Assessment of genetic diversity of *Dioscorea praehensilis* (Berth.) collected from Central Region, Ghana using simple sequence repeat (SSR) markers

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Abstract

Dioscorea praehensilis Berth is one of the wild yam species resistance to many yam disease (yam anthracnose disease and yam mosaic virus) grow in Ghana especially in the cocoa grown regions of the country. It is a crucial crop that has been known to contribute to poverty reduction and food gap. Genetic diversity in this yam species has been discovered to be eroding and neglected. In this study we evaluated the genetic diversity among 43 *D. praehensilis* collected from Ghana using simple sequence repeat (SSR). Using 11 SSR marker, a total of 99 number of alleles were generated with an average of 8.48 alleles per locus. The mean gene diversity was 0.81, mean polymorphism information content was 0.82 while mean Shannon information index was 1.94. Principal coordinate analysis (PCoA) revealed a contribution of 40.16% of the first three coordinate axes and grouped the 43 morphotypes into 2 groups while hierarchical cluster through UPGMA revealed the presence of 3 main clusters. Molecular variance (AMOVA) alongside the Fst revealed low genetic diversity and differentiation among accessions and population. Result of this study assess the genetic diversity and will facilitate the use *D. praehensilis* as sources of resistance gene into yam breeding program.

Key words: Genetic diversity, *Dioscorea praehensilis*, SSR markers, Ghana

Introduction

Yams (*Dioscorea* spp.) are significant food crops in West Africa where they contribute actively to food security and to poverty alleviation of the local farmers [1]. Ghana is one of the highest yams

producing nations after Nigeria [2]. The genus *Dioscorea* spp comprises of about 450 species [3]. Only 50-60 species are available for cultivation and wild-harvest [3]. Africa's most cultivated species are *D. alata* L., *D. bulbifera* L., *D. cayenesis* Lam, *D. esculenta* (Lour.) Burk, *D. rotundata* Poir and *D. trifida* L. [4]. The major wild yam species in Africa are *D. abyssinica*, *D. sagitifolia*, *D. preahensilis*, *D. liebrechstiana*, *D. mangenotiana* and *D. lecardi* [5]. Bush yam (*Dioscorea praehensilis*) which is one of the wild yams serves as a source of food and contributes greatly to the welfare of people in West Africa [6]. This species has a wide geographical range in Africa and occurs throughout the Western, Central and Eastern parts of the continent [5]. (*Dioscorea praehensilis)* is an edible wild yam that is mostly found around cocoa plantations in Ghana, has been known to fill the hunger gaps (food and income security) among cocoa farmers in Ghana for ages but is currently known to be disappearing [7]. The genetic variability level in these species of yams has been underutilized and understudied. There is the need to explore molecular markers of genotype characterization to determine the genetic variability level in *D. praehensilis*.

Molecular markers such as such as simple sequence repeat (SSR) [8-14], Rapid Amplified Polymorphic DNA (RAPD) [15], Amplified Fragment Length Polymorphism (AFLP) [16-18], among others had been employed to assess genetic variability of *Dioscorea spp.*

Simple sequence repeat markers are widely known due to their locus specificity, comprehensive genome coverage, elevated degree of polymorphism, co-dominant inheritance and convenience for simple automated scoring [19-20] thereby making them being increasingly used as the marker of choice in diversity analysis of different crop species. Several studies had been conducted on some other species of yam. Loko et al. [21] reported high genetic diversity among 64 yam landraces in Benin using Microsatellite markers. Otoo et al. [13] observed variability among 49 accessions of *D. alata* in Ghana using SSR markers. Silva et al. [22] observed little spatial structure and a considerable level of variability in *D. bulbifera* using SSR markers.

Some researchers had conducted genetic diversity on *Dioscorea* spp using molecular markers but few genotypes of *D. praehensilis* were involved. Bekele [23] reported genetic variability when he conducted genetic diversity on Dioscorea spp in Ethiopia using simple sequence repeat markers (SSRs) but only 5 genotypes of *D. praehensilis* were included.

There has been no rigorous study of the genetic diversity and genetic relationships currently in the collections of *D. praehensilis* using molecular markers. This research therefore aimed at evaluating genetic variability in *Dioscorea praehensilis* yam morphotypes using simple sequence repeat (SSR) markers and to understand the population structure in *D. praehensilis* for proper utilization for gene introgression.

Results

Genetic diversity of 43 *D. praehensilis* genotypes collected from Central Region, Ghana based on 11 SSR primers

The polymorphism and allelic variation of the 11 SSR primers used to assess the genetic diversity among 43 *D. praehensilis* genotypes is presented in Table 2. A total of 99 number of alleles were generated using 11 SSR primers in this study. The number of different alleles generated by each primer ranged from 5 to 13 with an average of 8.48 alleles per locus. The highest and lowest number of alleles was detected in primers YM18 and YM61 respectively. Major allele frequency ranges from 0.29 in YM30 and YM49 to 0.50 in YM18 and YM44. The observed heterozygosity per primer ranged from 0.00 to 0.5 with an average of 0.1 while the expected heterozygosity (gene diversity) per primer ranged from 0.58 in YM44 to 0.91 in YM61 with an average of 0.81. Polymorphism information contents (PIC) ranged from 0.7 in YM18 to 0.83 in YM30, YM31, YM49 and YM61 respectively with an average value of 0.82.

Genetic diversity within and among 43 *D. praehensilis* genotypes based on the populations

Genetic diversity within and among 43 *D. praehensilis* genotypes based on the populations is presented in Table 3. The inbreeding coefficient (F) recorded an average value of 0.89 with Nyame population recorded the lowest value of 0.84 while the highest value of 0.92 was recorded by Awo population. The highest Shannon's Information Index (I) of 2.36 was recorded in Awo population while the lowest value of 1.41 was recorded in Tetteh population. The mean Shannon's Information Index (I) recorded was 1.94.

Table 2: *Polymorphism and allelic variations of 11 SSR primers among 43 D. praehensilis genotypes*

Markers	N	Na	Ne	Ho	He	MAF	PIC
YM30	10.00	9.00	8.38	0.00	0.85	0.29	0.88
YM43	9.33	6.00	4.11	0.00	0.72	0.33	0.78
YM31	9.67	9.00	8.61	0.00	0.86	0.31	0.88
YM18	7.00	4.67	4.01	0.00	0.73	0.50	0.70
YM16	9.67	9.00	8.54	0.00	0.88	0.40	0.79
YM27	8.33	7.00	6.39	0.00	0.83	0.31	0.86
YM50	9.67	8.00	7.23	0.00	0.83	0.31	0.88
YM61	9.67	13.33	11.48	0.50	0.91	0.38	0.83
YM25	8.67	11.33	9.96	0.42	0.87	0.29	0.88
YM49	10.00	8.67	7.91	0.00	0.87	0.50	0.73
YM44	7.00	7.33	6.71	0.11	0.58	0.36	0.83
Total	99.00	93.33	83.33	1.03	8.92	3.98	9.05
Mean	9.00	8.48	7.58	0.09	0.81	0.36	0.82
SE	0.21	0.21	0.19	0.01	0.01	0.02	0.02
SD	0.69	0.70	0.63	0.04	0.03	0.08	0.06

N=Number of alleles per locus, Na=Number of different alleles, Ne=number of effective alleles, Ho=Observed heterozygosity, He=Expected heterozygosity, MAF=Major allele frequency, PIC=Polymorphism information content, SE=Standard error, SD=Standard deviation

Table 3: *Genetic diversity within and among the 43 D. praehensilis genotypes based on the populations*

Pop	N	Na	Ne	I	Ho	He	F	%P
Awo	13.45	11.73	10.27	2.36	0.07	0.89	0.92	100
Nyame	8.55	8.64	7.75	2.05	0.14	0.85	0.84	100
Tetteh	5.00	5.09	4.71	1.41	0.07	0.69	0.91	91

Total	27.00	25.45	22.73	5.82	0.28	2.43	2.68	291
Mean	9.00	8.48	7.58	1.94	0.09	0.81	0.89	97
SE	0.40	0.40	0.36	0.06	0.02	0.02	0.02	1.75
SD	0.69	0.70	0.63	0.11	0.04	0.03	0.04	3.00

N=Number of genotypes per population, Na=Number of different alleles, Ne=number of effective alleles, I=Shannon's information index, Ho=Observed heterozygosity, He=Expected heterozygosity, F=Inbreeding coefficient, content, %P=Percentage of polymorphic loci, SE=Standard error, SD=Standard deviation

The allelic analysis across populations of *D. praehensilis* revealed variability among individuals within populations with the highest heterozygosity (0.89) recorded among individual genotypes in Awo population while the least heterozygosity (0.69) was recorded among individual genotypes in Tetteh population (Figure 1).

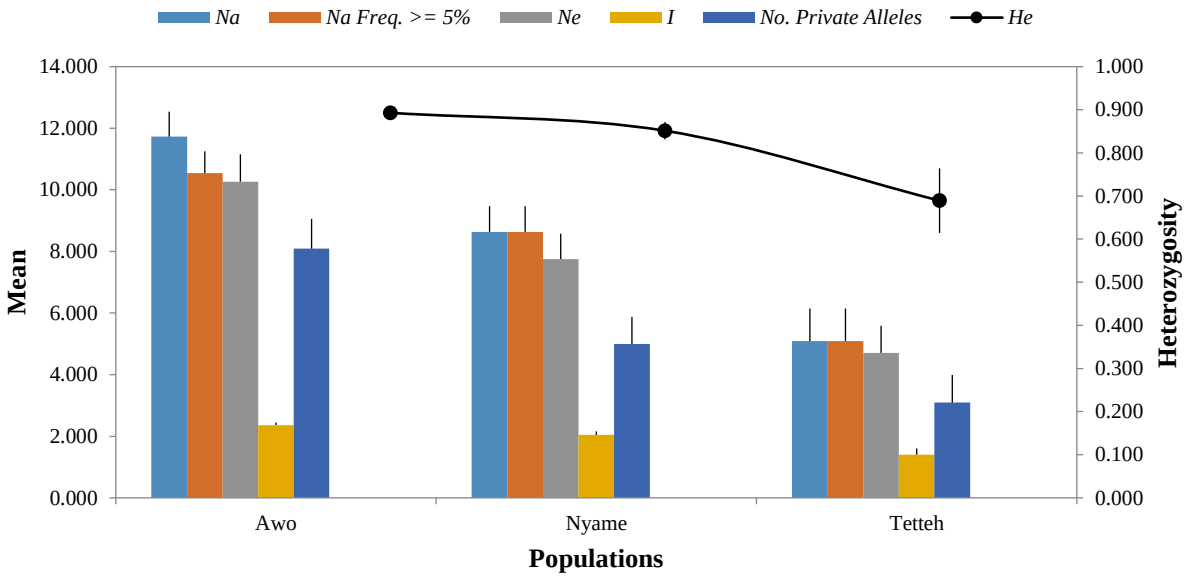

***Figure 1**: Allelic patterns across 3 populations of D. praehensilis*

Na=Number of different alleles, Na (Freq >= 5%) = No. of Different Alleles with a Frequency >= 5%, Ne = No. of Effective Alleles, I = Shannon's Information Index, No. Private Alleles = No. of Alleles Unique to a Single Population

Population differentiation and genetic structure

The molecular variance analysis (AMOVA) based on the FST (fixation index) values indicated that individual genotypes in the three populations accounted for most percentage variation of 86%. Low genetic diversity was observed among the three different populations as well as within

genotypes of the same population (Table 4). Fst value of 0.066 observed in this study is an indication of low genetic differentiation among the three populations. Fis value of 0.93 is an indication of high heterozygote deficits which may due to non-random mating within the populations.

The principal coordinate's analysis revealed a contribution of 40.16% of the first three coordinates and classified the 43 genotypes evaluated into two groups. Group I comprised of 27 mixture of genotypes from the three populations respectively and group II also comprised of 16 genotypes originated from three populations Awo, Nyame and Tetteh respectively (Figure 2).

Un-weighted pair group method with arithmetic mean (UPGMA) cluster analysis grouped the 43 genotypes of *D.praehensilis* into 3 clusters (A, B and C) (Figure 3). Cluster A was further grouped into 6 sub-clusters (I, II, III, IV, V and VI) (Figure 3). Sub-cluster I contained 22 genotypes in which 5 belonged to Awo population, 5 belonged to Nyame population and 12 belonged to Tetteh population. Sub-cluster II consisted of 6 genotypes in which 4 belonged to Awo population and 2 belonged to Nyame population. Sub-clusters III, IV and V contained 2 genotypes each with 1 belonged to Awo population and 1 belonged to Nyame population, respectively.

Sub-cluster VI consisted of 6 genotypes in which 3 genotypes belonged to Awo population, 2 genotypes belonged to Nyame population and 1 genotype belonged to Tetteh population. Cluster B consisted of 2 genotypes in which 1 each belonged Awo and Tetteh populations, respectively. Cluster C contained 1 genotype belonged to Nyame population. In each population identify the lowest genetic distance, the highest and the tendency to have duplicated.

***Table 4**: Analysis of molecular variance of 43* D. praehensilis *classified to 3 populations*

Source	df	SS	MS	Est. Var.	% Variation
Among Pops	2	35.147	17.573	0.315	7
Among Indiv	40	344.935	8.623	4.143	86
Within Indiv	43	14.500	0.337	0.337	7

Total	85	394.581	4.796	100
F-Statistics	**Value**	**P(≥0.001)**		
Fst	0.066	0.001		
Fis	0.925	0.001		
Fit	0.930	0.001		

df = Degree of freedom, SS = Sum of Square, MS = Mean of Square, Est. Var. = Estimated Variance, Fst = Total genetic differentiation, Fis = Inbreeding coefficient, Fit = Inbreeding Coefficient, P = Probability

***Table 5**: Eigen values and % variations observed at the first three axes of the PCoA*

Axis No.	Eigen Value	% Variation	Cum %
1	182.35	24.90	24.90
2	67.68	9.24	34.15
3	44.04	6.01	40.16

Cum % = Cumulative Percentage

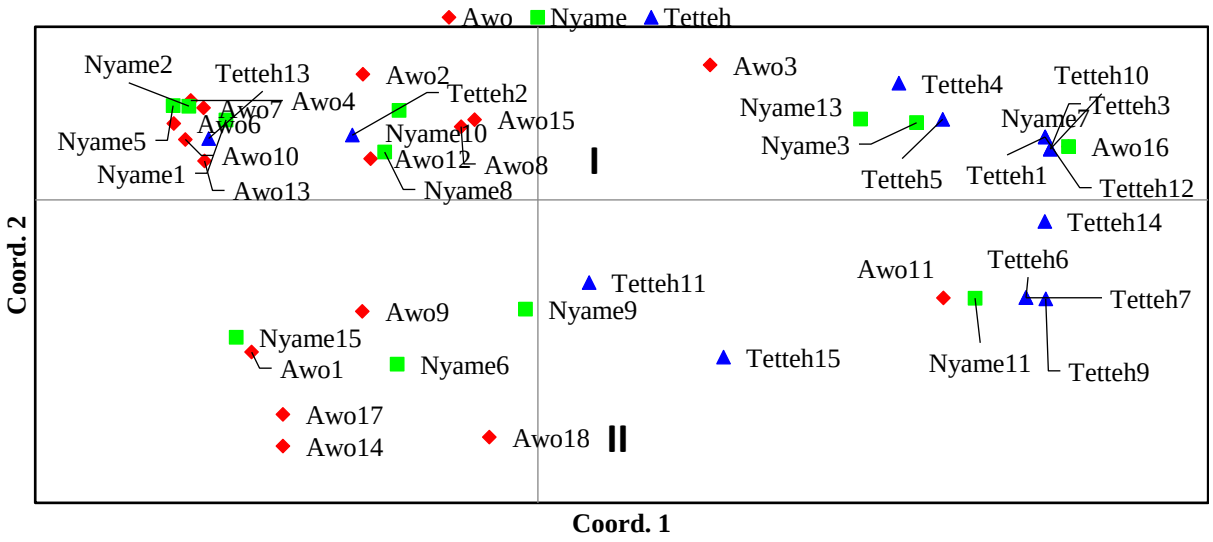

***Figure 2**: Principal coordinates analysis (PCoA) for genetic variability among 43 D. praehensilis genotypes*

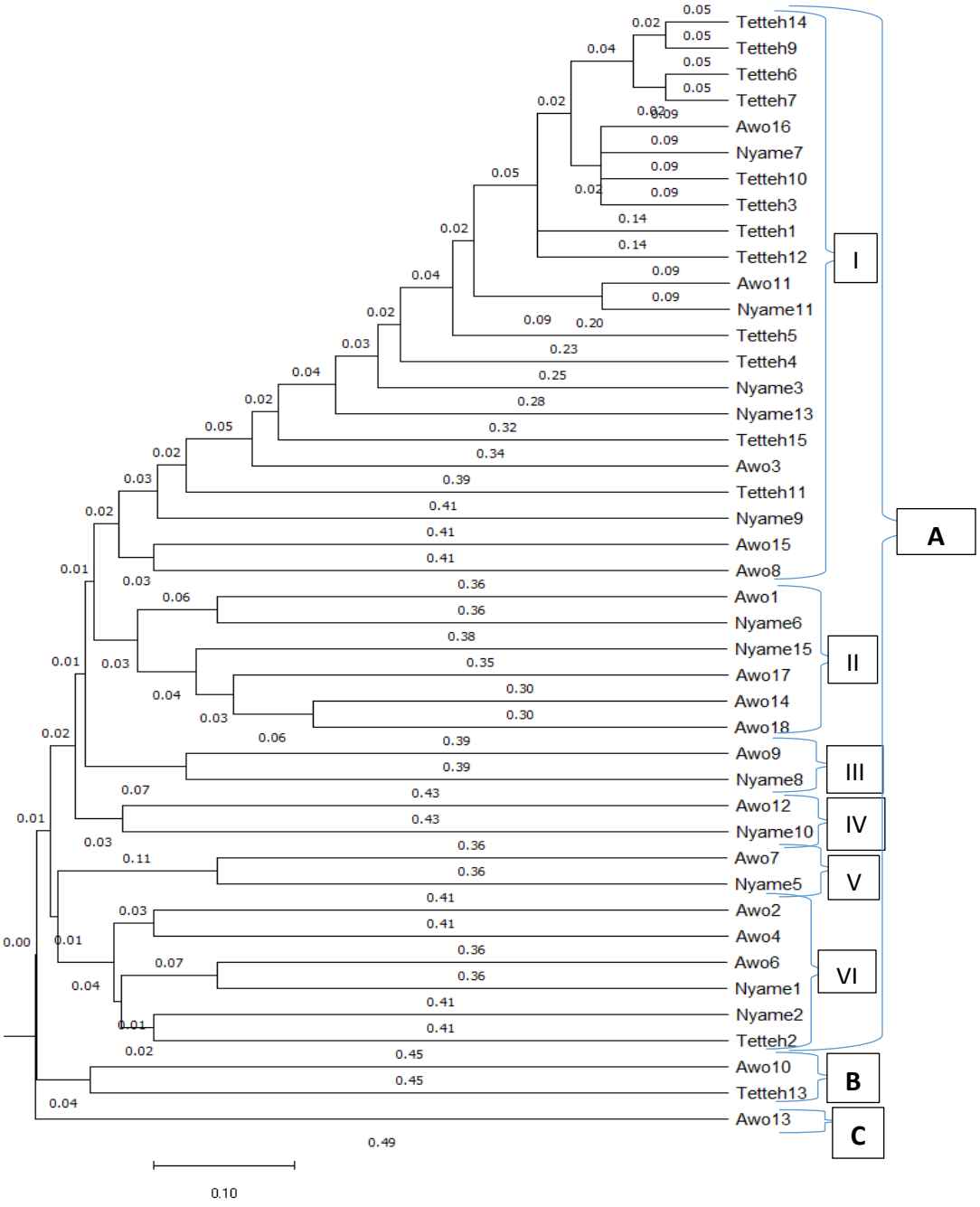

***Figure 3**: Dendogram showing genetic similarities among 43 genotypes of* D. praehensilis *based on 11 SSR primers using Euclidian similarity coefficients with UPGMA Clustering*

Discussion

Simple sequence repeat markers have been extensively employed in accessing genetic diversity among other species of yam [14, 21, 22, 13]. In this present study, the genetic diversity among populations were analyzed in three populations of *D.praehensilis* using eleven SSR markers.

The range and mean number of alleles detected per primer in this study (4.67-13.33 and 8.48) were similar to what was reported by Loko et al. [21] when using SSR markers to understand the genetic diversity and relationship among guinea yam germplasm in Benin republic. The mean value of 3.3 reported by Silva et al. [22] on genetic diversity of *D. bulbifera* using SSR markers was lower than the value from the present study. Siqueira et al. [12] also reported mean value of 5.1 on *D. alata* using 12 SSR primers.

It has been reported that high informative markers should have the polymorphic information contents (PIC) greater than 0.5 and lower than 0.95 [24]. The average PIC (0.82) detected in this study for all the 11 SSR markers agreed with report of Paal et al. [24]. The PIC for all the 11 SSR markers evaluated in this study were within the range of 0.5 – 0.95 reported by [24]. In characterization of yam using simple sequence repeat markers, higher PIC (0.62) had also been reported by Silva et al. [11] which was lower than the present value. High PIC is an indication that the markers used in this study are highly informative and they are useful for detecting genetic variability among *Dioscorea* spp.

Low average gene diversity (observed heterozygosity) of 0.09 was detected in this study compared to what had been reported by several authors who have worked on different species of yam. Loko et al. [21] reported a very high observed heterozygosity (0.72) in guinea yam with 13 SSR markers. Otoo et al. [13] also detected a very high average observed heterozygosity (0.77) when categorizing water or greater yam using SSR markers.

Mean expected heterozygosity (0.81) which is the measure of genetic variation in a population was observed to be very high in this present study when compared to what was reported on *Dioscorea* spp from Ethiopia [14]. High mean gene diversity in this present study might be due to high reproducible ability of the SSR primers used.

The mean Shannon index (I) of 1.94 reported in this study is higher than the mean Shannon index value of 0.4 reported on Amerindian yam (*Dioscorea trifidia*) [9]. Mean Shannon index value of 0.45 has also been reported in Ethiopia on diversity studies of *Dioscorea* spp landraces [14]. Bekele [23] reported mean Shannon index (0.49) when conducting research on the molecular genetic diversity of yam germplasm collections from Ethiopia. Higher Shannon information indices has been reported on guinea yam and water yam genotypes respectively [25,26]. High mean Shannon information index reported in this is an indication of low genetic diversity among the populations assessed i.e. the higher the Shannon information index the lower the diversity.

The mean inbreeding coefficient observed in this study was high (0.8) compared to what was reported by Tewodros et al. [14] which was 0.24. High inbreeding coefficient is an indication of movement of genetic materials between and within the populations resulting into large amount of duplications. This is an indication of low genetic diversity among the evaluated populations.

The percentage polymorphic loci range from 91% for the genotypes in Tetteh population to 100% in Awo and Nyame populations. The percentage polymorphic of 97% was observed in this study which was higher than 58.6% reported by [14]. Bekele [23] also reported percentage polymorphic loci of 81.21% which was lower that the value reported in this study. Higher value for percentage polymorphic loci is an indication of closely relatedness of the genotypes in each of the studied populations.

Analysis of molecular variance (AMOVA) revealed some low level of significance among the evaluated 43 genotypes ($P \geq 0.001$). Most genetic variation of 86% was attributed to among individuals in the three populations, while least genetic variation of 7% was accounted for between the populations. Low population genetic diversity observed among populations mighty be due to exchange of planting materials between the farmers resulting in increase in inbreeding. High population genetic diversity [25, 27,14] and low population genetic diversity [28] have been reported on several species of yam.

Fixation index (Fst), which is measure of the difference in allele frequency between populations was low (0.066). This implies that there is movement and sharing of genetic materials among the populations i.e. there is weak genetic differentiation among populations. This result is supported by the study conducted on genetic diversity and differentiation of *Juniperus thurifera* in Spain and Morocco as determined by SSR [29].

Principal coordinate analysis (PCoA) revealed that all the 43 genotypes were distributed into all the four groups in which they were grouped. Genotypes belonged to Awo population were found dominating in groups I and II. Genotypes belonged to Tetteh population were dominating groups III and IV while Genotypes belonged to Nyame population were scattered across all the groups. Widely distribution of the genotypes across the four groups is an indication that farmers are involving in the exchange of the planting materials among themselves which has resulted to the duplication of the genetic materials.

This study observed that the clusters were not far from one another. The weak genetic differentiation among the clusters may be attributed to low genetic distances among the populations. Weak population structure that exists among the populations allow the movement of genes resulting in effective communication between one another. Close similarities of these

genotypes among the populations is an indication of widely distribution of the genotypes of bush yam within this community. This agreed with Al Salameen et al. [30] who described that pairs of populations geographically close to each other will be more genetically similar because their seeds or pollen easily migrate within short distances. Absence of barriers have allow the movement of genetic materials within the locality and resulting to low genetic distances among genotypes within the populations.

Conclusion

Low level of genetic diversity and structural differences were observed among the evaluated genotypes in this study. High level of similarities were observed among the evaluated genotypes which might due to functional seed network or exchange of planting materials among the farmers within the study locality. This has resulted in the increase in planting the same genotypes but with different varietal names, based on the locality. This study has established limited information and research studies are available on the genetic diversity of bush yam (*Dioscorea praehensilis*). More studies need to be conducted using many regions and localities where bush yams are being cultivated to really understand the baseline of genetic diversity in this yam species.

Materials and Methods

Collection site and leaf sampling procedure

The germplasm studied consisted of 3 populations of *D. praehensilis* (Awo, Nyame and Tetteh). The populations have 17, 12 and 14 morphotypes respectively making a total of 43 *D. praehensilis* morphotypes. Plant materials were sourced from farmer's field at Amasamkrum village, Anomabo district, Central Region, Ghana (5° 37.8"N, 1° 33.3"E). Three piece of leaf samples were detached from each morphotype and placed inside covered plastic containers containing 10g of silica gel for preservation to remove moisture and also to prevent degradation of DNA from the leaves.

DNA extraction, quality and purity check procedures

DNA was extracted from leaves of all the 43 morphotypes using the modified cetyltrimethyl ammonium bromide (CTAB) procedure [31]. The concentrations and quality of DNA were measured following separation with a 1% agarose gel electrophoresis and a gel picture was captured using a UV light gel documentation system (Aplegen) to check the quality of the DNA. The DNA concentrations were estimated by measuring the absorbance at 260nm (A_{260}) and 280nm (A_{280}) in the Gene Quant pro spectrophotometer (Amersham Bioscience, Piscataway, NJ, USA). DNA Purity or quality was determined by calculating the ratio of absorbance at 260 nm and absorbance at 280 nm (A_{260}/A_{280}).

Polymerase chain reaction (PCR) procedure

To determine the genetic diversity among these morphotypes of *D. praehensilis*, eleven SSR primers were used (Table 1). DNA samples were diluted to a working solution of 50 ng/µl and was subjected to PCR reaction. Primer optimization was done initially to identify the best annealing temperature using first 8 genotypes and gradient protocol of optimizing PCR was used. The PCR cocktail had 10µl of the reagents (Ultra-pure water at 4.34µl, 10x NH4 (Reaction buffer) at 1µl, 50mM MgCl2 at 0.4µl, 25mM dNTPs at 0.2µl, DMSO at 1µl, 25ng/µl Forward primer at 0.5µl,

25ng/µl Reverse primer at 0.5µl, 5 U/ml Taq polymerase at 0.06µl and 50ng/µl DNA template at 2µl). The polymerase chain reaction followed an optimized program with initial denaturation at 94^{o}C for 3 min; denaturation at 94^{o}C for 1 min; annealing depending on the primers for 1 min; extension at 72^{o}C for 1 min; final extension at 72^{o}C for 10 min; and hold at 4^{o}C until the PCR products were removed from the thermocycler. The polymerase chain reaction products were electrophoresed on 2% agarose gel (2g agarose powder + 100ml 0.5X TBE buffer) with 1µg/ml ethidium bromide and ran in electrophoresis tank containing 0.5X TBE buffer at 100V for $1^{1/2}$ hours. Gel photographs were captured using a UV illuminator gel documentation system (Aplegen) and saved as TIFF images for easy uploading for gel analysis.

Gel scoring and molecular data analysis

Gel images were analyzed using Bio-rad image lab analysis software (version 6.0). The gel images were loaded into the software to generate the molecular size of the amplifications in base pairs of the respective markers. 50 base pair DNA molecular ladder (Biolab) was used as a ruler to estimate the molecular sizes of the DNA fragments. Where no amplification is detected was recorded as 0.

GenAlEx (Genetic Analysis in Excel) software version 6.503 [32] was used in estimating Number of Different Alleles (Na), Number of Effective Alleles (Ne), Shannon Information Index (I), Number of Observed Heterozygosity (Ho), Number of Expected Heterozygosity (He), Fixation Index (F), Allelic Pattern across the populations and Percentage of Polymorphic Loci (%P) across the three populations and the eleven SSR markers. Analysis of Molecular Variance (AMOVA) and Principal Coordinate Analysis (PCoA) were also computed via distance matrix using GenAlEx software version 6.503 [33]. The significance for AMOVA was determined at 9999 permutations. Major Allelic Frequency and Polymorphism Information Content (PIC) were estimated using PowerMarker software version 3.25. Cluster analysis was carried out using un-weighted pair group method with arithmetic mean (UPGMA) trees in Powermarker software version 3.25. The

dendogram was then generated using Molecular Evolutionary Genetic Analysis (MEGA-X) version 10.0.5.

Table 1: *Information of the 11 SSR markers used in this study*

Marker name	Marker sequence	Annealing Temp.°C	Observed marker Size in base pair
	F 5'- TGA AGA GAA TGT TGA GAT CGT ACC -3'		
YM16	R 5'- TAT CCG GCC CTC TCA TTG G -3'	56	87-180
	F 5'- GAC ATT GGG GAT CTC TTA TCA T -3'		
YM18	R 5'- TAG CAG CAG TAA CGT TAA GGA A -3'	48	259-304
	F 5'- GAT GGA GAT GAG GAG GCC G -3'		
YM25	R 5'- TTC GAA GCC AGA GCA AGT G -3'	57	197-269
	F 5'- TCC AGC TCT TTA GCA CAG G -3'		
YM27	R 5'- AGG AGC ATA GGC AAC AAG C -3'	55	215-236
	F 5'- CCA CAA CTA AAA ACA CAT GGA C -3'		
YM30	R 5'- GTG GTA GGG TGT GTA GCT TCT T -3'	49	212-250
	F 5'- AAG CCT AGT CGA TGG GTG G -3'		
YM31	R 5'- TGC TGT TCC AAC TTC CAA GC -3'	51	207-294
	F 5'- GCC TTG TTT TGT TGA TGC TTC G -3'		
YM43	R 5'- CCA GCC CAC TAA TCC CTC C -3'	52	178-225
	F 5'- CGC AAC CAG CAA AGG ATT TA -3'		
YM44	R 5'- ATT CTG TCT CTC AAA ACC CCT -3'	49	138-293
	F 5'- TGG GGT GAG AGA GTA AGT GG -3'		
YM49	R 5'- TCA CCG GGG ATC TTC TTG C -3'	52	116-146
	F 5'- TTG CCC TTG GGA TGT AGG G -3'		
YM50	R 5'- CAT CCC CGT TGT ATC CTG C -3'	52	184-296
	F 5'- AGT GGT GCT GTA GTA ACT GGA A -3'		
YM61	R 5'- CAT GAC TAC CTT TCC TCA ATC A -3'	50	217-290

F=Forward Primer, R=Reverse Primer

Author contributions

ASA, KJT, PAA and PAA^2 conceived the research topic. ASA, PAA^2 designed and executed the laboratory procedures, analyzed the data generated. ASA wrote the first draft manuscript and designed the tables and figures. KJT, PAA, PAA^2 and MOE read and edited the first draft. PAA^2 reviewed the final draft. All the authors agreed on the final draft.

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Conflict of Interest

The authors declare that they have no conflict of interest

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