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Seed Electrophorentic Characterization and Taxonomic Implications of some accessions of *Abelmoschus esculentus* L. (Moench) in Nigeria

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Abstract

Five accessions of *Abelmoschus esculentus* (okra) were subjected to electrophoretic analysis. Analysis of total soluble proteins in seeds was done by using one-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The main aim of the study was to discriminate the Okra accessions based on the nature of seed protein present in the seeds. On the basis of banding pattern zymogram was sketched, shows polymorphic which means presence of more than one band in each genotype. There are 11 zymotypes observed at different Rf values varied from 0.082-0.983. We scored the bands based on 0 for absence and 1 for presence of each band, then similarity coefficients was calculated which ranged from 0.62 to 1. Electrophoresis of seed proteins showed a total of 11 bands in NGB01288 and NGB01280; also all the intensity was presence, whereas for rest of the genotypes some bands were absent. The critical bands for identification were the bands at Rf values 0.877, 0.729, 0.491, 0.237, 0.147 and 0.082 which formed the 2nd, 4th, 7th, 9th, 10th and 11th bands respectively. Through the presence and absence of band in each of the accessions dendrogram was formed and it was clustered into different groups which revealed a close relationship among all the accessions. The SDS-page analysis of seed proteins showed not only the total number of bands, but also their intensity in term of thickness. Hence, the present study obviously indicated the use of SDS page profile through electrophoresis for discrimination of Okra genotypes.

Keywords: Electrophoresis, okra accessions, systematics

Introduction

Okra *Abelmoschus esculentus* L. (Moench), is an economically important vegetable crop grown in tropical and sub-tropical parts of the world. This crop is suitable for cultivation as a garden crop as well as on large commercial farms. It is grown commercially in India, Turkey, Iran, Western Africa, Yugoslavia, Bangladesh, Afghanistan, Pakistan, Burma, Japan, Malaysia, Brazil, Ghana, Ethiopian, Cyprus and the Southern United States. India ranks first in the world with 3.5 million tonnes (70% of the total world production) of okra produced from over 0.35 million ha of land (FAOSTAT, 2008).

Okra is known by many local names in different parts of the world. It is called lady's finger in England, gumbo in the United States of America, ila (Yoruba) Nigeria, guino-gombo in Spanish, guibeiro in Portuguese and bhindi in India. Okra is cultivated for its fibrous fruits or pods containing round, white seeds. The fruits are harvested when immature and eaten as a vegetable. The roots and stems

of okra are used for cleaning the cane juice from which gur or brown sugar is prepared (Chauhan, 1972). Its ripe seeds are roasted, ground and used as a substitute for coffee in some countries. Mature fruits and stems containing crude fiber are used in the paper industry. Extracts from the seeds of the okra is viewed as alternative source for edible oil. The greenish yellow edible oil has a pleasant taste and odor, and is high in unsaturated fats such as oleic acid and linoleic acid. The oil content of the seed is quite high at about 40%. Okra provides an important source of vitamins, calcium, potassium and other mineral matters which are often lacking in the diet of developing countries (IBPGR, 1990). Okra is said to be very useful against genito-urinary disorders, spermatorrhoea and chronic dysentery. Its medicinal value has also been reported in curing ulcers and relief from hemorrhoids (Adams, 1975).

Okra was earlier included in genus *Hibiscus*, section *Abelmoschus* in the family Malvaceae. The wider use of *Abelmoschus* was subsequently accepted in the taxonomic and contemporary literature. This genus is distinguished from the genus *Hibiscus* by the characteristics of the calyx, spathulate, with five short teeth, connate to the corolla and caduceus after flowering (Kundu and Biswas, 1973; Terrell and Winters, 1974). About 50 species have been described by taxonomists. The taxonomical revision undertaken by Borssum (1966) and its continuation by Bates (1968) constitute the most fully documented studies of the genus *Abelmoschus*.

In furtherance of taxonomic works on okra, an electrophoretic technique is employed in this work. Electrophoresis is the process of moving charged molecules in solution by applying an electric field across the mixture. It is an important analytical technique for the separation of biomolecules (proteins, amino acids, nucleic acids, lipids). Molecules in an electric field move with a speed dependent on their charge, shape and size. Electrophoresis has been extensively developed for molecular separations. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is a technique for separating proteins based on their ability to move within an electrical current, which is a function of the length of their polypeptide chains or of their molecular weight. This is achieved by adding SDS detergent to remove secondary and tertiary protein structures and to maintain the proteins as polypeptide chains. The SDS coats the proteins, mostly proportional to their molecular weight, and confers the same negative electrical charge across all proteins in the sample. Glycosylated proteins may not migrate at their expected molecular weights since their migration is based more on the mass of their polypeptide chains not the sugars that are attached (Sambrook *et al*., 1989). SDS-PAGE of proteins is the most commonly used method to discriminate the varieties. The protein banding pattern is unique for the particular genotype and is independent of seed vigor and physiological seed activity (Kamel *et al*., 2003).

The use of seed protein electrophoresis for taxonomic and evolutionary purposes has greatly increased and used within genera and families (Esen and Hilu, 1989, Henry and Taylor 1989, Mc Daniel, 1970, Ladizinsky and Hymowitz, 1976). Johnson and Hall (1965) have stated the role of seed protein electrophoresis in supporting genomic relationships and tracing the origin and evolutionary pathways of many grasses. This work aims at using presence or absence of soluble protein bands to determine the relationship among five Okra accessions in Nigeria. Also to determine using protein bands for the genetic diversity and relatedness in the five selected accessions.

Materials and Methods

Plant materials

Five accessions of okra seed namely 1. Okra NGB/06/083, 2. Okra NG/SA/DEC/07/0441, 3. Okra NGB 01322, 4. Okra NGB 01288, and 5. Okra NGB 01280 were obtained from National Center for Genetic Resources and Biotechnology (NACGRAB), Apata, Ibadan, Nigeria.

Seed proteins extraction

The seeds used for extraction of protein were dried in an oven and grounded to powder using a laboratory mortar and pestle, then the powder were homogenized with an extraction buffer containing 0.05M Tris-HCl pH 7.4 at 4^{o}C. The homogenate was centrifuged at 10,000g for 15mins at 4^{o}C and the supernatant

(total seed protein fraction) was used for electrophoresis.

One-dimensional gel electrophoresis (SDS-PAGE)

One dimensional electrophoresis (SDS-PAGE) was performed according to the procedure of Laemmli (1970), on a vertical slab gel (Mini-PROTEAN II Electrophoresis Cell) using a 12% gradient separating (acrylamide/bis acrylamide) gel and a 4% (w/v) stacking gel containing 0.1% SDS. Aliquots (10-15 µl) of each sample were applied onto each of the 20 gel wells. Wide range marker (6.500 to 200,000 kDa) was loaded onto the first well and was used as the standard. 1-DE SDS-PAGE was performed at constant voltage of 200 V for about 6hrs until the tracking bromophenol blue dye migrated to the bottom of the gel. At the end of the run, the gels were stained with Coomasie Brilliant Blue R250 in methanol/water/acetic acid (4:5:1 v/v/v) and destained with methanol/water/acetic acid (4:5:1 v/v/v) over night. Wide range molecular weight maker was used for the molecular weight estimation of the protein subunits. 12% SDS-gel was used for the run.

Electrophoresis technique was carried out by using a Mini-protean 11 Electrophoresis gel unit applying a constant current of 200mA until the tracking dye, bromophenol blue reached the bottom. The wide molecular weight sigma Maker containing 13 proteins from 6,500-205,000 daltons in size was used to trace the band size. The gels were taken out and stained using Coomasie blue stain solution overnight and de stained using methanol (40%) and acetic acid (10%) solution. Then using de-staining solution the gel was de-stained up to background become clear. The gels were placed in trans-illuminator and photographed.

To avoid ambiguity in data, only major protein bands between 6,500 and 205,000 daltons were considered for data recordings. For estimating the relationship between electrophoretic phenotype, similarities coefficients were calculated using (Nei's and Li, 1979) index of genetic similarity comparisons (Sxy) (Nei's and Li, 1979), which were:

$$Sxy = 2nxy/ (nx+ny)$$

Where, 2nxy = number of shared bands,

nx = number of bands in electrophoretic patterns of x, and

ny = number of bands and electrophoretic patterns of y.

Similarity coefficients were determined for all possible pairs of five Okra accessions ranging from 0.62 to 1. Similarity coefficient (Sxy) values of electrophoretic phenotypes were subjected to cluster analysis employing the unweighted pair group method using arithmetic means (UPGMA). Construction of dendrogram and cluster analysis was based on the value of 1 was put for the presence of the electrophoretic pattern and value 0 was used against the absence of the pattern of band for each genotypes.

Rf value a relative mobility or retention factor was calculated by the distance protein band migrated divided by the distance migrated by the dye front following the method of Mouemar and Gasquez 1983).

$$Rf = \frac{\text{Distance protein band moves}}{\text{Distance dye front moves}}$$

The data generated were analyzed statistically using software SPSS (Ver. 20.0).

Results

The total seed protein was extracted and separated by SDS PAGE method. The detailed electrophoretic profile of 5 okra genotype has been presented in Fig 1. The same polymorphism in their electrophoretic banding patterns and led to the detection of a total of 11 polypeptide bands. As can be seen, eleven polypeptide bands designated 1 to11 with molecular weight range of 6.500kDa to 200,000kDa could be distinguished. A close examination of the bands shows that the different accessions have the slight differences in their banding patterns with respect to the numbers and intensities. The detailed electrophoregram of total soluble seed protein has been presented in Fig 2. The entire protein was divided into five regions starting from A to E. This was in order of increasing Rf values and decreasing molecular of proteins. Among the five regions B and E were most useful to distinguish accessions due to clear banding appearance. The Rf value for all the bands found in the entire profile have been presented in Table 2.

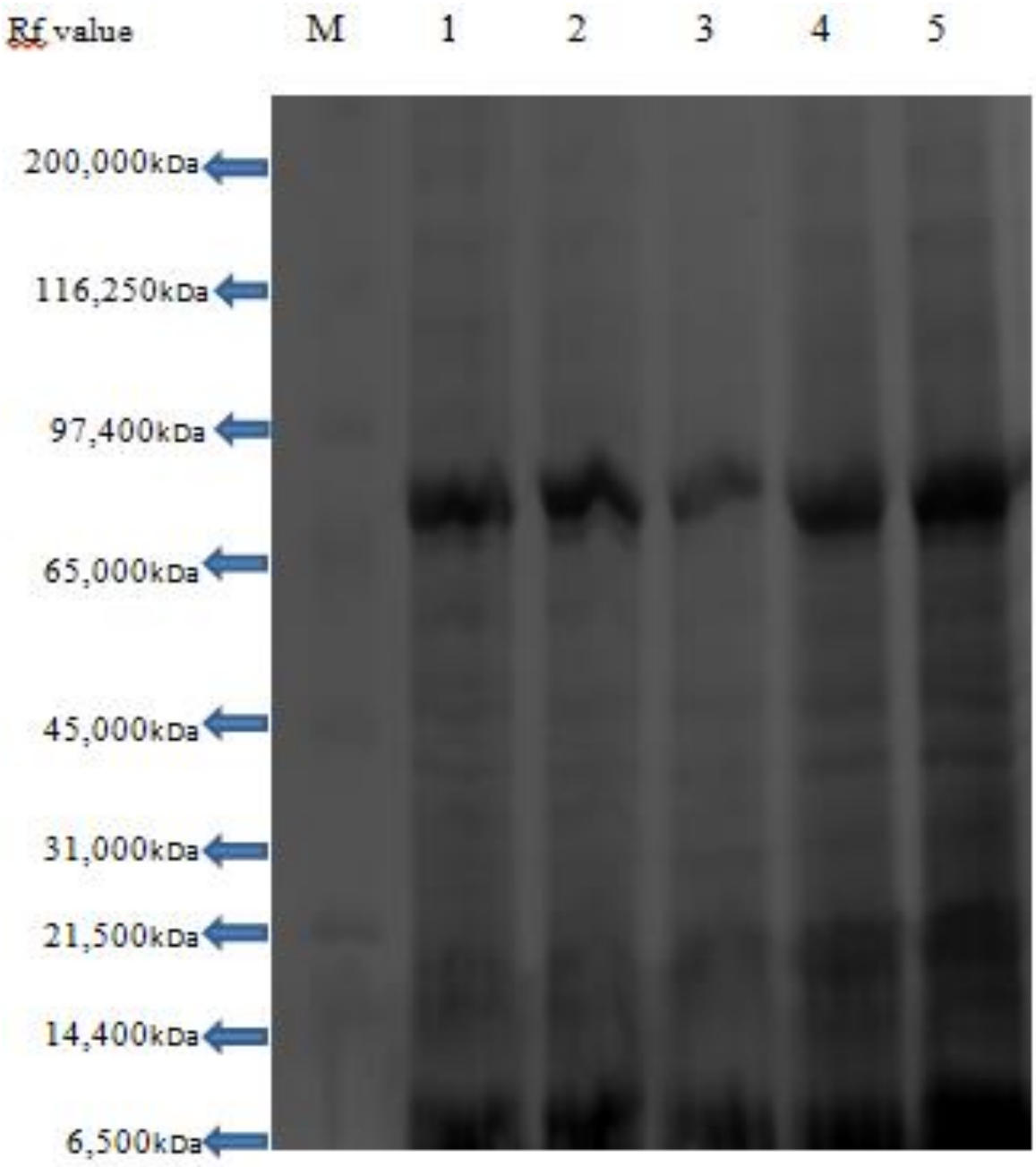

Fig. 1: Total seed protein profile of Okra genotype through SDS PAGE.

1: NGB/06/083, 2: NG/SA/DEC/07/0441, 3: NGB01322, 4:NGB01288, 5: NGB01280. M: Molecular weight (kDa).

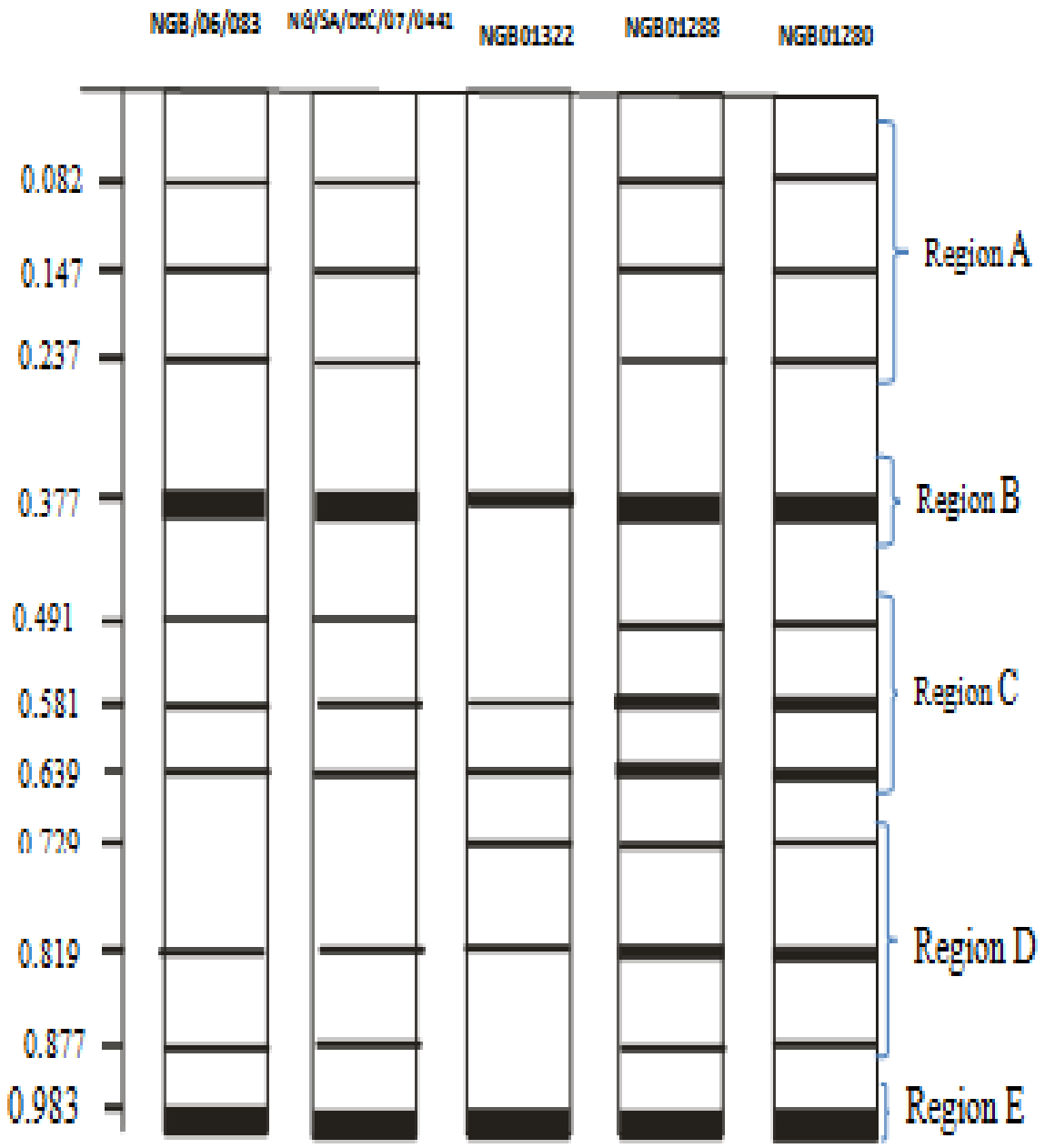

Fig. 2: Zygmogram of total soluble seed protein of Okra genotypes through SDS PAGE

KEY

High intensity

Medium intensity

Low intensity

The presence or absence of a band and the intensity of band were used for varietal characterization. The bands with low intensity of Rf value 0.082, 0.147 and 0.237 was categorized as Region A. Region B have only one band of medium and high intensity with Rf value of 0.377. Region C was appeared with the Rf values lies between 0.491, 0.581 and 0.639 with three bands of low and medium intensity. In Region D three bands with low and medium intensity whose Rf values lies between 0.729, 0.819 and 0.877. In the last Region which is E was characterized with one band of Rf value 0.983 with high intensity.

In the present investigation for total seed protein, five common bands (0.377, 0.581, 0.639, 0.819 and 0.983) were detected for all the five genotypes. The band at Rf value 0.082, 0.147, 0.237, 0.491 and 0.877 was present in all the accessions except NGB 01322. Similarly the band at Rf value 0.729 was present in NGB 01322, NGB 01288 and NGB 01280 while it was absent in NGB/C6/083 and NG/SA/DEC/07/0441. Thus the absence of the bands was effectively used for discriminating the Okra genotypes.

Cluster Analysis for the 5 Accessions of *Okra abelmoschus* Using Protein Profile Bands

The cluster analysis performed revealed 3 major clusters as evident from dendrogram (Fig.2) constructed from proximity matrix of Square Euclidean distance.

The first cluster consists of 2 genotypes which includes D, E (NGB01288 and NGB01280), the second cluster consists of 2 genotype which includes A, B (NGB/C6/083 and NG/SA/DEC/07/0441) while the third cluster consists of A, D (NGB/C6/083 and NGB01288).

From this dendrogram it was observed that sample D and E are more homogenous, sample A and sample B are also homogenous. For this cluster 1 comprising of sample D and E, cluster 2 comprising of sample A and B are more homogenous. There is one outlier in the dendrogram which is sample C mean that it was substantially different from other samples, because it does not form a cluster. However, the clustering scores among the accessions suggested that there is a strong relationship amongst individuals in a cluster (i.e. they are closely related).

Table 2: Rf values, intensity and position of protein bands for Okra accessions using SDS-page

Band	Rf value	NGB/ C6/083	NG/SA/DEC/ O7/0441	NGB 01322	NGB 01288	NGB 01280
1	0.983	+++	+++	+++	+++	+++
2	0.877	+	+	-	+	+
3	0.819	+	+	+	++	++
4	0.729	-	-	+	+	+
5	0.639	+	+	+	++	++
6	0.581	+	+	+	++	++
7	0.491	+	+	-	+	+
8	0.377	+++	+++	++	+++	+++
9	0.237	+	+	-	+	+
10	0.147	+	+	-	+	+
11	0.082	+	+	-	+	+

-: No intensity, +: Low intensity, ++: Medium intensity, +++: High intensity

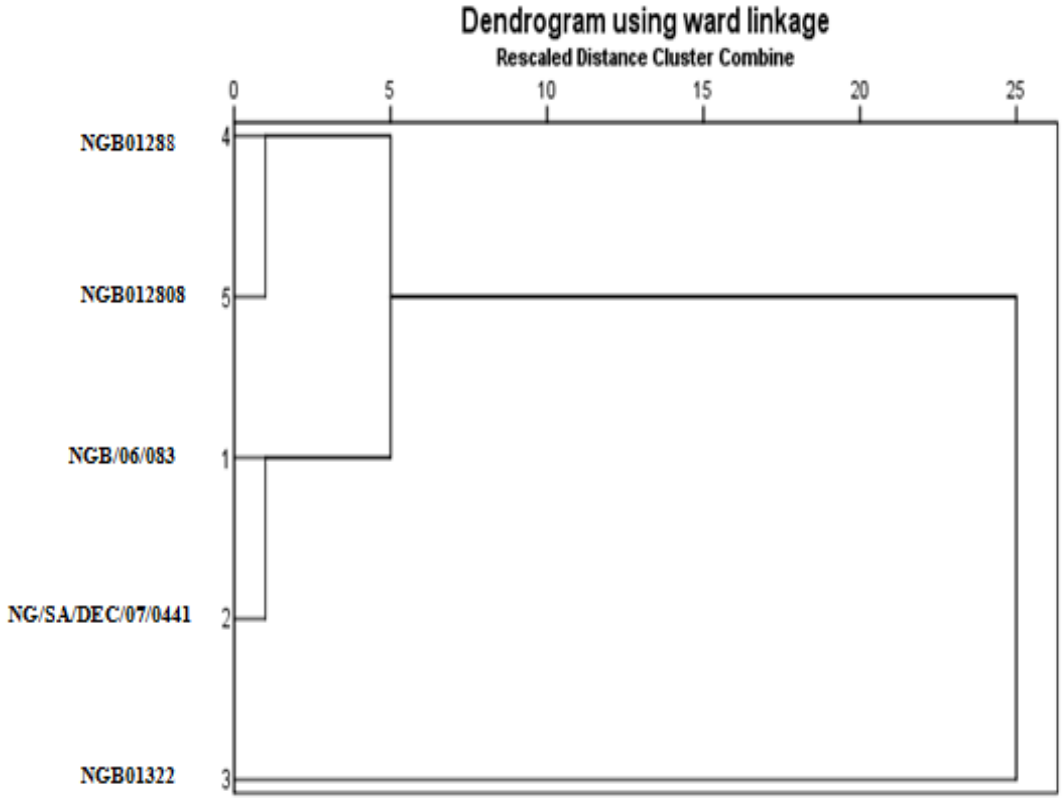

Figure 3: Hierarchical Cluster Analysis for the 5 accessions of *Abelmoschus esculentus* studied using protein profile

Discussion

Seed protein analysis by SDS-PAGE has proved to be an effective way of revealing the differences and relationships between taxa. The high stability of the seed protein profile and its additive nature make seed protein electrophoresis a powerful tool in elucidating the origin and the evolution of cultivated plants (Ladizinsky and Hymowitz, 1976). Proteins are considered to be direct products of genes and can be taken as markers of these genes (Ladizinsky, 1979). As such protein can be taken as additional means for characterizing systematic categories. Ladizinsky (1979) reported that seed protein profile often shows genetic affinities within a taxon or between different biological entities and that seed protein profile is species specific.

The present investigation revealed great similarities in the overall polypeptide profile of the seed proteins from the 5 accessions of the Okra studied. However, the analytical system revealed moderate differences in the accessions. This uniformity of seed protein profiles agreed with the findings of Ladizinsky and Alder (1975), which examined different cultivars of chickpea and concluded that seed protein, was a very conservative trait in chickpea. Similarly, Raymond *et al*. (1991) and De Vries (1996) also reported similar electrophoretic patterns of protein among the cultivars of sunflower and lettuce, respectively. Ladizinsky and Hymowitz (1976) also stated that taxonomic categories below the species level, despite morphological and ecological differences, still possess basically the same seed protein profiles.

Electrophoresis of proteins is a powerful tool to identify genetic diversity. However, the SDS-PAGE is particularly considered as a reliable technology because seed storage proteins are highly independent of environmental fluctuations (Valero *et al*., 2011). Seed protein patterns can also be used as a promising tool for distinguishing cultivars of particular crop species. The SDS-PAGE is considered to be a

practicable and reliable method for species identification (Miernyk and Hajduch, 2011).

The present study revealed that there was no difference in banding pattern on region A and E. accession 4 and 5 which is distinct and diverse can serve as sources for variability in characters for the improvement of the accessions studied. Meanwhile, crosses between accessions in a cluster may not produce meaningful improvement in the offsprings, since these accessions are expected to have similarities in gene and therefore may not introduce reasonable variation. This agrees with the report of Torkpol *et al*. (2006). Accessions 1, 2, 4 and 5 were the closest accessions with 98% similarity. High percentage of similarity could be related to these accessions which may have similar ancestral origin with a common gene.

The variation in number and intensity of the bands might be due to differential extraction or difference in solubility of protein or lack of separation of several proteins having similar migration rates (Ladizinsky and Hymowitz, 1976). Similar observations based on band intensity were reported by Chun *et al*. (1994, Asghar and Afzal (2003) and Varma *et al*. (2005) in maize genotypes, Devi (2000) in sunflower, Vijayan (2005) in rice, Paul and Datta (2006) in celery and ajowan, Nisha (2007) in wheat and Sumathi (2007) in oats. The two accessions (NGB01288 and NGB01280) show high resemblance in bands patterns and the same intensity to each other which seems to be further prove to their evolutionary similarity. These results were also confirmed by similarity matrix and dendrogram analysis (Fig. 3). In the same manner, Ladizisky and Hymowitz (1976) reported variation in number and position of bands in the accessions of the same species. They reported also differences in the darkness and thickness of various bands in the accessions of the same species, suggesting that bands characters are under control of quantitative gene systems.

The difference in banding pattern was mainly confined only to B, C and D regions (Fig 2). The region A and E of the total seed protein gel profile obviously indicated the usefulness of the high and low molecular proteins than the medium range molecular weight proteins for varietal discrimination of the Okra genotypes. The presence or absence of a band and the intensity of band were used for varietal characterization.

Electrophoresis of seed proteins showed a total of 11 bands in NGB01288 and NGB01280 whereas for rest of the genotypes some bands were absent. The critical bands for identification were the bands at Rf values 0.877, 0.729, 0.491, 0.237, 0.147 and 0.082 which formed the 2nd, 4th, 7th, 9th, 10th and 11th band, respectively. The intensity of the band also varied among all the genotypes. Hence the present study obviously indicated the use of SDS page profile through electrophoresis for discrimination of Okra genotypes.

In conclusion, seed proteins in Okra are useful characters to determine relatedness between accessions. In addition, these characters show no environmental and low evolutionary variability. The results show the close relationships between accessions studied. Also it clearly showed that it was impossible to discriminate various genotypes from each other, as they were characterized by almost the same banding patterns. However, it might be useful to distinguish diverse forms of it from one another. The present investigation revealed very limited variation in Okra genotypes studied.

The clustering scores among the accessions suggested that there is a strong relationship amongst individuals in the cluster analysis especially accession A, B (NGB/C6/083 and NG/SA/DEC/07/0441), accession D, E (NGB01288 and NGB01280) and also accession A, D (NGB/C6/083 and NGB01288). All these accessions are closely related (Fig. 3). Accessions 4 and 5 (NGB01288 and NGB01280) are homogenous in term of their band and intensity (Table 2; Fig. 2). Finally, accessions 4 and 5 (NGB01288 and NGB01280) are more distinct and diverse of all the accessions and can serve as sources for variability in character for okra improvement.

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