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Genetic Diversity of White Yam (*Dioscorea rotundata* Poir) Accessions Maintained in Tissue Culture and Cultivated in Field using Simple Sequence Repeat (SSR) Markers and Morphological Characterization

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Abstract

Dioscorea rotundata is a staple food crop for millions of people in the tropical and subtropical regions. *In vitro* germplasm conservation is a very useful tool in yam improvement strategies but very little is known about the genetic integrity and stability of *in-vitro* conserved yam plants. In this study, 42 accessions from *in vitro* and field populations were genotyped using 11 microsatellite markers and 23 morphological descriptors to assess variability within and between accessions. Out of the 23 morphological variables used, 13 were identified as most discriminate and were used to cluster the accessions into 4 clusters using the unweighted pair group arithmetic mean average (UPGMA). Accession maintained in field as well as in *in-vitro* showed high genetic similarity (R2 = 0.91, p-value: 1e-04). Out of the 42 accessions analyzed, nine accessions maintained in the field and *in-vitro* displayed different genetic profiles. This study provided basic information on the possible somaclonal variation of yam accessions maintained through *in-vitro*. Further study with advanced tools such as next-generation sequencing is required to elucidate the nature of the observed variation within clones.

Keywords: *Dioscorea rotundata*; Field; Genetic diversity; Morphological characterization; *In vitro*; Simple sequence repeats (SSR).

1. Introduction

Dioscorea rotundata is the most popular and economically important yams in West and Central Africa where they are indigenous and represent the largest depository of biodiversity [1]. The diversity in *Dioscorea rotundata* provides plant breeders with the necessary options to develop, through selection and breeding, new and more productive crops that are resistant to virulent pests and diseases, and adapted to changing environments as well as quality traits introgression. Breeders have put effort to address issues on yam production through many methods. However, the extent of genetic diversity and relationship of *Dioscorea rotundata* germplasm maintained at different growth conditions (tissue culture and field) has not been investigated using DNA based markers to understand possible variation of genetic material. Molecular marker information can help monitor the level of genetic diversity in breeding materials and assist breeders to more efficiently choose genetically diverse parents for breeding scheme. Such diversity assessment could provide a means for identifying potential gaps in the species collection and further guiding target collecting missions. Molecular markers such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSRS) have been applied in white yams (*Dioscorea rotundata*) for taxonomic, phylogenetic, diversity and mapping studies [2-9].

Plant tissue culture techniques are known to induce somaclonal variations. Frequency of these variations differ with the source of explants, their regeneration methods, composition of culture medium and cultural conditions [10]. DNA based markers have been used for individual identification, genome mapping, pedigree and phylogenetic diversity analysis in numerous taxa including yam. Molecular biological tools can accelerate artificial breeding processes and clarify variation between the germplasm [11]. The molecular marker technique efficiency is based on the amount of polymorphism it can detect in a given germplasm [12]. Specifically, this study evaluated the level of genetic diversity between *in vitro* and field - maintained *Dioscorea rotundata* and attempted to determine the main

cause(s) of this divergence, characterize genetic variability within and among populations (*in vitro* and field) of *Dioscorea rotundata* for the improvement of conservation strategies for further breeding purposes while addressing the issue of misslabelling within *Dioscorea rotundata* germ plasm from *in vitro* to field.

2. Materials and Method

The experiment was carried out on the experimental field of International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Ibadan is situated at the northern fringe of the tropical forest with mean annual rainfall of 1,200mm. Forty-two cultivars of *Dioscorea rotundata* were planted at a space of 1m x 1m in an augmented design with 5 plants per accessions. Each of the yam cultivar was represented by both *in vitro* and field maintained plant. Table1 shows the list of the materials used and their origin.

Table-1. List, status and origin of the clones evaluated

SN	Clones	Status	Origin
1	TDr8902665	Breeding line	Nigeria
2	TDr1956	Market variety	Cote d'Ivoire
3	Amula	Market variety	Nigeria
4	Danacha	Market variety	Unknown
5	TDr4697	Market variety	Benin
6	Hembakwase	Market variety	Unknown
7	TDr1918	Market variety	Ghana
8	TDr1100421	Breeding line	Nigeria
9	TDr1100582	Breeding line	Nigeria
10	TDr1100835	Breeding line	Nigeria
11	TDr1100101	Breeding line	Nigeria
12	TDr1100278	Breeding line	Nigeria
13	TDr1100396	Breeding line	Nigeria
14	TDr1100492	Breeding line	Nigeria
15	TDr1100497	Breeding line	Nigeria
16	TDr1100585	Breeding line	Nigeria
17	TDr8902672	Breeding line	Nigeria
18	TDr9601817	Breeding line	Nigeria
19	TDr8902157	Breeding line	Nigeria
20	TDr9902562	Breeding line	Nigeria
21	TDr0000371	Breeding line	Nigeria
22	TDr04-219	Breeding line	Nigeria
23	TDr9700793	Breeding line	Nigeria
24	TDr9501932	Breeding line	Nigeria
25	TDr9902607	Breeding line	Nigeria
26	0mi-efun	Market variety	Unknown
27	TDr9519177	Market variety	Nigeria
28	Pouna	Market variety	Unknown
29	Ogoja	Market variety	Unknown
30	Alumaco	Market variety	Unknown
31	TDr1765	GRC collection	Togo
32	TDr2540	GRC collection	Nigeria
33	TDr2076	GRC collection	Nigeria
34	TDr2595	GRC collection	Nigeria
35	TDr1652	GRC collection	Togo
36	TDr2225	GRC collection	Nigeria
37	TDr1634	GRC collection	Togo
38	TDr2271	GRC collection	Nigeria
39	TDr2337	GRC collection	Nigeria
40	TDr1908	GRC collection	Nigeria
41	TDr1720	GRC collection	Togo
42	TDr1820	GRC collection	Unknown

Morphological traits were phenotyped on individual plants of each genotypes following yam descriptors by the international plant genetic resources institute (IPGRI) and yam crop ontology were used for phenotyping. 23 yam morphological descriptors were used for morphological assessment of the genotypes (Table 2).

Table-2. Morphological descriptors used for the study and their codes

SN	Traits	Codes
1	Canopy architecture estimation	CAE
2	Flowering intensity estimation	FLRI
3	Inflorescence type estimation	Inf
4	Internode length measurement	IntL
5	Leaf apex shape estimation	LeA
6	Leaf shape estimation	LeS
7	Mature leaf color estimation	MLeCo
8	Petiole length measurement	PeL
9	Plant type estimation	GH
10	Plant vigor	PLNV
11	Spine base color estimation nominal	SBC
12	Spines on sprout presence estimation nominal	SSpC
13	Spines on stem estimation	Sstem
14	Springness estimation	Springness
15	Stem color estimation	**StCo**
16	Stem hairiness estimation	**StH**
17	Stems per plant computation per plant	**StP**
18	Twining habit estimation	TWNH
19	Upper surface vein color estimation	**USVCo**
20	Virus severity	VRSS
21	Yam anthracnose disease severity estimation	**YAD**
22	Young leaf color estimation	**YoL**
23	Cataphyle	**Ca**

2.1. DNA Extraction

DNA was isolated from Intermediate leaves using CTAB (Cetyl trimethyl ammonium bromide) method of extraction [13] with slight modification. The leaves were collected into a lyophilized bags and placed on ices, the samples were store at -80° C for 4 days and it was lyophilized at the lyophilization machine for 5 days. The lyophilized samples were grinded using the genogrinder at 3500 rpm for 2 minutes. Similarly, *in vitro* plantlets grown in Murashige and Skoog's (MS) medium (1962) were carefully removed from the test tubes and the leaves of the plantlets of each accessions were placed in a mortar with a volume of 5ml of liquid nitrogen for grinding.

1ml of Hepes buffer was added and centrifuged at 3500 rpm for 13 minutes and supernatant decanted. 400 µl of Cethyl Trimetyl Ammonium Bromide (CTAB) and 10 µl of proteinase K were added and incubated in the water bath for at 65°C for 1 hour, and at 10-minute intervals the samples were checked and shaken to ensure proper homogenization. Thereafter 600 µl of Chloroform Isoamyl Alcohol (CIA) 24:1 was added and centrifuged at 3500 rpm for 13 minutes. The aqueous phase was carefully transferred into another set of well-labelled extraction tubes and 500 µl of CIA added and centrifuged at 3500rpm for 13minutes. Then 300 µl of cold isopropanol and 50 µl of Nacl were added and shaken properly and incubated at -80°C for 1hour. The samples were centrifuged at 3500 rpm for 13 minutes and supernatant was decanted to obtain the pellet of DNA. Afterwards 500 µl of 70% ethanol was added and centrifuged for 10 minutes at 3500 rpm. The DNA pellets were dried for 30 minutes and 50 µl of low salt TE and 10 µl of RNase were added to suspend the DNA, and incubated at 4°C for 1 hour and later stored at -20°C.

2.2. Polymerase Chain Reaction

PCR was conducted using 10 µl volume in a 96 well micro titer plate with an automated thermal cycler. The reaction volume was 3 µl of DNA template, 2.54 µl of autoclaved distilled water, 1 µl of 10x reaction buffer, 0.6 µl of 2.5 mM dNtps, 0.8 µl of 50 mM MgCl2, 1µl of primer forward, 1 µl of primer reverse, 0.06 µl of taq polymerase enzymes. The PCR cycles consisted of denaturation at 94.0°C for 3 minutes, followed by 10 cycles of 94.0°C for 0.30 seconds at 60.0°C for 0.30 seconds and 72.0°C for 1.0 minutes and followed by 25 cycles at 94.0°C for 0.30 seconds, and at 50°C for 0.30 seconds the final extension step at 72.0°C for 15 minutes.

2.3. Data Analysis

Phenotypic data collected were analyzed using minitab software (version 9.3). Principal component analysis was performed to identify the first and important factors which were used for clustering the clones base on the proximity. Correlation as heat map was performed to identify the best traits for yam characterization. Data generated through phenotyping was also used for clustering analysis where all the clones was grouped using Ward method based on UPGMA [14].

Principal component analysis was generated using prcomp function in R. The matrix data generated through the phenotype was used to generate Eigen values, percentage of the variation accumulated by the PCA and the load coefficient values between the original characters and respective PCA.

Alleles were scored based on presence and absence (1/0) and converted to binary matrix for the eleven polymorphic SSRSs primers. Genetic parameters such as major allele frequency (MAF), heterozygocity (He),

polymorphic information content (PIC) and number of effective alleles (Ne) were performed using power marker software (version 4.0). Hierarchical Clustering Analysis (HCA) was constructed by unweighted paired group method using arithmetic average (UPGMA), in R software using hclust function based on shared common Allele. To assess the diversity of the two population (Field versus In-Vitro), Molecular Variance Analysis (AMOVA) was carried out using GeneAlex (version 6) software. Clones maintained both in *In-vitro* and in field were then pair and compared though their respective genetic distance.

3. Result

3.1. Phenotypic Trait Association

The first six principal components which accounted for 63.60% of the total variation (Table 3) and the first two were used to plot the two-dimensional dispersion or scatter diagram of the accessions. The scores on the first principal component (PC-1) which accounted for 17.3% of the variability in the data set were significantly correlated (correlation coefficient >0.3) with four traits namely canopy architecture estimation, internode length measurement, spines on sprout presence estimation nominal, cataphyle (Table 4). The second principal component (PC-2) accounted for 12.70% of the total variation and was highly associated with inflorescence type estimation (0.417), plant sex estimation (-0.416), spines on stem estimation (0.308), young leaf color estimation (0.350). The third components (PC-3) contributed 9.60% of the total (Table 4). While the fourth component (PC-4) was loaded with canopy architecture estimation (0.451), flowering intensity (-0.334), stem per plant computation (0.512). The fifth component (PC-5) was related to stem hairiness estimation (-0.416), twining habit (-0.452) and accounted for 8% of the variation (Table 4). Principal component six (PC-6) was dominated by springiness (0.506) and twining habit (0.383) and contributed 7% of the total variability (Table 3).

Table-3. Relationship between variable and vectors

Traits	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6
CAE	0.319*	0.071	-0.070	0.451*	-0.068	0.101
FLRI	-0.157	0.102	0.117	-0.334*	-0.279	0.150
Inf	-0.169	0.417*	0.031	0.205	-0.174	0.031
IntL	-0.316*	0.170	-0.098	0.111	-0.153	-0.140
LeA	0.084	0.166	-0.139	-0.042	-0.291	0.144
LeS	-0.031	-0.049	-0.301	0.131	0.137	0.197
MLeCo	0.208	0.139	-0.180	-0.268	-0.242	0.134
PeL	-0.252	0.063	-0.256	0.240	0.180	-0.144
PSD	0.222	0.416	-0.066	-0.223	0.144	0.064
PlV	-0.222	-0.328*	-0.154	0.111	-0.243	0.127
GH	-0.171	-0.252	-0.244	0.102	-0.015	-0.295
SSpC	0.244	0.121	-0.454*	-0.166	-0.157	0.102
Sstem	0.301*	-0.004	-0.434*	-0.110	0.011	-0.164
Spring	0.222	0.308*	-0.131	0.063	0.209	-0.180
StCo	-0.105	0.043	0.097	-0.046	-0.211	0.506*
StP	0.104	0.252	-0.065	-0.093	-0.014	-0.159
StH	0.194	-0.162	0.127	0.066	-0.416*	-0.278
StP	0.203	-0.078	0.006	0.512*	-0.048	0.255
TWN	0.187	-0.171	0.142	0.068	-0.452	-0.383*
VRSS	0.203	0.109	0.233	-0.211	0.296	-0.047
YAD	-0.123	-0.112	-0.343*	-0.126	-0.010	0.151
YoL	-0.027	0.350*	0.059	0.001	-0.081	-0.140
Ca	-0.331*	0.002	-0.198	-0.155	-0.046	-0.235
Eigen value	3.313	2.785	2.314	1.998	1.822	1.456
Variability	17.3	12.7	9.6	9.00	8.00	7.00
Cumulative	17.3	30	39.6	48.6	56.6	63.6

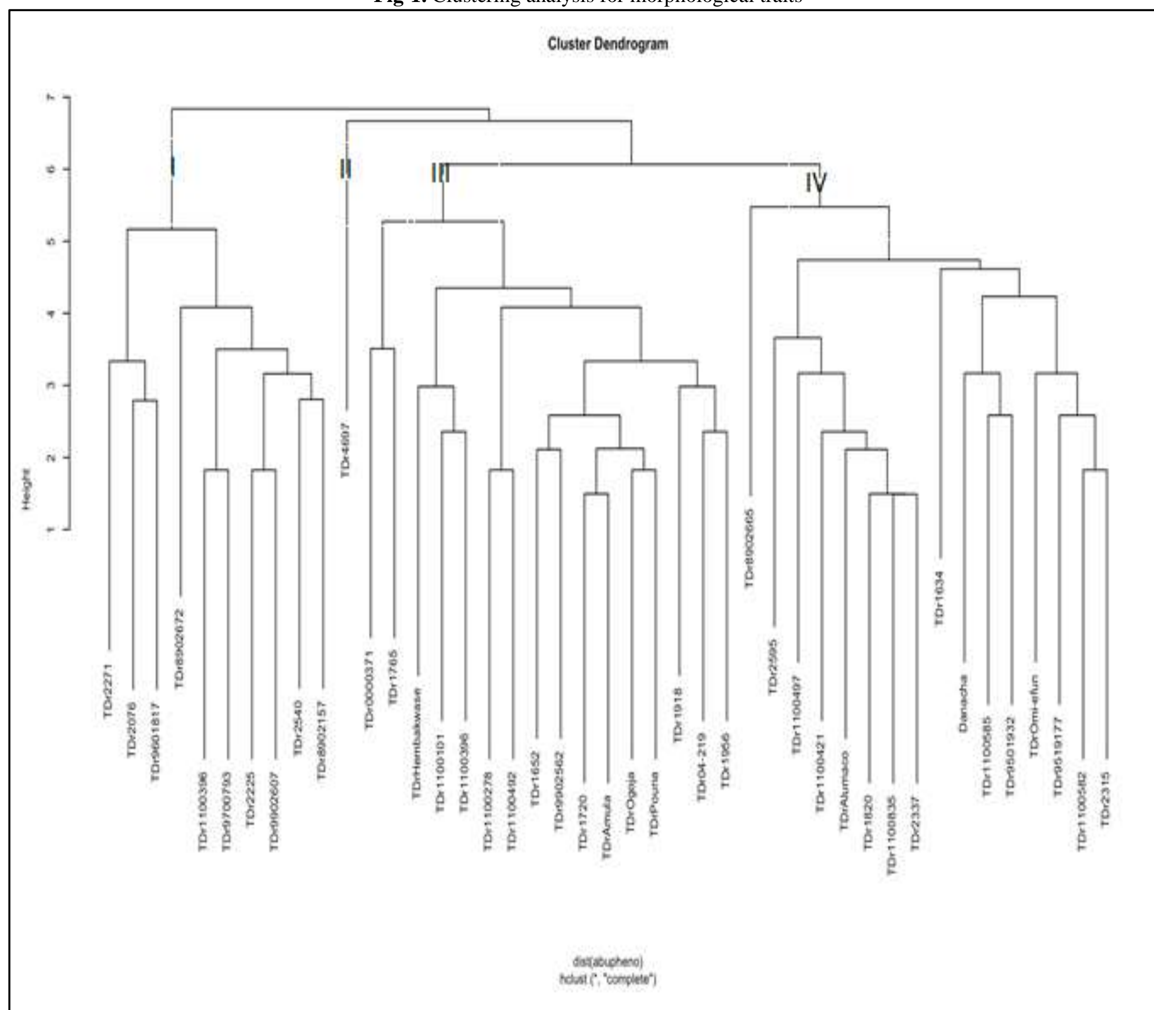
*significant correlated value (>0.30) CAE- canopy architecture estimate, FLRI-Flowering intensity, Inf - Inflorescence type, LeA - Leaf apex estimation, LeS - Leaf shape estimation, MLeCo - Mature lea colour estimation, PeL- Petiole length measurement, PSD – Plant sex, PlV – Plant vigour, SSpC – Spine on sprout, Sstem – Spine on stem, StCo – Stem colour, StP – Stems per plant, StH – Stem hairiness, TWN – Twinning habit, VRSS – Virus severity score, YAD – Yam anthracnose disease, YoL - Young leaf colour, Ca - Catapyl

Out of the 23 variables used for the phenotyping analysis, 13 showed high variation (>0.30) across all the first six principal components. Trait with high variation can be used as key variables for quick yam assessment in field.

Dendrogram for the Hierarchical Cluster Analysis (HCA) grouped the 42 accessions into 4 main clusters: I, II, III and IV with Euclidean distance dissimilarities ranging between 1.0 and 7.0 (Fig. 2); and it was truncated at the dissimilarities distance of 5.8 (Fig. 1). Cluster I comprise of 10 accessions including TDr2271, TDr2076, TDr1100396, TDr9700793, TDr9601817, TDr8900672, TDr2225, TDr9902607, TDr2540 and TDr8902157. Members of this cluster are characterized by dark green mature leaves, presence of spine on new sprouts and moderate tolerance to yam anthracnose disease. Cluster II which contains a single accession (TDr114007) is unique for exhibiting high tolerance to yam anthracnose disease. The third group (Cluster III) is made of 16 accessions

including TDr00000371, TDr1765, TDrHembakwase, TDr100101, TDr1100396, TDr1100278, TDr1100492 and TDr9902562. Other members of the cluster are Amula, Ogoja, Pouna, TDr1918, TDr04-219 and TDr1956. The members of this cluster are basically characterized by possession of acute leaf apex and a climbing nature. The cluster IV with 16 accessions have canopy characterized by several vines and many short branches with presence of spine base color. Members of this cluster include TDr892665, TDr2595, TDr1100497, TDr1100421, TDrAlumaco, TDr1820, TDr1100835, TDr2337, TDr1634, Danacha, TDr1100585, TDr9501932, TDrOmiefun, TDr9519177, TDr1100582 and TDr2315. (Figure 1).

Fig-1. Clustering analysis for morphological traits



3.2. Population Genetic Diversity for *In-vitro* and Field Populations

Eleven SSR markers were used to assess genetic diversity of 42 yam genotypes maintained both in Field and in vitro. A total of 97 alleles were recorded for 11 SSR markers. An average of 8.81 alleles was observed per marker, which varied from 6(DrM31) to 12 (DRM147) alleles (Table 4). The average allele frequency was 0.19 and varied from 0.05 (DrM69) to 0.25 (DRM32). Average polymorphic information content (PIC) value was 0.91 and ranged from 0.79 (DrM31) to 0.97 (DrM69) (Table 5).

Table-4. SSR primers used for the study

Marker	Allele Frequency	Allele No.	PIC
DrM 69	0.05	11	0.97
DrM 588	0.23	9	0.88
DrM 31	0.32	6	0.79
DrM 98	0.10	8	0.93
DrM 135	0.14	7	0.93
DrM 163	0.17	10	0.91
DrM 574	0.25	7	0.86
DrM 541	0.10	10	0.94
DrM 421	0.13	9	0.93
DrM 147	0.10	12	0.95
DrM 345	0.14	8	0.92
Average	0.19	8.81	0.91

DrM 69 had the lowest number of allele frequency ranging between 0.13 to 0.15 while DrM 421 had the highest number of allele frequency ranging from 0.76 to 0.76, the lowest number of different alleles were observed in DrM 163 and DrM 588 ranging between 1.600 to 1.888 while the highest number of different alleles was observed in DrM 421 which had 3.000 for both invitro and field populations, DrM 31 had the highest number of effective alleles ranging between 1.31 to 1.35 and the lowest was observed in DrM 421 had the highest number of effective alleles ranging between 1.71 to 1.74. DrM 69 had the lowest number of Shannon's information index ranging from 0.36 to 0.37 and the highest number of shannon's information index was observed in DrM 421 ranging between 0.77 to 0.78. DrM 69 had the highest the lowest number of genetic diversity ranging between 0.22 to 0.23 and the highest genetic diversity was observed in DrM 421 ranging between 0.50 to 0.52. DrM 69 had the lowest number of unbiased genetic diversity ranging between 0.22 to 0.23 while the highest number of unbiased genetic diversity was observed in DrM 421 which had the value 0.52 to 0.53.

Table-5. Populations genetic diversity parameters

Marker	Group	Frq	Na	Ne	I	H	Uh
DrM 69	Field	0.13	2.000	1.31	0.36	0.22	0.22
	In-vitro	0.15	2.000	1.35	0.37	0.23	0.23
DrM 588	Field	0.63	1.888	1.39	0.38	0.24	0.25
	In-vitro	0.65	1.888	1.45	0.41	0.27	0.28
DrM 31	Field	0.52	2.000	1.74	0.58	0.40	0.41
	In-vitro	0.49	2.000	1.71	0.53	0.36	0.37
DrM 98	Field	0.44	2.000	1.63	0.53	0.36	0.37
	In-vitro	0.44	2.000	1.62	0.52	0.35	0.36
DrM135	Field	0.46	2.000	1.66	0.53	0.36	0.37
	In-vitro	0.45	2.000	1.62	0.52	0.35	0.36
DrM 163	Field	0.46	1.600	1.37	0.36	0.23	0.23
	In-vitro	0.48	2.000	1.39	0.41	0.26	0.26
DrM 574	Field	0.49	2.000	1.43	0.44	0.27	0.28
	In-vitro	0.48	2.000	1.42	0.44	0.28	0.28
DrM 421	Field	0.77	3.000	2.38	0.78	0.52	0.53
	In-vitro	0.76	3.000	2.33	0.77	0.50	0.52
DrM 147	Field	0.29	2.000	1.67	0.55	0.38	0.38
	In-vitro	0.28	2.000	1.65	0.54	0.37	0.38
DrM 345	Field	0.41	2.000	1.53	0.48	0.31	0.32
	In-vitro	0.37	2.000	1.61	0.53	0.35	0.36
DrM 541	Field	0.51	1.900	1.65	0.52	0.36	0.37
	In-vitro	0.51	2.000	1.66	0.54	0.37	0.38

Frq=frequency, Na=no. of different alleles, Ne= no. of effective alleles, I=shannon's information index, h=genetic diversity, uh= unbiased diversity

3.3. Cluster Analysis based on Molecular Data Analysis from *in vitro* and Field Populations

To construct the genetic relationship between the two populations (field and *in vitro*) through hierarchical clustering by unweighted paired grouping method with arithmetic averages (UPGMA) high genetic profile of accession maintained both in field and through in-vitro (Figure 2). Using genetic comparison, out of the 42 accessions maintained both in field and In-vitro culrure 33 yam cultivars were identified to have the same genetic profile across the two conditions, and can be consided as true to type while nine accessions (TDr1100421, TDr1100101, TDr1100497, TDr1100585, TDrOmi-efun, TDrPouna, TDr2595, TDr1634, TDr1820) displayed different genetic profile. Mantel test for the correlation of similarity matrix of accessions from both *in vitro* and field was 0.91 indicating a strong association (Figure 3).

Figure-2. Molecular characterization of yam clone maintained in field and *in vitro* through SSR genotyping. The "I" after the clones name stand for in-vitro while the "F" stand for field

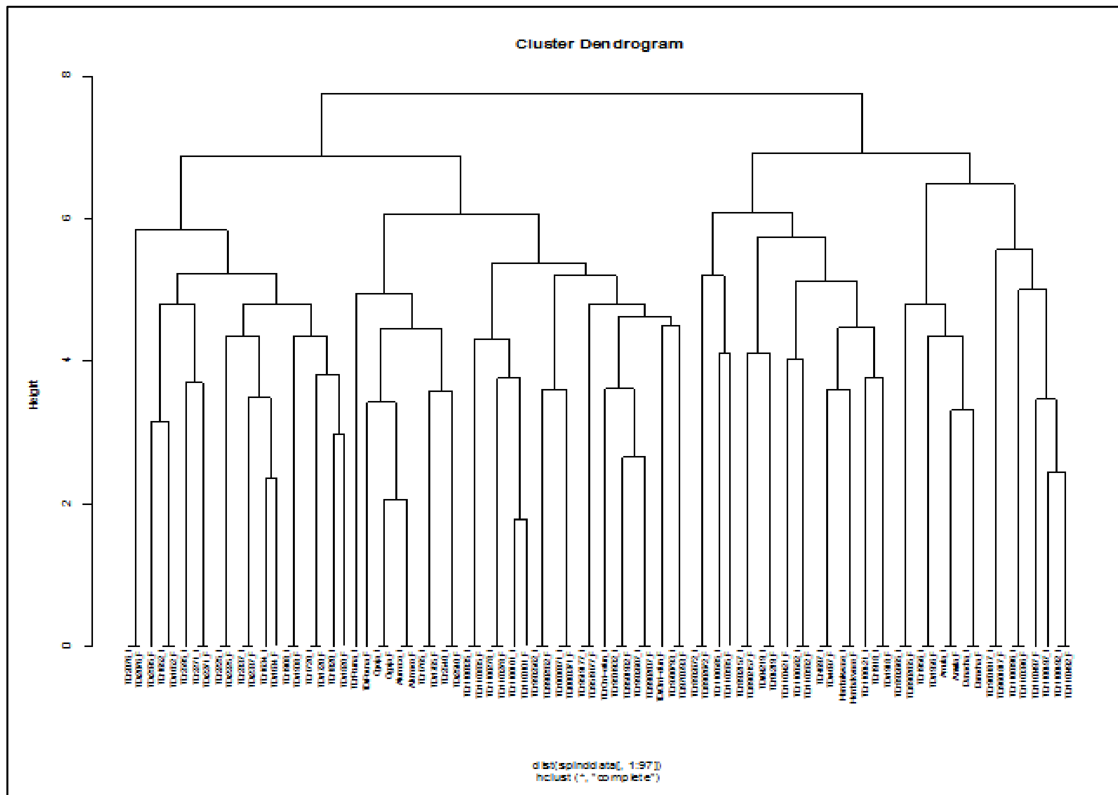
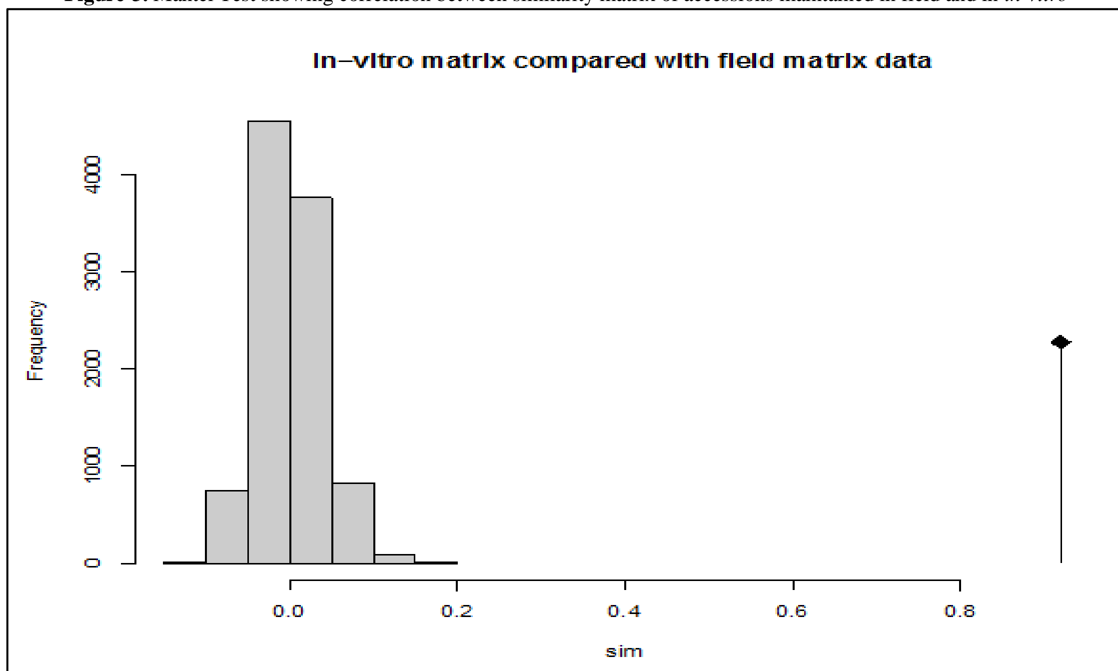


Figure-3. Mantel Test showing correlation between similarity matrix of accessions maintained in field and in *in-vitro*



4. Discussion

This study represents an attempt that investigated possible genetic variability of the same yam genotypes maintained *in vitro* and in the field using microsatellite primers and morphological descriptors with a view of ascertaining the stability of genotypes across the two conservation enviroments. Several other studies on genetic diversity have been conducted on this species using different types of molecular markers including random amplified polymorphism DNAs [2, 15], amplified fragment length polymorphism [3, 7, 16], and simple sequence repeat (SSRs) markers [4, 17, 18] and single nucleotide polymorphism [12]. However, none of these previous studies on yam have looked at the possible variation of the same clone from *In vitro* storage to field over time.

The eleven microsatellite primers used for this study were effective for identifying polymorphism and for evaluating the genetic relationship among the 42 accessions analyzed with regards to all SSRs loci being investigated, the primers gave a total of 97 polymorphic, reproducible and scorable bands. Though the eleven SSR primers used for discriminating the genetic diversity between these two sets of accessions is reasonable but further

study should involve more numbers of primers for effective and accurate result [19]. The number of alleles amplified per primer per locus is considerably high 6-12. This is in agreement with similar work done by [20], who reported a total number of 131 alleles when they analyzed 187 accessions of Kenya yam using 12 SSR markers. Obidiegwu, *et al.* [21] reported a total of 121 amplified alleles with 15 SSRs primers with the number of alleles observed per locus varying from 6 to 9 alleles when they analyzed 219 of Guinea yam germplasm from Benin, Congo, Cote d'Ivoire, Equatorial Guinea, Gabon, Nigeria, Sierra Leone and Togo. However low number of alleles was obtained by Otoo, *et al.* [17], who reported 27 alleles using 13 SSR primers in a study of Pona complex yam in Ghana. High diversity obtained from the breeding line can be explained by the progeny parentage and selection history during population development.

Out of the 42 accessions used in this study 33 were found to be true-to-type and 9 were off-type on the basis of SSR scoring pattern and genetic distance. This insight will enhance yam breeding program as it highlights the fact that same accessions maintained under different environmental conditions (*in vitro* to field) might be variable as a result of gene rearrangement this may be hinged on somaclonal variation, so further studies should involve more advanced type of primers such as DArT (Diversity Array Technology), SNP (Single nucleotide polymorphism) or whole genome sequencing that will fully establish the fidelity of materials distributed from *in vitro* to field. In this regard due precausion should be taken to eliminated variation that is due to mixtures of genotypes.

5. Conclusion

In this study, we employed 11 SSRs markers and 23 most discriminate morphological descriptors to assess variabilities between and within two sets (*in vitro* and field) of 42 genotypes of *Dioscorea rotundata*. 13 phenotipic traits contributed significantly to observed variations across six principal components. These traits will be useful for efficient phenotyping in yam vegetative characterization. Also dissimilarities were observed in 9 genotypes between *in vitro* and field clones. This could be associated to somaclonal variations or limitation in the number markers deployed for this study. The information obtained in this study will be an important insight towards development of effective *in vitro* germplasm conservation and management strategy for yam both for breeding and exchange purposes.

Author Contribution

David De Koyer, Ranjana Bhattacharjee and Paterne Agre designed the project, Alex Edemodu and Abu Gabriel, Managed the phenotypic data. Paterne Agre performed the analysis. Abu Gabriel, Alex Edemodu and Paterne Agre wrote the manuscript with the contributions of other authors.

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Consent for publication: All authors approve the publication of the study

Availability of data and material: Data used for the analysis are available upon request

Code availability: R code is available upon request

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