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RESEARCH ARTICLE

Genetic diversity and relationship of Guinea yam (*Dioscorea cayenensis* Lam.–*D. rotundata* Poir. complex) germplasm in Benin (West Africa) using microsatellite markers

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Abstract Guinea yam (*Dioscorea cayenensis–D. rotundata* complex) is an important tuber crop that highly contributes to food security and poverty alleviation in Benin. The knowledge and understanding of the extent of genetic variation of Guinea yam germplasm is important for planning of the genetic conservation, and the utilisation of this resource. The objective of this study was to assess the genetic diversity and relationships of 64 yam landraces whose dried chips are considered as resistant to insect attacks using 41 simple sequence repeat. Among these primers, 13 were found to be polymorphic, giving 113 polymorphic alleles. The number of alleles per locus ranged from 4 (Ym50) to 13 (Ym29), with an average of 8.69. Unique allele was observed with some landraces (Singou and Tchakatchaka) and can be considered as unique gene and use in yam breeding program. The mean polymorphic information content values for all markers used was 0.76 and ranged between 0.58 and 0.91 in loci YM3 and YM32 respectively. The genetic distance of yam landraces ranged from 0.45 (Yasoubagarou) to 0.04 (Assinapeira and Alahina), indicating that the yam germplasm has a high degree of genetic diversity supported by an averagely observed heterozygosity of 0.78. Cluster analysis using unweighted pair group method with arithmetic average grouped the 64 yam landraces into two distinct clusters. This tendency was also observed in the principal coordinate analysis. The analysis of molecular variance revealed that 96 % of the variation was found within the population and only 4 % between the populations. Genetic diversity and relationship assessments among the 64 yam landraces of Benin could provide useful information for efficient use of these materials, especially for genetic improvement.

Keywords Analysis of molecular variance · Benin · *Dioscorea cayenensis–D. rotundata* complex · Genetic diversity · Geographic pattern · SSR markers

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Introduction

Yam (*Dioscorea* spp.) is an economically important food crop in West Africa. Benin is the fourth world yam producer behind Nigeria, Ivory Coast and Ghana (FAO 2014). Several Dioscorea species are cultivated all over the countries where yam is priority agricultural food crop in Africa. Among these species, the

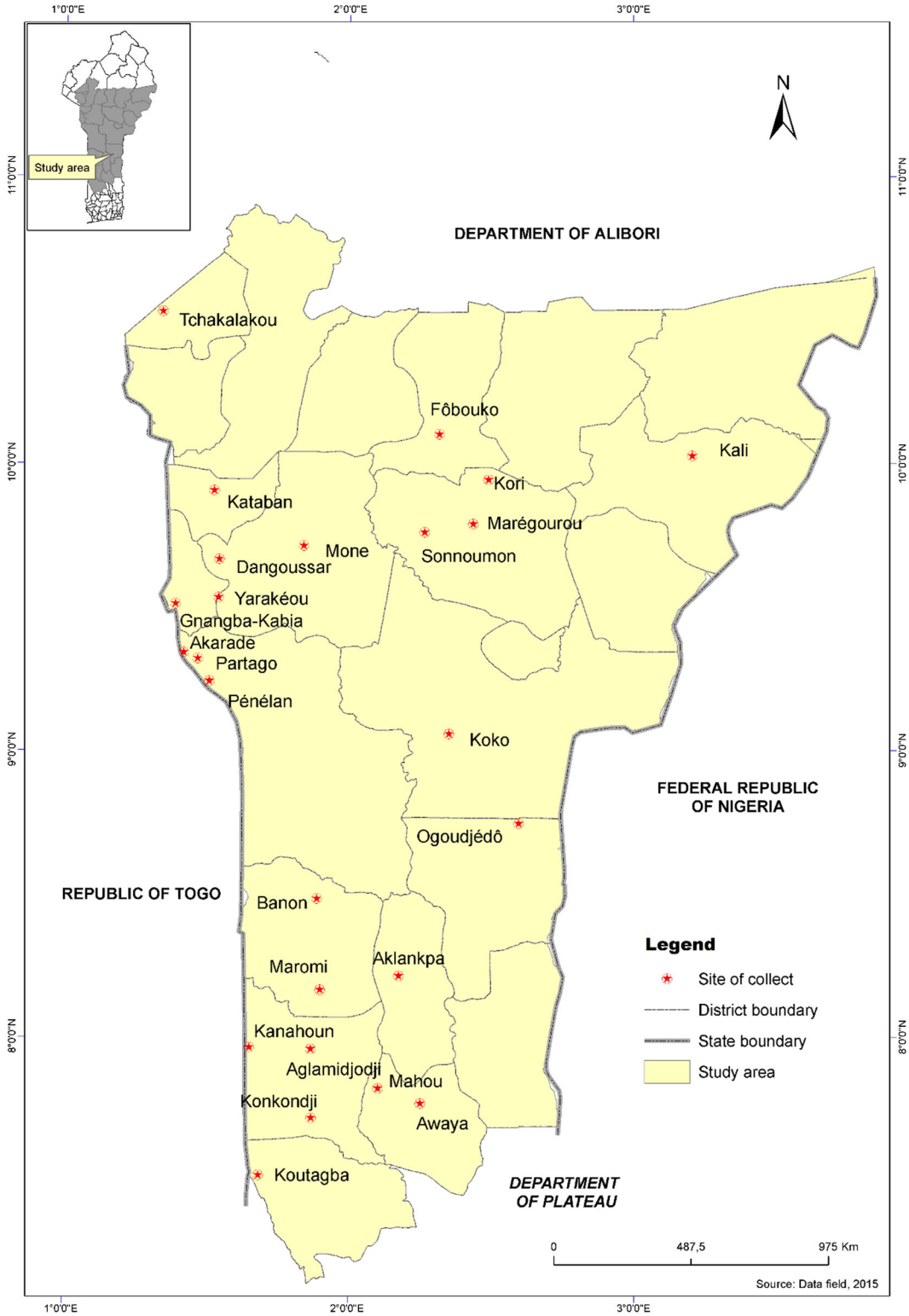

Fig. 1 Map of Benin showing the geographic distribution of villages were the 64 yam landraces have been collected

native *D. cayenensis–D. rotundata* complex (called Guinea yam) remains the most cultivated, since 95 % of total yam production relies on this complex (Dansi et al. 1999). This priority given to the cultivation of this yam complex is rooted in their preference in food consumption and therefore, the high economical potential its offers to small scale farmers (Loko et al. 2013a). Unfortunately, yam tubers are highly perishable with 65–85 % post-harvest losses being the major problem for farmers and traders preventing yearlong availability of yam for consumers (Umogbai et al. 2013). Overcome these issues, in order to enhance livelihood: increasing income generation and food security (Loko et al. 2013b).

The brown dough made with yam chips powder known as "Amala" in Yoruba, "Telibo" in Fon and Kanakou in Dendi is highly appreciated throughout the country (Bricas and Vernier 2000). Unfortunately, yam chips are often severely infested by insects, which can reduce whole stocks to dust within just a few months and strategies to control these pest often lead to food poisoning (Vernier et al. 2005; Loko et al. 2013b). The use of tolerant/resistant yam varieties will be the only sustainable strategy to limit the damage of insect pests on stored yam chips (Dansi et al. 2013).

Based on the hypothesis that those varieties would exist in the Benin traditional agriculture, a participatory variety evaluation survey was recently conducted throughout the country and yielded 64 landraces whose yam chips are resistant to insects infestation (Loko et al. 2015). Because of synonymies, it is very unlikely that all the farmer-named landraces correspond to genetically different cultivars. Morphological characterization of these 64 landraces revealed the existence of many duplicates (Loko et al. 2015). However, knowing that morphological traits have a number of limitations, such as low repeatability, late expression, and vulnerability to environmental influences (Smith and Smith 1992), the duplicate samples should be identified using molecular markers in order to have a set of unique individual (Otoo et al. 2009).

In yam research, a number of molecular markers have been applied to investigate the genetics of *Dioscorea* species. Therefore, Random Amplified Polymorphic DNAs (RAPDs) (Zannou et al. 2009), Amplified Fragment Length Polymorphism (AFLP) (Mignouna et al. 1998; Tamiru et al. 2007), Microsatellites or Simple Sequence Repeats (SSRs) (Tostain et al. 2007; Obidiegwu et al. 2009), and genotyping by sequencing (GBS) (Girma et al. 2014) were used to assess the genetic diversity and relationships of some landraces of Guinea yam. Despite the increasing popularity of genotyping by sequencing (GBS), SSRs markers remain competitive for genetic diversity studies because of their multi-allelic, highly polymorphic, co-dominant, high reproducibility character (Kawuki et al. 2009). Therefore, SSRs markers provide rich genetic information with good genome coverage (Lehka et al. 2010; Turyagyenda et al. 2012).

The objectives of this study are to assess the level of genetic diversity among 64 landraces of Guinea yam (*D. cayenensis–D. rotundata* complex) using single sequences repeats (SSRs), and to determine their genetic relationships in order to lay the foundation for the development of strategies for conservation and improvement of this crop.

Materials and methods

Plant material

Material of the 64 yam landraces were collected from 25 villages in the major yam cultivation regions in Central and Northern Benin (Fig. 1; Table 1). The accessions were planted in 30 cm size pots filled with sterilized loamy soil and maintained in a screen-house conditions at the International Institute of Tropical Agriculture of Ibadan (Nigeria). Overhead irrigation was used to ensure the establishment of the seedlings. After 2 months, samples of 5–10 young fresh leaves were collected per landrace and immediately put on ice for DNA extraction.

DNA extraction

DNA was extracted in the laboratory of Bioscience Center of International Institute of Tropical Agriculture of Ibadan (Nigeria) using standard procedures according to Dellaporta et al. (1983) with slight modifications. Freshly harvested young leaves of each individual were lyophilized for 72 h. About 250 g of lifolised leaves were put in a 2 mL tube and ground in geno-grinder (Spex certiprep genogrinder 2000) for 2 min to obtain the powder of each individual. Proteins and polysaccharides were precipitated by adding 500 µL of Hepes buffer mixed with pined for 5 min. The supernatant was decanted and about

Table 1 List of yam cultivars used for SSR analysis and origins

Code	Landrace name	Village	Region	Code	Landrace name	Village	Region
1	Kparokoume	Aglamidjodji	Centre	33	Singor	Fôbouko	Nord
2	Korou	Kanahoun	Centre	34	Gaboubaba	Koko	Nord
3	Allahina	Awaya	Centre	35	Tchinguita	Tchakalakou	Nord
4	Itikpa-agoudan	Konkondji	Centre	36	Tambouinrou	Sonnoumon	Nord
5	Kpinin	Koutagba	Centre	37	Kounto	Sonnoumon	Nord
6	Koukou	Konkondji	Centre	38	Tougbana	Akarade	Nord
7	Sega	Aglamidjodji	Centre	39	Porchahabim	Kali	Nord
8	Kokoro gbanbe	Ogoudjédô	Centre	40	Akoko	Fôbouko	Nord
9	Houbonon	Aglamidjodji	Centre	41	Tamba	Akarade	Nord
10	Djahe	Awaya	Centre	42	Babeterou	Kataban	Nord
11	Kokoro Lakolako	Banon	Centre	43	Deba	Koko	Nord
12	Agbale	Banon	Centre	44	Koumanan	Koko	Nord
13	Agadabangahi	Banon	Centre	45	Kinkerekou	Fôbouko	Nord
14	Adjawoungbo	Kanahoun	Centre	46	Gorougo	Kali	Nord
15	Tchakatchaka	Konkondji	Centre	47	Otoukpanan	Koko	Nord
16	Ayedou	Kanahoun	Centre	48	Dankpenai	Kataban	Nord
17	Tchadjo	Banon	Centre	49	Mouhame	Mone	Nord
18	Koriodjo	Banon	Centre	50	Gnombouanri	Sonnoumon	Nord
19	Adjogba	Kanahoun	Centre	51	Djessoume	Kataban	Nord
20	Alagbara	Mahou	Centre	52	Awonaou	Patargo	Nord
21	Adakada	Aklamkpa	Centre	53	Boniourésouan	Koko	Nord
22	Gbode	Maromi	Centre	54	Assinapeira	Akarade	Nord
23	Yasoubagarou	Fôbouko	Nord	55	Yakanougo	Koko	Nord
24	Aye	Fôbouko	Nord	56	Omonya	Koko	Nord
25	Iootchra	Kataban	Nord	57	Wonmangou	Fôbouko	Nord
26	Tamsam	Fôbouko	Nord	58	Bonioure	Kataban	Nord
27	Boiboi	Kori	Nord	59	Adada	Yarakéou	Nord
28	Kprakpra	Sonnoumon	Nord	60	Singou	Fôbouko	Nord
29	Ilorin	Pénélan	Nord	61	Aizoi	Gnangba-Kabia	Nord
30	Soanin	Marégourou	Nord	62	Bodi	Kanahoun	Nord
31	Kourakourogourouko	Koko	Nord	63	Assi	Mone	Nord
32	Gree	Dangoussar	Nord	64	Sowoukou	Koko	Nord

450 µL of 1 % preheated (65 °C) sodium dodecyl sulfate (SDS) buffer extract was added to each tube and the mixture homogenised. The tubes were incubated at 65 °C for 30 min with intermittent inversions and incubated at room temperature for 5 min to cool it down. To break down and make separation of DNA from proteins and other cellular debris, 450 µL of chloroform isoamyl alcohol (24:1) was added. The supernatant was carefully transferred to a new tube and 300 µL of ice-cold isopropanol was added and mixed by inverting gently to precipitate crude DNA. For properly activity of isopropanol, the mixture was incubated at −20 °C for 1 h and centrifuged at 13.2 rpm for 20 min. The supernatant was poured off and the last drops of isopropanol were removed using 70 % (300 µL) of ethanol, mixed gently and centrifuge at 13.2 rpm for 10 min after which, the supernatant was gently poored off and the DNA was dried by placing Eppendorf face down on paper towels for 30 min. After draining, 300 µL low salt TE was added to each sample followed by 3 µL RNAse. For DNA purification, the tubes were incubated at 37 °C for 1 h, 315 µL of Chloroform isoamyl alcohol (24:1) was added and the tubes were properly mixed and

were spined at 13.2 rpm for 20 min. The supernatant was collected in a new tube and about 315 µL of ethane sodium acetate PEG was added and the samples were incubated at −20 °C for proper DNA precipitation for 30 min. Samples were removed and cooled down at room temperature for 5 min and centrifuge at 13.2 rpm for 20 min. The supernatant was poured off and the remaining solution was removed using 70 % (300 µL) of ethanol, mixed gently and centrifuge at 13.2 rpm for 10 min after which, the supernatant was gently poor off and the DNA was dried by placing eppendorf face down on paper towels for 30 min. After draining, 100 µL of ultra-pure water was added to each sample. The concentration and purity of 64 DNA were checked by Nano-Drop spectrophotometer at A260 and A280, while the integrity of DNA was analysed by 1 % agarose gel electrophoresis in TBE buffer stained with ethidium bromide. The DNA samples were standardised to 25 ng/µL before PCR analysis.

Polymerase chain reaction and polyacrylamide gel electrophoresis

Polymerase chain reaction was carried out in a PCR system using forty-one (41) SSR primers (Table 2) developed by Tamiru et al. (2015). Each primer was screened with 8 yam landraces to choose the polymorphic SSR primers. The PCR was carried out in a total volume reaction of 10 µL mixture including 2 µL (25 ng/µL of DNA); 1 µL of 10X PCR buffer, 0.4 µL dNTPs (5 mM), 0.4 µL $MgCl_2$ (50 mM); 1 µL (5 mM) of each of the forward and reverse primers, 0.06 µL Taq polymerase and top it to 10 µL by adding ultra-pure water. The PCR were carried out using a thermocycler including pre-denaturation at 94 °C for 2 min followed for each 15 s at 93 °C, annealing at 55 °C for 20 s −1 °C/cycle, and an extension for 30 s at 72 °C. A final 24 cycles consisting of 15 s at 94 °C, 20 s at 45 °C, and 30 s at 72 °C and a final extension of 5 min at 72 °C. The PCR products were checked on agarose (2 % gel) electrophoresis stained with ethidium bromide in 1X TBE buffer at 100 V for 2 h and were visualised on Gel documentation system. 2 µL of PCR product and 2 µL of loading dye were mixed and subjected to 5 % polyacrylamide gel electrophoresis (no denature gel). The mixed solution was electrophoresed for 2 h at 100 W and DNA band was visualized after fixing the gel in ethidium bromide solution for 3 min.

Data analysis

Scoring of bands was done visually. The band of each SSR marker and sample were scored as 1 and 0, where 1 indicated the presence of a specific allele (band) and 0 its absence. Proportion of polymorphic loci, average number of alleles per locus, number of unique alleles per landrace and percentage of heterozygosity of each SSR locus were determined using Power Marker v3.0 and GenAlEx software (Liu and Muse 2005). To analyse the information of each primer, the polymorphism information content (PIC) was calculated using the formula:

$$\mathrm{PIC} = 1 - \sum \left(Pi^2\right) \quad (1)$$

where *pi* is the frequency of the *i*th allele detected in the germplasm. PIC values range from 0 (monomorphic) to 1 (very highly discriminative, with many alleles each in equal and low frequency).

Genetic similarity distance (SD) among samples analysed was calculated based on the band sharing coefficient (Lynch 1990), using the formula:

$$\mathrm{SD} = 2\mathrm{Niy}/\mathrm{Ni} + \mathrm{Ny} \quad (2)$$

where Ni and Ny are the number of bands in landrace i and y; Niy, the number of bands shared by the two landraces; and SD, standard deviation.

The genetic similarities between the 64 yam landraces were calculated with the ''Simqual'' subprogram using the DICE coefficient (NTSYS-pc version 2.1). Cluster analysis was performed based on the similarity matrices with the SAHN subprogram using the Un-weighted Pair Group Method with Arithmetic dendrogram (UPGMA) clustering method (Sneath and Sokal 1973) as implemented in the statistical software package, NTSYS-pc version 2.1 (Rohlf 2000). Furthermore, principal coordinate analysis (PCoA) (Gower 1966) using DCENTER and EIGEN procedures of NTSYS-pc software was performed to obtain graphical representations of the relationship structure of the accessions (McBenedict et al. 2016). This multivariate approach was used to complement the information obtained from cluster analysis because it is more informative regarding distances among major groups (Tar'an et al. 2005).

Table 2 Characteristics of the microsatellite loci developed by Tamiru et al. (2015) and used for analysis of genetic diversity and structure of populations of the 64 yam landraces

No.	Primer name	Repeat motif	Primers (5′–3′)	Temp. (°C)	GC content (%)	Product size (bp)
1	YM01	(AC)8	F: GTGTGTGGGATTTTGTCAATC	58	43	404
			R: AGGTTTACACACATCCCCTTT	57	43	
2	YM02	(AAG)6	F: TAGATTTCGCTTTTCCACTAGC	58	41	263
			R: CCTAATCATCATCATCGTCATC	57	41	
3	YM03	(GAT)6	F: TCACTCAAACAATGAGCGTAG	57	43	202
			R: GATGGCTGCTGCATGACTG	60	58	
4	YM04	(AC)21	F: AGTTCATCACAACTCATCCTCA	57	41	320
			R: CCTAGCAAGCATGTCAATCTAC	57	45	
5	YM05	(AAG)8	F: AGGATTATCACTGAAAGGGCT	57	43	140
			R: CCTTCCAATTACTCTCCAAGA	57	43	
6	YM06	(AAG)18	F: ACAGAGCTGTTGACACAAACA	57	43	398
			R: CCTCAATGAACCTTTGGTCTA	57	43	
7	YM07	(CTT)15	F: AGCATTGGGTCCTTTCATCC	59	50	203
			R: ACAATTCACACAAAGCATGGC	59	43	
8	YM08	(AG)24	F: TCTTAGGCTTTGGGCAGGG	60	58	166
			R: AGTATGCCTACCCTGTTCTTC	58	48	
	YM09	(CTT)12	F: AGGAACATTCCCACTCAGTTATG	59	43	193
			R: ATTGGGCAAGTGTGGTGTG	59	53	
10	YM10	(GAT)7	F: ACCCAAAATATTCTCCCCATTATAC	57	36	348
			R: TTGACACTCATCTTATATTGCTCC	57	38	
11	YM11	(AG)17	F: GGATGGCGTAGAGGAAGAGG	60	60	205
			R: GGATAAGACCACGAGTGTTGC	60	52	
12	YM12	(ATC)5…(AAC)8	F: TGAGCATTCTTGTTTTGCCG	58	45	215
			R: CTTTCAGGGCGTGCATGG	60	61	
13	YM13	(CTT)8	F: CCAATCACATCACGTCTAGTCT	57	45	328
			R: GACAATAGAAACTTCGAGACCC	57	45	
14	YM14	(GAT)10	F: TGACTTGAGTAGATCAGGTTGTC	58	43	196
			R: AAGTTGAAGCTTTCCTATAGACG	57	39	
15	YM15	(CTT)7	F: CCATCTCCTCCCTTATCTACAC	57	50	485
			R: GGGATTGAAGTTCCAGAGACTA	57	45	
16	YM16	(CT)13	F: TGAAGAGAATGTTGAGATCGTACC	59	42	150
			R: TATCCGGCCCTCTCATTGG	59	58	
17	YM17	(AC)8	F: TCCCTCAATTAAAGCATAGCCTC	59	43	181
			R: AGCCACCAAACATCTTGCTC	60	50	
18	YM18	(GT)19	F: GACATTGGGGATCTCTTATCAT	57	41	266
			R: TAGCAGCAGTAACGTTAAGGAA	57	41	
19	YM19	(CT)18	F: ACGGAAGCAGCAAGAGGAG	60	58	219
			R: GTGTCATCAGCATCTGGGC	59	58	
20	YM20	(CT)12	F: GTTGCCACACTTGGTGCC	60	61	249
			R: TGGTGAGACCTGAGAATAATTAATGG	60	38	
	YM21	(GAT)5	F: AATGATGCATCTGAGGATAGTG	57	41	340
			R: GATGCTATTACGACAACCTTGA	57	41	
22	YM22	(GTT)6	F: CGACTAGATTTTCTTGTTGGTG	57	41	282

Table 2 continued

No.	Primer name	Repeat motif	Primers (5′–3′)	Temp. (°C)	GC content (%)	Product size (bp)
			R: GGTCACTTTGTTCTAATGCAAG	57	41	
23	YM23	(AG)14	F: TTAAGACTTGCAGGGTTAAAGG	58	41	200
			R: GTGGCTAGTTTTTGTAGCTGGT	58	45	
24	YM24	(GTT)11	F: GGTGTTGTTGGGTTTCATTGTC	59	45	188
			R: TCCCTCTTCTCATTTCACTCCC	60	50	
25	YM25	(AG)30	F: GATGGAGATGAGGAGGCCG	60	63	237
			R: TTCGAAGCCAGAGCAAGTG	59	53	
26	YM26	(AG)22	F: CACTAGCTCCGAAGAAGAGAG	58	52	250
			R: AGGAGTGTTGGTGCTCATATC	58	48	
27	YM27	(GTT)8	F: TCCAGCTCTTTAGCACAGG	58	53	231
			R: AGGAGCATAGGCAACAAGC	59	53	
28	YM28	(CTT)8…(CTT)14	F: CCATTCCTATTTAAGTTCCCCT	58	41	333
			R: GATGAAGAAGAAGGTGATGATG	56	41	
29	YM29	(AAC)18…(AG)13	F: AAGGGCACCCTACATAATAAGA	57	41	352
			R: GAGATCTTGGAGATCATCACTG	56	45	
30	YM30	(GT)16	F: CCACAACTAAAAACACATGGAC	57	41	212
			R: GTGGTAGGGTGTGTAGCTTCTT	57	50	
31	YM31	(AAG)9	F: AAGCCTAGTCGATGGGTGG	60	58	221
			R: TGCTGTTCCAACTTCCAAGC	60	50	
32	YM32	(CT)24	F: GAGGTCTGCGACGGATTTG	59	58	244
			R: TCGCATTCTTCATCCTCTTCAC	59	45	
33	YM33	(AAG)13	F: ACCATGGGATGAAGGGAAGG	60	55	199
			R: GCATATGGTGCATGGGAGC	60	58	
34	YM34	(AG)16	F: GGTAATAGAGGGCAAAGTGGC	59	52	215
			R: AGACCTCCTACCATGCTCAAG	60	52	
35	YM35	(GT)8	F: GCTCTAGCAAACAATCCAATC	57	43	271
			R: CCCTATACGCATGAAAGTAACA	57	41	
36	YM36	(GAT)5	F: CCTTACCACCGGACTCCTC	60	63	156
			R: TGCAGCAATACACCGGAAC	59	53	
37	Ym43	(AAG)9(GA)7	F: GCCTTGTTTTGTTGATGCTTCG	60	45	178
			R: CCAGCCCACTAATCCCTCC	60	63	
38	YM44	(AG)20	F: CGCAACCAGCAAAGGATTTA	61	45	156
			R: ATTCTGTCTCTCAAAACCCCT	57	43	
39	YM49	(AG)26	F: TGGGGTGAGAGAGTAAGTGG	59	55	163
			R: TCACCGGGGATCTTCTTGC	60	58	
40	YM50	(CTT)9	F: TTGCCCTTGGGATGTAGGG	60	58	234
			R: CATCCCCGTTGTATCCTGC	59	58	
41	YM69	(CT)6…(AGTT)5	F: CTCTCTACCTCCCAACAAAAAC	57	45	229
			R: AATCTTGCACCACCTTTTCTAC	57	41	

Tm melting temperature, *GC* guanine-cytosine

To partition genetic diversity among the accession collected from the different region (North and Central Benin), an analysis of molecular variance (AMOVA) was computed with the software GenAlEx (Peakall and Smouse 2006), and significance was determined with 9999 permutations.

Table 3 Fingerprint patterns generated using the 41 microsatellite markers

No.	Primer name	Results			No.	Primer name	Results		
		Agarose (2 %)	Polyacrylamide gel (5 %)	Nature of the band			Agarose (2 %)	Polyacrylamide gel (5 %)	Nature of the band
1	YM1	++	+++	Scorable	22	YM22	–	–	Not scorable
2	YM2	+	–	Not scorable	23	YM23	–	–	Not scorable
3	YM3	++	+++	Scorable	24	YM24	–	–	Not scorable
4	YM4	++	–	Not scorable	25	YM25	+++	–	Not scorable
5	YM5	+	–	Not scorable	26	YM26	+++	–	Not scorable
6	YM6	++	–	Not scorable	27	YM27	+++	+++	Scorable
7	YM7	–	–	Not scorable	28	YM28	+++	+++	Scorable
8	YM8	–	–	Not scorable	29	YM29	+++	+++	Scorable
9	YM9	+	–	Not scorable	30	YM30	+++	–	Not scorable
10	YM10	–	–	Not scorable	31	YM31	+++	+++	Scorable
11	YM11	+	–	Not scorable	32	YM32	+++	+++	Scorable
12	YM12	++	–	Not scorable	33	YM33	++	+++	Scorable
13	YM13	++	+++	Scorable	34	YM34	++	+++	Scorable
14	YM14	+	–	Not scorable	35	YM35	++	+++	Scorable
15	YM15	–	–	Not scorable	36	YM36	++	–	Not scorable
16	YM16	+	–	Not scorable	37	YM43	+++	+	Not scorable
17	YM17	–	–	Not scorable	38	YM44	+++	+	Not scorable
18	YM18	–	–	Not scorable	39	YM49	++	+	Not scorable
19	YM19	–	–	Not scorable	40	YM50	++	+++	Scorable
20	YM20	–	–	Not scorable	41	YM69	+++	+++	Scorable
21	YM21	–	–	Not scorable					

– No amplification, + Low amplification, ++ Average amplification, +++ Good resolution of amplification

Results

Locus amplification

Among the 41 primers used for screening, only 29 presented good resolution of PCR product through agarose gel, while 13 were polymorphic (YM1, YM3, YM13, YM27, YM28, YM29, YM31, YM32, YM33, YM34, YM35, YM50, YM69) (Table 3). These 13 primers were then used to assess the genetic diversity and genetic relationship of 64 yam landraces known as tolerant to storage insects.

Genetic diversity of 64 yam landraces

A total of 113 alleles were found using these 13 SSR primers. The number of alleles produced by different primers ranged from 4 to 13 with an average of 8.69 alleles per locus (Table 4). The lowest number of allele was found with primer Ym50 and found out with Sega landrace while the highest number of the allele was obtained with primer Ym29 with Koukou landrace. Allele frequency varied from 0.15 and was observed with primer Ym31 while the highest value (0.57) of allele frequency was obtained with primer Ym3 (Table 4). The observed heterozygosity per primer ranged from 0.62 to 0.91 with an average of 0.78 suggesting a high degree of variation. Expected heterozygosity per primer ranged from 0.04 to 0.38 with an average of 0.2 (Table 4). The polymorphic information content (PIC) values had an average of 0.76. Marker Ym31 revealed the highest PIC of 0.91 while marker Ym3 had the lowest PIC of 0.58 (Table 4).

Genetic relationships among the 64 yam landraces

Based on the 113 shared alleles, genetic similarity coefficient was estimated for the 64 yam landraces and ranged from 0.04 to 0.45 (Fig. 3). The genetic

Table 4 Polymorphism detected by 13 SSR markers in 64 yam landraces (*D. rotundata–D. cayenensis*)

Marker	Allele frequency	Allele number	Abundant allele	Observe heterozygosity	Expected heterozygosity	PIC
Ym1	0.37	9	10.98	0.79	0.38	0.77
Ym3	0.57	5	10	0.62	0.34	0.58
Ym13	0.28	9	16	0.84	0.19	0.82
Ym27	0.37	10	8.7	0.71	0.04	0.67
Ym28	0.43	8	5.6	0.70	0.07	0.66
Ym29	0.35	13	23.92	0.84	0.23	0.83
Ym31	0.15	8	14	0.91	0.24	0.91
Ym32	0.26	9	18	0.88	0.29	0.87
Ym33	0.31	12	12.36	0.81	0.14	0.79
Ym34	0.31	8	14	0.87	0.19	0.87
Ym35	0.37	12	23.4	0.81	0.21	0.80
Ym50	0.5	4	5.48	0.65	0.12	0.61
Ym69	0.5	6	12	0.70	0.22	0.68
Mean	0.37	8.69	13.41	0.78	0.20	0.76

similarity matrix showed that the most closely related genotypes were found between Allahina and Kpinni all collected from the northern Benin with a genetic distance of 0.04 (Fig. 2) while the highest (0.45) was observed between Kinkerekou and Yassoubagarou. The UPGMA analysis regrouped the 64 yam landraces into 2 main clusters namely A and B and 5 sub-clusters (Fig. 2). Cluster A contained the majority of the 64 yam landraces and was sub-clustered into 4 group namely: I, II, III and IV (Fig. 2). Sub-cluster I included landraces collected in Northern Benin except the Gbode landrace (Fig. 2). Sub-cluster II contained 26 landraces whose 11 collected in Northern region. Sub-cluster III grouped 16 yam landraces whose 5 collected in Central (Tchadjo, Hounbonon) and Northern (Iootchra, Kounto, Gaboubaba) Benin are relatively similar with a genetic distance of 0.12 (Fig. 3). Sub-cluster IV included 3 yams landraces (Babétérou, Kinkérékou, Déba, Bodi, Porchahabim) from the Northern Benin. In this sub-cluster IV, Singor and Babeterou landraces are genetically similar with a genetic distance of 0.31 (Fig. 2). Cluster B included 14 yam landraces from the Northern Benin, except Ayédou, Kokoro lakolako and Agbale landraces. In this cluster, some of the yam landraces collected in Northern (Aizoi, Mouhame, Tougbana, Bodi) and Central (Kokoro lakolako, Agbale) Benin are relatively close with a genetic distance of 0.27 (Fig. 2). Also in this cluster, Ayedou from Central and Singou from Northern, and Tamba and Gnombouanri both from Northern Benin are relatively similar with respective genetic distances of 0.35 and 0.19.

The principal coordinate analysis provides an alternative view of the genetic distances among yam landraces compared to the UPGMA dendrogram (Fig. 3). The first thirteen Eigenvectors accounted for 52.08 % of the variation (Table 5). The first two dimensional vector plot separated the 64 yam landraces into two major clusters (Fig. 3). Unlike the UPGMA analysis, the yam landraces of sub-clusters 1 and 4 of the UPGMA dendrogram except the landraces Gbode and Soanin appear to be clustered together with cluster B (Fig. 4). The grouping of the rest of yam landraces identified by PCA analysis (Fig. 3) were comparable to those identified by the UPGMA tree cluster analysis (Fig. 2).

Analysis of molecular variance

The analysis of patterns across the population (Central and Northern) indicated a moderate level of diversity across the two different populations with heterozygoty of 0.19 and 0.21 in Central and Northern Benin, respectively (Fig. 4). Pairwise population comparisons examined using AMOVA indicated 96 % of molecular variation within populations, with lesser amounts among populations (4 %). Permutation tests (based on 9999 permutations) suggest that the overall

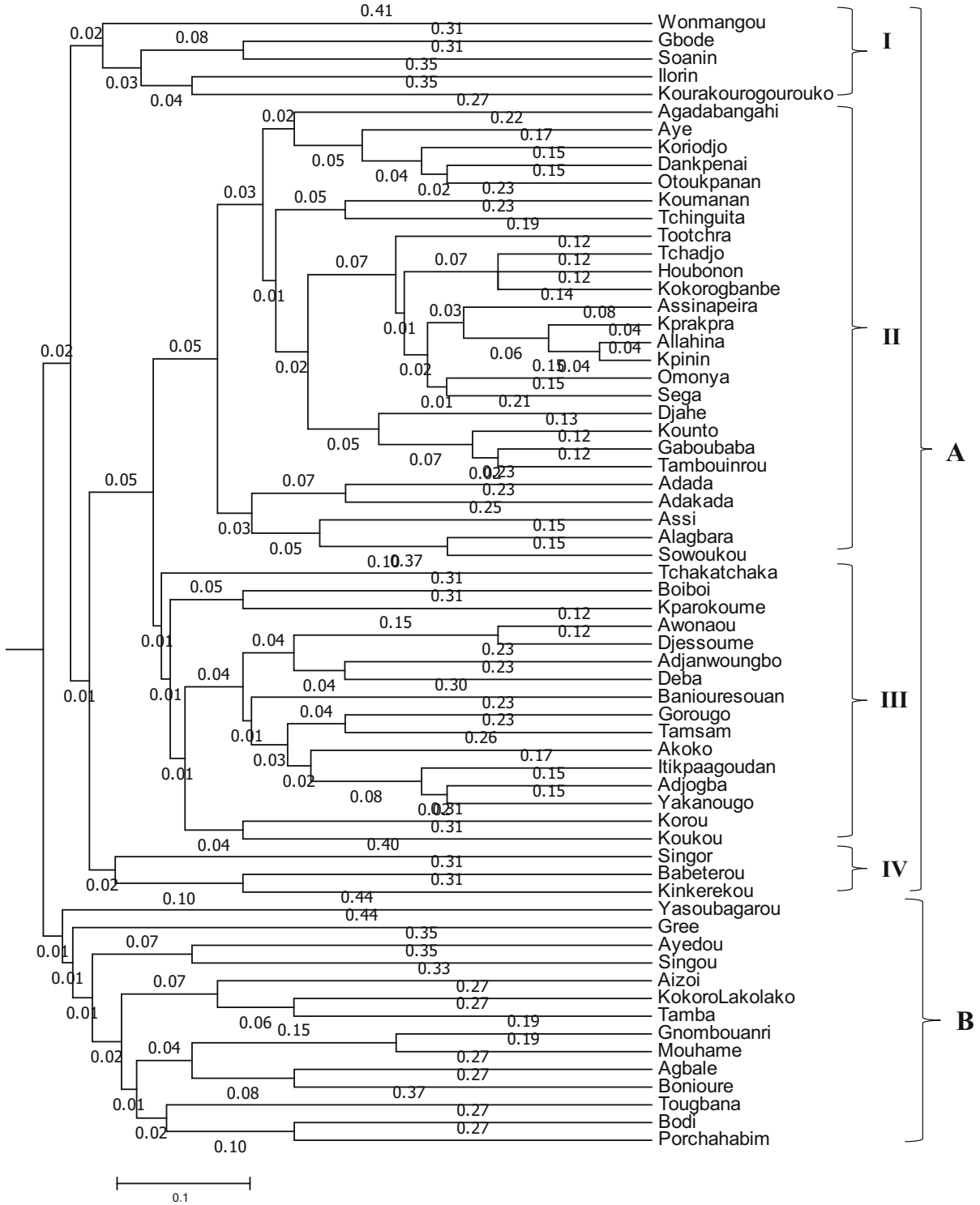

Fig. 2 Dendrogram of 64 yam landraces revealed by cluster analysis of genetic similarity estimates generated by UPGMA method based on 13 SSR markers (cluster: A, B; subcluster: I, II, III, IV)

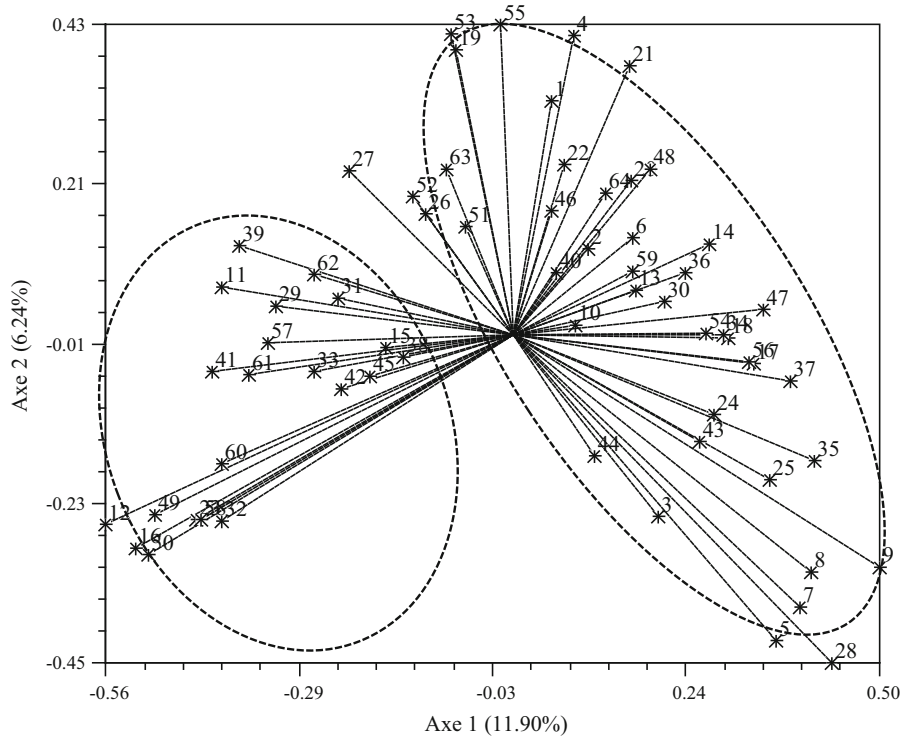

Fig. 3 Scatter plot of 64 yam landraces based on first and second components of principal coordinate analysis using SSR data

Table 5 Principal coordinates analysis (PCoA) of molecular data showing percentage of variation explained by each axis

Axes	Eigenvalue	Percent	Cumulative
	5.58	11.90	11.90
2	2.93	6.24	18.15
3	2.31	4.93	23.08
4	2.07	4.43	27.52
5	1.70	3.63	31.15
6	1.55	3.32	34.47
7	1.41	3.01	37.48
8	1.28	2.73	40.22
9	1.21	2.59	42.81
10	1.14	2.44	45.26
11	1.09	2.33	47.59
12	1.06	2.26	49.85
13	1.04	2.23	52.08
14	0.96	2.06	54.15
15	0.91	1.95	56.10

PhiPT was not significantly different from the null distribution (PhiPT = 0.043, $P = 0.052$) (Table 6), which indicates that the differences among cultivars are not significant. However, the number of non-common alleles is important in the Northern population (Fig. 4).

Discussion

Out of the 41 SSR markers used to characterize the 64 yam landraces, only 13 of them presented clear and reproducible scorable bands, giving 113 polymorphic alleles. The number of alleles amplified per primer pair varied from 4 to 13 with an average of 8.69 alleles per locus. Considering some works conduct on yam in Africa, High number of total alleles were reported by Muthamia et al. (2013), who found 131 alleles using 12 SSR markers with a minimum of 2 alleles and a maximum of 13 alleles per primer when analysed 187 accessions of Kenya yam. Obidiegwu et al. (2009) reported a total of 121 alleles amplified with 15 SSR markers with the number of alleles observed per locus varying from 6 to 9 alleles when analysed 219 accessions of Guinea yam germplasm from Benin, Congo, Côte d'Ivoire, Equatorial Guinea, Gabon, Ghana, Nigeria, Sierra Leone and Togo. However low number of alleles was obtained by Otoo et al. (2009)

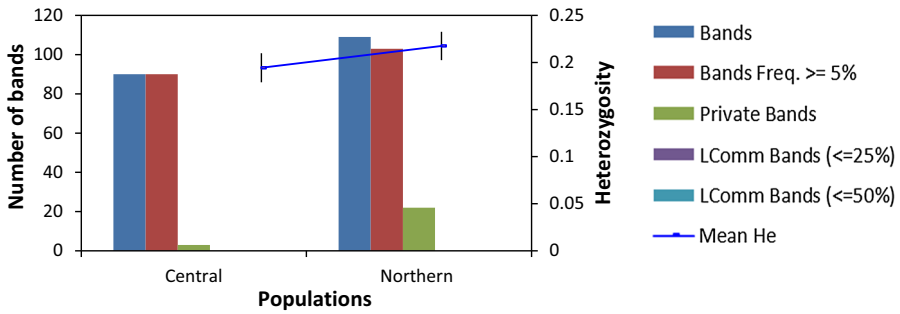

Fig. 4 Allele frequency in the two sampling regions. No. Bands = number of different bands; No. Bands Freq. ≥5 % = number of different bands with a Frequency ≥5 %; No. Private Bands = number of bands unique to a single population; No. LComm Bands (≤25 %) = number of Locally Common Bands (Freq. ≥5 %) Found in 25 % or fewer populations; No. LComm Bands (≤50 %) = number of locally common bands (Freq. ≥5 %) Found in 50 % or fewer populations; Mean He = mean of heterozygosity

Table 6 Analysis of molecular variance (AMOVA) considering variation between and within two sampling zone (Northern and Central Benin)

Source	Degree of freedom	Sum of squares	Mean square	Est. Var.	Percentage of total variation
Among populations	1	108,271	108,271	2184	4
Within populations	62	2945,164	49,086	49,086	96
Total	63	3053,435		51,270	100
Stat	Value	P(rand ≥ data)			
PhiPT	0.042	0.052			

who detected 27 loci using 13 SSR markers in a study of pona complex yam in Ghana.

The mean of polymorphism information content (PIC) obtained in this study is within the range of the previous studies. Siqueira et al. (2013) obtained an extremely high mean percentage polymorphism (92 %) among the Brazil water yam while Velasco-Ramírez et al. (2014) and Otoo et al. (2015) reported slightly lower PIC values of 48 and 53 % respectively on accessions from Mexico and West Africa, respectively. This high level of polymorphism associated with SSR markers may be function of a unique replication slippage mechanism responsible for generating SSR allelic diversity (Pejic et al. 1998). Relatively high level of polymorphism observed in Guinea yam of Benin could be due to the fact that wild yam domestication process still active in different regions of Benin regularly lead to new cultivars (Loko et al. 2013a).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 (Obidiegwu et al. 2009). Similar results were observed across the world especially in root and tuber crops like sweet potato (Karuri et al. 2009), Cassava (Tovar et al. 2015) and yam (Obidiegwu et al. 2009). However, due to the few or low SSR primer number which amplified our plant material, there is need for more work to design new yam primers.

Some of yam landraces presented a unique alleles and could be probably due to the high rate of mutation in SSR loci (Henderson and Petes 1992). These rare alleles could be of particular interest as they are a particular genotypes. Such alleles are important because they may be diagnosed for particular genotypes or for particular regions of the genome specific to a particular trait (Casa et al. 2005). The major rare alleles were observed in Northern Benin and this could explain the diversity of yam landraces of this region. As reported by Loko et al. (2013a), Northern Benin is the first yam producer and is considered as the yam pool diversity.

Yam landraces collected from different regions were grouped together in two clusters and there was no relationship between these yam landraces and their

geographical area of collection. In addition, analysis of molecular variance revealed that most of the genetic variability is concentrated within the 64 yam landraces. These results are consistent with those of Mignouna et al. (2003) on a collection of yam landraces from eight West African countries. As reported by Otoo et al. (2009) in Ghana and Tamiru et al. (2007) in Ethiopia, the non-repartition of diversity through regions is probably due to the exchange of plant material between farmers. Contrarily, Tostain et al. (2007) found that, the genetic diversity of yam landraces in Benin is organized according to cropping regions. It is so important to analyse more samples in each area to be able to characterize the overall structure throughout Benin.

The agromorphological characterisation has grouped the 64 analysed yam landraces in 24 morphotypes and revealed the existence of many duplicates (Loko et al. 2015). The comparison of molecular characterization with morphological characterization done by Loko et al. (2015) showed low correlation. In fact, this study allowed us to distinguish two cluster of yam landraces, with no distinctive morphological traits. This result could be due to the fact that morphological traits are influenced by environmental factors (Gixhari et al. 2014). Results of this study were congruent with results of Asare et al. (2011) and Mamba-Mbayi et al. (2014) on cassava, Karuri et al. (2010) and Koussao et al. (2014) on sweet potato, who suggested low correlations among molecular and morphological data. Therefore, many authors have been suggested the use of molecular markers which are not subject to either natural or artificial selection (Vieira et al. 2007; Karuri et al. 2010).

Conclusion

In conclusion, the present study revealed high genetic diversity and provided information on population structure among the 64 yam landraces whose dried chips are known as tolerant to storage insects in Benin. The genetic diversity of the investigated accessions is high, distributed over two clusters and 5 sub-clusters and exhibits a null level of association between genetic divergence and geographical origin of yam landraces. The information about genetic diversity will be very useful for proper identification and selection of appropriate parents for breeding programs.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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