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USE OF THE RANDOM AMPLIFIED POLYMORPHIC DNA TECHNIQUE TO CHARACTERIZE SOYBEAN (*Glycine max* (L.) Merrill) GENOTYPES

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ABSTRACT

We have started a breeding program to genetically eliminate the lipoxygenase isozymes (LOX) from soybean seeds. These enzymes are believed to be the main cause of off-flavors in soybean products. LOX are present in the seed as three isozymes encoded by three different genes, which are inherited in a simple mendelian fashion. Mutants lacking each one of these isozymes have been identified in the world germplasm. To introduce these null alleles into the Brazilian variety Cristalina, three mutant progenitors were chosen: PI 408.251 (LOX1 minus), PI 86.023 (LOX2 minus), and Ichigowase (LOX3 minus). The random amplified polymorphic DNA (RAPD) technique was used to characterize these progenitors, as well as lines lacking LOX1 (CR_1), LOX3 (CR_3), LOX1 and 3 ($CR_{1,3}$), and LOX2 and 3 (UFV 91-263, UFV 91-401 and UFV 91-717). The results enabled us to establish the fingerprint of each genotype and the genetic distances among them.

INTRODUCTION

In spite of its high protein content, soybean is mainly used as an oil source in Brazil (Panizzi, 1987). The main obstacle for soybean protein consumption in Western countries is the beany-flavor present in soybean products (Rackis, 1979). One of the major causes of these off-flavors is the association of short-chain carbonilic compounds with the protein fraction. These organic compounds are end products of a series of reactions which start with the hydroperoxidation of polyunsaturated fatty-acids catalyzed by lipoxygenases (Axelrod, 1974). These enzymes are dioxygenases which occur as three isozymes (LOX 1 to 3) in dry soybean seed. Each isozyme is encoded by a single dominant gene, inherited in a mendelian fashion (reviewed in Mack *et al.*, 1987). It has been suggested that the best way to improve flavor in soybean products is to genetically eliminate these enzymes from the seed (Kitamura *et al.*, 1983). Mutants lacking each of the isozymes have been identified (Hildebrand and Hymowitz, 1982; Kitamura *et al.*, 1983; Kitamura, 1984); however, no double or triple nulls have been found in nature (Hajika *et al.*, 1991). One of the reasons for this fact is the tight linkage between the genes encoding for LOX1 and LOX2 (Hajika *et al.*, 1991).

We have started at the Federal University of Viçosa (UFV), MG, Brazil, a breeding program to introduce LOX deficiency into Brazilian adapted soybean cultivars. So far, several lines lacking LOX1, LOX2, LOX3, LOX1 and LOX3, LOX2 and LOX3 have been obtained (Moreira *et al.*, 1993). Initially, only the variety Cristalina was used as recurrent progenitor, however, several other adapted cultivars have been included in this program.

We have used the RAPD (Random Amplified Polymorphic DNA) technique to characterize the progenitors used in our breeding program. This characterization should allow us to fingerprint each genotype and facilitate the recovering of the characteristics of the recurrent parent in a breeding program to introgress null LOX genes into Brazilian soybean varieties.

MATERIAL AND METHODS

The genotypes used were as follows: Cristalina (commercial variety, normal for LOX1, LOX2, and LOX3); PI 408.251 (LOX1 minus); PI 86.023 (LOX2 minus); Ichigowase (LOX3 minus), CR_1 (Cristalina-derived line lacking LOX1); CR_3 (Cristalina-derived line lacking LOX3); $CR_{1,3}$ (Cristalina-derived line lacking

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LOX1 and 3); and lines UFV 91-263, UFV 91-401, and UFV 91-717 (Cristalina-derived lines lacking LOX2 and 3). Lines CR_1 and CR_3 originated from crosses between Cristalina and PI 408.251 and Ichigowase, respectively, followed by three generations of backcrosses. $CR_{1,3}$ originated from a cross between CR_1 and CR_3 at BC_1F_2 followed by several cycles of selfing. Lines UFV 91-263, UFV 91-401, and UFV 91-717 were derived from crosses between CR_3 at BC_3F_2 with PI 86.023, F_2 seed selection for absence of both LOX2 and 3, followed by six cycles of outcross and selection for pure lines.

To confirm the presence or absence of individual LOX isozymes in the genotypes, seeds were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Kitamura, 1984). Once their phenotypes were confirmed, seeds were germinated and planted in the green house. Young leaves were harvested and kept at -80°C for DNA extraction (Saghai-Maroof *et al.*, 1984).

DNA extracted from leaves of the different genotypes was used as a template for amplification reactions. Each reaction (25 µl) contained: 50 ng of DNA; 100 µM of each deoxyribonucleoside triphosphate; 1.7 mM $MgCl_2$; 10 mM Tris-HCl, pH 8.3; 50 mM KCl; 0.4 µM of a single decamer primer (Operon Technologies), and one unit of Taq DNA polymerase (Perkin Elmer-Cetus Corp.). DNA amplification was performed in a thermocycler model 9600 (Perkin Elmer-Cetus Corp.) set for 40 cycles, each consisting of a denaturation step at 94°C for 15 seconds, an annealing step at 35°C for 30 seconds, and an elongation step at 72°C for one minute. After the 40th cycle a final elongation step at 72°C was performed for seven minutes. Amplification products were separated on 1.2% agarose gels containing 10 µg/ml ethidium bromide in TBE buffer (90 mM Tris-borate, 1 mM EDTA, pH 8.0). DNA bands were visualized under UV light and photographed with Polaroid film type 667.

The twenty-six random primer decamers used were as follows: OPA04, OPA07, OPA08, OPB08, OPC03, OPC04, OPC07, OPD01, OPE01, OPF01, OPF02, OPF04, OPF07, OPH02, OPH04, OPI01, OPI03, OPI04, OPJ01, OPJ03, OPV02, OPV03, OPX01, OPY03, OPW02, and OPW03. Only the most prominent DNA bands were scored. Genetic distances were determined by dividing the number of distinct DNA bands between two given genotypes and the total number of amplification products. Data were analyzed through a statistical software (SAEG) developed at the Federal University of Viçosa.

RESULTS AND DISCUSSION

The use of DNA-based markers for plant characterization is becoming very common in breeding programs for several crops (Hu and Quiros, 1991; Demeke *et al.*, 1992; Thormann and Osborn, 1992). We used RAPD markers to study the genetic diversity of progenitors and lines involved in a breeding program to genetically eliminate the lipoxygenase enzymes from soybean seeds.

Twenty-one out of the twenty-three primers tested generated amplification products (Figure 1); however, only 13 of them were informative, showing polymorphic bands between at least two of the initial progenitors used in the breeding program (Table I). Two primers generated no amplification products. Considering only the informative primers, one to five DNA bands (440 to 1,800 bp) were amplified per primer (Table I). These numbers are in the range obtained for soybean DNA by other authors (Tingey *et al.*, 1992). A total of 61 amplification products were generated, 30 of them being polymorphic (Table I). Pairwise genetic distances were between six and 36% (Table II). As expected, the largest distances were between the progenitor Cristalina, a Brazilian adapted cultivar, and the other three original progenitors involved in the program, which originated in Asia (Table II). The shortest genetic distances were among lines UFV 91-263, UFV 91-401, and UFV 91-717 (Cristalina-derived lines lacking LOX2 and 3) (Table II). This may be due to the type of selection made during the cycles of self-pollination which originated these lines. The genetic distances between cultivar Cristalina and lines CR_1 and CR_3 were well within the range expected for lines derived by three backcrosses, having Cristalina as a recurrent progenitor (Table II). The

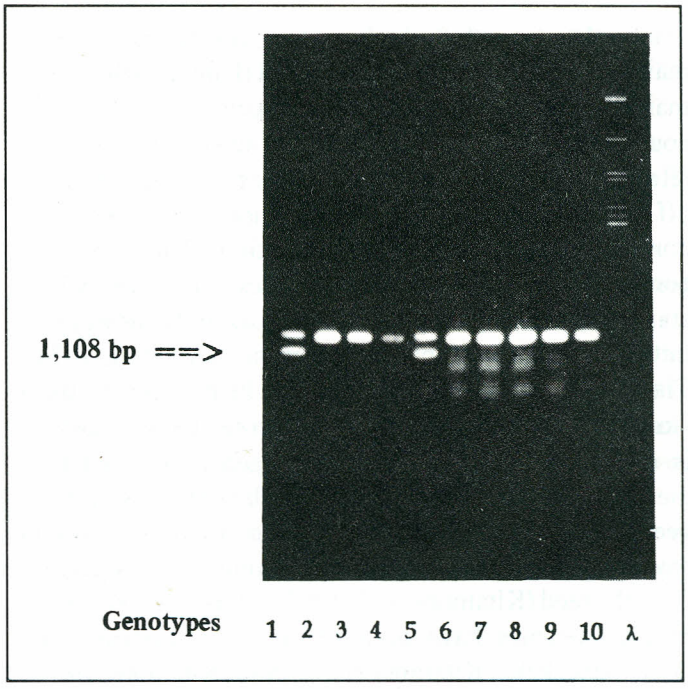

Figure 1 - Typical agarose gel showing RAPD amplified soybean DNA bands produced by primer OPW02. Lanes are as follows: 1, Cristalina; 2, PI 408.251; 3, PI 86.023; 4, Ichigowase; 5, CR_1; 6, CR_3; 7, $CR_{1,3}$; 8, UFV 91-263; 9, UFV 91-401; and 10, UFV 91-717. Arrow points to a polymorphic band obtained with primer OPW02. Lane lambda corresponds to lambda-phage DNA digested with endonucleases Eco RI, Bam HI, and Hind III.

Table I - Distribution of polymorphic DNA markers among soybean genotypes. After DNA amplification with different primers, each genotype was analyzed for the presence (1) or absence (0) of specific bands. Size range of the bands are indicated on the last column of the table.

Primers	Genotypes										No. of base pairs (bp)
	1	2	3	4	5	6	7	8	9	10	
OPA04 (I)	1	0	1	0	0	1	0	1	1	1	1,086
OPA04 (II)	0	1	1	0	1	0	0	0	0	0	960
OPA07 (I)	0	0	1	1	0	0	0	1	1	1	1,338
OPA07 (II)	1	0	0	0	1	1	1	0	0	0	771
OPA07 (III)	0	0	1	0	0	0	0	1	1	1	682
OPA07 (IV)	1	1	1	0	1	1	1	1	1	1	446
OPC03 (I)	1	0	0	0	1	1	1	0	0	0	1,798
OPC03 (II)	1	1	1	0	1	0	1	1	1	1	1,201
OPC03 (III)	1	0	1	1	1	1	1	1	1	1	834
OPC03 (IV)	1	1	1	0	0	0	1	1	0	1	698
OPC03 (V)	1	0	0	1	1	1	0	1	0	1	650
OPC04 (I)	0	1	0	0	0	1	1	1	0	1	857
OPC07 (I)	1	1	1	0	1	1	1	0	1	1	906
OPC07 (II)	1	0	0	0	1	1	0	0	1	1	594
OPE01 (I)	1	0	0	0	0	0	0	0	0	0	771
OPF04 (I)	1	1	0	1	1	1	0	0	0	0	650
OPI03 (I)	0	1	1	0	0	0	0	0	0	0	1,161
OPI03 (II)	0	1	1	1	0	0	0	0	0	0	682
OPI03 (III)	1	0	0	0	1	1	1	1	1	1	492
OPJ01 (I)	1	0	0	0	0	1	0	0	1	1	960
OPJ01 (II)	1	1	0	0	1	0	0	1	0	0	834
OPJ01 (III)	0	0	1	1	1	0	1	1	0	1	581
OPJ01 (IV)	0	0	1	1	0	0	1	1	0	0	446
OPH04 (I)	0	0	0	1	0	0	0	0	0	0	1,023
OPV03 (I)	0	1	1	0	0	0	0	0	0	0	1,273
OPV03 (II)	0	1	1	1	0	1	0	0	0	0	924
OPY03 (I)	1	0	0	0	0	0	0	0	0	0	1,517
OPY03 (II)	0	0	0	1	0	0	0	0	0	0	1,328
OPY03 (III)	1	1	1	0	1	1	1	1	1	1	961
OPW02 (I)	1	0	0	0	1	0	0	0	0	0	1,108

Table II - Pairwise genetic distances between soybean genotypes. Distances were calculated by dividing the number of polymorphic bands by the total number of amplification products and expressed as a percentage.

Genotypes	1	2	3	4	5	6	7	8	9	10
1	-									
2	28	-								
3	33	15	-							
4	36	27	23	-						
5	11	23	28	28	-					
6	13	24	29	26	15	-				
7	20	21	20	26	15	16	-			
8	23	24	16	23	21	23	13	-		
9	18	26	18	27	20	15	18	15	-	
10	18	26	18	27	20	15	15	08	06	-

relatively large genetic distances between cultivar Cristalina and lines $CR_{1,3}$ and UFV 91-263, UFV 91-401, and UFV 91-717, are also understandable because we had no intention to develop near-isogenic lines to the progenitor Cristalina by the end of the breeding process. These genetic distances were used for cluster analyses and allowed the separation of the genotypes into four distinct groups within 76% of relative genetic distance (Figure 2).

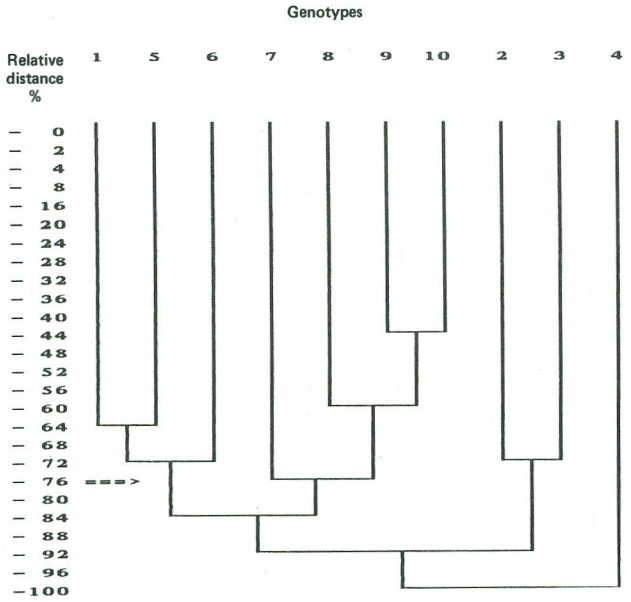

Figure 2 - Dendogram showing relative genetic distances among soybean genotypes. Pairwise genetic distances were calculated by dividing the number of polymorphic DNA bands by the total number of amplification products, and used for cluster analyses. Arrow indicates relative genetic distance (76%) used to define four distinct groups. Numbers corresponding to each genotype are as for Figure 1.

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RESUMO

Foi iniciado um programa de melhoramento para eliminar geneticamente as isozimas lipoxigenases (LOX) das sementes de soja. Acredita-se que essas enzimas constituam a principal causa de sabores indesejáveis nos produtos derivados da soja. LOX estão presentes nas sementes como três isozimas codificadas por três diferentes genes que são herdados através de herança mendeliana simples. Mutantes deficientes em cada uma dessas isozimas foram identificados no germoplasma mundial. Para introduzir esses alelos recessivos na variedade brasileira Cristalina, foram escolhidos três progenitores mutantes: PI 408.251 (sem LOX1), PI 86.023 (sem LOX2) e Ichigowase (sem LOX3). A técnica de DNA polimórfico amplificado ao acaso (RAPD) foi utilizada para caracterizar esses progenitores, bem como linhagens sem LOX1 (CR_1), sem LOX3 (CR_3), sem LOX 1 e LOX3 ($CR_{1,3}$) e sem LOX 2 e LOX3 (UFV 91-263, UFV 91-401 e UFV 91-717). Esses resultados permitiram estabelecer o "fingerpring" de cada genótipo e determinar a distância genética entre os mesmos.

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