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CRUDE PROTEIN ELECTROPHORESIS OF SOME SPECIES OF *ANNONA* IN NIGERIA

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Abstract

Seeds of four species of *Annona* were collected from different latitudes and special ecological zones such as the wet forest, dry forest and derived savanna areas of Nigeria and the crude proteins were extracted and analysed by electrophoretic fractionation. The result shows that the band at 3.3 cm, 4.2 cm and 4.7 cm are common to the *Annona* species. *Annona squamosa* and *A. reticulata* have the highest number of common bands. The band at 1.4 cm is common to both *A. reticulata* and *A. muricata*. The number and combination of protein bands were taxon-specific. These show the close genetic relationship of the species.

Keywords: *Annona*, Ecological, Nigeria, Electrophoretic, Fractionation.

1. Introduction

The family Annonaceae consist of 2,050 species which are distributed over 125 genera and are found mainly in the tropics (Mabberley, 1987 and Brummitt, 1992). The family comprises 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 *et al.*, 1996). The family is represented by 22 genera in Nigeria (Hutchinson and Dalziel, 1958). There are two species of *Annona* in Nigeria, the remaining species of *Annona* in Nigeria are introduced but may be naturalized. *Annona senegalensis* was collected from Gwagwalada along Abuja Road. *Annona squamosa, Annona reticulata* and *Annona muricata* were collected within the campus environment of Obafemi Awolowo University, Ile-Ife (Table 1).

The genus *Annona* has high economic value; their fruits considered exotic are of great commercial importance (Pino, 1997). Pino *et al.* (1998) analyzed the major flavoring components in fruits of four species of *Annona* such as soursop (*A. muricata*), Cherimoya (*A. cherimolia*), atemoya (*A. atemoya*) and bullock's heart (*A. reticulata*). Soluble solids, free reducing sugars and sucrose, total acidity and volatile compounds were evaluated. It was observed that the fruits have high sugar content and low acidity. The air dried seeds of *Annona squamosa* L. (sweet sop) contain water 10.0%, Oil 23.0%, minerals 1.89% and proteins 24-50% Ahmed *et al* (1996). The powdered seed is an irritant. It is applied as a pesticide and can cause conjunctivitis or even blindness (Burkill, 1935, Morton 1958). The seeds of *A. muricata* L. (sour sop) contain a yellow oil which in India and Mexico is applied to hair to kill lice, though it is irritant to the eyes (Burkill, 1935, Irvine, 1961).

Electrophoretic techniques for classification and identification have become a useful tool in studies of generic variability in plants. Several authors who have discussed the taxonomic significance of seed proteins using gel electrophoresis include Cherry and Ory (1972) on peanut cultivars, Okoli (1978) on *Andropogon* species and varieties, Pearce and Lester (1979) on *Solanum melongena*, Morakinyo (1984) on *Sorghum* species, Illoh (1986) on *Mangifera indica* (L.) varieties, Illoh (1990) on *Amaranthus* species, Illoh *et al.* (1993) on the genus *Sida*, Folorunso and Olorode (2002) on some species of Annonaceae.

Gottlieb (1971) recorded that variation in banding pattern can directly be equated to variation in genes coding for various proteins.

The objective of this paper is to provide useful information on the classification and identification of *Annona* species and identify both intraspecific and interspecific variations that exist among them and the highest protein richness in them.

2. Materials and Methods

The seeds of mature fruits were collected from different latitudes and special ecological zones such as the wet forest, dry forest and derived savanna areas of Nigeria (Table 1).

Protein of the dry seeds was extracted by grinding 1.5 g of the seeds with sterilized mortar and pestle. The seed proteins were extracted with 5 ml of 0.85 % sodium chloride (NaCl). The mixture was left

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Table 1: Collection data of the species of *Annona*

Species	Collection Number	Location
Annona senegalensis Pers.	094	Gwagwalada along Abuja Road
Annona squamosa Linn.	1275	Along road 19, C.A.U., Ile-Ife, Osun State
Annona reticulata Linn.	1280	Along road 7b, C.A.U., Ile-Ife, Osun State
Annona muricata Linn.	1420	Botanical garden. O.A.U., Ile-Ife, Osun State

Table 2: Upper and Lower gels preparation showing the compositions (volume in cm^3)

Chemical	Stacking Upper gel	Separating Lower gel
40%Acrylamide / Bisacrylamide	1.35	10.10
0.5M Tris HCL pH 6.8 (Upper gel buffer)	2.50	-
1.5M Tris HCL pH 8.8 (Lower gel buffer)	-	7.5
Distilled water	6.0	11.8
10% Sodium dodecylsulphate (SDS)	0.1	0.30
10% Freshly prepared Ammonium persulphate	0.1	0.30
TEMED (Tetramethylenediamine)	0.01	0.03

Table 3: The relationship between the species of Annona studied on the basis of the relative mobilities of the bands and their closeness to one another.

	Name of species	Total No of Bands	Fast Band	Intermediate Band	Slow Bands
			4.0 – 5.5cm	2.0 – 3.9cm	0 – 1.9cm
A	*Annona senegalensis*	4	1	2	1
B	*Annona squamosa*	7	4	2	1
C	*Annona reticulata*	6	4	1	1
D	*Annona muricata*	5	2	2	1
	Total	22	11	7	4

Table 4: Common Band relationship in *Annona* species (A – D).

		A	B	C	D
Annona senegalensis	A				
Annona squamosa	B	5	-		
Annona reticulata	C	5	6	-	
Annona muricata	D	3	3	4	

overnight to ensure thorough extraction of protein. It was then centrifuged at X 3000g for 15 minutes. The supernatants from this were then fractionated by disc electrophoresis following the method of Davis (1964) as modified by Ayeni (1984).

The resolution gel consisted of polyacrylamide at a concentration of 7.5 % in 1M Tris-glycine buffer at pH 8.3 according to the procedure of Weber and Osborn (1969). The composition of the gel is presented in Table 2. Coefficient of similarity was computed using the formula of Sokal and Sneath (1963).

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

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

3. Results

The pattern of protein distribution in the species of *Annona* studied is represented in Fig. 1 (A-D). A close examination of the bands shows that the different species have different patterns. Marked differences were recorded for number, combination of bands and intensity of bands between species. The bands range from 4 to 7 (Table 3). Most of the bands were found to be fast in movement (4.0 cm-5.5 cm), followed by intermediate moving bands (2.0-3.9 cm) and slow moving bands (0.0 cm-1.9 cm) respectively.

The bands of 3.3 cm and 4.7 cm are common to all the species and occur in three different intensities in all the species studied. The band at 4.2 cm is common to all the species but occur in two different intensities. The band at 1.4 cm is common to *A. reticulata* and *A. muricata* it is faint in *A. muricata* while it is thick in *A. reticulata*. The band at 0.5 cm and 3.6 cm are specific to *A. senegalensis* while the band at 1.1 cm and 2.0 cm are specific to *A. squamosa*. *A senegalensis*, *A. squamosa* and *A. reticulata* share common band at 5.1 cm.

Inter specific bands were observed between pairs of species in the taxa studied as shown in Table 4. *Annona squamosa* and *A. reticulata* have the highest number of common bands (six) while the following pairs have the least number of bands (3), *A senegalensis* and *A. muricata*, *A. squamosa* and *A. muricata*.

4.Discussion

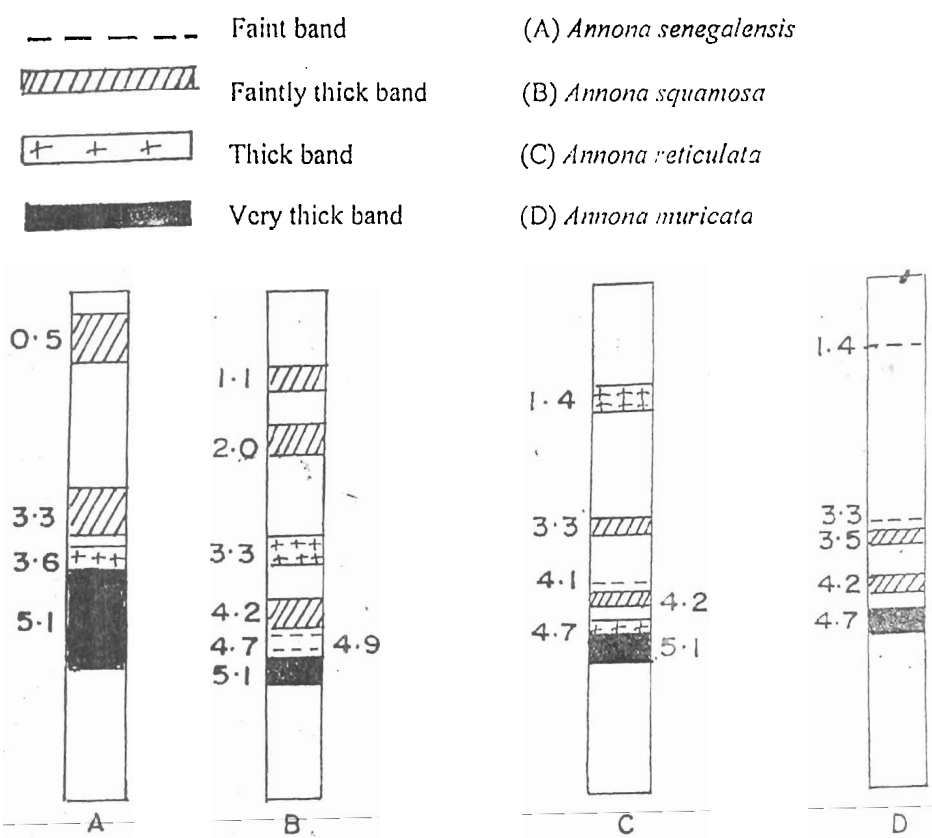
The variation in the patterns of electrophoretic mobility of protein was analysed from this work. Protein abundance sequence of the specimens are in the order Fig. 1 (B, C, D, A). Protein variation is an indication of protein polymorphism and this term forms the basis of the separation of individuals in a particular population into different taxa.

The electrophoretic studies as shown in Fig. 1 depict a measure of genetic divergence of *Annona* species over evolutionary time. The protein bands are taxonomically distinct as no two species have the same band distribution. This agrees with the opinion of Olsson (1967) that biogenetic relationships can best be indicated by quantitative results using chemotaxonomic methods.

The band at 3.3 cm, 4.2 cm and 4.7 cm is common to all the species. This presence of common bands among the various species of *Annona* (Table 4) shows evidence of common evolutionary origin and this is consistent with the high interspecific similarity value. Secondly, coming from the same parental stock their evolution is convergent, thereby making it possible for character traits to be shared in common. This supports the assertion of Gottlieb (1971) that when a band appears in all individuals in a population, it is assumed that the gene which codes the enzyme or protein, does not vary.

From the above results, bands with identical electrophoretic mobilities represent proteins with identical amino acid sequences and are therefore

Fig. 1: Diagramatic explanation of protein-bands of extracted protein in sodium dodecylsulphate polyacrylamide gel.



potentially homologous in their derivations (Scogin, 1972).

According to Cronquist (1968), the presence of a character is of greater taxonomic importance than its absence. Therefore, the band at 0.5 cm in *A. senegalensis* and at 1.1 cm, 2.0 cm in *A. squamosa* respectively could be useful in delimiting each of these two species from the other species of *Annona*. The band at 1.4 cm in both *A. reticulata* and *A. muricata* could contribute to the relatively big fruit size in them.

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

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