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Genetic Diversity and DNA Barcoding of Yam Accessions from Southern Nigeria

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Abstract

Knowledge of genetic diversity and barcoding of yam is lacking in Enugu and Ebonyi States of southern Nigeria. Therefore, DNA barcoding was used to facilitate identification and biodiversity studies of yam species from Southern Nigeria. Seventy five yam accessions were collected from Enugu and Ebonyi States, including International Institute of Tropical Agriculture for DNA extraction and amplification using a chloroplast DNA (cpDNA) ribulose-1,5-bisphosphate carboxylase (*rbc*L) marker. There was high level of similarity among the accessions and presence of 534 conserved and 7 variable sites. A transversional mutation of G/T at a consensus position of 335 was identified followed by transitions at 362 (A/G), 368 (A/G), 371 (C/T) and 391 (C/T) within the accessions. Phylogeny resolved the yam accessions into ten major groups with their bootstrap values ranging from 0 - 100. Phylogenetic diversity was highest in group X, followed by VII, VI and IX. The inter-group genetic distance based on Kimura 2-parameter model ranged from 0.5000 ± 0.4770 - 5.0560 ± 2.5760, while the intra-group had 0.5250 ± 0.5000 - 2.0103 ± 1.2579. The mean genetic diversity within the entire population was 0.7970 ± 0.06910. BLAST analysis of total bit score, query coverage, and percentage identity were in the ranges of 411 - 1011, 99% - 100% and 97% - 100%, respectively. However, the *rbc*L could not resolve the yam accessions well following the comparative assessment of some discrepancies in the detected number of species from phylogenetic groupings, genetic diversity indices and NCBI BLAST hits, thereby, exposing the inefficiency of this marker in dis-

criminating the yam accessions. It was demonstrated that *rbc*L is not an effective marker; therefore, it should not be recommended as a standard-alone marker of choice for DNA barcoding of yam accessions, especially, when accurate identification, discrimination and estimation of genetic diversity of this vital crop are of paramount importance for crop improvement and germplasm conservation.

Keywords

BLAST, Kimura 2-Parameter, Phylogenetic Diversity, *rbc*L, Transitional Mutation

1. Introduction

Yam (*Dioscorea* spp.) is a monocotyledonous, an annual or perennial stem tuber belonging to the family Dioscoreaceae of flowering plants. *Dioscorea* has been described as the largest genus with an estimated 600 species, 10 of which are cultivated and of economic importance [1] [2] [3]. It is the second most important crop after cassava in West Africa [4] [5] [6]. Important and cultivatable species of this vital crop include *D. cayenensis* Lam., *D. alata* L., *D. rotundata* Poir., *D. trifida* L. f., *D. bulbifera* L., *D. pentaphylla* L., *D. opposita* Thunb., *D. transversa* R. Br., *D. nummularia* Lam. and *D. esculenta* (Lour.) Burkill. [7]. Within Africa, the common species cultivated include *D. rotundata* (white yam), *D. alata* (water yam) and *D. cayenensis* (yellow yam), some of which have been reported to possess medicinal and ornamental values [8].

The crop ranks fourth after potato, sweet potato and cassava as the most important food tuber crop in the world [6]. Yam is important in the economic and social life of people in West Africa [9] [10]. As a starchy food, it provides a major source of cheap caloric energy food for millions of people in the tropical and sub-tropical regions of the world particularly in West Africa, the Caribbean, parts of Asia, South and Central America and the Pacific [8] [11]. Yam tubers are rich sources of energy, vitamin C, musin (glycoprotein), minerals (K, P, Ca, Mg, Fe, Cu, Co), phytosterols and steroidal saponins [12]. They are converted into different types of food products such as pounded yam, boiled yam, roasted yam, fried yam slices, yam balls, mashed yams, yam chips, and yam flakes [13]. Fresh yam tubers are also peeled, chipped, dried, and milled into flour that is used to prepare dough called "amala" or "telibowo" [14].

Yams are widespread in the tropics and subtropics. Nigeria is the leading producer of yam with 71% of the world production [15] [16] [17]. West Africa accounts for over 92% of the world's production (54.2 million tonnes) [6]. In Ghana and Nigeria, 26.2% and 31.8% of people, respectively rely on yam species for income generation and food security [4]. Despite the increasing demand for local consumption and export of yams, there has been a marginal decline in its production due to lack of proper identification of unique species for biodiversity

diversification for resistance to drastic changes in climate, introgression, cross hybridization and conservation processes to reduce genetic erosion [18]-[24]. To have adequate knowledge of these yam accessions, characterization to the species level, genetic richness and assessment of phylogenetic diversity (PD) are of utmost importance following the genetic resource preservation roles of PD in crop extinction [25], functionality in ecosystems [26] [27] and abiotic variability [28] and these can be achievable with accurate, sensitive and reliable methods.

Morphotaxonomy, the use of morphological characters to identify and classify plants, is currently the most widely used in yams in Nigeria. It entails using traits such as size, form and number of tubers per plant, bulbil formation, presence of spines on the stem, twining direction, fruit shape, and aerial bulbils, which could lead to misidentification of yam species [1] [3] [29]. Further, morphotaxonomy-based method requires cumbersome assessment of whole plants and the importance of this approach declines when specimens/tissue materials are utilized [30]. Use of molecular markers has become significant for accurate identification of these yams to the species level and to harness the genetic diversity inherent in them. Different markers including Restriction fragment length polymorphism (RFLP) [31], Random amplified polymorphic DNA (RAPD) [32], Simple sequence repeat (SSR) [32], Inter-simple repeat (ISSR) [12] and Amplified fragment length polymorphism (AFLP) [32] and gene sequencing [33] [34] have been applied in the characterization of yam species. The use of molecular tools to support morphotaxonomy-based identification is important to clear ambiguous species classification.

A DNA barcode facilitates taxonomic identification through the use of a standardized short genomic segment that is generally found in target lineages with adequate variations capable of discriminating living animals to the species level [35]. DNA barcoding techniques are useful tools in characterization as they allow more objective and rapid specimen identification, which can be cost-effective in providing a central catalog of species diversity. In general, DNA barcoding can improve biodiversity and genetic resource databases [36] [37]. Also, a phylogenetic diversity (PD) method possesses the merits of ease of reconstruction of phylogenetic relationships of species and as such it has resultant potential to enlighten effective taxonomic challenges [38]. MatK and rbcL which are the two plant barcode loci have been chosen for phylogenetic studies of *Dioscorea* [1] [39]. In this study, a barcoding marker of rbcL was used for identification and genetic characterization of *Dioscorea* accessions cultivated in southern Nigeria.

2. Materials and Methods

2.1. Sample Collection

Different yams were sampled from different locations across Eastern and Western Nigerian, including the ones in the germplasm collection at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria (**Table 1**). A total of

Table 1. List of yam samples collected from different locations and used for DNA barcoding.

Sample IDs	Location	LGA	State
1_TDa85.00250	IITA	Akinyele	Oyo
3_TDa3050	IITA	Akinyele	Oyo
4_TDb3050	IITA	Akinyele	Oyo
5_TDb3044	IITA	Akinyele	Oyo
6_TDb2857	IITA	Akinyele	Oyo
7_TDb3058	IITA	Akinyele	Oyo
8_TDb3690	IITA	Akinyele	Oyo
9_TDd3101	IITA	Akinyele	Oyo
10_TDd3829	IITA	Akinyele	Oyo
11_TDd3935	IITA	Akinyele	Oyo
12_TDd08-38-53	IITA	Akinyele	Oyo
13_TDdYellow	IITA	Akinyele	Oyo
14_TDd3100	IITA	Akinyele	Oyo
15_TDc0471-2	IITA	Akinyele	Oyo
16_TDc0497-4	IITA	Akinyele	Oyo
17_TDc2813	IITA	Akinyele	Oyo
18_TDc2796	IITA	Akinyele	Oyo
19_TDc2792	IITA	Akinyele	Oyo
20_TDc03-5	IITA	Akinyele	Oyo
21_TDc04-71-2	IITA	Akinyele	Oyo
22_TDm2938	IITA	Akinyele	Oyo
23_TDm3053	IITA	Akinyele	Oyo
24_TDm3052	IITA	Akinyele	Oyo
25_TDm3055	IITA	Akinyele	Oyo
27_TDes3035	IITA	Akinyele	Oyo
28_TDes3033	IITA	Akinyele	Oyo
29_TDes 3027	IITA	Akinyele	Oyo
30_TDes 3030	IITA	Akinyele	Oyo
31_TDesculenta	IITA	Akinyele	Oyo
33_TDaNwokporo	IITA	Akinyele	Oyo
34_Adakavariety	IITA	Akinyele	Oyo
35_Pepa	IITA	Akinyele	Oyo
36_Ke-emi	IITA	Akinyele	Oyo
37_Ame	IITA	Akinyele	Oyo
38_TDr 89.002665	IITA	Akinyele	Oyo
39_AlataTda 98.01176	IITA	Akinyele	Oyo
40_TDa00.00.94 41_Alata	IITA	Akinyele	Oyo
41_Tda00.00600	IITA	Akinyele	Oyo

Continued

42_OgojaVariety.1	IITA	Akinyele	Oyo
43_Gbangu_Variety.1	IITA	Akinyele	Oyo
44_ObioturuguVariety.1	IITA	Akinyele	Oyo
45_AmolaVariety .1	IITA	Akinyele	Oyo
46_OginiVariety	IITA	Akinyele	Oyo
47_Damieha	IITA	Akinyele	Oyo
48_Aloshivariety.1	IITA	Akinyele	Oyo
49_IghuUna	Osonu	Ezeagu	Enugu
51_Alata2	Osonu	Ezeagu	Enugu
52_Ighu_Dumenturum	Osonu	Ezeagu	Enugu
53_Ighu	Osonu	Ezeagu	Enugu
54_IghuUna.2	Osonu	Ezeagu	Enugu
57_2-WhiteYam-Iyo	Ukaka Ngwo	Enugu North	Enugu
59_D10WhiteNwopoko-Adaka	Agbalenyi Nachi	Oji-River	Enugu
60_D1Water-Nbana1	Agbalenyi Nachi	Oji-River	Enugu
61-6-EDO	Ukaka Ngwo	Enugu North	Enugu
62_3LeavedYam-Ono	Ukaka Ngwo	Enugu North	Enugu
65_WaterYam.Nbana	Ukaka Ngwo	Enugu North	Enugu
68_9ENEGBE	Ndibinagu Umuaga	Udi	Enugu
71_D1WaterYam-Nbana2	Agbalenyi Nachi	Oji-River	Enugu
72_1-Water_Yam-_ Nbana	Ndibinagu Umuaga	Udi	Enugu
73_Water yamji_mbala	Nkalagu	Ishielu	Ebonyi
76_OnaTDd	Ezzamgbo	Ohaukwu	Ebonyi
78_Obella	Ezzamgbo	Ohaukwu	Ebonyi
80_UtekpeVariety_2	Ezzamgbo	Ohaukwu	Ebonyi
81_WhiteYam-Nw-opoko	Amaeke Amaigbo Ozalla	Nkanu West	Enugu
82_Yellowyam_Akpukpu	Amaeke Amaigbo Ozalla	Nkanu West	Enugu
83_WaterYam-Mbuna	Amaeke Amaigbo Ozalla	Nkanu West	Enugu
84_BitterYam-Iwu_obe	Amaeke Amaigbo Ozalla	Nkanu West	Enugu
85_AerialYam_Edugbe	Amaeke Amaigbo Ozalla	Nkanu West	Enugu
86_3LeavedYam_Ona	Ede Oballa	Nsukka	Enugu
87_WaterYam-Mbana	Nru	Nsukka	Enugu
89_WhiteYam_Nwopoko	Ibagwa Aka	Igboeze South	Enugu
90_Yellowyam_Oku	Ihe Owerre	Nsukka	Enugu
91_TrifoliateYam_TDb	Ukana	Udi	Enugu
92_ChineseYam_TDes	Ukana	Udi	Enugu
93_YellowYam_TDes	Ukana	Udi	Enugu

IITA = International Institute of Tropical Agriculture; LGA = Local Government Area.

eleven Local Government Areas (LGAs), cutting across three States including Oyo (where IITA, Ibadan is located), Enugu and Ebonyi States were used for the yam collection (Figure 1). The IITA, Ibadan, has Genetic Resources Unit that contains many yam species from other parts of Nigeria.

2.2. DNA Extraction

Fresh young leaves of yam species weighing from 0.1 - 0.2 g were collected for DNA extraction using Silica resin method standardized by the DNA Learning Center (http://www.dnabarcoding101.org/lab/protocol-2.html) [40] In brief, fresh young yam leaf samples were weighed and homogenized in 300 µL of lysis solution using sterile mortar and pestle followed by incubation in a heat block at 65˚C for 10 minutes. Next, samples were centrifuged in a balanced configuration at maximum speed (13,000 rev/min) for 1 min to pellet debris. A 150 µL sample of the supernatant was transferred to fresh micro centrifuge tubes, being careful not to disturb the debris pellet. A 3 µL silica resin, was subsequently added to the respective supernatants, mixed well by pipetting up and down, and placed for 5 minutes in a heat block at 57˚C. The silica resin is a DNA binding matrix which in the presence of lysis solution binds readily to nucleic acids. After incubation, tubes were subject to centrifugation, with cap hinges pointing outward, for 30 seconds at maximum speed to pellet the silica resin, which was now bound to nucleic acid. Using a micropipette with fresh tip the supernatant was removed

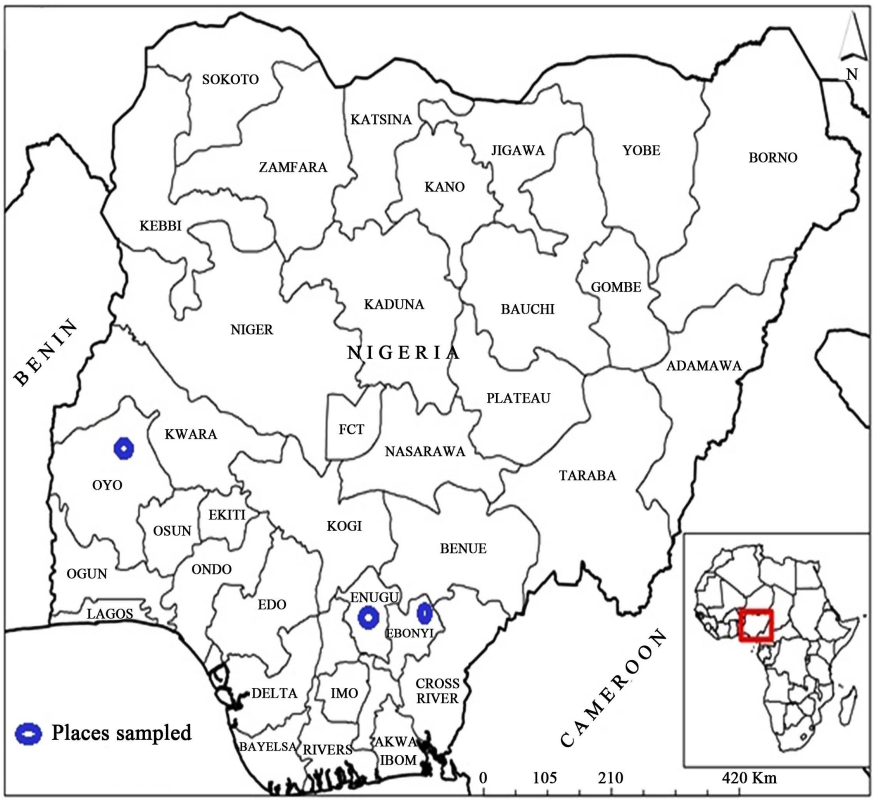

Figure 1. Map of Nigeria showing geographical areas for collection of yam accessions.

and 500 µL of ice cold wash buffer added to the pellet. The silica resin bound to nucleic acid was re-suspended by vortexing and centrifuged to repeat the wash procedure. The wash buffer removes contaminants from the samples while nucleic acids remain bound to the resin. A dry spin step after wash was performed to remove any remnant drops of supernatant with a micropipette. Finally, 100 µL of distilled water was added to the silica resin, mixed well by vortexing and incubated at 57˚C for 5 minutes. Samples were then centrifuged for 30 seconds at maximum speed to pellet the resin. This time 90 µL of the supernatant was transferred to fresh tubes as the nucleic acids eluate from the resin. The eluted DNA was stored to proceed to PCR step.

2.3. Polymerase Chain Reaction, Agarose Gel Electrophoresis and DNA Sequencing

PCR amplification was performed using Ready-To-Go PCR beads in a total volume of 25 µL: 2 µL of ~100 ng DNA and 23 µL of primer/loading dye mix for plant cocktail with rbcL primers (rbcLaf: 5'-TGTAAAACGACGGCCAGTATGTCACCACAAACAGAGACTAAAGC-3' and rbcLa-revM13: 5'-CAGGAAACAGCTATGACGTAAAATCAAGTCCACCRCG-3'). The PCR tubes were placed in a thermal cycler that had been programmed with the appropriate PCR protocol with initial step at 94˚C for 1 min., 35 cycles of 94˚C for 15 sec, 54˚C for 15 sec, and 72˚C for 30 sec., and 8 min final extension at 72˚C was maintained. The PCR products or amplicons were electrophoresed in a 1.5% agarose gel containing 0.5 mg/ml ethidium bromide and photographed on Transilluminator UV light (Omega G). The generated PCR amplicons sent to Genewiz LLC, New Jersey, USA, for DNA sequencing.

2.4. Data Analysis

The sequencing results generated from the Applied Biosystems Genetic automated sequencer (ABI Prism 3130X1, Froster City, CA 94404, USA) at Genewiz LLC were uploaded in the blue line of *DNA Subway* (https://dnasubway.cyverse.org/), which is an intuitive interface for analysing DNA barcodes. Using the Blue Line, the assembled sequences were end-trimmed, paired in their respective forward and reverse sequences to build consensus sequences. The consensus sequences from DNA subway were further edited, filtered and assembled for polymorphism detection using BioEdit software (BioEdit sequence aligner editor, version 7.6.2.1). Sequence alignment and percentage similarity searches were compared with GeneBank databases using NCBI web-based site, BLAST. Multiple alignments were done using the ClustalW [41] [42]. Phylogenetic tree reconstruction was performed using MEGA 6 software [43]. Phylogenies were constructed using the Maximum Parsimony and Maximum Likelihood options [44] [45] and the effectiveness of the trees was determined by bootstrapping up to 1000 replicates [46].

3. Results

3.1. Sequence Alignment of Sequences Generated from *Dioscorea* Spp. Using rbcL Barcoding Marker

A total length of sequence alignment, conserved sites, and variable sites of 525, 534 and 7 were respectively identified among the sequenced yam species. Different regions of polymorphisms and conserved regions at nucleotide level across the sequences exhibited variations among them. At a position of 335, 62_3LeavedYam_Ono and 76_Ona_TDd possessed a transversional mutation by having G nucleotide, while other samples had a T nucleotide (Additional file 1: Figure S1). At a consensus position of 362, yam species such as 3_TDa3050, 4_TDb3050, 5_TDb3044, 6_TDb2857, 7_TDb3058, 8_TDb3690, 61_6-EDO, 83_WaterYam-_Mbuna and 85_AerialYam_Edugbe showed a transitional mutation of A nucleotide, while the rest of the accessions had a G nucleotide. At a position of 368, accessions such as 1_TDa85.00250, 3_TDa3050, 4_TDb3050, 5_TDb3044, 6_TDb2857, 7_TDb3058, 8_TDb3690, 61_B-6-EDO, 83_WaterYam-_Mbuna, 85_AerialYam_Edugbe had a transitional mutation of A in place of G nucleotide possessed by other accessions at the same consensus position.. Also at 371 position, accessions including 35_Pepa, 36_Ke-emi, 37_Ame, 38_TDr.89.002665, 39_AlataTda98.01176, 40_TDa00.00.94, 42_OgojaVariety.1, 43_Gbangu_Variety.1, 44_ObioturuguVariety, 45_AmolaVariety.1, 46_OginiVariety, 47_Damieha, 48_Aloshivariety.1, 57_2-WhiteYam_Iyo, 61_6-EDO, 68_9ENEGBE, 78_Obella, 80_UtekpeVariety_2, 81_WhiteYam-Nwoopoko, 82_Yellowyam_Akpukpu, 83_WaterYam-Mbuna, 85_AerialYam_Edugbe, 89_WhiteYam_Nwoopoko and 93_YellowYam_TDes exhibited a transitional mutation by possessing a C nucleotide, while the remaining species had a T nucleotide. Also at a position of 391, 76_Ona_TDd possessed C, while other remaining yam species had T nucleotide.

3.2. Phylogenetic Tree Reconstruction (PTR) and Phylogenetic Diversity (PD)

Out of the 75 nucleotide sequences used for the analyses, a total of 270 codon positions including 1st, 2nd, 3rd, and non-coding regions as well as 4.3582% invariable (monomorphic) sites were found in the final dataset. From the phylogenetic tree analysis, the yam accessions were resolved into ten groups with variable phylogenetic diversities (PDs) (Figure 2). Group I with PD in the range of 0-27 consisted of twenty five accessions including 43_Gbangu_variety, 82_Yellowyam-Akpukpu, 81_Whiteyam-Nwopoko, 89_WhiteYam-Nwopoko, 24_TDm3052, 23_TDm3053, 20_TDc03-5, 19_TDc2792, 80_Utekpevariety, 17_TDc2813, 21_TDc04-71-2, 93_Yellowyam-TDes, 18_TDc2796, 68_9ENEGBE, 25_TDm3055, 15_TDc0471-2, 46_Oginivariety, 57_2-Whiteyam-Iyo, 45_Amolavariety, 40_TDa00.00.94, 38_TDr89.002665, 16_TDc0497-4, 78_Obella, 37_Ame and 35_Pepa grouping with *D. rotundata* obtained from NCBI data with a reference sequence accession of KR072483. The yam accessions were

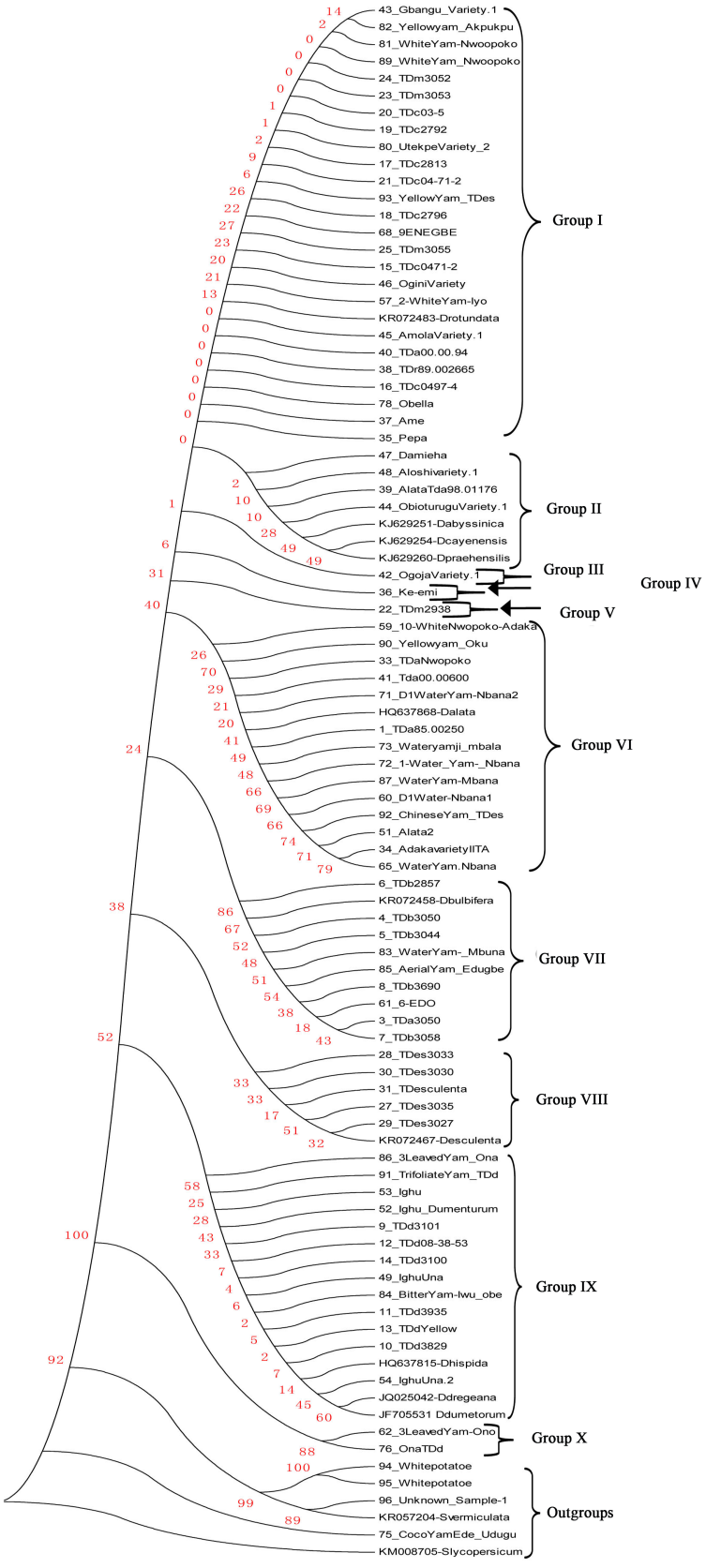

Figure 2. Phylogenetic tree of different yam species as revealed by rbCL barcoding marker.

collected from different locations including Enugu, Ebonyi and International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Group II (with PD of 2-49) contained four accessions such as 47_Damieha, 48_Aloshivariety, 39_Alata TDa98-01176, 44_Obioturuguvariety grouped together KJ629251-*D. abyssinica*, KJ629254-*D. cayenensis* and KJ629260-*D. praehensillis*. Group III (with PD of 1) contained only 42_Ogojavariety.

Groups IV (PD = 6) and V (PD = 31) had 36_Ke-emi and 22_TDm2938, respectively. Group VI (PD = 20 - 79) consisted fourteen including 59_10-Whiteyam-Nwopoko-Adaka, 90_YellowYam-Oku, 33_TDaNwopoko, 41_TDa00.00600, 71_D1WaterYam-Nbana2, 1_TDa85.00250, 73_WaterYam-Mbala, 72_1WaterYam-_Nbana, 87_WaterYam-Mbana, 60_D1WaterYam-Nbana 1, 92_ChineseYam-TDes, 51_Alata2, 34_AdakavarietyIITA, and 65_WaterYam-Nbana that grouped together with *D. alata* retrieved from NCBI database with an accession number of HQ637868. Group VII with PD value in the range of 18-86, had nine accessions including 6_TDb2857, 4_TDb3050, 5_TDb3044, 83_WaterYam-_Mbana, 85_AerialYam- Edugbe, 8_TDb3690, 61_6-Edo, 3_TDa3050 and TDb3058 grouped together with a known *D. bulbifera* species (with an accession No: KR072458) that was retrieved from NCBI database. Yam accessions 28_TDes3033, 30_TDes3030, 31_TDesculenta, 27_TDes3035 and 29_TDes3027 were in the same group VIII (PD = 17 - 51) identified as *D. esculenta* using a reference of *D. esculenta* (KR072467) obtained from the NCBI database. In group IX (PD = 2 - 60), 86_3leavedYam-Ona, 91_TrifoliateYam-TDd, 53_Ighu, 52_Ighu-*Dumenturum*, 9_TDd3101, 12_TDd08-38-53, 14_TDd3100, 49_IghuUna, 84_BitterYam-Iwu-obe, 11_TDd3935, 13_TDd-yellow, 10_TDd3829 and 54_Ighu-Una-2 were found grouping with *D. hispicia* (HQ637815), *D. dregeana* (JQ025042) and *D. dumenturum* (JF705531). Group X with PD of 88 had only 62_3leavedYam-Ono and 76_Ona-TDd, while outgroups (PD = 89 - 100) contained two *Ipomoea triloba* (trilobed (white potatoes), *Colocasia esculenta* (taro) *(*cocoyam) and *Coccinia quinqueloba* (96_unknown_sample) grouped together with *Solanum vermiculata* and *S. lycopersicum* with NCBI accession numbers KR057204 and KM008705, respectively.

3.3. Genetic Diversity Analysis

The analysis involved 75 nucleotide sequences between different groups. The highest inter-group genetic distance calculated based on K2P was 5.0560 ± 2.5760, while the lowest was 0.5000 ± 0.4770 (Table 2). The increment in genetic diversity started from the group combinations in ascending order: 0.5000 ± 0.4770 (**groups**, gps: I and II, I and III, I and IV, I and V) < 0.6700 ± 0.5500 (gps: II and VI, III and VI, V and VI) < 0.7510 ± 0.4240 (gps: II and IX) < 0.8100 ± 0.5500 (gps: III and IX, V and IX) < 0.8820 ± 0.5550 (gps: I and VI) < 0.9210 ± 0.4970 (gps: I and IX) < 1.0090 ± 0.9870 (gps: IV and VI) < 1.1390 ± 0.9170 (gps: IV and IX) < 1.2540 ± 0.6540 (gps: II and VIII) < 1.2770 ± 0.6800 (gps: III and VIII) < 1.3810 ± 0.8090 (gps: IV and VIII) < 1.5090 ± 1.4360 (gps: VII and VIII) < 1.5350 ± 0.8200 (gps: I and VIII) < 1.5820 ± 0.8660 (gps: VI and VIII) < 1.9060

Table 2. Genetic distances based on Kimura 2-parameter (K2P) between different groups of yam species.

Species 1	Species 2	Distance	Standard error
Group I	Group II	0.500	0.477
Group I	Group III	0.500	0.477
Group I	Group IV	0.500	0.477
Group I	Group V	0.500	0.477
Group I	Group VI	0.882	0.555
Group I	Group VII	3.020	1.704
Group I	Group VIII	1.535	0.820
Group I	Group IX	0.921	0.497
Group II	Group III	n/c	NC
Group II	Group IV	n/c	NC
Group II	Group V	n/c	NC
Group II	Group VI	0.670	0.550
Group II	Group VII	3.020	1.700
Group II	Group VIII	1.254	0.654
Group II	Group IX	0.751	0.424
Group III	Group IV	n/c	NC
Group III	Group V	n/c	NC
Group III	Group VI	0.670	0.550
Group III	Group VII	3.020	1.700
Group III	Group VIII	1.277	0.680
Group III	Group IX	0.810	0.550
Group IV	Group V	n/c	NC
Group IV	Group VI	1.009	0.987
Group IV	Group VII	3.020	1.700
Group IV	Group VIII	1.381	0.809
Group IV	Group IX	1.139	0.917
Group V	Group VI	0.670	0.550
Group V	Group VII	3.020	1.700
Group V	Group VIII	1.277	0.680
Group V	Group IX	0.810	0.550
Group VI	Group VII	5.056	2.576
Group VI	Group VIII	1.582	0.866
Group VI	Group IX	1.906	0.814
Group VI	Group X	2.276	1.792
Group VII	Group VIII	1.509	1.436
Group VII	Group IX	3.020	1.676

Continued

Group IX	Group VIII	2.456	1.458
Group VII	Group X	3.107	2.390
Group IX	Group X	1.746	1.479
Group I	Group X	2.718	2.076
Group V	Group X	2.790	2.130
Group VIII	Group X	1.664	1.491
Group IV	Group X	2.790	2.130
Group II	Group X	2.790	2.130
Group III	Group X	2.790	2.130

Group I = 43_Gbangu_variety, 82_Yellowyam-Akpukpu, 81_Whiteyam-Nwopoko, 89_Whiteyam-Nwopoko, 24_TDm3052, 23_TDm3053, 20_TDc03-5, 19_TDc2792, 80_Utekpevariety, 17_TDc2813, 21_TDc04-71-2, 93_Yellowyam-TDes, 18_TDc2796, 68_9ENEGBE, 25_TDm3055, 15_TDc0471-2, 46_Oginivariety, 57_2-Whiteyam-Iyo, 45_Amolavariety, 40_TDa00.00.94, 38_TDr89.002665, 16_TDc0497-4, 78_Obella, 37_Ame and 35_Pepa; **Group II** = 47_Damieha, 48_Aloshivariety, 39_Alata TDa98-01176, 44_Obioturuguvariety; **Group III** = 42_Ogojavariety; **Group IV** = 36_Ke-emi; **Group V** = 22_TDm2938; **Group VI** = 59_10-Whiteyam-Nwopoko-Adaka, 90_YellowYam-Oku, 33_TDaNwopoko, 41_TDa00.00600, 71_D1WaterYam-Nbana2, 1_TDa85.00250, 73_WaterYam-Mbala, 72_1WaterYam-_Nbana, 87_WaterYam-Mbana, 60_D1WaterYam-Nbana 1, 92_ChineseYam-TDes, 51_Alata2, 34_AdakavarietyIITA, and 65_YaterYam-Nbana; **Group VII** = 6_TDb2857, 4_TDb3050, 5_TDb3044, 83_WaterYam-_Mbana, 85_AerialYam-Edugbe, 8_TDb3690, 61_6-Edo, 3_TDa3050 and TDb3058; **Group VIII** = 28_TDes3033, 30_TDes3030, 31_TDesculenta, 27_TDes3035 and 29_TDes3027; **Group IX** = 86_3leavedYam-Ona, 91_TrifoliateYam-TDd, 53_Ighu, 52_Ighu-*Dumenturum*, 9_TDd3101, 12_TDd08-38-53, 14_TDd3100, 49_IghuUna, 84_BitterYam-Iwu-obe, 11_TDd3935, 13_TDd-yellow, 10_TDd3829 and 54_Ighu-Una-2; and **Group X** = 62_3leavedYam-Ono and 76_Ona-TDd, **N/C** = Not computable.

± 0.8140 (gps: VI and IX) < 1.6640 ± 1.4910 (gps: VIII and X) < 1.7460 ± 1.4790 (gps: 2.2760 ± 1.7920 (gps: VI and X) < 2.4560 ± 1.4580 (gps: VIII and IX) < 2.7180 ± 2.0760 (gps: I and X) < 2.7900 ± 2.1300 (gps: II and X, III and X, IV and X, V and X) < 3.0200 ± 1.7000 (gps: I and VII, II and VII, III and VII, IV and VII, V and VII, VII and IX) < 3.1070 ± 2.390 (gps: VII and X) < 5.0560 ± 2.5760 (gps: VI and VII). The intra-group genetic distance ranged from 0.5250 ± 0.5000 - 2.0103 ± 1.2579 and some intra-group genetic distances were not computable which were denoted by n/c (Table 3). Groups I, VI and X were found computable with their respective values of 0.5250 ± 0.5000, 0.5616 ± 0.4788, and 2.0103 ± 1.2579. The mean genetic diversity within entire population was 0.7970 ± 0.06910, while the transitional to transversional distances per site from mean interpopulational diversity calculations was $2.1478 \times 10^8 \pm 4.5300$. Also, the coefficient of differentiation of transitional to transversional distances per site was $1.1947 \times 10^8 \pm 6.9419 \times 10^7$.

3.4. BLAST Analysis of the Sequences Generated from the Yam Accessions Using rbcL Barcoding Gene

The output of the BLAST computations of the grouped sequences produced significant hits and some of the previously unknown sequences were fully identified (Table 4). The analysis identified ten putative species of yams including *Dioscorea alata*, *D. bulbifera*, *D. cayenensis*, *D. rotundata*, *D. wallichii*, *D. aspersa*,

Table 3. Genetic distances based on Kimura 2-parameter (K2P) within different groups of yam species.

Group name	Distance	Standard error
Group I	0.5250	0.5000
Group II	n/c	n/c
Group III	n/c	n/c
Group IV	n/c	n/c
Group V	n/c	n/c
Group VI	0.5616	0.4788
Group VII	n/c	n/c
Group II	n/c	n/c
Group III	n/c	n/c
Group X	2.0103	1.2579

Group I = 43_Gbangu_variety, 82_Yellowyam-Akpukpu, 81_Whiteyam-Nwopoko, 89_Whiteyam-Nwopoko, 24_TDm3052, 23_TDm3053, 20_TDc03-5, 19_TDc2792, 80_Utekpevariety, 17_TDc2813, 21_TDc04-71-2, 93_Yellowyam-TDes, 18_TDc2796, 68_9ENEGBE, 25_TDm3055, 15_TDc0471-2, 46_Oginivariety, 57_2-Whiteyam-Iyo, 45_Amolavariety, 40_TDa00.00.94, 38_TDr89.002665, 16_TDc0497-4, 78_Obella, 37_Ame and 35_Pepa; **Group II** = 47_Damieha, 48_Aloshivariety, 39_Alata TDa98-01176, 44_Obioturuguvariety; **Group III** = 42_Ogojavariety; **Group IV** = 36_Ke-emi; **Group V** = 22_TDm2938; **Group VI** = 59_10-Whiteyam-Nwopoko-Adaka, 90_YellowYam-Oku, 33_TDaNwopoko, 41_TDa00.00600, 71_D1WaterYam-Nbana2, 1_TDa85.00250, 73_WaterYam-Mbala, 72_1WaterYam-_Nbana, 87_WaterYam-Mbana, 60_D1WaterYam-Nbana 1, 92_ChineseYam-TDes, 51_Alata2, 34_AdakavarietyIITA, and 65_YaterYam-Nbana; **Group VII** = 6_TDb2857, 4_TDb3050, 5_TDb3044, 83_WaterYam-_Mbana, 85_AerialYam-Edugbe, 8_TDb3690, 61_6-Edo, 3_TDa3050 and TDb3058; **Group VIII** = 28_TDes3033, 30_TDes3030, 31_TDesculenta, 27_TDes3035 and 29_TDes3027; **Group IX** = 86_3leavedYam-Ona, 91_TrifoliateYam-TDd, 53_Ighu, 52_Ighu-*Dumenturum*, 9_TDd3101, 12_TDd08-38-53, 14_TDd3100, 49_IghuUna, 84_BitterYam-Iwu-obe, 11_TDd3935, 13_TDd-yellow, 10_TDd3829 and 54_Ighu-Una-2; and **Group X** = 62_3leavedYam-Ono and 76_Ona-TDd.

Table 4. BLAST outputs of total score, query coverage, e-value, percentage identity and accession number obtained from different yam accessions.

Sequence name	Hit in NCBI database	Total score	Query coverage	E-value	%Identity	Accession No
1_TDa85.00250	*Dioscorea alata*	852	852	0	100	KY710782
3_TDa3050	*D. bulbifera*	736	736	0	100	KR087030
4_TDb3050	*D. bulbifera*	771	100	0	100	KR087030
5_TDb3044	*D. bulbifera*	756	100	0	100	KR087030
6_TDb2857	*D. bulbifera*	839	100	0	100	KR087030
7_TDb3058	*D. bulbifera*	826	100	0	99	KR087030
8_TDb3690	*D. bulbifera*	737	100	0	100	KR087030
9_TDd3101	*D. dregeana*	1009	100	0	100	KR087039
10_TDd3829	*D. dregeana*	985	100	0	100	KR087039.1
11_TDd3935	*D. dregeana*	996	100	0	100	KR087039.1
12_TDd08-38-53	*D. dregeana*	1005	100	0	100	KR087039.1
13_TDdYellow	*D. dregeana*	996	100	0	100	KR087039.1
14_TDd3100	*D. dregeana*	1003	100	0	100	KR087039.1
15_TDc04-71-2	*D. wallichii*	835	100	0	100	MF142259.1
16_TDc04-97-4	*D. cayenensis*/*rotundata*	1005	100	0	100	KJ629254.1/ KJ490011.1
17_TDc2813	*D. rotundata*	743	100	0	100	KY679568.1

Continued

18_TDc2796	*D. rotundata*	715	100	0	100	KY679568.1
19_TDc2792	*D. wallichii*/ *rotundata*	739	100	0	100	MF142259.1/ KY679568.1
20_TDc03-5	*D. rotundata*	739	100	0	100	KY679568.1
21_TDc04-71-2	*D. rotundata*	758	100	0	100	KY679568.1
22_TDm2938	*D. cayenensis*/ *rotundata*	1002	100	0	100	KJ629254.1/ KJ490011.1
23_TDm3053	*D. rotundata*	739	100	0	100	KY679568.1
24_TDm3052	*D. rotundata*	739	100	0	100	KY679568.1
25_TDm3055	*D. wallichii*/ *rotundata*	824	100	0	100	MF142259.1/ KY679568.1
27_TDes3035	*D. esculenta*	941	100	0	100	KJ956696.1
28_TDes3033	*D. esculenta*	828	100	0	100	KJ956696.1
29_TDes3027	*D. esculenta*	998	100	0	100	KJ956696.1
30_TDes3030	*D. esculenta*	736	100	0	100	KJ956696.1
31_TDesculenta	*D. esculenta*	734	100	0	100	KJ956696.1
33_TDaNwopoko	*D. alata*	1003	100	0	100	KY710782.1
34_Adakavariety.IITA	*D. alata*	1000	100	0	100	KY710782.1
35_Pepa	*D. cayenensis*/ *rotundata*	1003	100	0	100	KJ629254.1/ KJ490011.1
36_Ke-emi	*D. cayenensis*/ *rotundata*	981	100	0	100	KJ629254.1/ KJ490011.1
37_Ame	*D. cayenensis*/ *rotundata*	1000	100	0	100	KJ629254.1/ KJ490011.1
38_TDr89.002665	*D. cayenensis*/ *rotundata*	1005	100	0	100	KJ629254.1/ KJ490011.1
39_AlataTda_98.01176	*D. cayenensis*/ *rotundata*	1007	100	0	100	KJ629254.1/ KJ490011.1
40_TDa00.00.94	*D. cayenensis*/ *rotundata*	1005	100	0	100	KJ629254.1/ KJ490011.1
41_Tda00.00600	*D. rotundata*	992	100	0	100	KY710782.1
42_OgojaVariety.1	*D. cayenensis*/ *rotundata*	998	100	0	100	KJ629254.1/ KJ490011.1
43_GbanguVariety.1	*D. praehensilis*/ *cayennensis*/ *rotundata*	955	100	0	99	KR072476.1/ KJ629254.1/ KJ490011.1
44_ObioturuguVariety.1	*D. cayenensis*/ *rotundata*	1011	100	0	100	KJ629254.1/ KJ490011.1
45_AmolaVariety.1	*D. cayenensis*/ *rotundata*	1005	100	0	100	KJ629254.1/ KJ490011.1
46_OginiVariety	*D. wallichii*/ *rotundata*	857	100	0	100	MF142259.1/ KY679568.1
47_Damieha	*D. cayenensis*/ *rotundata*	1005	100	0	100	KJ629254.1/ KJ490011.1

Continued

48_Aloshivariety.1	*D. cayenensis/ rotundata*	1007	100	0	100	KJ629254.1/ KJ490011.1
49_IghuUna	*D. dregeana/ hispida*	992	100	0	99	KR087039/ HQ637815.1
51_Alata.2	*D. alata*	736	100	0	100	KY710782.1
52_Ighu_Dumenturum	*D. hispida*	774	100	0	100	KY710783.1
53_Ighu	*D. dumetorum/ hispida*	830	100	0	100	KY710783.1
54_IghuUna.2	*D. hispida/ dumetorum*	756	100	0	100	KY710783.1
57_2-WhiteYam-_Iyo	*D. rotundata*	872	100	0	100	KR072483.1
59_10-WhiteNwopoko-Adaka	*D. spicata/ intermedia/ wallichii/ rotundata/ oppositiflia*	534	100	4.00E−148	100	KY457460.1/ KY457459.1/ KY679569.1/ KY679568.1/ KY679566.1
60_D1Water-Nbana1	*D. alata*	778	100	0	100	KY710782.1
61_6-EDO	*D. bulbifera*	737	100	0	100	KR087030.1
62_3LeavedYam-Ono	*D. aspersa/ petelotii/ daunea*	665	99	0	97	HQ637816.1/ AY904802.1/ AY904793.1
65_WaterYam.Nbana	*D. alata*	730	100	0	100	KY710782.1
68_9ENEGBE	*D. rotundata*	822	100	0	100	KR072483.1
71_D1WaterYam-Nbana2	*D. alata*	989	100	0	100	KY710782.1
72_1-WaterYam-_Nbana	*D. alata*	798	100	0	100	KY710782.1
73_Wateryamji_mbala	*D. alata*	852	100	0	100	KY710782.1
76_OnaTDd	*D. aspersa*	612	100	2.00E−171	97	HQ637816.1
78_Obella	*D. cayenensis/ rotundata*	992	100	0	100	KJ629254.1/ KJ490011.1
80_UtekpeVariety_2	*D. wallichii/ rotundata*	741	100	0	100	MF142259.1/ KY679568.1
81_WhiteYam-Nwoopoko	*D. rotundata*	737	100	0	100	KR072483.1
82_Yellowyam_Akpukpu	*D. rotundata*	806	100	0	100	KR072483.1
83_WaterYam-_Mbuna	*D. bulbifera*	750	100	0	100	KR087030.1
84_BitterYam-Iwu_obe	*D. dregeana/ hispida*	998	100	0	100	KR087039/ HQ637815.1
85_AerialYam_Edugbe	*D. bulbifera*	739	100	0	100	KR087030.1
86_3LeavedYam_Ona	*D. hispida*	861	100	0	100	KU865503.1
87_WaterYam-Mbana	*D. alata*	782	100	0	100	KY710782.1
89_WhiteYam_Nwoopoko	*D. rotundata*	739	100	0	100	KR072483.1
90_Yellowyam_Oku	*D. alata*	963	100	0	100	KY710782.1
91_TrifoliateYam_TDd	*D. hispida*	837	100	0	100	KU865503.1
92_ChineseYam_TDes	*D. alata*	739	100	0	100	KY710782.1
93_YellowYam_TDes	*D. rotundata*	697	100	0	100	KY710782.1

D. trifida, *D. dregeana*, and *D. mangenotiana*. The total bit score obtained in all ranged from 411 - 1011. The query coverage spanned between 99 and 100%, while the expected values (e-values) were $9e^{-111}$ or less. The percentage sequence identity ranged from 97% - 100%. Some accessions with acronyms including TDa, TDc and TDm denoting *D. alata*, *D. cayenensis* and *D. manganotiana* were found to be *D. bulbifera*, *D. rotundata* or *cayenensis*, respectively. Some of the sequences had NCBI hits ranging from two to four sequences with synonymous values of total bit score, query coverage, e-value, percentage identity but different accession numbers. For instance, 16_TDc04-97-4, 22_TDm2938, 35_Pepa, 36_Ke-emi, 37_Ame, 38_TDr.89.002665 and many others in this category had hits of *D. cayenensis* and *D. rotundata*. For accessions of 19_TDc2792, 25_TDm3055, 46_OginiVariety and 80_UtekpeVariety_2 had *D. wallichii* and *D. rotundata* as their hits with similar values in all the BLAST indices. Also, three species of yam including *D. praehensilis*, *D. cayenensis* and *D. rotundata* were obtained with a yam accession of 43_Gbangu_Variety.1 in the process of BLAST analysis, while 62_3LeavedYam-Ono produced *D. aspersa*, *D. petelotii and D. daunea* that had same values of total bit score, query coverage, e-value, percentage identity but different accession numbers. The yam accession, 59_D10 White-Nwopoko-Adaka, had five different NCBI hits of *D. spicata*, *D. intermedia*, *D. wallichii*, *D. rotundata* and *D. oppositifolia* with three having similar accession number, while the remaining two had a separate accession number as revealed by BLAST analysis.

4. Discussion

DNA barcoding has become an effective method for species discrimination of flowering plants in the Polygonaceae [35] [47] and Fabaceae families [39], and other land plant species [35] [42] [48] [49]. While mitochondrial cytochrome oxidase 1 (CO1) has proven a standardized animal DNA barcoding for necessary discrimination, no single barcode sequence works across all plants [49]. In the present work, the candidate barcoding marker, rbcL satisfied the DNA barcoding process, regarding the ease of amplification and sequencing Hollingsworth *et al.* [49]. However, this barcoding marker, rbcL, was not able to achieve the basic quality of discriminating different yam species in this study. Sequence alignment showed low degree of polymorphisms among the sequences. This study of genetic diversity in yam accessions is also dependent on the nucleotide variations occurring within the genome that are informative for the identification of different species. The discriminatory level of the rbcL marker has been linked to other researches, which contradict its potential for use as a universal DNA barcode for plants [50] [51] [52]. This low resolution of different accessions of yams into their respective species level could be attributed to the poor efficiency of rbcL marker when not jointly applied with other plastid markers. It has been reported that the joint application of rbcL+matK as a marker of choice in species resolution was based on clear recovery of the region of rbcL and discriminatory efficiency of fast evolving coding region of matK [53].

In this study, 525 bp distinct total lengths of sequence alignment, 534 conserved sites, and variable sites of 7 were identified in the sequenced yam species. The alignment of 525 bp out of the total lengths of 568 bp, followed by the existence of similar regions (conserved sites) and low points of variations (variable areas) among the sequences demonstrate the low level of informativeness of rbcL in DNA barcoding of yam species. These findings are not in complete agreement with a previous report on yam species [30], where 568 bp, 538, and 30 as total lengths of sequence alignment, conserved sites and variable sites were identified among accessions. Also, the sequence alignment length, conserved sites excluding the variable sites detected in this work correlate with the findings of Sun *et al.* [54] in which 553 bp, 522 bp and 31 of alignment length, conserved sites and variable sites were found among the accessions of *Dioscorea* species. The difference in the variable sites could have emanated from the number of samples studied.

Phylogenetic reconstruction of the generated *Dioscorea* species using rbcL marker resolved them into ten groups and this indicates different existing isolated groups inherent in the accessions. The existence of these different accessions among the collections could be attributable to lack of exchange of yam tubers by farmers among villages thereby resulting in a stronger heterozygosity among species compared to wild ones as reported by Ngo Ngwe *et al.* [24]. A contribution of evolutionary biology regarding conservation is the knowledge of diverse phylogenetic diversities, defined by the sums of branch lengths of the evolutionary trees connecting a set of taxa or individuals [55]. In this present work, group X had the highest PD value of 88, followed by groups VII, VI, IX, and VIII with their respective PDs of 86, 79, 60 and 51. The highest PD was identified in a group containing wild species of *D. aspersa* and this is in agreement with a previous report though in a different wild species wild called *D. praehensilis* [24]. When compared with other unrelated crops, the highest was observed in Cocoyam and other crops which were deliberately included to access the accuracy of this marker. The group with the lowest PD value *D. rotundata* clustered with other species and they were collected from a given single region. In this way, a given set of taxa will have a greater PD if they are widely spread out on a phylogenetic tree. Lack of or total loss of PD is generally assumed as a declining signal in the degree of biodiversity [56]. Furthermore, PD is associated with functional diversity since it is a measure of features also due to the fact that evolutionarily distant species are more likely to possess variable molecular functions in an ecosystem [28] [57] [58]. Also in group I, some accessions including 45_Amolavariety, 40_TDa00.00.94, 38_TDr89.002665, 16_TDc0497.4, 78_Obella, 37_Ame, and 35_Pepa had a PD value of 0 and this could be attributed to lack of sequence divergence. It could also be attributable to occurrence of common ancestral sequence homology [59] or poor resolving power of the rbcL DNA barcoding marker in yams [54]. Most of the accessions were accurately grouped according their species. For instance in group VIII, all the *D. esculenta* species was

grouped with a known reference sequence from NCBI database. Also, group VII had all the accessions classified as *D. bulbifera* thereby identifying correctly an accession, 3_TDa3050, which was regarded as *D. alata*. A particular yam species was given different names as *Ighu* or *Una* (*Ona*) at a village in Enugu State but it was found to be just one species called *D. dumetorum* through the use of rbcL thereby resolving the issue of multiple names for the plant. Group IX had three reference sequences as *D. dregeana*, *D. hispida* and *D. dumetorum* but most of the accessions in the group are D. *dumetorum* and this could be as a result of their genetic relatedness [59]. However, accessions in group I of the trees were not correctly resolved following the existence of different yam species in various distinct subclades and non-grouping of any of the retrieved yam sequences from NCBI database. This may possibly be linked to sample contamination or a deficiency on the part of the rbcL resolution.

The identified genetic distances (0.5000 ± 0.4770 - 5.0560 ± 2.5760) based on K2P model regarding the inter-groups were in agreement with the previous works of other researchers in yams [24] [54] and in authentication of native plants [60]. High genetic diversity indices were obtained from between group calculations, producing 5.0560 ± 2.5760 with the highest in two combined groups (groups VI and VII) and this demonstrates higher interspecific diversity than intraspecific one within the yam accessions as obtained in an earlier report involving ornamental plants with interspecific value of 3.080 [61]. Assessment of genetic diversity within the groups (intra-group genetic diversity) could not be computed in most of the groups except three groups (groups I, VI and X), where group I had the lowest value of 0.5250 ± 0.5000, while X had the highest value of 2.0103 ± 1.2579. These values are higher than the ones obtained by Sun *et al.* [54]. The mean genetic diversity within entire population was 0.7970 ± 0.06910 and this is higher than the one (0.00266 ± 0.0044) obtained by Sun *et al.* [54].

BLAST hits obtained in this study showed some degrees of similarity matches to the ones already annotated and deposited in NCBI database and some were not purely specific. The percentage sequence identity ranged from 97% - 100%, demonstrating low efficiency of this tool in identification of unknowns in yam species. However, some of the yams sampled from different regions were differently identified from what they were previously known to be using this method, indicating the potential of rbcL barcoding marker to resolve misclassification encountered via morphotaxonomy based approach despite the low discriminatory power. For instance, yam accessions with acronyms including TDa, TDc and TDm denoting *D. alata*, *D. cayenensis* and *D. manganotiana* were found to be *D. bulbifera*, *D. rotundata* or *cayenensis*, respectively. Furthermore, 62_3LeavedYam-Ono and 76_Ona_TDd sequences were correctly identified as *D. aspersa*. In the community where the two species (*D. aspersa* and *D. dumetorum*) were collected, they were misclassified by the villagers who generally called them *D. dumetorum* due to their similar morphological features. According to the villagers, the ones in group X which were later identified as *D. aspersa* are normally boiled and eaten directly, while the other ones (*D. dumetorum*,

which had similar values of NCBI hits with *D. hispida*) are usually boiled, processed to remove bitterness in them before they are consumed. The discriminatory level of the rbcL marker in plants as a potential universal DNA barcode is demonstrated in this study as reported in other researches [50] [51]. However, some of the yam sequences had two, three or five NCBI hits of different species of yams with synonymous values of total bit score, query coverage, -value and percentage identity with different accession numbers except in one that had five BLAST outputs with three having similar accession numbers and two with different accession numbers. For instance, the yam accession, 59_D-10-White-Nwopoko-Adaka, had five different NCBI hits of *D. spicata, D. intermedia, D. wallichii, D. rotundata* and *D. oppositifolia* with three (*D. wallichii, D. rotundata, D. oppositifolia)* having similar accession number (KY679569), while the remaining two (*D. spicata and D. intermedia*) had separate accession numbers of KY457460 and KY457459, respectively, after the BLAST analysis. Also, sequences generated from accessions 45_Amolavariety, 40_TDa00.00.94, 38_TDr89.002665, 16_TDc0497.4, 78_Obella, 37_Ame, and 35_Pepa hit two (*D. cayenensis and D. rotundata*) sequences with similar values of query coverage, e-value and percentage identity, while total bit score ranged from 1000-1005. This is possible due to existence of common ancestral homology as opined by Pearson [59] or due to redundancy, which in bioinformatics is observed when one or more homologous or synonymous sequences are found in the same set of data [62]. It could also be attributable to the low discriminatory potency of rbcL marker to correctly resolve species as previously reported in yams [54] and ornamental plants [61].

5. Conclusion

The candidate barcoding marker, rbcL, was found to be ambiguously discriminatory in DNA barcoding process of yam accessions. Some of the accessions were not correctly identified to the species level and low polymorphisms were detected and this further demonstrates the low distinguishing potency of rbcL barcoding marker. The use of phylogenetic diversity (PD), which is associated with functionality in biodiversity and which was applied in the computational processes for the estimation of phylogenetic groups with lowest and largest collections in terms of diversity was of great potential. The highest phylogenetic diversity was in *D. aspersa*, while some were not computable due to the low efficacy of the marker. The group with the lowest PD value, *D. rotundata* clustered with other indistinguishable species and they were collected from a given single region. The accessions with high PD within the yam accessions should be considered for use in breeding programme to enhance biodiversity of *Dioscorea* species within the studied region. However, the rbcL could not resolve the yam accessions well following some noted discrepancies in the detected number of species from phylogenetic groupings and NCBI BLAST hits possibly due to inefficiency of the marker. Therefore, the rbcL may not be a marker of choice for species identification, discrimination and estimation of genetic diversity of yam

accessions. The marker should be used in combination with other chloroplast markers for accurate DNA barcoding of yams for their improvement and germplasm conservation.

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Ethics Approval and Consent to Participate

Consent was obtained from farmers before using their individual farms for sample collection.

Consent for Publication

Not applicable.

Availability of Data and Materials

All data generated during this study are included in this published article. Sequence data were deposited in NCBI GenBank with accession numbers ranging from MH078114 to MH078188 to match the individual yam accessions in the list of supplementary Table S1.

Authors' Contributions

All authors were involved in project design. GNU, DOI, JM, OO, JH, DB, CA and OC did the literature search process, extracted data elements, and carried out study compilation. Data analyses were performed by DOI, MO, CE, VC, MU and CO and reviewed by GNU, GA, JO and AD. DOI developed the first draft of the manuscript. All authors read the manuscript and approved the final copy of it.

Conflicts of Interest

The authors declare that they have no competing interests.

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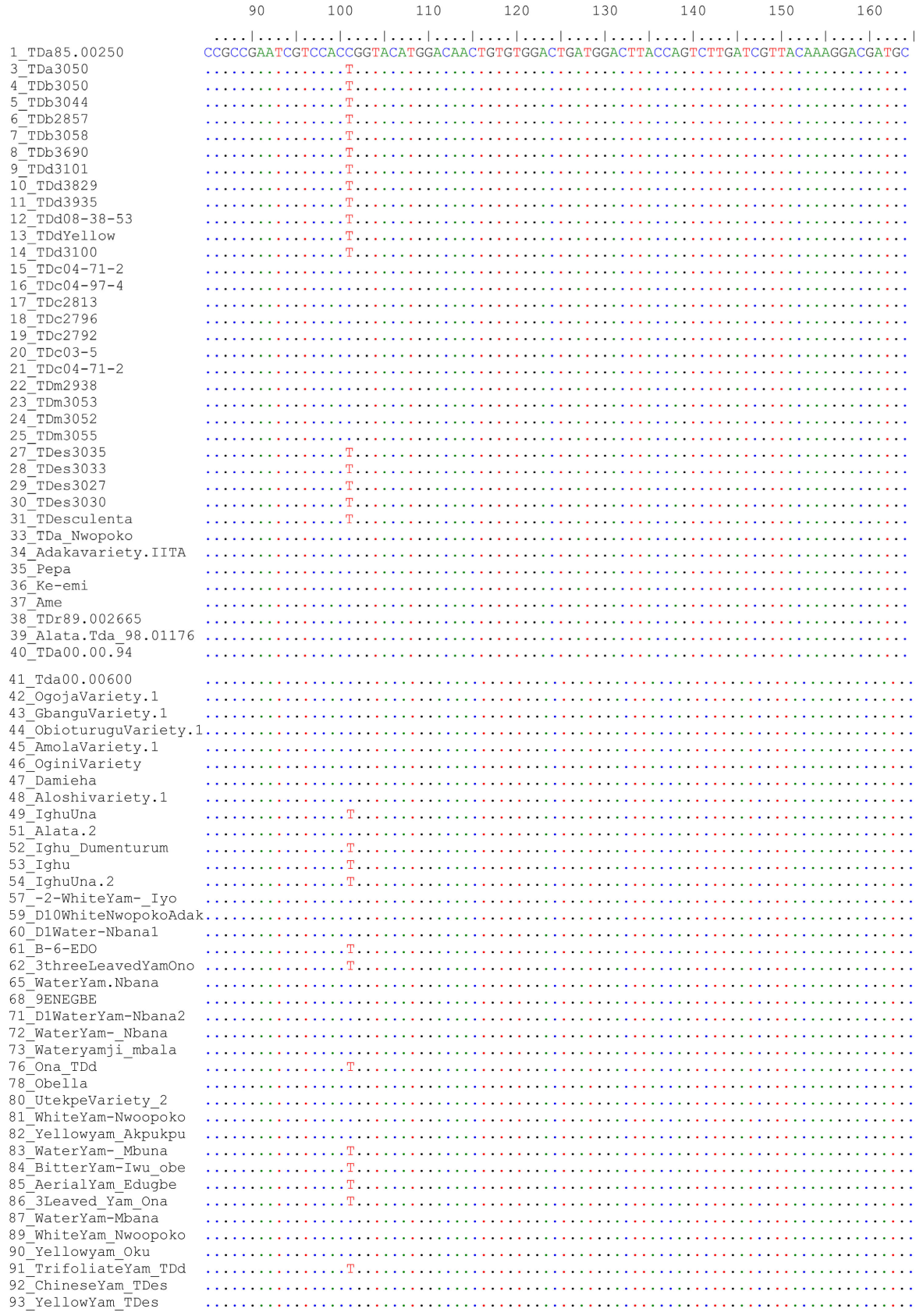
Supplementary File 1



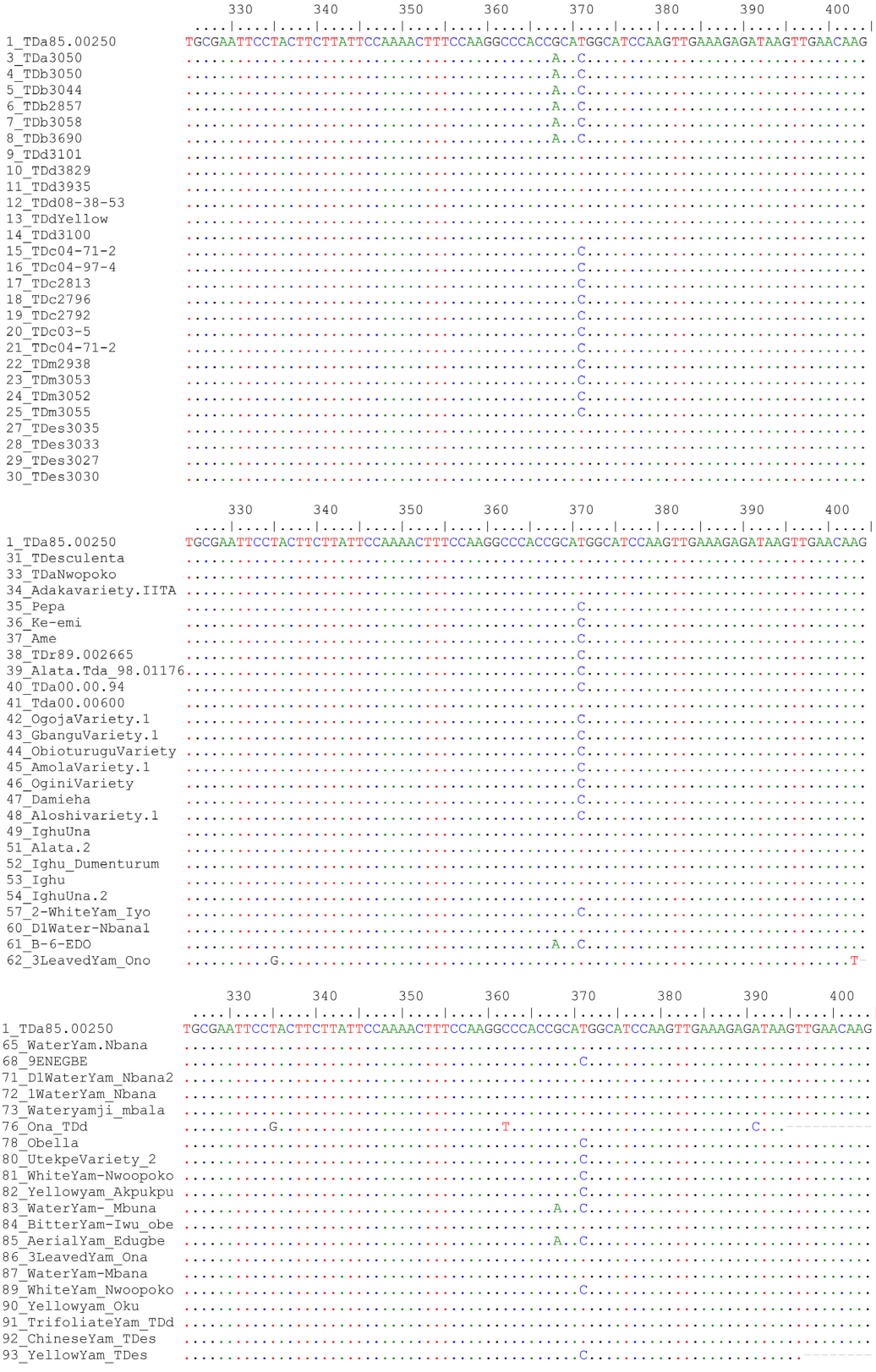


Figure S1. Consensus sequence of rbcL gene for *Dioscorea* species and its associated consensus points of polymorphisms (variations). Note that the dotted line (…) in the sequence alignment indicates similarity of nucleotide to the nucleotide of TDa-85.00250 that serves as a reference sequence of TDa-85.00250.

Supplementary File 2

Table S1. List of sequenced yam species collected from different locations and their GenBank accession numbers.

Sample IDs	Location	LGA	State	GenBank No
1_TDa85.00250	IITA	Akinyele	Oyo	MH078115
3_TDa3050	IITA	Akinyele	Oyo	MH078154
4_TDb3050	IITA	Akinyele	Oyo	MH078155
5_TDb3044	IITA	Akinyele	Oyo	MH078156
6_TDb2857	IITA	Akinyele	Oyo	MH078157
7_TDb3058	IITA	Akinyele	Oyo	MH078158
8_TDb3690	IITA	Akinyele	Oyo	MH078159
9_TDd3101	IITA	Akinyele	Oyo	MH078163
10_TDd3829	IITA	Akinyele	Oyo	MH078164
11_TDd3935	IITA	Akinyele	Oyo	MH078165
12_TDd08-38-53	IITA	Akinyele	Oyo	MH078166
13_TDdYellow	IITA	Akinyele	Oyo	MH078167
14_TDd3100	IITA	Akinyele	Oyo	MH078168
15_TDc0471-2	IITA	Akinyele	Oyo	MH078170
16_TDc0497-4	IITA	Akinyele	Oyo	MH078127
17_TDc2813	IITA	Akinyele	Oyo	MH078141
18_TDc2796	IITA	Akinyele	Oyo	MH078142
19_TDc2792	IITA	Akinyele	Oyo	MH078171
20_TDc03-5	IITA	Akinyele	Oyo	MH078143
21_TDc04-71-2	IITA	Akinyele	Oyo	MH078144
22_TDm2938	IITA	Akinyele	Oyo	MH078128
23_TDm3053	IITA	Akinyele	Oyo	MH078145
24_TDm3052	IITA	Akinyele	Oyo	MH078146
25_TDm3055	IITA	Akinyele	Oyo	MH078172
27_TDes3035	IITA	Akinyele	Oyo	MH078175
28_TDes3033	IITA	Akinyele	Oyo	MH078176
29_TDes 3027	IITA	Akinyele	Oyo	MH078177
30_TDes 3030	IITA	Akinyele	Oyo	MH078178
31_TDesculenta	IITA	Akinyele	Oyo	MH078179
33_TDaNwokporo	IITA	Akinyele	Oyo	MH078116
34_Adakavariety	IITA	Akinyele	Oyo	MH078117
35_Pepa	IITA	Akinyele	Oyo	MH078129
36_Ke-emi	IITA	Akinyele	Oyo	MH078130
37_Ame	IITA	Akinyele	Oyo	MH078131
38_TDr 89.002665	IITA	Akinyele	Oyo	MH078132
39_AlataTda 98.01176	IITA	Akinyele	Oyo	MH078133
40_TDa00.00.94 41_Alata	IITA	Akinyele	Oyo	MH078134

Continued

41_Tda00.00600	IITA	Akinyele	Oyo	MH078147
42_OgojaVariety.1	IITA	Akinyele	Oyo	MH078135
43_Gbangu_Variety.1	IITA	Akinyele	Oyo	MH078188
44_ObioturuguVariety.1	IITA	Akinyele	Oyo	MH078136
45_AmolaVariety .1	IITA	Akinyele	Oyo	MH078137
46_OginiVariety	IITA	Akinyele	Oyo	MH078173
47_Damieha	IITA	Akinyele	Oyo	MH078138
48_Aloshivariety.1	IITA	Akinyele	Oyo	MH078139
49_IghuUna	Osonu	Ezeagu	Enugu	MH078184
51_Alata2	Osonu	Ezeagu	Enugu	MH078118
52_Ighu_Dumenturum	Osonu	Ezeagu	Enugu	MH078185
53_Ighu	Osonu	Ezeagu	Enugu	MH078182
54_IghuUna.2	Osonu	Ezeagu	Enugu	MH078183
57_2-WhiteYam- Iyo	Ukaka Ngwo	Enugu North	Enugu	MH078148
59_D10WhiteNwopoko-Adaka	Agbalenyi Nachi	Oji-River	Enugu	MH078114
60_D1Water-Nbana1	Agbalenyi Nachi	Oji-River	Enugu	MH078119
61-6- EDO	Ukaka Ngwo	Enugu North	Enugu	MH078160
62_3LeavedYam-Ono	Ukaka Ngwo	Enugu North	Enugu	MH078180
65_WaterYam.Nbana	Ukaka Ngwo	Enugu North	Enugu	MH078120
68_9ENEGBE	Ndibinagu Umuaga	Udi	Enugu	MH078149
71_D1WaterYam-Nbana2	Agbalenyi Nachi	Oji-River	Enugu	MH078121
72_1-Water_Yam-_Nbana	Ndibinagu Umuaga	Udi	Enugu	MH078122
73_Water yamji_mbala	Nkalagu	Ishielu	Ebonyi	MH078123
76_OnaTDd	Ezzamgbo	Ohaukwu	Ebonyi	MH078181
78_Obella	Ezzamgbo	Ohaukwu	Ebonyi	MH078140
80_UtekpeVariety_2	Ezzamgbo	Ohaukwu	Ebonyi	MH078174
81_WhiteYam-Nw-opoko	Amaeke Amaigbo Ozalla	Nkanu West	Enugu	MH078150
82_Yellowyam_Akpukpu	Amaeke Amaigbo Ozalla	Nkanu West	Enugu	MH078151
83_WaterYam- Mbuna	Amaeke Amaigbo Ozalla	Nkanu West	Enugu	MH078161
84_BitterYam-Iwu_obe	Amaeke Amaigbo Ozalla	Nkanu West	Enugu	MH078169
85_AerialYam_Edugbe	Amaeke Amaigbo Ozalla	Nkanu West	Enugu	MH078162
86_3LeavedYam_Ona	Ede Oballa	Nsukka	Enugu	MH078186
87_WaterYam-Mbana	Nru	Nsukka	Enugu	MH078124
89_WhiteYam _Nwopoko	Ibagwa Aka	Igboeze South	Enugu	MH078152
90_Yellowyam_Oku	Ihe Owerre	Nsukka	Enugu	MH078125
91_TrifoliateYam_TDb	Ukana	Udi	Enugu	MH078187
92_ChineseYam_TDes	Ukana	Udi	Enugu	MH078126
93_YellowYam_TDes	Ukana	Udi	Enugu	MH078153

IITA = International Institute of Tropical Agriculture; LGA = Local Government Authority.