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Characterisation of Okra (Abelmoschus [medik.]) Accessions Using Dehydrogenase Isozymes and Protein

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ABSTRACT *Okra (Abelmoschus [Medik.]) is cultivated in distant farms and home gardens on a commercial or subsistence scale in tropical and subtropical regions of the world. Several diverse cultivars exist but they all possess a characteristic mucilaginous substance. Isozymes are enzymes composed of different structural amino acid sequence but may catalyse similar chemical reaction. In chemosystematics isozymes and other phytochemicals may be used to characterize plant germplasm. This study employed dehydrogenase isozymes and total protein to characterize Abelmoschus accessions. Five Abelmoschus accessions including 2 A. esculentus (NG/OA/03/12/157 and NG/OA/05/12/159) and 3 A. caillei (NG/OA/03/12/158, NG/SA/DEC/07/0475 and NG/SA/DEC/07/0482) species were collected from the National Center for Genetic Resources and Biotechnology Ibadan and cultivated at the Experimental fields of University of Benin, Nigeria. At maturity, fruits were harvested for analysis at the Biochemistry Division, Nigeria Institute of Medical Research, Lagos. Results showed that variation exist in the quantity of total protein in the five accessions with accessions, NG/SA/DEC/07/0475, NG/SA/DEC/07/0482 and NG/OA/03/12/158 having higher values (33.076 g, 31.210 g and 28.992 g respectively) compared to NG/OA/03/12/157 and NG/OA/05/12/159, which had low values of total protein content (21.227 g and 9.429 g respectively). Isozyme activities of alcohol, malate and isocitrate dehydrogenases showed distinctive results. Significance results (at $P < 0.05$) were obtained with accessions NG/OA/03/12/157 and NG/OA/05/12/159 having higher values when compared with accessions NG/SA/DEC/07/0482, NG/OA/03/12/158 and NG/SA/DEC/07/0475. When homogeneity was compared accessions NG/SA/DEC/07/0482, NG/OA/03/12/158 and NG/SA/DEC/07/0475 were found to be in the same category, while accessions NG/OA/03/12/157 and NG/OA/05/12/159 in separate categories. The degree of intra specific variation differ for the five accessions. Grouping of the A. caillei accessions in one cluster suggest a within species similarity. Nevertheless, the separation of a single cluster for A. esculentus accessions may suggest a considerable degree of total protein content and isozymatic variation within the species. The isozyme characterization has proven effective in the identification and characterization of these Okra accessions but activities of these isozymes only cannot be used as an indication of evolutionary advancement and classification at the generic and species level.*

Keywords: Okra (Abelmoschus), Chemosystematics, Isozyme, Protein, Dehydrogenase, Plant characterization

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Introduction

Okra (Abelmoschus [Medik.]) is cultivated in distant farms and home gardens on a commercial or subsistence scale in tropical and subtropical regions of the world (Osawaru and Ogwu, 2013). India is the largest producer (67.1 %), followed by Nigeria (15.4 %) and Sudan (9.3 %) with total world area under cultivation of 0.43 million hectares and production of 4.54 million tons (Varmudy, 2011). Cultivated and wild species of okra clearly show overlapping in Southeast Asia, which is considered as the centre of diversity (Aladele et al., 2008). Diverse hypothesis exist regarding the geographical origin of A. esculentus. Some scientists argue that one putative ancestor (A. tuberculatus) is native of Northern India, suggesting that the species originated from this geographic area. On the basis of ancient cultivation in East Africa and the presence of the other putative ancestor (A. ficulneus), others suggest that the area of domestication is Ethiopia or North Egypt, but no definitive proof is available today (Benchasri, 2012). Abelmoschus caillei is found only in West and Central Africa, hence, it is difficult to suggest an origin outside. It originated as an allopolyploid hybrid between Abelmoschus manihot and Abelmoschus esculentus (Benchasri, 2012). Growth habit of Abelmoschus spp is vast. Some grow annually (herbs) while others perennially (shrubs or trees). There are several cultivars ranging from those where the skin is completely smooth to those which bear prickly spines. They all possess mucilaginous characteristics substance (Edwin et al., 2006).

Okra provide important source of mineral nutrients, which are often lacking in the diet in developing countries. Okra also contains iron and iodine which are important vegetable source of viscous fibre but it is reportedly low in sodium, saturated fat and cholesterol (Kendall and Jenkins, 2004). The composition of okra leaves per 100g edible portion is reported in Gopalan and Balasubramanian (2007). Carbohydrates are mainly present in the form of mucilage (Kumar et al., 2009). Siemonsma and Hamon (2002) reported that Okra mucilage has been used as plasma replacement or blood volume expander. Also, midwifery in Southern Edo state of Nigeria have used it during child labour to rub the female external reproductive organ to expel fetus faster (Obire, 2002). The leaf buds and Flowers are also edible (Doijode, 2001). Okra seeds contain about 20% proteins and 20% oil (Tindall, 1983). Okra seed oil has potential hypocholesterolemic effect (Rao et al., 1991). Okra seed flour could also be used to fortify cereal flour (Adelakun, et al., 2008). Its ripe seeds are roasted, ground and used as a substitute for coffee in some countries (Moekchantuk and Kumar, 2004). Mature fruits and stems containing crude fibre which may be useful in the paper industry (Osawaru et al., 2016). Edible okra oil can be obtained from okra seeds, which has pleasant taste and odour, and is high in unsaturated fats and has biofuel potential and properties (Franklin and Martin, 1982; Farooq et al., 2010). Its medicinal value has also been reported in Adams (1975); Felter et al. (2011); Adetuyi et al. (2008); Moaward et al. (1984); Kumar et al. (2009).

The methods used in identifying different Okra cultivar are based on conventional or phenotypic expressions of the plant parts, seeds or period of sowing and harvesting. These expressions are strongly influenced by the environment in which they grow. Such conventional means have gradually

being replaced by biochemical and molecular means such as DNA profiling, cytology, proteins/enzyme-electrophoresis (Osawaru et al., 2015). The characterization of diverse organisms into group by the application of biochemical method is known as chemosystematics. Several research characterizing Okra species have applied this means which greatly enhance their identification and characterization including Osawaru et al. (2011) Nwangburuka et al. (2011); Osawaru et al. (2013a); Osawaru et al., (2014). Isozymes are enzymes composed of different structural amino acid sequence but catalyse similar chemical reaction. They are produced by different genes and are not redundant despite their similar functions. One of the most extensively studied isozyme systems are dehydrogenases including malate dehydrogenase, alcohol dehydrogenase and isocitrate dehydrogenase. These isozymes have been used for various research purposes for characterization and delimitation of phylogenetic relationships, to estimate genetic variability and taxonomy, to characterization in plant genetic resources management and plant breeding, studying population structure and divergence as well as for fingerprinting purposes (Osawaru et al., 2014; Freville et al., 2001; Efisue, 2013). Alcohol dehydrogenase (ADH) is an enzyme whose activity is much expressed during episodes of oxygen shortage or insufficiency (anoxia). Under these conditions, ADH acts in the terminal step of anaerobic glycolysis, or fermentation, converting acetaldehyde to ethanol (Saches and Freeling, 1978). Although the activity of alcohol dehydrogenase does not seem essential to growth, under anoxia it seems absolutely necessary (Lemke-Keyes and Sachs, 1989). Malate dehydrogenase (MDH) is occur in animals, plants and micro- organisms. MDH catalyses the oxidation (dehydration) of L-malate to oxaloacetate in the presence of NAD+ as a cofactor. Mitochondrial MDH functions as part of the Krebs cycle, while soluble (cytoplasmic) MDH can be involved in acid metabolism in plant tissues, in autotrophic carbon dioxide fixation in higher plants and in more other metabolic pathways (Lehninger, 2000; Ting et.al. 1975; Scandalios, 1974). In plant tissues, MDH has additionally been found in glyoxysomes, peroxisomes and microsomes (Longo and Scandalios, 1969; Yang and Scandalios, 1974; Sadunishvili and Nutsubidse, 1980; Yunina and Levites, 2007 and 2008). Isocitrate dehydrogenase is an enzyme which catalyses the reversible oxidative decarboxylation of isocitrate to -ketoglutarate via the formation of an enzyme bound intermediate (oxalosuccinate) in presence of a coenzyme NAD+ or NADP+ (as oxidant) and a divalent metal ion (Pramod et al.,2010).

This present study aims to use isozymes activities to characterize Okra species and contribute to the identification of different Okra species, for better understanding of the different species and enhance conservation and breeding strategies. Isozyme activity may proffer a reliable approach to Okra cultivar classification, phylogeny and overall systematics. The study will also contribute to understand the similarities and differences among and within Okra species by using isozymes from seeds.

Materials and Methods

Collection of Materials

Okra (Abelmoschus) accessions were obtained from active collection at National Center for Genetic Resources and Biotechnology, Ibadan. The passport data of these accessions are presented in Table 1. The collections include 2 A. esculentus (NG/OA/03/12/157 and NG/OA/05/12/159) and 3 A. caillei (NG/OA/03/12/158, NG/SA/DEC/07/0475 and NG/SA/DEC/07/0482) species.

Table1

Identity of Okra accessions used

S/N	Accessions Number	Status	Location	Source
1	NG/OA/157/12/03	Landrace	0 7.4N and 0 3.84E	NACGRAB Ibadan
2	NG/OA/158/12/03	Landrace	0 7.4N and 0 3.84E	NACGRAB Ibadan
3	NG/SA/DEC/0475/07	Landrace	0 7.4N and 0 3.84E	NACGRAB Ibadan
4	NG/SA/DEC/0482/07	Landrace	0 7.4N and 0 3.84E	NACGRAB Ibadan
5	NG/OA/159/12/05	Landrace	0 7.4N and 0 3.84E	NACGRAB Ibadan

Study Area

The five accessions were grown simultaneously at the Experimental field of Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria (6.20 0N; 5.73 0E). The climate includes high rainfall up to 2000 mm – 3000 mm of bimodal pattern with peaks at July and September respectively, high temperature ranging between 20 – 400C and high atmospheric humidity (Omuta, 1980). Radiation is fairly high and varies according to different period of the year; above 1,600 hours per year have been reported (Onwueme and Singh, 1991). Detailed description of the study area including soil characteristics can be found in Osawaru et al. (2013b; 2013c); Osawaru and Ogwu (2014); Ogwu and Osawaru (2015).

Crop Husbandry

A simple seed test was carried out to on the seeds to determine purity and viability. Three seeds per accession were planted at random into holes of 3 cm depth on five different ridges. Among these numerous stands, 10 stands were tagged from 1 - 10. On the five ridges, each ridges had 10 tagged stands totally 50. These stands were counted for study during the field trials and under the same climatic conditions. Watering of the plants was rain fed. Agronomic practices such as mulching and fertilizer were not applied. The accessions were sown in July 2013 and harvested in February 2014 before shatter stage. Weeding was done normally as at when due. Roguing was carried out on all suspected off-types on each row.

Preparation of Isozymatic Assay and Isozyme Analysis

After the seeds of each accession have been extracted from the fruit pod, they were sun-dried and kept in tightly sealed containers for enzymatic analysis. This analysis was done at Biochemistry division, Nigeria Institute of Medical Research, Yaba, Lagos. Seeds were blended using a pulverized seed mill machine. 10 g of each accession was measured out using an electronic weighing

machine and then kept in different conical flask labelled with their accession numbers on masking tape. 50 ml of Tris buffer was added to each flask and shaken, this was filtered using Whitman filter paper. The supernatant was decanted into beaker and pallet discarded. Ammonium sulphate was added to the supernatants and left to stand for hour, this was done to precipitate out the protein. The resulting suspension was centrifugation at 2500 rmp for 10 mins. Decant the supernatant from the pallet and add 1 ml of phosphate buffer then shaken properly and centrifuge again. Thereafter supernatant were decanted leaving the pallet. To the pallet 1 ml of phosphate buffer was added and used as the assay. Before assaying for each isozymatic activity, a total protein analysis was carried out as it will be required for obtaining further results on isozymatic analysis. Determination of total protein was done using biuret method (Table 2) thus;

R1 = sodium hydroxide with copper II sulphate (ml)

R2 = potassium sodium tartrate (ml)

Table 2

Showing test procedure for the determination of total protein analysis

	Blank	Standard	Sample
R1 (ml)	1.02	1.00	1.00
R2 (ml)	1.00	1.00	1.00
Standard (μl)	-	20.00	-
Sample (μl)	-	-	20.00

Test was allowed to stand for 10 mins at room temperature and readings were taken at 550nm.

$$\text{Total protein (grams)} = \frac{\text{absorbance (assay)} \times \text{standard concentration (0.122g)}}{\text{Absorbance of standard (6)}}$$

Alcohol dehydrogenase

0.1M of sodium pyrophosphate buffer (pH 9.2)

2M of ethanol. Dilute 12.1ml of 95% to 100ml of distilled water

0.025M of NAD

0.1M phosphate buffer, (pH 7.5)

Malate dehydrogenase

0.1M of potassium phosphate buffer (pH 7.4)

0.006M of oxaloacetic acid, freshly prepared in 0.1M of phosphate buffer.

0.00375M NADH

Isocitrate dehydrogenase

The NADH is formed as a result of oxidation of isocitrate. The appropriately diluted enzyme solution (0.01ml) was added to 0.79 ml of reaction mixture containing isocitrate (2.25mM), NAD+ (0.62mM) and MgCl2 (3.75mM) in 2.5 ml assay buffer (50mM phosphate buffer, pH 7.5).

Absorbance reading of enzymes

The Isozymes activities were spectrophotometrically determined in triplicate

of each accession at 340nm at 300C at a minute interval, by monitoring the rate of formation of NADH or NAD+.
The enzymes activity was calculated using:

$$\text{Activity} = \frac{340/\text{min} \times \text{cuvette volume} \times \text{enzyme dilution}}{6.22 \times \text{sample volume}}$$

Sample volume =1cm, cuvette volume =1cm, enzyme dilution =30

$$\text{Specific Activity} = \frac{\text{Enzyme activity}}{\text{Total protein}}$$

Table 3

Absorbance readings on activity of Malate dehydrogenase

TEST	0482	158	0475	157	159
1	0.041	0.047	0.041	0.044	0.146
2	0.023	0.037	0.020	0.022	0.038
3	0.027	0.020	0.046	0.010	0.038

Table 4

Absorbance readings on activity of Isocitrate dehydrogenase

TEST	0482	158	0475	157	159
1	0.027	0.035	0.027	0.038	0.039
2	0.011	0.006	0.019	0.021	0.046
3	0.012	0.006	0.019	0.022	0.046

Table 5

Absorbance readings on activity of Alcohol dehydrogenase

TEST	0482	158	0475	157	159
1	0.067	0.107	0.080	0.144	0.207
2	0.058	0.087	0.073	0.134	0.197
3	0.108	0.101	0.073	0.126	0.199

Statistical Analysis

Multivariate statistical analyses were applied in this study to assess possible relationship in isozymatic characters malate dehydrogenase, alcohol dehydrogenase and isocitrate dehydrogenase in the Abelmoschus accessions.

Data collected were analysed using SPSS (version 16.0) and Squared Euclidean distance, which was used as a measure of distance for cluster formation after standardization of quantitative and qualitative data. Analysis of variance was done for quantitative attributes and all isozymatic characters to locate significant difference among the accession. Pair-wise distance matrixes between accessions were derived using Statistic XL Excel.

Results and Discussion

Total protein content: The results of total protein content of each accession is presented in Table 6 Variation exist in the quantity of total protein present in the five accessions. Amongst the accessions, NG/SA/DEC/07/0475, NG/SA/DEC/07/0482 and NG/OA/03/12/158 showed higher values for total protein (33.076 g, 31.210 g and 28.992 g respectively), compared to NG/OA/03/12/157 and NG/OA/05/12/159 which had low values for total protein content (21.227 g and 9.429 g respectively).

Table 6
Total protein (g) content of each accession

S/N protein (g)	Accession No	Total
1	NG/OA/03/12/157	21.227
2	NG/OA/03/12/158	28.990
3	NG/SA/DEC/07/0475	33.076
4	NG/SA/DEC/07/0482	31.210
5	NG/OA/05/12/159	9.429

Proteins are important for breakage of seed dormancy. Most of the activities that occur in seeds before germination are products of protein (Hilman, 1962).The more protein content in a seed, the faster seedlings emerge.The total protein of the five accessions revealed to a large extent, the quality of the protein content in the seeds. Although proteins are necessary for the germination of seed both under favourable and unfavourable condition, but the condition under which the species seed grows well/favourable is essential. Total protein of accessions NG/SA/DEC/07/0482, NG/OA/03/12/158 and NG/SA/DEC/07/0475 (A. caillei) were observed to be high but did not emerge fast compared to accessions NG/OA/03/12/157 and NG/OA/05/12/159 (A. esculentus) that had low total protein content. This may be due to conditions favourable to their growth. However accession NG/SA/DEC/07/0482 possessed high total protein content like it other A. caillei, it emerged even faster than A. esculentus. Absence of growth in accessions NG/OA/03/12/158 and NG/SA/DEC/07/0475 along with others proves them to be true A. caillei as they hardly throve in anoxia condition. The delay may represent the time necessary for those enzymes, which are involved in aerobic metabolism express themselves (Pramod et al., 2010) in the seeds. The emergence of accession NG/SA/DEC/07/0482 with other A. esculentus maybe a diversion as both morphological and total protein

content prove it is a true A. caillei having high protein content with others.

Isozymatic Characterization: Characterization of these species using their isozyme activities is easily affected by environment. These isozymes are part of metabolic pathway for all living things which is necessary for growth of the genus, Abelmoschus.

The results of isozyme activities are presented in Table 7. Isozyme activities of alcohol, malate and isocitrate dehydrogenases showed distinctive results as shown in Table 7. A level of significance was obtained at P < 0.05 with accessions NG/OA/03/12/157 and NG/OA/05/12/159 having higher values for mean ± STD error of 0.135 ± 0.005 and 0.201 ± 0.003 respectively when compared with accessions NG/SA/DEC/07/0482, NG/OA/03/12/158 and NG/SA/DEC/07/0475. However, when homogeneity was compared within the accessions to check the cause of variation, accessions NG/SA/DEC/07/0482, NG/OA/03/12/158 and NG/SA/DEC/07/0475 were found to be in the same category, while accessions NG/OA/03/12/157 and NG/OA/05/12/159 in separate categories.

Table 7
Enzymatic activities for the different enzymes

Accession Number	Isocitrate Dehydrogenase	Malate Dehydrogenase	Alcohol Dehydrogenase
NG/OA/03/12/157	0.017 ± 0.005	0.030 ± 0.005	0.077 ± 0.015
NG/OA/03/12/158	0.016 ± 0.010	0.035 ± 0.008	0.098 ± 0.059
NG/SA/DEC/07/0475	0.022 ± 0.003	0.036 ± 0.008	0.098 ± 0.059
NG/SA/DEC/07/0482	0.027 ± 0.006	0.025 ± 0.010	0.135 ± 0.005
NG/OA/05/12/159	0.044 ± 0.002	0.074 ± 0.036	0.201 ± 0.003
	*	NS	*

Results represent mean ± standard error * Significant at P<0.05 NS : Not significant

Specific activity of the Isozyme was used to construct a phylogenetic relationship dendrogram among the five accessions. It revealed a genetic diversity and relationships among the accessions.

Alcohol Dehydrogenase

From Table 7: a level of significance was obtained at P<0.05 with accessions NG/OA/03/12/157 and NG/OA/05/12/159 having higher values for mean ± STD error of 0.135 ± 0.005 and 0.201±0.003 respectively when compared with accessions NG/SA/DEC/07/0482, NG/OA/03/12/158 and NG/SA/DEC/07/0475. However, when homogeneity was compared within the accessions to check the cause of variation, accessions NG/SA/DEC/07/0482, NG/OA/03/12/158 and NG/SA/DEC/07/0475 were found to be in the same category, while accessions NG/OA/03/12/157 and NG/OA/05/12/159 in separate categories

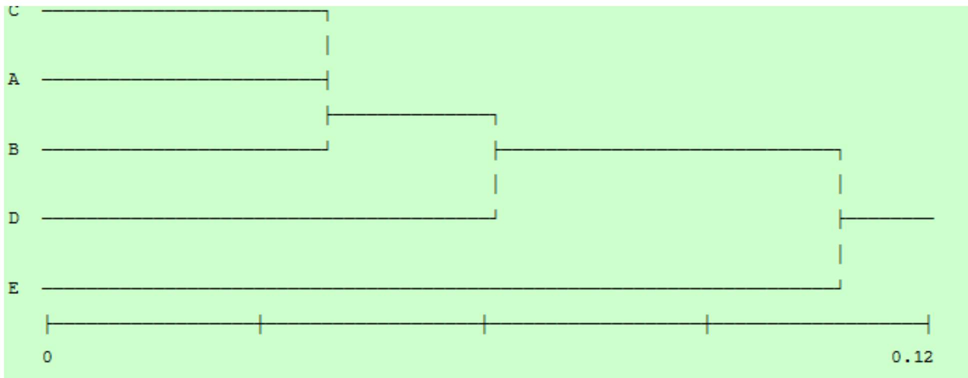

Figure 1: Dendrogram showing the clusters relationship among the five Okra accessions revealed by Alcohol dehydrogenase activity

A = NG/OA/03/12/157 B = NG/OA/03/12/158
C = NG/SA/DEC/07/0475 D = NG/SA/DEC/07/0482 E = NG/OA/05/12/159

At about 0.040 levels of coefficient of similarity, a genetic cluster was revealed, while at 0.041 level of coefficient of similarity, clusters 2 was observed comprising of three accessions, though distance not greatly observed. Cluster 3 was revealed at 0.065 level of coefficient of similarity, while cluster 4 was observed at 0.115 level of coefficient of similarity (Table 8).

Table 8
Clustering strategy for alcohol dehydrogenase

Cluster	1st item	2nd item	Distance
1	C	A	0.040
2	Cluster 1	B	0.041
3	Cluster 2	D	0.065
4	Cluster 3	E	0.015

Alcohol dehydrogenase activity tends to be higher during anoxia and is hypothesized that it may affect some morphological attributes. The only factor that can pose anoxic condition in okra ecology is well irrigated/flooded soil. McManmon and Crawford, (1971) proposed that tolerance to anoxia is based upon the control of glycolysis through the inductive properties of alcohol dehydrogenase. A. caillei is commonly grown in the high rainforest zone of West Africa. In contrast, A. esculentus is commonly grown under irrigation in areas

with less rainfall (Schipper, 1998). To survive this condition, accessions should possess morphological features to aid expelling of water faster than normal.

Colom and Vazzana, (2001) reported that during water stress, total leaf area decreased significantly in Eragrotis curvula and in Sorghum (Yavad et al., 2005). The adjustment of plant to use of water depends greatly on the area/size of the leaves. Similar results were observed under drought stress in Abelmoschus esculentus (Bhatt and Srinivasarao, 2005). The reverse would be observed in flooded condition which may result in anoxia. Morphological features like; petiole length, leaf width and number of leaves were higher in some accession compared to the others.

The statistical analysis done using Version 5.0 of SPSS (ANOVA) showed a level of significance at P<0.05 with accessions NG/SA/DEC/07/0482 and NG/OA/05/12/159 when compared with accessions NG/OA/03/12/157, NG/OA/03/12/158 and NG/SA/DEC/07/0475. When homogeneity was compared within the accessions to check the cause of variation, accessions NG/OA/03/12/157, NG/OA/03/12/158 and NG/SA/DEC/07/0475were found to be in the same category, while accessions NG/SA/DEC/07/0482and NG/OA/05/12/159 in separate categories. The high activities of this enzyme in accession NG/SA/DEC/07/0482 and NG/OA/05/12/159 did not place them the same category proving their ancestry is far or activity may have been disturbed by environmental factor.

When a cluster diagram was constructed, it formed three clusters. Cluster 1 (NG/OA/03/12/157 and NG/SA/DEC/07/0475) revealed great similarity compared to cluster 2 (cluster 1 + NG/OA/03/158). Cluster 3 revealed similarities between clusters 2 and NG/SA/DEC/07/0482. Although accession NG/OA/03/12/157 from morphological attribute which was A. esclentus is now located among A. caillei, but accession NG/SA/DEC/07/0482 still maintained its deviation amongst A. caillei. Cluster 4 revealed similarities between clusters 1, 2, 3 and NG/OA/05/12/159 but closer in distance to accession NG/SA/DEC/07/0482. This close distance is as a result of both possessing higher isozymatic activity than the other. Althoughthis may have been affected by other environmental factor yet, accession NG/SA/DEC/07/0482still proved a true A. caillei from ancestry.

Isocitrate dehydrogenase

From Table 7, a level of significance was obtained at P<0.05 with accessions NG/OA/03/12/157 and NG/OA/05/12/159 having higher values for mean ± STD error with 0.027±0.006 and 0.044±0.002 respectively when compared with accessions NG/SA/DEC/07/0482, NG/OA/03/12/158 and NG/SA/DEC/07/0475. However, when homogeneity was compared within the accessions to check the cause of variation, accession NG/OA/03/12/157 was found to be the cause of variation appearing in both categories.

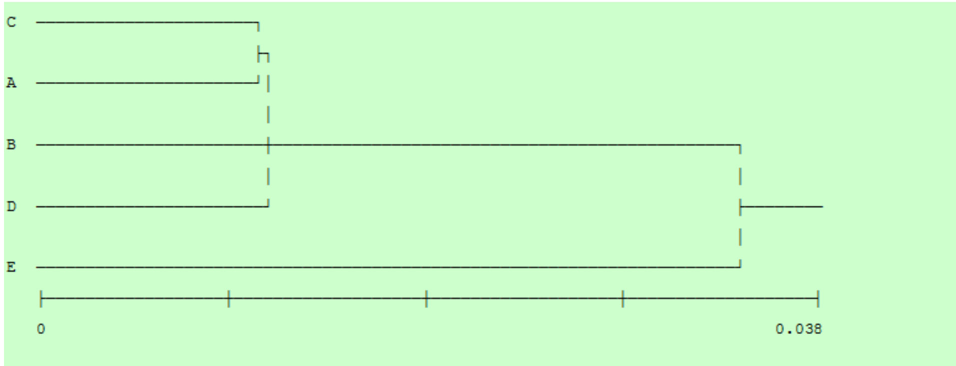

Figure 2: Dendrogram showing the cluster relationship among the five Okra accessions revealed by Isocitrate dehydrogenase activity.

A=NG/OA/03/12/157 B=NG/OA/03/12/158

C=NG/SA/DEC/07/0475 D=NG/SA/DEC/07/0482

E=NG/OA/05/12/159

Specific activity of the Isozyme was used to construct a phylogenetic relationship dendrogram among five accessions. It revealed a genetic diversity and relationships among the accessions (Figure 2).

Table 9
Clustering strategy for isocitrate dehydrogenase

Cluster	1st item	2nd item	Distance
1	C	A	0.011
2	Cluster 1	B	0.011
3	Cluster 2	D	0.012
4	Cluster 3	E	0.035

A genetic cluster was revealed for accessions NG/OA/03/12/157, NG/OA/03/12/158 and NG/SA/DEC/07/0475 at about 0.011 levels of coefficient of similarity, forming clusters 1 and 2. At 0.012 level of coefficient of similarity, cluster3 was observed comprising of four accessions with accession NG/SA/DEC/07/0482 bridging distance between clusters 1, 2 and 3. At 0.035 level of coefficient of similarity, cluster 4 was observed.

A statistical analysis was done using Version 5.0 of SPSS (ANOVA) which showed a level of significance at $P<0.05$ with accessions NG/OA/03/12/157 and NG/SA/DEC/07/0482having higher values for mean ± STD error of 0.027±0.006 and 0.044±0.002 respectively when compared with accessions NG/OA/05/12/159, NG/OA/03/12/158 and NG/SA/DEC/07/0475. When homogeneity was compared within the accessions to check the cause of variation, accessions NG/SA/DEC/07/0482was found to be the cause of variation,

appearing in both categories. This may have contributed to it deviation from A. caillei at morphological state. Its morphological traits expressed those of A. caillei and A. esclentus.

When a cluster diagram was constructed, it formed four clusters. Cluster 1 and 2 having same distance. Cluster3 was observed comprising of four accessions with accession NG/SA/DEC/07/0482 bridging distance between cluster 1, 2 and 3. Cluster 4 revealed similarity with the other clusters. Though accession NG/OA/05/12/159 still proved a true A. caillei from ancestry. Accession NG/OA/03/12/157 from morphological attribute which was A. esclentus was located among A. caillei, but accession NG/SA/DEC/07/0482 did not maintain its deviation amongst A. caillei completely. The high activities possessed by accessions NG/SA/DEC/07/0482 and NG/OA/05/12/159 (A. esculentus) ought not to be, however metabolism under anoxic condition still proceed toward Kreb cycle where this isozyme is a key enzyme in the metabolic pathway.

Malate dehydrogenase

From the result obtained for malate dehydrogenase (Table 7), no significant difference was recorded at P<0.05. However, when compared with a phylogenetic dendrogram (Figure 3), it revealed a genetic diversity and relationships among the accessions.

Table 10

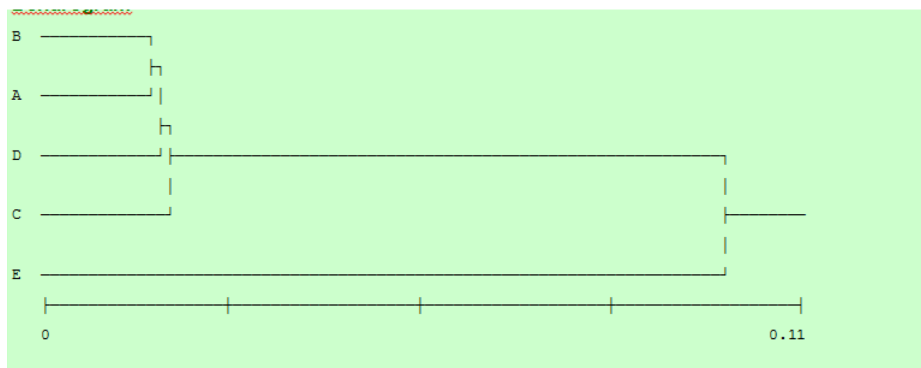

Figure 3: Dendrogram showing the clusters among five (5) Okra accessions revealed by Malate dehydrogenase activity.

A=NG/OA/03/12/157
B=NG/OA/03/12/158
C=NG/SA/DEC/07/0475
D=NG/SA/DEC/07/0482
E=NG/OA/05/12/159

Clustering strategies for malate dehydrogenase

Cluster	1st item	2nd item	Distance
1	C	A	0.017
2	Cluster 1	B	0.017
3	Cluster 2	D	0.019
4	Cluster 3	E	0.0101

A genetic cluster was revealed for accessions A, B and D at about 0.017 levels of coefficient of similarity, forming clusters 1 and 2. At 0.019 level of coefficient of similarity, cluster 3 was observed comprising of four accessions. At 0.101 level of coefficient of similarity, cluster 4 was observed (Table 10).

A statistical analysis was done using Version 5.0 of SPSS (ANOVA) which showed no level of significance at P<0.05. When a cluster diagram was constructed, it formed four clusters. Cluster 1 and 2 having same distance. Cluster3 was observed comprising of four accessions with accessions NG/SA/DEC/07/0482 and NG/SA/DEC/07/0475seperated from each other though closely related by distance. Cluster 4 revealed similarity with the other clusters been the most ancestral of all. No level of significance may have resulted as malate; the substrate to malate dehydrogenase is a prominent metabolite for both anoxic and aerobic condition. This is supported by McManmon and Crawford (1971) who reported that in both anoxia tolerant and anoxia intolerant species possess this enzyme.

Conclusion

The degree of intra specific variation differs for the five accessions. A. caillei formed two clusters: NG/SA/DEC/07/0475 and NG/OA/03/12/158 one cluster while NG/SA/DEC/07/0482 forms another. Grouping of the A. caillei accessions in one cluster indicate a similarity within the species. Nevertheless, the separation of a single cluster NG/SA/DEC/07/0475 and NG/OA/03/12/158 from NG/SA/DEC/07/0482 shows a considerable degree of total protein content and isozymatic variation within the species. Also, the A. esculentus accessions formed a single cluster: NG/OA/03/12/157 and NG/OA/05/12/159. This shows that there is a close relationship among them. At a higher Euclidian distance, four distinct clusters were formed for each of the isozymes used. In all, accession NG/OA/05/12/159proved to be a true A. esculentus. Accession NG/SA/DEC/07/0482 possessed attribute of both A. esculentus and A. caillei. This is an indication of inter specific morphological and isozymatic dissimilarity between the two groups of species. Further analysis should be carried out on accession NG/SA/DEC/07/0482 as it real identity still proof unstable. All cluster diagrams (dendrogram) reveals that the accessions with the highest level of relatedness were NG/SA/DEC/07/0475 and NG/OA/03/12/158. However crosses between such accessions may be a waste, since the result of such a cross may not produce much difference (i.e. they possess similar gene).

The isozyme characterization has proven effective in identification and characterization but activities of these isozymatic characters only cannot be used as an indication of evolutionary advancement and classification at the generic and species level.

References

Adams, C. F. (1975). *Nutritive value of American foods in common units,* U.S. Department of Agriculture, Agric Handbook. pp29.

Adelakun, O.E., Oyelade, O.J., Ade-Omowaye, B. I. O., Adeyemi, I.A., Van M. (2008). *Influence of pretreatment on yield, chemical and antioxidant properties of Nigerian okra seed (Abelmoschus esculentus Moench) flour.*

Adetuyi, F.O., Osagie, A.U., Adekunle, A.T. (2008). Effect of Postharvest Storage Techniques on the Nutritional Properties of Benin Indigenous Okra Abelmoschus esculentus (L) Moench. *Pakistan Journal Nutritive, 7*: 652-657.

Aladele, S. E., Ariyo, O. J. and Lapena, R. (2008). Genetic relationships among West African okra (Abelmoschus esculentus). *Indian Journal of Biotechnology, 7*(10): 1426 – 1431.

Benchasri, S. (2012). Okra (Abelmoschus esculentus (L.) Moench) as a valuable vegetable of the world. *Rather Povrt., 49*: 105 – 112.

Colom, M. R. and Vazzana, C. (2001). Drought stress effects on three cultivars of Eragrostis curvula: photosynthesis and water relations. *Plant growth regulation 34*: 195-202.

Doijode, S. D. (2001). *Seed Storage of Horticultural Crops:* Food products Press, New York. USA. Pp. 342-347.

Edwin, R., Sekar, T., Sankar, P. and Munusamy, S. (2006). *Botany.* In: Pandian, T.T., Rathina kumar, S. S., (eds). Tamil Nadu text Book Corporation. Tamil Nadu, India. 264p.

Efisue A. A. (2013).Isozymes-based genetic fingerprinting of Manihot sp. *Nigeria Journals of Biotechnology, 26*: 1-10

Farooq, A., Umer, R., Muhammad, A., Muhammad, N. (2010). Okra (Hibiscus esculentus) seed oil for biodiesel production. *Applied Energy 87* (3): 779–785.

Felter, H., Wickes, L. and John, U.(1898). *King's American Dispensatory.*

Franklin, W. and Martin, O. (1982). Okra; Potential Multiple-Purpose Crop for the Temperate Zones and Tropics. *Economic Botany 36* (3): 340–345.

Freville, H., Justy, F. and Olivieri, I. (2001) Comparative allozyme and microsatellite population structure in a narrow endemic plant species, Centaurea corymbosa Pourret (Asteraceae), *Molecular Ecolology, 10*:879–889

Gopalan, C., Sastri, S.B.V. and Balasubramanian, S. (2007). *Nutritive value of Indian foods,* National Institute of Nutrition (NIN), ICMR, India

Kendall, C.W.C. and Jenkins D.J.A. (2004). A dietary portfolio: maximal reduction of low-density lipoprotein cholesterol with diet. *Current Atherosclerosis Reports, 6*:492-498.

Kumar, R., Patil M.B., Patil S.R., Paschapur M.S. (2009). Evaluation of

Abelmoschus esculentus mucilage as suspending agent in paracetamol suspension. *International Journal of Pharmaceutical Technology Research, 1*: 658-66.

Kumar, S., Dagnoko S., Haougui A., Ratnadass A., Pasternak D., Kouame C.(2010). Okra (Abelmoschus sp.) in West and Central Africa: potential and progress on its improvement. *African Journal. of Agricultural. Research, 5*: 3590-3598.

Lehninger, A. L., Nelson D. L. and Cox, M. (eds) (2000). *Principles of Biochemistry.* Worth publisher. New York.

Lemke-Keyes, C.A. and Sachs, M.M. (1989). Anaerobic tolerance null: a mutant allows Adh1 nulls to survive anaerobic treatment. *Journal Heredity, 80*:316-319.

Longo, C.P. and Scandalios J.G. (1969) Nuclear gene control of mitochondrial malic dehydrogenase in maize. *Proceedings of the National Academy of Sciences, 62*: 104-111.

McManmon, M. and Crawford, R. M. M. (1971). A metabolic theory of flooding tolerances: the significance of enzyme distribution and behavior. *New Phytol, 70*:299-306.

Moaward F.G., Abdelwhab B.M., Abdelnahun F.M. and Shehaya F.W. (1984).*Annual of Agricultural science, 21*: 603 – 613.

Moekchantuk, T. and P. Kumar, (2004). *Export okra production in Thailand Inter-country Programme for vegetable IPM in South and SE Asia Phase II.* Pp. 111 – 128.

Nwangburuka, C. C., Kehinde, O. B., Ojo, D. K., Popoola, A. R., Oduwaye O., Denton, O. A. and Adekoya, M. (2011). Molecular Characterization of Twenty-nine Okra Accessions Using the Random Amplified Polymorphic DNA (RAPD) Molecular Marker. *Acta SATECH, 4*(1): 1 - 14

Obire, I.O. (2002). *Ethnobotanical survey of West African Okra Abelmoschus caillei (A. Chev) Stevels) in Southern Edo State.* B.Sc. thesis. University of Benin, Benin City, Nigeria. 39p.

Ogwu, M. C and Osawaru, M. E. (2015). Soil characteristics, microbial composition of plot, leaf count and sprout studies of Cocoyam (Colocasia [Schott] and Xanthosoma [Schott], Araceae) collected in Edo State, Southern, Nigeria. *Science Technology and Arts Research Journal, 4*(1): 34 - 44

Omuta, G. E. D. (1980). *A profile of Development of Bendel State of Nigeria publication in Geography 1 no. 2.*Department of Geography and Regional Planning, University of Benin, Benin City, Nigeria. 67p.

Onwueme, I. C. and Singh, T. D. (1991).*Field crop production in tropical Africa.* CTA, Ede,the Netherlands 480p.

Osawaru M.E. and Ogwu, M.C. (2014). Ethnobotany and germplasm collection of two genera of Cocoyam (Colocasia [Schott] and Xanthosoma [Schott], Araceae) in Edo State Nigeria. *Science Technology and Arts Research Journal, 3*(3): 23-28

Osawaru, M. E. and Ogwu, M. C. (2013). Collecting West African Okra

(Abelmoschus caillei (A. Chev.) Stevel.) germplasm from traditional agriculture in parts of Southwestern Nigeria. *The Bioscientist, 1*(2):171 - 181

Osawaru, M. E., Ogwu, M. C. and Aiwansoba, R. O. (2015). Hierarchical approaches to the analysis of genetic diversity in plants: a systematic overview. *University of Mauritius Research Journal, 21*: 92 -127

Osawaru, M. E., Ogwu, M. C. and Aiwansoba, R. O. (2016). Wood properties of Abelmoschus caillei [A. Chev.] Stevels. *JORMAR, 10* (1): 23 - 32

Osawaru, M. E., Ogwu, M. C. and Dania-Ogbe, F. M. (2013a). Morphological Assessment of the Genetic Variability among 53 Accessions of West African Okra [Abelmoschus caillei (A. Chev.) Stevels] from South Western Nigeria. *Nigerian Journal of Basic and Applied Science, 21*(3): 227-238

Osawaru, M. E., Ogwu, M. C. and Dania-Ogbe, F. M. (2013b). Morphological assessment of the genetic variability among 53 accessions of West African Okra [Abelmoschus caillei (A. Chev.) Stevels] from South Western Nigeria. *Nigerian Journal of Basic and Applied Science, 21*(3): 227-238

Osawaru, M. E., Ogwu, M. C. and Omologbe, J. (2014).Characterization of three Okra [Abelmoschus (L.)] accessions using morphology and SDS-PAGE for the basis of conservation. *Egyptian Academic Journal of Biological Science, 5*(1): 55 –65

Osawaru, M. E., Ogwu, M. C., Ogbeifun, N. S. and Chime, A. O. (2013c). Microflora diversity on the phyloplane of wild Okra (Corchorus olitorius L. Jute). *Bayero Journal of Pure and Applied Sciences, 6*(2): 136 – 142

Osawaru, M.E., Dania-Ogbe, F.M., Chime, A.O. and Ogwu, M.C. (2011). Epidermal morphology of West African Okra Abelmoschus caillei (A. Chev.) Stevels from South Western Nigeria. *Science World Journal, 6*:15-23.

Pramod, K. S., Govind, K. S. and Siddhartha S. (2010).Isolation and Characterization of NADP+-Linked Isocitrate Dehydrogenase in Germinating Urd Bean Seeds (Phaseolus mungo).*Journal of Proteins and Proteomics, Vol 1*, pp25-32.

Rao, P.S., Rao, P.U., Serikeran, B. (1991). Serun cholesterol, triglycerides and total total fatty acid of rates in response to okra (Hibiscus esculentus) seed oil. *JAOCA journal, 68*:433- 446

Sachs, M.M. and Freeling, M. (1978).Selective synthesis of alcohol dehydrogenase during anaerobic treatment of maize..*Molecular and General Genetics, 161*:111-115.

Sadunishvili, T.A. and Nutsubidse, N.N. (1980) Intra cellular location of malate and glutamate dehydrogenases in lemon citrus-limon leaves. *Soobsheniya Akademii Nauk Gruzinskoy SSR, 100*:693-696.

Scandalios, J.G. (1974) Isoenzymes in development and differentiation. *Annual Review of Plant Physiology, 25*.225-258.

Schippers, R. R. (1998). *African indigenous vegetables – An overview of the cultivated species.* Chatham, UK: Natural Resources Institute/ACP-EU

Technical Center for Agricultural and rural cooperation, pp. 103- 118.

Siesmonsma, J.S. and Hamon, S. (2002). *Abelmoschus caillei (A. chev) Stevels.* In: Oyen, L.P. A. and Lemmens R. H. M. (eds) Plant Resources of Tropical Africa. Precusor PROTA Programs. Wageningen, Netherlands. pp27 - 30.

Tindall, H. D. (1983). *Vegetable in the tropics.* Macmillan press ltd London first edition. 89p

Ting, I.P., Fuhr I., Curry, R. and Zschoche, W.G. (1975). *Malate dehydrogenase isozymes in plant: Preparation, properties and biological significance.* In: Isozymes, (eds C.L. Markert). Academic Press, New York, pp 369-384.

Varmudy, V. (2011). *Marking survey need to boost Okra exports.* Department of Economics, Vivekananda College, Puttur, Karnataka, India.45p

Yang, N.S. and Scandalios, J.G. (1974). Purification and biochemical properties of genetically defined malate de- hydrogenase in maize. *Archives of Biochemistry and Biophysics, 161*: 335-353.

Yudina, R.S. and Levites, E.V. (2007). Malate dehydrogenase isozymes as markers of organelles physiological state sugar beet (Beta vulgaris L.). *Sugar Tech, 9*, 67-71.

Yudina, R.S. and Levites, E.V. (2008) Subcellular localization of isozymes of NAD-dependent malate dehydrogenase in sugar beet Beta vulgaris L. *Russian Journal of Genetics, 44,* 1419 - 1423.