

Value of Seed Protein Profile in the Taxonomy of cultivars of *Capsicum* in Nigeria.

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Abstract:

Over a decade, the taxonomy of the genus *Capsicum* in Nigeria has remained largely unrevised, unclassified and unidentified. As such, there is a dearth of information on the proper identification of *Capsicum* spp and relatives found in the country. The aim of this study was to re-examine the taxonomic status of the *Capsicum* in Nigeria in order to establish genetic diversity between them for proper identification and classification. Sodium dodecyl polyacrylamide gel electrophoresis of total seed protein was performed on five varieties of Nigerian *Capsicum* spp., following standard procedures. Six protein bands were observed across the five cultivars of *Capsicum*, of which 12-14 Kda was the only polymorphic band. Only *C. frutescens* var. *ijosi* and *C. frutescens* var. *sombo* were unique for manifesting 20-24 and 15-16 Kda bands respectively. Dendrogram of analysis obtained resolved the taxa into two distinct groups. In the first group were cultivars of *C. frutescens* var. *ijosi* and *sombo* while in the second group were *C. chinense*, which was distinctly separated from *C. frutescens* var. *bawa* and *C. annum*. Artificial dichotomous key was constructed for the identification of members of the genus *Capsicum* available in Nigeria based on the protein profiles of their seeds.

Keywords: *Capsicum*, seed protein, electrophoresis, identification, diversity.

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Introduction

In West Africa, peppers are widely grown and are used in a number of ways. They occupy third position in Nigeria among the cultivated vegetables being utilized in the dry state as spice due to their capsaicin content (an alkaloid which is a digestive stimulant) and as vegetable, when supplied for their vitamin content and aroma (USDA, 2015). The crop is utilized both as condiment and food. The thick sweet fleshy or non-pungent varieties are used

in salads or stuffed with meat and cooked (Arnason, 2015). The chemical in chilli peppers (i.e. *Capsicum*) responsible for the burning sensation is capsaicin which affects only mammals, but not birds. Capsaicin extract is used to make pepper spray, a useful deterrent against aggressive mammals (Grubben and El Tahir, 2004). Pepper fruit accounts for a large portion of vitamins A and C in many Nigerian diets; the most common species of pepper in Nigeria are *Capsicum annum* L., *C. chinense*

Jaqc. and *Capsicum frutescens* L. (DeWitt and Bosland, 2009).

The genus *Capsicum* in Nigeria has not been thoroughly revised, identified and classified. There is therefore a dearth of information on the exact number of *Capsicum* spp and varieties found in the country. In addition, some reported works on Nigerian *Capsicum* misrepresented some *Capsicum* species due to lack of proper identification. For instance, Aziagba et. al., (2014), erroneously assigned the local name 'shombo' (Yoruba name for a cultivar of *C. frutescens*) to *C. annuum* and 'atarugu' (Hausa name for *C. chinense*) to *C. annuum*. When the classification of taxa is confused, so is the nomenclature and literally, any information about such taxa is not specific and therefore, less useful. *Capsicum* has long been regarded as a taxonomically difficult genus by many workers (Pickersgill et. al., 1979; Eshbaugh, 1970, 1975, 1980; Heiser and Pickersgill, 1975; Edeoga et. al., 2010; Zhigila et. al., 2014). In fact, there is no agreement yet among workers with regard to the number of species of *Capsicum* present in Nigeria.

Presently, there is no satisfactory revision of the taxonomical status of the Nigerian genera of *Capsicum*. . Moreso, some species of the genus being considered in this study (i.e. *Capsicum*) are difficult to distinguish because members of the genus have been reported to possess morphological similarities and some are highly phenotypically plastic (Moscone et. al., 2007; Walsh and Hoot, 2001). So far, only one report (Olatunji and Morakinyo, 2015) could be traced on taxonomic markers based on biochemical contents or SDS-PAGE analysis with respect *Capsicum* species and varieties in Nigeria. Olatunji and Morakinyo (2015) claimed to have worked on *C. frutescens* and *C. annuum* varieties only, leaving out *C. chinense*, which is a commonly used variety in Nigeria. The results of their study also indicated some levels of confusion, as there were reports of consistencies between the protein profile from the leaves when compared with those of the seeds, in their study. These problems clearly justify the necessity for a proper identification of all members of the Nigerian *Capsicum* taxon by their seed protein composition, especially because a preliminary

survey of the wild and cultivated Nigerian species of the *Capsicum* genus revealed several cases of confusion regarding uncertainty in names and genetic distances.

The aim of this study was to evaluate the seed proteins of the plant species using Sodium Dodecyl Polyacrylamide Gel Electrophoresis (SDS PAGE) with a view to establish genetic diversity between species and also document those protein markers that could be utilized for diagnosing the varieties and species of *Capsicum* in Nigeria.

Materials and Methods

Sample Collection and regeneration:

Seeds of the Nigerian species of *Capsicum* investigated were collected from cultivated field, identified with the assistance of Prof. H.C. Illoh at Obafemi Awolowo University Herbarium (OAUH) and thereafter grown (for the purpose of a balance in environmental conditions) at the Botanical garden, Ladoke Akintola University of Technology, Ogbomoso. The seeds obtained from the regenerated plants were used as material for Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) analysis which was carried out at the Molecular Biology and Biotechnology department, Nigerian Institute of Medical Research (NIMR), Yaba, Lagos.

Protein extraction

About 200mg seeds from each genotype was homogenized with mortar and pestle using 0.01M Tris-Hcl buffer (pH 7.5). The resulting homogenates were centrifuged at 15000rpm for 10 minutes, the supernatants were filtered with 541 Whatmann filter paper. The residues were boiled at 90°C for five minutes with 1:1 ratio of 1.0M Tris (pH 6.8), 10% SDS; 2% β-mercaptoethanol, 10% glycerol and 0.002% bromophenol blue (following the method of Kumar and Tata (2010) with gel composition as indicated in Table 1.

Electrophoresis

SDS-PAGE of total seed protein was carried out in vertical slab gel in discontinuous

buffer system following the method that was modified by Essiet and Illoh (2008) as follows: On cooling to room temperature, three drops of 10% Sodium dodecyl sulfate (SDS), 1% of 2-mercaptoethanol and sucrose crystals was added to the sample in order to weigh down the protein molecules. Then, one drop of 0.05% bromophenol which served as a tracer dye was added. Four drops of the resultant mixture

obtained from the product was directly added to the gels. The tubes were then placed in column acrylamide gel apparatus with tri-glycine buffer in both the upper and the lower vessels. A current of 1.5mA per gel was applied. The current was thereafter increased to 3mA per gel until the dye front was 1 cm from the bottom of the gel.

Table 1: Gel Composition adopted for SDS-PAGE in the study.

Chemical	Upper Gel (cm ³)	Lower Gel (cm ³)
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 Dodecyl Sulphate (SDS)	0.10	0.30
Ammonium per Sulphate	0.10	0.30
TEMED	0.01	0.03

Source: Essiet and Illoh (2008).

Staining and destaining

After electrophoresis, the gels were stained with Coomassie Brilliant Blue (R250) for 30-40 minutes with continuous shaking, then shifted to another container with destaining solution of methanol and acetic acid for 45 minutes. The gels were further de-stained until the back ground was clear enough for bands scoring. Marker proteins (RPMW): Bovine Serum Albumin (66 kilodaltons), Egg Ovalbumin (45 kilodaltons), Pepsin Porcine Stomach Mucosa (34.7 kilodaltons). Bovine Trypsinogen (24 kilodaltons) and B- Lactoglobulin (18.4 kilodaltons) were used as references. Protein marker used was in form of "MW-SDS-70 Kit" from Sigma Chemical Company, USA. Molecular weights of protein bands were estimated by their relative mobilities. In order to eliminate differences in electrophoretic conditions as a cause of variation in the protein profiles, each genotype protein sample was separated from three independent electrophoretic runs and two separate extractions (Kumar and Tata, 2010). In

analyzing the data obtained, presence of a band was scored "1" while absence of the band was scored "0" to generate binary matrix which was used to perform statistical analysis.

Cluster Analyses

The scores of the protein fragment in relative to the standard marker were used as characters to perform a cluster analysis on the species; each of which was taken as operational taxonomic units (OTU). Dendrograms were obtained by adopting a hierarchical cluster analysis using *Ward's method* applying *squared Euclidean Distance* (as the distance or similarity measure), both of which could be combined using PAST (Paleontological Statistics Software Package) by Hammer et. al. (2001).

Construction of Dendrogram and Artificial key

An artificial dichotomous key was constructed for the purpose of diagnosing the species in each genus, using the characters obtained. While the qualitative characters were directly

used, as observed, the means of quantitative characters were first subjected to statistical significance across the species in each genus to determine which ones were truly diagnostic. The statistical tool used for dendrogram construction was SPSS version 21.

Results

The results of SDS PAGE analysis of the seed proteins in the plants studied are shown in Plate 1 and Table 2. A total of six protein bands was observed across the five cultivars of *Capsicum* i.e. B1, B2, B3, B4, B5 and B6. The molecular weight of the protein were 35kDa, 28-32 kDa,

25-27 kDa, 20-24 kDa, 15-16 kDa and 12-14 kDa respectively (Table 2).

Among these bands, 12-14 kDa is the polymorphic band for the *Capsicum* species as all the taxa contained the protein with that range of marker. The 35 kDa band was present in only *C. frutescens* (var. *bawa*) and *C. annuum*; 28-32 kDa band was found in only *C. frutescens* (var. *ijosi* and var. *sombo*); 25-27 KDa band was observed in two varieties of *C. frutescens* (i.e. *sombo*, *bawa* and in *C. annuum*; 20-24 KDa band was discovered only in *C. frutescens* var. *ijosi* while the 15-16 KDa band was observed in all but *C. frutescens* var. *sombo*.

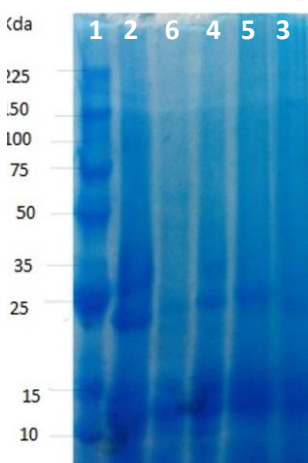


Plate 1: Plate 4.13: SDS-PAGE Gel electrophoresis analysis of seed protein of five cultivars of *Capsicum* in Nigeria. (1=Protein Markers, 2= *C. frutescens* var. *ijosi*, 3= *C. frutescens* var. *sombo*, 4= *C. frutescens* var. *bawa*, 5= *C. annuum*, 6= *C. chinense*).

Table 2: SDS PAGE Score of seed protein contents in the *Capsicum* spp studied.

Taxa	Bands (kDa)					
	35	28-32	25-27	20-24	15-16	12-14
IJO	0	1	0	1	1	1
SOM	0	1	1	0	0	1
BAW	1	0	1	0	1	1
ANN	0	0	1	0	1	1
CHI	1	0	0	0	1	1

0 = absent; 1= present. (IJO= *C. frutescens* var. *ijosi*, SOM= *C. frutescens* var. *sombo*, BAW= *C. frutescens* var. *bawa*, ANN= *C. annuum* and CHI= *C. chinense*).

The dendrogram which was constructed based on the UPGMA shows distinct separation of the Nigerian varieties of *Capsicum* in Nigeria into two major groups (HC and LC) at 23% genetic distance. Among the two major clusters obtained, the higher cluster (HC) has *C. frutescens* var. *ijosi* and *C. frutescens* var. *sombo* at 13% genetic distance while the lower cluster (LC) consists of two sub-clusters (LC1 and LC2) at 11% distance. LC1 (occurring at 11% distance) consists of *C. chinense* while LC2 consists of *C. frutescens* var. *bawa* and *C. annuum*.

Discussion

In consonance with the results obtained from morphological, leaf epidermal and wood anatomical evaluation of the cultivars of *Capsicum* (Adepoju, 2018), the dendrogram obtained from seed protein data (Fig 1) resolved the taxa into three distinct groups. In the first group were two cultivars of *C. frutescens* clustered (i.e. varieties *ijosi* and *sombo*); in the second, *C. frutescens* var *bawa* and *C. annuum* clustered; while *C. chinense* stood as a distinct cluster on its own.

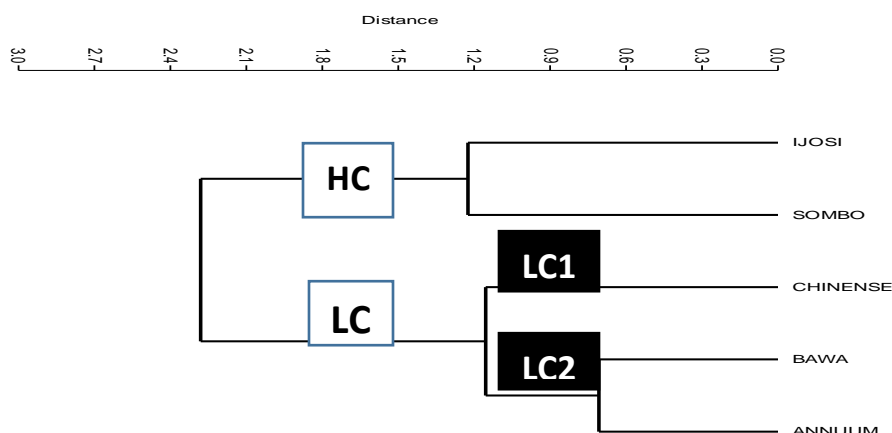


Fig 1: Dendrogram based on the cluster Analysis of seed protein data recorded on the five Nigerian cultivars of *Capsicum* studied (IJO= *C. frutescens* var. *ijosi*, SOMBO= *C. frutescens* var. *sombo*, BAWA= *C. frutescens* var. *bawa*, ANNUUM= *C. annuum* and CHINENSE= *C. chinense*).

Biosystematic Implications of the seed protein molecular weights observed in *Capsicum*

It is clear that the dendrogram in Fig 1, and those obtained from morphological and leaf epidermal data of these *Capsicum*

Adepoju (2018) are similar in all respects, indicating that the classificatory value of morphological, leaf epidermal and seed protein characters in this genus are the same. Also, if one places the dendrogram obtained by Adepoju (2018) for wood anatomical characters of the cultivars side by side with Fig 1, one finds out some similarity between the two, particularly as regards the close clustering of *C. frutescens* var. *bawa* and *C. annuum*.

Again, the results as depicted in Figure 1 closely align with capsaicin content profile of the fruits of these plants (Nwokem et. al., 2010 and Zeid et. al., 2011). In the first place, the two cultivars with low fruit capsaicin contents clustered together (i.e. *C. frutescens* var. *bawa* and *C. annuum*) while the two cultivars of *C. frutescens* with high content of this chemical (i.e. var. *ijosi* and var. *sombo*) clustered as a group. Lastly, *C. chinense* which is acknowledged to possess an intermediate value of capsaicin content between the two extremes stood alone as a cluster between the earlier two groups (Fig 1).

McLeod et. al. (1982) reported a close distance of clustering between *C. chinense* and the two varieties of *C. frutescens* and the present study agreed with the authors report. Also, Bhat and Kudesia (2011) studied the protein profile of 5 species of the family Solanaceae (i.e. *Solanum melongena*, *S. xanthocarpum*, *Datura alba*, *Lycopersicon esculentum* and *Capsicum annum*) using SDS-poly acrylamide gel electrophoresis. Their results revealed that the genus *Lycopersicon* was very close to the genus *Solanum* and that the species *Datura alba* and *Solanum melongena* were closer at molecular level compared to other species. Furthermore, similarity index was higher for *Capsicum annum* and *Solanum xanthocarpum* (22.22%) which are cultivated and wild types respectively, as compared to two exclusively wild species of *Datura alba* and *Solanum xanthocarpum* (in which their similarity index was only 11.11%). Yousaf et. al., (2006) also investigated the taxonomical status of 42 accessions belonging to 7 species of 4 different genera (*Datura*, *Hyoscyamus*, *Withania* and *Atropa*) from the family Solanaceae by Poly Acrylamide Gel Electrophoresis. A dendrogram constructed based on UPGMA revealed the generic status and inter relationship of *Hyoscyamus*, *Atropa*, *Withania* and *Datura*. The specimens of *Withania somnifera* collected from Panjgur (109717, 109718, and 109710) did not only show their morphological variation but also, variations based on their protein profiles. Based on the total seed protein profiles, close association was noticed by between *Withania/Datura* and *Atropa/Hyoscyamaus* but

they maintained their generic status, as there was no species intermixing.

Olatunji and Morakinyo (2015) carried out SDS-PAGE of leaf and seed protein in four Nigerian *Capsicum* varieties. Their results showed distinct electrophoretic banding patterns with a total of 38 bands. They concluded based on the results of their work that the differences and similarities observed in the protein profiles among the *Capsicum* species studied were indicative of genetic diversity, that Electrophoresis (SDS-PAGE) of seed and leaf proteins can be used as effective technique in plant characterization, identification and differentiation, would be of importance for broadening the *Capsicum* gene pool and that it may be used in hybridization in breeding programmes.

From the foregoing account, it can be said that analysis of seed protein profiles have been useful for resolving ambiguous taxonomic boundaries in the genus *Capsicum*. Moreover, these data have sufficient diagnostic potential among the five cultivars of the Nigerian *Capsicum* studied as evident from the entries in Tables 3 and 4.

In *Capsicum*, the seed protein profile has similar classificatory value in line with only those clusters based on fruit capsaicin content. This study has established for the first time, some concordance between fruit capsaicin content (or fruit hotness) in *Capsicum* and infrageneric taxonomic groupings based on such conventional characterization approach, as seed protein profile. Variations in seed protein profile on Nigerian species of *Capsicum* have been documented in form of unambiguous artificial keys for proper identification of the species.

Table 3: A Numerical key on seed protein profile for identification of five cultivars of *Capsicum* in Nigeria.

TAXA	1 (35 kDa)	2 (28-32kDa)	3 (25-27kDa)	4 (20-24kDa)	5 (15-16kDa)
IJO	0	2	0	4	5
SOM	0	2	3	0	0
BAW	1	0	3	0	5
ANN	0	0	3	0	5

CHI	1	0	0	0	5
(IJO= <i>C. frutescens</i> var. <i>ijosi</i> , SOM= <i>C. frutescens</i> var. <i>sombo</i> , BAW= <i>C. frutescens</i> var. <i>bawa</i> , ANN= <i>C. annum</i> and CHI= <i>C. chinense</i>).					
Table 4: A seed protein-based dichotomous key for identification of five cultivars of <i>Capsicum</i> in Nigeria.					
1a. Protein bands 28-32 kda molecular weight detected in seed	2				
2a. Protein Bands 20-24 and 15-16 present in plant seed	<i>C. frutescens</i> var. <i>ijosi</i>				
2b. Protein Bands 20-24 and 15-16 absent in plant seed	<i>C. frutescens</i> var. <i>sombo</i>				
1b. Protein bands of 28-32 kda molecular weight not detected in seed	3				
3a. 25-27 kda band of seed protein detectable	4				
4a. 35kda protein band discovered in plant seed	<i>C. frutescens</i> var. <i>bawa</i>				
4b. 35kda protein band discovered in plant seed	<i>C. annum</i>				
3b. 25-27 kda band of seed protein not detectable	<i>C. chinense</i>				

In conclusion, out of the six seed protein bands observed in *Capsicum*, the band with the molecular weight of 12-14kda was polymorphic in *Capsicum*. Also, the seed protein profile has similar classificatory value in line with only those clusters based on fruit capsaicin content.

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