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Genetic Fingerprinting of Sweet Potato [*Ipomoea batatas (L.) Lam]* as Revealed by Isozyme Electrophoresis Analysis

Efisue, A. A.
Department of Crop and Soil Science, University of Port Harcourt P.M.B. 5323 Choba, Port Harcourt, Rivers State, Nigeria.

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Abstract

Sweet potato is an important staple crop and many varieties have been released into farmers' fields in Nigeria, but no reliable means in tracking their identity, thus causing multiple naming of these varieties among farmers. The objective of the study is to establish objectively and reliable means of identifying released, local and elite sweet potato genotypes available in farmers' fields. Thirty genotypes of sweet potato (*Ipomoea batatas*) species comprised IITA elites, local landraces and adaptable farmers' varieties were planted at a space of 1m x 1m in a randomized complete block design in two replications. High genetic diversity was observed among the sweet potato genotypes examined. The morphological data revealed three distinctive clusters. In cluster I, purple vine, green petiole and light pink storage root colour were the dominant traits. While cluster II had dark green leaf at emergence and later changed to light green when fully expanded and the root cortex and pulp were white and cream colour, respectively. Cluster III exhibited different morphological characters. Two of the four isozyme markers examined, Aspartate amino transferase (AAT) and 6-phosphogluconate dehydrogenase (6-PGD) were more effective to discriminate sweet potato genotypes. AAT had 9 loci while 6-PGD had 10 loci and polymorphism ranged from 10.0% - 90.0% for AAT and 10.0% - 96.7% for 6-PGD. Isozyme data analysis revealed four clusters and insignificant correlations were observed between the isozyme and morphological analyses. The results could assist breeders in genetic diversity study of this crop and for its improvement.

Key words: Electrophoresis, isozyme markers, sweet potatos, morphology and polymorphisms

***Correspondence**: andyefisue@yahoo.com*

Introduction

Sweet potato (*Ipomoea batatas*) is the only economically important member of the family *convolvulaceae*. It is not known in the wild state but probably originated in Central America, where it has long been cultivated. It is now grown extensively throughout the tropics and subtropics, and particularly in the central region of Nigeria. It is a perennial herb with a vine-like habit and great variation in leaf form, but commonly cultivated as annual. Sweet potato is grown in all part of Nigeria, but grows best with an average temperature of 24^0C, an average rainfall of 750- 1,250mm, and matures at 4-6 months after planting. In some areas sweet potato are grown as a perennial crop but usually they are regularly harvested and replanted by root stem cuttings. They are planted on mounds or ridges. Many sweet potato varieties have been released in Nigeria, but no reliable means in tracking their identity, thus causing multiple naming of these varieties.

Morphological characterization is an important tool in the identification of duplicate cultivars, detection of major traits and types of cultivars to be conserved to conserve storage space and enhanced plant selection by breeders (Reed *et al*., 2004). However, this technique is environmental dependent, and has become unreliable in cultivar identification and

classification. The use of morphological characters to study the variability in sweet potato and hybrid progenies have been reported and observed the inert-relationship of different characters such as yield in association with length of petiole, number of tubers and the mean tuber weight (Thankamma *et al.* 1990, Arslanoglu *et al.* 2011; and Fongod *et al.,* 2012). Reports also showed that morphological characterization has been used for studies of genetic diversity patterns, cultivar identification and correlation with characteristics of agronomic importance (Nwankwo *et al*., 2012; Karuri *et al.,* 2010 and Tairo *et al.,* 2008). Vimala and Nair (1988) reported the variability observed in the hybrid progenies and segregation pattern for the different morphological characters of 20 selected cultivars of sweet potato (*Ipomoea batatas*), reported the existence of continuous and overlapping variation that points towards the quantitative nature for all morphological characters studied.

DNA fingerprinting has become an important tool for cultivar identification in plant breeding and for germplasm management. A number of different molecular assays have been applied in sweet potato (Karuri *et al.,* 2010; and Sossah *et al.,* 2014). Sofy *et al.* (2013) differentiated wild sweet potato species based on their reaction to potato spindle tuber viroid (PSTV) disease and reported differential response to PSTV disease. Isozyme technique is an important screening tool used by scientists for genetic diversity studies in cultivars and as well response to biotic and abiotic stresses (Jang *et al.,* 2004; Efisue, 2013 and Kennedy and Thompson, 1991). The use of isozyme markers for cultivar identification and genetic studies is relatively inexpensive method and gives comparative reliable results as other DNA markers (Rao, 2004 and Oppong-Konadu *et. al*. 2005). The objective of the study is to establish objectively and reliable means of identifying released, local and elite sweet potato genotypes available in farmers' fields.

Materials and Methods

The experiment was carried out on the field at International Institute of Tropical Agriculture (IITA) experimental station, Ibadan. Ibadan is situated at latitude 7^0 23' 16" N and longitude 3^0 53' 47" E with mean annual rainfall of 1,200mm, mean monthly temperature of about 26.6^0C and high relative humidity of about 80%. Thirty genotypes of *Ipomoea batatas* comprising of IITA elites, local landraces and adaptable farmers' varieties were planted at a space of 1m x 1m in a randomized complete block design in two replications. Seven plants per genotype per replicate were planted and five plants per genotype per replication were tagged for record taking, a total of six plots of size $224m^2$ per plot were used. The Plots were ploughed and ridged at a distance of 1m apart, and the total experimental farm size was 0.134 ha. Data collected were based on International Plant Genetic Resources Institute (IPGRI, 1990). The genetic materials used in this study, status and country of origin are indicated in Table 1.

Morphological data: Observations were made on the following parameters: colour of leaves at emergence, unexpanded and first expanded leaves and vein colour at the first month after planting. Length and width of the central lobe and the petiole length were measured using calibrated ruler and leaf area at two months after planting was take using a leaf area meter (Li-3100C, Lincoln, Nebraska), while the pubescence of the young stems and leaves, flowering and flower colour, petiole and vine colour and pubescence of the petiole were observed weekly. At maturity, tuber characteristics were taken such as shape, out and inner skin colour and the flesh colour. Data collected were based on International Plant Genetic Resources Institute (IPGRI, 1990) evaluation system.

Stock solutions and constituents: Slab polyacrylamide gel used was made up of the following stock solutions.

A = Tris - HCL buffer, Trizma base = 36.60g + distilled H_2O at pH 8.9 to 100 mL at room temperature.

B = Tris – HCl buffer, Trizma base = 5.0g + distilled H_2O to 100 mL at room temperature.

C = Acrylamide = 28.00g, Bis- acrylamide = 0.735g + distilled to 100 mL at room temperature. D= Acrylamide = 10.00g, Bis-acrylamide = 2.50g + distilled H_2O to 100mL at room temperature.

E= Ammonium Persulphate at 10g/100 mL prepared fresh weekly.

Table 1: Genotype used, their status and source

Genotype code No.	Genotype	Status	Source
1	Tis 9068	Improved	IITA
2	Tis 8209	Improved	IITA
3	Tis 84/0471	Improved	IITA
4	Tis 8417	Improved	IITA
5	Tis 2154	Improved	IITA
6	Tib 11	Land race	Nigeria
7	Tib 4	Land race	Nigeria
8	Tib 9291	Improved	IITA
9	Tis 8267	Improved	IITA
10	Tis 9172	Improved	IITA
11	Tis 8409	Improved	IITA
12	Tis 8504	Improved	IITA
13	Tis 84/0247	Improved	IITA
14	Tis 82/0405	Improved	IITA
15	Tis 81/0286	Improved	IITA
16	Tis 82/0201	Improved	IITA
17	Tis 3180	Improved	IITA
18	Tis 70150	Improved	IITA
19	Tis 2153	Improved	IITA
20	Tis 2347	Improved	IITA
21	Tis 80/089	Improved	IITA
22	Tis 80/723	Improved	IITA
23	Tis 81/530	Improved	IITA
24	Tis 9191	Improved	IITA
25	Tis 80/733	Improved	IITA
26	Tis 83/176	Improved	IITA
27	Tis 8429	Improved	IITA
28	Tis 9217	Improved	IITA
29	Tis 82/0602	Improved	IITA
30	Tis 70054	Improved	IITA

The separating gel: Contains (A = 7.50 mL, C = 15.0 mL, distilled H_2O = 37.5 ml, E = 0.41 mL and TEMED = 18.75mL) of the stock solutions

The Stacking gel: Contains stock solutions (B = 2.5 mL, D = 5.0 mL, distilled H_2O = 12.5 mL, E = 60.0 µL and TEMED = 24. 0µL)

Sample extraction: Samples of young fresh leaves from the field were collected with test tubes placed in an ice container and then taken into the laboratory. The samples were crushed in extraction buffer solution trizma base = 1. 211g, Potassium Chloride = 0. 725g, Ethylene Diaminetetraacetic acid (EDTA) = 0.298g, Sucrose = 3.420g, 2M-E(Mercaptoethanol) = 60.0µl, and distilled H_2O to 100 mL at pH 7.5 with 200 mg of Polyviylpolypyrrolidone (PVPP)of ratio 1:5 (W/V)) per sample. After crushing, the sample was centrifuged at 4^0 C for 10 min. at 10,000 x g. about 20.0µL of the supernatant was loaded into each slot. Two standard genotypes were

included on each slab to provide reference bands. The stock electrode buffer was Davis 10x of Tris/glycineat pH 8.57 at room temperature and 10x concentration. The Hoeffer Scientific Instrument model SE700 electrophoresis tank and model PS2500 power supply unit were used for electrophoretic analysis. Slab polyacrylamide gels were used which were made up of the following stock solutions. For electrophoresis, an initial voltage between 200-300V and a constant current of 60mA for 3 hours at 4^0 C was used. The gels were then stained according to the following recipes:

Enzyme markers and constituents:
AAT: Aspartate amino transferase (E.C.2.6.1.1.): Modified Vallejos method (1983) Tris-HCL pH 8 (0.1M) = 10 mL, H_{2O} (distilled) = 90 mL, Aspartic acid = 0. 30g,
a- ketoglutaric acid = 0.15 g, pyridoxal -5-phosphate = 0.01 g or pinch and Fast blue BB salt = 0.15 g

6-PGD: 6-phosphogluconate dehydrogenase (E.C.I.I.I.44): Modified Vallejos method (1983) Tris –HCl pH7.5 (0.1M) = 50 mL, $MgCl_2$ (0.1M) = 0.25 mL, 6-P-gluconic acid = 10 mg,
NADP (20 mg/mL) = 0.38 ml, MTT (10 mg/mL) =1.0 mL and PMS (10 mg/mL) = 0.2 mL

G-6-PDH: Glucose-6-phosphate dehydrogenase (E.C.I.I.I.49): Modified Vallejos method (1983). Tris- HCL pH 7.5 (0.1M) = 50 mL, $MgCl_2$ (0.1M) = 0.5 mL, Glucose -6-phospate = 20 mg, NADP (20 mg/mL) = 0.38 mL, MTT (10 mg/mL) = 1.0 mL and PMS (10 mg/mL) = 0.2 mL

*SKDH: shikimate dehydrogenase (E.C.I.I.I. 25)***:** Modified Vallejos method (1983). Tris-HCL pH 7.5 (0.1M) = 50 mL, Shikimic acid = 50 mg, NADP (20 mg/mL) = 0.38 mL, MTT (10 mg/mL) = 1.0 mL and PMS (10 mg/mL) = 0.2mL

The gels were equilibrated with the staining buffer for approximately 10 min in the incubator at 37°C. This was replaced with the complete staining solution and incubated in the dark at 37°C for 30 min or until the bands developed.

Statistical analysis: The morphological data were subjected to hierarchical cluster analysis in GENSTAT computer programme which drew dendrograms. For the isozyme analysis, numerical code for scoring enzyme phenotypes was combined with copies of banding. Enzyme loci were evaluated for polymorphism and enzyme activity. The allelic frequencies for each genotype were recorded based on the repeatability and interpretation of the dendogram. To study the genetic relationships among the genotypes, the presence or absence of isozyme bands transformed into a binary character matrix (1 = presence and 0 = absence of isozyme band). Thus, isozymic variability was created and a binary matrix was derived. Pair-wise distance matrixes between genotypes were again derived using the numerical taxonomy and multivariate analysis system (NTSYS-PC), Version 2.1 (Rohlf, 2000) and the Jaccard coefficient of similarity (Jaccard, 1908). Genetic diversity dendogram for the genotypes was created by unweighted pair group method with arithmetic mean (UPGMA) cluster analysis (Sneath and Sokal, 1973; Swofford and Olsen, 1990).

Results

Morphological characters were highly variable among the genotypes studied. The most distinguishing morphological traits for sweet potato are petiole and stem colour, pubescence of young leaf and stem and storage root colour Table 2.

Three distinct clusters were identified from the morphological character analysis. At 0.44 levels of coefficient of similarity index, cluster I was revealed, while cluster II and cluster III were identified at 0.55 levels of coefficient of similarity. There were 5, 4 and 10 genotypes for cluster I, II and III, respectively. Tib 4 is distinct and did not form cluster while more genotypes exhibited individuality at 0.69 levels of coefficient of similarity index (Fig.1)

The most common morphological traits found among the genotypes in cluster I were purple vine, green petiole and light pink storage root colour (Fig. 1 and Table 2). While cluster II had dark green leaf at emergence and later changed to light green when fully expanded. The root cortex and pulp were white and cream colour, respectively. Cluster III comprised 10 genotypes exhibiting different morphological characters. However, the dominant characters are dark green colour of leaves at emergence, green colour vine and the petiole is green colour (Fig. 1 and Table 2).

Table 2. Major distinguishing morphological features among 30 sweet potato genotypes.

		Distinguishing Characters												
Genotype	Genotype code No.	1	2	3	4	5	6	7	8	9	10	11	12	13
Tis 9068	1	DG	DG	LG	P	G	1	NL	G	LP	HP	DP	W	C
Tis 8209	2	DG	DG	LG	V	G/V	1	NL	V	NP	NP	W	W	C
Tis 84/0471	3	DG	DG	LG	V	G	1	NL	G	LP	HP	P	W	W
Tis 8417	4	DG	DG	V	V	V	1	NL	V	P	HP	P	P	W
Tis 2154	5	DG	DG	V	V	V	1	NL	V	NP	NP	P	P	W
Tib 11	6	P	P	GP	P	G	5	SL	G	HP	NP	LP	C	DP
Tib 4	7	DG	DG	DG	V	G/V	7	DL	G/V	P	NP	LP	C	P
Tib 9291	8	DG	DG	LG	V	G	1	NL	G	NP	NP	-	-	-
Tis 8267	9	LG	LG	LG	G	G	1	NL	G	NP	NP	LP	W	W
Tis 9172	10	GP	GP	GP	P	G	1	NL	G	HP	NP	LP	W	C
Tis8409	11	DG	DG	DG	G	G	1	NL	G	NP	NP	W	C	Y
Tis 8504	12	DG	DG	LG	V	V	5	DL	V	LP	P	--	--	--
Tis 84/0247	13	V	V	V	V	V	2	DL	G/V	P	HP	P	W	W
Tis 82/0405	14	DG	DG	LG	V	GV	4	SL	G/V	NP	NP	W	W	C
Tis 81/0286	15	V	V	V	G	G	3	SL	G	NP	HP	P	W	W
Tis 82/0201	16	DG	DG	LG	V	G	3	SL	G	LP	HP	P	W	W
Tis 3180	17	V	V	V	V	G	3	SL	G/V	NP	P	W	W	W
Tis 70150	18	DG	DG	LG	G	G	1	NL	V	P	P	DP	P	P
Tis 2153	19	DG	DG	LG	G	G	1	NL	G	LP	LP	DP	P	W
Tis 2347	20	DG	DG	LG	G	G	3	SL	G	NP	NP	W	W	Y
Tis 80/089	21	DG	DG	LG	G	P	3	NL	P	NP	NP	LP	W	W
Tis 80/723	22	GP	GP	GP	P	G	6	SL	G	P	HP	W	W	C
Tis 81/530	23	GP	GP	GP	P	G	1	NL	G	HP	HP	LP	W	W
Tis 9191	24	DG	DG	LG	G	G	1	NL	P	LP	LP	W	W	C
Tis 80/733	25	P	P	P	P	G	4	SL	G	NP	LP	LP	W	W
Tis 83/176	26	DG	DG	GP	P	G	5	SL	G	NP	NP	P	W	W
Tis 8429	27	DG	DG	LG	P	V	3	SL	G	P	P	-	-	-
Tis 9217	28	DG	DG	LG	G	G	1	NL	G	NP	P	DP	P	W
Tis 82/0602	29	DG	DG	LG	G	G	1	NL	G	NP	NP	W	W	C
Tis 70054	30	DG	DG	LG	P	P	3	NL	V	NP	NP	P	W	C

Character codes

1) Colour of leaves at emergence: colour of unexpanded apical leaves: Light green (LG), Dark green (DG), Green purple (GP), Purple (P), and violet (V)
2) Colour of unexpanded apical leaves: Light green (LG), Dark green (DG), Green purple (GP), Purple (P) and Violet (V)
3) Colour of first fully expanded leaf: Colour of unexpanded apical leaf: Light green (LG), Dark green (DG), Green purple (GP), Purple (P), and violet (V)
4) Vine colour: Green(G), Purple (P) and Violet (V)
5) Petiole colour: Green(G), Purple (P), Green violet (GV) and Violet (V)
6) No of toothing of the leaf
7) Type of lobe: No lobe (NL), Shallow lobe (SL), and Deeply lobe(DL)
8) Stem colour: Green (G), Purple (P), Green violet (GV) and Violet (V)
9) Pubescence of young stem: Highly pubescence (HP), Pubescence (P), Less pubescence (LP), and Non pubescence (NP)
10) Pubescence of young leaf: Highly pubescence (HP), Pubescence (P), Less pubescence (LP), and Non pubescence (NP)
11) Storage root colour: White(W), Light pink (LP), Pink (P) and Deep pink (DP)
12) Colour of cortex: White (W), Cream (C) and Pink (P), Pulp colour: White (W), Cream (C) Pink (P), Deep pink (DP) and Yellow (Y)

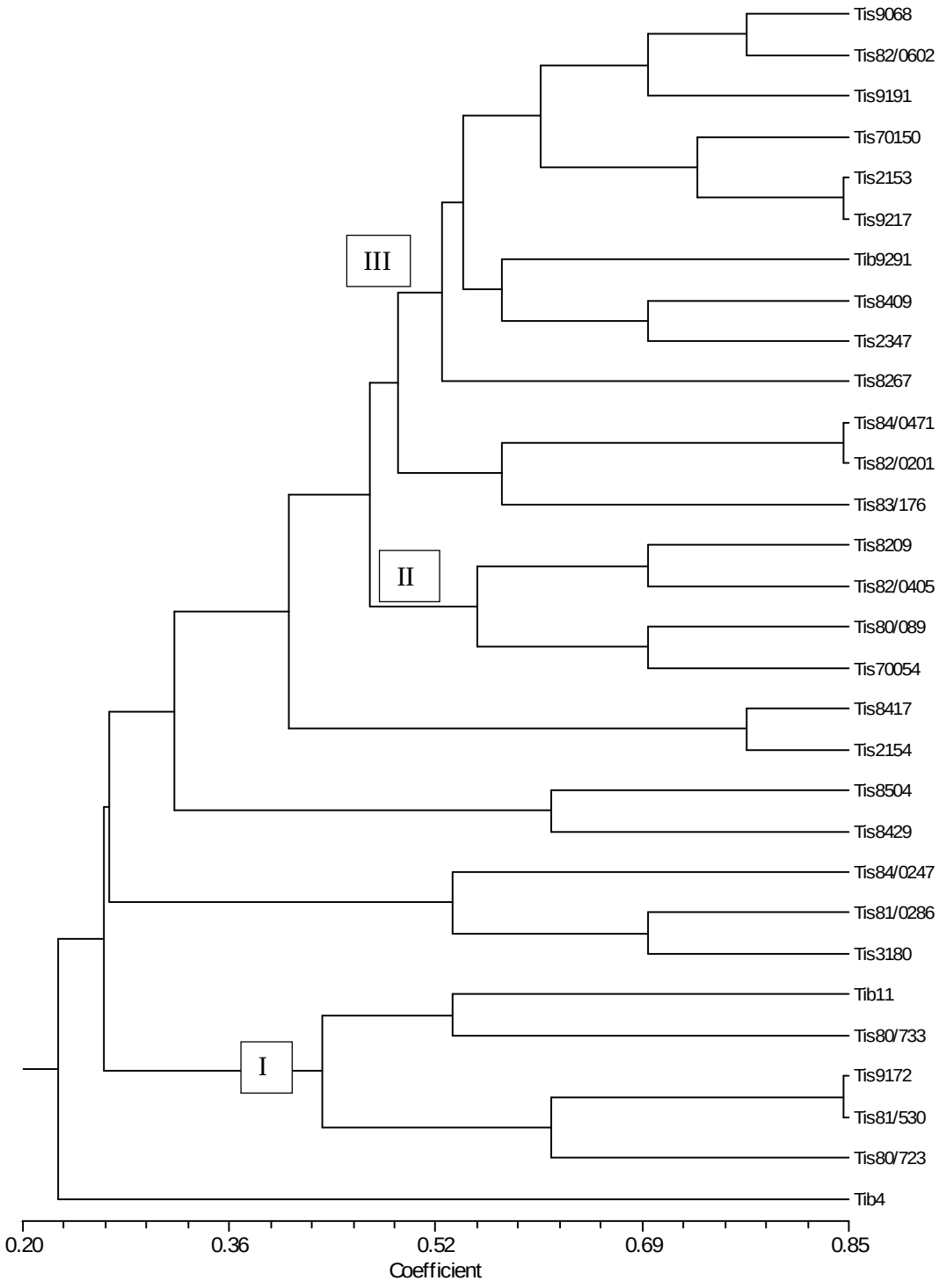

Figure1: Dendrogram showing genotypes clusters among 30 *Ipomoea* sp as revealed by morphological characters**.**

Isozymes markers.

In this study, only two isozyme markers were useful enough to discriminate sweet potato among the markers examined. AAT had 9 loci while 6-PGD had 10 loci and polymorphism ranged from 10.0-90.0% for AAT and 10.0-96.7% for 6-PGD. Locus with low percentage polymorphism indicates that the locus appears in most of the genotypes examined like L-6 of AAT with 10.0% and L-3 and L-6 of 6-PGD with 10.0% polymorphism (Table 3)

There were four cluster groups derived from 19 isozyme loci used in constructing the phylogenetic relationship dendogram among the 30 genotypes of sweet potato used in this experiment. Clustering becomes visible at 0.70 levels of coefficient of similarity. Cluster I and II had 3 genotypes each, while cluster III and IV had 13 and 11 genotypes, respectively. The common trait in cluster I is light green colour of the first expanded leaf. Cluster II had Tib 4 that do not cluster with other genotypes based on morphological traits (Fig. 1) this may be due to its peculiar traits such as deeply lobe, cream colour cortext and pink pulp colour. Other common morphological traits in this group were less pubescence of young leaves, the vine and stem were more violet in colour. Cluster III and IV had varying morphological traits amongst the genotypes. Phylogenetic clustering of the aforementioned clusters may be due to 6-PGD revealing more loci than AAT across the genotypes (Table 3). In 6-PGD, L-3, L-6 and L-9 were present in all the genotypes as well L-6 and L-9 for AAT.

Table 3. Isozyme markers that revealed polymorphism among 30 *Ipomoea* spp.

S/N	***Isozyme Marker***	***Loci***	***% Polymorphism***
1	AAT	L-1	46.7
		L-2	86.7
		L-3	40.0
		L-4	40.0
		L-5	90.0
		L-6	10.0
		L-7	90.0
		L-8	90.0
		L-9	16.7
2	6-PGD	L-1	20.0
		L-2	30.0
		L-3	10.0
		L-4	70.0
		L-5	56.7
		L-6	10.0
		L-7	46.7
		L-8	66.7
		L-9	13.3
		L-10	96.7

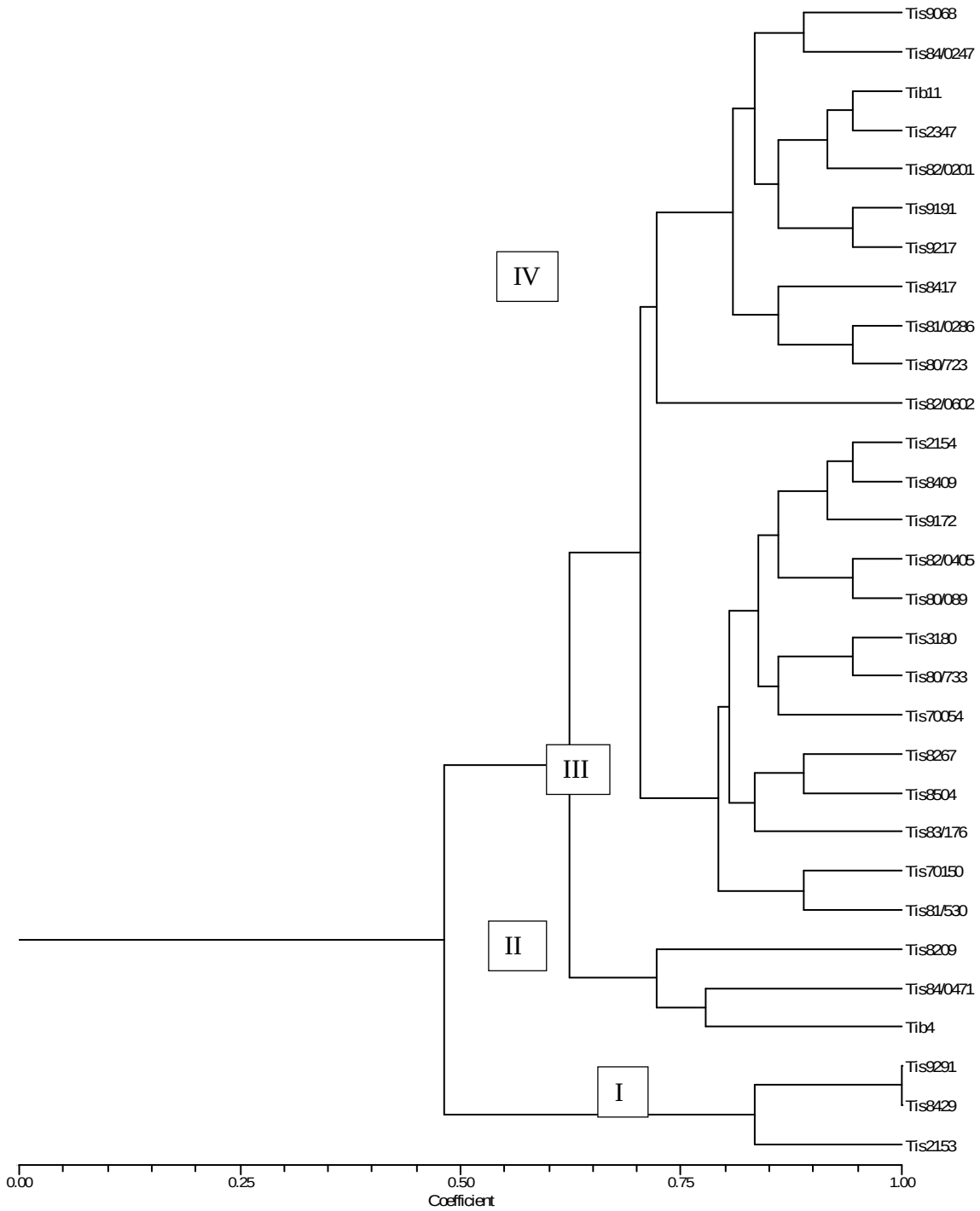

Figure 2. Dendrogram showing genetic diversity among 30 sweet potato sp as revealed by isozyme markers

Discussion

Sweet potato is one of the most widely grown root crops in Sub-Saharan Africa and in north central Nigeria (Low *et al*., 2009). Based on morphological characters, high genetic diversity was observed among the sweet potato genotypes examined. This however, should not be used as absolute indication of genetic

diversity, but other environmental factors as biotic and abiotic stresses and weather conditions may also influenced results in subsequent experiments. The reports by (Yada *et al*., 2010 and Morakinyo and Ajibade, 1998) corroborated the above observations.

Three major morphological clusters were observed and cluster I had predominant pink storage root surface colour, varied cream to white cortex colour and pink pulp colour for the Tib 4 and 11, these characters could be used as markers in genetic studies of these species. In cluster II, the pulp colour of the storage root is cream. Genotypes in this cluster could serve as parents in population improvement for high carotene content in sweet potato. Beta carotene rich cream fleshed sweet potato is reported to be an excellent source of provitamin A and may play a role in controlling vitamin A deficiency (van Jaarsveld *et al.,* 2005). In cluster II, the leaves were highly toothed, which was the major distinguishing morphological characters from cluster I (Fig.1 and Table 2).This toothing ability could serve as a marker for genetic studies in the breeding programme. Cluster III exhibiting different morphological characters, which could serve as gene pool for population development. There were continuous and overlapping variation, which may point towards the quantitative nature of all morphological characters studied.

Due to global warming resulting to erratic environmental conditions and as well mutation potential in sweet potato, it is very necessary to complement morphological characterization with molecular characterization. The clustering of genotypes based on isozyme electrophoresis data is close to the one obtained using morphological characters. In this study, two of the four isozyme markers examined (AAT and 6-PGD) were effective to discriminate sweet potato genotypes. Kennedy and Thompson (1991) reported shikimate dehydrogenase (SKDH) and 6-phosphogluconate dehydrogenase (6-PGD) as the effective discriminant markers, but used different buffer systems and sources of germplasm. Efisue (2013) earlier reported four effective isozyme markers that were good discriminant for cassava, which included AAT and 6-PGD. The isozyme 6-PGD differentiated the genotypes without any overlap, thus confirming the high genetic variability among sweet potato clones. There was overlap both in morphological and isozyme clusters, but the morphological characters revealed more clusters than the electrophoretic isozyme markers and insignificant correlation between the isozyme and morphological characters observed, could be due to high discriminating ability of the two isozyme markers used, this was also observed by Tairo *et al*.(2008). However, Maquia *et al.* (2013) reported high correlation between morphological and molecular data, but with different enzymes systems.

Conclusion

Sweet potato is an important staple crop in Nigeria and research to its genetic diversity will be useful to the breeding and improvement of the crop. The two isozyme markers Aspartate amino transferase (AAT) and 6-phosphogluconate dehydrogenase (6-PGD) were the most effective discriminant of sweet potato for genetic diversity study in this experiment. The three morphological clusters and four from isozymes analysis indicated some peculiar agronomic traits of the genotypes, which could be used for population improvement of the crop.

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