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Genetic relationship among four varieties of pineapple, *Ananas comosus*, revealed by random amplified polymorphic DNA (RAPD) analysis

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ABSTRACT

RAPD analysis was used to estimate relationships of four pineapple cultivars: 'Perola', 'Smooth Cayenne', 'Primavera', and 'Perolera'. Smooth Cayenne and Primavera were most closely related, followed by Perolera. Perola was more distant from the other three cultivars than they were from each other. These results confirmed genetic relationships derived by analyses of morphological and agronomic characters.

INTRODUCTION

The genus *Ananas* (Bromeliaceae) includes several economically important species whose center of origin is Brazil. Some species are important for their edible fruits (pineapple) while other species are important for their fibers, which are used in the manufacture of cloth, string, and rope. The species of greatest economic importance is *A. comosus* which is cultivated for commercial pineapple prodution. The fruits are seedless with a minimum length of 15 cm., abundant flesh, good flavor, and a stout peduncle (Py *et al*., 1987). The most important cultivated varieties are 'Perola', 'Smooth Cayenne', 'Primavera', and 'Perolera'. Unfortunately, the pedigrees of these varieties are unknown. They apparently arose by spontaneous mutation, followed by possible hybridization of unknown parents, and natural and artificial selection.

The emergence of simple techniques for rapid identification of polymorphic DNA markers has made possible the application of DNA marker analysis to determine phylogenetic relationships.

MATERIAL AND METHODS

Plant materials

Leaves from five plants each of four pineapple cultivars, Perola, Smooth Cayenne, Primavera, and Perolera, were obtained from the germplasm bank of EMBRAPA, Cruz das Almas, Bahia, Brasil.

DNA extraction

For DNA extraction the procedure described by Dellaporta *et al*. (1983) and Fairbanks *et al*. (1993) was used. After DNA extraction, the isopropanol precipitation step was repeated and the suspension was centrifuged again to pellet the DNA. To precipitate impurities from the DNA suspension, 100 μl of 5 M

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NaCl, 100 µl of 0.5 M EDTA, and 300 µl of sterile distilled water were added and the pellet was suspended in this mixture. The suspension was incubated at 4°C for 20 min., and then it was centrifuged for 10 min. at 13,000 g in a microcentrifuge. The supernatant was transferred to a new microcentrifuge tube and 1 volume of isopropanol was added to precipitate the DNA, after which the suspension was centrifuged again for 5 min. The isopropanol was removed and after air drying, the DNA was resuspended in 50 µl of TE (pH = 8.0). DNA concentration was estimated using a Hoefer TKO 100 mini fluorometer in accordance with the manufacturer's instructions. DNA samples were diluted to a concentration of 4 ng/µl.

DNA amplification

Each amplification reaction used 4 ng template DNA, and was made according to Transue *et al.* (1994). Following amplification, samples were maintained at 4°C until electrophoresis. For each primer, a control reaction that lacked template DNA, but contained all other reagents, was included. Amplified products were separated in ethidium bromide stained 1.4% agarose gels and photographed under transilluminated 302 nm UV light.

Data analysis

Each gel contained a lane of standard DNA size markers of the following sizes in kilobase pairs (kbp) listed in descending order: 2.7, 2.0, 1.6, 1.4, 1.0, 0.7, 0.5, 0.4. Amplified DNA fragments were assigned molecular weights by scanning the gel photograph by using DeskScan v. 1.5.2 for Macintosh on a Hewlett Packard Scanjet II C flatbed scanner. The scanned image was saved as a TIFF file and imported into Collage v. 2.0 for Macintosh. A cubic regression was used to fit standard DNA markers to a linear equation and the program automatically estimated the molecular weight of each fragment, based on the regression equation.

A total of 75 DNA markers from 17 primers (Table I) with molecular weights ranging from 0.25 to 2.6 kb were scored in all genotypes as present (1) or absent (0). The data matrix was read by NTSYS-pc (Numerical Taxonomy and Multivariate Analysis System for Personal Computers, v. 1.80) on an IBM compatible computer and analyzed using SIMQUAL (similarity for qualitative data) with Jaccard's similarity coefficients. A dendrogram was constructed employing UPGMA (unweighted pair-group method with arithmetic averages) with the SAHN (sequential, agglomerative, hierarchical, and nested clustering) routine.

Table I - Decanucleotide primer sequences for the 17 primers used to generate RAPD markers.

Operon designation	DNA sequence
OPAA-06	5'GTGGGTGCCA3'
OPAE-01	5'TGAGGGCCGT3'
OPAE-02	5'TCGTTCACCC3'
OPAE-10	5'CTGAAGCGCA3'
OPAE-11	5'AAGACCGGGA3'
OPAE-12	5'CCGAGCAATC3'
OPAE-14	5'GAGAGCCTCC3'
OPAE-15	5'TGCCTGGACC3'
OPAE-17	5'GGCAGGTTCA3'
OPAE-18	5'CTGGTGCTGA3'
OPAE-20	5'TTGACCCCAG3'
OPAF-03	5'GAAGGAGGCA3'
OPAF-04	5'TTGCGGCTGA3'
OPAF-07	5'GGAAAGCGTC3'
OPAF-10	5'GGTTGGAGAC3'
OPAF-13	5'CCGAGGTFAC3'
OPAF-14	5'GGTGCGCACT3'

RESULTS AND DISCUSSION

A total of 75 RAPD markers were used to determine the relationships of four cultivars of *A. comosus* (Figures 1 and 2). Twenty-eight markers were shared by all four cultivars, while the remaining 47 markers were polymorphic. Of the polymorphic fragments, 15 were shared among three cultivars, 17 between two cultivars, and 15 were unique to a single cultivar. The dendrogram based on these markers revealed that Perola was more distant from the other three cultivars than they were from each other (Figure 2). Primavera and Smooth Cayenne were the most similar. These results confirm the relationships proposed by Py *et al.* (1987) on the basis of morphological characteristics, including general plant appearance, shoot formation at fruit harvest, plant dimensions, leaf spines, peduncle length, and fruit characteristics, including size, weight, shape, diameter, flesh, and flavor. The same conclusion was reached by Loison-Cabot (1992), whose analysis of

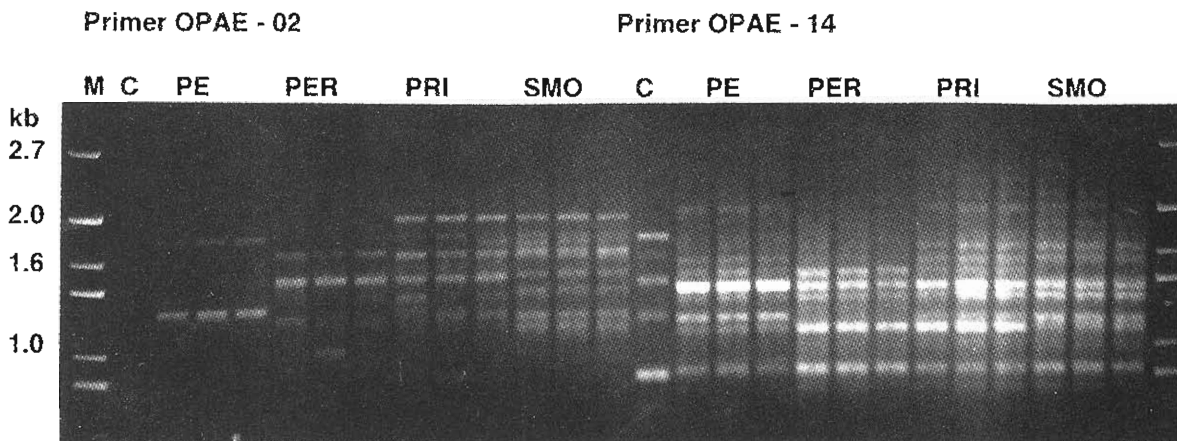

Figure 1 - RAPD polymorphism in four varieties of *Ananas comosus* with primers OPAE-02 (5'TCGTTCACCC3') and OPAE-14 (5'GAGAGCCTCC3'). Each variety is represented three times. M represents PUC DNA markers; C controls lacking template DNA; PE variety *Perola*; PER variety *Perolera*; PRI variety *Primavera*; SMO variety *Smooth Cayenne*.

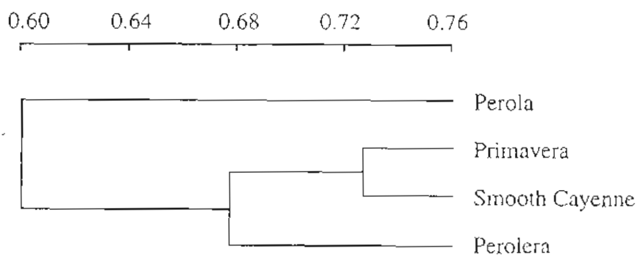

Figure 2 - Dendrogram generated from 75 RAPD markers screened in four pineapple cultivars. The scale portrays a similarity index based on Jaccard's coefficient.

agronomic characteristics indicated that Perola (referred to as 'Pernambuco') and Smooth Cayenne (referred to as 'Cayenne') were quite distant. However, our results contradict those of Garcia (1988) who placed Perolera and Primavera in one group while Perola and Smooth Cayenne fell into a second group on the basis of eight isozyme systems.

The similarity of results obtained with RAPD analysis and those obtained by morphological and agronomic evaluations indicated that RAPD analysis can be accurate in estimating genetic diversity in plant genetic resources. Andersen and Fairbanks (1990) proposed that RAPD analysis could be used to efficiently evaluate plant genetic resources for genetic diversity if the diversity predicted by RAPD analysis corresponded with more traditional methods. Our observations in pineapple indicate that this is the case. On the other hand, the results of Garcia (1988) suggest that isozyme analysis with a limited number of markers may not provide accurate estimates of genetic relationships.

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RESUMO

A origem dos cultivares mais importantes de abacaxi não é conhecida até o momento. Estimativas da relação e diversidade genética destes cultivares seria de grande ajuda, para avaliação de recursos genéticos. Análise por RAPD foi usada com o objetivo de estimar a relação entre quatro cultivares de abacaxi: 'Perola', 'Smoth Cayenne', 'Primavera' e 'Perolera'. Os cultivares Smooth Cayenne e Primavera foram os mais estreitamente relacionados, seguidos por Primavera. Perola foi o cultivar com maior distância dos outros três cultivares. Estes resultados concordam com a relação genética sugerida pela análise de características morfológicas e agronômicas, mostrando que a análise por RAPD pode ser usada de maneira eficiente para caracterização de recursos genéticos no gênero *Ananas*.

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