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CRUDE PROTEIN ELECTROPHORESIS OF SEEDS OF SOME SPECIES OF *HIBISCUS*

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ABSTRACT

Seeds of ten species of *Hibiscus* L. were collected from different locations within South Western Nigeria and the crude proteins were extracted and analysed by electrophoretic fractionation. The species of the genus have one common band. Interspecific bands varying from eight to nine are common between pairs of species. These show the close genetic relationship of the species. The number and combination of protein bands were taxon-specific.

INTRODUCTION

The genus *Hibiscus* L. containing over 300 species is found mostly in the tropics ''(Walsh, 1975; Heywood, 1978)''. The genus belongs to the tribe Hibisceae of the family Malvaceae. Species of the genus range in habit from trees to shrubs to herbs. They may be annuals or perennials.

''Hutchinson and Dalziel (1958)'' have listed thirty- three species in West Africa. According to ''Nielsen (1965)''. exotic species introduced into West Africa include three common species namely, *H. mutabilis* Linn. (Blushing Hibiscus), *H. rosa-sinensis* Linn. (the most commonly grown), and *H. schizopetalus* (mast.) Hook. f. (with very frilly lobed petals). *Hibiscus* species have been characterised by attributes such as flower colour, leaf size, margin of leaves and leaf shape. Many character differences exist among different species and the different character states are combined in so many ways in the various species that it is difficult to define sizeable definitive natural groups. A wide range of chromosome and ploidies have been reported from 2n = 28 to 2n = 180. The numbers have previously been arranged in ten series with basic numbers x= 7, 8, 9, 11, 12, 15, 17, 19, 20 and 39 ''(Darlington and Wylie, 1956)''.

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 electrophoresis 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)''.

This study is aimed at using gel electrophoretic technique to evaluate the taxonomic relationships among ten species of *Hibiscus* (listed on Table 1).

Table 1: *Relative Mobilities of Bands*

	Name of Species	Slow bands (0-1.9cm)	Intermediate Bands (2.0-3.9cm)	Fast Bands (4.0-5.9cm)	Total No. of Bands	Unique Bands (cm)
A.	*H. rostellatus*	1	10	5	16	-
B.	*H. tiliaceus*	2	9	4	15	5.5
C.	*H. sabdariffa*	3	9	3	15	2.1
D.	*H. vitifolius var. vitifolius*	3	7	3	13	-
E.	*H. lunarifolius*	3	9	3	15	-
F.	*H. acetosella*	5	8	3	16	
G.	*H. physaloides*	2	10	5	17	4.4 & 4.7
H.	*H. surattensis*	4	8	3	15	-
I.	*H. scotellii*	4	8	5	17	
J.	*H. sterculiifolius*	2	11	4	17	3.8

MATERIALS AND METHODS

Seeds of mature fruits of the genus were collected from different locations within South Western Nigeria and bagged in small envelopes which were properly labelled.

One gramme of the seed of each species was ground in a porcelain mortar in 10ml of 0.09% sodium chloride solution. The mixture was allowed to settle inside test tubes immersed in ice-bath for about an hour. The supernatants were then centrifuged at x3000g for fifteen 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) polyacrylamide was carried out on 7.5% gels in 1M Tris-glycine buffer at pH 8.3 according to the procedure of "Weber and Osborn (1969)".

Ten drops each of the centrifuged supernatants of the proteins were taken in other test tubes and 3 drops of glycerol, 1% 2-mercaptoethanol and sodium dodecyl sulphate (SDS) were added and boiled in a water bath for ten minutes. On cooling to room temperature, three drops of glycerol were added to each sample to weigh down the protein molecules. This was followed by a drop of 0.05% bromophenol blue which served as a tracer dye. 0.2ml of the resultant mixture were added directly to the gels. The tubes were placed in a column acrylamide gel apparatus with tris glycine buffer in both upper and lower vessels.

A current of $1\frac{1}{2}$ mA per gel was applied and after the protein was stacked in the lower gel, the current was increased to 3mA per gel. The current was stopped after about 3 hours when the dye front had migrated a distance of about 5cm from the anode and the gels were then removed from the tubes. The gels were stained with 0.05% comasic brilliant blue for 15-20 minutes after which destaining commenced at intervals of three hours for about 48 hours. Photographs of the gels were taken and schematic diagrams were also drawn.

RESULTS

The patterns of protein in the different species of *Hibiscus* studied are shown in Plate 1 and Fig. 1. 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. Most of the bands were found to be intermediate in movement

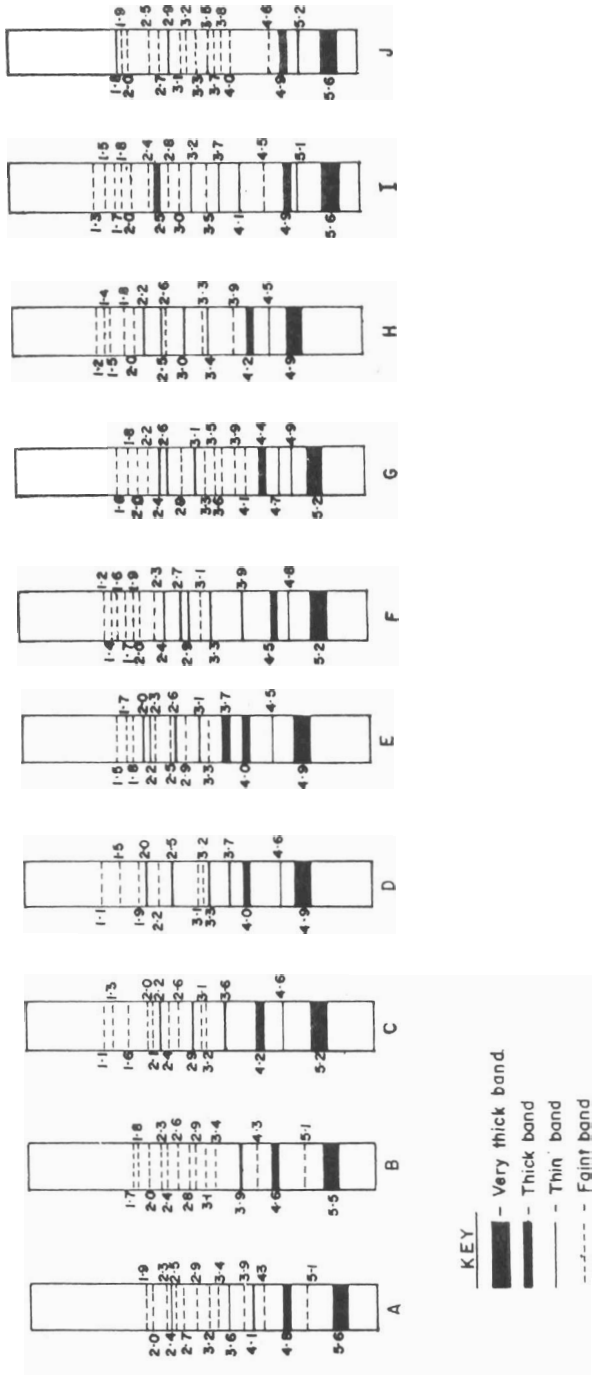
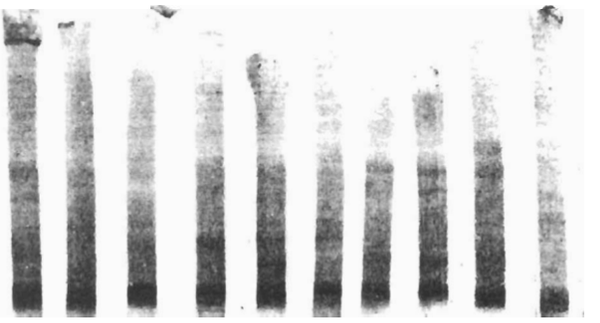


Fig. 1: The intensity of the bands that are not clearly shown in plate 1. (A - J as indicated in Table 1)

(2.0cm-3.9cm), followed by fast moving bands (4.0cm-5.9cm) and slow moving bands (0.0-1.9cm) respectively.

The band at 2.0cm is common to all the species and occur in two different intensities in all the species studied. Some bands are widespread in the genus, for example, the protein bands at 2.9cm and 3.1cm are present in seven species. The bands at 5.5cm, 2.1cm, 4.4cm, 4.7 cm and 3.8cm are unique to the species in which they appeared as shown in Table 1.

Interspecific bands were observed between pairs of species in the taxa studied as shown in Table 2. The following pairs of species have the highest number of common bands (nine). *H. vitifolius* var. *vitifolius* and *H. lunarifolius; H. lunarifolius* and *H. surattensis; H. sabdariffa* and *H. physaloides; H. lunarifolius* and *H. sterculiifolius.* The least number of bands (three) occur between *H. tiliaceous* and *H. vitifolius* var *vitifolius.*



A B C D E F G H I J

Plate 1: Electrophoretic characteristic of *Hibiscus* species studied, showing their protein distribution (A - J as indicated in Table 1)

The protein bands at 1.2cm and 1.4cm are unique to the two species, *H. acetosella* and *H. surattensis.* In addition, the two species have four other bands in common at 2.0cm, 3.3cm 3.9cm and 4.5cm.

Table 2: *Common Band relationship in* Hibiscus *Species (A - J as in Table 1)*

	A	B	C	D	E	F	G	H	I	J
A	-									
B	8	-								
C	5	6	-							
D	4	3	6							
E	4	7	5	9	-					
F	8	7	6	4	7	-				
G	6	7	9	5	8	8	-			
H	4	5	4	6	9	6	7	-		
I	7	6	4	6	8	4	6	6	-	
J	7	5	6	9	9	7	8	5	8	-

DISCUSSION

Species-specific bands of seed proteins were observed as illustrated in Plate 1 and Fig. 1. The degree of variation in the bands depicts the genetic divergence of *Hibiscus* species 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 supports the opinion of "Olsson (1967)" that biogenetic relationships can best be indicated by quantitative results using chemotaxononic methods.

According to "Gottlieb (1971)", 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 band at 2.0cm as a generic band in that it is present in all the species studied. The presence of common bands varying from eight to nine at the same distances from the anode reflects some level of affinity among the taxa and depicts two things. Firstly, it is the evidence of common evolutionary origin of the *Hibiscus* species. Secondly, 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.

The number of bands from 13 to 17 recorded for *Hibiscus* species studied shows that wide variations occur in their protein content. 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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