Evaluation of Genetic Diversity within Some Cucurbitaceae Seeds from Nigeria using Electrophoresis

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ABSTRACT

Taxonomic relationships within seven species of *Cucurbitaceae*; melon seeds were examined at the molecular level with the help of seed storage proteins using electrophoretic methods. Water soluble seed proteins were resolved into 99 and 218 bands respectively under Native- and SDS-PAGE. Similarly 132 and 123 bands respectively were observed for buffer soluble proteins. For water soluble proteins under Native- PAGE, the Jaccard's similarity indexes indicated that *Lagenaria siceraria/Cucurbita moschata* showed highest similarity index (~96%) while *Cucumis sativus/Colocynthis vulgaris* showed the lowest similarity index (~35%). For buffer extracts, highest similarity index (~81%) was exhibited by *C. sativus/C. maxima* while *C. sativus/C. mannii* showed lowest similarity index (~51%). For water soluble proteins under SDS-PAGE, highest similarity index (~78%) was found between *C. maxima/C. vulgaris* and the lowest similarity index (34%) was observed between *C. sativus/L. siceraria*. For buffer extracts, highest similarity index (~98%) was exhibited by *C. mannii/C. moschata* while *C. maxima/C. vulgaris* showed the lowest similarity index (~41%). However, from the dendograms constructed, buffer soluble proteins under SDS-PAGE proved to be the best method as it was the only one that grouped *C. mannii* and *C. edulis* according to their taxonomic classification.

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INTRODUCTION

Melon with a fibrous and shallow root system is a herbaceous leguminous plant belonging to the *Cucurbitaceae* (gourd) family. It grows in tropical, subtropical, arid deserts and temperate locations and consist of 118 genera with about 825 species (Whitaker and Davis, 1962). Melon is one of the most important grain legume crops in the Sub-Saharan regions of Africa where it is interplanted with early maize, bean and yam (Coble, 1957; Bates *et al.*, 1990). Most *Cucurbits* demonstrate exuberant ethno-medicinal and agronomical chattels and are consumed as vegetal crops by humankind. Several parts of melon such as dry or fresh seeds, the mature pods, and the leaves are used for human consumption. The melon seeds are usually

decorticated, ground and used as a thickener of soups or prepared into a nutritious melon cake and cooked. For this purpose, melon is a valuable source of income for farmers and traders in most African countries.

The *Cucurbitaceae* seeds contain a high protein content that makes them valuable as good and cheap sources of proteins (Nwokolo and Sim, 1987; Fokou *et al.*, 2002; Achu *et al.*, 2005; 2013). Studies on *Cucurbitaceae* seeds showed they have a high protein content with high levels of most essential amino acids except lysine (El-Adawy and Taha, 2001; Akpambang *et al.*, 2008; Horax *et al.*, 2011; Ogundele *et al.*, 2012; Achu *et al.*, 2013; Penuel *et al.*, 2013). Lal *et al.*, (1983) showed that the oil- free kernel meals of different Cucurbit species had 60 to 70% of proteins. Jacks (1986)

reviewed the usefulness of Cucurbit seeds and showed that globulins account for 70 to 90% of the protein. Achu *et al.*, (2005; 2013) showed that these seeds and their defatted cakes have protein contents of 28 to 40.49% and 61 to 73.59% respectively. Kanar *et al.*, (2006) reported that *Cucumis sativus* and *Lagenaria vulgaris* seeds contained 31.2 to 31.8% crude proteins and that heat treatment reduced the trypsin inhibitor and lectin activities in both samples to negligible levels. Yanty *et al.*, (2007) showed that the crude protein content of Malaysian *Cucumis melo* seeds was 25% while Loukou *et al.*, (2007) found similar results with Cucurbit seeds from Côte d'Ivoire. Ojeh *et al.*, (2008) showed that *Citrullus lanatus* has a crude protein content of 23.4% and competes favourably with protein rich legumes such as soybean, cowpeas and pigeon peas (Akpambang *et al.*, 2008; Horax *et al.*, 2011; Ogundele *et al.*, 2012).

The *Cucurbitaceae* family has a tremendous genetic diversity, extending to vegetative and reproductive characteristics (Ng, 1993). Information on genetic diversity and relationships among crop species is essential for the efficient explanation of taxonomic relationships and to assist plant breeders to select species to be utilized in hybridization programmes (Ghafoor *et al.*, 2002). Presently, several molecular approaches are deployed to assess genetic diversity and taxonomic relationships. These include among others isoenzymes or random amplified polymorphic DNA (RAPD), and generate data faster than restriction fragment length polymorphism (RFLP) or the use of microsatellites (Chan and Sun, 1997). Proteins can serve as genomic markers since they are the primary products of the genetic system and thus can give an insight into the genome structure and genotype specificity as a whole. The most commonly used proteins are seed storage proteins, which are polymorphic with respect to size, charge, or both and are scorable, from inviable organ or tissues (Cooke, 1984; Marcone and Yada, 1998; Martinez *et al.*, 1997). Storage proteins are found in seeds of dicotyledon plants and in cereal grains. The storage proteins of dicotyledon plants are mainly globulins and albumins (Segura-Nieto *et al.*, 1999; Duranti and Gius, 1997; Muchova *et al.*, 2000; Marcone and Yada, 1998; Martinez *et al.*, 1997; Nakamura *et al.*, 1998). Storage seed proteins are suitable genetic markers for species/cultivars identification purposes in crops because they are highly polymorphic, their polymorphism is genetically determined and the molecular sources of their polymorphism are known, they are not sensitive to environmental fluctuations, they are conserved and their banding pattern is very stable (Sadia *et al.*, 2009).

Electrophoretic analysis of seed storage proteins is a reliable method for species identification. It is practically simple, inexpensive and a method of choice in plant breeding experiments (Sadia *et al.*, 2009). Seed storage protein profiling based on SDS-PAGE can be deployed for various purposes, such as characterization of germplasm (Javid *et al.*, 2004; Iqbal *et al.*, 2005), varietal identification (Sheidai *et al.*, 2000), biosynthetic analysis and the determination of genetic diversity and phylogenetic relationship between different species (Sadia *et al.*, 2009; Sammour, 1991; Ghafoor *et al.*, 2002; Lukong *et al.*, 2014). Genetic diversity and the pattern of variation in many dicotyledon plants have been evaluated with seed protein (Ghafoor *et al.*, 2002;

Lukong *et al.*, 2014) including the *Cucurbitaceae* (Singh and Ram, 2005; Kandasamy, 2014; Lukong *et al.*, 2015; Barman *et al.*, 2015). To our knowledge, no studies have yet been carried in Nigeria on the diversity of indigenous *Cucurbitaceae* germplasm based on protein electrophoresis. So the present study aimed, to evaluate the protein polymorphisms in some Nigerian melon species and to clarify the genetic nature of polymorphic bands. These results would assist plant breeders and producers to understand the genetic variability and relationship among the various national melon species so as to facilitate the transfer of useful genes among cultivated species and maximizing the use of available germplasm resources.

MATERIALS AND METHODS

Materials: Sodium dodecyl sulfate (SDS), acrylamide, polyacrylamide, Coomassie Brilliant Blue R-250 and molecular weight markers (14-78 kDa) used were of analytical grade and were purchased from Sigma-Aldrich Chemical Co, St Louis, MO, USA. All reagents were freshly prepared unless otherwise stated and deionized water was used for all reagent preparation.

Sample Sources and Characteristics: The germplasms of seven different species of mature *Cucurbitaceae* seeds (*Cucumis sativus*, *Cucurbita maxima*, *Lagenaria sicerani*, *Colocynthis vulgaris*, *Cucumeropsis mannii*, *Cucurbita moschata* and *Cucumeropsis edulis*) were obtained from Ihiala main market in Anambra State, South-East region of Nigeria. The seeds were coded 1, 2, 3, 4, 5, 6 and 7 respectively. All species were identified by Onyeukwu, C.J. from the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. The seeds were dehulled and well ground using a warring commercial blender (Smart Grind, Black and Decker, Towson, MA, USA). The flour was defatted as described by Sammour *et al.*, (1995) in three hexane extractions (10 ml hexane/g flour), each for 2 hours with slow stirring at 4°C. After, the n-hexane layer was discarded and the flour was air-dried. With the aim to remove the impurities and to obtain a uniform product, the whole flour was sieved through a net with mesh size of 75 µm. Flour samples were packaged in sealed low density polyethylene bags and stored in the refrigerator prior to analysis.

Methods

Protein Extraction: Water and buffer soluble seed protein extracts were prepared from the seven species of melon as described by Lukong *et al.*, (2015). The buffer soluble seed protein extracts were prepared when a portion (30 mg) of defatted flour was mixed with 0.5 ml of 50 mM Tris-HCl buffer pH 8.0 in an Eppendorf tube overnight at room temperature and then centrifuged in micro-centrifuge machine (Eppendorf) at 23 000 x g for 15 min at 15 °C. The residue was re-extracted twice under the same conditions. All the extracts were combined and stored at -10 °C until used. Water soluble seed protein extracts were prepared in a similar manner and the buffer was replaced with deionized water. Total protein in all the extracts was estimated by the Lowry *et al.*, (1951) method using bovine serum albumin as standard protein.

Electrophoresis: Protein separation was carried out in vertical slabs using the TV50 Camlab (England) Vertical Electrophoresis Unit. Gel electrophoresis of the extracted water and buffer soluble seed proteins were performed using 5 % stacking and 12.5 % separating gels according to the method of Laemmli (1970) with our modifications (Lukong *et al.*, 2015).

The polymerization mixture for native PAGE contained 16.7 ml of 30 % acrylamide, 10 ml of 4x resolving gel buffer (pH 8.8), 13.2 ml deionized water, 200 µl of 10 % ammonium persulfate and 13.3 µl of TEMED. The stored buffer-or water- soluble seed protein extracts (10 µl) were solubilised separately in sample buffer consisting of 4x stacking gel buffer (pH 6.8), deionized water; 10 % glycerol and 0.1 % bromophenol Blue and 20 µl was applied to the gel.

The polymerization mixture for SDS PAGE contained 16.7 ml of 30 % acrylamide, 10 ml of 4x resolving gel buffer (pH 8.8), 0.4 ml of 10 % SDS, 12.8 ml of deionized water, 200 µl of 10 % ammonium persulfate and 13.3 µl of TEMED. The stored buffer-or water- soluble seed protein extracts (10 µl) were solubilised separately in sample buffer consisting of 4x stacking gel buffer (pH 6.8), 10 % SDS; 10 % glycerol and 0.1 % bromophenol Blue. The mixture was heated in a boiling-water bath for 5 min and was placed on ice until 20 µl of the mixture was applied to the gel. Pharmacia low-molecular weight (14 to 100 kDa) protein marker was used along with the sample to determine the molecular weight of separating fractions.

The gels were electrophoresed using a current of 15 mA and a voltage of 300 V (using Consort E844 power pack) until the bromophenol blue tracker dye reached the bottom of the gel. Gels were fixed and stained with 0.2 % Coomassie Brilliant

blue R-250 in methanol: acetic acid: deionized water (5:4:1, v/v/v) overnight. Afterwards, the gels were destained by using the solvent of the stain mixture; methanol: acetic acid: deionized water (5:4:1, v/v/v) until protein bands became clearly visible.

Gel documentation and analysis. The electrophoregrams of protein profile were prepared after which the gel was photographed and documentation was carried out with the obtained results. The band pattern of each species profile from the electrophoregrams was coded as 0 for absence and 1 for presence in the computer analysis. For the numerical analysis the PyElph (version 1.4) software was used to generate the similarity matrix for all possible pairs of species. The similarity index, based on the total seed protein patterns of melon species was calculated from the similarity values in the matrix (Silva and Russo, 2000) and using the Unweighted Pair-Group Method Analysis (UPGMA) algorithm proposed by Sokal and Michener (1958) cluster analyses were conducted and the resulting clusters were expressed as dendrograms. Average polymorphism was calculated as a ratio of total number of polymorphic bands (TNPB) to total number of bands (TNB) multiplied by 100 ($TNPB/TNB \times 100$) while Jaccard's similarity index was calculated as ratio of similar bands to total bands between two species multiplied by 100 (Jahangir *et al.*, 2014).

RESULTS

Native-PAGE of total water and buffer soluble proteins yielded a total of 99 and 132 polypeptide bands respectively for all the species under study (Figure 1) while a total of 218 and 123 protein bands respectively were detected for all the species using SDS-PAGE (Figure 2).

Figure 1: Electrophoregram of buffer- (1 - 7) and water- (1' - 7') soluble seed storage proteins in seven *Cucurbitaceae* species using Native-PAGE. 1, *C. sativus*; 2, *C. maxima*; 3, *L. siceraria*; 4, *C. vulgaris*; 5, *C. mannii*; 6, *C. moschata*; 7, *C. edulis*. Lanes marked 1 - 7 are buffer soluble proteins of the same species as that of water soluble proteins marked 1' - 7'.

Figure 2: Electrophoregram of buffer (1 - 7) and water (1' - 7') soluble seed storage protein in seven *Cucurbitaceae* species using SDS-PAGE. 1, *C. sativus*; 2, *C. maxima*; 3, *L. siceraria*; 4, *C. vulgaris*; 5, *C. mannii*; 6, *C. moschata*; 7, *C. edulis*; M, Pharmacia low molecular weight marker. Lanes marked 1 - 7 are buffer soluble proteins of the same species as that of water soluble proteins marked 1' - 7'.

To further distinguish between the species, the band pattern generated (Figure 1 and 2, lanes 1-7 and 1'-7') were given codes (0 for absent and 1 for present) which were used to

construct the data matrix table (Table 1) for the densitometry analysis of the bands.

Table 1: Data matrix for the codes given to the seed protein characters of seven *Cucurbitaceae* species

Band No.	Melon species/electrophoretic condition													
	Native-PAGE of BSP							Native-PAGE of WSP						
	1	2	3	4	5	6	7	1'	2'	3'	4'	5'	6'	7'
1	1	1	0	0	0	1	1	1	1	0	1	1	1	1
2	1	0	0	1	1	1	0	0	1	1	0	1	0	0
3	1	1	1	0	1	1	0	0	1	1	1	1	1	0
4	1	1	1	1	0	0	0	1	0	0	0	0	0	0
5	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	1	0	0	0	1	0	1	1	0	0	0	0	0	1
7	0	0	0	1	0	1	1	1	0	0	0	0	0	0
8	1	0	0	0	0	1	1	0	1	1	1	1	1	1
9	0	0	0	0	1	0	0	1	1	0	0	0	1	0
10	0	0	0	0	1	0	0	1	1	0	0	0	0	1
11	1	1	1	1	1	1	1	1	0	1	1	1	1	1
12	1	0	0	0	0	0	1	1	0	0	0	0	0	1
13	1	0	0	0	0	0	0	1	1	0	1	1	0	0
14	0	0	0	0	1	0	0	0	1	1	1	1	1	1
15	1	1	1	1	1	1	1	1	0	0	0	0	0	0
16	1	0	0	0	0	0	0	1	1	1	1	1	1	1
17	0	1	1	1	1	1	1	1	0	1	0	0	0	0
18	1	0	0	0	0	0	0	1	1	0	0	0	0	0
19	0	0	0	0	0	0	1	0	1	1	1	1	1	1
20	1	1	1	1	1	1	1	1	0	0	0	0	0	0
21	1	1	0	0	0	0	0	1	1	1	0	1	1	1
22	1	0	0	0	0	0	0	1	0	0	0	0	0	0
23	1	1	1	1	1	1	1	1	1	1	1	1	1	1
24	0	0	0	0	1	1	0	1	1	1	1	1	1	1
25	1	1	0	0	0	1	0	1	0	0	0	0	0	0
26	1	1	1	1	1	1	1	1	1	1	1	1	1	1
27	1	1	1	1	0	1	1	1	0	1	0	1	1	1
28	1	0	1	1	1	1	1	1	1	1	1	1	1	1
29	0	1	0	0	1	0	0	1	1	1	1	1	1	1
30	1	1	1	0	0	1	1	1	1	1	1	1	1	1
31	1	0	0	0	0	0	0	1	0	0	0	0	0	0
32	1	1	0	1	1	1	1	1	0	0	0	1	0	0
33	1	1	1	0	1	0	1	1	0	0	0	0	0	1
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1, *C. sativus*; 2, *C. maxima*; 3, *L. siceraria*; 4, *C. vulgaris*; 5, *C. mannii*; 6, *C. moschata*; 7, *C. edulis*; BSP, Buffer soluble proteins and WSP, Water soluble proteins. Lanes marked 1' – 7' are water soluble proteins of the same species as that of buffer soluble proteins marked 1 - 7.

In Native- PAGE a maximum number of 33 protein bands were observed in all the different melon genotypes for their buffer soluble proteins while a maximum number of 28 protein bands were observed in all the different melon genotypes for their water soluble proteins (Table1). Similarly, in SDS-PAGE a maximum number of 31 protein bands were observed for buffer soluble proteins while a

maximum number of 50 were observed for water soluble proteins (Table1).

The banding patterns of the seven *Cucurbitaceae* species observed for Native- and SDS-PAGE data matrix were analysed for average polymorphism (Table 2) as proposed by Jahangir *et al.*, (2014). In all the seven *Cucurbitaceae* species

studied a total of 99 and 132 protein bands were detected respectively for water and buffer soluble proteins under Native-PAGE while a total of 218 and 123 protein bands were detected respectively under SDS-PAGE (Table 2).

Table 2: Average polymorphism of the seven *Cucurbitaceae* under Native-PAGE and SDS-PAGE

Experimental condition	TNB	TNPB	AP (%)
Native-PAGE(WSP)	99	21	21.21
Native-PAGE(BSP)	132	42	31.82
SDS-PAGE(WSP)	218	35	16.06
SDS-PAGE(BSP)	123	49	39.84

TNB, Total number of bands; TNPB, Total number of polymorphic bands; AP, Average polymorphism; WSP, Water soluble proteins; BSP, Buffer soluble proteins

The Jaccard's similarity index which tells more about the relationship among the species is shown in Table 3 and 4. Under Native- PAGE and considering water soluble proteins, the similarity index for the species varied from 95.65% to 35.30% (Table 3). *L. siceraria* /*C. moschata* showed the highest similarity index (~96%) followed by *C. maxima* /*C. mannii* (~93%). The lowest similarity index (~35%) was observed between *C. sativus* / *C.vulgaris* followed by *C.sativus* / *L. siceraria* (40%). For buffer extract, the Similarity Index for species varied from 81.25% to 51.06% (Table 3). The highest similarity index (81%) were exhibited by *C. sativus* / *C. maximal* and *C.maxima* / *L. siceraria* while *C. sativusi*, *C.mannii* and *C. sativus* / *C.vulgaris* showed the lowest similarity index (51% and 56%) respectively.

Table 3. The similarity index of seven *Cucurbitaceae* species based on protein data under Native-PAGE using Jaccard's similarity index (%)

Sp	Native-PAGE(WSP)							Native -PAGE(BSP)						
	1	2	3	4	5	6	7	1	2	3	4	5	6	7
1	100							100						
2	48.65	100						69.57	100					
3	40.00	46.15	100					81.25	81.25	100				
4	35.30	88.00	86.95	100				55.81	66.67	75.86	100			
5	48.65	92.86	92.30	88.00	100			51.06	59.45	60.60	58.82	100		
6	52.94	88.00	95.65	90.90	88.00	100		66.67	68.42	64.71	57.14	66.67	100	
7	54.05	57.14	53.85	48.00	50.00	56.00	100	68.06	70.27	72.72	70.59	57.89	68.42	100

Sp, species; WSP, water soluble proteins; BSP, buffer soluble proteins; 1, *C. sativus*; 2, *C. maxima*; 3, *L. siceraria*; 4, *C.vulgaris*; 5, *C. mannii*; 6, *C. moschata*; 7, *C. edulis*.

Under SDS-PAGE, the similarity index for the species varied from 77.00% to 34.04% (Table 4) for water soluble proteins. The highest similarity index (~77%) was found between *C. maxima*/*C. vulgaris* and the lowest similarity index (~36% and 34%) were observed between *C. sativus*/*C. maxima* and *C. sativus* /*L. sicerania* respectively. For buffer extract, the similarity index for the species varied from 97.12% to

41.02% (Table 4). The highest similarity index (~97%) was exhibited by *C. manni* /*C. moschata* this was followed by 91% for *L. siceraria* /*C. moschata* and *C. mannii* /*C. moschata*. The lowest similarity index (~41% and ~45%) were observed between *C. maxima* /*C. vulgaris* and *C. maxima*/*C. moschata* respectively.

Table 4. The similarity index of seven *Cucurbitaceae* species based on protein data under SDS-PAGE using Jaccard's Similarity Index (%).

Sp	SDS-PAGE(WSP)							SDS -PAGE(BSP)						
	1	2	3	4	5	6	7	1	2	3	4	5	6	7
1	100							100						
2	36.84	100						66.67	100					
3	34.04	61.53	100					75.68	51.28	100				
4	57.14	77.01	74.19	100				64.86	41.02	70.59	100			
5	71.43	70.00	58.18	66.67	100			75.68	51.28	88.24	70.59	100		
6	50.91	68.50	64.70	71.43	69.84	100		68.42	45.00	91.43	74.29	91.43	100	
7	49.12	66.67	71.43	75.00	64.62	76.62	100	79.94	60.00	74.29	68.57	97.14	72.22	100

Sp, species; WSP, water soluble proteins; BSP, buffer soluble proteins; 1, *C. sativus*; 2, *C. maxima*; 3, *L. siceraria*; 4, *C.vulgaris*; 5, *C. mannii*; 6, *C. moschata*; 7, *C. edulis*

According to UPGMA analysis, dendrograms were constructed (Figures 3, 4, 5 and 6) to group the 7 melon species on the basis of the similarity or dissimilarity in their protein banding patterns. For Native-PAGE, the 7 melon

species were classified into 2 major clusters based on their water soluble proteins (Figure 3) and into 3 major clusters based on their buffer soluble proteins (Figure 4).

Figure 3: UPGMA dendrogram depicting genetic diversity of seven *Cucurbitaceae* species based on Native-PAGE water soluble seed protein profile.

Figure 4: UPGMA dendrogram depicting genetic diversity of seven *Cucurbitaceae* species based on Native-PAGE buffer soluble seed protein profile.

The dendrograms produced by the analysis of SDS-PAGE profiles classified the 7 species into 2 major clusters based on both water soluble (Figure 4) and buffer soluble (Figure 6) seed proteins.

Figure 5: UPGMA dendrogram depicting genetic diversity of seven *Cucurbitaceae* species based on SDS-PAGE water soluble seed protein profile.

Figure 6: UPGMA dendrogram depicting genetic diversity of seven *Cucurbitaceae* species based on SDS PAGE buffer soluble seed protein profile.

DISCUSSION

Several biochemical and molecular marker loci are currently available for elucidation of genetic variation even between closely related germplasm stocks. However, no single method is adequate for assessing genetic variation as each of these differ in terms of sample variation at different level and difference in their power of genetic resolution, as well as in the quality of information content (Sultana and Ghafoor, 2008). In the present study, Native- and SDS-PAGE of water and buffer soluble seed proteins of seven different *Cucurbitaceae* species were used as biochemical markers to

investigate their genetic diversity. The results of protein banding (Figure 1 and 2) established that seed proteins of the investigated species are heterogeneous and reveals the extensive polymorphism of total seed proteins (Ranjan *et al.*, 2012).

All the species investigated can be distinguished from each other since the band patterns (Figure 1 and 2, lanes 1-7 and 1'-7') indicated wide differences among the investigated species in number and position of the bands as reveal by densitometry analysis. The protein banding pattern in Native- and SDS-PAGE was characterized by two distinct zones viz.,

A and B depending on whether the band was present or absent in the water or buffer soluble seed proteins. In Native-PAGE, the 33 protein bands for buffer and 28 protein bands for water soluble proteins observed in the 7 melon species were categorized into zones A and B. The zone A represented protein bands (1-26) found in both buffer and water soluble proteins while zone B represented protein bands (27-33) found only in buffer soluble proteins. Similarly, in SDS-PAGE the 31 protein bands for buffer and 50 protein bands for water soluble proteins observed in the 7 melon species were categorized into two zones. The zone A represented protein bands (1-31) found in both buffer and water soluble proteins and represented heaviest molecular weight proteins ranging from above 97 KDa to 45 KDa while the second and the lowest region, zone B represented protein bands (32-50) found only in water soluble protein. The B zone represented the proteins ranging from 25 KDa to 10 KDa. The presence and absence of protein bands have also been reported in several melon species (Dudwadkar *et al.*, 2015; Mashilo *et al.*, 2016).

Analysis for average polymorphism revealed that water and buffer seed storage protein profiling showed a low level of polymorphism of protein patterns between the 7 species. This was as a result of the fact that out of the 99 bands for water soluble proteins under Native-PAGE, only 21 were polymorphic while 42 out of the 132 bands were polymorphic for the buffer soluble proteins (Table 2). With SDS-PAGE, out of 218 water soluble protein bands only 35 were polymorphic while 49 were polymorphic out of the 123 buffer soluble protein bands detected (Table 2). These observations are similar with the results of Lukong *et al.*, (2014) in which buffer soluble proteins also demonstrated a higher similarity index in bean species. The low polymorphism seen in water extract was equally observed by Lukong *et al.*, (2014) in which water extract showed no polymorphism in the studied bean species. These observations demonstrated that water soluble proteins are not “good” enough to study genetic diversity. Also previous studies have shown that most genetic diversity studies are carried out using electrophoretic separation of buffer soluble proteins (Sammour, 1991; Javid *et al.*, 2004; Iqbal *et al.*, 2005; Sheidai *et al.*, 2000 Sadia *et al.*, 2009; Ghafoor *et al.*, 2002).

The dendrogram constructed by the UPGMA method (Figure. 3) shows that the examined species are distinguished in two groups under Native-PAGE (water soluble); the first group comprising the two species: *C. sativus* and *C. edulis* and the other group including the five species of which *C. moschata* and *L. siceraria* were clustered together and *C. mannii* and *C. maxima* equally clustered together while *C. vulgaris* is alone. In the buffer soluble proteins, species *L. siceraria*, *C. maxima*, *C. edulis*, *C. moschata*, and *C. vulgaris* are of the same cluster of which *L. siceraria* and *C. maxima* were grouped together and *C. moschata* and *C. edulis* grouped together. *C. sativus* and *C. mannii* are on a separate cluster respectively (Figure. 4). This conforms to the result of the similarity index in Table 2.

Dendrograms produced by the analysis of SDS-PAGE profiles of seed (Figure 5 – 6) had a topology which generally

does not resemble that of the trees constructed under Native-PAGE (Figure 3 - 4) in the examined species. In these dendrograms, there were two clusters: cluster 1 comprising *C. edulis*, *C. vulgaris*, *C. maxima*, *C. moschata*, and *L. siceraria*; cluster 2 contains *C. mannii* and *C. sativus* for water soluble proteins while in buffer soluble proteins, there was only one cluster in which *C. edulis*, *C. mannii*, *L. siceraria*, *C. moschata*, *C. sativus* and *C. vulgaris* were clustered together while *C. maxima* is on a separate cluster.

It is evident from this investigation that SDS-PAGE profiles of the seed proteins demonstrated the highest % polymorphism and similarity index. The dendrogram constructed under SDS-PAGE buffer extract grouped *C. mannii* and *C. edulis* according to their genus which is in agreement with the taxonomic classification of these species. SDS-PAGE profiles of buffer soluble seed proteins is thus the best method for the study of genetic diversity and has been utilized by other investigators to study genetic diversity in a number of crops (Panda *et al.*, 1986; Panella *et al.*, 1993; Sammour, 1991; Javid *et al.*, 2004; Iqbal *et al.*, 2005; Sheidai *et al.*, 2000 Sadia *et al.*, 2009; Ghafoor *et al.*, 2002; Koenig *et al.*, 1990; Karihaloo *et al.*, 2002; Lioli *et al.*, 2005; Aiken *et al.*, 1998; Yüzbaşıoğlu *et al.*, 2008).

CONCLUSION

Our finding demonstrated that electrophoretic method can be used to study genetic diversity at molecular levels. Out of the two methods used, SDS-PAGE of buffer extracted proteins proved to be the best method since it provided results that conformed to the taxonomic classification of *C. mannii* and *C. edulis*. Hence, for the study of genetic diversity at a molecular level, electrophoretic profiling of buffer soluble proteins subjected to SDS-PAGE should be adopted.

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