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Crude Protein Profiling of Varieties of Capsicum annuum and Capsicum frutescens using SDS-PAGE.

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Abstract: *The study was aimed at assessing the genetic variation and relatedness among four Capsicum varieties through electrophoretic separation of their leaf and seed proteins by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Total seed and leaf proteins were extracted and separated on 12% polyacrylamide gels using standard protocols. Distinct polymorphism in electrophoretic banding patterns of seed and leaf proteins was observed in the four Capsicum varieties through a total of thirty-eight (38) polypeptide bands in the seeds and a total of seventeen (17) polypeptide bands in the leaves analyzed. Variation existed not only in the number of bands but also in the intensity of bands in both the leaf and seed samples studied. Sokal and Sneath's coefficient of similarity revealed a generally high level of similarity in the seed protein bands of the four varieties studied ranging from 50% to 100%. It also revealed an average level of similarity in the leaf protein bands of the four varieties of Capsicum studied ranging from 16.7% to 83.3%. A dendrogram constructed based on the Single Linkage Cluster Analysis(SCLA) using the relative mobility values of seed and leaf proteins of the four varieties revealed two major clusters. From the seed, cluster 1 contains (C. annuum var. abbreviatum and C. annuum var. acuminatum), and cluster 2(C. annuum var. grossum, and C.frutescens var.baccatum). Also, two prominent clusters were revealed in the leaf sample, with cluster 1 containing (C. annuum var. abbreviatum, C. annuum var. acuminatum and C.frutescens var.baccatum) and cluster 2(C. annuum var. grossum). Significance of the findings has been discussed. Plant breeders can use the information on variation and relatedness among the Capsicum species for cultivar development in pepper.*

Keywords: *Genetic diversity, Capsicum varieties, Single Linkage Cluster Analysis (SCLA), Dendogram, Similarity index, Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE), Seed protein , Leaf protein , Schematic diagram, Protein bands*.

I. Introduction

The family *Solanaceae* comprises of many economically important food and industrial crops such as potato, tobacco, tomato, garden egg, petunia and pepper (Capsicum). *Solanaceae* is an important source of almost 300 alkaloids (Al-Wadi, 2007). Solanine, scopolamine, atropine and hyoscyamine are the key alkaloids of the family (Kalifa et al., 1998). The genus *Capsicum* consists of approximately 22 wild and 5 domesticated species. The domesticated species include *C. annuum, C.frutescens, C. Chinenses, C. baccatum,* and *C.pubescens*. (Bosland and Votava, 2000). Most *Capsicum* fruits are pungent, because the placenta accumulates capsaicinoids (e.g., capsaicin) an alkaloid that is a digestive stimulant, and an important ingredient of daily diet with many other medicinal properties. (Zewdie and Bosland, 2001; Thompson et al., 2005). *Capsicum* species can be divided into several groups based on fruit/pod characteristics such as pungency, colour, shape, flavor, and size. Despite their vast trait differences most cultivars of peppers commercially cultivated in the world belong to the species *C. annuum* L. (Smith et al., 1987; Bosland, 1992). The fruit colour is due to the presence of total carotenoid pigments which consist mainly of capsanthin and capsorubin in red fruits and β–carotene and Violaxanthin in yellow – orange fruits (Bosland and Votava 2000; Kumar et al. 2003). The pharmaceutical utilization of capsaicinoids is attributed to its antioxidant, anticancer, antiarthritic, and analgesic properties (Bosland and Votava, 2000). In addition, pepper fruits are valuable on account of their richness in ascorbic acid, which is an important vitamin.

Capsicum was domesticated at least five times by prehistoric peoples in different parts of South and Middle America. Three out of the five domesticated species, namely: *Capsicum annuum, Capsicum frutescens and Capsicum chinense* grow well in many communities of Nigeria and constitute important spice in most foods. Pepper is one of the major revenue sources in Nigeria and it serves as the world, most crucial and most used condiment (Showemimo and Olanrewaju, 2000).

Knowledge of morphological, biochemical and molecular relationships between plant species is very useful in planning effective breeding strategies, thereby producing fruitful genomic reconstructions and improved cultivars. Determination of genetic diversity of any crop species is a preliminary step for improvement of the crop as it generates baseline data to guide selection of parental lines and design of an appropriate breeding scheme. It is therefore an important step to assess the genetic variability among the experimental plants.

Traditionally, morphological descriptors like plant height, flower colour, fruit length and orientation and seed characteristics are routinely used to distinguish pepper genotypes (Sitthiwong et al., 2005). However, morphological information alone may not provide an accurate assessment of genetic diversity existing in a species because of environmental influences. Moreover, field evaluation of plant material is often laborious and time consuming, in particular, when a large number of accessions are involved. Consequently, introduction of biochemical techniques has provided a more accurate and less laborious way to evaluate genetic variation, bringing greater precision to measures of genetic diversity.

Among numerous techniques available for assessing the genetic variability and relatedness among crop germplasm, seed protein analysis presents a valid alternative and/or improved approach to varietal identification (Mennella et al. 1999) because, protein markers are highly polymorphic and environmental influence on their electrophoretic pattern is limited (Sadia et al. 2009). Protein electrophoresis has been used to estimate diversity among accessions in genetic resources collection and species inter-relationships (Gardiner & Forbe, 1992; Badr, 1995). Its applications include analysis of genetic diversity within and among plant populations, plant domestication in relation to genetic resources conservation and breeding, genome relationships especially in polyploid series, and as a tool in plant breeding (Gepts, 1990).

The banding patterns produced by seed and leaf protein electrophoresis have been used to effectively characterize cultivars of pasture grasses and legumes (Sheidai et al., 2000). Multiple domestication centres have been suggested based on results of seed protein electrophoresis of different wild and cultivated accessions of common bean (Gepts et al., 1986). In recent years, Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) has become an economical, simple and extensively used biochemical technique for describing the seed protein diversity of crop germplasm (Das and Mukarjee, 1995; Iqbal et al., 2005). SDS-PAGE of total seed and leaf proteins and cytological analyses have found wide application in resolving genetic diversity and for intra and interspecific studies.

Considering the foregoing, the present study seeks to determine the identities of and assess the variation among four varieties of *Capsicum* species using protein profiling with a view to appraising genetic diversity and phylogeny within the genus *Capsicum* in Nigeria.

II. Materials And Methods

This study was carried out in the Biotechnology Laboratory, Department of Animal Science, Obafemi Awolowo University, Ile-Ife, Osun State.

Viable seeds of 4 *Capsicum species (Capsicum annuum* Var. *abbreviatum* –Sample A, *Capsicum annuum* Var. *acuminatum*-Sample B, *Capsicum frutescens* Var. *baccatum*-Sample C and *Capsicum annuum* Var. *grossum*-Sample D) were obtained from Ojatuntun, Ilorin. They were grown at the University of Ilorin Botanical Garden.

Electrophoretic study of the protein variations of seeds and leaves of *Capsicum* varieties were carried out using 12% Polyacrylamide gels. The species were screened for total protein banding pattern using a modified method of Laemmli (1970), described by Aguegia et al.,(1994), Omitogun et al., (1999) and Tokpo et al.,(2006).

Dried seeds of each variety were ground in porcelain mortar and 2g each of the ground samples was weighed into separately labelled test tubes. 7ml of 0.6M NaCl (extraction buffer) was added to each sample in the test tubes and covered. This was left for about 12hours. The samples were then centrifuged for 10mins at 3000 revolutions per minute (rpm). Each sample was denatured for 4mins at 95ºC and then allowed to cool for 1hour.

Fresh leaves of *Capsicum* varieties for the study were carried in fresh and moist polythene nylon to Ile –Ife and labelled appropriately. The leaves were weighed and washed in extraction buffer PBS (Phosphate buffer) and ground in mortar with pestle. For Sample A, 1.5ml of freshly prepared buffer (PBS) was added to the ground leaves and mixed together using the pestle; for Sample B, 0.94ml of freshly prepared buffer was added; for Sample C, 1.32ml of freshly prepared buffer was added; for sample D, 0.95ml of freshly prepared buffer was added. The extract from each sample was poured into their labelled test tubes and centrifuged at 3000 revolution per minute (rpm) for 10 minutes. The supernatants from each sample was collected using a Pasteur pipette into an ependorf vial , closed and kept at 4ºC in the freezer. Sample buffer (Mercapto ethanol) was used to dilute the supernatant from each sample in the ratio 1:3 i.e. 15µl of sample buffer: 45µl of sample. The mixture for each sample was heated for 4mins at 95ºC and also cooled for 1hour in the freezer.

The supernatant from the seeds and leaves were subjected to SDS-PAGE (Sodium Dodecyl Sulphate - Poly Acrylamide Gel Electrophoresis). 10µl of each sample were loaded in the designated well. The gel was allowed to run at 150mini volts for 1hour.

After the electrophoretic separation, the gels were stained in Comassie brilliant blue overnight. Destaining was done in a mixture of 40 ml Methanol dissolved in 10ml Acetic acid and 50ml of Distilled water. This was done overnight in order to visualize the protein bands for subsequent scoring. The data obtained for

SDS were scored for the presence (1) and absence (0) of bands. Photographs of the gels were taken and schematic diagrams also drawn.
Single Linkage Cluster Analysis (SCLA) was carried out on the data using Paleontological Statistics (PAST). Coefficient of similarity of Sokal and Sneath (1963) was used to show the level of similarity of protein profile in the species.

III. Result

The pattern of the seed protein electrophoresis distribution of the four varieties of *Capsicum* studied is presented in Plate 1, while the schematic diagram is shown in Figure 1.

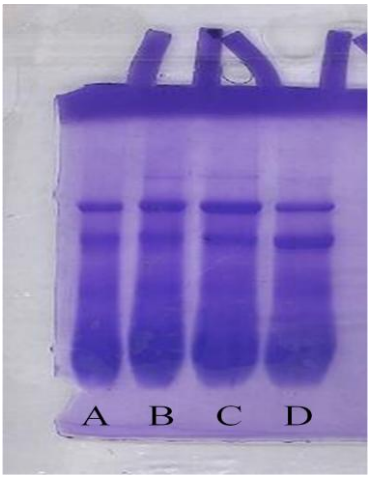

Plate 1: Pattern of protein distribution in the seed of Capsicum species studied in the order of Sample A, B, C, D. A (*C.annuum* var. *abbreviatum*), B (*C.annuum* var.*acuminatum*), C, (*C.frutescens* var. *baccatum*) D (*C.annuum* var. *grossum*)

SDS-PAGE of seed protein of four varieties showed distinct electrophoretic banding patterns resulting in the detection of a total of 38 bands (Tables 1 and 2). There were qualitative and quantitative variations in numbers, positions and intensities of bands detected, all of which are common to the two species, except two that are specific to *C.annuum* Var.*abbreviatum* (Table 2 and Figure 1).

Table 1: Band relationships in the seeds of *Capsicum* species studied

SAMPLE	B1	B2	B3	B4	B5	B6	B7	B8	B9	B1O
A	1	1	1	1	1	1	0	1	0	1
B	1	1	1	1	1	1	1	1	1	1
C	1	1	1	1	1	1	1	1	1	1
D	1	1	1	1	1	1	1	1	1	1

A (*C.annuum* var. *abbreviatum*), B *(C.annuum* var.*acuminatum*), C (*C.frutescens* var. *baccatum*) D (*C.annuum* var. *grossum*)

Table 2: Protein band distribution in seeds of the four varieties of *Capsicum* studied

Variety	Slow bands Unique bands (0-3.0cm)	Intermediate bands (3.1-6.0cm)	Fast bands (6.1-9.0cm)	Total number of bands
A	4	3	1	8	2
B	4	5	1	10	0
C	4	5	1	10	0
D	4	5	1	10	0
Total	16	18	4	38	

A (*C.annuum* var. *abbreviatum)*, B (*C.annuum* var.*acuminatum*), C(*C.frutescens* var. *baccatum)* D (*C.annuum var. grossum*)

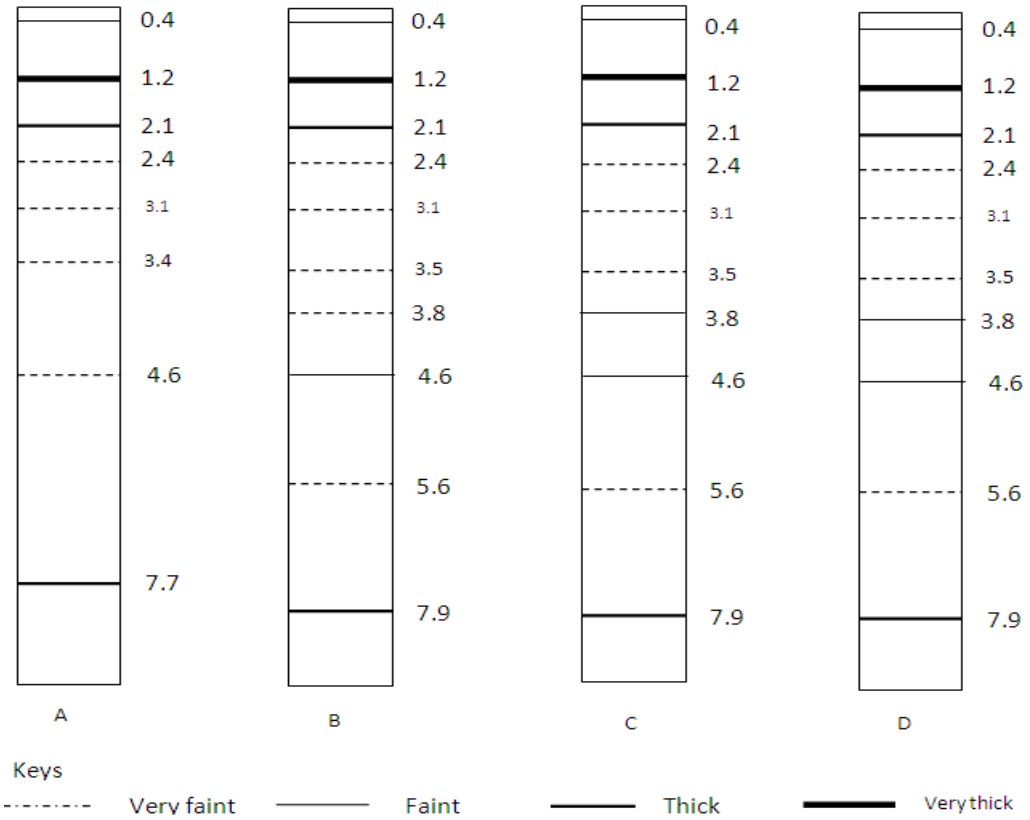

Figure 1: Schematic diagram of stained protein bands recognized after gel electrophoresis of seed proteins of *Capsicum* species studied. (**A**) Ata rodo *C.annuum* var. *abbreviatum* Fingerh, (**B**) Ata Sombo *C.annuum* var. *acuminatum* Fingerh, (**C**) Ata wewe *C . frutescens* var. *baccatum* L. , (**D**)Ata tatase, *C. annuum* var. *grossum* (L.) Sendt.

The result further shows that four (4) bands (10.5%) were fast bands, 18(47.4%) were intermediate in movement while 16 bands (42.1%) were slow moving protein bands. Generic bands occur at 0.4, 1.2, 2.1, 2.4, 3.1, and 4.6 though with varying degree of intensity. 8 bands were observed in *C.annuum* var. *abbreviatum*, 10 bands in *C.annuum* var. *acuminatum*, 10 bands in *C.frutescens* var. *baccatum* and 10 bands in *C.annuum* var. *grossum.*

Apart from the generic bands, inter -specific bands were widespread in the species as observed in the bands at 3.5 (3 varieties), 3.8 (3 varieties), 5.6 (3 varieties) and 7.9(3 varieties). Unique bands occur at 3.4 and 7.7 (figure 1). The highest number of inter –specific bands (3) was found among sample (Sample B, *C.annuum* var. *acuminatum*, Sample C, *C. frutescens* var. *baccatum* and Sample D, *C.annuum* var.*grossum*) .

Sokal and Sneath's (1963) coefficient of similarity revealed a generally high level of similarity in the seed protein bands of the four varieties studied and ranged between 50% and 100% (Table 3). The highest co-efficient of similarity occurred between sample B (*C.annuum* var.*acuminatum*) and C(*C.frutescens* var. *baccatum*) (100%), B(*C.annuum* var.*acuminatum*) and D(*C.annuum* var. *grossum*) (100%), and D(*C.annuum* var. *grossum*) and C(*C.frutescens* var. *baccatum)* (100%).

Table 3: Sokal and Sneath's similarity index for *Capsicum* species of seed based on the relative mobility (Rm) values in (%)

SAMPLES	A	B	C	D
A	-	50	50	50
B	50	-	100	100
C	50	100	-	100
D	50	100	100	-

A (*C.annuum* var. *abbreviatum)*, B (*C.annuum* var.*acuminatum*), C, (*C.frutescens* var.*baccatum*) D (*C.annuum* var. *grossum)*

The Single Linkage Cluster Analysis (SCLA) dendrogram of the relative mobility (Rm) value of protein bands is presented in Figure 2.

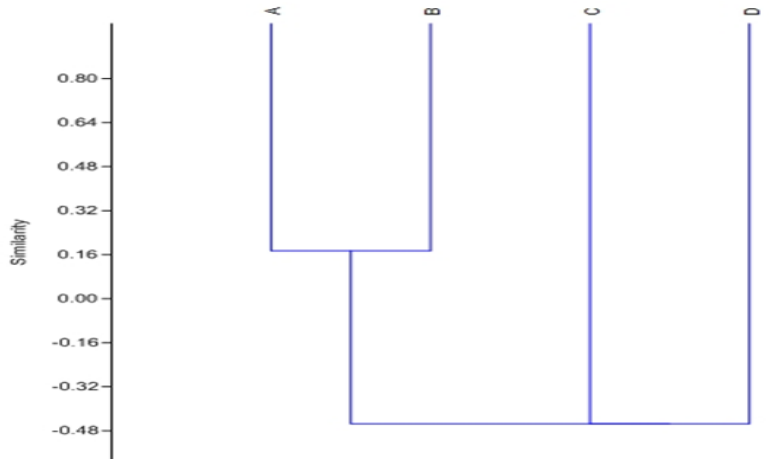

Figure 2: Single linkage Cluster Analysis (SCLA) dendogram of relative mobility (Rm) values for seed protein in the four varieties of *Capsicum* studied. A (*C.annuum* var. *abbreviatum*), B (*C.annuum* var.*acuminatum),* C, (*C.frutescens* var. *baccatum*) D (*C.annuum* var. *grossum*)

The SCLA diagram shows the 4 varieties separated into two main groups, with samples A and B in the first main cluster and sample C and D in the second main cluster. Sample A and B are more closely related than Samples C and D which appear more distantly related to each other and to A and B.

The pattern of the leaf protein electrophoresis distribution of the four varieties of *Capsicum* studied is presented in Plate 2, while the schematic diagram is shown in Figure 3.

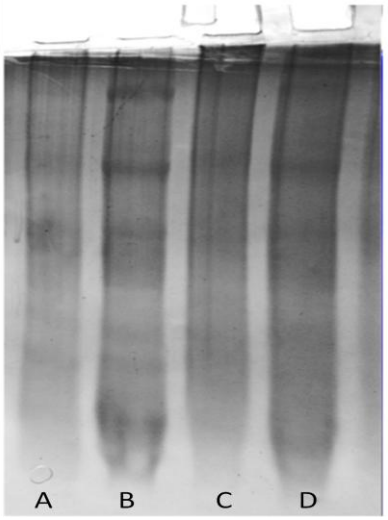

Plate 2: Pattern of protein distribution in the leaves of Capsicum species studied in the order of Sample A, B, C, D. A (*C.annuum* var. *abbreviatum*), B (*C.annuum* var.*acuminatum*), C, (*C.frutescens* var. *baccatum*) D (*C.annuum* var. *grossum*)

SDS-PAGE of leaf protein of four varieties showed distinct electrophoretic banding patterns resulting in the detection of a total of seventeen (17) bands (Table 4 and 5), and protein banding in each species of *Capsicum* studied was species specific as no two varieties had completely the same profile in band number, mobility and intensity.

Table 4: Band relationships among *Capsicum* species studied

SAMPLE	B1	B2	B3	B4	B5	B6
A	1	1	1	1	1	0
B	1	1	1	1	1	1
C	0	1	0	0	0	1
D	1	1	0	0	1	1

A (*C.annuum* var. *abbreviatum*), B (*C.annuum* var.*acuminatum*), C, (*C.frutescens* var.*baccatum*) D (*C.annuum* var. *grossum*)

Table 5: Protein band distribution in leaves of the four varieties of *Capsicum* studied

Variety	Slow bands (0-3.0cm)	Intermediate bands (3.1-6.0cm)	Fast bands (6.1-9.0cm)	Total number of bands	Unique bands
A	3	0	2	5	0
B	3	0	3	6	0
C	1	0	1	2	0
D	2	0	2	4	0
Total	9	0	8	17	

A *(C.annuum* var. *abbreviatum*), B *(C.annuum* var.*acuminatum*), C, (*C.frutescens* var.*baccatum*) D (*C.annuum* var. *grossum)*

The result further shows that eight (8) bands (47.1%) were fast bands, 9bands (52.9%) were slow moving protein bands. Generic bands occur at 0.8 though with varying degree of intensity. Apart from the generic band, inter -specific bands were widespread in the species as observed in the bands at 0.1 (3 species), 2.2(2species), 6.1 (2 species) and 10.7(3 species), 16.7 (3 species). 5 bands were observed in *C.annuum* var. *abbreviatum*, 6 bands in *C.annuum* var. *acuminatum*, 2 bands in *C.frutescens* var. *baccatum* and 4 bands in *C.annuum* var. *grossum*.

Sokal and Sneath's (1963) coefficient of similarity revealed a lower level of similarity in the leaf protein bands of the four varieties studied ranging from 16.7% to 83.3% (Table 6). The highest co-efficient of similarity occurred between samples A and B (83.3%), compared with the 50% coefficient of similarity detected between these two varieties using seed protein SDS-PAGE. In general, SDS-PAGE resolution of the crude leaf proteins in the four varieties of pepper was relatively weak resulting in much fewer diagnostic bands.

Table 6: Sokal and Sneath's similarity index for *Capsicum* species based on the relative mobility (Rm) values in (%) of leaf proteins

SAMPLES	A	B	C	D
A	-	83.3	16.7	50
B	83.3	-	33.3	66.7
C	16.7	38.3	-	50
D	50	66.7	50	-

A (*C.annuum* var. *abbreviatum),* B *(C.annuum* var.*acuminatum),*
C, *(C.frutescens* var.*baccatum)* D *(C.annuum* var. *grossum)*

The Single Linkage Cluster Analysis (SCLA) dendrogram of the relative mobility (Rm) value of leaf protein bands leaf is presented in Figure 4.

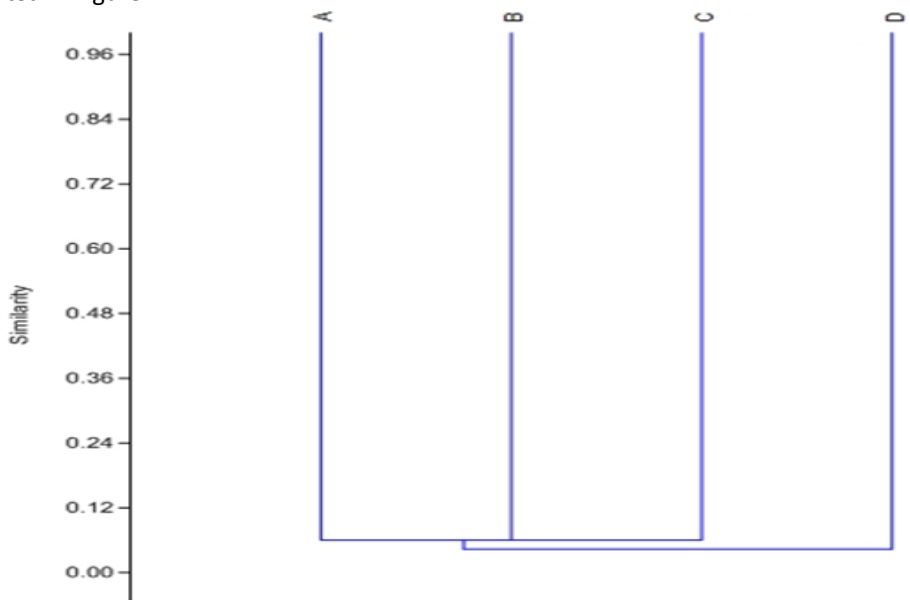

Figure 4. Single Linkage Cluster Analysis (SLCA) dendogram of relative mobility (Rm) values for leaf protein in the species of *Capsicum* studied. A (*C.annuum* var. *abbreviatum*), B (*C.annuum* var.*acuminatum*), C, (*C.frutescens* var. *baccatum*) D (*C.annuum* var. *grossum*)

The SLCA diagram shows the 4 varieties separated into two main groups, with samples A, B and C in the first main cluster and sample D in the second cluster all appearing closely related.

IV. Discussion

Electrophoretic analysis of the seed and leaf proteins have direct relationship to the genetic background of the proteins that reveal genetic diversity. Such analysis can be used to certify the genetic makeup of germplasm (Javid et al., 2004. and Igba et al., 2005).

The results from this study show variation in the pattern of electrophoretic mobility of the crude proteins. Protein variation is an indication of protein polymorphism and thus phenotypic variation which forms the basis of separation of individuals in a particular population into different groups. Protein bands (8-10) recorded in the seed, and (2-6) recorded in the leaf of *Capsicum* showed variation among the plants of *Capsicum.* Since SDS separated the protein into their polypeptide sub –units, it may be assumed that when two accessions exhibit the same band, they have the same form of gene coding for the polypeptide. Therefore, plants with similar banding pattern can be considered as being genetically similar.

According to Hubby and Lewontin, (1966) and Gottileb (1971), when a particular electrophoretic band appears in all the individuals examined in a population, it is assumed that the gene coding the enzyme does not vary. This can be used to label the generic band 0.4, 1.2,2.1,2.4,3.1 and 4.6 in the seed protein profile and the band 0.8 in the leaf protein profile (figure 1 and 3), with varying degree of intensity in the species, since these bands tend to prove that the species are from the same parental stock.

The presence of common bands among the varieties is an evidence of evolutionary relationship of the *Capsicum* plants in which the genes for the common bands have been conserved. These may be adaptive genes which have become fixed in the species over evolutionary time.

The numerous inter and intra specific bands at the same distances from the anode among the species studied reflect, to a large extent, a measure of affinity among varieties. This agrees with the idea of Daas and Nybom (1967) that the concept of 'biochemical distances' among species of known genetic relationship are measures of affinity.

Large intra and inter specific differences were not found in genotypes of *Capsicum* studied based on protein profiles. This may be due to genetic homogeneity or purity (Odeigah et al., 1999).

The highest co-efficient of similarity occurred between samples B and C (100%), B and D (100%), and D and C (100%).Such higher percentage similarities were also reported in cultivars of *C. annuum* L. by Anu and Peter (2003).

The dendrogram as a whole revealed low genetic diversity because most of the varieties are in the same cluster. Fufa et al. (2005) reported that the genetic diversity estimates based on seed storage protein were lowest because they were the major determinants of the end-use quality, which is a highly selected trait.

The information gathered from cluster analysis are useful to identify genetic variability among parents for the purpose of hybridization in crop improvement (Maity et al., 2009). Clustering of the genotypes signifies close genetic affinity between/among species. Based on distance between species of different clusters, contrasting parents may be identified and used in hybridization programme for generating wider variability for selection and crop improvement. The clustering has however, not differentiated the two species of *Capsicum* (*C.frutescens* and *C.annuum*) in this study. This suggests overlap and mutual exchange of genes as recorded between *C.frutescens* var. *baccatum* and *C.annuum* var. *acuminatum.*

Gepts (1989) suggested the loss of genetic diversity upon domestication of a crop species. From the four varieties of *Capsicum* studied, those with high average similarity index are considered domesticated and widely cultivated e.g. *C. annuum, C. chinense*, and *C.frutescens* (Pickersgill, 1991). In this study, the varieties of pepper used were not traced to their geographical origins but were clearly identifiable as the common cultivated varieties in Nigeria with possible exchange of genes in cultivation and overlapping genetic variability.

V. Conclusion

Electrophoresis (SDS-PAGE) of seed and leaf proteins can be used as effective technique in plant characterization, identification and differentiation. The differences and similarities observed in the protein profiles among the *Capsicum* species studied are indicative of genetic diversity and would be of importance for broadening the *Capsicum* gene pool and may be used in hybridization in breeding programmes.

Competing Interest

The authors declare that they have no competing interest in whatsoever form in research design, execution, finance, manuscript preparation and choice of journal.

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