NJB, Volume 34 (2), December, 2021

CRUDE PROTEIN ELECTROPHORESIS OF SIX SPECIES OF ASPLENIUM L.

[1]Bamigboye, R. A. and [2]Oloyede, F. A.
[1]Natural History Museum, Obafemi Awolowo University, Ile-Ife, Nigeria
[2]Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria
Correspondence: bamigboyeadebola@yahoo.com

Received 18th November; accepted 5th December, 2021

Abstract

Protein electrophoresis of leaflet was carried out using six species of *Asplenium*, to separate and identify different protein bands in each species. Young leaflets of each species were collected from Southwestern Nigeria for gel electrophoresis. The leaflets were washed with distilled water, macerated with sterile mortar and pestle in 7.5 ml of 0.9% NaCl. Schematic electrophoregram of the electrophoretic gel of the six species showing clear protein profile with different protein bands was drawn. The distribution of protein bands was divided into three types which include the slow - moving band, intermediate-moving band and fast-moving band. The unique bands that distinguished each species from one another and interspecific bands which two or three species had in common were identified. All the species had 0.6 band with the same intensity except *A. barterii*. The dendrogram showed that among the six taxa *A. barterii, A. monanthes* and *A. unilaterale* were clustered into one main group, indicating that they are closely related. *A. nidus, A. sceipei* and *A. scolopendrium* were in a different cluster and that they are distantly related to the other species. Leaflet electrophoresis revealed taxonomic characters which showed the relationship among the taxa studied.

Key words: Electrophoretic gel; generic bands; leaflets; protein bands; protein layers; taxonomic characters
https://dx.doi.org/10.4314/njbot.v34i2.9

INTRODUCTION

The genus *Asplenium* is the largest and most prominent genus in the family Aspleniaceae. It is one of the largest fern genera worldwide with 700 species widely distributed in the tropics (Reichstein, 1981). Oloyede and Odu (2011) reported seven species of *Asplenium* in Southwestern Nigeria. The six species of *Asplenium* studied include: *Asplenium barterii, A. monanthes, A. nidus, A. scheipei, A. scholopendrium* and *A. unilaterale.* Some species of *Asplenium* are commonly used in horticulture as ornamental and decorative plants; some local people use it in worship and in folk medicine to treat asthma. Maidenhair spleenwort (*Asplenium trichomanes*) is used as cough medicine. The sprout of *Asplenium nidus* is edible (Nwosu, 2002; Oloyede, 2012).

Gel electrophoresis is a useful diagnostic tool for preliminary experimentation of more complicated analytical project. The major advantage is that it is a visual method of confirming the presence of DNA, RNA, protein or specific sizes of these molecules unlike other detection methods that give the precise quantity but not the actual size (Gordon *et al*., 2001). Gel electrophoresis has an advantage of equating variation in protein banding patterns to genes coding of various proteins. It is used in the characterisation of the amount and type of some genes, thereby establishing taxonomic classification. Denaturing gel electrophoresis is often more accurate for size identification, whereas native gel electrophoresis is usually used to identify larger protein complexes. Native gel electrophoresis usually keeps RNA or protein in its native structure while running it through a gel. Denaturing gel electrophoresis attempts to reduce the RNA or protein into its most linear structure before or during gel electrophoresis. In gel electrophoresis where separation of proteins is carried out, sodium dodecyl sulphate (SDS) electrophoresis is always encountered. This shows how widespread SDS electrophoresis is in proteomics. It can be used as the sole protein separation technique for digestion (Lasonder *et al*., 2002; Schirle *et al*., 2003), ion exchange chromatography (Szponarski *et al*., 2004), denaturing electrophoresis (Macfarlane, 1989; Rais *et al*.,

2004) and isoelectric focusing for high-resolution 2D electrophoresis. In addition to promoting protein denaturation, SDS also confers a very strong negative charge on proteins. In systematic research on ferns, cytotaxonomical and enzyme electrophoretic data have revealed cryptic species in many lineages such as *Adiantum, Botrichium* and *Pityrogramma* species (Wagner and Wagner, 1989). Recently, it has become easier to collect DNA nucleotide sequence data from wild plants using polymerase chain reaction (PCR) and direct sequencing techniques. The characters of DNA sequences might be useful in discovering cryptic species in ferns especially in *Asplenium*. This study was aimed to show protein layers and to determine various types of protein bands of six species of *Asplenium* studied, and to use the differences and similarities in the protein contents as taxonomic indices.

MATERIALS AND METHODS

Mature, healthy, fresh leaflets of six species of *Asplenium* were collected from different locations in southwestern Nigeria. The reagents were prepared according to the required concentrations and stored in the refrigerator. The species were screened for total protein banding patterns by using the modified method described by Aguegia *et al*. (1994), Omitogun *et al*. (1999) and Tokpo *et al*. (2006). The leaflets of each of the six species were washed with distilled water, air-dried in the laboratory and macerated with sterile mortar and pestle.

From the macerated leaflets, 2.0 g of the leaflets of each of the six species was homogenised in 7.5 ml of 0.9% of NaCl with a porcelain mortar. The crude homogenate extracts were centrifuged at 3000 revolutions per minutes (rpm) for 15 minutes to remove cellular debris. The resultant supernatants were poured into sampling bottles, identified and analysed in five replicates. The mixture was left for proper extraction for 24 hours and kept in the refrigerator till use.

From each protein supernatant, 1.0 g was measured with a pipette into 1.5 ml micro tubes. Four drops of 10% 2-mercaptoethanol was added, heated in boiling water bath at 95°C for five minutes and introduced into each gel tube. The reducing agent, 2-Mercaptoethanol, was used. It breaks disulphide bridge before they adopt the random-coil centrifugation necessary for separation by size and to expose the protein to sodium dodecyl sulphate (SDS) action. The heating was to allow for proper separation of the polypeptide chains and complete denaturation. Glycerol and bromophenol blue were added to the solution on cooling. Glycerol was added due to its high molecular weight which increases the density of protein extract and makes it denser than the running buffer. Glass gel tube and single gel with ten walls were used to prepare 4% stacking gel and 10% running gel for SDS-PAGE (Table 1). A single gel was prepared by mixing the component solution in the correct proportions. N,N,N,N-Tetramethylethyldiamine (TEMED) and ammonium persulphate (APS) were added to the mixture for polymerisation of the acrylamide monomers. The gel was allowed to polymerise for another 30 minutes. Table 2 shows composition of the buffer solution used for 10% SDS-PAGE.

The composition was dissolved in distilled water and made up to 600 ml, stored at 4°C and warmed up to room temperature before it was used. The separation of protein was carried out with the aid of cleaver electrophoresis power supply Model CS-300V at 150 Volts for 45 minutes. Electrophoretic run was done with cleaver scientific electrophoresis pack which was connected to the power supply and run at 150 V for three hours. The schematic electrophoregram representing the six taxa studied was drawn. Sokal and Sneath (1963) was used to calculate the similarity indices for all the taxa studied.

TABLE 1: Composition of solution for stacking gel and running gel

STOCK SOLUTIONS	RUNNING GEL	STACKING GEL
Acrylamide/Bis Acrylamide	4.5 ml	0.45 ml
Tris buffer (1.5M Tris HCl, PH 8.8)	2.5 ml	-
Tris buffer (0.5M Tris HCl, PH 6.8)	-	0.8 ml
Distilled water	3.5 ml	2.0 ml
10% Ammonium persulphate	50 µl	18 µl
TEMED	10 µl	10 µl

Table 2: Composition of buffer solution used for 10% SDS-PAGE

Sample Buffer	SDS-reducing Buffer (ml)	Running Buffer (g)
Deionized water	3.8	Tris base 9.0
0.5M Tris-HCl, PH 6.8	1.0	Glycine 72.2
Glycerol	8.0	SDS 3.0
10% (W/V) SDS	1.6	
1% (W/V) Bromophenol blue	0.4	

RESULTS

Results showed that the *Asplenium* species studied have varied protein bands (Table 3). The migration of Protein bands was divided into three parts: slow-moving bands, ranged from 0-2.0 cm; the intermediate-moving bands ranged from 2.1-4.0 cm and the fast-moving bands ranged from 4.1-6.0 cm. The gel of the leaf protein electrophoresis of the six species is presented in the schematic diagram as shown in Figure 1. The pattern of protein banding in the six *Asplenium* species revealed a total of 21 bands (Table 4). The protein banding in each species was species-specific as no two species had exactly the same profile. Distinct differences were observed not only in the number of bands and combination of bands but also in the intensity of bands. The results further showed that seven bands (33.3%) were fast-moving; three bands (14.2%) were intermediate in movement while 11 bands (52.4%) were slow-moving. A generic band occurred at 0.6 with the same intensity in all the taxa except *Asplenium barterii* which had only one protein band at 1.3. Apart from the generic band, interspecific bands were widespread in the different taxa as observed in the bands at 1.3 (2 species), 4.5 (2 species), 5.4 (3 species); unique bands occurred at 4.3, 2.6, 1.5, 3.4, 4.6, 2.0, 1.2, 2.4 and 1.8. (Figure 1). The highest number of interspecific bands (5.4) was observed in *A. nidus*, *A. schelpei* and *A. scolopendrium.*

The result of the dendrogram showed that there was only one main cluster which contained *A. barterii, A. monanthes* and *A. unilaterale* indicating that they are closely related while the other three groups (comprising *A. nidus*, *A. schelpei* and *A. scolopendrium*) are distantly related (Figure 2). Sokal and Sneath co-efficient of similarity values (Table 5) for the six species of *Asplenium* in this study ranged from 12.5% (between *A. nidus* and *A. barterii* and *A. scheipei* and *A. nidus*); 14.3% (between *A. scolopendrium* and *A. barterii*); 20% (between *A. unilaterale* and *A. barterii*) as well as *A. unilaterale* and *A. scolopendria*, respectively.

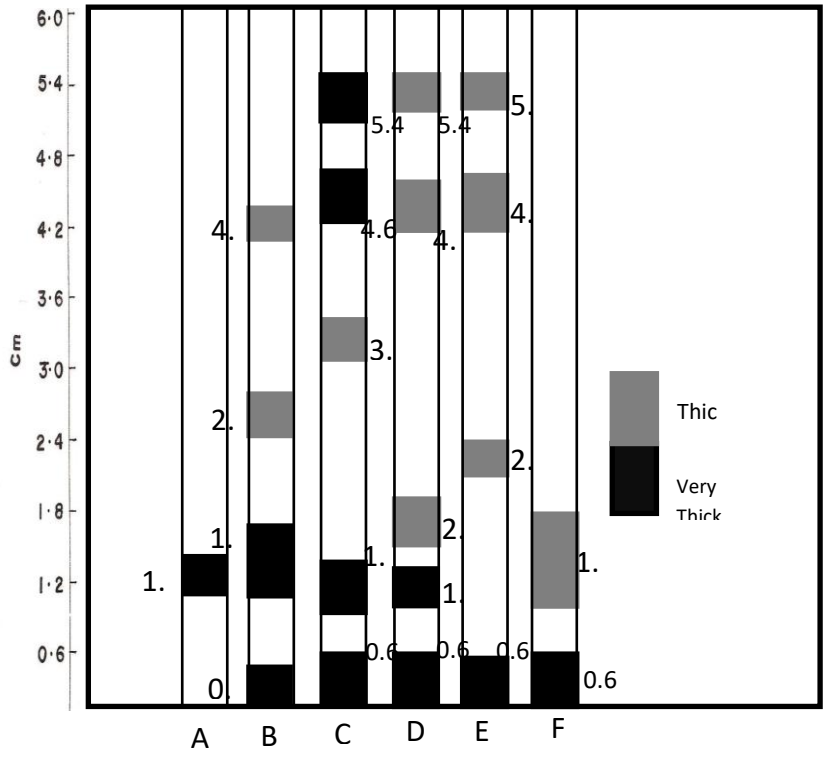

Fig 1: Schematic electrophoregram of six species of *Asplenium*
A*: A. barteri,*B: *A. monanthes,* C: *A. nidus,* D: *A. scheipei,* E: *A. scolopendrium*, F: *A. unilaterale*

Table 3: Relative mobility of the six species of *Asplenium*

SPECIES	Slow-moving bands (0-2.0 cm)	Intermediate moving bands (2.1-4.0 cm)	Fast-moving bands (4.1-6.0 cm)	Total number of bands	Unique bands
A. barteri	2	1	1	4	3
A. monanthes	1	-	-	1	-
A .nidus	2	1	2	5	2
A. scheipei	3	-	2	5	2
A .scolopendrium	1	1	2	4	1
A. unilaterale	2	-	-	2	1
Total	11	3	7	21	

Table 4: Number of common bands between pairs of *Asplenium* species

SPECIES	*A.barteri*	*A.monanthes*	*A. nidus*	*A.scheipei*	*A.scolopendrium*	*A.unilaterale*
A. barteri	-	-	1	1	1	1
A. monanthes	-	-	1	-	-	-
A .nidus	1	1	-	2	2	1
A. scheipei	1	-	2	-	3	1
A. scolopendrium	1	-	2	3	-	1
A. unilaterale	1	-	1	1	1	-

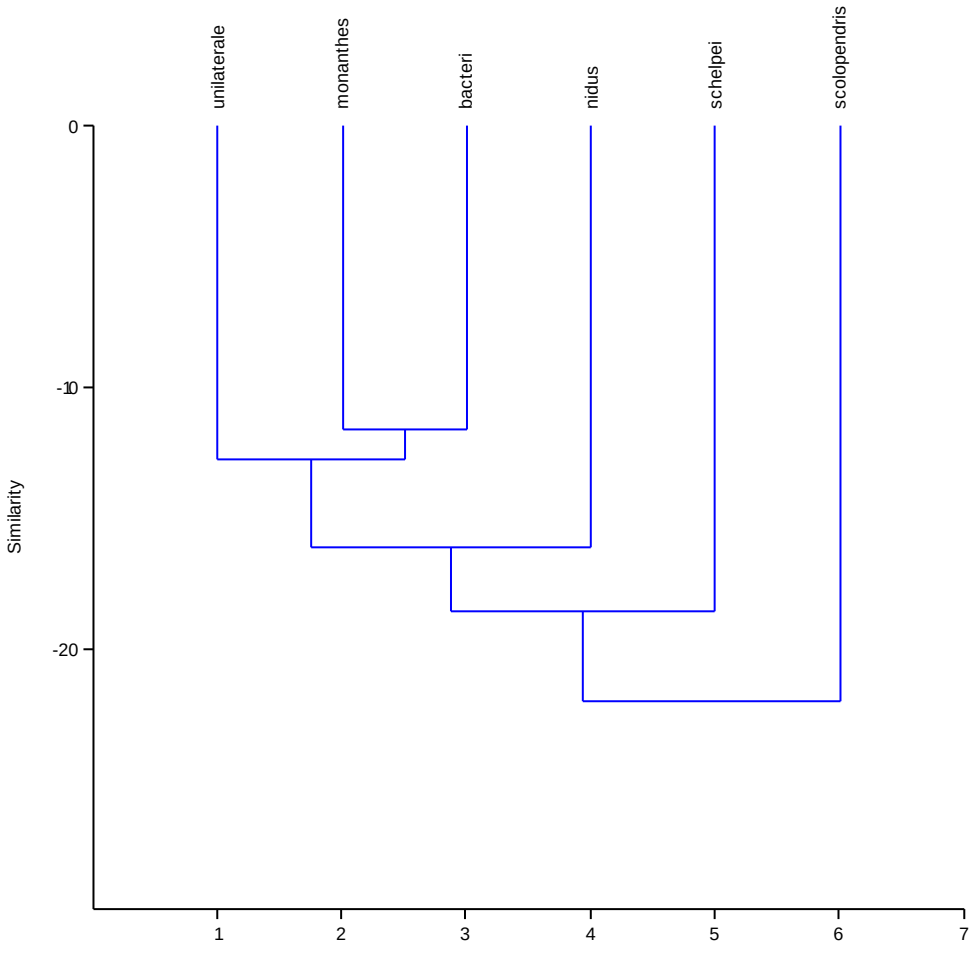

Fig 2: Dendogram showing the phylogenetic relationship between the *Asplenium* species studied.

Table 5: Sokal and Sneath, similarity of coefficient among *Asplenium* species

SPECIES	*A.barteri*	*A.monanthes*	*A.nidus*	*A.scheipei*	*A.scolopendrium*	*A.unilaterale*
A.barteri	-	0%	12.5%	12.5%	14.3%	20%
A.monanthes	0%	-	20%	0%	0%	0%
A.nidus	12.5%	20%	-	25%	28.6%	16.7%
A.scheipei	12.5%	0%	25%	-	50%	16.7%
A.scolopendrium	14.3%	0%	28.6%	50%	-	20%
A.unilaterale	20%	0%	16.7%	16.7%	20%	-

DISCUSSION

The identification of plant species through protein bands allows variation to be differentiated and identified. This is because each of the six *Asplenium* species studied had different protein bands which made it easy to differentiate each species compared to other methods of differentiation and identification such as measurement and morphological description like habitats, leaves, stems and floral characteristics. Protein bands through gel electrophoresis is a useful method for identifying variations among species.

Through gel electrophoresis, it has been shown that many isoenzymes and polymorphic proteins are widely distributed in plants. Proteins are considered to be direct products of genes and can be taken as markers of these genes (Ladizinsky, 1983). The variation observed in the distribution of protein bands in this genus is species-specific. This corroborates the report of Olson (1967) that biogenetic relationship can be best revealed by quantitative results emanating from chemotaxonomic methods. Similarly, the degree of variations observed in the protein profile of these taxa is indicative of the generic divergence among the taxa studied. Gotlieb (1971) reported that the presence of common bands in a group of taxa reflect evolutionary relationship. In this study, *A. monanthes* and *A. nidus* showed a high level of inter-specific relationship, just like *A. nidus, A. schelpei* and *A. scolopendrium,* using the number of common bands between pairs of *Asplenium* shown in Table 4. The presence of the same generic bands in these taxa shows that they have the same evolutionary origin. Generic band is the band common to all the species which belong to the same genus. There are three bands that were unique to *A. monanthes* (1.5, 2.6 and 4.3), two were unique to both *A. nidus* (3.4 and 4.6) and *A. schelpei* (1.2 and 2.0) while one band was unique to *A. barterii* (1.3), *A. scolopendrium* (2.4) and *A. unilaterale* (1.8) (Figure 1).

Unique bands are bands that distinguish one plant species from the other plant species; it is always common to a particular species, and distinguishes a from the other species. Interspecific bands are bands that two species have in common; interspecific bands are present in two different species of the same genus. However, not all species form interspecific bands Schneller (1981) reported pre-zygotic isolation between two *Athyrium* species and *Dryopteris filix-mas*. The differences observed in the protein profiles of the taxa studied are indicative of generic diversity and thus may be useful in the taxonomic delimitation of the members of the genus as reported by Oladipo and Illoh (2012). The coefficient values showed generally low level of similarity in the leaf protein bands of the six species of *Asplenium* studied ranging from 0- 50%. The highest level of coefficient of similarity (50%) was observed between *A. schelpei* and *A. scolopendrium* while the lowest level of similarity (0%) was observed between *A. barterii* and *A. monanthes*; *A. monanthes* and *A. scheipei* as well as between *A. monanthes and A. unilaterale.* The dendrogram shows that the six species of *Asplenium* were separated into four main groups with *A. nidus, A. scheipei, A. scolopendrium* being the only taxon on the first, second and third main clusters while *A. barterii, A. monanthes* and *A. unilaterale* form the fourth main group and the only one main cluster (Figure 2). *A. barterii, A. monanthes* and *A. unilaterale* appeared to be the most closely related as they clustered to the highest

level. Dendrogram was used to show the level of similarity of protein profile of the taxa. Generally, the degree of variation in the bands has been reported by Folorunsho and Olorode (2002) to depict the genetic diversity of the genera as in the case of the family *Annonaceae.* Furthermore, it has been observed that the variation in the combination of protein bands at the anode is taxon-specific and no two species had the same protein band distribution.

CONCLUSION

This study confirmed that the six *Asplenium* species studied contained proteins that distinguish them from other species of the same genus (the unique protein bands) as well as protein bands that relate them genetically with other species of the same genus. These are called generic bands. Even though they may differ morphologically in leaflet shape and spore arrangement, they still have some common protein bands. Leaf protein electrophoresis showed different types of protein bands, indicating a close relationship and distinctiveness of members of the genus in this work. It is, therefore, recommended as a useful technique in taxonomic research.

ACKNOWLEDMENT

The authors acknowledge the financial support by the Tertiary Education Trust Fund (TETFund) through the grant No TETFUND/DESS/UNI/ILE-IFE2016/RP/VOL1.

REFERENCES

Aguegia, A., Fatokun, C. A. and Hahn, S. K. (1994). Leaf protein analysis of ten cocoyam [*Xanthosoma sagitifolium* (L.) Schott] and *Colocasia esculenta* (L.) Schott genotypes. *Proceedings of the Fifth Symposium ISTRC-AB*, 348-353.

Folorunsho, A.E. and Olorode, O. (2002). Electrophoresis of crude protein of seeds of some genera of *Annonaceae*. *Nigerian Journal of Botany,* 7: 916-919.

Gordon, S., Stanley, W., Fisher, S. W. and Raymond, R. H. (2001). Preparative Agarose Gel Electrophoresis of ribonucleic acid. *Journal of Crystal Growth,* 232(1-4): 610-617.

Gotlieb, L.D. (1971). Gel electrophoresis. New approach to study of evolution. *Bioscience,* 21(18): 939-944.

Ladizinsky, G. (1983). Study of Evolutionary Problems by Means of Seed Protein. *Isreal Journal of Botany,* 22: 73-91.

Lasonder, E., Ishihama, Y., Andersen, J. S., Vermunt, A. M., Pain, A., Sauerwein, R. W., Elling, W. M., Hall, N., Waters, A. P., tunnenberg, H. G. S. and Mann, M. (2002). Analysis of the *Plasmodium falciparum* proteome by high-accuracy mass spectrometry. *Nature,* 419: 537-542.

Macfarlane, D. E. (1989). Two dimensional benzyldimethyl-n-hexadecylammonium chloride-sodium dodecyl sulphate preparative polyacrylamide gel electrophoresis: a high capacity and high resolution technique for the purification of proteins from complex mixtures. *Analytical Biochemistry,* 176: 457-463.

Nwosu, M. O. (2002). Ethnobotanical studies of some Pteridophytes of Southern Nigeria. *Economic Botany,* 56 (3): 255-259.

Oladipo, O.T. and Illoh, H. C. (2012). Leaf protein Electrophoresis and Taxonomy of Species of *Jatropha* L. (Euphorbiaceae). *Notable Science Biology,* 4(3): 92-96.

Oloyede, F.A. (2012). Survey of Ornamental ferns, their Morphology and Uses for Environmental Protection, Improvement and Management. *Ife Journal of Science*, 14(2): 012-020.

Oloyede F. A. and Odu, E. A. (2011). Taxonomic evaluation of Homosporous Leptosporangiate Ferns in Southwestern Nigeria. *International Journal of Current Research*, 2: 009-017.

Olson, U. (1967). Chemotaxonomic analysis of some cytotypes in *Mentha x Verticillate complex* (*Labiatae*). *Botaniska Notiser,* 20: 255-267.

Omitogun, O.G., Jackal, L. and Thottappilly, G. (1999). Isolation of insecticidal lectin-enriched extracts from African yam bean (*Sphenostylis sternocarpon*) and other legume species. *Entomologia Experimentalis et Applicata,* 90: 301-311.

Rais, I., Karas, M. and Schagger, H. (2004). Two-dimensional electrophoresis for the isolation of integral membrane proteins and mass spectrometric identification. *Proteomics,* 4: 2567-2571.

Reichstein, T. (1981). Hybrids in European Aspleniaceae (Pteridophyta). *Botanica Helvetica*, 91: 89-139.

Schirle, M., Heurtier, M. A. and Kuster, B. (2003). Profiling core proteomes of human cell lines by one-dimensional PAGE and liquid chromatography-tandem mass spectrometry. *Molecular and Cellular Proteomics,* 2: 1297-1305.

Schneller, J.J. (1981). Evidence for intergeneric incompatibility in ferns. *Plant Systematics and Evolutionary,* 137: 45-56.

Sokal, R. R. and Sneath, P. H. A. (1963). *Principles of numerical taxonomy*. 1st edition, W. H. Freeman and Co, San Francisco. New York, Wiley. 190p.

Szponarski, W., Sommerer, N., Boyer, J. C., Rossignol, M. and Gibrat, R. (2004). Large-scale characterisation of integral proteins from *Arabidopsis vacuolar* membrane by two-dimensional liquid chromatography. *Proteomics,* 4: 397-406.

Tokpo, E.Y., Danquah, E. Y., Ottfei, S. K. and Bla, E. T. (2006). Esterase, total protein and seed storage protein diversity in okra (*Abelmoschus esculentus* L.). *West African Journal of Application Ecology,* 9: 177-183.

Wagner, F.S. and Wagner, H.W. (1989). Cryptic species, species delimitation and taxonomic practice in the homosporous ferns. *American Fern Journal,* 79: 46-54.