ISOENZIMATIC VARIABILITY AMONG FIVE PEANUT CULTIVARS ([1])

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ABSTRACT

Genetic variability within and among different samples of peanut (*Arachis hypogaea* L.) from the cultivars Roxo, Tatu Branco, Tatu Vermelho, Tatuí Vermelho and Tatuí (white skin seeds) was evaluated using polyacrilamide gel electrophoresis by studying leucine aminopeptidase (LAP), aspartate aminotransferase (ATT) and peroxidase (PER) enzymatic systems. Seeds were obtained from local farms located in Marília, Presidente Prudente and São Manuel cities, State of São Paulo, Brazil. Three enzymatic bands called LAP-A, LAP-B and LAP-C were observed in the patterns of the leucine aminopeptidase. Aspartate aminotransferase patterns showed three anodic bands, AAT-A, AAT-B and AAT-C. The peroxidase system showed fifteen bands, including eight anodics (PER-A to PER-H) and seven cathodics (PER-I to PER-P). The peroxidase and leucine aminopeptidase enzymatic systems were not discriminative among the different samples from the five cultivars analysed. Only the aspartate aminotransferase enzymatic system showed one characteristic pattern compound by AAT-B and AAT-C bands. This pattern was observed in Tatu Branco from Presidente Prudente and Tatuí Vermelho from Presidente Prudente and São Manuel.

Index terms: electrophoresis, isoenzymes, peanut cultivars, genetic variability.

RESUMO

AVALIAÇÃO DA VARIABILIDADE ISOENZIMÁTICA DE CINCO CULTIVARES DE AMENDOIM

A variabilidade genética foi avaliada dentro e entre amostras de diferentes cultivares de amendoim, *Arachis hypogaea* L., conhecidos como Roxo, Tatu Branco, Tatu Vermelho, Tatuí Vermelho e Tatuí (sementes com película branca), fornecidos por fazendas situadas nas regiões dos municípios de Marília, Presidente Prudente e São Manuel. Para tal análise, foi utilizada a técnica de eletroforese horizontal em gel de poliacrilamida, para os sistemas da leucil-aminopeptidase (LAP), aspartato aminotransferase (ATT) e peroxidase (PER). No sistema da leucil-aminopeptidase, foram observadas três bandas enzimáticas, denominadas LAP-A, LAP-B e LAP-C. Os padrões de bandas obtidos para o sistema da aspartato-aminotransferase mostraram

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a existência de três bandas anódicas, AAT-A, AAT-B e AAT-C. No sistema da peroxidase (PER), foram observadas quinze bandas, sendo oito anódicas (PER-A a PER-H) e sete catódicas (PER-I a PER-P). Os sistemas enzimáticos da peroxidase e leucil-aminopeptidase não foram discriminativos para as amostras analisadas dos diferentes cultivares obtidos nas diversas regiões. O sistema da aspartato-aminotransferase apresentou um padrão composto pelas bandas AAT-B e AAT-C, que se mostrou característico e discriminativo para as amostras do cultivar Tatu Branco, procedente de Presidente Prudente, e do 'Tatuí Vermelho', proveniente de Presidente Prudente e São Manuel.

Termos de indexação: eletroforese, isoenzimas, cultivares de amendoim, variabilidade genética.

1. INTRODUCTION

The genus *Arachis* is from South America (Krapovickas, 1973; Gregory et al., 1980) and extends over more than 2.6 million quilometers squared on the continent. This genus includes the cultivated groundnut, *Arachis hypogaea* L., one of the most widespread and important food legume in the world.

Arachis hypogaea has seeds rich in oil and protein, that are very important to human nutrition. This species is widely distributed in the tropical and subtropical areas of the world (Norden, 1980; Syalker, 1985).

Qualitative and quantitative isoenzymatic analysis have been utilized in biochemical, physiological, chemotaxonomy and genetic variability investigation in a large number of plant populations, cultivars and species (Cherry & Ory, 1973a; Fedak, 1974; Huhns & Fretz, 1978; Nielsen & Johansen, 1986; Ramirez et al., 1986).

Genetic variability and characterization of peanut germplasm have been estimated by isoenzymes (Cherry & Ory, 1973a,b; Grieshammer & Wynne, 1990; Lacks et al., 1991) and by molecular markers (Halward et al., 1991, 1992; Kochert et al., 1991). As an important cultivated species, *A. hypogaea* displays a great interest in characterizing and understanding its genetic structure.

The objectives of the present study were to evaluate the genetic variability within and among samples from the cultivars Tatu Branco, Tatu Vermelho, Tatuí Vermelho, Tatuí and Roxo, using isoenzyme polymorphism as genetic markers.

2. MATERIAL AND METHODS

The cultivars utilized in this study were Roxo, Tatu Branco, Tatu Vermelho, Tatuí Vermelho and Tatuí (white skin seeds) grown in different cities from São Paulo State (Brazil).

The cultivars Roxo, Tatu Branco and Tatu Vermelho belong to the Valencia type, which corresponds to *Arachis hypogaea* subsp. *fastigiata* var. *fastigiata*, Tatuí Vermelho and Tatuí cultivars are marketed as Spanish type (*Arachis hypogaea* subsp. *fastigiata* var. *vulgaris*).

Seeds of each cultivar were provided by local farms from the following counties: Marília, Presidente Prudente and São Manuel. Fifteen samples of each cultivar from each county were analysed (45 samples), except for the cultivar Tatuí (white skin seeds) obtained only from Marília, totalizing 195 samples.

Young leaves were collected from 15 to 20 cm height seedlings grown in a greenhouse ([3]). The leaves were macerated at 4°C in 100 µl extraction buffer with some antioxidants (Galgaro, 1987), listed in table 1. This procedure was required in order to prevent phenol oxidation and to obtain an appropriate enzyme pattern.

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Electrophoresis was performed in 6% horizontal polyacrylamide gel (Cyanogum-40, Sigma) with 9 parts of 0.20 mol/L Tris-Citrate pH 8.3 buffer and 1 part of 0.20 mol/L Lithium-Borate pH 8.3 buffer, according to Chao & Scandalios (1972). Electrode buffer consisted of lithium-borate gel buffer (Chao & Scandalios, 1972).

The isoenzymatic systems analysed were leucine aminopeptidase (LAP, E.C. 3.4.1.1), peroxidase (PER, E.C. 1.1.1.1.7) and aspartate aminotransferase (AAT, E.C. 2.6.1.1) stained with the solutions described by Scandalios (1969).

3. RESULTS AND DISCUSSION

The isoenzymatic patterns obtained for leucine aminopeptidase (LAP), asparte aminotransferase (AAT) and peroxidase are shown in Figure 1.

Three anodic leucine aminopeptidase isoenzymatic bands were observed in the patterns of the five cultivars analysed (Figure 1a). These bands were named LAP-A, LAP-B and LAP-C. All the samples from each cultivar exhibited this phenotype. This date shows a large genetic uniformity for LAP in peanuts. Monomorphic LAP patterns were also observed in peanuts by Cherry & Ory (1973a) and Grieshammer & Wynne (1990).

Qualitative variation within and among the different accessions of the commercial cultivars were not observed in any zymograms obtained from leucine aminopeptidase. Otherwise, quantitative differences were observed within each sample, detected by an increase of enzymatic activity from LAP-C to LAP-A bands.

Aspartate aminotransferase patterns showed three anodic bands, with staining quantitative differences (Figure 1-b), indicating variation in activity among these isoenzymes. According to the Rms, the bands were called AAT-A, AAT-B and AAT-C from the fastest to the slowest one. The AAT-A band was observed in all the studied cultivars, except for Tatu Branco from Presidente Prudente and Tatuí Vermelho from Presidente Prudente and São Manuel (Figure 1-b2). AAT-B and AAT-C bands were detected in all the samples from the four cultivars studied and AAT-A was the only discriminative band betweeb the samples.

The peroxidase system showed fifteen bands, including eight anodic bands and seven cathodic bands. The cathodic ones were observed only in this enzymatic system (Figure 1-c). According to the patterns and the Rms observed, the eight anodic bands were named PER-A to PER-H, from the faster to the slowest one, and the seven cathodic bands were named PER-I to PER-P (Figure 1-c) from the slowest to the fastest one. The peroxidase system was also very uniform, and no qualitative nor quantitative variation were observed within and among all samples analysed from the five cultivars studied.

Table 1. Buffer with appropriate antioxidant mixtures utilized to extract each enzyme

Enzymes	Buffer	Antioxidants
Peroxidases	0.10mol/L Tris-HCl pH 7.0	0.20mol/L Natrium tetraborate
and Aspartase		0.25mol/L L-ascorbic acid
aminotransferases		0.02mol/L Natrium bissulfite
		0.02mol/L DIECA([1])
		0.02mol/L Ditiothreitol
		0.02mol/L L-cisteina
Leucyl aminopeptidases	0.10mol/L Tris-HCl pH 7.0	0.20mol/L Natriun tetraborate
		0.02mol/L Ditiothreitol
		0.02mol/L L-cisteina

([1]) Natrium disthylditiocarbamate.

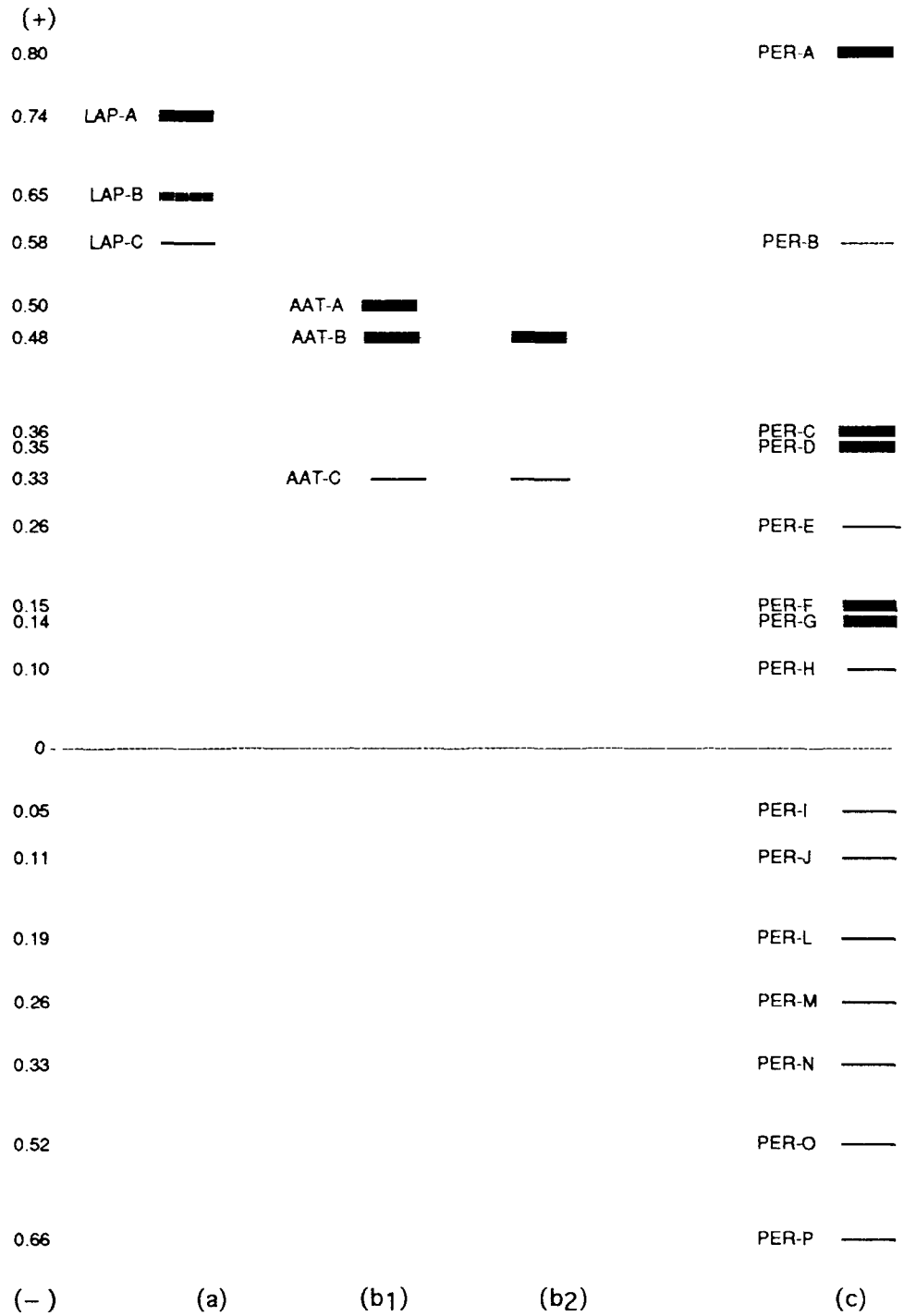

Figure 1. Banding patterns observed in the three enzymatic systems. (**a**) Leucine aminopeptidase patterns with the three anodic bands, showing the increase of activity from C to A isoenzymes. (**b_1**) Aspartate aminotransferase pattern, with the three anodic enzymes observed in most of the samples. (**b^2**) Banding pattern of aspartate aminotransferase observed in Tatu Branco from Presidente Prudente and Tatuí Vermelho from Presidente Prudente and São Manuel; note the absence of AAT-A band. (**c**) Peroxidase pattern showing the eight anodic and seven cathodic bands observed in all the samples analysed.

Peroxidase and leucine aminopeptidase enzymatic systems were not discriminative neither among cultivars nor among collect areas. Only the aspartate aminotransferase enzymatic system provided one discriminatory pattern to Tatu Branco from Presidente Prudente to Tatuí Vermelho from Presidente Prudente and São Manuel.

Cherry & Ory (1973a,b) also observed no variation among cultivars of *Arachis hypogaea* from different areas, working with seven enzymatic systems, such as INT oxidase, catalase, esterase, acid phosphatase, alcohol dehydrogenase, besides leucine aminopeptidase and peroxidase utilized in the present research.

Other studies using molecular markers (Halward et al., 1991, 1992; Kochert et al., 1991) have also found that cultivated peanut showed very little genetic variation.

Besides the high homozigosity commonly observed in a self-pollinated specie as *A. hypogaea*, the intensive selections in the breeding programs may have contributed to the high uniformity exhibited by the peanuts cultivars.

According to the data with brazilian peanut cultivars and the results obtained by other authors working with american peanut cultivars, it was concluded that *A. hypogaea* has a narrow germplasm bases. Based on this, it was proposed the exploration of new sources of genetic variability from closely related wild species to peanut improvement or to their own improvement.

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