

COMPARISON OF NATURAL VARIANTS OF THE SOYBEAN CULTIVAR PARANA BY ISOENZYME ANALYSIS

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ABSTRACT

The natural variants of the soybean cultivar Paraná, Paranagoiana, SS-1 and Pirapó-78, are considered mutants of the original cultivar. However the morphology of cultivar Pirapó-78 raises doubts about its origin through mutation. To clarify this point the electrophoretic profiles of the total protein, acid phosphatase, alcohol dehydrogenase, esterase, malic dehydrogenase, peroxidase and urease of seed extracts of the four cultivars were determined. These did not permit the cultivars Paranagoiana and SS-1 to be distinguished from the original cultivar. However peroxidase, esterase and malic dehydrogenase profiles of cultivar Pirapó-78 were different from the respective profiles of the other cultivars. The data are consistent with origin of cultivars Paranagoiana and SS-1 by mutation but suggest a different origin for Pirapó-78.

INTRODUCTION

Comparisons of plant genotypes have been made traditionally by the analysis of easily identifiable external characters. However in recent years the determination of profiles of soluble proteins and the isoenzyme composition of various enzymes through electrophoresis has permitted the establishment of a complementary system for the discrimination of genotypes. Applications in plant genetics and breeding have been reviewed by various authors (in Tanksley and Orton, 1983). Probably the earliest application to soybean was the comparison of seed proteins of varieties reported by Larsen (1967). In the following year the report of isoenzyme compositions of peroxidase of various cultivars (Buttery and Buzzell, 1968) initiated a very active

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period of studies using isoenzyme data, much of which has been reviewed by Kiang and his collaborators (Kiang and Gorman, 1983; Doong and Kiang, 1987).

In South America many variants of soybean cultivars have been selected and commercialized. The variants of the cultivar Paraná include cultivars Paranagoiana, SS-1 and Pirapó-78. "Paranagoiana" and "SS-1" originated through natural mutations for time to flowering (Kiihl *et al.*, 1984 and OCEPAR/EMBRAPA, 1988) and "Pirapó-78", according to information from the Estação Experimental de Pirapó, Paraguai, is also a mutant for time to flowering from cultivar Paraná. However because "Pirapó-78" has, as well as the difference in the flowering cycle, a semi-determinate growth habit in contrast to the determinate habit of the original cultivar, its origin through natural mutation can be questioned.

The objective of the present work was to compare cultivar Paraná and the three variants by electrophoretic separations of various isoenzymes.

MATERIAL AND METHODS

Mature seeds of the cultivars Paraná, SS-1, Paranagoiana and Pirapó-78 were produced in the Centro Nacional de Pesquisa de Soja, EMBRAPA, Londrina, Paraná.

Protein was extracted from seed meals approximately five months after harvest by stirring 100 mg meal in 1 ml 0,1M acetate buffer pH 5.5 or in 1 ml of distilled water, at 4°C for 5 minutes. Insoluble material was removed by centrifugation at 23,000 g for 30 minutes. The extracted proteins were separated by electrophoresis in polyacrylamide gels (7% monomer) under discontinuous alkaline conditions as described by Davis (1964). Proteins were stained non-specifically with 0.05% Coomassie Brilliant Blue in a mixture of methanol, acetic acid, and water (25:7:68). Proteins with acid phosphatase, alcohol dehydrogenase, esterase, malic dehydrogenase, peroxidase or urease activity were stained respectively with the following combinations of substrates and stains: α and β -naphthylphosphate/Fast red B salt (Shaw and Prasad, 1970); ethanol/nitrobluetetrazolium (Shaw and Koen, 1965); α -naphthylacetate/Fast blue RR (Shaw and Prasad, 1970); sodium malate/nitrobluetetrazolium (Shaw and Koen, 1964); hydrogen peroxide/o-dianizidine (Brewbaker *et al.*, 1968); and urea/cresol red (Buttery and Buzzell, 1971).

RESULTS AND DISCUSSION

No difference was detected among the four cultivars when the profiles of soluble protein (non-specific protein), acid phosphatase, alcohol dehydrogenase and urease were determined (Figure 1A). On the other hand two types of profiles of non-specific protein (Larsen, 1967), two of urease (Buttery and Buzzell, 1971), three of

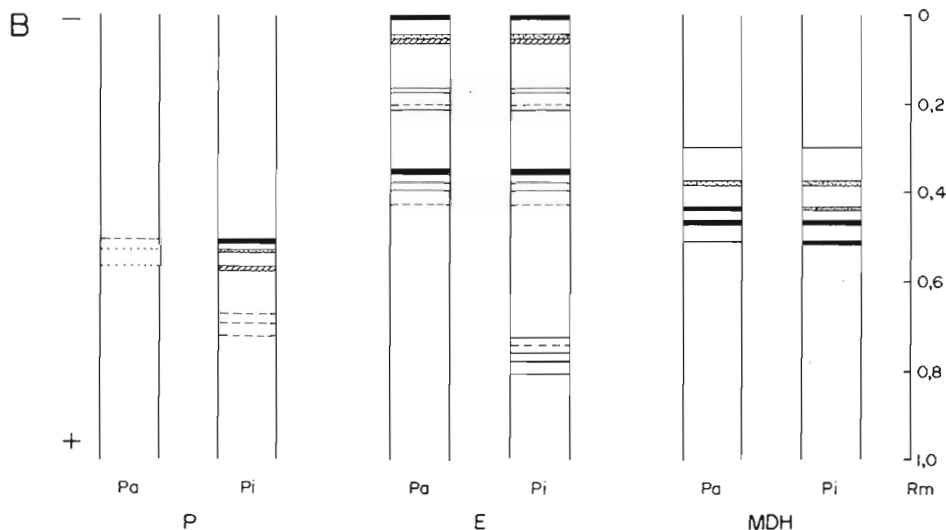
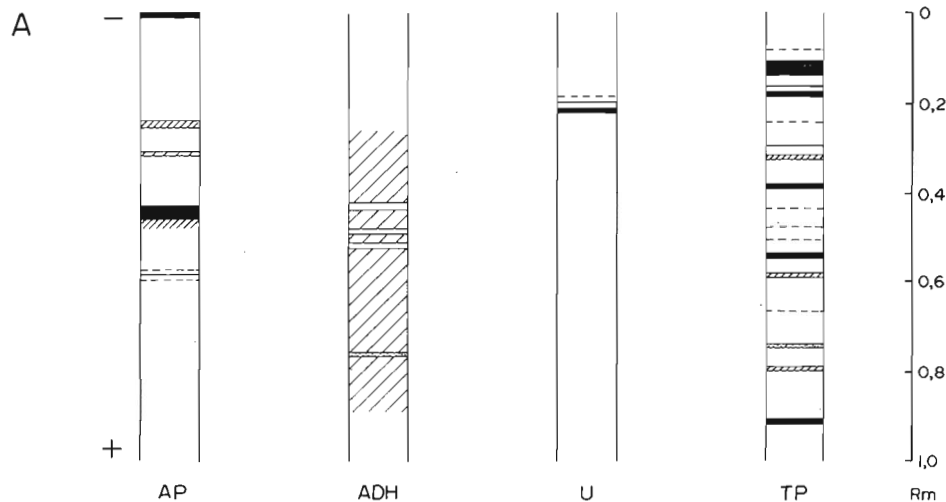


Figure 1 - Electrophoretic profiles of seed proteins of the soybean cultivar Paraná and three natural variants. A - profiles common to cultivars Paraná, SS-1, Paranagoiana and Pirapó-78; B - profiles which distinguish cultivar Pirapó-78 (Pi) from the other three cultivars (Pa). AP = acid phosphatase, ADH = alcohol dehydrogenase, U = urease, TP = total protein, P = peroxidase, E = esterase, MDH = malic dehydrogenase; R_m = relative mobility.

alcohol dehydrogenase and four of acid phosphatase (Kiang and Gorman, 1983) have been distinguished among other varieties of soybean.

The profiles of esterase, malic dehydrogenase and peroxidase also were similar among the cultivars Paraná, SS-1 and Paranagoiana. However the profiles of these enzymes obtained from cultivar Pirapó-78 were different from those of the other three cultivars (Figure 1B). The profiles of esterase and peroxidase of this cultivar each contained an additional set of bands, of relatively high mobility (esterase R_m 0,71-0,80, peroxidase R_m 0,68-0,74), and the peroxidase profile presented a much higher level of activity than that observed in the other profiles. In accord with the observations of Buttery and Buzzell (1968) peroxidase activity was confined to the seed coat in all four cultivars. As to malic dehydrogenase, the relative intensities of two isoenzymes (R_m 0,44 and R_m 0,51) differed between the profiles of cultivar Pirapó-78 and those of the other three cultivars. No qualitative variation of the isoenzymes of malic dehydrogenase was observed among the samples and none has been reported in other studies (Kiang and Gorman, 1983; Cedeño and Perez, 1985; Doong and Kiang, 1987).

The similarity of isoenzymes among the cultivars Paraná, Paranagoiana and SS-1 is consistent with the existence of a close genetic relationship among them, and hence supports the view that, based on field characters (Kiihl *et al.*, 1984 and OCEPAR/EMBRAPA, 1988), "Paranagoiana" and "SS-1" originated from cultivar Paraná by mutation. By contrast cultivar Pirapó-78 differs from the other cultivars in three of the seven protein characters examined and its genetic relationship to Paraná thus appears to be more distant. In the absence of evidence of linkage among the genes responsible for the synthesis of the three enzymes which distinguish cultivar Pirapó-78, and considering that the four cultivars were grown in the same environment, it can be concluded, independently of other types of data, that at least three genetic differences exist between this cultivar and the others. As only 19 years passed between the selections of "Paraná" and "Pirapó-78", it is very unlikely that the observed differences resulted from natural mutations. It is more probable that cultivar Pirapó-78 resulted from a natural cross involving the Paraná genotype.

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RESUMO

Os variantes naturais do cultivar Paraná de soja "Paranagoiana", "SS-1" e "Pirapó-78", são considerados mutantes do cultivar original. Entretanto, a morfologia do cultivar Pirapó-78 levanta dúvidas com respeito a sua origem por mutação. Para se obter informação auxiliar foram determinados os perfis eletroforéticos de proteína total, fosfatase ácida, desidrogenase alcoólica, esterase, desidrogenase

málica, peroxidase e urease de extratos de sementes dos quatro cultivares. Nenhum dos perfis permitiu diferenciar os cultivares Paranagoiana e SS-1 do cultivar original. Entretanto, os perfis da peroxidase, esterase e desidrogenase málica do cultivar Pirapó-78 foram diferentes dos respectivos perfis dos outros cultivares. Os dados são consistentes com a origem dos cultivares Paranagoiana e SS-1 por mutação, mas sugerem uma origem diferente para o cultivar Pirapó-78.

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