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Identification of Cowpea Cultivars (*Vigna unguiculata* (L) Walp) by Isoenzyme Electrophoresis

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ABSTRACT. *The differences among cowpea (Vigna unguiculata (L) Walp) cultivars were identified using electrophoretically detected isozymic variability. The enzymes studied in the selected 10 cultivars of cowpea were Diaphorase (DIAP), Phospho Gluco Isomerase (PGI), 6 Phospho Gluconate Dehydrogenase (6PGD), Phospho Gluco Mutase (PGM), Esterase (EST), Hexose Kinase (HK) and Glucose Oxalo Acetate Transaminase (GOT). Starch gel electrophoresis was used to analyze extracts prepared from young leaf tissues of seven day old seedlings of each cultivar grown under greenhouse conditions. Three enzymes GOT, HK, and PGM exhibited two patterns, while three different patterns were observed for EST and 6PGD. Phospho Gluco Isomerase showed four different patterns, and DIAP appeared to be the most polymorphic with eight patterns. Distinct variety specific zymograms were observed for several enzymes. Unique combinations of isozymic variants of two enzyme systems assayed (DIAP and PGI) could differentiate all the cultivars. Sufficient variability was present in cowpea cultivars to allow the use of isozyme electrophoresis as a method for cultivar identification. Therefore, it could be used in variety identification to complement the morphological characters.*

INTRODUCTION

Cowpea is a leguminous crop especially adapted to semi-arid regions. It has little dependence upon nitrogen fertilizer for optimum yield, since it can fix nitrogen symbiotically with Rhizobium. Cowpea is nutritionally important in cereal based diets because the seeds have a high (23%) protein content (Norton *et. al.*, 1985).

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The increased economic importance of this crop has stimulated the development of new cultivars. Therefore, identification of varieties became very important in seed certification and crop breeding programmes to screen different plant genotypes. At present, morphological features are commonly used to identify crop cultivars. For some plant species, identification based on plant and seed morphology has been unreliable, because morphological characters can be affected by environmental conditions (Ansary and Smith, 1976; Hamil and Camlin, 1984; and Gottschalk, 1985). Moreover, a cultivar also must be judged by an individual who possesses a thorough knowledge of the cultivar at the precise time (Wagner and McDonald, 1982). For morphological characterization, the plant must be grown to flowering or fruiting stage, which is space and time consuming. Therefore, it is desirable if a cultivar identification system could be developed, based on biochemical techniques.

Electrophoresis of seed proteins and plant enzymes is more rapid than field testing (Wagner and McDonald, 1982). It is more capable of differentiating varieties. The swiftness of analysis, ease of operation and ability to analyze simple protein samples repeatedly, contribute to the feasibility of cultivar identification. Electrophoresis has been efficiently used to identify several crop cultivars, such as, soybean (Wagner and McDonald, 1982; Larsen and Cadwell, 1968; Gorman and Kiang, 1977 and Cardy *et. al.*, 1984) and many crops belonging to Graminae (Cooke, 1983). However, current literature does not provide much information regarding the identification of available cowpea cultivars by electrophoresis.

Therefore, the objective of this study was to find out the potential use of electrophoresis in cowpea cultivar identification.

MATERIALS AND METHODS

Cowpea seeds from 10 cultivars (Lanka Kadala, IT-82D-513-1, IT-82D-889 (Warunee), Iita, IT-82D-789 (Vijaya), Bombay Cowpea, Tru 946, Arlington, MI-35, MICP-44) were obtained from research stocks maintained at the Field Crops Research and Development Institute, Maha-Illuppallama. The first true leaves of seven day old seedlings grown in green house conditions at 28+3°C and with adequate soil moisture were used for the analysis. Plant leaf tissues were used in this experiment, since a greater number of banding patterns can be achieved with leaf materials (Nehara *et. al.*, 1991). In addition, leaf tissues are preferred for isoenzyme analysis of

plants, because of their availability throughout the growing season. Fresh leaf tissues (approximately 0.5 g) were thoroughly crushed using a mortar (9 cm top diameter) and pestle with 0.5 ml of the extraction solution, as described by Peiris (1986). The homogenate was centrifuged at 4500 RPM for 20 minutes at 4°C. The clear supernatant was collected and stored at -10°C, prior to being used for electrophoresis.

Horizontal starch gels were prepared using 25 g of hydrolyze potato starch in 220 ml of buffer for the specific enzyme under investigation, as described by Weeden (1982). Gels were cast in a rectangular gel mould (16.5x13.0 cm) and allowed to cool for one hour. After cooling, gels were covered with a plastic wrap to prevent desiccation and left to set overnight at room temperature (28±3°C). Filter paper wicks (0.8x0.5 cm No. 470, Schleicher and Schuell) were used to absorb samples of thawed supernatants for insertion into a slit cut 4.0 cm from the cathodal edge of a gel. All the cowpea varieties were placed into the slit, spaced at 1.5 cm intervals. A wick containing red dye marker was placed at one edge of the gel, as a marker to follow isoenzyme migration.

A fresh electrode buffer (Peiris, 1986) was used for each electrophoretic run. The gel was placed in an electrophoretic tank kept in a refrigerator at 4.0°C, and a 50 mA constant current was applied for 20 minutes. The wicks were removed thereafter, and electrophoresis was resumed at a 40 mA constant current, until the dye marker had migrated 7.5 cm from the point of wick insertion. After electrophoresis, 1 mm thick slices were cut from the gels by drawing nylon thread horizontally through the gel over successive layers of 1.2 mm thick glass strips placed along the slab. Isoenzyme banding patterns were revealed for seven enzymes (Esterase (EST), Diaphorase (DIAP), Glucose Oxalo Acetate Transaminase (GOT), Hexose Kinase (HK), Phospho Gluco Mutase (PGM), Phospho Gluco Isomerase (PGI), Phospho Gluconate Dehydrogenase (6PGD)), by immersing 1 mm thick gel slices in specific staining solutions (Peiris, 1986) and incubating at 37°C for 1 h. The exception was for esterase, where gels were incubated at room temperature to reveal the esterase activity.

After incubation and optimum stain development, the solutions were removed and the gels were fixed with a mixture of ethanol, water, acetic acid and glycerol (in the ratio of 5:4:2:1) was used for stains which did not contain MTT (Thyozolyl Blue). The banding patterns were visually recorded, and each slice was photographed for permanent records. However, photographic documentation present difficulties due to the low

contrast of paint bands against the background stain of the electrophoretic lane. Photographic films did not distinctly record faint bands. Thus, the electrophoregrams were mapped for clear presentation.

The relative migration (Rf values) of each band was measured in each zymogram for every variety tested using the following equation:

$$Rf\ Value = \frac{Distance\ migrated\ by\ the\ enzyme\ band\ from\ the\ cathodal\ edge}{Distance\ migrated\ by\ the\ dye\ marker}$$

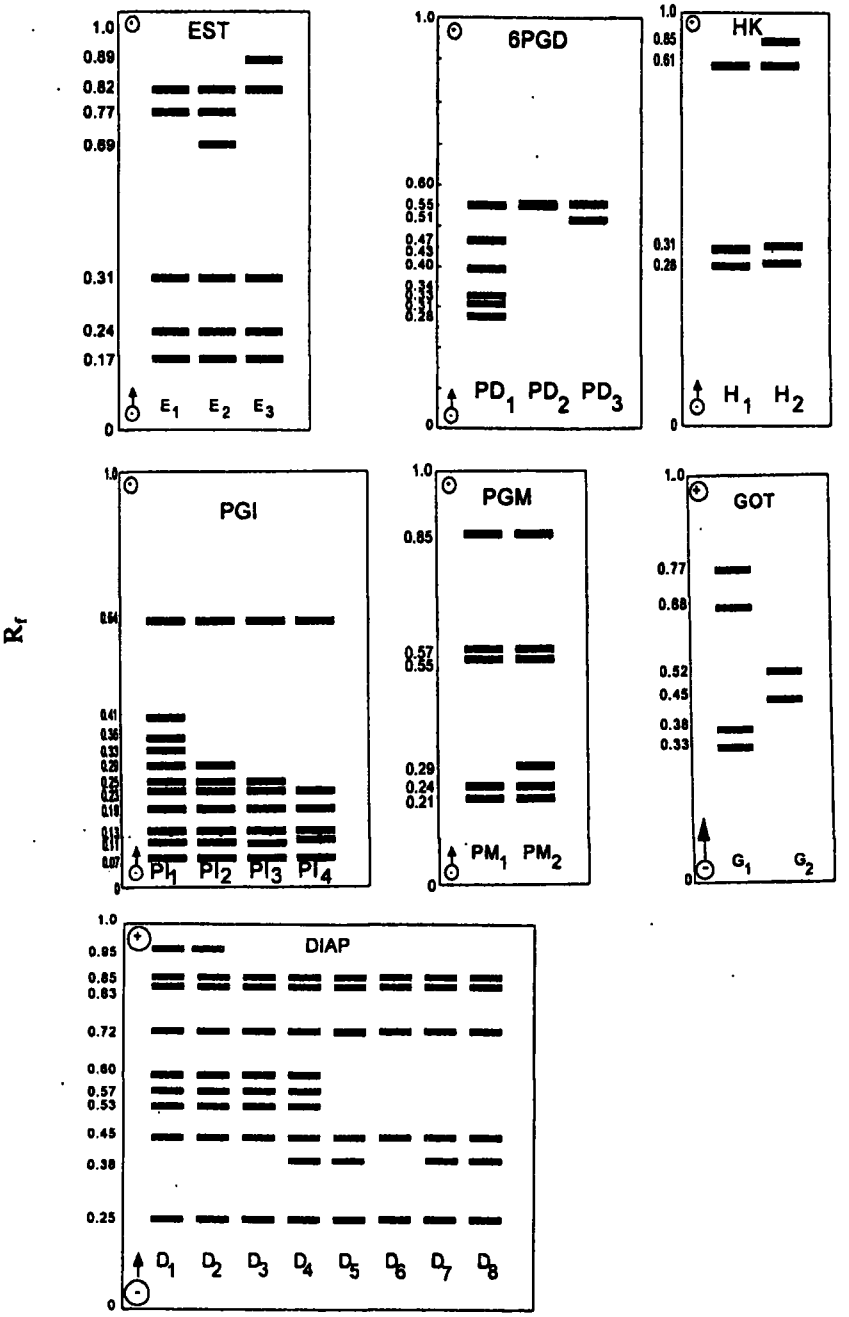
After preparing schematic diagrams (zymograms), isoenzyme banding patterns were identified according to the number of bands and the location (Rf value) of the band.

RESULTS AND DISCUSSION

Three enzymes GOT, HK, and PGM exhibited two patterns, while three different patterns were observed for EST and 6PGD (Figure 1). Phospho Gluco Isomerase showed four different patterns and DIAP appeared to be the most polymorphic with eight patterns.

Seven of the ten varieties examined for isozymic banding patterns produced variety specific zymograms, at least for one of the enzyme systems evaluated (Table 1). Six out of eight banding patterns observed for DIAP activity were variety specific zymograms. Phospho Gluco Mutase did not exhibit a variety specific zymogram, while the other five enzymes, PGM, 6PGD, EST, HK and GOT produced one variety specific banding pattern for each enzyme.

The variety MICP-44 exhibited four distinctly different isozymic banding patterns for enzymes, DIAP, EST, HK and GOT. Varieties IT-82D 513-1, Iita and IT82D-889 (Warunee) did not exhibit specific banding patterns for any of the enzymes examined. However, all 10 cowpea varieties could be identified with the help of two enzymes systems, DIAP and PGI. This implied that all the enzymes evaluated are not necessary to identify the cowpea varieties examined in this experiment (Table 2). If more varieties are to be identified all the enzymes may be required for variety identification. Therefore, isozymic finger prints for 10 cowpea varieties were prepared (Table 1).



Letters indicated at the base represent banding patterns.

Figure 1. Diagrammatic representation of Electrophoretic Banding Patterns of 7 enzymes of cowpea cultivars.

Table 1. Isozymic finger prints for ten cowpea varieties.

Variety	DIA	PGI	6PGDH	PGM	EST	HK	GOT
MI-35	D7*	PI3*	PD1	PM1	E1	H1	G1
Lanka Kadala	D1	PI2	PD2*	PM1	E1	H1	G1
MICP-44	D8*	PI4	PD1	PM1	E2*	H2*	G2*
IT82D-513-1	D1	PI4	PD3	PM2	E1	H1	G1
IT82D-789 (Vijaya)	D3*	PI2	PD3	PM1	E1	H1	G1
Iita	D2	PI2	PD3	PM2	E1	H1	G1
IT-82D 889 (Warunee)	D2	PI1	PD3	PM1	E1	H1	G1
Arlington	D6*	PI2	PD3	PM1	E3	H1	G1
Tru 946	D5*	PI1	PD3	PM1	E3	H1	G1
Bombay Cowpea	D4*	PI1	PD3	PM1	E3	H1	G1

* variety specific banding patterns.

Table 2. Differentiation of cowpea varieties based on the Diaphorase and Phospho Gluco Isomerase banding patterns.

DIAP	PGI	Varieties
D1	PI2	Lanka Kadala
	PI4	IT-82D-513-1
D2	PI1	IT-82D-889 (Warunee)
	PI2	Iita
D3	PI2	IT-82D-789 (Vijaya)
D4	PI1	Bombay Cowpea
D5	PI1	Tru 946
D6	PI2	Arlington
D7	PI3	MI-35
D8	PI4	MICP-44

Banding patterns produced by the enzymes were found to be very stable and reproducible by repeating the analysis. The presence of sufficient genetic diversity among the varieties for isoenzyme patterns is another advantage in variety identification.

The results of this study clearly showed the feasibility of isozyme electrophoresis in cowpea variety identification. However, there are many unanswered questions regarding the use of electrophoresis in seed certification and breeding programs. In many cases electrophoretic characters are largely independent of the agronomically important characters. Therefore, it is necessary to assess whether a variety maintains its uniformity and stability of the agronomically important characters through seed multiplication.

CONCLUSIONS

The results of this experiment clearly indicate that electrophoretic analysis can be used in identifying cowpea varieties, especially those with the related parents and no morphological differences. Further studies are needed to identify all the available cowpea varieties by isozyme electrophoresis.

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