PLANT SCIENCES RESEARCH COMMUNICATIONS
2(1): 48 - 52 (2004)

ELECTROPHORETIC STUDY OF CRUDE PROTEIN DIVERSITY IN THE SEEDS OF *ABELMOSCHUS ESCULENTUS* (L) MOENCH AND *A. MOSCHATUS* MOENCH

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ABSTRACT: Seeds of two species of *Abelmoschus* were collected from the National Horticultural Research Institute (NIHORT), Ibadan. The crude proteins were extracted and analysed by electrophoretic fractionation. The species of the genus have three common bands. This shows the close genetic relationship of the species and evidence of a common evolutionary origin. Also the band at 3.5cm is specific to *A. moschatus* and could be useful in delimiting the two species.

1. INTRODUCTION: Okra, *Abelmoschus*, is a member of the family Malvaceae. It is a fast-growing annual herb, cultivated for its young edible fruits, and it is one of the most important vegetables in tropical and sub-tropical regions *[1,2,3,4]*. Okra is a potential multipurpose crop for the temperate zones and the tropics *[2]*. According to Martins and Ruberts *[8]*, the high mucilage content of okra makes it useful in the curing of ulcers and in the relief of hemorrhoids.

Okra shows a high variability in its vegetative and fruit characters *[5]*. Ariyo and Oken'ova *[6]* reported that there are many lines of okra, each with striking uniformity, suggesting that the okra population under consideration had a wide genetic base. Unfortunately, in Nigeria, genetic studies of okra are very limtied *[7]*.

Electrophoretic techniques for identification and classification have become a useful tool in studies of genetic variability in plants. Authors who have discussed the taxonomic significance of seed proteins, using gel electrophoresis, include Cherry and Ory *[9]* on peanut cultivars, Okoli*[10]* on *Andropogon* species, Pearce and Lester *[11]* on *Solanum melongena*, Morakinyo *[12]* on *Sorghum* species, Illoh *[13]* on *Mangifera indica* (L) varieties, Illoh *[14]* on *Amaranthus* species, Illoh *et al [15]* on the genus *Sida*, Akinwusi and Illoh *[16]* on *Hibiscus* species, and Folorunso and Olorode *[17]* on the seeds of some genera of Annonaceae. Gottlieb *[18]* reported that variation in banding pattern can directly be equated to variation in genes coding for various proteins.

The objective of this study was to provide useful information on the identification and classification of two *Abelmoschus* species and the variation that may exist between them, and on the high protein richness in them.

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2. MATERIALS AND METHODS: The seeds of *Abelmoschus esculentus* (L) Moench and *A moschatus* (Moench) used for this study were obtained from the National Horticultural Research Institute (NIHORT), Ibadan. They were grown on sandy loam soil in the garden around the screen house in Botany Department, Obafemi Awolowo University. The plants were nurtured until mature seeds of the fruits were collected for electrophoretic analysis.

Proteins of the dry seeds were extracted by grinding 1.5g of the seeds with sterilized mortar and pestle. The seed proteins were extracted with 5ml of 0.85% sodium chloride (NaCl). The mixture was left overnight to ensure thorough extraction of proteins; it was then centrifuged at 3000g for 15 minutes. The supernatants from this were fractionated by disc electrophoresis following the method of Davis *[19]* as modified by Ayeni *[20]*. For resolution, sodium dodecyl sulphate (SDS) polyacrylamide electrophoresis was carried out on 7.5% gels in 1M Tris-glycine buffer at pH 8.3 according to the procedure of Weber and Osborn *[21]*.

Photographs of the bands were taken and schematic diagrams were also drawn. The similarity index between the species was computed. The similarity index between any 2 taxa was 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 was expressed as a percentage.

3.0 RESULTS: The pattern of protein distribution in the species of *Abelmoschus* studied is represented in Fig. 1. The results show that there are variations in the number, position and intensity of the bands. The bands range from 3 in *A. esculentus* to 4 in *A. moschatus* (Table 1). Most of the bands manifested in the runs were found to be intermediate (2.8cm to 4.8cm); the remaining bands are lower bands (0.8cm).

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

Species	Total No. of Bands	Higher Band 4 to 5.5cm	Intermediate Band 2 to 3.9cm	Lower Band 0 to 1.9cm
A. moschatus	4	1	2	1
A. esculentus	3	1	1	1
Total	7	2	3	2

The bands at 0.8cm, 2.8cm and 4.8cm are common to both species. The band at 2.8cm is common to the species and occurs in two different intensities in the two species. Hence common band relationships in the two *Abelmoschus* species are 3. The band at 3.5cm is specific to *A. moschatus*. The similarity index based on the electrophoregram of *Abelmoschus* species is shown in Table 2.

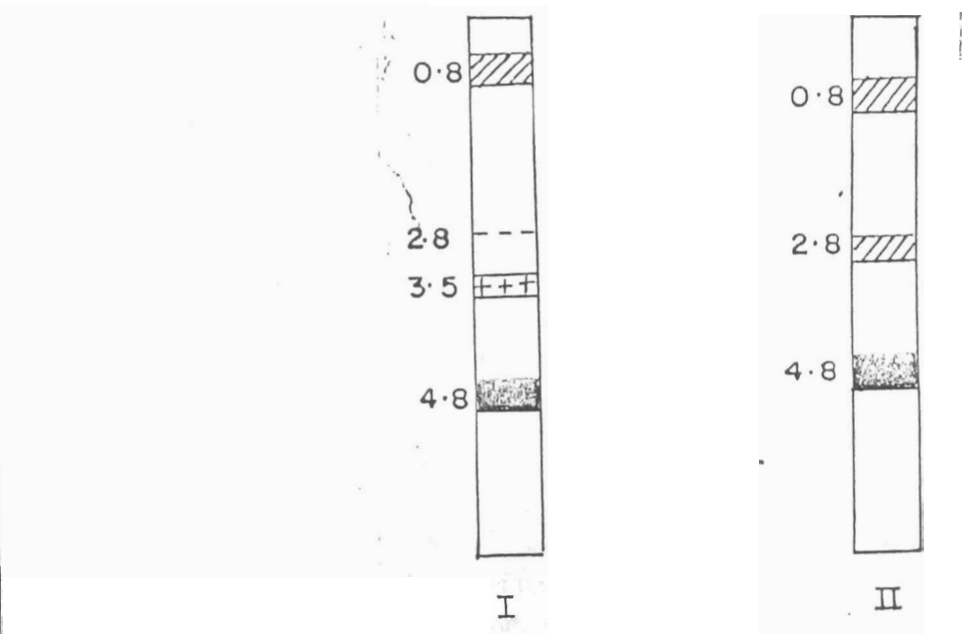

Figure 1: Diagramatic explanation of protein-bands of extracted protein in sodium dodecylsulphate polyacrylamide gel.

4. DISCUSSION: Species - specific bands of seed proteins were observed as illustrated in Fig. 1. The degree of variation in the bands depicts the genetic divergence of *Abelmoschus* species over evolutionary time. The variation in combination of protein bands at various distances from anode is taxon-specific; no two species have the same band distribution. This supports the opinion of Olsson *[22]* that biogenetic relatioships can best be indicated by quantitative results using chemotaxonomic methods.

Table 2: Similarity index based on electrophoregram of *Abelmoschus* species

	I	II
I	-	
II	75.0	-

I - A. moschatus II - *A. esculentus*

According to Gottlieb *[18]*, 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. This assessment can be used to tag the bands at 0.8cm, 2.8cm and 4.8cm as generic bands in that they are present in both species of *Abelmoschus*. The presence of common bands among the various species of *Abelmoschus* shows evidence of common evolutionary origin. This is consistent with the high interspecific similairty index value (Table 2). Furthermore, coming from the same parental stock, their evolution is convergent thereby making it possible for character traits to be shared in common.

According to Cronquist *[23]*, the presence of a character is of greater taxonomic importance than its absence. Therefore, the band at 3.5cm in *A, moschatus*, which is absent in *A. esculentus*, could be useful in delimiting the two species in that it could contribute to the pedicel being as long as the fruit or longer, and to other characters. Its absence in *A. esculentus* could also contribute to the pedicel being much shorter than the fruit, and to other characters.

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

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