Electrophoresis of crude protein of seeds of some genera of Annonaceae.

by

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ABSTRACT

Seeds of five genera of Annonaceae were collected from different latitudes and special ecological zones of Nigeria, the crude proteins were extracted and analysed by electrophoretic fractionation with a view to establishing whether they have evolutionary relationships and also to know the highest protein richness in them.

0.5 ml of each protein sample was loaded into the gel tubes. A current of 1.5 m A per gel was applied until stacking. The current was increased to 3 m A per gel. The elecctrophoresis was allowed to run until the dye front was about 1 cm from the bottom of the gel. ***The result shows that the band at 5.2 cm is common among the genera except in Dennettia tripetala. Intergeneric bands were observed between pairs of gnera in the family. The highest intergeneric band is between Greenwayodenron suaveolens and Xylopia aethiopica at 0.2cm, 5cm and 5.1. The presence of common bands among the genera of Annonacea shows evidence of common evolutionary origin in them.***

Key Words: *Annonaceae, Electrophoretic, Ecological, Band Nigeria.*

INTRODUCTION

The family Annonaceae consist of 2,050 species which are distributed over 125 genera and are found mainly in the tropics (Mabberley, 1987; Brummitt, 1992). The family compariesses woody trees, shrubs and lianas which are found in almost all vegetation types in the Neotropics.

Some species of this family (among other species) constitute the shrub community in the old world tropics (Krishnan and Priya, 1996). The family is represented by 22 genera in Nigeria (Hutchinson and Dalziel, 1958).

Although the Annonaceae as a family is well characterized, its infra-familial categories has always been problematic (Kessler, 1995). Classifications are far from being comparable with one another, although they all contain valuable insights. Some tribes of Annonaceae, especially those containing genera from South America, are very distinct and represent clear-cut and well-defined natural groups. Asiatic Australian genera with their reticulate distribution of primitive and advanced characters create difficulties for a classification at the tribal level (Kessler, 1995).

Many genera of this family are of high economic importance. The fruits and seeds of Xylopia aethiopica are hot to taste and are sold as a spice and as a substitute for African black pepper (piper nigrum L. Fam. Piperaceae). The crushed seeds rubbed on the forehead cure headache and neuralgia; a decotion of the fruit is used as a lotion for boils and eruptions. An extract of the bark is used in Hausaland as an ointment for sores (Irvine,1991). The oil from Xylopia aethiopica is semi-dry, with relatively high saponification values and used for making alkyd resins which could be used for making paints (Ajiwe et al., 1998). After roasting and grinding, the seeds of Monodora tenuifolia are rubbed on the skin for unspecified skin diseases (Irvine, 1961).

The advantage of electrophoresis according to "Gottlieb (1971)" is that variation in banding pattern can directly be equated to variation in genes coding for various proteins. Gel elecrophoresis of enzyme and protein have shown that many isoenzyme or polymorphic proteins are widely distributed in higher plants "(Cherry and Ory, 1972)" Such analyses have been carried out on Andropogon species and varieties "(Okoli, 1978)", Sorghum species (Morakinyo, 1984); Amaranthus species "(Illoh, 1990)" on the genus Sida (Illoh et al, 1993)

This study is aimed at using gel electrophoretic technique to evaluate the taxonomic relationships among five genera of Annonaceae. And also, the variation that exist among them and the highest protein richness in them.

MATERIALS AND METHODS

The seeds of mature fruits of the different genus were collected from different latitudes and special ecologi-

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Table 1. **The relationship between the genera of Annonaceae studied on the basis of band distribution.**

	Name of species	Total No of Bands	Higher Band	Intermediate Band	Lower Band
			4.0 - 5.5cm	2.0 - 3.9cm	0 - 1.9cm
i	*Monodora tenuifolia*	5	2	2	1
ii	*Greenwayodendron suaveolens*	4	2	-	2
iii	*Dennetita tripetala*	6	2	1	3
iv	*Xylopia aethiopica*	4	2	1	1
v	*Cleistopholis patens*	2	2	-	-
	Total	21	10	4	7

cal zones such as the wet forest, and dry forest area of Nigeria.

Protein of the dry seeds were extracted by grinding 1.5gm of the seeds with sterilized mortar and pestle . The seed proteins were extracted with 5ml of 0.9% Sodium Chloride (NaCl) as described by "Gottlieb (1971) " and reported by (Essiett and Illoh, 1997). The mixture was left overnight to ensure thorough extraction of protein, it was then centrifuged at X3000g for 15 minutes. The supernatants from this were then fractionated by disc electrophoresis following the method of Davis (1964) as modified by Ayeni (1984). For resolution, sodium dodecyl sulphate (SDS) polyarcylamide was carried out on 7.5% gels in IM Tris-glycine buffer at pH8.3 according to the procedure of Weber and Osborn (1969).

Photographs of the gels were taken and schematic diagrams were also drawn. The similarity index between the species was computed. Where similarity index between any two taxa is computed as the ratio of common/identical bands to the total number of bands in the taxon with the higher number of bands between the two. It is expressed as a percentage.

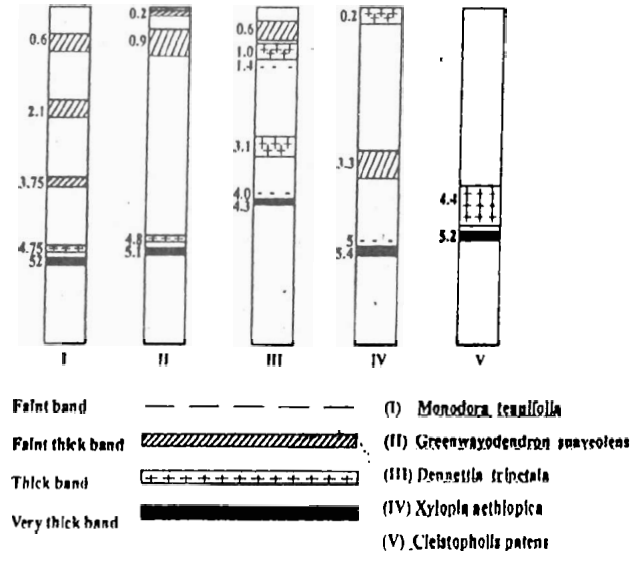

Figure 1: Diagrammatic explanation of protein - bands of extracted protein in sodium dodecysulphate polyacryamide gel

RESULTS

The pattern of protein distribution in the genera of Annonaceae studied is represented in Fig.1 (I-V). A close examination of the bands shows that there are different patterns of band distribution in the genera. Marked differences were recorded for number, combination of bands and intensity of bands between species . The bands range from 2 to six (Table 1). Most of the bands were located between (4,0 –5.5cm). followed by another group located between (0 –1.9cm) and intermediate band located between (2.0 – 3.9) respectively.

The band at 5.2cm is common to all the genera except Dennettia tripetala . Xylopia aethiopica and Cleistopholis patens have two common bands at 5cm and 5.2cm respectively. Apart from Monodora tenuifolia , Dennettia tripetala and Xylopia aethiopica that have all the band distribution. Greenwayodendron suaveolens have two higher bands.

Intergeneric bands were observed between pairs of genera in the family as shown in Table 2. Dennettia tripetala share one common band with both Monodora tenuifolia and Greenwayodendron suaveolens at 0.6cm respectively. Xylopia aethiopica and Dennettia tripetala share one common band at 3.1cm. Cleistopholis patens and Monodora tenuifolia also share one common band at 5.2cm.

The highest intergeneric band is between Greenwayodendron suaveolens and Xylopia aethiopica at 0.2cm, 5cm and 5.1cm.

DISCUSSION

Intergenetic bands of seed proteins were observed as illustrated in Fig. 1. The degree of variation in the bands depicts the genetic divergence of the genera of Annonaceae studied over evolutionary time. The variations in combination of protein bands at various distances from the anode is taxon-specific, no two species have the same band distribution. This support the opinion of "Olsson (1967)"

2. Common Band relationship some genera of Annonaceae.

	Monodora Tenuifolia	*Greenwaydendron suaveolens*	*Dennettia tripetala*	*Xylopia aethiopica*	*Cleistopholis patens*
Monodora tenuifolia	-				
Greenwayodendron suaveolens	2	-			
Dennettia tripetala	1	1	-		
Xylopia aethiopica	2	3	1	-	
Cleistopholis patens	1.	2	2	2	-

that biogenetic relationship can best be indicated by quantitative results using chemotaxonomic methods.

According to "Gottlieb (1971)" when a band appears in a population, it is assumed that the gene which codes the enzyme or protein does not vary. This assessment can be used to tag the band at 5.2cm as a family band in that it is present in all the genera studied except in Dennettia tripetala.

It is also noted that the genera having the band at 5.2cm inhabit the dry forest areas of Nigeria while Dennettia tripetala is found naturally in the wet forest areas of Nigeria hence ecological factor might have interracted with the generic factor in the production of the band.

The highest intergenetic band recoded for Greenwayodendron suaveolens and Xylopia aethiopica depicts two things. Firstly, it is the evidence of common evolutionary origin of the two species. Secondary, the similarities in the band patterns suggest that the proteins are under the control of the same genes. These may be adaptive genes which have evolved, become dispersed and fixed in the species over evolutionary time [Akinwusi and Illoh, 1995}.

The evidence from the variation in protein bands indicates that the genera are distinct with board-based relationship occurring between them.

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