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# RAPD-PCR characterization of varieties of the common bean (*Phaseolus vulgaris* L.) used to identify races of Anthracnose fungus (*Colletotrichum lindemuthianum*)

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## ABSTRACT

The Random Amplified Polymorphic DNA (RAPD) technique was used to fingerprint and determine the genetic distances among the twelve genotypes of *Phaseolus vulgaris* L. used internationally to differentiate races of *Colletotrichum lindemuthianum*. Leaf DNA was extracted from plants from each cultivar and used as a template for the amplification reactions. Twenty-four different oligonucleotide primer decamers were used, and 74 reproducible amplification products were obtained, 59 of them being polymorphic. Genetic divergence was determined based on the presence (1) or absence (0) of the DNA bands in the individuals analyzed. Cluster analysis was performed by the method of Tocher, based on the arithmetical complement of the Jaccard index and by the method of the nearest neighbour. Both gave the same results, grouping the cultivars in similar clusters. The bidimensional graphic dispersion of the individuals demonstrate that the largest genetic distance was between cultivars PI 207262 and AB 136, and cultivars Perry Marrow and Dark Red Kidney, while the shortest distance was between Dark Red Kidney and Perry Marrow.

## INTRODUCTION

Anthracnose is one of the most important diseases affecting the common bean worldwide. It is caused by the fungus *Colletotrichum lindemuthianum* (Sacc. et Magn.) Scrib. which infects susceptible cultivars grown in regions with high relative humidity and temperatures ranging from moderate to cold (Sartorato, 1988). Losses may reach 100% if infected seeds are planted under favorable conditions (Chaves, 1980). Control of the disease is normally achieved by the use of resistant cultivars. However, the existence of many physiological races of the pathogen represents an obstacle for the development of cultivars harboring resistance genes. In Brazil, several races of *C. lindemuthianum* have been identified in different regions (Oliari *et al*., 1973; Oliveira *et al*., 1973; Pio Ribeiro and Chaves, 1975; Paradela Filho and Pompeu, 1975; Paradela Filho *et al*., 1981; Menezes *et al*., 1982; Menezes, 1985).

Classification of new isolates of *C. lindemuthianum* into physiological races is usually

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made by analyzing the response of differential common bean cultivars to the isolate. The following 12 international differential cultivars are normally used: Michelite, Michigan Dark Red Kidney (MDRK), Perry Marrow, Cornell 49242, Widusa, Kaboon, Mexico 222, PI 207262, To, Tu, AB 136 and G 2333 (Pastor-Corrales, 1988). To our knowledge, no attempt has been made so far to characterize these cultivars by means other than the analysis of morphological characteristics. The random amplified polymorphic DNA (RAPD) technique (Williams *et al.*, 1990) has been used to characterize plants from different species (Hu and Quiros, 1991; Demeke *et al.*, 1992; Thormann and Osborn, 1992).

## MATERIAL AND METHODS

All the genetic material used in this work was provided by the Department of Plant Sciences of the Federal University of Viçosa, Viçosa, MG, Brazil, and by the Agronomy Institute of Paraná, Paraná, Brazil (Table I). Seeds were germinated and plants were grown in the greenhouse. At the stage of the first trifoliolate leaves, young leaves were collected, frozen in liquid nitrogen and kept at -80°C for DNA extraction, according to the procedure of Saghai-Maroof *et al.* (1984).

Each amplification reaction of 25 µl contained: 50 ng of DNA, 100 µM of each deoxynucleoside triphosphate, 1.7 mM $MgCl_2$, 10 mM Tris-HCl, pH 8.3, 50 mM KCl; 0.4 µM of one primer decamer (Operon Technologies, Inc., Alameda, CA, USA), and 1 unit of Taq DNA polymerase (Perkin Elmer-Cetus Corp., Norwalk, Conn., USA). Reactions were performed in a thermocycler model 9600 (Perkin Elmer-Cetus Corp., Norwalk, Conn., USA) programmed for 40 cycles, each with one denaturation step at 94°C for 15 sec, one annealing step at 35°C for 30 sec, and one elongation step at 72°C for 60 sec. A final elongation step was performed for seven min at 72°C. DNA amplification products were analyzed on 1.2% agarose gels immersed in TBE buffer (90 mM Tris-borate, 1 mM EDTA, pH 8.0) containing ethidium bromide (10 µg/ml). DNA bands were visualized under UV light and photographed with polaroid film type 667. The primers used were as follows: OPI02, OPI03, OPI06, OPI07, OPI09, OPJ04, OPJ07, OPJ08, OPK01, OPK03, OPK04, OPK06, OPN08, OPN09, OPN10, OPN11, OPN12, OPN13, OPO01, OPO02, OPO03, OPP01, OPP02, and OPP03.

The most prominent DNA bands were scored as 1 (presence) or 0 (absence) and these data were used for determination of genetic distances by the Jaccard similarity index (Sneath and Sokal, 1973). Cluster analysis was done by the nearest neighbour method and by the method of Tocher (Singh and Chaudhary, 1979) based on the arithmetical complement of the Jaccard index. Genetic dissimilarity values were also used to locate the cultivars on bidimensional space (Cruz and Viana, 1994). Size markers used are described in the legend of Figure 1.

**Table I** - List of the 12 common bean cultivars internationally used to differentiate races of *Colletotrichum lindemuthianum*. The cultivars were classified into races based on personal information by Dr. Shree Singh from CIAT (Cali, Colombia).

| Number | Cultivar | Race |
|---|---|---|
| 1 | Michelite | Mesoamerica |
| 2 | MDRK | Nueva Granada |
| 3 | Perry Marrow | Chile |
| 4 | Cornell 49242 | Mesoamerica |
| 5 | Widusa | Durango |
| 6 | Kaboon | Nueva Granada |
| 7 | Mexico 222 | Durango |
| 8 | PI 207262 | Mesoamerica |
| 9 | To | Durango |
| 10 | Tu | Durango |
| 11 | AB 136 | Mesoamerica |
| 12 | G 2333 | Mesoamerica |

## RESULTS AND DISCUSSION

Table II shows the distribution of DNA amplification products obtained for the 12 common bean differential cultivars with 24 different primers. Only strong, reproducible DNA bands were considered (Figure 1). Seventy-four bands ranging from 820 to 2,340 bp were amplified, 59 of them being polymorphic. All but one primer (OPO02) generated polymorphic bands.

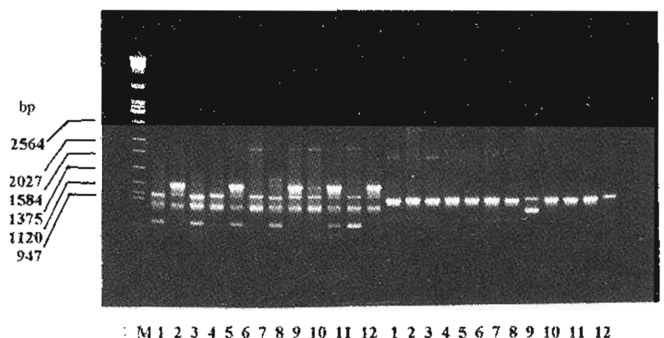


**Figure 1** - Agarose gel showing typical pattern of RAPD amplification products with primers OPP01 and OPP02. Lanes are as follows: M, Lambda phage DNA digested with *Eco*RI, *Hin*dIII, and *Bam*HI (size markers); 1, Michelite; 2, Dark Red Kidney; 3, Perry Marrow; 4, Cornell 49242; 5, Widusa; 6, Kaboon; 7, Mexico 222; 8, PI 207262; 9, To; 10, Tu; 11, AB 136 and 12, G 2333.

**Table II** - List of polymorphic DNA amplification products among the 12 common bean differential cultivars with different primers. Presence of a band is indicated by (1) and absence by (0). Sizes of the bands are indicated in the last column of the table.

| Primer/ DNA band | | | Common bean differentials* | | | | | | | | | | | | Base pairs (bp) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | |
| 1 | I02 | A | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1,528 |
| 2 | | B | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1,280 |
| 3 | I03 | A | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1,691 |
| 4 | | B | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1,605 |
| 5 | | C | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1,184 |
| 6 | I06 | A | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 2,300 |
| 7 | I07 | A | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1,694 |
| 8 | | B | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1,431 |
| 9 | | C | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1,119 |
| 10 | | D | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 903 |
| 11 | I09 | A | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1,100 |
| 12 | J04 | A | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 2,307 |
| 13 | | B | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1,479 |
| 14 | | C | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1,079 |
| 15 | J07 | A | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1,673 |
| 16 | | B | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1,264 |
| 17 | | C | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1,180 |
| 18 | J08 | A | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 873 |
| 19 | | B | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 838 |
| 20 | K01 | A | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 2,307 |
| 21 | | B | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1,038 |
| 22 | K03 | A | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 1,108 |
| 23 | | B | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1,020 |
| 24 | K04 | A | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2,341 |
| 25 | | B | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1,972 |
| 26 | | C | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1,502 |
| 27 | | D | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1,275 |
| 28 | K06 | A | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 1,313 |
| 29 | | B | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1,180 |
| 30 | N08 | A | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1,786 |
| 31 | | B | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1,528 |
| 32 | | C | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1,280 |
| 33 | N09 | A | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1,702 |
| 34 | | B | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1,111 |
| 35 | | C | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 867 |
| 36 | N10 | A | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1,425 |
| 37 | | B | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 937 |
| 38 | | C | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 867 |
| 39 | N11 | A | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 2,213 |
| 40 | | B | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1,870 |
| 41 | | C | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1,741 |
| 42 | | D | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1,094 |
| 43 | | E | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1,029 |
| 44 | N12 | A | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1,029 |
| 45 | N13 | A | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 2,025 |
| 46 | | B | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1,386 |
| 47 | | C | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1,131 |
| 48 | O01 | A | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 2,025 |
| 49 | | B | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1,870 |
| 50 | O03 | A | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1,094 |
| 51 | | B | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 926 |
| 52 | | C | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 905 |
| 53 | | D | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 850 |
| 54 | P01 | A | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1,434 |
| 55 | | B | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1,079 |
| 56 | P02 | A | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1,236 |
| 57 | | B | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1,128 |
| 58 | P03 | A | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 926 |
| 59 | | B | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 818 |

*Common bean differentials: 1 - Michelite, 2 - MDRK, 3 - Perry Marrow, 4 - Cornell 49242, 5 - Widusa, 6 - Kaboon, 7 - Mexico 222, 8 - PI 207262, 9 - To, 10 - Tu, 11 - AB 136, and 12 - G 2333.

**Table III** - Pairwise genetic dissimilarity estimates (arithmetical complement of the Jaccard index) among the 12 common bean cultivars, based on RAPD data. Identification numbers for the common bean cultivars are according to Table I.

| Cultivar | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.76 | 0.78 | 0.21 | 0.47 | 0.70 | 0.51 | 0.43 | 0.51 | 0.62 | 0.43 | 0.41 |
| 2 | | 0.20 | 0.76 | 0.72 | 0.41 | 0.44 | 0.82 | 0.70 | 0.37 | 0.79 | 0.77 |
| 3 | | | 0.78 | 0.78 | 0.44 | 0.51 | 0.84 | 0.69 | 0.45 | 0.84 | 0.80 |
| 4 | | | | 0.46 | 0.64 | 0.53 | 0.34 | 0.47 | 0.63 | 0.30 | 0.36 |
| 5 | | | | | 0.55 | 0.54 | 0.58 | 0.57 | 0.57 | 0.54 | 0.47 |
| 6 | | | | | | 0.39 | 0.68 | 0.64 | 0.36 | 0.68 | 0.66 |
| 7 | | | | | | | 0.57 | 0.50 | 0.40 | 0.54 | 0.51 |
| 8 | | | | | | | | 0.57 | 0.73 | 0.38 | 0.35 |
| 9 | | | | | | | | | 0.63 | 0.50 | 0.51 |
| 10 | | | | | | | | | | 0.70 | 0.73 |
| 11 | | | | | | | | | | | 0.35 |

Studies based on crop evolution, morphological features, isozymes, and storage proteins allowed the definition of two large groups of the common bean: the Middle American and the Andean South American groups (Singh *et al.*, 1991). Each one of these groups can be further divided into three races: Mesoamerica, Jalisco, and Durango (Middle American), and Nueva Granada, Peru, and Chile (Andean South American) (Singh *et al.*, 1991). According to the RAPD data we obtained, cultivars Perry Marrow (race Chile) and Dark Red Kidney (race Nueva Granada), both of South American origin, were the closest ones with 20 DNA bands in common (1,1) and 91.5% identity (1,1 or 0,0) (Table II). On the other hand, the cultivar pairs Perry Marrow (race Chile) and AB 136 (race Mesoamerica), and Perry Marrow, and PI 207262 (race Mesoamerica) were the most dissimilar ones sharing only seven bands with each other, and showing 35.6% identity (Table II). Cultivars AB 136 and PI 207262 (both race Mesoamerica), which demonstrate high resistance levels to several races of *C. lindemuthianum* (Menezes, 1985), had 76.3% identity among the 59 polymorphic loci analyzed, with 23 DNA bands in common. These data were confirmed by the genetic dissimilarity index (arithmetical complement of the Jaccard index) (Table III).

Cluster analyses based on the Tocher method (Table IV) and the method of the nearest neighbour (Figure 2) gave similar results, grouping the 12 cultivars into four distinct groups. All five cultivars in group II (Table IV) belong to race Mesoamerica. However, group I includes cultivars from races Durango (Tu and Mexico 222), Nueva Granada (Dark Red Kidney and Kaboon), and Chile (Perry Marrow). Groups III and IV each include only one cultivar from race Durango, Widusa and To, respectively (Table IV). Cultivars from distinct origins (Middle America and Andean South America) were grouped together (Table IV, group I), while cultivars from the same race (Durango) were scattered into three different groups (Table IV). These discrepancies may be due to the fact that the present race classification is mainly based on morphological data (Singh *et al.*, 1991), while the groups obtained in this work were exclusively based on RAPD data. However, we believe the small number of cultivars analyzed in this work do not allow any further speculation.

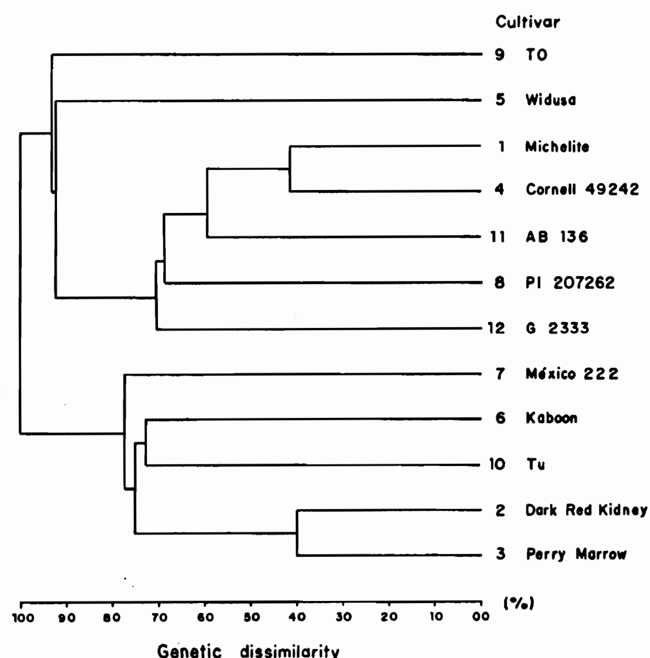


**Figure 2** - Dendrogram of the 12 common bean differential cultivars based on the genetic dissimilarity determined by the method of the nearest neighbour.

**Table IV** - Cluster analysis (Tocher method), based on the genetic dissimilarity matrix (arithmetical complement of the Jaccard index).

| Group | Cultivar |
|---|---|
| I | Dark Red Kidney, Perry Marrow, Tu, Kaboon, Mexico 222 |
| II | Michelite, Cornell 49242, AB 136, G 2333, PI 207262 |
| III | Widusa |
| IV | To |

Figure 3 gives a bidimensional graphic dispersion of the cultivars based on the genetic dissimilarity among them. This type of analysis shows that the correlation between the original and the estimated dissimilarity values is 0.926, with a graphic distortion of 27.24%. The greatest genetic distance were between cultivars PI 207262 and Perry Marrow, AB 136 and Perry Marrow, and PI 207262 and Dark Red Kidney. Cultivars Dark Red Kidney and Perry Marrow were the most similar.

All three types of analyses performed, i.e., cluster analysis by the nearest neighbour and the Tocher method, and the bidimensional graphic dispersion gave very similar results, grouping the 12 cultivars into four distinct groups (Figures 2 and 3, and Table IV). This demonstrates that the RAPD technique is very potent to determine genetic diversity among individuals, and could help orient crosses in a breeding program.

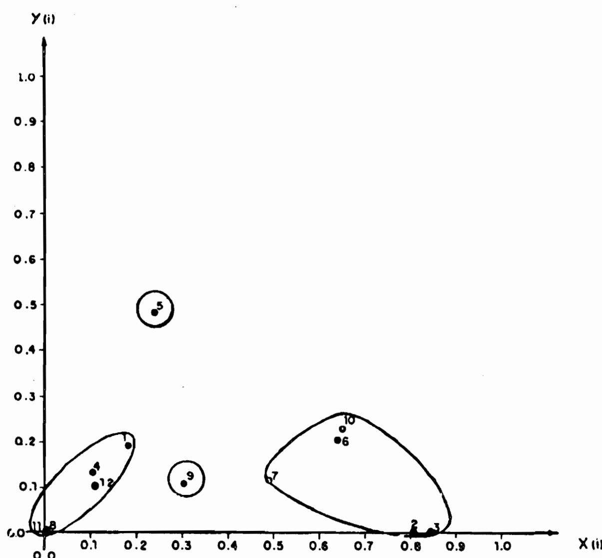


**Figure 3** - Dispersion of the 12 common bean differential cultivars in two dimensional space, based on a dissimilarity matrix obtained by the Jaccard index arithmetic complement. Identification numbers for the common bean cultivars are according to Table I.

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## RESUMO

A técnica do DNA Polimórfico Amplificado ao Acaso (RAPD) foi utilizada para a caracterização e a determinação de distâncias genéticas entre doze genótipos de *Phaseolus vulgaris* L. que são utilizados internacionalmente para diferenciar raças de *Colletotrichum lindemuthianum*. DNA de folha foi extraído de plantas de cada cultivar e usado como molde para as reações de amplificação. Vinte e quatro diferentes oligonucleotídeos iniciadores ("primers") foram usados e 74 produtos de amplificação reprodutíveis foram obtidos, 59 dos quais eram polimórficos. A divergência genética foi determinada com base na presença (1) ou ausência (0) de bandas de DNA nos indivíduos analisados. Análise de agrupamento foi feita pelo método de Tocher, com base no complemento aritmético do índice de Jaccard e pelo método do vizinho mais próximo. Ambos deram os mesmos resultados e agruparam os cultivares em grupos similares. A dispersão gráfica bidimensional dos indivíduos demonstra que a maior distância foi entre os cultivares PI 207262 e AB 136 e os cultivares Perry Marrow e Dark Red Kidney, enquanto que a menor distância foi entre Dark Red Kidney e Perry Marrow.

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