gene pool is found in Ecuador, Peru, Bolivia, and Argentina. The two domesticated gene pools appear to converge in Colombia (Gepts and Bliss 1986). A third, ancestral gene pool, based on a novel phaseolin type originated in southern Ecuador and northern Peru. The two major domestication events, Middle America (possibly west-central Mexico) and southern Andes (Andean) diverged into three races within each of the two major domesticated gene pools (Singh et al. 1991). The Middle American gene pool, consisting of races Durango, Jalisco, and Mesoamerica is represented by the medium and small seeded pinto, pink, black, white, and some snap beans. The Andean gene pool, consisting of races Nueva Granada, Peru, and Chile, is represented by the large-seeded kidney, cranberry, and many snap beans among others.

Knowledge of genetic diversity in a crop species is fundamental to its improvement. Evaluation of genetic diversity among adapted, elite germplasm can provide predictive estimates of genetic variation among segregating progeny for pure-line cultivar development (Manjarrez-Sandoval et al. 1997), and may estimate the degree of heterosis in progenies of some parental combinations (Cox and Murphy 1990; Barbosa-Neto et al. 1996).

The advancement of molecular biology has created new opportunities for estimating the genetic diversity at a molecular level; however, genetic studies are often restricted, both by the limited number of polymorphic markers and by the low level of variability within self-pollinated species. Assessing genetic diversity within a narrow genetic pool of novel breeding germplasm could lead to more efficient crop improvement by marker directed accumulation of desired alleles. Marker-assisted selection is likely to speed up the breeding process and decrease the amount of plant material that needs to be screened in such experiments. A variety of molecular, chemical, and morphological descriptors are used to characterize the genetic diversity among and within crop species. The development of gene-and/or expressed sequence tags-derived molecular markers provides opportunities for the assessment of molecular diversity at selected loci. Such gene-targeted markers can contribute to the study of genetic resources and to ecological studies (Van Tienderen et al. 2002). For all of these approaches, the availability of highly polymorphic and user friendly

DNA markers is vital. Genetic variation in common bean has been reported based on restriction fragment length polymorphism (RFLP) (Becerra-Velasquez and Gepts 1994), random amplified polymorphic DNA (RAPD) (Duarte et al. 1999; Galván et al. 2001), simple sequence repeats (SSR) (Lioi et al. 2005; Diaz and Blair 2006; Blair et al. 2006, 2007; Benchimol 2007), and amplified fragment length polymorphism (AFLP) (Tohme et al. 1996; Beebe et al. 2001).

The first essential question regarding whether any species can be improved for a particular trait is to determine the degree of variability which exists for this trait within the species. The existing genetic diversity of a species in gene banks enables plant breeders to choose the most suitable strategy for improving the species (Lefort-Buson et al. 1988). Thus, the availability of diverse common bean accessions represents a valuable resource for the improvement of this species, since co-adapted genes of different accessions can convey similar response if selected for a specific trait (Harlan 1975). The knowledge of genetic diversity patterns can increase the efficiency for conservation, utilization and genetic improvement of common beans (Beebe et al. 2000b; Singh 2001).

Zinc (Zn) and iron (Fe) are essential micronutrients for human growth, development, and maintenance of the immune system. Zinc is needed for growth and for maintenance of immune function, which enhances both the prevention of and recovery from infectious diseases (Black 2003). Iron is needed for psychomotor development, maintenance of physical activity and work capacity, and resistance to infection (Stoltzfus 2001).

Common bean is an important source of protein and minerals, especially Zn and Fe. Genetic differences have been reported for seed Zn and Fe concentrations among genotypes and landraces (Singh et al. 1991; Graham et al. 1999; Moraghan and Grafton 2001). Beebe et al. (2000a) evaluated a core collection of over 1000 accessions of common beans in the field and found a range in Fe concentrations from 34 to 89 mg g^{-1}, with an average of 55 mg g^{-1}. Zinc concentrations in these same accessions ranged from 21 to 54 mg g^{-1}, with an average of 35 mg g^{-1}.

The genetics of Zn and Fe content appears to be complex. Analysis of three RIL populations at the International Center for Tropical Agriculture (CIAT) revealed that both Zn and Fe contents had continuous distribution, suggesting that these mineral contents were quantitatively inherited (Beebe et al. 2000a, Blair et al. 2005). Beebe et al. (2000a) and Blair et al. (2005) also observed that the parental accessions were very close to the extremes of the populations, with little evidence of transgressive segregation, suggesting that most of the favorable alleles came from the high-mineral content parent. A recent inheritance study (Cichy et al. 2005) demonstrated that a single gene controls seed Zn in navy bean and that high seed Zn is dominant over low seed Zn. Cichy et al. (2005) also observed transgressive segregation for seed Zn content in the F_2 generation and speculated that this was the effect of additional minor genes. Forster et al. (2002) observed a similar result in a cross between two navy bean cultivars, Voyager and Albion. Singh and Westermann (2002) reported that a single dominant gene controls the resistance to soil Zn deficiency in common bean. Guzmán-Maldonado et al. (2003) reported two QTLs for seed Fe content and one for Zn content responsible for 25% and 15% phenotypic variation, respectively, from a

cultivated×wild *P. vulgaris* cross. Almost all of these studies were based on seed mineral contents from plants grown in field conditions subject to various influences of soil characteristics and environments.

We conducted our study under greenhouse conditions with an array of common bean genotypes consisting of various genetic and geographical backgrounds. Our objectives were (1) to assess genetic diversity among 29 common bean genotypes using molecular markers developed from various genomic sources, and (2) to select parental genotypes suitable for mapping loci affecting mineral content in common bean.

MATERIALS AND METHODS

Plant Material

The 29 common bean cultivars/lines in this study consisted of 14 genotypes from CIAT, 13 from the United States of America, and one each from Brazil and India. Among the cultivars, 13 represent parents of one or more mapping populations developed for various interests, and vary in terms of growth habit and geographic origin. All common bean cultivars/lines, and country of origin, are listed in Table 1.

Greenhouse Experiment

Common bean genotypes were grown in the greenhouse in 18–19 cm pots filled with Sunshine mix 1 (Sun Gro Horticulture Canada Ltd., formulated with Canadian sphagnum peat moss, coarse grade perlite, gypsum, and dolomitic lime) as substrate. The seeds were planted on 2007 Mar. 27 following a randomized complete block design with three replications. Two seeds for each entry were placed in each pot for germination, but one plant was allowed to grow until harvest of the seeds. Pots were watered periodically with tap water to the approximate field capacity to facilitate normal plant growth. No additional fertilizer or pesticide was applied during the period of experimentation.

Chemical Analysis

After harvesting, seeds from each pod of individual plant were mixed thoroughly, and 10 seeds were randomly taken for chemical analysis. Seeds were washed with deionized water containing Joy® detergent (Proctor and Gamble, Cincinnati, OH), and later rinsed with deionized water only. Samples were oven-dried at 70°C for 48 h, weighed, and ground in an agate mortar with an agate pestle (Brinkmann Instruments Co., Westbury, NY). A 300-mg aliquot of the ground material was processed for concentrated nitric acid digestion, followed by 30% hydrogen peroxide, before Fe and Zn concentrations were measured in parts per million (ppm) using atomic absorption spectroscopy (Moraghan and Grafton 2001).

DNA Isolation and PCR Primers

Total genomic DNA was extracted and purified from leaf tissue of 2- to 3-wk-old greenhouse-grown plants using the CTAB method as described by Doyle and Doyle (1987).

A total of 49 primer pairs from three different sources were used for genetic diversity analysis. The primer names, primer sequences and genomic locations in the Phaseolus core linkage map BAT 93/Jalo EEP558 (BJ) (Freyre et al. 1998) are presented in Table 2.

Primers Collected from Reference Map—Twenty-three SSR primer pairs used in this study were developed from coding and non-coding sequences of common bean (Blair et al. 2003). McConnell et al. (2006) analyzed *P. vulgaris* TC (tentative consensus) sequences and identified 322 primer pairs with their polymorphism. In our study, we used 16 of these primer pairs.

Primers Developed from NBS-LRR Gene Sequences—Common bean NBS-LRR type disease resistance gene sequences were downloaded from NCBI GenBank and were aligned with multiple sequence alignment software CLUSTALX (1.81). Phylogenetic trees were generated to evaluate the relationship among different sequences using Neighbor-Joining options of the software. Initially, four NBS-LRR complete coding sequences were chosen to design a series of primers based on sequence alignment. The large (~5 to 6 kb) gene sequences were divided into small ordered overlapping fragments, and a total of 37 primer pairs were designed to amplify genomic DNA of ~500 bp using the web-based PCR-primer designing program 'Primer 3'. Ten of these 37 primer pairs were selected and included in this study.

PCR Amplification

Each 20 μL amplification reaction consisted of 1 ×PCR buffer, 1.88 mM $MgCl_2$, 200 μM of each deoxyribonucleotide triphosphate, 1 μM of primer, 1 unit of *Taq* polymerase, and 150 ng of template DNA. Amplification was performed in a 96-well BioRad thermal cycler.

The PCR program for SSR primers and primers developed from NBS-LRR gene sequences consisted of one cycle of 95°C for 3 min; 40 cycles of 95°C for 1 min, from 55 to 57°C for 1 min, and 72°C for 2 min; and one cycle of 72°C for 10 min. For primers developed from TC sequences, the PCR protocol was one cycle of 95°C for 3 min, followed by 40 cycles of 95°C for 20 s, from 52 to 58°C for 20 s, and 72°C for 2 min, one cycle of final extension for 72°C for 7 min. In all cases, the amplified products were separated on 2% agarose gel with 60 V and run for 200 min.

Data Collection and Statistical Analysis

Seed Zn and Fe content data were subject to analysis of variance (ANOVA) for a treatment effect. ANOVA were performed using general linear model of the statistical package, Minitab® Release 13.2 (Minitab Inc.). If a significant difference among the genotypes was detected with ANOVA at P 0.05, means comparison was performed (α=0.05) using Fisher's least-significant-difference (LSD) test.

After gel electrophoresis of the PCR products the presence or absence of each single fragment was coded by 1 or 0, respectively, and scored for a binary data matrix. The resulting matrix was used to estimate genetic similarity (GS) among all pairs of lines by Dice coefficient of similarity (Nei and Li 1979) as follows:

$$GS_{ij} = \frac{2N_{ij}}{N_i + N_j}$$

Where N_{ij} is the number of alleles (scored bands) shared by lines i and j, and N_i and N_j are the total number of scored bands in lines i and j, respectively.

Polymorphism information content provides an estimate of the discriminatory power of a locus, or loci, by taking into account not only the number of alleles that are expressed, but also the relative frequencies of those alleles. Polymorphism information content values were calculated by the algorithm as described by Anderson et al. (1993):

$$PIC = 1 - \sum_{i=1}^{n} (Pi)^2$$

Where P_i is the frequency of the ith allele in the population.

Polymorphism information content values range from 0 (monomorphic) to 1 (very highly discriminative, with many alleles each in equal and low frequency).

Genetic diversity analyses were conducted using numerical taxonomy and the multivariate analysis system, NTSYSpc v. 2.2 (Rohlf 2000). Genetic similarity values were computed between all possible pairs with the SIMQUAL option and ordered in a similarity matrix. The similarity matrix was run by sequential, agglomerative, hierarchical, nested (SAHN) clustering (Sneath and Sokal 1973) with the unweighted pair group with arithmetic averaging (UPGMA) method as an option (Sokal and Michener 1958). The dendrogram and cluster groupings were constructed by the UPGMA clustering algorithm from the SAHN option of NTSYSpc v.2.2. Principal coordinate analysis was performed using EIGEN and graphs were plotted using MXPLOT module, again in the same software package.

An estimate of the confidence limits for the grouping produced by each dendrogram was obtained by performing 2000 bootstrap re-samplings in WinBoot (Yap and Nelson 1996).

RESULTS

The Zn and Fe contents in the seeds of the 29 common bean genotypes are presented in Table 1. There were significant differences for both Zn ($P < 0.05$) and Fe ($P < 0.01$) content of the seeds among genotypes. In general, the Zn and Fe content of the Middle American genotypes were 16.1 and 11.3%, respectively, higher than the Andean genotypes.

The variability for seed Fe content among the genotypes was high, ranging from 8.9 to 112.9 ppm. The highest seed Fe content was observed in the USA navy bean cultivar Vista and one of the CIAT lines, NUA 56-1770, bred for high iron content in the redmottled seed class of the Andean gene pool. The Brazilian breeding line, Jalo EEP558, a parent of the common bean core mapping population, contained the lowest seed Fe content from the set studied.

The genetic location of SSR primers and primers developed from TC sequences were known and used in this study to give a uniform coverage for the common bean genome. These markers detected a total of 153 polymorphic alleles (Table 2). The number of alleles per locus ranged from two to nine, with an average of 3.1. The PIC values ranged from 0.19 for the size-indel 2685 and SNP 2285 to 0.99 for pv 2758 (Table 2). In general, the magnitude of PIC value is higher in primers developed from NBS-LRR gene sequences followed by SSR primers and primers developed from common bean TC sequences.

A GS matrix based on all possible pairs of genotypes ranged from 14.0 to 91.4% (Table 3). The lowest pairwise GS was observed between the Andean common bean genotype Jalo EEP558 and the Mesoamerican common bean genotype Dorado. The highest pairwise GS was observed between two related Mesoamerican genotypes MIB 465 and MIB 152. Among the Andean genotypes, the GS values ranged between 32.7 and 89.1%, while they were between 31.7 and 91.4% among Mesoamerican genotypes. However, the mean GS within the Mesoamerican genotypes (60.5%) was higher than among the Andean genotypes (55.5%), indicating a higher overall diversity within the Andean group, similar to observations by Tofiño et al. (2007).

For a better understanding of the genetic relationship among common bean genotypes, the GS values of Table 3 were submitted to hierarchical clustering by UPGMA. The cluster analysis primarily separated the cultivars in close correspondence to two major clusters representing their primary gene pools (Fig. 1). Principal coordinates analysis revealed the global structure similar to the dendrogram analysis, but the distribution of these accessions was shown more clearly in three dimensions. Principal coordinate analysis showed that the first three eigen values explained 73.8% of the cumulative variation. These values were then plotted to identify the diversity of the genotypes (Fig. 2). Overall, the clustering pattern of the genotypes in the principal coordinates analysis corresponds with the dendrogram derived from UPGMA (Fig. 1). The Andean common bean cultivars were separated from the Middle American cultivars convincingly by PC1 (principal coordinate 1).

DISCUSSION

The highest seed Zn content was observed in our trial among genotypes MIB 465 and MIB 466, both belonging to the Middle American gene pool. The MIB lines were developed by the CIAT breeding program for improved nutritional quality. Beebe et al. (2000a) analyzed the seed Zn and Fe contents of over 1000 accessions in the CIAT core collection and suggested that the seed Fe content in the Andean gene pool tended to present higher values than those from the Mesoamerican pool under field grown conditions. The NUA lines were also developed by CIAT breeding program for high Fe content. These NUA lines previously observed with high Fe content at CIAT were variable in our trial. We conducted our study in greenhouse conditions, where there is no influence of soil differences as usually observed under field trial conditions. The variability for Zn and Fe contents in our study also could be due to the small sample size and inclusion of some genotypes selected for high Zn and Fe contents.

The UPGMA clustering analysis divided the genotypes into two major clusters. Two main branches of the dendrogram showed more than 50% GS, suggesting that genetic diversity is low between two distinct *P. vulgaris* gene pools. The genotypes Jalo EEP558, G122, NY 6020-4, Benton, and all four NUA breeding lines from CIAT originating from Andean gene pool, formed a distinct cluster separating from the other large group constituting the Middle American gene pool. Within the Andean group, the NUA lines, as expected, formed a subgroup. These CIAT lines were developed for high seed Fe content and share the same backcross pedigree, which includes one Mesoamerican bean parent, but seed class and plant selection for the Andean morphology. Likewise, within the large Mesoamerican group, the genotypes clearly formed three sub-groups. In the first sub-group, the genotypes BAT 93, Dorado, Aztec, BelNeb RR-1, Voyager and Albion, all are being used as parents of mapping populations developed for disease resistance and other trait attributes. Among these parents, the pinto bean cultivar Aztec and navy bean cultivar Voyager were bred for commercial cultivation in the United States of America. In the second sub-group, most of the black beans grouped together with a few pinto and navy bean cultivars. The six MIB accessions selected for nutritional quality traits in CIAT were placed into this second group and the other three formed a separate third group. MIB lines 465 and 152 grouped very closely, as they originated from the same pedigree. The other light-tan colored CIAT accession, MIB151 also has the same pedigree as of MIB465 and MIB152. However, a genetic shift appears to have occurred between these MIB lines, as these accessions clustered in two separate groups. This shift may be associated with selection for favorable alleles.

Several breeding strategies can be derived from the results of our genetic diversity analysis; however, our interest at present is to develop populations to map Zn and Fe content traits and to breed common bean cultivars with enhanced Zn and Fe contents. Substantial genetic variation existed among the genotypes under study that could be exploited for selection for mineral content. The clustering of the Andean accessions separate from the Mesoamerican accessions suggests that these common bean genotypes represent distinct germplasm and could be used to construct highly polymorphic populations. CIAT developed nutritionally enriched common bean lines clustered in both gene pools. Broad-based populations could be constructed by crossing selected cultivars from the two gene pools as well from combinations of several of the elite cultivars within each gene pool. We are currently using both approaches to develop common bean populations for further breeding and selection.

Selection of genotypes for breeding purpose based on micronutrient variability and genetic diversity has been suggested in many crop species including common bean (Beebe et al. 2000a), rice (Gregorio 2000), wheat (Monasterio and Graham 2000), and maize (Banziger and Long 2000). We have designed crossing programs based on seed Zn and Fe contents of the genotypes and their genetic divergence at the molecular level. The crossing program for seed Zn content was designed to cross genotypes with high Zn content, Jalo EEP558, MIB465, and MIB466, with the low Zn content genotypes, NUA56-1770 and NUA59 (Table 1). The high Zn content MIB lines belong to the Mesoamerican gene pool, while both the low Zn content NUA genotypes and the high Zn content genotype, Jalo EEP558, belong to the Andean gene pool. The GS values of genotypes NUA56-1770 with Jalo EEP558, MIB465, and MIB466 are 51.9, 35.5, and 49.1%, repectively, (Table 3) and those of NUA59 with Jalo EEP558, MIB465, and MIB466 are 43.4, 34.9, and 46.6%, respectively. These

values indicate that the selected genotypes are very divergent at the molecular level and could result in better segregation and recombination of the desired alleles in successive generations during population development.

For the crossing plan to enhance seed Fe content, our choices for high Fe are genotypes Vista and NUA 56-1770 (Table 1), which belong to the two divergent gene pools (Fig. 1). The genotypes Jalo EEP558, NY6020-4, XAN176 and Mayflower have low seed Fe content (Table 1) and are included in the crossing program. Both the genotypes Jalo EEP558 and NY6020-4 are from the Andean gene pool, while the genotypes XAN176 and Mayflower represent the Mesoamerican gene pool. The selection of parents from both gene pools enables us to study inter- and intra-gene pool recombination events. The GS values of genotype Vista with the low Fe genotypes, Jalo EEP558, NY6020-4, XAN176 and Mayflower are 34.9, 42.1, 69.0, and 78.3%, respectively (Table 3). The high GS value between the genotypes Vista and Mayflower is obvious as both of them are navy bean cultivars adapted and selected for commercial cultivation in the United States of America. Although, these two genotypes belong to the same market class and are not genetically divergent, they differ significantly for seed Fe content. It can be expected that the introgression of a desired trait would be easier in this cross without sacrificing valuable trait(s) and/or adding unwanted trait(s) as a consequence of linkage drag. The GS of other high Fe genotype, NUA56-1770, with the low Fe genotypes, Jalo EEP558, NY6020-4, XAN176 and Mayflower, are 51.9, 33.0, 45.0 and 34.8%, respectively, (Table 3) indicating that the selected high Fe content Andean parent is genetically widely divergent from both Andean and Mesoamerican genotypes selected as low Fe content parents. The extent of genetic diversity present within the selected genotypes suggests that the introgression of genes for mineral nutrient might also benefit in the recombination of other economically important traits, especially when some of these selected genotypes (Jalo EEP558, NY 6020-4 and XAN 176) are being used as parents in mapping populations known to segregate for important biotic and abiotic resistance genes.

In order to generate advanced breeding lines with higher seed Zn and Fe contents, additional crossing programs may be considered. The Andean genotypes, Jalo EEP558 and Benton, and the Mesoamerican MIB genotypes, 151, 152, 465 and 466, have high Zn content in their seeds (Table 1). Similarly, the genotypes NUA45, NUA56-1770, Benton of the Andean gene pool and the genotypes MIB154, MIB465, Dorado, Voyager and Vista in the Mesoamerican gene pool have high Fe content in their seeds (Table 1). Crossing combinations with genotypes between and within gene pools having high genetic diversity and mineral content would be expected to accumulate positive alleles derived from unique sources and generate breeding lines with even higher seed mineral contents.

Acknowledgments

Abbreviations

CIAT	International Center for Tropical Agriculture
GS	genetic similarity
PIC	polymorphism information content
SAHN	sequential, agglomerative, hierarchical, nested
SSR	simple sequence repeats
TC	tentative consensus
UPGMA	unweighted pair group with arithmetic averaging

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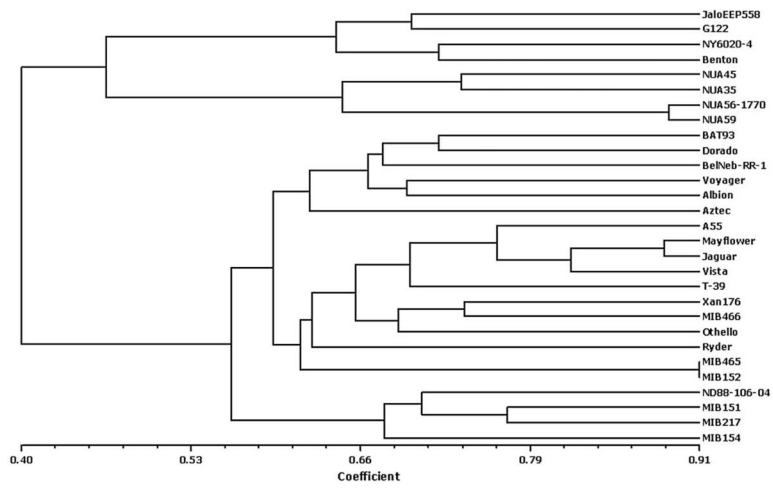
Fig. 1.
Dendrogram of common bean cultivars based on Dice coefficient of similarity matrix data using UPGMA.
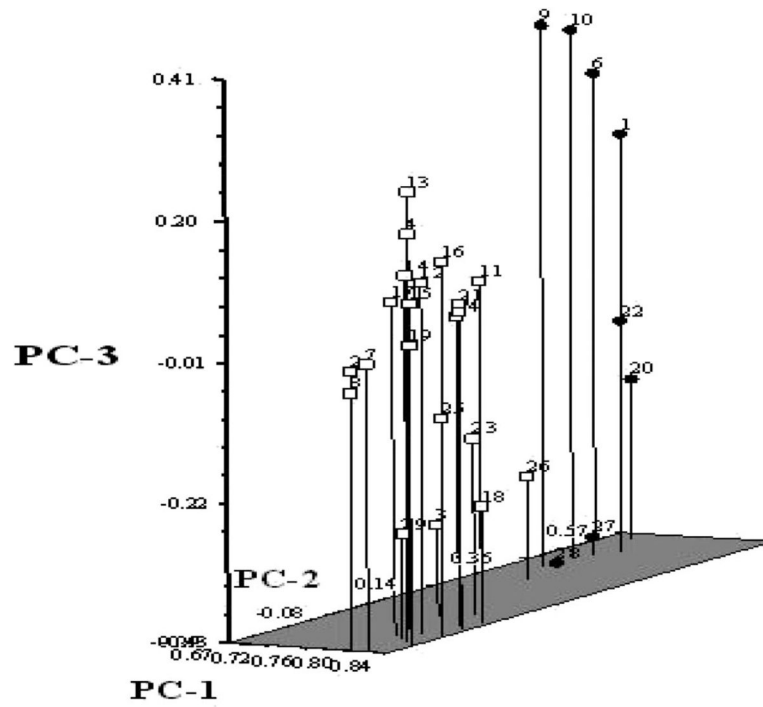
Fig. 2.
Principal coordinate analysis of genetic diversity in 29 common bean cultivars. Open square symbols: Mesoamerican genotypes; closed circle symbols: Andean genotypes.

Table 1

Common bean cultivars, gene pool group, origin, seed color, zinc and iron contents in the seeds

No.	Cultivar/line	Gene pool	Origin	Market class	Seed color	Seed Zn (ppm)	Seed Fe (ppm)
1	Jalo EEP558	Andean	Brazil	–	Cream	59.5^{a-c}	8.9^{k}
2	NUA 45	Andean	CIAT breeding line	–	Purple mottled	40.8^{f-h}	74.6^{cd}
3	NUA 35	Andean	CIAT breeding line	–	Purple mottled	47.4^{c-g}	22.9^{i-k}
4	NUA 56-1770	Andean	CIAT breeding line	–	Purple mottled	34.5^{gh}	110.6^{ab}
5	NUA 59	Andean	CIAT breeding line	–	Purple mottled	30.9^{h}	34.2^{f-k}
6	G 122	Andean	Indian landrace	Cranberry	Red mottled	41.1^{f-h}	42.0^{e-j}
7	NY6020-4	Andean	USA breeding line	Snap	White	47.0^{c-g}	20.6^{i-k}
8	Benton	Andean	USA	Snap	White	53.6^{a-f}	74.5^{cd}
9	Dorado	Mesoamerican	CIAT	Small red	Dark red	42.4^{e-h}	84.3^{a-d}
10	BAT 93	Mesoamerican	CIAT breeding line	–	Cream	54.7^{a-f}	42.1^{e-j}
11	A 55	Mesoamerican	CIAT breeding line	Black	Black	52.6^{a-f}	22.2^{i-k}
12	XAN 176	Mesoamerican	CIAT breeding line	Black	Black	44.0^{c-h}	17.2^{i-k}
13	MIB 151	Mesoamerican	CIAT breeding line	–	Light Cream	58.9^{a-d}	64.4^{c-f}
14	MIB 152	Mesoamerican	CIAT breeding line	–	Black	58.3^{a-e}	66.7^{c-e}
15	MIB 154	Mesoamerican	CIAT breeding line	–	Brown mottled	48.6^{b-g}	92.8^{a-c}
16	MIB 217	Mesoamerican	CIAT breeding line	–	Black	42.8^{e-h}	34.2^{f-k}
17	MIB 465	Mesoamerican	CIAT breeding line	–	Black	64.4^{ab}	84.7^{a-d}
18	MIB 466	Mesoamerican	CIAT breeding line	–	Pink with black specks	64.6^{a}	57.6^{d-h}
19	ND88-106-04	Mesoamerican	USA	Navy	White	49.8^{a-g}	27.4^{h-k}
20	Aztec	Mesoamerican	USA	Pinto	Cream mottled	53.9^{a-f}	24.3^{i-k}
21	Voyager	Mesoamerican	USA	Navy	White	43.4^{d-h}	80.9^{b-d}
22	Albion	Mesoamerican	USA	Navy	White	53.9^{a-f}	28.8^{g-k}
23	Mayflower	Mesoamerican	USA	Navy	White	53.6^{a-f}	12.2^{jk}
24	T-39	Mesoamerican	USA	Black	Black	45.0^{c-g}	29.0^{g-k}

No.	Cultivar/line	Gene pool	Origin	Market class	Seed color	Seed Zn (ppm)	Seed Fe (ppm)
25	Jaguar	Mesoamerican	USA	Black	Black	51.8^{a-f}	55.2^{d-h}
26	Othello	Mesoamerican	USA	Pinto	Cream mottled	45.1^{c-h}	58.9^{d-g}
27	Ryder	Mesoamerican	USA	Small red	Red	54.9^{a-f}	33.3^{g-k}
28	Vista	Mesoamerican	USA	Navy	White	49.2^{a-g}	112.9^{a}
29	BelNeb-RR-1	Mesoamerican	USA breeding line	Great Northern	White	49.6^{a-g}	43.3^{e-i}
Mean±SE						49.5 ± 1.49^{z}	50.4 ± 5.46^{y}
$LSD_{0.05}$						15.94	30.8

[z]Significance at the 0.05 probability level on the basis of F tests with $n-1$ degrees of freedom.

[y]Significance at the 0.01 probability level on the basis of F tests with $n-1$ degrees of freedom.

[a–k]Means having common letter do not differ significantly.

Table 2

Primer name, primer sequence, polymorphic information content (PIC), amplified alleles, and linkage group of 49 primers

Primer name	5′ to 3′ Left primer sequence	5′ to 3′ Right primer sequence	PIC	No. of alleles	Linkage group
SSR primers					
BM139	TTAGCAATACCGCCA TGAGAG	ACTGTAGCTCAA ACAGGGCAC	0.68	5	B02
BM154	TCTTGCGAC CGAGCTTCTCC	CTGAATCTGAGGAA CGATGACCAG	0.77	5	B09
BM157	ACTTAACAAGGAATAG CCACACA	GTTAATTGTTTCCAATA TCAACCTG	0.43	2	B10
BM160	CGTGCTTGGCGA ATAGCTTTG	CGCGGTTCTGATCG TGACTTC	0.57	3	B07
BM164	CCACCACAAGGAGAA GCAAC	ACCATTCAGGCCG ATACTCC	0.44	2	B02
BM170	AGCCAGGTGCAAGACC TTAG	AGATAGGGAGCTGGT GGTAGC	0.50	2	B06
BM175	CAACAGTTAAAGGTCG TCAAATT	CCACTCTTAGCA TCAACTGGA	0.66	3	B05
BM181	ATGCTGCGAGTTAA TGATCG	TGAGGAGCAAAACAGA TGAGG	0.78	5	B03
BM211	ATACCCACATGC ACAAGTTTGG	CCACCATGTGCTCATGA AGAT	0.50	2	B08
BMd2	AGCGACAGCAAG AGAACCTC	CAACAAACGGTGAT TGACCA	0.76	7	B02
BMd9	TATGACACCACTGGC CATACA	CACTGCGACATGAG AGAAGA	0.49	2	B04
BMd16	ATGACACCACTGGCC ATACA	GCACTGCGACATGAG AGAAA	0.74	4	B04
BMd17	GTTAGATCCCGCCCA ATAGTC	AGATA GGAAGGGCGTGGTTT	0.66	3	B02
BMd25	GCAGATCGCCTACTC ACAA	CGTTGACGAGAAGCA TCAAG	0.47	2	B08
BMd33	TACGCTGTGAT GCATGGTTT	CCTGAAAGTGC AGAGTGGTG	0.72	5	B11
BMd36	CATAACATCGAAGCC TCACAGT	ACGTGCGTACGAATAC TCAGTC	0.49	2	B03
BMd42	TCATAGAAGATTTGT GGAAGCA	TGAGACACGTA CGAGGCTGTAT	0.48	2	B10

Primer name	5′ to 3′ Left primer sequence	5′ to 3′ Right primer sequence	PIC	No. of alleles	Linkage group
BMd53	TGCTGACCAAGGAAATTCAG	GGAGGAGGCTTAAGCACAAA	0.50	2	B05
GATS91	GAGTGCGGAAGCGAGTAGAG	TCCGTGTTCCTCTGTCTGTG	0.49	2	B02
K03289	ATGACACCACTGGCCATACA	GCACTGCGACATGAGAGAAA	0.36	2	B04
X74919	GTTGCCACCGGTGATAATCT	GTTGAGGCAAGAAGCCTTCAA	0.43	2	B05
X80051	AGTTAAATTATACGAGGTTAGCCTAAATC	CATTCCCTTCACACATTCACCG	0.49	2	B09
X96999	CAAATCGCAACACCTCACAA	GTCGGAGCCATCATCTGTTT	0.49	2	B03
Primers developed from tentative consensus (TC) sequences					
755-size	TCCATGTCCCCTACAGATTAAGGA	TCCAAAGCCTACAAATGGAACCTA	0.40	2	B4
762-allele-indel	GGATTTGTACAGTTGAAGTGACTTCTG	CAAGCCCTTGAATACTGAGTGTGA	0.29	2	B3
822-SNP	GAAGGGAAGTAGCTCAAAGAGAAAGGCG	AGAAGCCATGAAGATGAAGAACTAGACCAATAGAAG	0.43	2	B1
909-SSR	GAGTGACATCAAGGAACAGATTCTAAATTACC	CACCCACCCCCGAATTAAGTTGC	0.47	2	B1
1148-size	GACTGGAAACATGTTGAGAGAATGG	AATGTGCAACTACAACAGAGTGCAA	0.43	2	B2
1181-SNP	TTGTGGAGTTGGCGAGGGCG	AATTTTGGTTTTGGTTATTGTTGTTATTTTCCCAG	0.47	2	B8
1286-SNP	ATGATGCCTTATGGAATGCCCCGA	TCCCCGTAGTACTAATGTGCAGATCTCAAGAAT	0.40	2	B9
1564-CAPS	TGTTTCATTTGATCCCAAGCCTAT	TTCAACCATACAGAGAACACAGAAGG	0.42	2	B4
1639-SNP	AATCTTTGTTGGGTGTTGTAAAGTTTGGATAAAGA	CAAGAAGTGTGAGACGTTCTTCCGCTG	0.45	2	B6
2285-SNP	GATATACAGGGAAGCATTAGCACTCAATCCTGAT	ACATGATGAGTTACCCAAGCCCAAAAGAA	0.19	2	B11
2298-SSR	ATATCTACCGATCATAAGCCTTATTAAG	AAATGGAGAAAGGGTTACCTTC	0.37	2	B7

Primer name	5′ to 3′ Left primer sequence	5′ to 3′ Right primer sequence	PIC	No. of alleles	Linkage group
2422-allele-indel	GGACCATTGGTACAT ATTACAGTATATA	GACTACACAAGAAT GGACTATTAAGG	0.45	2	B5
2551-size-indel	GAATCTCGTTTTCCTG CTCAG	GATCTTGTAAGACT TTACTTTTACATTAGGAG	0.49	2	B6
2647-allele-indel	TATTACCTTTTCATC CTATTTTACACCATG	ATATGAAGCAGAA CCAATGACTTGTG	0.46	2	B1
2654-SSR	CTATGTCTCAA GTACCGCAAATTGG	GGTTCCTCCATTTCTTT GTCCAACTC	0.33	2	B3
2685-size-indel	GTGCTTGGAATT CGAATTGGAGGTG	CGAAACCATGCTATCA ACACACATCAAC	0.19	2	B4

Primers developed from nucleotide-binding site–leucine-rich repeat (NBS-LRR) sequence

Primer name	5′ to 3′ Left primer sequence	5′ to 3′ Right primer sequence	PIC	No. of alleles	Linkage group
0436	GGCACCATCTC AGAAAAACAA	TGATGAATGAA CGAATGGGA	0.60	3	–
0536	TTTAGGAAATTCAGA TTGAGATATTG	TGATGAATGAA CGAATGGGA	0.67	3	–
2353	GCATCCATTCCGAA CAGAGT	GCTGCATGTTT AGATTTTTCCA	0.98	9	–
2555	CAGCTCTTCCAAAGTTGGTG	GAATTTGTTGA GCAGTGCGA	0.95	6	–
2356	GCATCCATTCCGAA CAGAGT	AAAGCATAAGC ACATTGTTCCA	0.89	6	–
2657	TGGATACAC CCATTCTTCTTG	GCAGATTGATTTTTA TTCAACGG	0.98	7	–
2758	GGAGAACCTCCT GCAACTGA	TCTTTAAG AGAATTCGCGGC	0.99	6	–
2859	GTGAAGGTGGTTTG GCATTT	TGCAGGAGGTTCTC CAGATT	0.85	7	–
2960	ATCTGGAGAACCTCCT GCAA	ATTCGCGGCCTG TTAAATC	0.84	4	–
3061	GCATCCATTCCGAAG AGAGT	AAATGCCGAAGCCT TGAATA	0.47	2	–

Table 3

Similarity matrix (%) of 29 common bean genotypes based on 153 polymorphic bands

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
1.	Jalo EEP558	100																												
2.	NUA 45	63.5	100																											
3.	NUA 35	61.1	73.3	100																										
4.	NUA56-1770	**51.9**	72.7	61.4	100																									
5.	NUA 59	**43.4**	67.3	55.4	89.1	100																								
6.	G 122	69.5	48.5	53.3	40.4	35.6	100																							
7.	NY6020-4	57.1	49.5	46.0	**33.0**	37.8	66.0	100																						
8.	Benton	69.2	53.7	46.5	42.6	32.7	62.6	71.6	100																					
9.	Dorado	**14.0**	28.8	33.0	34.6	39.6	23.2	41.9	26.9	100																				
10.	BAT 93	20.0	28.1	32.4	40.4	48.3	28.6	45.2	36.8	71.6	100																			
11.	A 55	43.0	47.8	38.5	46.0	44.2	37.3	44.6	48.6	59.8	63.2	100																		
12.	XAN 176	23.9	41.4	37.3	**45.0**	42.5	19.6	37.5	41.4	67.3	70.1	70.2	100																	
13.	MIB 151	44.2	49.6	40.4	49.6	48.7	37.0	44.1	47.9	57.1	52.0	63.3	53.3	100																
14.	MIB 152	37.2	39.6	45.4	32.1	35.2	37.1	44.9	37.7	56.9	58.9	58.7	55.0	62.9	100															
15.	MIB 154	51.4	55.7	50.5	57.4	53.9	44.2	40.4	44.2	47.1	50.8	47.1	36.2	55.9	67.2	100														
16.	MIB 217	33.9	37.9	35.2	44.8	49.2	29.7	32.5	32.8	70.3	67.2	65.5	58.8	64.9	76.8	69.4	100													
17.	MIB 465	40.8	37.4	42.9	**35.5**	34.9	34.7	42.6	44.9	54.4	56.6	60.0	58.2	67.2	**91.4**	55.4	64.9	100												
18.	MIB 466	43.6	42.1	50.0	**49.1**	**46.6**	26.7	36.5	40.4	66.1	63.3	65.0	73.5	63.4	67.9	55.5	68.9	70.8	100											
19.	ND88-106-04	31.2	46.8	42.7	54.1	56.6	25.5	41.1	41.4	62.3	59.8	61.4	62.1	68.3	45.9	65.5	72.3	50.9	58.1	100										
20.	Aztec	30.4	27.1	27.6	35.4	40.8	32.2	41.2	43.8	63.0	64.7	56.6	60.6	41.9	44.7	31.7	51.9	44.2	54.9	52.5	100									
21.	Voyager	38.5	41.4	43.1	45.0	47.8	49.0	57.1	48.6	66.0	68.4	64.9	63.8	61.7	58.7	51.7	60.5	58.2	61.5	62.1	60.6	100								
22.	Albion	29.1	37.4	36.7	41.1	42.2	34.7	50.0	44.9	66.0	63.7	61.8	67.3	62.1	59.0	41.1	57.4	67.9	61.9	56.4	53.6	69.1	100							
23.	Mayflower	43.2	38.3	41.5	**34.8**	37.6	32.1	41.4	47.0	57.7	61.2	76.3	67.8	61.3	61.9	50.0	66.7	64.9	67.8	57.6	52.4	57.6	57.9	100						
24.	T-39	30.8	40.7	37.6	37.0	32.7	26.3	42.2	46.3	59.0	56.1	66.7	68.5	62.7	62.3	49.1	59.8	67.9	65.5	62.5	52.1	57.7	59.8	69.6	100					
25.	Jaguar	42.3	40.7	42.4	40.0	40.0	32.3	45.9	48.1	53.8	59.6	77.5	66.7	61.5	56.6	49.6	58.6	57.9	70.2	50.4	58.3	57.7	56.1	88.7	72.2	100				
26.	Othello	32.7	27.8	38.4	46.3	41.8	22.2	38.5	46.3	53.8	61.4	46.8	68.5	54.7	50.9	40.7	51.7	59.8	68.4	48.6	67.3	59.5	61.1	57.7	59.1	63.0	100			
27.	Ryder	27.5	53.8	52.6	59.6	58.5	27.4	40.0	60.0	65.5	56.1	60.0	62.4	58.4	50.8	53.2	69.6	54.4	69.1	59.5	46.8	62.4	59.0	55.9	60.6	55.8	62.3	100		
28.	Vista	**34.9**	33.6	40.4	46.0	45.2	30.8	**42.1**	58.7	67.2	67.2	74.1	62.3	66.7	49.2	66.7	61.2	64.3	72.3	51.7	59.5	62.1	62.5	**69.0**	51.5	69.0	62.1	**78.3**	100	
29.	BelNeb-RR-1	28.3	35.0	36.2	33.0	32.4	34.0	53.8	46.6	67.9	67.2	66.7	64.2	46.4	45.5	40.7	52.3	47.1	55.0	49.1	66.7	64.2	68.6	60.6	58.3	62.7	58.3	64.1	57.9	100