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CRUDE PROTEIN ELECTROPHORESIS OF SEEDS, OF EIGHT SPECIES OF *CROTALARIA* L.

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ABSTRACT

Seeds of eight species of *Crotalaria* were collected and the proteins were analysed by electrophoretic fractionation. On the basis of number, intensity and mobility of the protein bands, no definite relationship can be drawn among the eight species except that, on a broad base, seven species, *Crotalaria retusa, C. goreensis, C. doniana, C. cylindrocarpa, C. comosa, F. naragutensis and* C. spectatilis are more related to each other than to *C. spectatilis*

INTRODUCTION

The genus *Crotalaria* L., containing over 500 named species, is found predominantly in the tropics. The genus belongs to the family Fabaceae and subfamily Papilioniodeae. Members of the genus range in habit from shrubs to annuals and tend to be pioneers mostly in disturbed places over a wide range of habitats.

A reasonable subgeneric grouping was worked out by Hepper (1958). Hutchinson and Dalziel (1956) have listed over 56 species in West Africa. Some difficulties exist in the classification of *Crotalaria* species above the species level and this is mainly due to the vast number of species and the reticulate nature of the interspecific variations. Many character differences e.g. flower colour, seed colour, leaf size etc. exist among different species but the different character states are combined in so many ways in the various species that it is difficult to define sizeable definitive natural groups. However, the character states are usually species specific.

It is generally believed that chemical identification of specific compounds will provide a greater insight into the relationships and differences between plant taxa (Swain, 1963). Such analyses have been carried out on other plants (Grant and Whetter, 1966; Alston, 1967). The amino acid profiles of a number of *Crotalaria* species have been studied (Polhill, 1968; Pilbeam and Bell, 1979; Pant and Fales, 1974). The presence of alkaloids in some species of *Crotalaria* have also been reported (Culvenor and Smith 1959).

Pilbeam, Polhill and Bell (1979) used chemical methods (electrophoresis) to characterize 163 species of *Crotalaria* of America, Asia and Australia. On the basis of their study, these species which hitherto posed classification problems were assigned positions in the classification of the genus. Polhill (1981) produced an improved classification of the African species of the genus but some discrepancies still exist in this classfication. The value of chemical analysis in *Crotalaria* genetics is great. This work is a contribution to the pool of information on the crude protein relationships of the dominant species of the genus *Crotalaria* in Nigeria. The work throws some light on the inter-specific relationship of the species studied.

MATERIALS AND METHODS

Seeds of the dominant species of *Crotalaria* were collected from various locations in Nigeria and bagged in small envelopes which were properly labelled. The colours of the seeds were recorded.

Ten grams of seeds of each species were ground in a porcelain mortar and shaken in 10 mls of 1.5M phosphate buffer (pH.8.5). The mixture was allowed to settle for ten minutes and then filtered. The supernatant was fractionated by disc electrophoresis following the method of Davis (1964).

The resolving gel consisted of polyacrylamide at a concentration of 7.5% containing 1M Tris—glycine buffer at a running pH of 8.3. The solutions were prepared as follows:

Acrylamide A	7.0% (10.00 cm^3)
Ammonium persuilfare	0.14M (o.30 cm^3)
Separation gel buffer (Tris = Hcl IM)	p^G 8.9 9(7.50 cm^3)
SDS	10% (0.30 cm^3)
TEMED	3.00% (0.10 cm^3)
Distilled Water	11.00 cm^3

Spacer gel:

Acrylamide A	7.0% (1.35 cm^3)
Ammonium persulfate	0.147 (0.10 cm^3)
Spacer gel buffer (Tris — Hc1 IM	p^H .87 (2.50 cm^3)
SDS	10% (0.10 cm^3)
TEMED	3.0% (0.10 cm^3)
Distilled water	6.00 cm^3.

TABLE 1

Number of protein bands and seed coat colour in eight species of Crotalaria L

Species	*Nubmber of Protein Band*	*Seed coat colour*
Crotalaria spectabillis	10	Black
C. donimaa	0	Yar/brow
C. retusa	6	Yellow/brown
C. comosa	7	Brown
C. nargutensis	10	Green
C. calycina	8	Greenish brown
C. Cylindrocarpa	6	Yellow
C. Goreensis	10	Reddish.

All solutions were prepared fresh and the gels were set in 75 mm x 5 bore runing tubes at 24°C (lagoratory temperature.

One ml of each sample were added to 1% SDS and 2—mercaptoethanol buffer solution. Two drops of 0.05% bromophenol blue stain were added astracers. The samples were then introduced into the gel tubes which were then fixed in the electrophoresis chamber.

Satisfactory separation was accomplished in about 2.5 hours at the running power of 1.5 UA per gel and when the dye fron was o.c5 cm from the anode end of the gels. At the end of the run, the gels were removed from the tubes with the aid of a syringe and fixed in 10 ml of 20 TCA for 10 minutes. Staining was done with 0.05% Coomassie Amido Black for about 2 hours. The gels were destained over 48 hours in several changes of methanol acetic acid solution' Photographs of the gels were taken and the schematic representation of the bands was made.

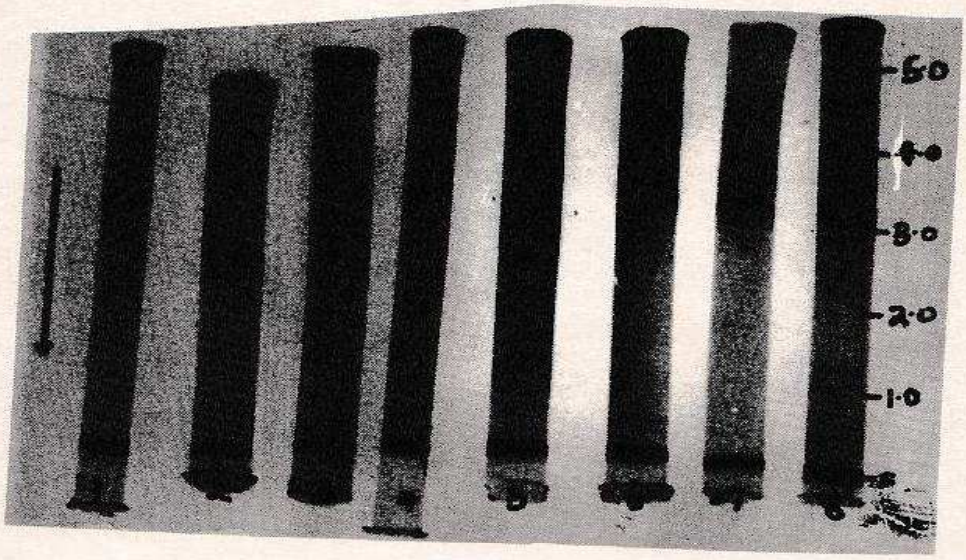

Figure 1 = Gel. electrophoresis of eight species of *Crotalaria L.*

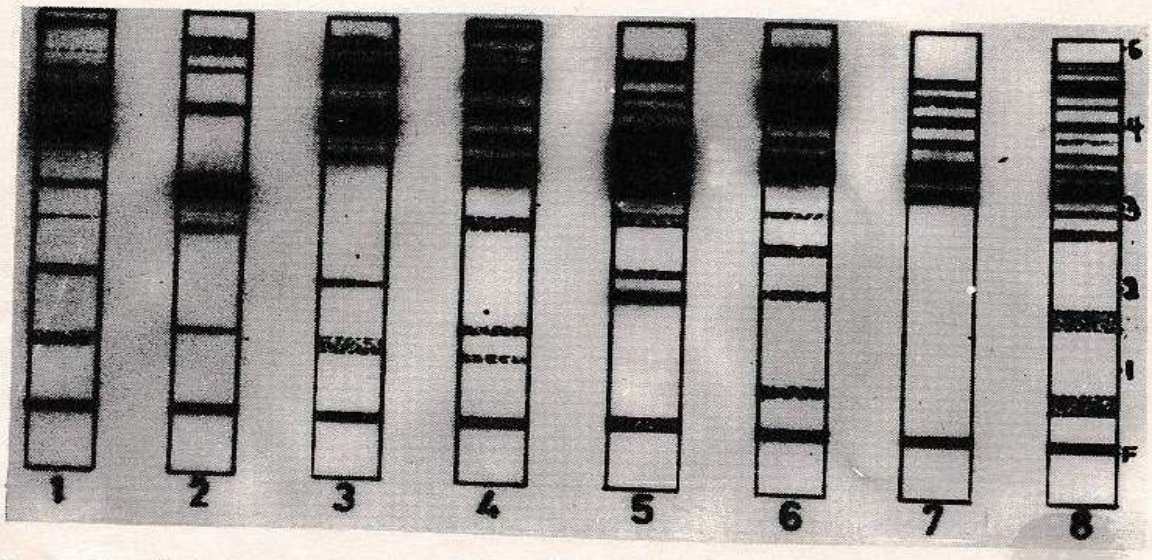

Figure 2 = Schemmatic representation of the gel patterns.

1 = *C. spectabilis;* 2 = *C. doniana;* 3 = *C. retusa;* 4 = *C. comosa;* 5 = *C. naragutensis* 6 = *C. cylindrocarpa;* 7 = *C. calycina;* 8 = *C. goreensis;*

F = dye front, Arrrow indicates direction of mobility Vertical numbers 1–5 reperesent graduation in cm.

RESULTS

The results of the crude protein fractionation from seeds of the eight species are shown in figures 1 and 2, A close examination of the bands shows that the different species have different patterns. Marked differences were recorded for number, position and intensity of bands between species. However, some similarities in bands were recorded between different species.

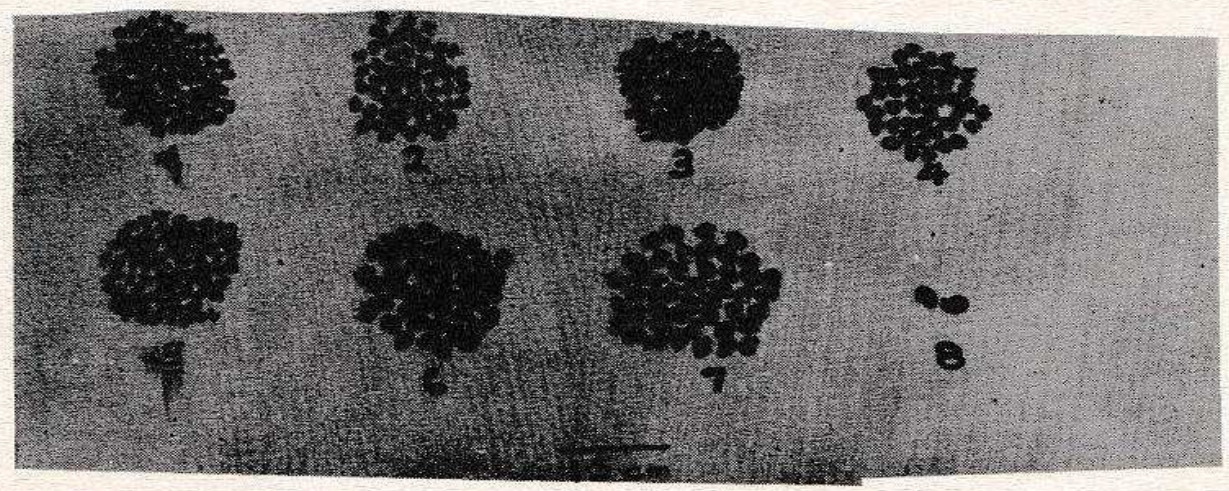

Figure 3 = Seed colour variation of eight species of *Crotalaria L.*

1 = *Crotalaria naragutensis;* 2 = *C. cylindrocarpa;* 3 = *C. comosa;* 4 = *C. retusa;* 5 = *C. goreensis;* 6 = *C. spectabilis;* 7 = *C. doniana;* 8 = *C. calycina* F = dye front.

Vertical number 1 – 5 represent graduations in cm.

Crotalaria spectabilis (Fig 1.1.), *C. retusa* (Fig 1.3) and *C. naragutensis* (Fig 1.5) have one common band in terms of tensity and position (4.5 cm) from the dye front. Similarly *C. cylindrocarpa* (Fig. 1.6) *C. goreensis* (Fig 1.8) and *C. calycina* (Fig 1.7) have a major band common to them about 3.4 cm from the dye front. Between *C. retusa* (Fig. 1.3) and *C. comosa* (Fig 1.4). there is one common band 4.6 cm and another 1.0 cm from the dye front.

The only major band in *C. doniana* (Fig 1.2) corresponds in position to a minor band 4.0 cm from the dye fronts in *C. spectabilis* (Fig 1.1), *C. naragutennis* (Fig 1.5) and *C. goreensis* (Fig 1.8).

The variation in number of bands is shown in Table I and the relative positions and intensity are schematically presented in Figure 2. Seed colour variation is presented in Table I and Figure 3 and shows a continuum of relationship. On the whole, there is a broad based relationship recognised among all the species except *C. calcine* which separates out as least related to the group.

DISCUSSION

The substances studied are proteins which are primary products of gene action, hence minimising the influence of environmental changes. Thus, the work attempts to get close to the observation of the genotype itself. The number of genes can be equated to the number of protein bands. The evidence from the variation in protein bands in this study indicates that the species are discrete with only broad based relationships occuring between them. Except for one

or two corresponding bands between one or two species, no definite patterns of variation among them can be deducted. However in terms of number of bands and mobility *C. calycina* (Fig 1.7) appears distinct from the remaining species. It lacks fast moving proteins and shows only six bands which are clustered at the cathode end of the gel.

The variation in seed colour (Fig 3) is probably due to accumulation of biochemicals controlled by genetic factors. concretely manifested in biochemical pathways which result in their production and accumulation. These pathways are characterised by specific sequential reactions directed by genes, the number of which vary with the number of reactions themselves (Birch, 1963).

From the evidences of the crude protein profile and seed colour assessment *C. spectabilis, C. retusa C. goreensis, C. domiana, C. cylindrocarpa, C. naragutensis* and *C. comosa* are more closely related while *C. calycina* is less related to the group.

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