

Determination of genetic diversity within Brazilian soybean germplasm using random amplified polymorphic DNA techniques and comparative analysis with pedigree data

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ABSTRACT

We used the random amplified polymorphic DNA (RAPD) technique to evaluate genetic diversity among 38 Brazilian soybean (*Glycine max* L. Merrill) cultivars. The analysis allowed the separation of the cultivars into five distinct groups based on the genetic distances among them. Comparison between these results and pedigree data were coherent for most cases. The data confirmed the use of the RAPD technique as a potent tool to aid the study of genetic diversity among individuals of the same species and should allow Brazilian soybean breeders a more rational choice of progenitors to be used in specific breeding programs.

INTRODUCTION

Soybean (*Glycine max* L.) is one of the most important crops in the world today. Brazil is presently the second largest world producer, surpassed only by the United States. Soybeans are planted in a vast range of latitudes in Brazil, from the state of Rio Grande do Sul in the southern part of the country to the states of the northern region.

Although the number of available cultivars is large in Brazil, the genetic base of the Brazilian germplasm is extremely reduced. One hundred per cent of the gene pool of the cultivars recommended for the agricultural year 1983/84 were derived from only 26 ancestors. Nine of these ancestors were responsible for more than 80% of that pool, and only four among these ancestors were the source of about 50% of the gene pool

(Hiromoto and Vello, 1986). This situation is not different from that of the United States and Canada (Delannay *et al.*, 1983).

Genetic variability is essential for the success of any breeding program. Traditionally, morphological characteristics have been used to estimate the genetic diversity among individuals. More recently, molecular markers, such as isozymes and other proteins, have also been used for this purpose (Singh *et al.*, 1991a; Singh *et al.*, 1991b). Both these systems present disadvantages because of the limited number of markers available. This is particularly important for soybean and other cultivated species with a narrow genetic base.

Several types of DNA-based markers have been developed in the last few years. Restriction fragment length polymorphisms (RFLP) (Beckmann and Soller, 1986) and random amplified polymorphic DNA (RAPD) (Williams *et al.*, 1990) are the more popular ones. The use of these markers have increased the resolution in studies of genetic diversity because the number of

markers is practically unlimited, and because they are not affected by the environment, unlike morphological markers.

Genetic diversity studies among soybean cultivars have been undertaken by different groups using RFLP markers (Apuya *et al.*, 1988; Keim *et al.*, 1989; Tingey *et al.*, 1991; Chen *et al.*, 1993). The results of these studies confirm the low genetic diversity in soybean. RFLP markers have several advantages, including the larger number of available markers, reproducibility, and codominant inheritance. On the other hand, the technique is very laborious and involves a large number of steps, which makes automation almost impossible (Newbury and Ford-Loyd, 1993). In addition, RFLPs mainly cover the regions of the genome represented by unique or low copy number sequences.

The RAPD technique is a modification of the polymerase chain reaction developed by two independent groups (Williams *et al.*, 1990; Welsh and McClelland, 1990). In the RAPD technique discrete DNA fragments of the genome are amplified by the use of one single primer of arbitrary sequence. A specific fragment is amplified whenever a primer attaches to two annealing sites in the opposite strands of the template DNA, as long as these sites are not separated too far apart (less than 2 to 3 kb, in general). Polymorphisms are detected due to mutations in the annealing site(s), insertions, or deletions in the region flanked by these sites. Among the advantages of RAPDs one can cite the large number of markers available, the possibility of covering unique as well as repetitive regions of the genome, and the simplicity of the steps involved.

The main goal of this study was to evaluate the use of RAPDs to determine the genetic diversity among Brazilian soybean cultivars, which are currently being used in breeding programs of the Federal University of Viçosa, and other research stations in Brazil. To validate the RAPD data, they were compared to known genealogy information of the cultivars studied. Taken together, these data should help soybean breeders in Brazil in the choice of progenitors to be used for specific breeding programs.

MATERIAL AND METHODS

Genetic material

The 38 soybean cultivars used in this work were supplied by the Departamento de Fitotecnia of the Federal University of Viçosa (UFV), Minas Gerais, Brazil (Table I). Seeds from each cultivar were germinated and

planted in the greenhouse and young leaves from one plant from each cultivar were collected and stored at -80°C for DNA extraction. Preliminary RAPD data from our laboratory revealed a certain degree of variability among individuals from each cultivar which had been collected in different locations. However, this variability was negligible when plants from the same location were considered (data not shown).

Table I - List of the 38 soybean cultivars analyzed and their genealogies.

Cultivar	Genealogy
1. BR-1	Hill x L-356
2. BR-2	Hill x Hood
3. BR-6	Bragg(3) x Santa Rosa
4. BR-9 (Savana)	Selection in pop. LoB74-21
5. BR-11 (Carajás)	UFV-1 x IAC 73-2736-10
6. Campos Gerais	Arksoy x Ogden
7. Doko	Progenie F7, obtained from pop. RB-72-1, derived from six crosses (E 70-46 x Viçoja, E 70-47 x Viçoja, Hill x E 70-47, E 70-46 x Pickett, E 70-47 x F65-1376 and Davis x IAC-79308)
8. EMGOPA-301	IAC-4 x Júpiter
9. FT-11	UFV-1 x Campos Gerais
10. FT-Cometa	FT 420 x Williams
11. FT-Cristalina	Natural cross in UFV-1
12. IAC-8	Bragg x (Hill x PI 240664)
13. IAC-12	Paraná x IAC 73-231
14. IAS-4	Selection in R60-390 (Hood x Jackson)
15. IAS-5	Hill x Hood
16. Ivaí	Majos x Hood
17. OCEPAR-8	Selection in Paraná
18. OCEPAR-9-SSI	Natural mutation in Paraná
19. Paraná	Hill x (Roanoke x Ogden)
20. Paranagoiana	Natural mutation in Paraná
21. Paranaíba	Davis x [Davis x (Hill x PI 240664)]
22. Planalto	Hood x Kedeele STB no. 452
23. Santa Rosa	D49-772 x La41-1219
24. Tropical	Hampton x E 70-51
25. UFV-Araguaia	Hardee x IAC-2
26. UFV-1	Selection in Viçoja
27. UFV-2	Hardee x IAC-2
28. UFV-3	Hardee x Improved Pelican
29. UFV-5	Mineira x UFV-1
30. UFV-6 (Rio Doce)	Santa Rosa x UFV-1
31. UFV-7 (Juparanã)	(Hardee x IAC-2) x UFV-1
32. UFV-8 (Monte Rico)	(IAC-2 x Hardee) x UFV-1
33. UFV-9 (Sucupira)	Selection in UFV-1
34. UFV-10 (Uberaba)	Santa Rosa x UFV-1
35. UFV-14 (Itamarati)	Paraná x Viçoja
36. UFV-15 (Uberlândia)	Paraná x UFV-1
37. União	D65-2874 x Hood
38. Viçoja	D49-2491 x Improved Pelican

DNA extraction

DNA was extracted as suggested by Keim *et al.* (1988). Briefly, ca. two grams of lyophilized leaves were powdered in a mortar and pestle with liquid nitrogen. The powder was transferred to a propylene tube with 15 ml of extraction buffer (50 mM Tris-HCl, pH 8.0, containing 50 mM EDTA, 0.7 M NaCl, and 1% (v/v) 2-mercaptoethanol). CTAB (cetyltrimethylammonium bromide) was added to a final concentration of 1% (w/v), and the tubes were incubated at 65°C for one hour. After incubation, proteins were extracted by addition of one volume of chloroform:isoamylalcohol (24:1, v/v) followed by centrifugation at 3,500 x g. The aqueous phase was transferred to a clean tube and the nucleic acids were precipitated by addition of 2/3 volume of cold isopropanol. After a new centrifugation at 3,500 x g, the supernatant was discarded and the pellet was washed with cold 80% (v/v) ethanol, vacuum-dried and resuspended in TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). RNA was removed by addition of RNAase A (40 µg/ml) and incubation at 37°C for 30 min. The DNA was reprecipitated by addition of two volumes of cold 95% ethanol (v/v) and centrifugation. The final pellet was washed with 80% (v/v) ethanol, vacuum-dried, resuspended in 0.75 ml TE, and quantified spectrophotometrically at 260 nm.

DNA amplification

A total of 23 primer decamers (Operon Technologies, Alameda, CA, USA) were used to amplify the DNA extracted from the 38 cultivars. These primers were chosen because in preliminary studies they revealed at least one polymorphic DNA band among the cultivars analyzed. Each amplification reaction of 25 µL contained: 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2 mM MgCl₂, 100 µM of each deoxyribonucleoside triphosphate, 0.4 µM of one specific primer decamer, one unit of Taq DNA polymerase, and 40 ng of DNA. Reactions were performed in a thermocycler model 9600 (Perkin Elmer, Norwalk, Conn., USA) programmed for 45 cycles; each cycle consisting of one denaturation step at 94°C for 15 sec, one annealing step at 35°C for 30 sec, and one polymerization step at 72°C for one min. After the 45th cycle a final elongation step for seven min at 72°C was performed. The amplification products were separated on 1.3% agarose gels immersed in TBE (0.09 M Tris-Borate, 2 mM EDTA), stained with ethidium bromide, and photographed under UV light with polaroid film 667.

Pedigree analysis

Pedigree analysis for most cultivars was based on Hiromoto and Vello (1986). For the remaining cultivars the calculations were based on their genealogy (Table I). It was not possible to determine the pedigree data for cultivars FT-Cometa and IAC-12 because we were unable to trace back the genealogy of one of their progenitors.

Data analysis

Reproducible DNA bands, normally the most intense ones, were scored as 1 (presence) or 0 (absence). Pairwise genetic distances were determined by dividing the number of polymorphic bands between two given cultivars and the total number of bands analyzed. Cluster analyses were done by the method of the nearest neighbor with the aid of a statistical software (SAEG) developed at the Federal University of Viçosa, Viçosa, MG, Brazil.

RESULTS

For this study, only primers that generated at least one polymorphic amplification product were considered (Figure 1). Ninety-four amplification products (220 to 2500 bp) were obtained with the 23 primers used, an average of 4.1 DNA bands per primer. Forty-two of these bands were polymorphic and 52 were monomorphic (Table II).

Pairwise genetic distances based on the presence or absence of the DNA bands were calculated (Table III). These distances varied between 0% (cultivars OCEPAR-9-SS1 and Paranagoiana) and 31% (cultivars Tropical and UFV-6). The distances calculated are somewhat overestimated because only primers generating at least one polymorphic band were considered. The average distance obtained was 17.4% and the average genetic distance between each cultivar and the other 37 cultivars was between 14.2% (IAS-4) and 20.5% (Tropical) (Table IV).

Cluster analyses divided the cultivars into two large groups, group A with 14 cultivars, and group B with 24 cultivars (Figure 2). Considering an upper limit of 76% of relative genetic distance, these two groups can be further divided into five distinct subgroups (A1 and A2, and B1, B2, and B3) (Figure 2).

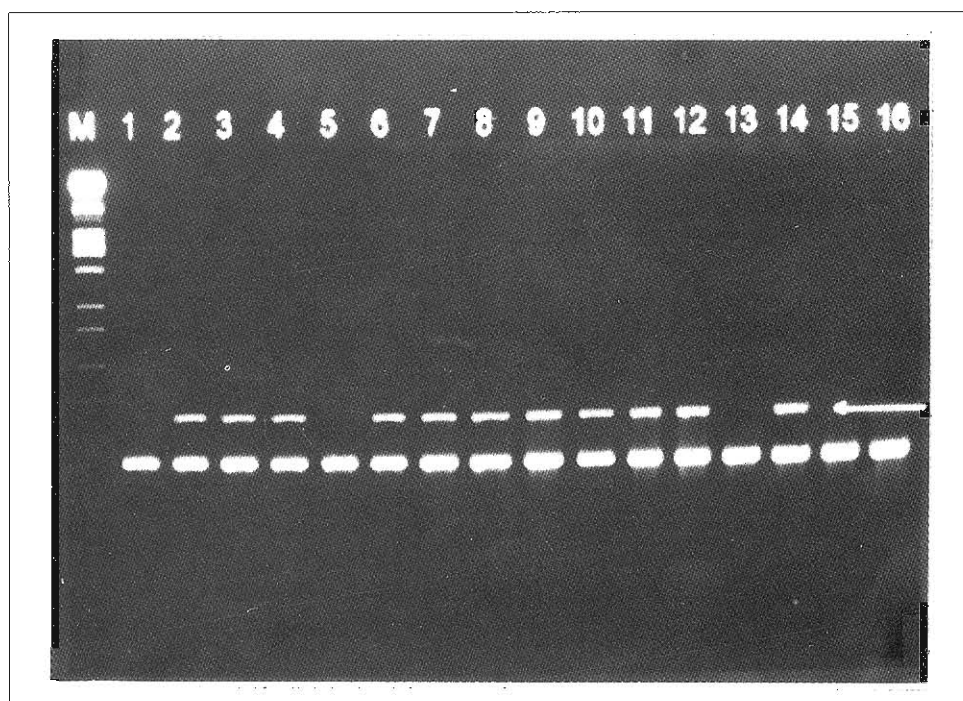


Figure 1 - Typical DNA amplification pattern obtained with primer OPN03 for 16 of the cultivars analyzed. Lanes are as follows: M, λ phage DNA digested with *EcoRI*, *BamHI*, and *HindIII*; 1, FT Cristalina; 2, UFV-2; 3, Tropical; 4, BR-2; 5, IAS-5; 6, UFV-9; 7, UFV-6; 8, Santa Rosa; 9, UFV-1; 10, UFV-10; 11, FT-11; 12, Campos Gerais; 13, UFV-14; 14, Viçosa; 15, Paraná; 16, UFV-15. The arrow points to a 840-base-pair polymorphic DNA band.

Table II - List of oligonucleotide primers used, their sequences, and the size range of DNA fragments obtained.

Primer	Sequence (5'-3')	Poly-morphic	Mono-morphic	Size range (base pairs)
OPA04	AATCGGGCTG	2	0	982-1225
OPA05	GGTCCCTGAC	1	4	631-1516
OPA07	GAAACGGGTG	2	4	217-1638
OPB01	GTTTCGCTCC	2	1	502-1259
OPB03	CATCCCCCTG	2	5	443-1653
OPC03	GGGGTCTTT	3	1	703-2132
OPC04	CCGCATCTAC	3	2	672-1510
OPC06	GAACGGACTC	1	2	559-1511
OPC07	GTCCCGACGA	1	2	594-1119
OPC18	TGAGTGGGTG	1	4	391-1456
OPF04	GGTGATCAGG	2	5	546-1718
OPH04	GGAACTCGCC	1	0	480
OPI01	ACCTGGACAC	3	2	381-873
OPI04	CCGCTAGTC	2	1	524-884
OPJ01	CCCGGCATAA	2	0	1055-1465
OPK03	CCAGCTTAGG	1	1	660-700
OPL02	TGGGCGTCAA	3	3	483-1529
OPM02	ACAACGCCCTC	3	7	511-2518
OPM04	GGCGCTTGTTC	2	2	600-1585
OPN03	GGTACTCCCC	1	1	660-840
OPV03	CTCCCTGCAA	2	1	466-878
OPX01	CTGGCCACGA	1	2	620-999
OPW02	ACCCCGCCAA	1	2	615-967
Total		42	52	217-2518

DISCUSSION

The present study confirmed the narrow genetic base of Brazilian soybean germplasm. According to Keim *et al.* (1989), the most probable reason for this fact is the autogamous nature of this species and the limited number of ancestors of cultivated soybean. The high resolution of the technique used here allowed a more detailed study of the genetic diversity of a group of 38 cultivars. Although the average genetic distances between each cultivar and the other 37 materials did not vary considerably (Table IV), the individuals could be perfectly distinguished from the others on a pairwise basis (Table III). Comparison between the data on Tables III and IV suggest that cultivars Tropical and IAC-8 have several alleles in common and at the same time present several alleles which are absent in other materials. These two cultivars have genetic distances superior to the average genetic distance (17.4%) for 30 out of the 37 possible comparisons (Table III). Cultivar IAS-4, on the other hand, has alleles that are common to the majority of the cultivars (Table IV). According to Table III, cultivars originated by selection from the same genetic background have very short genetic distances among themselves. For instance, the genetic distances among

Table III - Pairwise genetic distances (%) among the 38 soybean 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	28	29	30	31	32	33	34	35	36	37		
2		22																																					
3		18	19																																				
4		21	20	22																																			
5		12	19	20	18																																		
6		16	20	16	20	13																																	
7		22	17	13	22	20	18																																
8		19	20	18	15	19	13	19																															
9		17	23	20	16	10	12	26	16																														
10		15	14	12	15	16	11	18	15	16																													
11		21	20	24	11	13	14	24	16	13	19																												
12		22	22	12	19	22	17	12	16	18	15	23																											
13		13	14	16	15	19	16	20	22	17	9	21	21																										
14		18	15	13	12	12	15	17	18	16	12	14	20	12																									
15		21	13	14	14	13	17	16	18	18	13	14	22	13	3																								
16		15	12	11	15	18	15	18	16	14	12	18	18	14	12	12																							
17		16	18	16	13	16	18	16	19	22	13	15	21	11	7	9	14																						
18		18	16	14	15	15	17	14	17	21	11	15	19	11	7	6	15	2																					
19		17	17	15	14	15	17	15	18	21	12	16	20	10	6	8	15	1	1																				
20		18	16	14	15	15	17	14	17	21	11	15	19	11	7	6	15	2	0	1																			
21		24	16	19	14	22	16	14	16	24	14	20	14	17	15	13	22	15	13	14	13																		
22		25	13	15	16	19	16	17	20	20	16	16	20	20	9	11	10	16	16	15	16	22																	
23		15	22	16	26	18	16	24	24	22	19	23	23	19	18	22	17	21	23	22	23	23	24																
24		18	23	13	18	21	18	15	17	20	16	22	3	20	19	20	17	18	18	19	18	15	23	20															
25		13	16	20	26	9	17	22	22	18	22	22	26	22	18	21	17	25	25	24	25	27	19	12	25														
26		11	22	18	23	9	15	22	16	10	17	15	21	21	20	22	18	23	21	22	21	29	22	21	24	15													
27		15	17	18	25	10	18	20	22	20	24	23	26	24	16	19	16	24	24	23	24	27	17	15	25	2	17												
28		15	19	14	22	15	11	22	17	18	15	20	21	19	15	18	15	21	21	20	21	19	19	4	21	10	18	13											
29		13	18	16	28	11	16	27	24	17	19	26	26	19	18	22	15	26	26	24	26	27	20	11	24	3	15	6	10										
30		17	22	20	23	16	15	27	16	12	19	17	28	23	20	23	17	26	26	24	26	29	20	15	31	13	11	16	11	15									
31		12	23	20	22	7	20	22	19	12	22	16	27	22	16	20	20	19	19	18	19	29	20	19	28	11	5	12	17	15	12								
32		16	21	17	22	11	16	21	19	15	22	20	27	22	17	19	16	22	22	21	22	28	19	14	28	7	14	6	11	12	12	9							
33		11	24	20	23	10	16	22	16	13	17	19	21	21	20	24	20	23	23	22	23	27	22	17	24	12	9	13	13	13	9	10	14						
34		17	18	18	21	18	15	22	15	14	17	15	26	19	16	18	15	21	21	20	21	25	16	15	29	16	11	20	9	17	6	11	16	13					
35		18	16	13	14	15	19	14	18	18	10	16	18	11	9	9	13	4	2	3	2	15	15	25	19	24	18	23	22	25	23	16	19	20	20				
36		19	20	23	14	13	17	18	21	16	17	6	24	18	11	13	21	10	11	11	11	21	17	23	23	22	13	23	21	25	18	13	22	18	14	12			
37		22	12	20	20	22	20	15	24	26	16	22	24	16	13	12	14	14	14	13	14	16	8	25	25	22	27	23	20	23	26	25	25	27	20	16	18		
38		13	20	18	23	9	13	20	14	10	15	15	19	19	20	22	18	21	19	20	19	27	22	23	22	18	2	19	20	17	13	8	16	9	13	16	13	27	

Table IV - Average genetic distances (%) between each cultivar and the other 37 materials.

24. Tropical	-20.5	34. UFV-10	-17.2
12. IAC-8	-20.3	13. IAC-12	-17.2
21. Paranaíba	-20.0	1. BR-1	-17.2
37. União	-19.6	31. UFV-7	-17.2
23. Santa Rosa	-19.4	38. Viçoja	-17.1
7. Doko	-19.1	36. UFV-15	-17.0
30. UFV-6	-18.8	3. BR-6	-16.9
29. UFV-5	-18.8	28. UFV-3	-16.7
4. BR-9	-18.7	17. OCEPAR-8	-16.2
27. UFV-2	-18.5	6. Campos Gerais	-16.1
2. BR-2	-18.4	16. Ivaí	-15.7
8. EMGOPA-301	-18.1	20. Paranagoiana	-15.6
25. UFV-Araguaia	-18.1	15. IAS-5	-15.6
32. UFV-8	-17.8	18. OCEPAR-9-SSI	-15.6
11. FT-Cristalina	-17.8	10. FT-Cometa	-15.6
33. UFV-9	-17.8	19. Paraná	-15.5
22. Planalto	-17.6	35. UFV-14	-15.4
9. FT-11	-17.4	5. BR-11	-15.1
26. UFV-1	-17.2	14. IAS-4	-14.2

*These distances were calculated based on the pairwise genetic distances depicted in Table III.

OCEPAR-8, OCEPAR-9-SSI, Paranagoiana and Paraná are between 0 and 2%. The first three cultivars were selected in Paraná, probably derived from natural mutations in this background. The distance between cultivars Viçoja and UFV-1 (2%) may be also explained by the same reason. UFV-1 is a natural mutant of Viçoja. Cultivars FT-Cristalina and UFV-9 are described in the literature as selections of UFV-1. The distances between those cultivars and UFV-1 are 15 and 9%, respectively. These results support the assumption that the former two cultivars are not natural mutants of UFV-1, but were derived by outcrosses in a population of UFV-1. Based on differential reactions to pathogens, it is believed that FT-Cristalina is derived from a natural cross between UFV-1 and DAVIS, and that UFV-9 is derived from a cross between UFV-1 and Santa Rosa (Dr. Carlos S. Sediyaama, personal communication).

Certain groups of cultivars in this study have ancestors in common which contributed equally to their genetic background. This is the case for UFV-Araguaia and UFV-2; UFV-5, UFV-6, UFV-9 and UFV-10; UFV-7 and UFV-8; UFV-14 and UFV-15; and BR-2 and IAS-5 (Table I). The genetic distances within these groups varied considerably, from 2 to 17% (Table III), indicating that the distances among cultivars with common ancestors depend directly on the selection pressure the cultivar was submitted to after the initial cross.

According to the same reasoning, the cultivars Santa Rosa and UFV-3, which have common ancestors which contribute differently to their gene pools, are similar to one another (4% dissimilarity, Table III).

There were cultivars included in this study with no common ancestors; i.e., zero parentage coefficient, but with genetic distances within the range of those cultivars with common ancestors. This is the case for cultivar Campos Gerais in relation to BR-1, Doko, UFV-1, and Viçoja. The genetic distances between Campos Gerais and these other cultivars are between 13 and 18% (Table III), indicating that these cultivars are not so distant from Campos Gerais, as the parentage coefficient indicates. Explanation for this apparent discrepancy resides in the fact that upon calculating parentage coefficients one assumes that there is no relationship among the ancestors, and that each progenitor is considered to supply 50% of the nuclear genes of the offspring; i.e., allelic selection is absent (Vello *et al.*, 1988). A more realistic study should perhaps take into account the genetic distances among the progenitors. Recently, a genetic diversity study among several ancestors of North American soybean cultivars showed similarity coefficients varying from 0 to 0.88 (Gizlice *et al.*, 1993).

Cluster analyses based on the genetic distances among the cultivars grouped them into two large groups: group A, with 14 cultivars, and group B, with 24 cultivars. Considering an upper limit of 76% of relative genetic distance in the dendrogram depicted in Figure 2, group A can be further subdivided into subgroups A1 and A2, and group B into B1 through B3. Cultivars clustered in group A have a greater frequency of common alleles, as suggested by the information in Table V. All but one of the cultivars in this group have at least 50% of their gene pool derived from only two ancestors, PI 60406 and Tanloxi. The majority of the cultivars in group B do not have these two genotypes as ancestors, or have a low contribution from them to their genetic backgrounds, 25% at most (Table V).

Subgroup B1 includes 14 cultivars, all of which have common ancestors. Roanoke, Tokyo, and PI 54610 contributed at least 37.5% to the gene pool within this subgroup. The only exception is UFV-14, which received only a 25% genetic contribution (Table V). The majority of the cultivars included in the other subgroups received at most 31.25% contribution from these three ancestors; the exceptions being Campos Gerais (50%), Paranaíba (46.9%), and Santa Rosa (37.5%), which were included in subgroups B3, B2, and group A, respectively (Table V).

All four cultivars in subgroup B2 have PI 240664 as one common ancestor, and four of six cultivars in

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RESUMO

A técnica de DNA polimórfico amplificado ao acaso (RAPD) foi utilizada na avaliação da diversidade genética entre 38 cultivares de soja brasileiros. A análise permitiu a separação dos cultivares em cinco grupos distintos de acordo com as distâncias genéticas entre eles. A comparação entre esses resultados e dados de genealogia foram bastante coerentes para a maioria dos casos. Isso atesta a técnica de RAPD como uma ferramenta potente no estudo da diversidade genética entre indivíduos de uma mesma espécie. Os dados obtidos neste trabalho irão permitir ao melhorista de soja uma escolha mais racional de progenitores a serem usados em programas de melhoramento específicos.

REFERENCES

- Apuya, N.R., Frazier, B.L., Keim, P., Roth, E.J. and Lark, K.G.** (1988). Restriction fragment length polymorphisms as genetic markers in soybean, *Glycine max* (L.) Merrill. *Theor. Appl. Genet.* 75: 889-901.
- Beckmann, J.S. and Soller, M.** (1986). Restriction fragment length polymorphism and genetic improvement of agricultural species. *Euphytica* 35: 111-124.
- Chen, L.F.O., Chen, G.C., Lin, S.F. and Chen, S.C.G.** (1993). Polymorphic differentiation and genetic variation of soybean by RFLP analysis. *Bot. Bull. Acad. Sin.* 34: 249-259.
- Delannay, X., Rodgers, D.M. and Palmer, R.G.** (1983). Relative genetic contributions among ancestral lines to North American soybean cultivars. *Crop Sci.* 23: 944-949.
- Gizlice, Z., Carter, T.E. and Burton, J.W.** (1993). Genetic diversity in North American soybean: I. Multivariate analysis of founding stock and relation to coefficient of parentage. *Crop Sci.* 33: 614-620.
- Hinomoto, D.M. and Vello, N.A.** (1986). The genetic base of Brazilian soybean (*Glycine max* (L.) Merrill) cultivars. *Rev. Brasil. Genet.* 9: 295-306.
- Keim, P., Olson, T.C. and Shoemaker, R.C.** (1988). A rapid protocol for isolating soybean DNA. *Soybean Genet. Newsl.* 15: 150-152.
- Keim, P., Shoemaker, R.C. and Palmer, R.G.** (1989). Restriction fragment length polymorphism diversity in soybean. *Theor. Appl. Genet.* 77: 786-792.
- Newbury, H.J. and Ford-Loyd, B.V.** (1993). The use of RAPD for assessing variation in plants. *Plant Growth Regulation* 12: 43-51.
- Singh, S.P., Gutiérrez, J.A., Molina, A., Urrea, C. and Gepts, P.** (1991a). Genetic diversity in cultivated common bean: II. Marker-based analysis of morphological and agronomic traits. *Crop Sci.* 31: 23-29.
- Singh, S.P., Nodari, R. and Gepts, P.** (1991b). Genetic diversity in cultivated common bean: I. Allozymes. *Crop Sci.* 31: 19-23.
- Tingey, S.V., Rafalski, J.A. and Williams, G.K.** (1991). Soybean genome analysis: DNA polymorphisms are identified by oligonucleotide primers of arbitrary sequence. In: *Plant Molecular Biology 2* (Hermann, R.G. and Larkins, B.A., eds.). Plenum Press, New York, pp. 263-268.
- Vello, N.A., Hiromoto, D.M. and Azevedo Filho, A.J.B.V.** (1988). Coefficient of parentage and breeding of Brazilian soybean germplasm. *Rev. Brasil. Genet.* 11: 679-697.
- Welsh, J. and McClelland, M.** (1990). Fingerprinting genomes using PCR with arbitrary primers. *Nucl. Acids Res.* 18: 7213-7218.
- Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A. and Tingey, S.V.** (1990). DNA polymorphism amplified by arbitrary primers are useful as genetic markers. *Nucl. Acids Res.* 18: 6531-6535.

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