Original Research Article

EVALUATION OF GENETIC DIVERSITY OF SEVEN SPECIES OF SOLANUM IN NIGERIA USING SDS-PAGE

ABSTRACT

SEED PROTEIN PROFILE OF 12 ACCESSIONS BELONGING TO 7 SPECIES OF THE GENUS *SOLANUM* OF THE FAMILY SOLANACEAE WERE INVESTIGATED THROUGH POLY ACRYLAMIDE GEL ELECTROPHORESIS. THE ACCESSIONS WERE SELECTED BASED ON THEIR OVERLAPPING MORPHOLOGICAL ASSOCIATIONS OBSERVED ON THE FIELD. OCCURRENCE OF TWO PROTEIN BANDS (2 AND 7) IN ALL ACCESSION SHOWED INTERSPECIFIC RELATIONSHIPS AND COULD BE USED AS GENERIC BANDS AMONG THE GENUS *SOLANUM*. ALL MORPHOLOGICALLY SIMILAR ACCESSIONS MANIFESTED SIMILARITIES IN THEIR PROTEIN BANDS EXCEPT *S. MELONGENA*. DENDROGRAM BASED ON UPGMA ALSO REVEALED THE GENERIC STATUS AND INTRASPECIFIC RELATIONSHIPS OF THE ACCESSION OF *S. AETHIOPICUM* AND *S. MACROCARPON*.

Keywords: Genetic diversity, Nigeria, SDS-PAGE, Solanum

1. INTRODUCTION

The domestication of *Solanum* vegetables in Africa has been influenced by the system of agriculture practiced. Gepts [1] suggested that domestication of a crop species may lead to the loss of genetic diversity. Introduction of new cultivars has been mainly through hybridization and breeding programme [2]. The introduced species, especially those of economic importance are considered as new species rather than a cultivar. Early distinctiveness, uniformity and stability (DUS) of any taxa rely on morphological methods; but that these however, may be influenced by environmental conditions [3]. The electrophoresis of seed storage protein is reliable because it is not sensitive to environmental fluctuations; therefore banding patterns are very stable and can be used for cultivar identification [4,5]. Yousaf *et al.* [6] reported that most of the taxonomic information on the genus *Solanum* rely on morphological markers. These characters are important for inter-generic studies. However, they become less important for detailed intra-generic studies. Flowering-plant Taxonomists traditionally also place a heavy reliance on floral characters in assessing relationships and in arriving at taxonomic conclusion, whereas inflorescence of the members of this genus have morphological similarities. For instance, *S. americanum* and *S. nigrum* show resemblance in plant habit, flower, and fruit. Thus, morphological markers which in the past were sufficient for their correct and proper identification would now require more distinguishing features for critical delimitation.

Several researchers have used SDS-PAGE in separating cultivar of species in the family Solanaceae; *Capsicum annum* [7,8,9], *Solanum* [6,10,11]. Their findings however revealed varying relationships among the species studied. This present study is therefore, encouraged to find out further genetic diversity among some accessions of seven species of *Solanum* with conspicuously overlapping morphological characters using SDS-PAGE.

2. MATERIAL AND METHODS

Seeds and seedlings of 7 species of *Solanum* were collected from different locations namely roadsides, abandoned plots, refuse dumps, farms and market places. Collection from each location was taken as an accession. The seeds were germinated in polythene bags and transplanted directly into the soil at the Botanical garden of Federal University of Agriculture Abeokuta. The plants were nurtured until matured, morphological observations were made and recorded. Seeds of the matured fruits were collected and stored for analytical use.

Specimen seeds of the accessions were separately ground into flour. Seed proteins were extracted from 0.3g of seed flour using 800µm extraction buffer that contained 0.1M Tris-HCl pH7.6. Seed flour was thoroughly mixed with buffer by vortexing for 1min. The extracted proteins were separated by centrifuging the sample at the rate of 10000rpm for 10mins after which the supernatants were extracted. Electrophoresis was carried out in a discontinuous SDS-PAGE using 12.5% acrylamide gel. Running buffer was loaded to cover the wells. Electrophoresis was run at 180°V for 50mins. The gel was then stained in the staining solution containing 44% methanol, 6% acetic acid, 500ml distilled water and 2.25g of Coomassie brilliant blue for 45 mins. Destaining was done with several changes in a solution containing 20% methanol, 5% acetic acid and 750ml of distilled water until the background colour disappeared and protein bands were clearly visible.

Protein bands were scored depending on their presence (1) or absence (0). Hierarchical clustering was constructed by UPGMA using computer software SPSS v 17.0.

3. RESULTS

3.1 Field observations

In the accessions of *S. aethiopicum*, unripe fruit colour was mosaic and green. More variation was observed in *S. macrocarpon* (stem colour: green and purple, unripe fruit colour: green and mosaic, fruit orientation: blossom-end upright - blossom end points upward, pendulous orientation - blossom end points downward and porrect blossom orientation - blossom end is at 90° to the stem). Also, two species: *S. dasyphyllun* and *S. torvum* possessed spines on their vegetative and reproductive parts. The observations in the morphological characters were tested for variability by electrophoresis.

3.2 Protein profiling

The protein patterns obtained in the 12 accessions studies are shown in Plate 1. The accessions showed variations in number, position and intensity of bands. The marker which formed the standard had nine protein bands. The weights of the marker which was numbered from the top from the highest to lowest were 150kDa, 100kDa, 70kDa, 50kDa, 35kDa, 25kDa, 20kDa, 18.4kDa, and 14.7kDa respectively (Plate 1).

Accessions of *S. macrocarpon* had the highest number of bands (8) while *S. dasyphyllum* had the least (5) (Table 1). Two bands (2 and 7) were observed in all the accessions and can be considered genus specific bands. Accessions of *S. aethiopicum* (2 and 3) and *S. macrocarpon* (5, 6, 7, 8 and 9) had similar and patterns in terms of number and intensity and the bands could be identified as species specific bands (Plate 1). Dendrogram differentiated two main groups (Fig.1). The first group consisted of *S. melongena* with the morphological similar *S. nigrum* and *S. americanum*. The second group was further divided into two subgroups with two clusters in each group. The first subgroup comprised of all the accessions of *S. macrocarpon* (at the same Euclidean distance) except accession 5 (porrect fruit orientation) which maintain a distinct and higher Euclidean distance).This difference was also observed in the number of ands patterns (7) possessed by the accession as against the number (8) possessed by other accessions of the species. The second subgroup comprised of two clusters. The first consisted of the accessions of *S. aethiopicum* (2 and 3) while the second consisted of the two wild species with spiny character: *S. dasyphyllun* (1) and *S. torvum* (4) (Fig. 1).

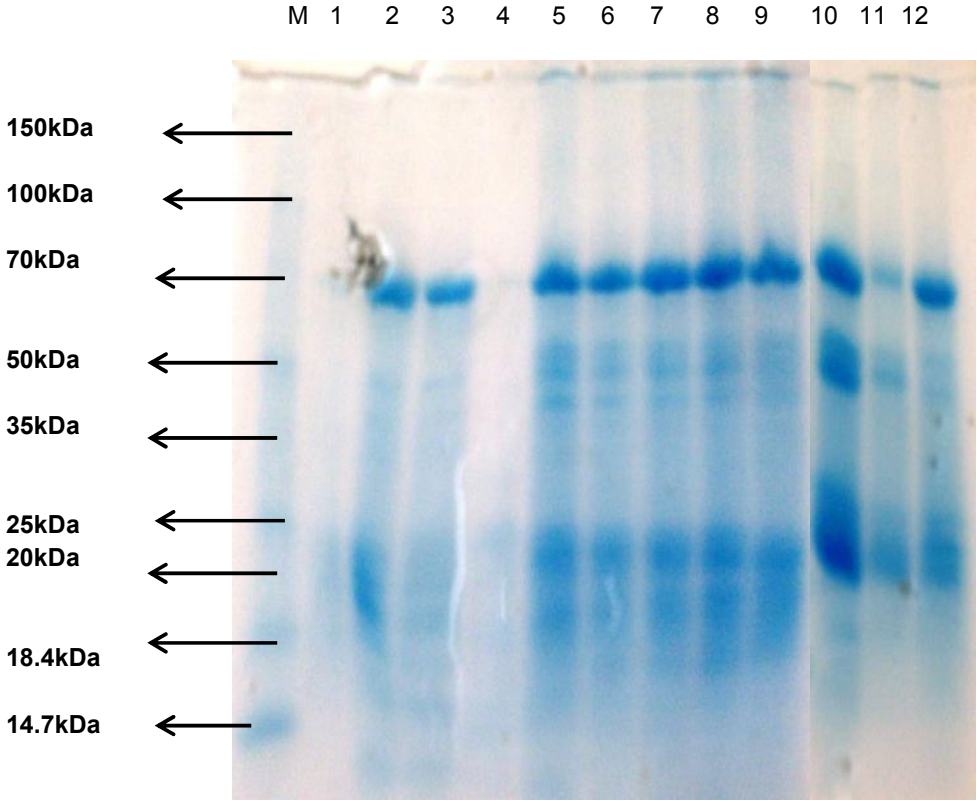

Plate 1: Electrophoretic banding pattern of seed protein of *Solanum* species generated by SDS- PAGE

Key:

M - Marker
1 – *S. dasyphyllum*
2 – *S. aethiopicum* (green fruit)
3 – *S. aethiopicum* (mosaic fruit)
4 – *S. torvum*
5 - *S. macrocarpon* (porrect fruit)
6 - *S. macrocarpon* (pendulous fruit)
7 - *S. macrocarpon* (white fruit)
8 - *S. macrocarpon* (Purple stem)
9 - *S. macrocarpon* (blossom-end upright fruit)
10 - *S. americanum*
11 - *S. nigrum*
12 - *S. Melongena*

Plant species	Accession number	Total number of bands
S. dasyphyllum	1	5
S. aethiopicum	2	7
S. aethiopicum	3	7
S. torvum	4	6
S. macrocarpon	5	7
S. macrocarpon	6	8
S. macrocarpon	7	8
S. macrocarpon	8	8

S. macrocarpon	9	8
S. americanum	10	7
S. nigrum	11	7
S. melongena	12	7

Table 1: Total number of protein bands in SDS-PAGE of *Solanum* species analysed

Table 2: Similarity index for the *Solanum* accessions studied

Accession Number	1	2	3	4	5	6	7	8	9	10	11	12
1	1	0.66*	0.66*	0.82**	0.66*	0.50	0.50	0.50	0.50	-0.22	-0.22	-0.22
2		1	1.00**	0.80**	0.52	0.22	0.22	0.22	0.22	-0.43	-0.43	-0.43
3			1	0.80**	0.52	0.22	0.22	0.22	0.22	-0.43	-0.43	-0.43
4				1	0.80**	0.61	0.61	0.61	0.61	-0.09	-0.09	-0.09
5					1	0.76*	0.76*	0.76*	0.76*	0.05	0.055	0.05
6						1	1.00**	1.00**	1.00**	0.22	0.22	0.22
7							1	1.00**	1.00**	0.22	0.22	0.22
8								1	1.00**	0.22	0.22	0.22
9									1	0.22	0.22	0.22
10										1	1.00**	1.00**
11											1	1.00**
12												1

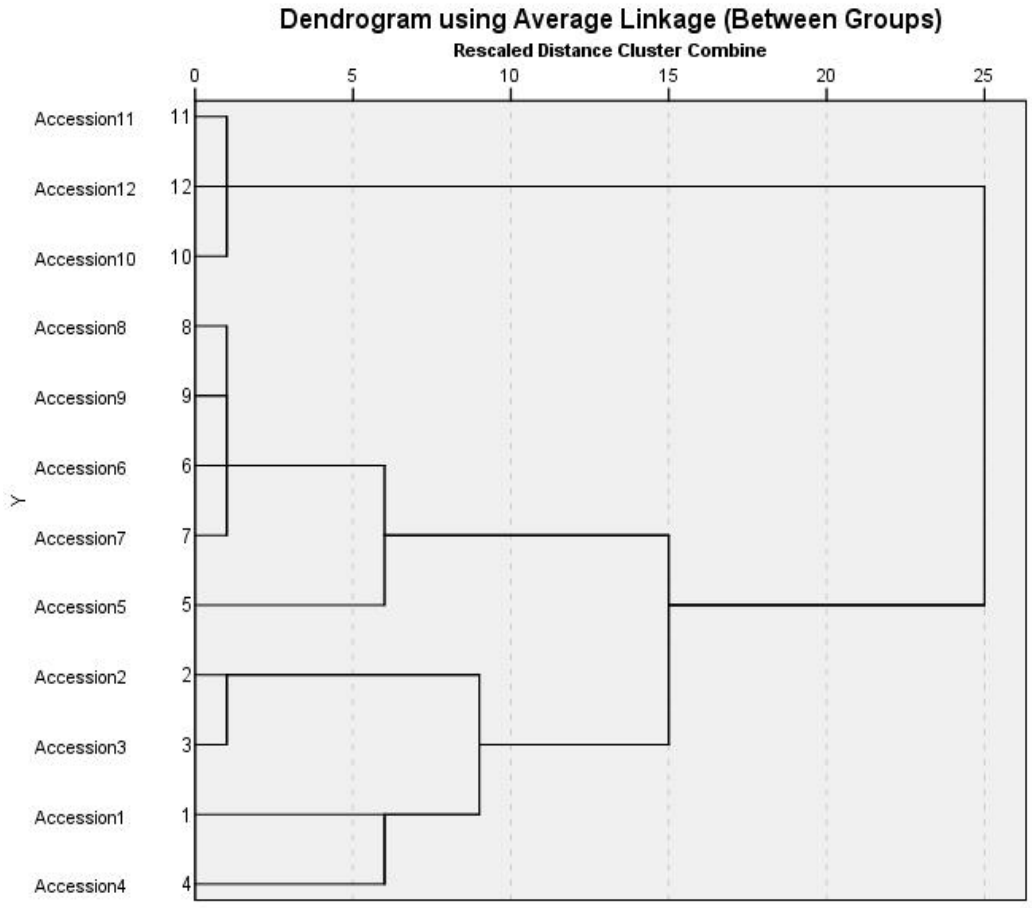

Fig. 1: Dendrogram showing relationships based on gel electrophoresis

4. DISCUSSION

Morphological similarities of characters contributed a lot toward the difficulty in identification of *Solanum* species. Seed protein patterns obtained using the PAGE method have been successfully used to elucidate the taxonomy and evolutionary relationship of several species because banding patterns are species specific and completely dependent on the genotype [12]. The banding patterns observed in this study showed the relationship among the accessions despite the variations in their fruit colour and stem colour and fruit orientation, varietal identification was also possible in the accessions of the same species (*S. macrocarpon* and *S. aethiopicum*) which confirmed the origin of new cultivars. Presence of common bands in varieties was also observed in *Capsicum* species by Olatunji and Morakinyo []this suggests the evidence of evolutionary relationship of the plant.

However, seed protein could still not taxonomically distinguish the morphologically similar species, *S. americanum* and *S. nigrum*. It is therefore necessary to employ more delimiting molecular methods such as random amplified polymorphic DNA (RAPD) to ascertain the relationship between the species. Also seed protein revealed *S. melongena* being closely related to *S. americanum* and *S. nigrum,* although the species differ greatly morphologically. This observation contradicts the earlier report of Pearse and Lester [13] (based on serological data), Sakata *et al.* [14] (based on chloroplast DNA) and Ona *et al*. [10] (based on seed proteins) that *S. melongena* is most closely related *S. aethiopicum* and maintained a distant relationship with *S. nigrum*. However the result is in accordance with Yousaf *et al*. [6] who suggested based on electrophoresis the same group for *S. nigrum, S. americanum* and *S. melongena*. The result of SDS-PAGE favours the two wild species (*S. torvum* and *S. dasyphyllum)* as both possessed similar and patterns and also grouped together by the dendrogram. While some investigators proposed that seed protein was insufficient for the discrimination at the cultivar level [15], the findings in this study indicated that SDS-PAGE of seed

protein supplied additional information on delimiting the species of the genus *Solanum* and could also be used for genetic improvement of the species.

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