CHEMOTAXONOMIC ANALYSES AND CHEMICAL CONTENT OF SOME VARIETIES OF ONION (*ALLIUM CEPA* L.)

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Abstract

Eight (8) varieties of onions (*Allium cepa*) were characterized for total proteins using agarose gel electrophoresis (AGE-PAGE) and proximate chemical components. Total proteins were electrophorecally separated on 12% agarose gels by standard protocols. A total of 4 polypeptide bands were observed, of which 2 (25%) were polymorphic and 6 (75%) were monomorphic, with molecular weight ranging from 13.5 to 100 kDa. Results for the proximate analyses showed percentage contents of moisture 86.08±4.398, ash 4.45±1.386, fibre 15.01±3.184, lipids 7.44±1.101, protein 8.15±1.829 and carbohydrate 69.795±4.977. The Ex-Kudan variety gave the highest nutritional value while the Wuyan Bijimi variety gave the lowest. Dendrogram based on dissimilarity matrix using unweighted pair group method with arithmetic averages (UPGMA) separated all onion varieties into three main groups. Overall a low to medium level of genetic variability was observed for SDS-PAGE (single dimension). As AGE-PAGE alone did not reveal high level of genetic variability, hence 2-D gel electrophoresis along with other advanced type DNA markers and more number of onion varieties from all over the country are recommended for the future genetic evaluation. Our investigation will significantly support the classification, development, genetic evaluation and cultivation of onion in Nigeria.

Keywords: Onions, Polymorphic, Monomorphic, Ex-Kudan, Ex-Wuyan Bijimi

Introduction

Allium cepa also known as bulb onion belongs to the family of *Liliaceae* (Boukary *et al.*, 2012). It is a plant that is cultivated and used around the world and not known in the wild but it has been bred and cultivated for over 7,000years. The Egyptians in particular are known to have cultivated it for about 2,000 years. Its other local names in Nigeria are Ayim (Ibibio), Ayo (Igbo), Alubusa (Yoruba), Albasa (Hausa), Albasara (Chinene). It is an herbaceous annual crop grown for its edible bulb (Ogoleke *et al.*, 2016). Common onions are normally available in three colours, red (brown), and yellow or white. The stem of the plant is flattened disc at the base and tabular leaves form a pseudostem where their sheaths overlap. The leaves are either erect or oblique and there are 3-8 per plant. The onion plant produces pink or white flowers clustered on stalks. The bulb is formed just above the flattened stem of the plant by overlapping leaves. The bulb is made up of several layers, each corresponding to a leaf. They are generally oval but shape can be variable and occur in clusters of 3-18 to a plant. The bulb is protected by a membrane which turns to a papery coat. Onion plants can reach a height of 50 cm (20 in) and are grown as annuals, harvested after one growing season. Onion may also be referred to by cultivar and these include red or purple onion, shallots and spring onions or scallions (Azoom *et al.*, 2014).

Extensive studies have been done on onions and widely reported. A related study to this work was by Grubben and Denton (2004) which reported that the nutritional composition of onions may depend on type of variety, developmental stage and period of storage. Accordingly, fully developed onion bulb usually contains 85.0-88.6% moisture, 1.2-2.0 % protein, 0.1-0.3% fat, 0.6-1.0% fibre, 0.4-0.7% mineral elements and 10.2- 12.1% carbohydrates. In another related study by Yahaya *et al*., (2010) reported the proximate chemical content of *Allium cepa* and found a moisture content of 71.00±0.95%, protein 6.48±1.3%, carbohydrate 64.53±0.98%, ash content 4.26±0.22%, crude fibre 13.56±0.95%, and crude lipid 11.13±1.28%. However Bhattacharjee *et al*. (2013) determined the composition and energy values of two varieties onions bulbs of different origin and found out the moisture content to be 82.99±0.05% and 82.77±0.07%, crude protein 2.62±0.3 and 1.489±0.4%, crude fibre 2.646±0.3% and 1.659±0.8%, ash content 0.205±0.08% and 0.248±0.1, total carbohydrate 14.146±0.07 and 14.772±0.04.

The science of chemotaxonomy or chemical taxonomy is used for the classification of plants on the basis of their chemical constituents which are often specific and restricted to taxonomically related plants and hence useful in classification. This method of classification is considered better in comparison to traditional method because the biochemical properties of plants are stable, unambiguous and consistent. In chemotaxonomic classification, the phenolics, alkaloids, terpenoids and non-protein amino acids, are the four important and widely exploited groups of compounds utilized for chemotaxonomic classification (Smith, 1976). These groups of compounds exhibit a wide variation in chemical diversity, distribution and function (Smith, 1976 and Hegnauer, 1986). The system of chemotaxonomic classification relies on the chemical similarity of taxon (Atal,1982 and Rasool *et al*., 2010). Three broad categories of compounds are used in chemotaxonomy: Primary metabolites are the compounds that are involved in the fundamental metabolic pathways. Most of the primary metabolites are of universal occurrence and utilized by the plant itself for growth and development (Singh, 2010 and 2012). These compounds are ubiquitous in nature and hence play little role in chemotaxonomic classification. However, these molecules sometimes serve as useful chemotaxonomic behaviour on the basis of their quantities. For example, carbohydrate sedoheptulose is present in genus sedum in large quantity. Therefore, the accumulation of sedoheptulose in the species of genus sedum serves as a useful chemical character in chemotaxonomy (Singh, 2010). With the advancement of analytical techniques, today so many groups of plants are there in which phytochemical data has contributed to extensive taxonomic improvements. The presence or absence of a particular phytochemical in a plant along with the knowledge of its biochemical synthetic pathways can be used to assign its taxonomic position (Singh, 2016).

The aims of this study was to determine the chemotaxonomic variations using gel electrophoresis and proximate analyses within and between the varieties of *Allium cepa* being historically assigned to the family Liliaceae, Amarylliaceae, Alliaceae and recently reclassified into different families based on morphological variations.

Materials and methods

Plant samples

Leaves and bulbs of 8 varieties of *Allium cepa* namely: Ex-Dutse, Ex-Huguma, Ex-Kudan, Ex-Kura, Ex-Romi, Wuyan Bijimi, Ex-Kwadon and Waɓe were collected from a randomly. The leaves were exclusively used for the electrophoresis and the bulb used for the analyses of the chemical composition.

DNA extraction

DNA was isolated from the leaves of plants few weeks old in accordance with the modified protocol for CTAB (Cetyltrimethyl ammonium bromide) for isolation of DNA (Soltis laboratorije, http://www.ihcworld.com/_protocols/lab_ protocols/soltis-lab-protocols.htm). About 50 mg of fresh leaf was crushed in liquid nitrogen into a fine powder that was then homogenized with 500µl extraction buffer (STAB buffer) and incubated for 1 hour at 55ºC. Chloroform and isoamyl alcohol in the ratio 24:1 was then added and the mixture was centrifuged for 5 minutes at maximum speed (Eppendorf Microcentrifuge). Aqueous phase (top layer) was then transferred into new tubes and DNA was precipitated with cold ammonium acetate and isopropanol. After precipitation the DNA pellet was washed with cold 70% ethanol and dried well in the oven at 35ºC and then dissolved in 200µl TE buffer (Pavlović *et al*., 2012).

DNA amplification involved preheating at 94°C for 3 minutes, 44 amplification cycles (each consisting of 94°C for 20 s, 38°C for 40 s, 72°C for 1 minute) and an extension phase of 72°C for 7 min. The amplified fragments were run on 1.5% agarose gel in 0.5 M tris-borate EDTA (TBE) buffer at 100 V for 3 hrs (Adesoye *et al*., 2012). The resulting gel was visualized under ultraviolet (UV) light and photographs were taken.

Data analysis

The DNA profiles were scored manually and directly from photographs of the gels. The data were analyzed using the Numerical Taxonomy and Multivariate Analysis System (NTSYSPC) (version 2.02) computer package (Rolf, 1998). Each RAPD fragments was treated as a unit character and was scored presence or absence of the band (1 or 0). The 1/0 matrix was prepared for all fragments scored and the data were used to generate Jaccard's similarity coefficients for RAPD bands (Jaccard, 1908). The Jaccard's coefficients were subjected to unweighted pair-group method using arithmetical averages (UPGMA) to generate a dendrogram using linkage procedure.

Determination of the chemical components

Moisture content

The moisture content was determined following a method adopted by Udo and Oguwele (1986) in which about 2g of the fresh sample of *Allium cepa* was placed into a pre-weighed crucible (W_0) and weighed (W_1) and then placed into an oven at 105ºC for 24 hrs. The crucibles were removed, cooled in a desiccator and weighed. The process was repeated until a constant weight (W_2) of the sample onion and the crucible was obtained. This was to ensure the crucibles were completely dry. The weight loss due to moisture was obtained by the following equation:

$$\text{Mositure } (\%) = \frac{W1-W2}{W1-W0} X\ 100$$

Where:
W_0 = Weight (g) of the empty crucible
W_1 = Weight (g) of fresh onion sample + empty crucible
W_2 = Weight (g) of dried sample + empty crucible

Ash content

About 5-10g of the onion samples were aired dried and grinded in a wooden mortar with a pestle. Then the ash content was determined by following a method by James (1995), in which 2 g of the powdered sample was weighed (W_1) into pre-weighed empty crucibles (W_0) and placed into a Lenton furnace at 600ºC for 3 hours after which it was removed, cooled in a desiccator and weighed (W_2). The weight of the ash was determined by the difference between the weight of the powdered sample, and the ash.

$$\%\ \text{Ash } = \frac{W2-W0}{W1-W0} X\ 100$$

Where:
W_0 = Weight of empty crucible (g)
W_1 = Weight of crucible + powdered sample (g)
W_2 = Weight of crucible + ash sample (g)

Crude lipids

Determination of crude lipid content of the samples was done using the Soxhlet direct solvent extraction method (Nwinuka, *et al* 2005) using petroleum ether (boiling range of 40^0C and 60^0C) as the solvent. 3.0g of the air dried sample was dried in an oven as in the determination of the ash content was weighed and secured in Soxhlet extraction thimble. The thimble was then put into $20cm^3$ capacity soxhlet extractor. A clean oven-dried $100cm^3$ round-bottomed flask was weighed into which and approximately $60cm^3$ of the petroleum ether were added to it. The flask was then mounted on the heating mantle and connected to the extractor (with a condenser) and extraction carried on for four hours. At the end of extraction, the solvent was evaporated and

the flask dried in the oven at 60^0C. The flask was then cooled and reweighed. The percentage crude lipid was calculated using the formula:

$$\text{Crude lipids (\%)} = \frac{Mex}{Ms} X\ 100$$

Where;
M_{ex}= mass of extract (g)
M_s = Mass of sample (g)

Crude fibre

The percentage crude fibre was determined by a literature method (Udo and Oguwele, 1986), in which about 2g of ground sample was weighed (W_0) into a 1 dm^3 conical flask. Distilled water (100 cm^3) and 20 cm^3 (20% H_2SO_4) were added and boiled gently for 30 minutes. The content was filtered through Whatman No. 1 filter paper. The residue was scrapped back into the flask with a spatula. Water (100 cm3) and 20 cm^3 (10% NaOH) were added and allowed to boil gently for 30 minutes. The contents were filtered and the residue washed thoroughly with hot distilled water; then rinsed once with 10% HCl; twice with ethanol and finally three times with petroleum ether. It was allowed to dry and scrapped into the crucible and then dried overnight at 105ºC in an oven. The sample was then removed and cooled in a desiccator. The sample was weighed (W_1) and ashed at 600ºC for 90 minutes in a Lenton muffle furnace after which it was removed and finally cooled in a desiccator and weighed again (W_2). The percentage crude fibre was calculated using the equation

$$\text{Crude fibre (\%)} = \frac{W1-W2}{W0} X\ 100$$

Where:
W_0 = Weight of sample (g)
W_1 = Weight of dried sample (g)
W_2 = Weight of ash sample (g)

Protein:

The crude protein was determined by the popular Kjeldal method in which the nitrogen present in the protein was converted to an ammonium salt and then titrated against an acid. The nitrogen present is calculated and multiplied by 6.25 to give the amount of protein present.

Carbohydrates (CHO):

The method by James (1995) was adopted where the total proportion of carbohydrate in the sample was obtained by difference i.e. the carbohydrate content was obtained by subtracting the % sum of food nutrients; % protein, % crude lipids, % crude fibre and % ash from 100%. CHO (%) = 100% - (% protein + % crude lipid + % fibre + % ash)

Results and Discussion

A total of twenty bands were scored among the 8 onion varieties evaluated. Of these 2 bands, (70%) were polymorphic and 6 (30%) were monomorphic (Figure 1). Size of the protein bands generated by AGE-

PAGE (measured by Unstained Protein Molecular Weight Marker ranging from 14.4 to 116 kDa) ranged from 13.5 to 100 kDa. The bands 1, 2, 5, 6 and 8 were present in all the differed from one another in their minor bands (Fig. 1). Variability in intensity was viewed in many protein bands that showed the amount of protein peptides mounting up at a specific molecular weight. The nucleic acid concentration and the DNA purity of the extract were relatively lower in varieties Ex-Kwadon (286.6 ng/µl) and (5.732 A260) respectively whereas Ex Huguma recorded the highest (537.2 ng/µl) concentration and (10.743 A260) purity.

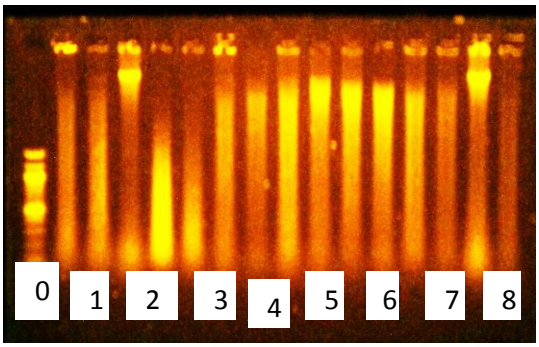

Figure 1: RAPD bands on agarose gel electrophoresis of the eight varieties viz. 0 = DNA ladder; 1 = Ex-Dutse; 2= Ex-Huguma; 3= Ex-Kudan; 4= Ex-Kura; 5= Ex-Romi; 6 = Wuyan Bijimi; 7 = Ex-Kwadon and 8 = Waɓe

These diagnostic features are good taxonomic information among the varieties. The AGE-PAGE technique is mostly thought as a reliable means for the reason that total proteins are mainly free of environmental variations (Javaid *et al*., 2004; Iqbal *et al*., 2005). Biological markers are simply used to determine genetic diversity in plants (Rabbani *et al*., 2001; Akbar *et al*., 2014). Protein types and their diversity varied among a variety of crop species, which may assist us for the early detection of species at seed level and to varieties, whereas band 3 was present in Ex-Kudan out of 8 onion varieties and band 4 was missing in Ex-Huguma only. It was examined that protein profile of most onion varieties acquire the information on clarity of genetic assets (Rehman and Hirata. 2004). Our results exposed that limited level infra-specific diversity was present in the evaluated onion varieties. Variations based on major bands were present in a few varities such as Ex-Huguma, and Ex-Kwadon, but diversity based on minor bands were available in most of the varieties. The equality in major bands among a variety of accessions specifies that the genes coding these proteins are preserved (Ali *et al.*, 2009). Our results were supported by Ghafoor *et al*. (2005); Mehrani (2002) and Akbar *et al*. (2014) who reported a limited level intra–specific variation for seed protein among in pea and chickpea. Our results did not support the findings of Nisar *et al*., (2011), who reported a high level of infra-specific variation for seed protein among local and exotic chickpea germplasm. The disagreement is in fact due to use of different gene pools from both sources. Results of AGE-PAGE revealed that this technique provided a means for steady genotypes discrimination based on genetic variation in protein comparison in onion varieties, but no other relationship among the onion varieties observed. Onion varieties showed the same banding pattern may be duplicated; it should be verified through the use of advanced molecular markers. Over all a low to medium level of genetic diversity was observed. In the present investigation infra-specific difference was narrow and it was noticed that AGE-PAGE technique only did not show high level of infra-specific dissimilarity; so, different genotypes based on AGE-PAGE are suggested to be obtained

from a variety of sources, to make a wide based gene pool with maximum diversity.

The progression of analytical techniques of phytochemical data has contributed to far-reaching taxonomic improvements in many groups of plants (Singh, 2016). The presence or absence of a particular phytochemical (Table 1) in a plant along with the knowledge of its biochemical synthetic pathways can be used to assign its taxonomic position.

Moisture content

Results for the analyses of the chemical content are presented under Table 2. The results show that the moisture content of the 8 varieties are generally within the range of about 84- 89%. However, only the value for the Ex-Kudan variety agrees exactly with the literature of about 89%.(Grubben and Denton, 2004) reported values within range of 85-88.6% and argued that the nutritional values of onions depends several factors such as the onion variety, developmental stage, and period of storage. The onion varieties undertaken in this study were fully developed but the period of storage was not taken seriously into account i.e the determinations were not done at certain intervals of storage. Hence it could be said that the slight variation of the other varieties are in order.

Ash content

Results for the ash content for all the 8 varieties undertaken fall within the range of 2-5% with the highest values of about 6% obtained for both the Ex-Kudan and Ex-Kwadon varieties. But on the other hand results of about 0.2% reported by (Bhattacharjee et al., 2013) were rather low.

Crude fibre

There was no general agreement for the results obtained for the crude fibre content of the different varieties studied. They values range from about 9 -16% with the highest fibre content of about 16% found for the Ex-Kwadan and Ex-Kura varieties. These were totally much higher than the reported values of about 2% and 1% by Bhattacharjee *et al* (2013) and Grubben and Denton (2002) respectively. But they were fairly close to that by Yahaya *et al.* (2010) of about 14%.

Crude protein

The results obtained for the crude protein were fairly consistent for 3 varieties where a value of about 6% was found. Three other varieties Ex-Kudan, Ex-Kura and Ex-Kwadon all had fibre content of approximately 9%. These values are totally in disagreement with those by Bhattacharjee *et al.* (2013) and Grubben and Denton (2002) of about 2% but close to that of Yahaya *et al.*, (2010) that reported a value of about 6%. The Recommended Dietary Allowance (RDA) for children, adult males, adult females, pregnant women and lactating mothers are 28, 63, 50, 60, 65 g of protein daily (Ganong, 2003). For 100 g of *Allium cepa* L. to provide those values of proteins, then it indicates that the samples are poor sources of daily proteins.

Crude Lipids

Like the values for the crude protein there was no consistency. The values range from about 5-9% except for EX-Dutse that had a value of about 11%. This latter value agrees pretty well than that reported by Yahaya *et al.* (2010).

Total carbohydrate

There was wide a range (62-75%) in the values obtained for the carbohydrate content of the samples. Carbohydrate and lipids are principal sources of energy. The values of about 14% and 10-12% reported by Bhattacharjee *et al.* (2013) and Grubben and Denton (2002) respectively are much lower but close to that by Yahaya *et al.* (2010) of 64%

Table1:Proximate analyses of eight varieties of onion, Allium cepa L

S/N	Varieties	Moisture Content (%)	Ash Content (%)	Crude Fibre (%)	Crude Protein (%)	Crude Lipids (%)	Total Carbohydrate (%)
1.	Ex-Dutse	86.5±0.40	3.9±0.29	13.56±0.95	6.48±1.23	11.13±1.28	64.92±0.01
2.	Ex-Huguma	88.3±0.23	3.7±0.42	9.33±1.41	6.74±0.78	6.56±1.13	73.67±0.02
3.	Ex-Kudan	89.0±0.40	6.4±0.28	15.68±0.01	8.75±0.07	8.99±0.83	75.87±0.01
4.	Ex-Kura	86.16±1.00	2.3±0.24	15.71±0.04	8.76±0.01	10.21±0.47	63.04±0.02
5.	Ex-Romi	85.5±0.40	3.4±0.14	11.46±0.01	6.05±0.01	6.00±2.04	73.07±0.02
6.	Wuyan Bijimi	86.6±0.47	3.3±0.24	9.21±0.01	6.74±0.78	6.85±1.8	73.6±0.18
7.	Ex-Kwadon	84.0±0.40	5.8±0.42	15.13±0.03	8.53±1.74	7.29±0.83	62.79±0.08
8.	Waɓe	83.6±1.18	5.8±0.92	14.98±0.05	2.26±0.05	5.16±0.77	71.4±0.37

Pooling these features into coded data matrix to form a dendrogram clustered the varieties into 3 major groups visa-viz: A, B and C (Fig. 2). Group A consisted of two sub-groups or sub-clusters A1 and A2. Cluster A1 consisted of two varieties (Ex-Romi and Wuyan Bijimi); A2 consisted of variety Ex-Huguma. Group B consisted of two varieties namely – Ex-Dutsi and Ex-Kura. Group C was made up of 2 minor clusters. C1 consisted of Ex- Kudan and Waɓe while C2 consisted of Ex- Kwadon. Similarity coefficients ranged from 0.50 to 25.00.

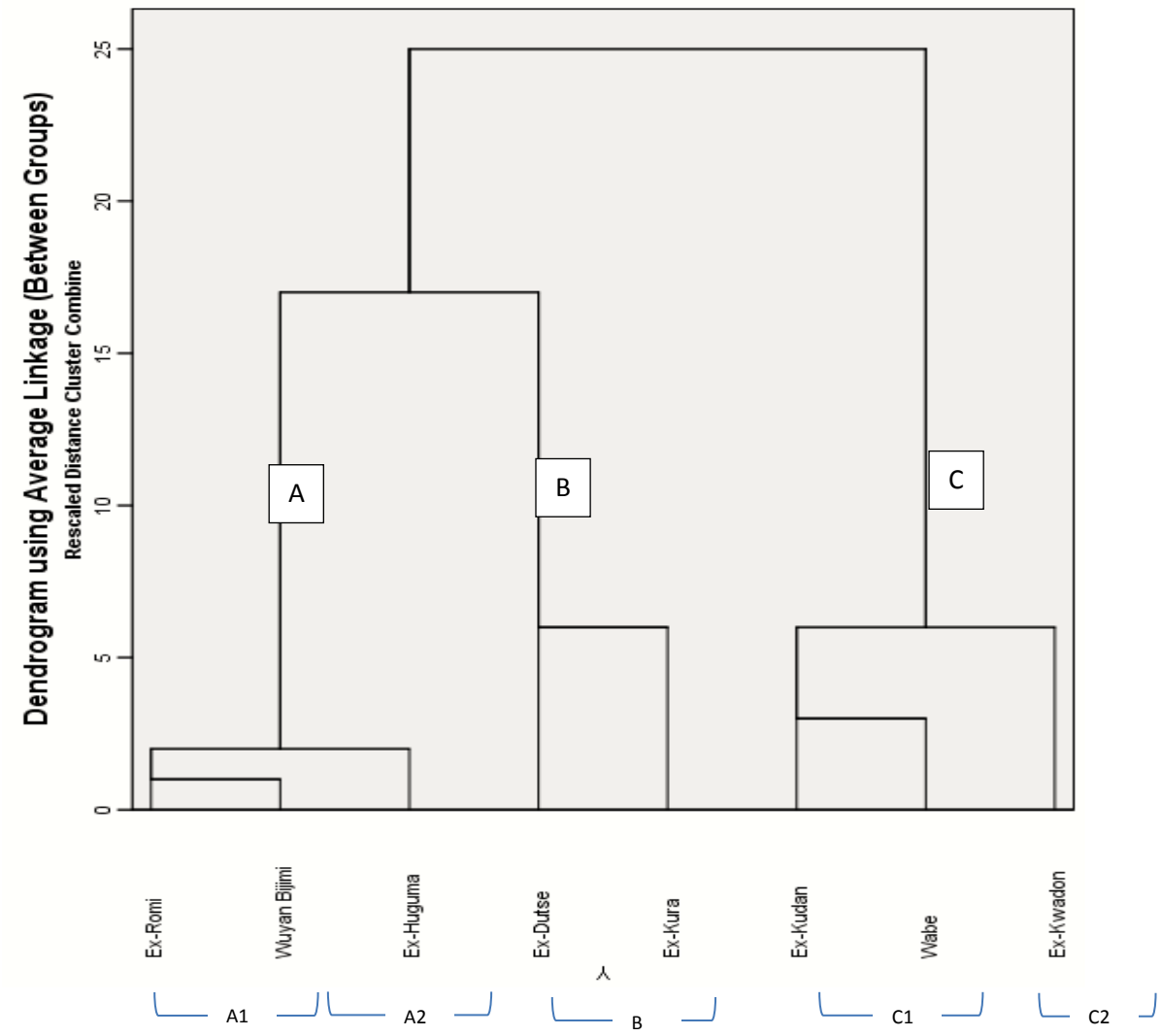

Figure 2: Dendrogram showing infra-specific phenetic relationship among eight onion varieties using agarose gel electrophoresis and proximate chemical markers

Conclusion

This research work has shown that the onion varieties studied were diverse with relatively low infraspecific variations among some varieties and this knowledge can aid in the stability of genotypes before entering into a breeding programme. It is, therefore, recommended that interspecific hybridization between Ex-Kudan and Ex-Kwadon be considered for increased phytochemicals in *A. cepa* cultivar with big bulb size which is usually preferred by consumers.

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