RAPD ANALYSIS OF GENETIC DIVERSITY IN *Phaseolus vulgaris* L.

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INTRODUCTION

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. This type of information can be used to orient the choice of progenitors and ultimately define the outcome of the program.

Molecular markers have been suggested as a potent tool to characterize and determine genetic distances within and among plant species (Hu and Quiros, 1991; Vilarinhos et al., 1994) These markers, especially the ones based on the DNA, are very abundant, are inherited in a mendelian fashion, and are not affected by the environment (Williams et al., 1990)

The main objective of this work was to characterize and determine the genetic distances among 28 potential common bean progenitors for the breeding program of the Federal University of Viçosa (UFV), MG, Brazil. For this purpose we utilized SDS-polyacrylamide gel electrophoresis for phaseolin analysis and the random amplified polymorphic DNA technique (RAPD) (Williams et al., 1990).

MATERIALS AND METHODS

The 28 common bean cultivars used in this work were supplied by the germplasm collection of the Departamento de Fitotecnia, UFV, MG, Brazil. 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.

For phaseolin analysis, 100 mg of seed meal was extracted in a mortar and pestle in 1 ml of 0.05M Tris-HCl, pH 6.8 and 10 µl aliquots of the extracts were loaded on a 7.5 to 15% Laemmli gel (Laemmli, 1970).

For RAPD analysis, leaf DNA was extracted based on a modified procedure of Keim et al., 1988 (Vilarinhos et al., 1994). Amplification reactions were performed in a thermocycler model 9600 from Perkin-Elmer (Norwalk, Conn., USA) according to Vilarinhos et al. (1994). The amplification products were separated electrophoretically on 1.2% agarose gels immersed in TBE (0.09 M Tris-Borate, 2mM EDTA). The DNA bands were visualized under UV light and photographed with a polaroid camera.

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.

RESULTS AND DISCUSSION

Eighteen of the cultivars presented "S" type phaseolin and small seeds, typical of Middle American germplasm (group A, Table 1). The other 10 cultivars were typically Andean, with "T" type phaseolin and medium-to-large seeds (group B, Table 1). Forty-five oligonucleotide decamers generated 276 amplification products, 144 of them being polymorphic. All primers revealed at least one polymorphic DNA band. These data allowed the determination of the pairwise genetic distances among the cultivars, which were used to cluster the cultivars into different groups. The cluster analysis divided the cultivars into two large groups (A and B) which coincide with the ones based on the types of phaseolin The 18 Middle American cultivars were clustered in one group and the 10 Andean cultivars formed the other group (Table 1). Cultivars Ouro Negro and US Pinto 111 could be distinguished from the other Middle American cultivars at 42% of relative genetic distance. 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 Ouro Negro, on the other hand, indicated to the states of Minas Gerais and Espirito Santo, is very productive, grows well during the winter period and in association with corn, and has excellent cooking qualities. The largest genetic distances were between cultivars of the two groups (16 to 21%). The shortest genetic distances were found within the two groups: 1 to 5% in the Andean group, and 1 to 13% in the Middle American group This is in well accordance to the trend already observed that polymorphisms are higher between than within common bean gene pools (Haley et al., 1994).

A1. Ouro Negro	A4 CNF 5547	A5. Caraota 260	B. Manteigão Fosco 11
	Negrito 897	RAB 94	Diacol Calima
A2. Ouro	Cornell 49-242		Jalo EEP 558
Milionário 1732	ESAL 633	A6. US Pinto 111	Diacol Andino
Carioca	Costa Rica 1031		Ouro Branco
Seleção Cuva	Venezuela 350		ICA Tundama
ESAl. 648	2172		Antioquia 8
	Rio Tibagi		Dark Red Kidney
A3 Ricopardo 896			Mineiro Precoce
			Preto 60 Dias

Table 1. Cluster analysis based on RAPD data.

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