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Assessment of Genetic Diversity of Dioscorea Praehensilis (Berth.) Collected from Central Region, Ghana Using Simple Sequencerepeat (SSR) Markers

Research article

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Abstract

In Ghana, Dioscorea praehensilis (Berth.) is one of the semi-wild yam cultivated speciesespecially in the cocoa grown regions. Recently, D.praehensilis has been reported to contribute to food security of the small households in the region. In this studywe evaluated the genetic diversityof 43 D. praehensiliscollected from three regions in Ghana using simple sequence repeat (SSR) markers. Using 11 SSR markers, 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 Genotypes into 2 groups while hierarchical cluster through UPGMA revealed the presence of 3 main clusters. Molecular variance (AMOVA) revealed low genetic diversity among the three genetic populations. Results from this study revealed the need to conduct extensive germplasm collection across the country where D. praehensilis are cultivated as source of food to really understand the genetic baseline for its proper utilization in the breeding program as source of novel genes.

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 many households [1]. Yam production in Ghana is estimated at about 5.8 million metric tonsand Ghana is considered as the second highest producing country after Nigeria [2]. The genus Dioscorea spp comprises of about 450 species among which, 50-60 species are considered as cultivated and semi-wild [3]. In Africa, the most cultivated species are areD. rotundata Poir D. alata L., D. bulbifera L., D. cayenesis Lam, D. esculenta (Lour.)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]. Dioscorea praehensilis is considered as wild yam in many countries in West and Central Africa however, this species has been introduced into yam production system recently due to his adaptation to climate change and is ability to tolerate yam disease such as yam anthracnose disease and yam mosaic virus [6]. Despite its nature of being wild or semi wild, D.praehensilis is an edible crop mostly found

around cocoa plantations in Ghana, and known to fill the hunger gaps (food and income security) among cocoa farmers in Ghana [7]. Due to the demographic pressure, many yam varieties of this species were erode and it genetic variability has been understudied. There is the need to explore molecular markers of genotypic characterization to determine the genetic variability levelin 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 genotypes 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 variabilitywhen 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 beenno rigorous study of the genetic diversity and genetic relationships currently in the collections of D. praehensilis using molecular markers. This research thereforeaimedat evaluating genetic variability in Dioscorea praehensilis yam genotypes using simple sequence repeat (SSR) markers and to understand the population structure in D. praehensilis for proper utilization for gene introgression.

Materials and Methods

Collection site and leaf sampling procedure

The germplasm studied consisted of 3 populations of D. praehensilis (Awo, Nyame and Tetteh). Awo population genotypes were gotten from germplasm survey conducted in 2016 season at Western and Eastern Regions of Ghana. Nyame population genotypes were seeds yam tuber collected from farmers at Central Region, Ghana while Tetteh population genotypes accessions of D. praehensilis maintained at the Department of Crop Science, University of Cape Coast, Central Region, Ghana. The populations have 17, 12 and 14 genotypes respectively making a total of 43 D. praehensilis genotypes. The 43 accessions were planted at cocoa farm 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 genotype 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 Genotypes using the modified cetyltrimethyl ammonium bromide (CTAB) procedure [24]. The concentrations and quality of DNA were measured following separation with a 1% agarose gel electrophoresis anda 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 (A260) and 280nm (A280) 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 (A260/A280).

Polymerase chain reaction (PCR) procedure

To determine the genetic diversity among these Genotypes of D. praehensilis, eleven SSR primers were used (Table 1). DNA samples were diluted to a working solution of 50ng/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 94oCfor 3 min; denaturation at 94oCfor 1 min; annealing depending on the primers for 1 min; extension at 72oCfor 1 min; final

extension at 72oC for 10 min; and hold at 4oCuntil 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 11/2 hours. Gel photographs were captured using a UV illuminator gel documentation system (Aplegen) and saved as TIFF images for easy uploading for gel analysis.

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

F=Forward Primer, R=Reverse Primer

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 [25] 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 [25]. 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).

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.

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	99.01	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

Genetic diversity among 43 D. praehensilisgenotypes based on the populations

Genetic diversity among 43 D. praehensilisgenotypes based on the populations is presented in Table 3. An average value of 0.89 was recorded as inbreeding coefficient (F) with the lowest value observed in Nyame population (0.84) while the highest value of 0.92 was recorded by Awo population. The highest Shannon's Information Index (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. The level of genetic diversity observed among 43 D. praehensilis genotypes of the three populations was very low. Nyame population recorded observed heterozygosity values of 0.14 while Awo and Tetteh populations recorded heterozygosity values 0.07 each, respectively.

Population differentiation and genetic structure

Molecular variance analysis (AMOVA) based on the Fst revealed low genetic variability (7%) among the three population evaluated while 86% value was recorded among individuals or genotypes. Fst value of 0.066 observed in this study indicated low genetic differentiation among the three population. Fis and Fst values of 0.93 each, respectively indicating high heterozygote deficits which may due to non-random mating within genotypes in the populations (Table 3).

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

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	14.5	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	0.93	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

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 16Genotypes originated from three populations Awo, Nyame and Tetteh respectively (Figure 1). Through the PCA, itis aclear indication of non-classification of the genotypes based on the three different populations.

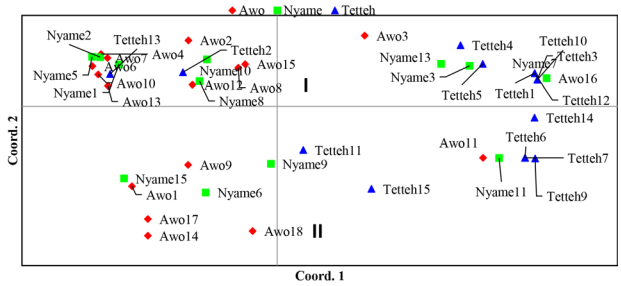

Figure 1: Principal coordinates analysis (PCoA) for genetic variability among 43 D. praehensilis genotypes

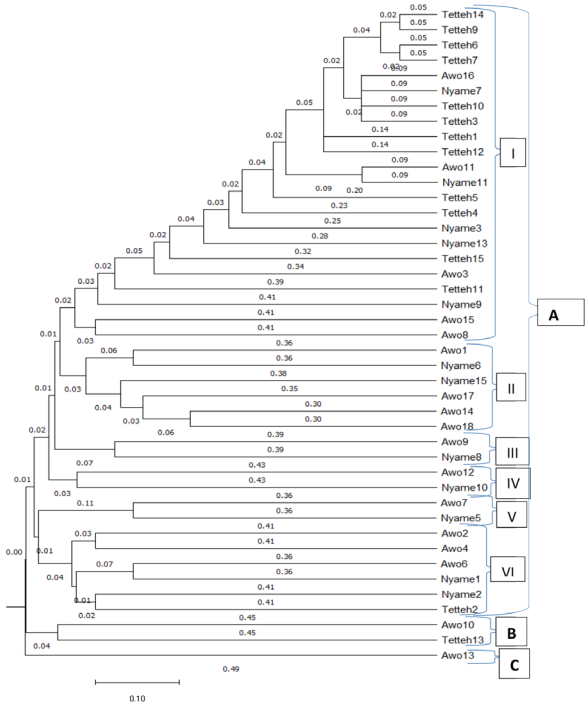

Figure 2: Dendogram showing genetic similarities among 43 genotypes of D. praehensilis based on 11 SSR primers using Euclidian similarity coefficients with UPGMA Clustering

Using the shared alleles dissimilarity matrix and based on the 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 2). Cluster A was further grouped into 6 sub-clusters (I, II, III, IV, V and VI) (Figure 2). Sub-cluster I contained 22 genotypes in which 5 belonged to Awo population, 5 belonged to Nyame population and 12 belonged to Tetteh population. In this sub-cluster, highest genetic distance (0.41) was observed in genotypes Awo8, Awo15 and Nyame9 while the least (0.05) was in Tetteh6, Tetteh7, Tetteh9 and Tetteh14. Sub-cluster II consisted of 6 genotypes in which 4 belonged to Awo population and 2 belonged to Nyame population with the highest genetic distance (0.38) was observed in Nyame6 while the least (0.35) was obtained in Awo14 and Awo18. Sub-clusters III, IV and V contained 2 genotypes each with 1 belonged to Awo population and 1 belonged to Nyame population, respectively. Both genotypes in each of the 3 sub-clusters (III, IV, and V) have the same genetic distances 0.39, 0.43 and 0.43, 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 with the highest distance (0.41) was obtained in Awo2, Awo4, Nyame2 and Tetteh2 while the least genetic distance (0.36) was obtained in Awo6 and Nyame1 . Cluster B consisted of 2 Genotypes in which 1 each belonged Awo and Tetteh populations with the same genetic distance (0.45), respectively. Cluster C contained 1 genotype belonged to Nyame population with genetic distance (0.49). In each population, low genetic distance was identified, the highest tendency for the genotypes to have been duplicated.

Discussion

Simple sequence repeat markers have been extensively employed in accessing genetic diversity among other species of yam [14,21,22,]. In this present study, the genetic diversity of 43 yam Genotypes of D. praehensiliswere analyzed 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. Higher number of alleles reported in this present study indicated the high reproducible ability of the 11 SSR primers. This result was later confirmed with the high polymorphism information content (0.82) obtained in this study.

In this study, we reported very low gene diversity (observed heterozygosity) of 0.09 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. The low genetic diversity observed in this present study might be due to the domestication of very few D.praehensilis over the time by the farmer and the continuous exchange of planting materials among the relatives and neighbors across the collection sites of these genotypes. Through the AMOVA, most of the genetic variability (86%) was attributed to individuals in the three populations, while least genetic variation of 7% was accounted for among the populations. Indeed, in Africa especially in West Africa, where there is no yam formal system, good planting materials are exchanged by neighboring farmers resulting in continuous duplication of the same materials across the growing areas.

Principal coordinate analysis (PCoA) revealed clear separation of the genotypesinto two major groups of genetically close genotypesconsisted of the three populations. However, PCoA and UPGMA analyses could not distinctly separate the 43 genotypesanalyzed based on their populations. As hypothesized, these could be as a result of some of theD.praehensilisgenotypes were domesticated from one region of the country which was later spread across other regions through informal seed system exchange.

Close similarities of these genotypes among the populations is an indication of widely distribution of the genotypes of D. praehensilis around the collection areas which has been introduced in the yam production system in Ghana. This agreed with Al Salameen et al. [26] 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 among the populations.

Our results showed low level of genetic differentiation within this species of yam, probably due to unlimited gene flow as a result of random mating. This results showed the genotypes from the three populations are genetically similar.

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 andresearch 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

Author contributions

ASA, KJT, PAA and PA conceived the research topic. ASA, PA designed and executed the laboratory procedures, analyzed the data generated. ASA wrote the first draft manuscript and designed the tables and figures. KJT, PAA, PA and MOE read and edited the first draft. PA corrected the whole manuscript and approved. 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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