



## Genetic characterization of some water yam (*Dioscorea alata* L.) accessions in West Africa with simple sequence repeats

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### Abstract

A collection of 89 water yam (*Dioscorea alata* L.) accessions from Benin, Congo, Côte d'Ivoire, Equatorial Guinea, Gabon, Ghana, Nigeria, Sierra Leone and Togo was assessed for genetic diversity using thirteen microsatellite loci. These 89 are some of the *D. alata* accessions conserved by the International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria. A total of 97 alleles were detected with an average allele number of 7.46 per locus. Polymorphism information content (PIC) mean value of 0.65 showed existence of variability among the accessions. Accessions from Nigeria showed highest gene diversity of 0.678 while those from Côte d'Ivoire had lowest diversity with 0.596. Observed mean heterozygosity value of 0.469 was observed. Cluster and principal coordinate analysis showed 8 major cluster groups. There was no relationship between relatedness of the accessions and their geographical area of collection. SSR markers proved to be effective to characterise studied *D. alata* germplasm.

**Key words:** Core germplasm, *Dioscorea alata*, genetic diversity, microsatellite.

### Introduction

Food yams (*Dioscorea* spp.) are economically important starchy staples in West Africa, Asia, Far East, the Pacific and the Caribbean regions. These regions are considered main centres of yam domestication and diversity<sup>1</sup>. The yam belt of West Africa accounts for 95% of the global annual production of yams now estimated at over 51 million metric tons<sup>2</sup>. Yam is a multi-species tuber crop with *Dioscorea alata* L. (water yam) being the most widely distributed species globally because of its agronomic flexibility and productive potential. Despite the importance of *D. alata* in the diets of millions of poor people, and its contribution to national economies, several cultivars are susceptible to pests and diseases and lack the aesthetic values of smooth skin and elegant tuber shape that appeal to consumers in the market. These problems and farmers' expectations underscore the need to develop improved varieties that combine higher and more sustainable yields with market preferred attributes. The International Institute for Tropical Agriculture (IITA, Ibadan, Nigeria) holds in trust in its genebank, one of the largest collections of *D. alata* germplasm. An understanding of the diversity structure of these collections provides a needed baseline data for efficient breeding scheme. The genetic diversity and relationships in the *D. alata* germplasm can be more reliably assessed with DNA based markers. Various molecular assays have been applied in yam research including RAPDs<sup>3</sup>, AFLPs<sup>4,5,6,7,8</sup> and SSRs<sup>4,9,10</sup>. Our aim in this study was to assess the diversity of some *D. alata* germplasm held in trust by IITA using SSR markers.

### Materials and Methods

**Plant material:** Eighty-nine accessions of *Dioscorea alata* L. (landraces and breeder's lines) representing entries from nine countries (Table 1) were used for the study. Plants were cultivated in 30 cm size pots filled with sterilized top soil and maintained in a screenhouse at IITA, Ibadan, Nigeria.

**DNA extraction and quantification:** Genomic DNA was extracted from fresh leaf apex of young leaves using modified CTAB procedure as described by Mignouna *et al.*<sup>11</sup>. The quality and concentration of DNA was assessed by gel electrophoresis using 1% agarose with known concentrations of undigested lambda DNA (Sigma, St Louis, MO, USA). Quantification of DNA was done using a spectrophotometer (Beckman Coulter DU530) at 260 nm. Extracts were diluted in water to obtain DNA concentration of 25 ng/μl.

**Polymerase chain reaction and fragment analysis:** A total of thirteen SSR primer pairs as described by Mignouna *et al.*<sup>4</sup> and Tostain *et al.*<sup>12</sup> were used in the study (Table 2). PCR reaction was conducted in a 20 μl volume in a 96-well microtiter plate using an automated thermal cycler (Peltier Thermal Cycler 200). The reaction volume contained 25 ng of template DNA, 100 μM each of dNTP, 2.5 mM MgCl<sub>2</sub>, 0.5 μM each of fluorescently labelled forward primer and unlabelled reverse primer, 1X reaction buffer and 2 units of Taq DNA polymerase (Invitrogen). The forward primer was 5'-labelled with one of the four fluorochromes PET,

**Table 1.** List and country of collection of the 89 accessions of *D.alata* analysed in the present study.

Accession number	Local name	Country of collection
TDa 1178	BE 136	Benin
TDa 1190	BE 114	Benin
TDa 1240	BE 112	Benin
TDa 3898	Sanse	Benin
TDa 3901	Biowonkourou	Benin
TDa 3919	Doutierou	Benin
TDa 3922	Souanrou	Benin
TDa 3936	Garko	Benin
TDa 3944	Agba	Benin
TDa 3701	Mpalakala	Congo
TDa 3703	Unkown	Congo
TDa 1194	IC 20	Côte d'Ivoire
TDa 1196	IC 28	Côte d'Ivoire
TDa 1198	IC 48	Côte d'Ivoire
TDa 1239	IC 28	Côte d'Ivoire
TDa 1250	IC 3	Côte d'Ivoire
TDa 3925	N'zasegula	Côte d'Ivoire
TDa 3128	EQ-89 - 23	Equatorial Guinea
TDa 1313	PS/89/168	Gabon
TDa 1297	Dansiesumne 1	Ghana
TDa 1328	Sieduble	Ghana
TDa 1333	Akwa	Ghana
TDa 2839	Borlobolo	Ghana
TDa 2844	Dansiesumne 2	Ghana
TDa 2846	Adiamawoba	Ghana
TDa 2849	Kronkosi	Ghana
TDa 2851	Akwa 2	Ghana
TDa 3269	Alamunkpiti	Ghana
TDa 3275	Mensmfi	Ghana
TDa 1286	Fayinka	Nigeria
TDa 1347	85/0253	Nigeria
TDa 1391	UM 680	Nigeria
TDa 1404	(29) 40	Nigeria
TDa 1416	D3	Nigeria
TDa 1429	UYT TDa 96	Nigeria
TDa 1430	PYT 85/0236	Nigeria
TDa 1431	86/00611	Nigeria
TDa 1441	UYT TDa 96	Nigeria
TDa 1442	HT 86/88682	Nigeria
TDa 1454	87/0305 - 19	Nigeria
TDa 1467	86/00611	Nigeria
TDa 3743	BN 301	Nigeria
TDa 3911	Obonse	Nigeria
TDa 3914	Souwanrou	Nigeria
TDa 3920	Aluwinrin	Nigeria
TDa 4041	Weredede	Nigeria
TDa 4127	Unkown	Sierra Leone
TDa 4134	Unkown	Sierra Leone
TDa 4139	Yamsigboi	Sierra Leone
TDa 3962	Ngaobule	Sierra Leone
TDa 4146	Yamssiegbolie	Sierra Leone
TDa 2806	Betebete	Togo
TDa 1032	Gnagnassion	Togo
TDa 1045	Kabanga S-1 639	Togo
TDa 1060	Tonfou	Togo
TDa 1065	Unkown	Togo
TDa 1066	Fanamawe T25	Togo
TDa 1069	Gnalabi	Togo
TDa 1087	Sakata S 85	Togo
TDa 1089	Olancodje Hoe	Togo
TDa 1096	Kaki A 86	Togo
TDa 3902	Fabangasot 135	Togo
TDa 1108	Lambor mande KN 82	Togo
TDa 1123	Tabere T-39	Togo
TDa 1124	Kere 2B 65	Togo
TDa 1130	Ogbo 248	Togo
TDa 4152	Sova2B 50	Togo
TDa 1159	Awe 71	Togo
TDa 1164	Fetiou BH 34	Togo

**Table 1.** (Continued).

Accession number	Local name	Country of collection
TDa 1165	640	Togo
TDa 1201	Coulou	Togo
TDa 1209	Tsrokpa784	Togo
TDa 1210	Kpent 112	Togo
TDa 1157	226	Togo
TDa 1295	TcussenKN 19	Togo
TDa 1379	Akooa	Togo
TDa 2869	Guete 43	Togo
TDa 3152	Otsrope 407	Togo
TDa 3163	Kabanga S-1 639	Togo
TDa 3168	Tsrorpa154	Togo
TDa 3177	Akpoyide 68	Togo
TDa 3187	Tfigou D-9 - 1156	Togo
TDa 3195	Lotossou 399	Togo
TDa 3202	Kaki AB-6 972	Togo
TDa 3207	Tifiou BH-32 1125	Togo
TDa 3215	Ogbo 186	Togo
TDa 3221	Afassetchissem KN-91	Togo
TDa 3231	Gnagnassi40	Togo
TDa 3234	Adigo 303	Togo

6-FAM, NED and VIC. The PCR programme consisted denaturation at 94°C for 4 min, followed by 34 cycles of 94°C for 30 s, 51 or 58°C for 1 min and 72°C for 1 min, with a final extension step at 72°C for 7 min. Capillary electrophoresis with a semi-automated system ABI PRISM 3100 Genetic Analyser was used to separate amplified PCR products. Samples for amplified product separation were prepared by adding 1 µl of diluted PCR products to 9.4 µl formamide and 0.1 µl GenSize-500 LIZ. This was dispensed in ABI 96-well plates and were denatured at 94°C for 5 min and allowed to cool down on ice.

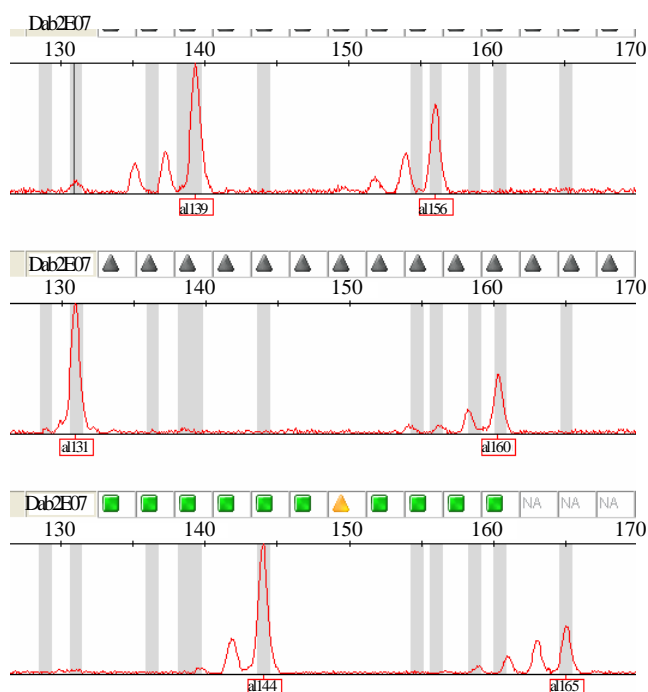
**Data analysis:** Observed allelic data were binned into discrete units and SSR fragment sizes were called using Genemapper v.3.7 software (Fig. 1). The fragment sizes in base pairs for each genotype across SSR markers were converted to binary data where alleles were transformed into presence (1) or absence (0) of an SSR band. Missing data accounted for less than 5% (i.e. marker × genotype) of the entire data set. The genetic diversity parameters (Table 2) such as number of alleles per locus, percent of polymorphic loci, observed heterozygosity and gene diversity according to Nei<sup>13</sup> were estimated with FSTAT v. 2.9.3 software<sup>14</sup>. The tree structure (Fig. 2) of the genetic diversity was constructed using DARwin 5.0 software using OTUs distance. A principal component analysis (PCA) with binary data was also performed using the SAS v 9.1<sup>15</sup>.

## Results

A total of 97 alleles were amplified with 13 SSR loci analyzed in 89 accessions, with the number of alleles observed per locus varying from 3 to 9 alleles (Table 2). The observed heterozygosity average of 0.469 varied from 0.238 (Da1F08) to 0.804 (Dab2E07). A total diversity of 0.651 was observed for the studied accessions. Polymorphism was observed in all thirteen microsatellite loci analysed (Table 2). Polymorphic information content (PIC) ranged from 0.30 detected in locus Dab2C05 to 0.82 detected in locus Dpr3D06. Average PIC value was 0.65. The unweighted neighbour joining derived radial phylogram constructed for the accessions provides an overview of the diversity structure (Fig. 2) resulting in eight distinct clusters. Accessions from different countries were fairly represented within each cluster. The scatter graph from the

**Table 2.** Primer sequences (forward/reserve) used in the SSR analyses and their respective annealing temperature (Ta), number of alleles per locus (A), observed heterozygosity (Hobs) and polymorphic information content (PIC).

Microsatellite name	5' to 3' Primer sequence	T <sub>a</sub> (°C)	A	H <sub>obs</sub>	PIC
Da1F08	AATGCTTCGTAATCCAAC -F CTATAAGGAATTGGTGCC -R	51	6	0.238	0.58
Dab2C05	CCCATGCTTGTAGTTGT -F TGCTCACCTCTTACTTG -R	51	9	0.288	0.30
Dab2D08	ACAAGAGAACCGACATAGT -F GATTTGCTTTGAGTCCTT -R	51	8	0.503	0.60
Dab2E07	TTGAACCTTGACTTTGGT -F GAGTTCCTGTCCTTGGT -R	51	9	0.804	0.80
Dpr3B12	CATCAATCTTCTCTGCTT- F CCATCACACAATCCATC -R	51	9	0.862	0.79
Dpr3D06	ATAGGAAGGCAATCAGG -F ACCCATCGTCTTACCC -R	51	9	0.241	0.82
Dpr3F12	TCCCCATAGAAAACAAAGT -F TCAAGCAAGAGAAAGGTG -R	51	9	0.345	0.77
Dpr3F04	AGACTCTTGCTCATGT -F GCCTTGTTACTTTATTC -R	51	6	0.511	0.58
Da1A01	TATAATCGGCCAGAGG - F TGTTGGAAGCATAGAGAA -R	51	7	0.489	0.72
YM5	AATGAAGAAACGGGTGAGGAAAGT -F CAGCCCAGTAGTTAGCCCATCT -R	58	6	0.512	0.65
YM13	TTCCCTAATTGTTCTCTTGTG -F GTCCTCGTTTTCCCTCTGTGT -R	58	3	0.361	0.56
YM15	TACGGCCTCACTCCAAACACTA -F AAAATGGCCACGTCTAATCCTA -R	58	7	0.373	0.76
YM26	AATTCGTGACATCGGTTTCTCC -F ACTCCCTGCCACTCTGCT -R	58	9	0.572	0.57



**Figure 1.** Examples of SSR profiles obtained for 3 yam accessions with marker *Dab2E07* using analysis software GeneMapper v. 3.7 (Applied Biosystems, USA).

first three principal components in the PCA analysis, which explained 34% of total variation, also indicated the genetic variability of the accessions when examining the distribution of the eight groups (Fig. 3). This graph agrees with the cluster analysis described above.

## Discussion

Knowledge of genetic diversity of the of *D. alata* germplasm using DNA based markers like SSR is important for efficient conservation and utilization. Our study detected an average of 7.46 alleles per locus. Gene diversity values of 0.651 on average were also observed. The results demonstrate genetic polymorphism in *D. alata* germplasm from Benin, Congo, Côte d' Ivoire, Equatorial Guinea, Gabon, Ghana, Nigeria, Sierra Leone and Togo. This genetic variation offers high potential for genetic improvement because it implies high amount of genetic variance upon which selection could be made for breeding. Polymorphism was observed in all thirteen microsatellite loci analysed (Table 2). Polymorphic information content ranged from 0.30 (Locus Dab2C05) to 0.82 (Locus Dpr3D06) with an average value of 0.65. Our result shows that available SSR markers are discriminatory enough in this species. The 0.469 mean observed value for heterozygosity in this vegetatively propagated crop is expected due to the fact that yams are dioecous and implies that spontaneous hybridization must have contributed to the ancestry of some of the accessions and improvement by farmers must have been far more often by selection of somatic mutants. Gene diversity was highest (0.669) within accessions collected from Nigeria and suggests that the country is a regional centre of diversity. An alternative explanation could be that the numerous introductions that were made over the years by IITA and National Root Crops Research Institute Umudike, both in Nigeria, might have influenced the diversity in the country. Accessions from Côte d'Ivoire, with 0.596 gene diversity, represent the least in diversity amongst the countries. It is interesting to note that in Côte d'Ivoire *D. alata* is a major staple and constitutes 65% of yams grown in the country<sup>16</sup>. The relatively low diversity observed suggests the dominance of a narrower range of cultivars suited to the utilization pattern of yam in the country over the years.

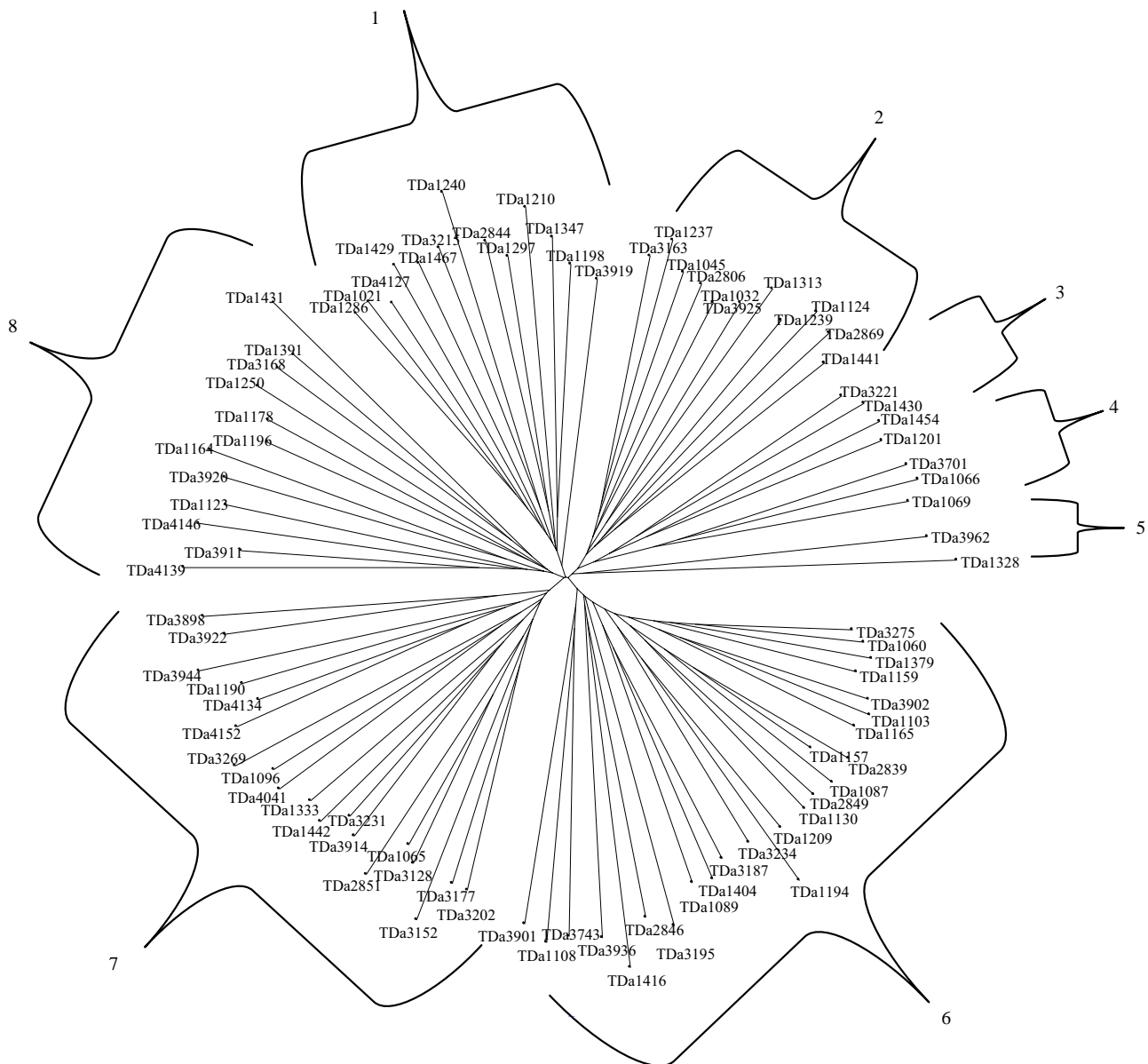


Figure 2. Radial dendrogram of 89 accessions of *D. alata* based on unweighted neighbour joining cluster analysis.

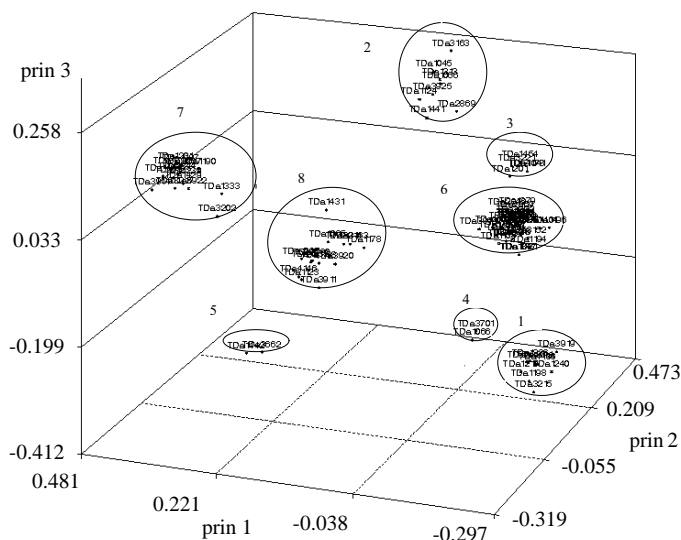


Figure 3. Three-dimensional plot of 89 accessions of *D. alata*, on principal component axes I, II and III.

The non-distinction between country cultivars of *D. alata* as shown in the clustering pattern strengthens the inference that clones must have been widely distributed and agrees with previous studies of Malapa *et al.*<sup>5</sup>, Egesi *et al.*<sup>6</sup> and Tamiru *et al.*<sup>8</sup> that used AFLP markers. *D. alata* is indigenous to Asia and most varieties cultivated in West Africa were introduced to this sub-region in the 16<sup>th</sup> century. These accessions must have been distributed over great distances as clones during centuries of human migration and it is possible that some of them share common origins. The majority of accessions within clusters are most likely clones of a common source. The cluster group variation suggests that the mutation rate is really high and that lots of mutations are not neutral, i.e. have an effect on the phenotype. This is expressed in morphological variation among the cultivars presenting different colours and shapes for both their aerial and underground organs.

**Table 3.** Country gene diversity per locus, total diversity and overall mean total diversity.

Locus	Benin	Côte d'Ivoire	Ghana	Sierra Leone	Nigeria	Togo
Da1F08	0.708	0.579	0.750	0.556	0.534	0.507
Dab2C05	0.504	0.232	0.167	0.367	0.458	0.303
Dab2D08	0.595	0.500	0.700	0.711	0.656	0.636
Dab2E07	0.773	0.750	0.867	0.828	0.721	0.681
Dpr3B12	0.839	0.622	0.833	0.717	0.784	0.786
Dpr3D06	0.913	0.894	0.900	0.839	0.862	0.841
Dpr3F12	0.764	0.711	0.717	0.750	0.750	0.740
Dpr3F04	0.605	0.514	0.633	0.533	0.623	0.613
Da1A01	0.809	0.622	0.733	0.756	0.634	0.722
YM5	0.364	0.417	0.633	0.569	0.647	0.634
YM13	0.564	0.643	0.617	0.618	0.621	0.507
YM15	0.777	0.666	0.650	0.625	0.798	0.723
YM26	0.424	0.595	0.633	0.639	0.727	0.544
Total diversity	0.665	0.596	0.679	0.654	0.678	0.634
Mean total diversity	0.651					

Note. - Only countries with more than two entries were included in this analysis.

### Conclusions

The information generated in this study would be valuable for breeding and conservation of the species in addition to providing insights on the phylogeny and evolution of the genus *Dioscorea*.

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