GLOBAL JOURNAL OF PURE AND APPLIED SCIENCES VOL 14, NO. 3, 2008: 257 - 261

 ISSN 1118-0579

CRUDE PROTEIN ELECTROPHORESIS OF SEEDS OF TEN SPECIES OF *Solanum L*.

U. A. ESSIETT AND H. C. ILLOH

(Received 30, August 2007; Revision Accepted 27, March 2008)

ABSTRACT

Seeds of mature fruits of ten species of *Solanum* were collected from the gardens near the screen house, Botany Department, Obafemi Awolowo University, Ile Ife, Osun State, Nigeria. Crude seed proteins were extracted from them and characterised using polyacrylamide gel electrophoresis. Inter and intra specific qualitative and quantitative protein bands depicting some degree of relationships among the taxa were observed. The degree of variation in bands as a measure of genetic divergence of *Solanum* species is discussed. Electrophoretic mobility of seed proteins revealed different combinations of bands at various distances from the anode was taxon specific and could be used in separating *Solanum* species.

KEYWORDS: *Solanum* species, Polyacrylamide electrophoresis, Taxonomy.

INTRODUCTION

The family Solanaceae comprises 84 genera of which *Solanum* (L) is the largest and most whidespread with about 1,700 species in the world (Gbile, 1987). Heine (1963) listed 15 species of *Solanum* for Nigeria, but *S. clerodendroides* (Hutch. and Dalz.) has since been excluded as the record was based on erroneous label (Gbile, 1987).

The genus contains cultivated and wild species. The cultivated species are sources of very important, leafy vegetables and edible fruits which are rich in proteins, minerals and vitamins (Oke, 1965 and Oyenuga, 1968).

The potential value of electrophoretic patterns in the systematic of plants was discussed exhaustively by Boutler *et al*. (1966). Odetola *et al*. (2004) reported Hypolipidaemic potentials of *Solanum melongena* and *Solanum gilo* on Hypercholesterolemic rabbits. Illoh *et al*. (1993) carried out an electrophoretic study of protein diversity in the seeds of the genus *Sida* in Nigeria. Inyang (1992) worked on electrophoretic characterization of crude proteins in some species. Akpabio (1988) also reported crude protein electrophoresis of seeds of eight species of Crotalaria L. Essiett (1996) on taxonomic studies of some Nigerian *Capsicum* species and varieties.

Gel electrophoresis has shown that many isoenzymes and polymorphic proteins are widely distributed in plants (Cherry and Ory, 1972). Electrophoresis has an advantage that it can directly equate variation in protein banding patterns to genes coding various proteins (Gottlieb, 1971).

The present paper attempts to employ gel electrophoresis of crude proteins in the seeds of *Solanum* species to evaluate the established taxonomic relationships among the species occurring in Nigeria.

MATERIALS AND METHODS

Seeds of mature fruits of the taxa were collected from the garden near the screen house, Botany Department, Obafemi Awolowo University, Ile Ife, Osun State, Nigeria. One gramme of the seeds of each taxon was ground in 5ml of 0.9% sodium chloride solution. The mixtures were allowed to settle inside test tubes immersed in an ice-bath for about one to one and a half hours. The supernatants were filtered and centrifuged for 15 minutes at 10,000 rpm. The supernatants obtained from centrifuging were 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 10% in 1m Tris-glycine buffer at pH 8.3. The gels were prepared as shown in Table 1.

Table 1: Composition of Polyacrytamide Gels (Volume in cm^3)

Chemical	Stacking Gel Upper Gel	Lower Gel
Acrylamide A.	1.35	13.53
Upper gel buffer (4x)	2.50	-
Lower gel buffer (4x)	-	7.50
Distilled water	6.00	8.57
10% Sodium Lauryl sulphate (SDS)	0.10	0.30
Ammonium per sulphate	0.10	0.30
TEMED	0.01	0.03

Source: Ayeni, 1984.

U. A. Essiett, Department of Botany and Ecological Studies, University of Uyo, Uyo Akwa Ibom State, Nigeria.
H. C. Illoh, Department of Botany, Obafemi Awolowo University of IIe Ife, Osun State, Nigeria.

Half a ml of each extract was mixed with sucrose crystals, 3 drops of 1% 2-mercaptoethanol and 10% SDS and 1 drop of Bromo phenol and warmed in a water bath for 10 minutes. On cooling to room temperature 3 drops of 10% SDS, 1% 2-mercaptoethanol and sucrose crystals were added again to each sample to weigh down the protein molecules. This was followed a drop of 0.05% bromophenol which served as tracer dye. The resultant mixture (4 drops) was added directly to gels. The tubes were placed in column acrylamide gel apparatus with tris-glycine buffer in both upper and lower vessels.

A constant current of 1½mA per gel was applied and after the protein was stacked in the lower gel, the current was increased to 3ml per gel. The current was stopped when the dye front was about 1.0cm from the bottom of the gel. The gels were removed from the tubes and stained with 0.05% Coomasia brilliant blue after which distaining was commenced at intervals of 3 hours four 24 – 36 hours.

Photographs of the gels were taken and schematic diagram were also drawn.

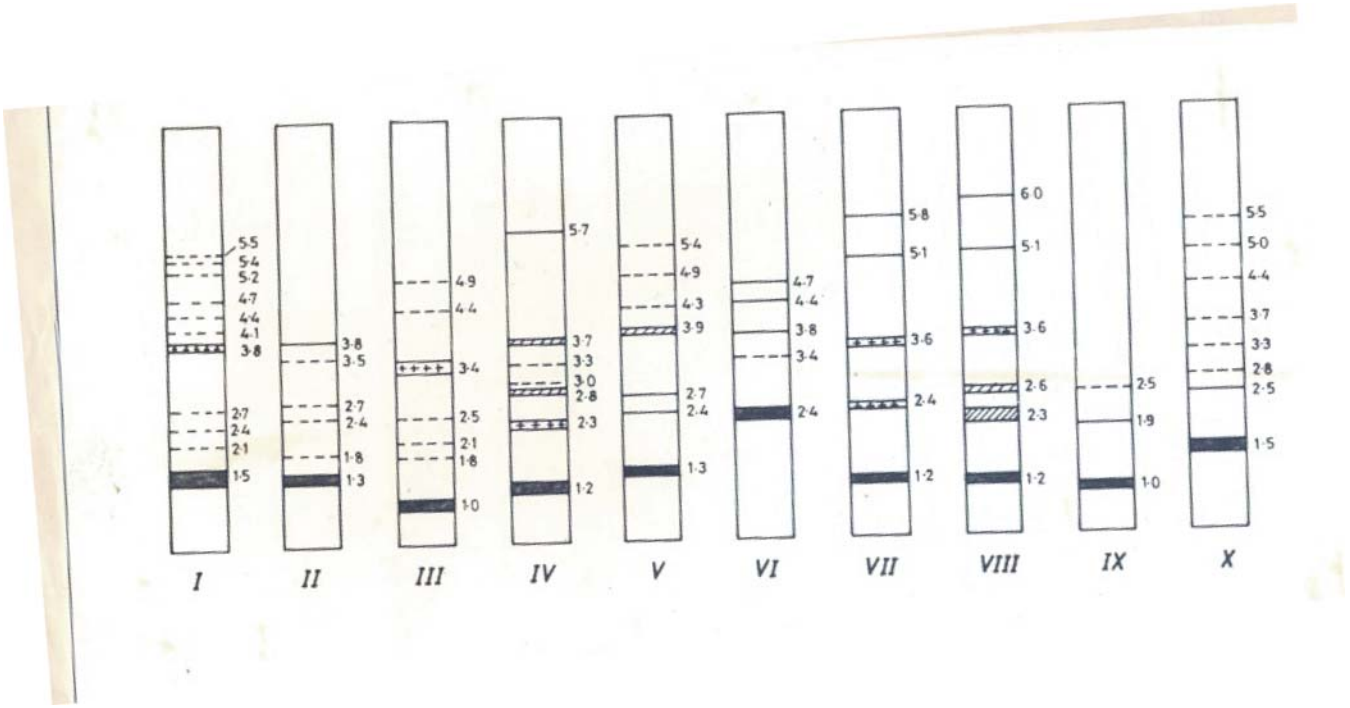
RESULT

Plate 1 and Fig. 1 show protein band similarities among the *Solanum* species. From the Figure, the highest number of interspecific bands (four) for section *Oliganthes* subgenus *Leptostemonum* were recorded for the pairs *S. aethiopicum* (Fig. 1(i) and *S. gilo* (Fig. 1(iv) at 2.4cm, 3.8cm, 4.4cm and 4.7cm. *S. aethipicum* (Fig. 1(i) share three bands with *S. aethiopicum* x *S. gilo* (Fig. 1(v) at 2.4cm, 4.4cm and 5.4cm. Similarly, two bands are shared between *S. aethipicum* (Fig. 1(i) and *S. aethipicum* x *S. dasyphyllum* (Fig. 1(iii) at 2.1cm and 4.4cm respectively. *S. aethiopicum* x *S. dasyphyllum* (Fig. 1(iii) has two bands in common with *S. gilo* (ex Jos) (Fig. 1(vi)) at 3.4cm and 4.4cm. Only one common band was fond between *S. aethipicum* x *S. gilo* (Fig. 1(v)) and *S. gilo* (ex Jos) (Fig. 1(vi)) at 2.4cm. The bands at 1.3cm, 3.9cm, 4.3cm and 4.9cm are unique at *S. aethiopicum* x *S. gilo* (Fig. 1(v)).

In the section *Melongena* subgenus *Leptostemonum* three common bands were recorded for the pair *S. macrocarpon* (Fig. 1(vii)) and *S. melongena* (Fig. 1(viii) at 1.2cm, 3.6cm and 5.1cm respectively.

In the section *Torva* subgenus *Leptostemonum*, *S. torvum* (Fig. 1(x)) and *S. anomalum* (Fig. 1(ii)) have no band in common but there are some bands in *S. torvum* (Fig. 1(x)) at 3.7cm, 2.8cm and 2.5cm which arae close to some of those bands in *S. anomalum* (Fig. 1(i)) at 3.8cm, 2.7cm and 2.4cm.

Inter-sectional bands common to species in sections *Melongena* and *Oliganthes* were also observed. One band was common to *S. aethipicum* (Fig. 1(i)) and *S. macrocrapon* (Fig. 1(vii)) at 2.4cm, while *S. aethiopicum* x *S. gilo* (Fig. 1(v)) has a band at 2.4cm common with *S. macrocarpon* (Fig. 1(vii) but this band is also common to *S. gilo* (Fig. 1(vi) and *S. macrocarpon* section *Melongena* (Fig. 1(vii)). Three bands at 1.5cm, 4.4cm and 5.5cm are common between *S. torvum* (Fig. 1(x)) section *Torva* and *S. aethiopicum* (Fig. 1(i)). Also, two bands at 2.5cm and 4.4cm were recorded for *S. torvum* (Fig. 1(x)) section *Torva* and *S. aethiopicum* x *S. dasyphyllum* (Fig. 1(iii)) section *Oliganthes*. Only one band at 4.4cm is common to *S. torvum* (Fig. 1(x)) section *Torva* and *S. gilo* (ex Jos) Fig. 1(vi) section *Oliganthes*. Three common bands were observed at 2.8cm, 3.3cm and 3.7cm in both *S. erianthum* (Fig. 1(iv)) section *Brevantherum* subgenus *Brevantherum* and *S. torvum* (Fig. 1(x)) section *Torva*. *S. erianthum* section *Brevantherum* also has band shared between *S. melongena* (Fig. 1(viii)) section *Melongena* at 1.2cm and 2.3cm but a band at 1.2cm common to *S. macrocarpon* (Fig. 1(vii) and both of them belong to section *Melongena*. Band at 2.5cm common to *S. nigrum* subsp *nigrum* (Fig. 1(ix)) section *Solanum* subsection *Morella* subgenus *Solanum* and *S. torvum* (Fig. 1(x)) section *Torva*.



(I) *S. aethiopicum*; (II) *S. anomalum*; (III) *S. aethiopicum* x *S. dasyphyllum*; (IV) *S. erianthum*; (V) *S. aethiopicum* x *S. gilo*; (VI) *S. gilo*; (VII) *S. macrocarpon*; (VIII) *S. melongena*; (IX) *S. nigrum* subsp. *nigrum*; (X) *S. torvum*.

Fig. 1: Diagrammatic representaqtion of stained protein in bands (cm) recognised during gel electrophoresis of seed extracts. Details of names of plants I – X as follows:

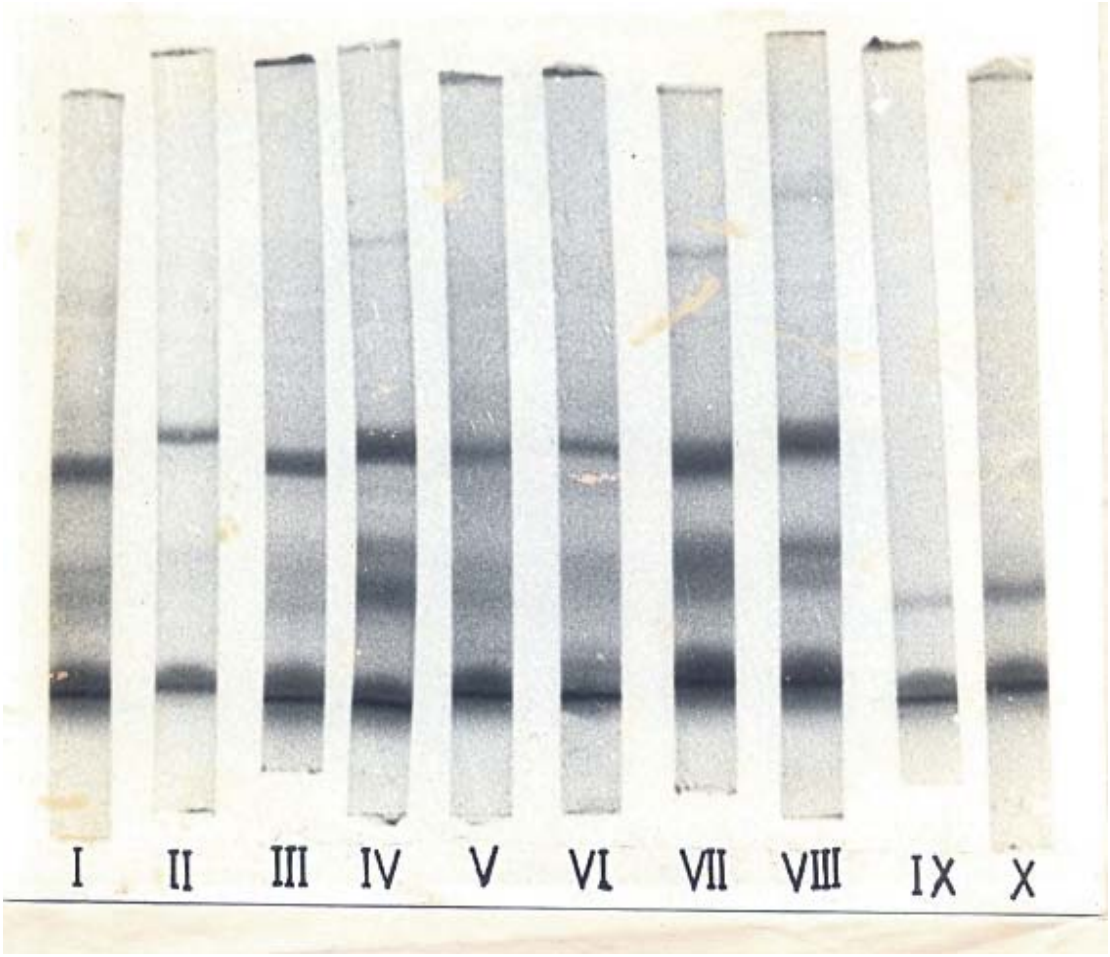


Plate 1: Gels showing band distribution in the proteins seed extract of 10 species of *Solanum*. Details of names of plants I – X as follows:

(I) *Solanum aethiopicum*; (II) *S. anomalum*; (III) *S. aethiopicum* x *S. dasyphyllum*; (IV) *S. erianthum*; (V) *S. aethiopicum* x *S. gilo*; (VI) *S. gilo* (ex Jos); (VII) *S. macrocarpon*; (VIII) *S. melongena*; (IX) *S. nigrum* subsp. *nigrum*; (X) *S. torvum*.

In the section *Oliganthes*, three species (*S. aethipicum*, *S. aethiopicum* x *S. gilo* and *S. gilo* (ex Jos) share a common band at 2.4cm. the band at 4.4cm was observed for *S. aethipicum* (Fig. 1(1)), *S. aethipicum* x *S. dasyphyllum* and *S. gilo* (ex Jos). The bands at 2.4cm and 4.4cm are most widespead in the taxa. The bands at 2.4cm, 2.7cm and 3.8cm is common to *S. anomalum* (Fig. 1(ii)) and *S. aethiopicum* (Fig. 1(i)) while *S. anomalum* (Fig. 1(ii)) shares a band at 1.8cm with *S. aethiopicum* x *S. dasyphyllum* (Fig. 1(iii)) and shares three bands at 1.3cm, 2.4cm and 2.7cm with *S. aethiopicum* x *S. gilo* (Fig. 1(v)) and also shares two baqnds with *S. gilo* (Fig. 1(vi)) at 2.4cm and 3.8cm. The band at 2.4cm was recorded in the three sections (*Melongena*, *Oliganthes* and *Torva*) of the subgenus *Leptostemonum*. Table 3 is presented to illustrate the number of interspecific band relationships observed among the taxa.

Table 2: Relative mobilities of seed protein bands of *Solanum* spp on polyacrylamide gels

	Name of Species	Total No. of Bands	Fast Bands (0-2.50 cm)	Inter-mediate Bands (2.60 – 5.0cm)	Slow Bands 5.1cm and above	Unique Band (cm)
I	*Solanum aethiopicum*	11	3	5	3	(2) 5, 2, 4.1
II	*S. anomalum*	6	3	3	0	(1) 3.5
III	*S. aethiopicum* x *S. dayphyllum*	7	4	3	0	-
IV	*S. erianthum*	7	2	4	1	(1) 5.7
V	*S. aethiopicum* x *S. gilo*	7	2	4	1	(2) 4.3, 3.9
VI	*S. gilo* (ex. Jos)	5	1	4	0	-
VII	*S. macrocarpon*	5	2	1	2	(1) 5.8
VIII	*S. melongena*	6	2	2	2	(2) 2.6, 6.0
IX	*S. nigrum* subsp. *Nigrum*	3	3	0	0	(1) 1.9
X	*S. torvum*	8	2	5	1	(1) 5.0
	Total	**65**	**24**	**31**	**10**	

Table 3: Intra and interspecific protein band relationships among the *Solanum* spek./cies

		I									
I	*Solanum aethiopicum*	-	II								
II	*S. anomalum*	3	-	III							
III	*S. aethiopicum* x *S. dayphyllum*	2	1	-	IV						
IV	*S. erianthum*	-	-	-	-	V					
V	*S. aethiopicum* x *S. gilo*	3	3	-	-	-	VI				
VI	*S. gilo* (ex. Jos)	4	2	2	-	1	-	VII			
VII	*S. macrocarpon*	1	1	-	1	1	1	-	VIII		
VIII	*S. melongena*	-	-	-	2	-	-	3	-	IX	
IX	*S. nigrum* subsp. *Nigrum*	-	-	2	-	-	-	-	-	-	X
X	*S. torvum*	3	-	2	3	-	1	-	-	1	-

DISCUSSION

These investigations revealed that electrophoresis can be used for identification of some Solanum species. Hutchinson and Dalziel (1963) and Gbile (1979, 1985) used vegetative and floral qualitative and quantitative characters while Metcalfe and Chalk (1950) used anatomical characters for identification of some *Solanum* species. These characters may be prone to environmental variation within the same species but electrophoresis can take care of these variations, since protein structure are hardly affected by environmental factors.

The patterns of protein distribution in different species of *Solanum* studied are presented in Plate 1(I – X) and Figs. 1(i – x). Observations from the results show that there are both qualitative and quantitative variations in the number, position and intensity of bands. The bands range from three to eleven. Most of the bands manifested in the runs were found to be intermediate in movement (2.60cm – 5.0cm) follwoed by faqst moving bands (0cm – 2.5cm) and few slow-moving bands (5.1cm and above). Table 2 shows that all species in the genus *Solanum* studied,; except *S. aethiopicum*, *S. dasayphyllum* and *S. gilo* (ex Jos) recorded unique bands. Some bands were found common to two or more species.

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

The concept of common bands has been reported by Dass and Nybom (1967) that “biochemical distances” among species of known genetic relationship are measures of affinity.

The band at 2.4cm which is common to species of the three sections *Melongena*, *Oliganthes*, and *Torva* of the subgenus *Leptostemonum* is specific for the subgenus. Also, the band at 4.4cm is specific for the section *Oliganthes*. The two species (*S. macrocarpon* and *S. melongena*) in the section *Melongena* have three common bands. The presence of common bands among the various species of *Solanum* shows evidence of common evolutionary origin. Secondly, coming from the same parental stock their evolution is convergent, thereby making it possible for character traits to be shared in common. These results are in consonance with 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.

Omidiji (1982) have shown by crossability tests that *S. anomalum* showed close affinity to series *Aethiopica* (section *Oliganthes*) than with other members of section *Torva*. Pearce and Lester (1979) also showed this affinity serologically. In this work, *S. anomalum* in the same section with *S. torvum* shared bands in common with members of section *Oliganthes* and had none common with *S. torvum*. With evidence from this work supporting earlier findings of Omidiji (1982) and Pearce and Lester (1979), *S. anomulum* should be transferred to section *Oliganthes* as proposed by these two authors.

S. erianthum section Brevantherum subgenus *Brevantherum* has three bands in common with *S. torvum* section *Torva* at 2.8cm, 3.3cm and 3.7cm. This shows that there may be genetic affinity between them because they possess shrubby habit, globose fruits, cymose inflorescence, ovate leaf shape, single arc-shaped amphicribral bundle in the tranverse section of the midrib and cortical sclerenchyma in the transverse section of the stem.

Omidiji (1975, 1983, 1986) reported that hybridization can take place among some *Solanum* species. This therefore may lead to reticulate transfer of traits (protein inclusive) from one species to the other and this may explain the common bands relationships observed among the species.

From the above results, bands with identical electrophoretic mobilities represent proteins with identical amino acid sequences and are therefore potentially homologous in their derivations (Scogin, 1972). Although this contradicts Shaw (1965) that two different molecules can migrate to the same point and result in some mistaken identity, electrophoresis can be reasonably used in the taxonomic studies of the genus *Solanum*.

Investigations based on electrophoretic techniques from the ten species of *Solanum* show some significant differences that can be used for classificatory and identification purposes.

Evidence of the relationships of the species of *Solanum* from the electrophoretic studies shows that the band at 2.4cm and 4.4cm are most widespread in the taxa but the former was recorded in the three sections (*Melongena*, *Oliganthes* and *Torva*) of the subgenus *Leptostemonum*. The present experimental investigations have indicated that *S. anomalum* (section *Torva*) is most closely related to series *Aethiopica* (section *Oliganthes*) than to section *Torva* where it is now placed.

On this basis of the evidence from electrophoresis, it seems necessary to reconsider the taxonomic position of *S. anomalum*.

The present deductions from this study are not intended to be a more natural classification of *Solanum*, but only represents a possibly more, realistic interpretation to enhance the electrophoretic similarities and differences as found in some Nigerian *Solanum*.

REFERENCES

Akpabio, K. E., 1988. Crude protein electropheresis of seeds of eight species of *Crotalaria* L. Nigerian Journal of Botany, 1: 106 – 110.

Ayeni, M. A., 1984. Quarternary structure of *kinizys erosa* haemoglobin. M. Sc. (Biochemistry) *Thesis* submitted to Obafemi Awolowo University, Ile-Ife (formerly University of Ife).

Boutler, D., Thurman, D. A. and Turner, B. C., 1966. The use of electrophoresis in plant protein systematics. *Taxon* 15(4):135 – 143.

Cherry, J. P. and Ory, R. T., 1972. Electrophoresis of six selected enzymes of peanut cultivars. *Phytochemistry*. 12: 283 – 289.

Dass, H. and Nybom, N., 1967. In Chemotaxonomic analysis of some ecotypes in *Mentha X verticillata* Complex (Labiatae). *Botaniska Notiser*. 120: 255 – 267.

Davis, B. J., 1964. Disc Electrophoresis II: Method and application to human serum proteins. Annals of New York Academy of Science 121: 404 – 407.

Essiett, U. A., 1996. Taxonomic studies of some Nigerian Capsicum species and varieties. Trans. Nig. Soc. Bio. Conserv, 4, (1):11 – 18.

Gbile, Z. O., 1979. *Solanum* in Nigeria. The Biology and Taxonomy of the Solanaceae (ed. J. G. Hawkes, R. N. Lester and A. D. Skelding). Academic Press, London, Ch. 4, 113 – 120.

Gbile, Z. O., 1985. Taxonomic Study in Nigerian Species of *Solanum* Linn. I studies of the generic and infrageneric statuses. Nigerian Journal of Science 19(1 & 2): 57 – 61.

Gbile, Z. O., 1987. Nigerian *Solanum* species. In the Nigerian Field, 52 (Parts 1 – 2): 19 – 26.

Gottlieb, L. D., 1971. Gel electrophoresis: New approach to the study of evolution. Bioscience 21(18): 939 – 944.

Heine, H., 1963. Solanaceae. In Flora of West Tropical Africa (ed. Hutchinson, J. and J. M. Dalziel; 2nd ed.; edited by F. N. Hepper), Vol. II, pp. 325 – 335, published by the Governments of Nigeria, Ghana, S. Leone and Gambia.

Hutchinson, J. and Dalziel, J. M., 1963. In Flora of West Tropical Africa. Clarendon Press, Oxford. Vol. II, pp. 325 – 335.

Illoh, H. C., Ijogun, O. O. and Bakare, O. A., 1993. An electrophoretic study of protein diversity in the seeds of the genus sida in Nigeria. Nigerian Journal of Botany, 6, 13 – 20.

Inyang, U. E., 1992. Biosystematic Studies of Some Nigerian *Solanum* Species. M.Sc. Thesis submitted to Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria.

Metcalfe, C. R. and Chalk, L., 1950. *Solanaceae*. Anatomy of the Dicotyledons, Vol. II. Oxford University Press, pp. 965 – 978.

Odetola, A. A., Irantoye, Y. O. and Akinloye, O., 2004. Hypolipidaemic potentials of *Solanum melongena* and *Solanum gilo* on Hyperchotesterolemic rabbits. Pakistan Journal of Nutrition. 3(3): 180 – 187.

Oke, O. L., 1965. Chemical studies of some Nigerian vegetable. Expl. Agric. 1: 125 -129.

Olsson, U., 1967. Chemotaxonomic analysis of some ecotypes in *Mentha X verticillata* Complex (Labiatae). *Botaniska Notiser*. 20: 255 – 267.

Omidiji, M. O., 1975. Interspecific hybridization in the cultivated, non-tuberous *Solanum* species. *Euphytica* 24: 341 – 353.

Omidiji, M. O., 1982. Interrelationships of *Solanum* species in different series of the subgenus *Leptostemonum* (Dun.) Bitt, Hort. Res. 22: 3 – 12.

Omidiji, M. O., 1983. Cytomorphological Studies of *S. aethiopicum x S. macrocarpon* F. hybrid. *Cytologria* 48: 35 – 40.

Omidiji, M. O., 1986. The role of hybridization in the evolution of species in *Solanum* subgenus *Leptosttemonum*. In *Solanaceae:* The Biology and Systematics. Edited by William C. D'Arcy. Columbia University Press, New York, Chap. 33: 469 – 476.

Oyenuga, V. O., 1968. Nigerian food and feeding stuffs. Their composition and nutritive value. 3rd edition. Ibadan: University Press.

Pearse, K. and Lester, R. N., 1979. Chemotaxonomy of the cultivated egg plants; a new look at the taxonomic relationships of *S. melongena* Linn. In 'The Biology and Taxonomy of the Solanaceae" (ed. J. G. Hawkes, R. N. Lester and A. D. Skelding). Academic Press, London, Ch. 48, 615 – 628.

Scogin, R., 1972. Protein in the genus lithops (Aizoaceae): Developmental and comparative studies. Journal of African Botany, *39*(1): 55 – 61.

Shaw, E. R., 1965. Electrophoretic variations in enzymes. Science 149: 936 – 943.