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Comparative Study of Protein Profiles of the Leaves of Wild *Manihot glaziovii* Mueller and the Cultivated Species, *Manihot esculenta* Crantz by SDS-polyacrylamide Gel Electrophoresis

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Abstract: Polyacrylamide gel electrophoresis was conducted on leaf protein extracts of *Manihot esculenta* and the wild relative, *Manihot glaziovii*. *Manihot esculenta* recorded the highest number of protein bands which might be from the past hybridizational processes which had taken place between it and the wild relatives, among which is *Manihot glaziovii*. In order to further increase the protein content of the edible *Manihot esculenta,* the two protein bands between 5.0 and 5.9 cm characteristic of *Manihot glaziovii,* as revealed in the rod gels, by electrophoresis, could be transferred to *Manihot esculenta* through hybridization. It is also possible that in the process, resistance to insects, diseases and drought may be transferred.

Key words: Polyacrylamide gel electrophoresis, *Manihot esculenta, Manihot glaziovii,* hybridization, protein extracts

Introduction

The genus Manihot belongs to the Euphorbiaceae and consist about 98 species ranging from sub-shrubs, shrubs to trees (Rogers and Appan, 1973). *Manihot esculenta* is a popular food in Africa as it gives a feeling of fullness but it has low protein content. The need for interspecific hybridization of *Manihot esculenta* with the wild relatives *Manihot glaziovii* has been suggested by several authors among which are Nassar and Dorea (1982), Nassar and Grattapaglia (1986). Lost genes can be restored to the gene pool of the cultigen by interspecific hybridization with wild relatives which possess these genes. *Manihot glaziovii*, a typical example of wild species of cultivated crops have been frequently used as an important source of genetic diversity and have been employed effectively in a variety of breeding programmes (Nassar 1985; 2000).

Electrophoretic technique has been employed on a number of plant groups to show that many isoenzymes or polymorphic proteins are widely distributed in higher plants and also to compare protein distribution of the wild relative of plants to the cultivated ones (Illoh, 1990; Illoh *et al*., 1993; Folorunso and Olorode, 2002). So far, there has been no report on the comparative study of the electrophoretic protein profile of the cultivated *Manihot esculenta* with the wild species. The present research therefore aims to study and compare the total soluble protein profiles of the cultivated *M. esculenta* with the wild species.

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Materials and Methods

Materials

Acrylamide, N,N,N',N'-tetramethylenediamine (TEMED), ammoniumpersulphate, sodiumdodecylsulphate (SDS), bovine serum albumin, ovalbumin, chymotrypsinogen A and lysozyme were obtained from Sigma Chemical Company, St. Louis, M.O. USA. All other reagents were of analytical grade and were obtained from Pierce Chemical Company, Rockford, Illinois, USA or BDH Chemical Co. Ltd. Poole, England.

Fresh leaves of *M. esculenta* and *M. glaziovii* were collected from different locations within Obafemi Awolowo University Campus, Ile-Ife, Nigeria.

Methods

Protein Extraction

The proteins of the fresh leaves were homogenized with 0.9% NaCl solution. The 50% homogenate was left overnight to ensure thorough extraction of all the soluble proteins. The homogenate was then centrifuged at 6,000 g for 30 min. The clear supernatant was removed and used as the crude protein sources.

Sodium Dodecyl Sulphate-polyacrylamide Gel Electrophoretics (SDS-PAGE)

SDS-PAGE was carried out on the crude proteins on 7.5% phosphate gels according to the method of Weber and Osborn (1975), along with a mixture of standard proteins for the determination of subunit sizes of the crude protein mixtures. The standard proteins used and their molecular weights were bovine serum albumin (67,000 dal), ovalbumin (45,000 dal), chymotrypsinogenA (25,000 dal) and hen egg-white lysozyme (15,000 dal). The coefficient of similarity was computed using the formula of Sokal and Sneath (1963).

$$C_s = \frac{a}{a + b + c}$$

where, a = # of band(s) present in both taxa being compared
b = # of band(s) present in taxon 1 and absent in taxon 2
c = # of band(s) absent in taxon 1 and present in taxon 2.

Results

The Electrophoretic separation of the leaf protein in the species of *Manihot* studied is presented in Fig. 1 and Fig. 2 (A and B). The patterns reveal distinct quantitative and qualitative inter-specific variation with respect to position and intensity of crude protein bands. The bands are defined as fast migrating bands (4.0-7.5 cm), intermediate migrating bands (2.0-3.9 cm) and slow moving bands (below 2.0 cm) (Table 1). Marked differences were recorded for number, combination of bands and intensity of bands between species. The bands range from 4 to 10 (Table 1). *Manihot esculenta* has the highest number of bands while *Manihot glaziovii* recorded the lowest number of bands. Slow moving bands and intermediate bands have the same number of bands and this is the highest number of bands recorded. There are 2 fast moving bands.

Table 1: The relationship between the species of *Manihot* studied on the basis of the relative mobilities of the bands and their closeness to one another

Names of species	Total No. of bands	Fast band 4.0-7.5 cm	Intermediate band 2.0-3.9 cm	Slow band 0-1.9 cm
Manihot glaziovii	4	1	3	-
Manihot esculenta	10	1	3	6
Total	14	2	6	6

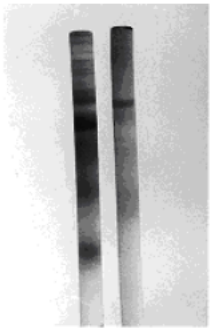

Fig. 1: The electrophoretic separation of leaf profien in the *Manihot* species, A-*Manihot glaziovii*, B-*Manihot esculenta*

The bands at 2.2, 2.7 and 3.7 cm are commonly shared between the two species and occur in different intensities. This means that the common band relationship between the two species is 3. The band between 5.0 and 5.9 cm is characteristic of *Manihot glaziovii*. The coefficient of similarity between the two species is 63.6%.

Discussion

Interspecific bands of leaf proteins were observed as illustrated in Fig. 1. The wild *Manihot* species showed variability in morphology growth habit and geographic distribution. This variation has been shown to be reflected in the electrophoretic profiles by differences in number and intensity of visible bands (Nasar 2000). The bands at 2.2, 2.7 and 3.7 cm, common to the two species showed that the gene which codes for the protein does not vary (Gottlieb, 1971).

From the above results, bands with identical electrophoretic mobilities represent proteins with identical amino acid sequences and are therefore potentially homologous in their derivations (Scogin, 1972).

The highest number of bands recorded for *Manihot esculenta* might have been accumulated through series of hybridization occurring naturally between wild *Manihot* species and cassava (Nassar, 1984; 1989).

As noted by Nassar (1985), wild species of cultivated crops have been frequently used as an important source of genetic diversity in a variety of breeding programs. Controlled introgression of genes could alleviate stress problems in cassava in view of the availability of wild relatives which exhibit diversity in adaptation and attributes (Nassar, 1985).

In conclusion, in comparison to *Manihot esculenta*, *Manihot glaziovii* is generally seen to be more resistant to insects, diseases and drought and some proteins are know to confer these qualities on

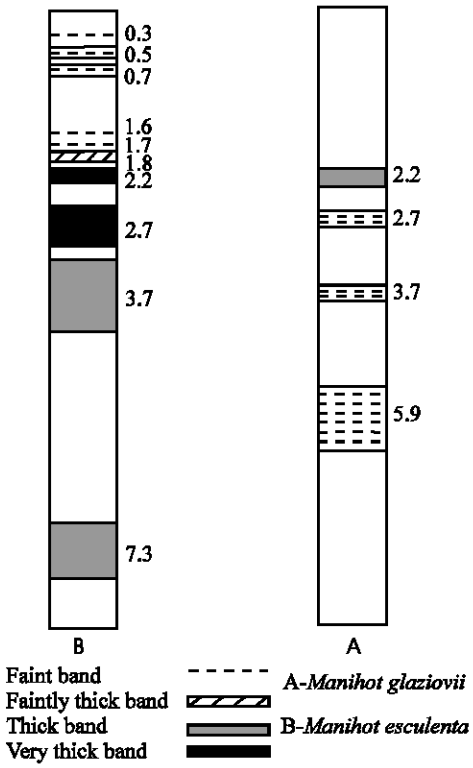

Fig. 2: Diagrammatic explanation of protein-bands of extracted protein in sodium dodecylsulphate polyacrylamide gel

plants. *M. glaziovii* has unique protein bands between 5.0 and 5.9 cm. It remains to be established whether hybridization of this wild cassava with *M. esculenta*, will in addition to increasing the protein content, also confer resistance to insects, diseases and drought.

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