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Genetic diversity of the common bean *Phaseolus vulgaris* L. determined by DNA-based molecular markers

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ABSTRACT

We selected 28 potential common bean (*Phaseolus vulgaris* L.) progenitors to determine their genetic diversity using RAPD technique. They were divided into two groups based on their type of storage protein (phaseolin); 18 of them had type "S" phaseolin and the other 10 had type "T" phaseolin. DNA samples of all the cultivars were amplified *in vitro* with oligonucleotide primers of random sequences. A total of 276 amplification products, 144 of them being polymorphic, were obtained with 45 different primers. Multivariate analysis of these data divided the cultivars into two groups and, in addition, allowed a more precise intra and intergroup distinction.

INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is the main source of vegetable protein in Latin America, particularly among the poorer population. Annual world production is about 15 million tons, Brazil being one of the major producing countries (FAO, 1991). Although in many Brazilian states modern techniques have significantly increased productivity, the national average yield is still very low, ca. 445 kg/ha (IBGE, 1991). This is because the common bean is a subsistence crop in Brazil, and small farmers rarely use either appropriate planting techniques or genetically superior seeds, and they prefer crop association systems.

In Latin America, common bean breeders have traditionally developed new cultivars by selection and adaptation of superior lines. However, breeding programs based on oriented crosses or hybridization are becoming more common. In this context, a previous knowledge of the genetic distances among potential progenitors to be crossed in the breeding program is of paramount importance.

Molecular markers have been suggested as a potent tool to characterize and determine genetic distances within and among plant species (Hu and Quiros, 1991; Haley *et al.*, 1994; Vilarinhos *et al.*, 1994). These markers, especially those based on DNA, are very abundant, are inherited in a Mendelian fashion, and are not affected by the environment (Williams *et al.*, 1990). We utilized the random amplified polymorphic DNA technique (RAPD) (Williams *et al.*, 1990; Welsh and McClelland, 1990).

MATERIAL AND METHODS

Genetic material

The 28 common bean cultivars used in this work were supplied by the germplasm collection of the Departamento de Fitotecnia, Universidade Federal de

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Viçosa, MG, Brazil (Table I). Seeds from each cultivar were divided into two lots, one was used for electrophoretic analysis of phaseolin and the other was treated with fungicide, germinated in a sand bed, and planted in the greenhouse. Young leaves from one plant of each cultivar were collected and kept separately at -80°C for DNA extraction.

Table I - List of the 28 common bean cultivars used.

Cultivar	Origin	Seed color	Seed size
01. Ouro Negro	Honduras	Black	Small
02. Ouro	CIAT*	Cream	Small
03. Milionário 1732	CIAT	Black	Small
04. Manteigao Fosco 11	Brazil	Beige	Large
05. Carioca	Brazil	Beige/brown	Small
06. Diacol Calima	Colombia	Beige/red	Large
07. Ouro Branco	CIAT	White	Large
08. CNF 5547	CNPAF**	Beige/purple	Small
09. Caraota 260	Venezuela	Black	Small
10. Jalo EEP 558	Brazil	Yellow	Large
11. Negrito 897	Costa Rica	Black	Small
12. Ricopardo 896	Costa Rica	Brown	Small
13. Cornell 49-242	Venezuela	Black	Small
14. Diacol Andino	Colombia	Pink/red	Medium
15. ICA Tundama	Colombia	White/red	Large
16. Antioquia 8	Colombia	White/red	Medium
17. Dark Red Kidney	USA	Red	Large
18. ESAL 633	Brazil	Beige/brown	Small
19. US Pinto 111	USA	Beige/brown	Small
20. RAB 94	***	Red	Small
21. Costa Rica 1031	Costa Rica	Black	Small
22. Venezuela 350	Venezuela	Black	Small
23. Seleção Cuva	***	Black	Small
24. ESAL 648	Brazil	Beige/brown	Small
25. 2172	***	Purple	Small
26. Rio Tibagi	Brazil	Black	Small
27. Mineiro Precoce	Brazil	Beige	Large
28. Preto 60 Dias	Brazil	Black	Large

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***Unknown.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

Five seeds of each cultivar were powdered in a coffee mill and 100 mg of the meal was extracted with a mortar and pestle in 1 ml 0.05 M Tris-HCl, pH 6.8. After centrifugation at 13,000 rpm in an Eppendorf microcentrifuge, aliquots of the supernatant were mixed with equal volumes of sample buffer (0.05 M Tris-HCl, pH 8.0, containing 0.2% SDS, 5 M urea, 5% 2-mercaptoethanol, and 0.05% bromophenol blue) and 10-µl aliquots of these mixtures were loaded on a 7.5 to 15% Laemmli gel (Laemmli, 1970). Electrophoresis was performed at 100 volts for 7 h. The gel was stained with 0.1% Coomassie blue R-250, and destained in a solution containing 7.5% acetic acid and 25% methanol.

DNA extraction

The leaves were powdered in liquid nitrogen and the DNA was extracted based on a procedure of Keim *et al.* (1988) as modified by Vilarinhos *et al.* (1994). DNA concentration was determined spectrophotometrically at 260 nm (Sambrook *et al.*, 1989).

DNA amplification

Amplification reactions were performed in a thermocycler model 9600 from Perkin-Elmer (Norwalk, CT, USA). The amplification reactions consisted of 40 cycles each of one denaturation step (94°C for 15 sec), one annealing step (36°C for 30 sec), and one elongation step (72°C for 1 min). After the last cycle, an extra elongation step at 72°C for 7 min was performed. Each reaction mixture (25 µl) contained: 10 mM Tris-HCl, pH 8.0, 50 mM KCl, 2 mM $MgCl_2$, 100 µM of each deoxyribonucleoside triphosphate (dATP, dCTP, dGTP, and dTTP), 0.4 µM of one primer decamer (Operon Technologies, Inc., Alameda, CA, USA), one unit of Taq DNA polymerase, and 25 ng of DNA. The amplification products were separated electrophoretically on 1.2% agarose gels immersed in TBE (0.09 M Tris-borate, 2 mM EDTA). The DNA bands were visualized under UV light and photographed with a polaroid camera.

Data analyses

The DNA bands were scored as 1 (presence) or 0 (absence). Only intense, reproducible bands were considered. These data were used to determine the pairwise genetic distances among the cultivars by the complement of Nei and Li's similarity index (Nei and Li, 1979). The genetic distances were used to cluster the cultivars into defined groups by the single ligation method. Multivariate analysis allowed the projection of the cultivars onto bidimensional space (Cruz and Viana, 1994). The analyses were performed with the aid of the statistical softwares SAEG and GENES I developed at the Universidade Federal de Viçosa.

RESULTS AND DISCUSSION

Common bean cultivars are often divided into two large groups based on the size of their seeds and on the type of storage protein they bear. Small seeds with type "S" phaseolin are characteristic of Middle American cultivars while large or medium seeds with "T" type phaseolin are found in Andean cultivars (Gepts *et al.*, 1986; Singh *et al.*, 1991). As a first approach to characterize the 28 common bean potential progenitors, we analyzed their storage protein. Eighteen of them presented "S" type phaseolin and the other 10 bore "T" type phaseolin (data not shown). Pereira and Souza (1992) analyzed the phaseolin present in 192 common bean land races collected in Brazil and determined that the majority of them (80.6%) bore "S" type phaseolin while 19.4% had "T" type phaseolin.

We used the RAPD technique to study the genetic diversity within the groups defined by the phaseolin analysis. Forty-five oligonucleotide decamers generated 276 amplification products, 144 of them being polymorphic (Figure 1). All primers revealed at least one polymorphic DNA band. These data allowed the determination of pairwise genetic distances among the cultivars (Table II), which were used to cluster the cultivars into different groups (Figure 2). The cluster analysis divided the cultivars into two large groups which coincided with the ones based on the types of phaseolin. The 18 Middle American cultivars with small seeds and "S" type phaseolin were clustered in one group and the 10 Andean cultivars with medium-to-large-size seeds and "T" type phaseolin formed the other group. The shortest genetic distance was 1%, between cultivars of the same group. The largest genetic distances (11 to 13%) were found between Middle American and Andean cultivars (Table II). Cultivars Ouro Negro and US Pinto 111 cold be distinguished from the other Middle American cultivars at 38% of relative genetic distance (Figure 2, arrow). This indicates that these two cultivars are good candidates as sources of genes in hybridization programs involving the other genotypes in this group. However, US Pinto 111 is not being used in breeding programs in Brazil, perhaps due to its known susceptibility to rust (*Uromyces appendiculatus*). Ouro Negro, however, is very productive in the States of Minas Gerais and Rio de Janeiro, resistant to rust and anthracnose (*Colletotrichum lindemuthianum*), grows well during the winter period and in association with corn, and has excellent cooking qualities (Araújo *et al.*, 1991). Our data support those obtained by Haley *et al.* (1994) who observed that the polymorphism between common bean gene pools is larger than the polymorphism within races.

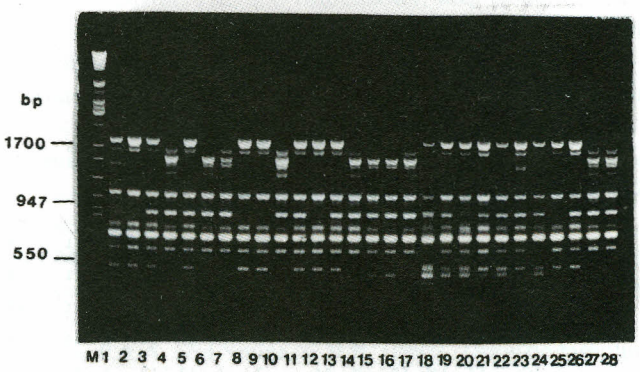

Figure 1 - Electrophoretic analysis of DNA amplification products obtained with primer OPG-19. Lane M corresponds to DNA from bacteriophage λ digested with endonucleases *Eco*RI, *Bam*HI, and *Hin*dIII, used as size markers. Lanes 1 through 28 correspond to the amplification products of the 28 cultivars, as identified in Table I.

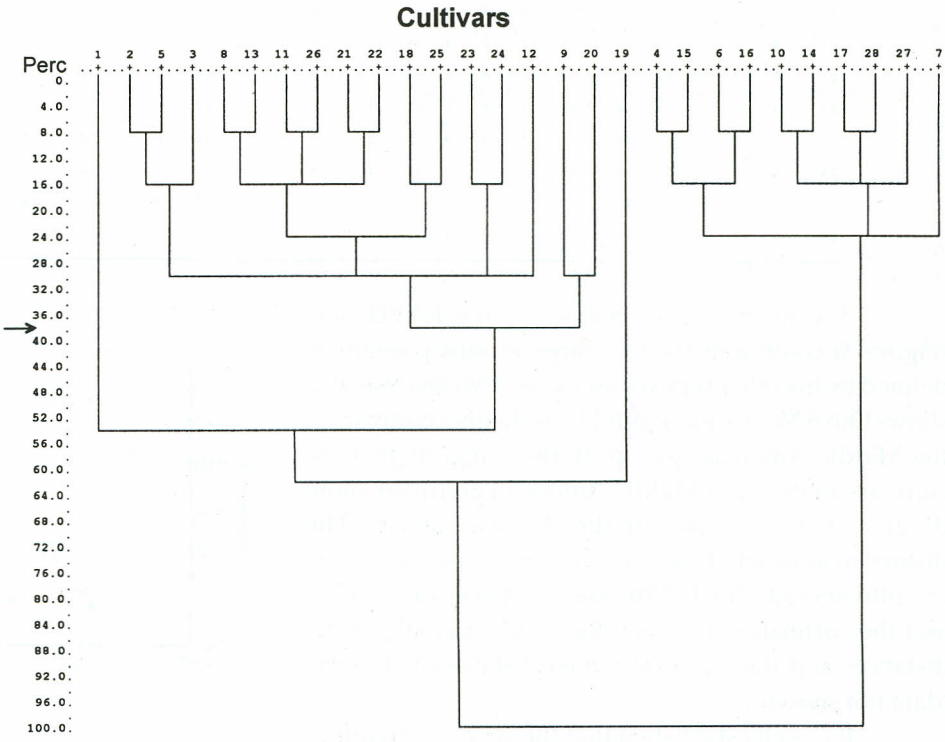

Figure 2 - Clustering of the 28 cultivars based on RAPD data. Identification of the cultivars is according to Table I.

Table II - Pairwise genetic distances (%) among the 28 common bean cultivars. Identification of the cultivars is according to Table I.

	CULTIVARS																										
	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
2	4																										
3	4	2																									
4	11	11	11																								
5	5	1	2	11																							
6	10	10	10	2	10																						
7	9	10	10	2	10	3																					
8	5	2	3	12	3	11	11																				
9	7	4	4	11	4	11	10	3																			
10	10	11	11	2	11	2	2	12	11																		
11	5	2	2	12	3	11	11	2	3	12																	
12	6	4	4	11	3	9	9	4	5	10	3																
13	4	1	2	11	2	10	10	1	3	10	1	3															
14	10	10	10	2	10	2	3	11	11	1	11	9	10														
15	11	10	11	1	10	1	2	11	10	2	11	10	10	3													
16	9	10	10	2	10	1	3	10	10	3	10	9	9	2	2												
17	12	12	12	2	11	2	3	13	12	1	12	10	11	2	2	3											
18	6	3	3	12	4	11	11	3	4	11	2	3	2	10	11	11	12										
19	8	6	6	10	6	10	10	5	6	10	6	7	5	10	11	10	11	4									
20	7	3	4	11	4	12	11	2	4	12	4	5	3	11	12	11	12	3	4								
21	5	3	3	11	3	10	10	2	4	11	2	4	1	10	11	10	11	3	6	3							
22	4	3	2	11	3	11	11	2	4	11	2	4	1	11	11	11	12	2	6	4	1						
23	5	3	2	10	2	11	10	4	4	11	3	4	2	10	10	10	11	3	5	4	2	2					
24	4	4	3	11	4	11	11	4	5	11	3	4	3	10	11	10	12	4	6	5	3	3	2				
25	5	3	3	12	4	11	11	3	4	11	2	3	2	11	12	11	12	2	5	3	3	2	3	3			
26	4	3	2	11	3	11	11	2	3	11	1	4	1	11	11	10	12	2	5	3	2	1	2	2	1		
27	11	11	11	1	11	2	2	12	12	1	11	10	11	2	2	2	2	11	11	12	11	12	11	11	11	11	
28	12	12	12	2	11	3	3	13	12	1	12	10	11	2	3	3	1	12	11	12	11	12	11	11	11	12	1

The multivariate analysis of the RAPD data (Figure 3) confirmed the two large groups previously defined by the other types of analyses. This analysis also shows Ouro Negro and Pinto 111 as distinct cultivars in the Middle American group. It also suggests that the cultivars analyzed in Middle American group are more diverse than the ones in the Andean group. The distortion associated with the graphic dispersion of the 28 cultivars onto the bidimensional space was 11.17%, and the correlation between the 2,561 original genetic distances and the 2,275 estimated distances was 0.983 (data not shown).

It is well established that the genetic variability within the Middle American and Andean groups is very narrow. Consequently, the possibility of genetic gain by

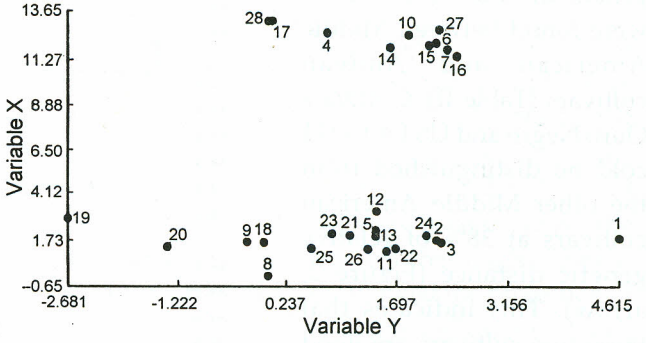

Figure 3 - Projection of the 28 common bean cultivars onto bidimensional space. Identification of the cultivars is according to Table I.

hybridization within these groups is very low. Our data support the idea suggested by Singh *et al.* (1991) of the need for a search for new sources of genes in wild-type material as well as in crosses between cultivars originated in different domestication centers, even though this latter procedure, in some cases, may lead to hybrid weakness (Koinange and Gepts, 1992).

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RESUMO

Vinte e oito cultivares de feijão comum, progenitores potenciais em programas de melhoramento, foram selecionados para análise de diversidade genética usando a técnica RAPD. Eles foram inicialmente divididos em dois grandes grupos com base no tipo de proteína de reserva (faseolina) que eles possuem. Dezoito deles apresentaram faseolina do tipo "S" e dez, do tipo "T". Amostras de DNA dos cultivares foram amplificadas *in vitro* pelo uso de oligonucleotídeos iniciadores de seqüências aleatórias. Um total de 276 produtos de amplificação, sendo 144 deles polimórficos, foram obtidos com 45 diferentes tipos de iniciadores. Análise multivariada desses dados novamente agrupou os cultivares em dois grupos e além disso, possibilitou uma distinção mais precisa dentro desses grupos e entre eles.

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