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RELATIONSHIP WITHIN AND BETWEEN *SOLANUM L.* SPECIES BASED ON SDS-POLYACRYLAMIDE GEL ELECTROPHORESIS OF SEED PROTEINS

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ABSTRACT

The relationship within and between 20 cultivars of five *Solanum* L. species was investigated. Results from the SDS-polyacrylamide gel electrophoresis of the seed proteins revealed 47 polypeptide bands with molecular weight from 2.5 to 105.5 kilodaltons (KD). The cultivars could be characterized by the presence and or absence, staining intensities (degree of manifestation of proteins) and electrophoretic mobilities of the bands. The bands were assigned to 3 zones based on the electrophoretic mobilities – fast, intermediate and slow moving band with majority of the bands in the slow moving zone. There was a common inter and intra-specific band at 3.5cm (44.5KD). There was no correlation between the banding pattern and the shape or colour of the fruits. Similarity indices shows that cultivars within the same species are more closely related than the ones between species. Data presented shows that SDS-polyacrylamide gel electrophoresis of seed storage proteins is useful in classifying *Solanum* germplasm and that it helps in establishing the relationship within and between *Solanum* species.

Key words: *Solanum* species, Seed proteins, Polyacrylamide gel electrophoresis, Cultivars, Polypeptides, Genetic diversity.

INTRODUCTION

The family Solanaceae is a large family of about 20 genera and over 2000 species. It is a large family cultivated in tropical and subtropical areas of the world including West Africa (Dutta, 1979). They are mainly herbs or small shrubs often clothed with thorns. Among the Solanaceae the largest genera is *Solanum* which has about 1,500 species, other genera include *Nicotiana, Capsicum, Lycopersicum, Physalis* etc. The genus *Solanum* is represented by vegetable crops, tuber producing crops and weedy species. In West Africa, 21 species of *Solanum* have been described by Heine (1963) as cited by Gbile (1983) while 19 Nigerian species have been described by Gbile (1983) of which 15 are indigenous. In Nigeria, several *Solanum* species are grown as leafy and fruit vegetables. Some are food plants – *S. Melogena* L., *S. gilo* Raddi and *S. macrocarpon* L. Others are used as medicinal plants – *S. nigrum* L. A few wild species serve as a source of solasidine which is used in the synthesis of steroid hormones (Okoli, 1988). Some are ornamental or hedge plants *S. mammosum* L. and some are weeds *S. aerianthum* (Gbile, 1985). The vegetative Solanaceae are represented by several species which are usually characterized by their fruits e.g. *S. gilo* which has different cultivars based on its fruit shape and size, *S. nigrum* characterized based on its fruit size and *S. macrocarpon* is characterized based on the fruit texture. Although, the taxonomy in *Solanum* is based on the nature of the inflorescence and the presence or absence of prickles, Gbile (1985) was of the opinion that classification of the genus *Solanum* from the West African region has been uncertain because of the variations that exist within the taxa and the possibility of hybridization between and within species. The basic chromosome number in the genus *Solanum* has been observed to be 12 (Okoli, 1988; Nsowah, 1969). Karyotype studies revealed that differences exist among the species in chromosome sizes and even within the genome of each species variation in chromosome sizes were observed (Okoli, 1988). It was observed that most varieties in *S. melogena, S. toruum* and *S. indicum* all have diploid number (2n = 24) of chromosomes while *S. nigrum* had 2n = 48/72 (Okoli, 1988).

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In order to have an effective breeding strategy the existing Nigerian lines of *Solanum* species need to be screened. Electrophoresis of seed proteins has proved to be a useful and quick screening method for varietal characterization in pepper even though most biuochemical characterization studies in cultivars and varieties of vegetable crops such as *Solanum, Lycopersicon* and *Capsicum* species have used mainly enzymatic and RFLP markers (Odeigah, *et. al.*, 1999). We hereby report an investigation on the used of seed protein electrophoresis to determine relationships between and within *Solanum* L. species.

MATERIALS AND METHODS

Mature fruits from 5 different species of *Solanum* were collected from various locations in Southern Nigeria. Table 1 give a description of the *Solanum* species used in this study based on their fruit characteristic. Seeds were extracted from the fruits, air-dried and subsequently packaged. A minimum of 10 fruits were sampled per cultivar. Samples of the plants and flowers were also collected and brought to the herbarium for proper identification. Seeds were also collected from the Nigerian Institute for Horticultural Research (NIHORT) for each of the identified cultivars and a few others for comparative purposes.

Seeds of about 0.5g from the same fruit were ground to a fine powder and 100mg of the extract was defatted by washing with 3 changes of cold acetone for 6 hours. The acetone was removed by filtration and samples were air-dried at room temperature (29 ± 2^0C). Extraction of seed protein was carried out using a technique that has previously been described by Odeigah *et. al.* (1999). 10% SDS-polyacrylamide was carried out using a discontinuous gel technique as described by Odeigah and Osanyinpeju (1996). The gels were removed, stained for 20 minutes with 2% (w/v) Coomasic blue at 65^0C in a water bath, destained for 20 minutes and left overnight in 8% (v/v) acetic acid at room temperature for complete destaining.

Schematic diagrams of the bands were carefully traced out by placing the electrophoregrams on a light box. Percentage similarity of cultivars were determined using the similarity index proposed by Vaughan (1973). The distance migrated by each band was also measured and used to calculate the electrophoretic mobility according to Webber and Osborn (1969). This was then used to determine the apparent molecular weights of the proteins resolved by the gel.

RESULTS AND DISCUSSIONS

The banding patterns of the seed proteins in the different species and cultivars of *Solanum* L. used are presented in Fig. 1. There were no intra-cultivar differences for each fruit sample within a cultivar, thus results are presented per cultivar. In this study, the *Solanum* seed proteins were highly heterogeneous and consisted of 47 polypeptide bands with apparent molecular weights ranging from 2.5 kilodaltons (KD) to 105.5KD. The polypeptide bands differed in number, degree of manifestation of protein (staining intensity) and band combinations within and between species. One of the cultivars used (SG3) however did not reveal any banding pattern.

SMA 3 had the highest number of bands with 21 polypeptide bands resolved while SN 3 had the lowest with 7 polypeptide bands (Fig. 2). The band at 3.5cm with an apparent molecular weight of 44.5KD was common to all species and cultivars although they appeared with different staining intensities. Amongst the *S. gilo* cultivars, there was no other band common to all

cultivars apart from the band at 3.5cm (Fig. 3). However, the band at 1.3cm (84.5KD) and 1.8cm (73.5KD) was unique to SG 1.

In the *S. melogena* group, in addition to the band at 3.5cm, bands at 0.1cm (105.5KD), 3.0cm (52.5KD), 4.0cm (35.5KD) and 9.5cm (2.5KD) were common to all cultivars in this group. SME 1 had more deeply stained bands than all other members in this group, with the least number of 12 polypeptide bands in the group found in SME 2. Results from *S. macrocarpon* cultivars showed that 11 polypeptide bands were common to all the 3 cultivars analysed in this group. The cultivars in this group showed more bands than any other species and most were deeply stained.

Table 1: Description of *Solanum* Species and Cultivars used in the study

SPECIES	SERIAL NUMBER	CULTIVAR CODE	DESCRIPTION OF FRUITS AND LOCAL NAMES
S. gilo	1	SG 1	Green, round, medium size
	2	SG 2	Green, round, big size
	3	SG 3	White, round with bold green stripes
	4	SG 4	Cream, oblong, big with faint strips
	5	SG 5	Small creamy, oblong, no stripes, pointed at both end.
	6	SG 6	Green, round, no stripe.
	7	SG 7	Green, oblong, medium size.
	8	SG 8	"Ikan" seed from NIHORT, NH94/37 – 1
S. melongena	9	SME 1	Big/giant purple.
	10	SME 2	Purple, medium fluted shape.
	11	SME 3	Green, medium fluted shape.
	12	SME 4	Black, beauty seed from NIHORT, NH94/1 - 1
S. macrocarpon	13	SMA 1	Medium size, smooth ripe fruit (Round).
	14	SMA 2	Medium size, wrinkled ripe fruit (Round).
	15	SMA 3	"Igbo" seed from NIHORT, NH94/2.
S. nigrum	16	SN 1	Big green fruits turns purple when natured.
	17	SN 2	Small green fruits turns purple when mature.
	18	SN 3	"Ogunmo" seeds from NIHORT.
S. aethipicum	19	SA 1	Green round turns yellow at maturity. "OSUN".
	20	SA 2	Green round medium turns red at maturity.

TABLE 2: Similarity indices between different seed protein electrophoregrams of twenty (20) cultivars of *solanum*

20																			
19																			
18																			100
17																		100	19
16																	100	32	33
15																100	25	43	13
14															100	52	16	37	14
13														100	43	42	14	33	23
12													100	21	20	25	16	27	25
11												100	45	33	29	35	15	21	17
10											100	42	32	22	26	22	17	11	23
9										100	50	29	23	22	33	32	27	24	20
8									100	44	29	30	29	40	25	30	24	48	26
7								100	50	32	31	20	27	42	33	40	20	42	17
6							100	42	42	26	30	30	29	27	19	21	23	35	20
5						100	35	41	44	29	50	32	15	20	23	20	21	20	13
4					100	26	42	33	47	14	19	23	22	12	17	26	26	29	18
3				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2			100	-	39	31	50	47	56	30	22	23	25	35	22	26	25	43	21
1		100	24	-	32	8	26	16	20	27	23	29	32	25	24	40	25	36	31
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18

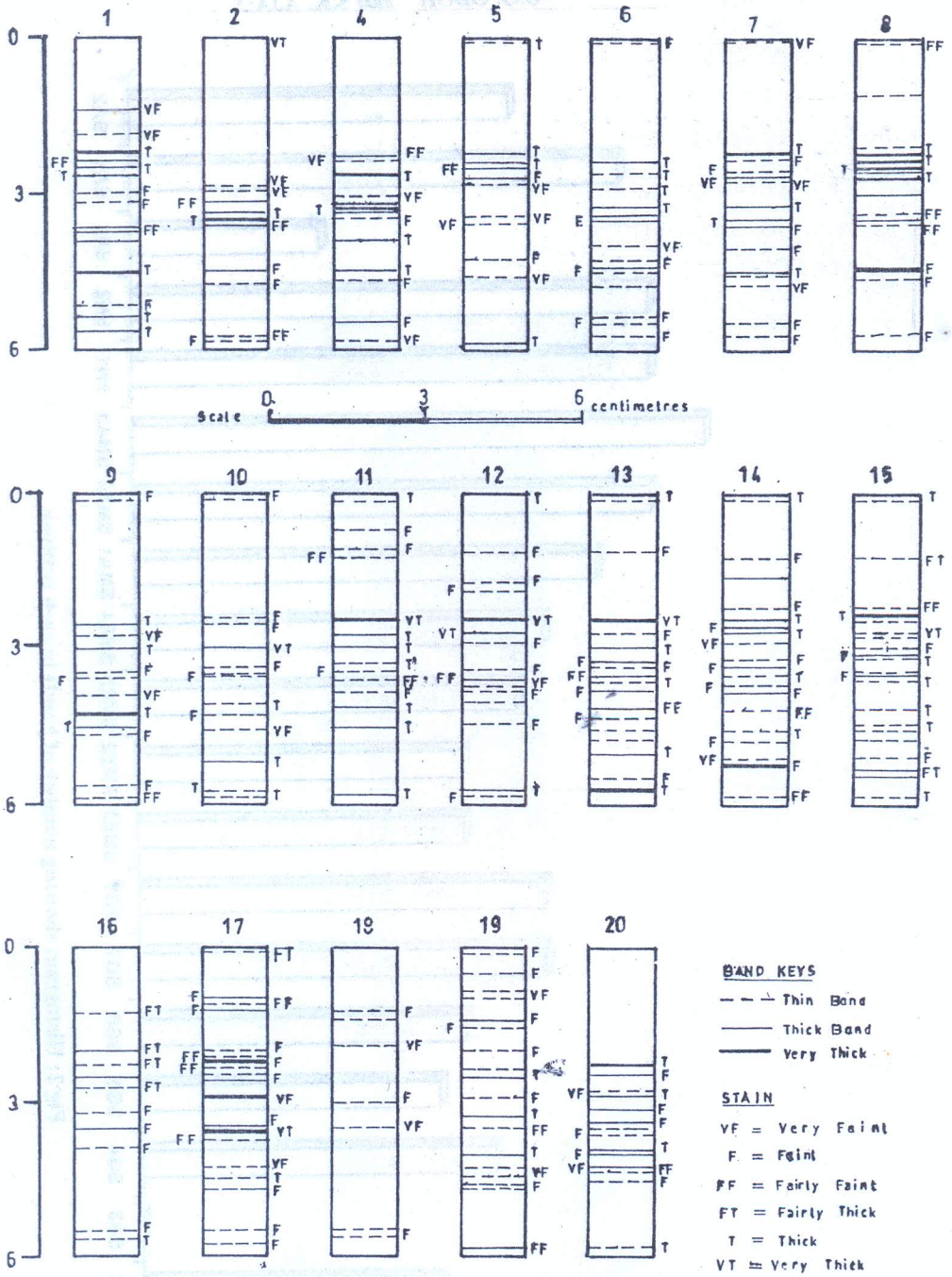

Fig. 1: Schematic representation of the various protein bands resolved for the Solanum species using gel length (in cm)
LANE 1–8 = S. gilo; 9–12 = S. melongena;
13–15 = S. macrocarpon; 16–18 = S. nigrum;
19–20 = S. aethiopicum.

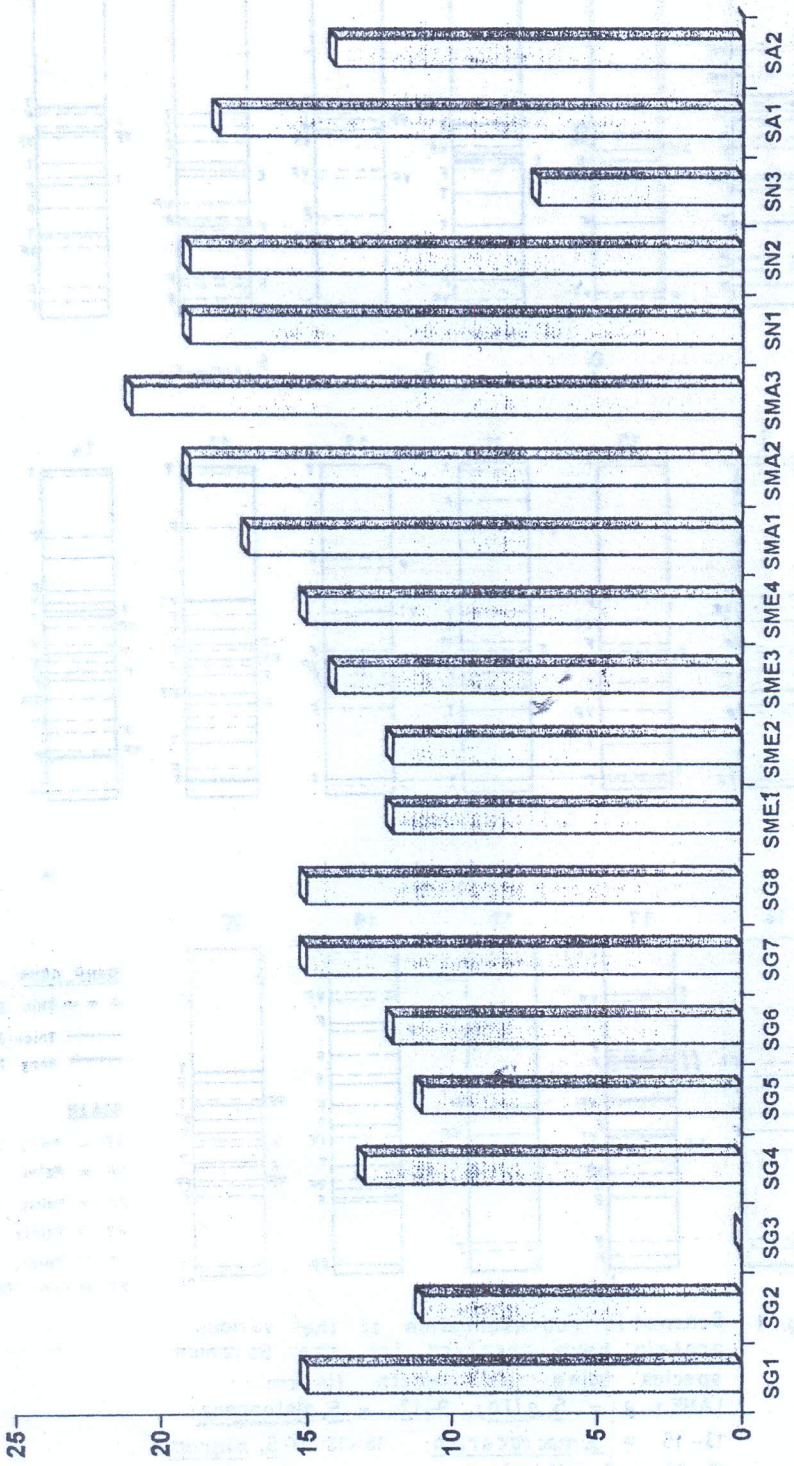

Fig 2: Histogram showing number of bands in each cultivar

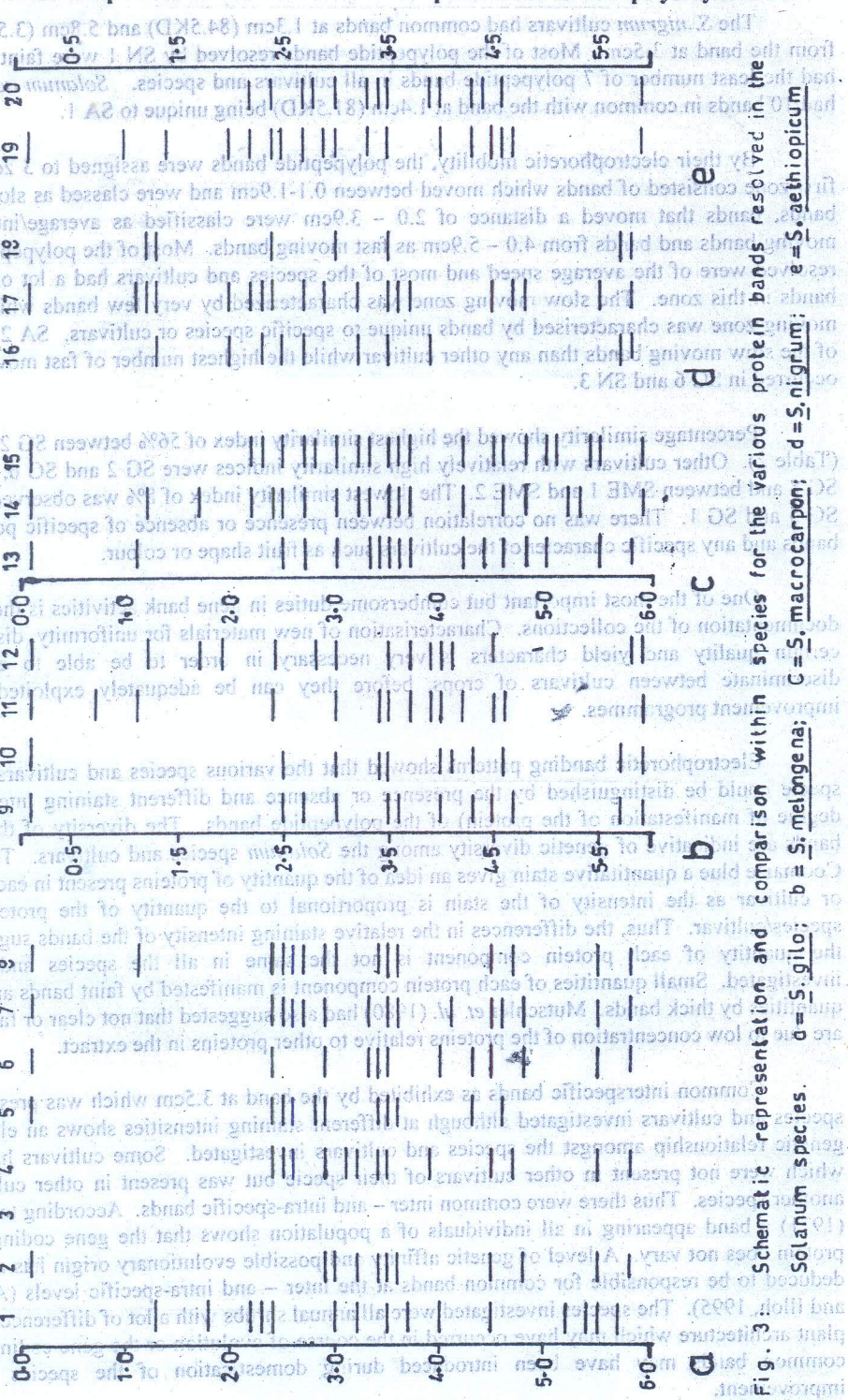

Fig. 3: Schematic representation and comparison within species for the various protein bands resolved in the Solanum species. a=S. gilo; b = S. melongena; C=S. macrocarpon; d=S. nigrum; e=S. aethiopicum

The *S. nigrum* cultivars had common bands at 1.3cm (84.5KD) and 5.8cm (3.5KD) apart from the band at 3.5cm. Most of the polypeptide bands resolved by SN 1 were faint and SN 3 had the least number of 7 polypeptide bands in all cultivars and species. *Solanum aethiopicum* had 10 bands in common with the band at 1.4cm (81.5KD) being unique to SA 1.

By their electrophoretic mobility, the polypeptide bands were assigned to 3 zones. The first zone consisted of bands which moved between 0.1-1.9cm and were classed as slow moving bands, bands that moved a distance of 2.0 – 3.9cm were classified as average/intermediate moving bands and bands from 4.0 – 5.9cm as fast moving bands. Most of the polypeptide bands resolved were of the average speed and most of the species and cultivars had a lot of common bands in this zone. The slow moving zone was characterized by very few bands while the fast moving zone was characterised by bands unique to specific species or cultivars. SA 2 had more of the slow moving bands than any other cultivar while the highest number of fast moving bands occurred in SG 6 and SN 3.

Percentage similarity showed the highest similarity index of 56% between SG 2 and SG 8 (Table 2). Other cultivars with relatively high similarity indices were SG 2 and SG 6, SG 7 and SG 8 and between SME 1 and SME 2. The lowest similarity index of 8% was observed between SG 5 and SG 1. There was no correlation between presence or absence of specific polypeptide bands and any specific character of the cultivars such as fruit shape or colour.

One of the most important but cumbersome duties in gene bank activities is the accurate documentation of the collections. Characterisation of new materials for uniformity, distinctness, certain quality and yield characters is very necessary in order to be able to effectively discriminate between cultivars of crops, before they can be adequately exploited in crop improvement programmes.

Electrophoretic banding patterns showed that the various species and cultivars within a specie could be distinguished by the presence or absence and different staining intensities (a degree of manifestation of the protein) of the polypeptide bands. The diversity of the protein bands are indicative of genetic diversity among the *Solanum* species and cultivars. The use of Coomaise blue a quantitative stain gives an idea of the quantity of proteins present in each species or cultivar as the intensity of the stain is proportional to the quantity of the protein in the species/cultivar. Thus, the differences in the relative staining intensity of the bands suggest that the quantity of each protein component is not the same in all the species and/cultivars investigated. Small quantities of each protein component is manifested by faint bands and higher quantities by thick bands. Mutschler *et. al.* (1980) had also suggested that not clear or faint bands are due to low concentration of the proteins relative to other proteins in the extract.

Common interspecific bands as exhibited by the band at 3.5cm which was present in all species and cultivars investigated although at different staining intensities shows an element of genetic relationship amongst the species and cultivars investigated. Some cultivars had bands which were not present in other cultivars of their specie but was present in other cultivars of another species. Thus there were common inter – and intra-specific bands. According to Gottlieb (1971) a band appearing in all individuals of a population shows that the gene coding for the protein does not vary. A level of genetic affinity and possible evolutionary origin has also been deduced to be responsible for common bands at the inter – and intra-specific levels (Akinwusi and Illoh, 1995). The species investigated were all annual shrubs with a lot of differences in their plant architecture which may have occurred in the course of evolution or the gene coding for the common bands may have been introduced during domestication of the species or crop improvement.

In this study, there was no correlation between protein banding patterns and colour or shape of the fruit. There was no specific protein band(s) found in the purple, green, cream or red fruits neither was there any for oval, cylindrical, oblong or globose fruits. Thus, these characteristics may be influenced by multiple genes. High similarity indices among the *S. gilo* cultivars suggest a close remembrance and this was noticed more in the fruit colour and shape as most were green and oblong/oval in shape. The *S. melogena* cultivars had 5 common bands which may be responsible for their habit and fruit size and colour. The fruits were bigger than fruits in any other group and were predominantly purple in colour. The major bands in the *S. macrocarpon* cultivars were the same and this may be responsible for their close affinity as they differed only in the slow moving bands. Fruit shape, colour and sizes were the same except for fruit texture which remains smooth in SMA 1 when ripe and wrinkles in SMA 2 on ripening. There were marked differences in the *S. nigrum* cultivars, while *S. aethiopicum* which was assumed to be the same had low similarity indices.

CONCLUSION

In Nigeria, similar cultivars have been given different names in different localities creating a problem in the effort at identifying, collection and conservation of the *Solanum* species. It was however concluded by this study that the samples collected across the Southern part of Nigeria differed from those obtained at NIHORT. A wide range of genetic diversity has been found to exist in the Nigerian *Solanum* germplasm with a close relationship existing among cultivars of the same *Solanum* species and a wide diversity between *Solanum* species. Thus seed protein electrophoresis can be effectively used to characterize the germplasm collections and it would also help in tracing genetic relationships among the species of the genus *Solanum.*

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