Genetic Diversity of Yam (*Dioscorea* spp.) Landraces from Ethiopia Assessed by Morphological and Microsatellite Markers

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Abstract

Genetic diversity present within and between populations is crucial for breeding and conservation. The objective of this study was to assess the genetic diversity in yam landraces by using agro-morphological and microsatellite markers. Phenotypic diversity of 36 landraces collected from southwest Ethiopia was determined using diversity indices, principal component and cluster analyses. High phenotypic diversity indices were recorded, ranging from 0.53 to 1.50, with a mean of 0.985. Principal component analysis identified seven PCAs which contributed 88.4% of the total phenotypic variation among the landraces. The test primers amplified a total of 30 fragments, of which 80% was polymorphic. The number of alleles detected per locus ranged from 1 to 5, with a mean of 3. Number of effective alleles ranged from 1 to 3.57. Gene diversity ranged from 0.00 to 0.80 with a mean of 0.53. The mean polymorphic information content ranged from 0.00 to 0.72, with a mean of 0.30. The Simple Sequence Repeat markers and phenotypic traits showed similar clustering patters of landraces except some differences. The results obtained in this study are useful for future yam breeding and conservation program.

Keywords: Breeding, heterozygosity, microsatellite markers, phenotypic traits, yam

Introduction

Yam is a multi-species crop that belongs to the genus *Dioscorea* and family *Dioscoreaceae* (Tamiru et al., 2007). It is found in Africa, India, Southeast Asia, Australia and South America comprising of 600 species (Mignouna et al., 2002; Loko et al., 2015). All species are tropical origin and cultivated for their edible starchy tubers (enlarged, fleshy, usually underground storage stems) (FAO, 2010). Yam has great potential to combat food insecurity and for local and regional markets (Sesay et al., 2013). Globally, yam is the fourth most important tuber crop after potato (Solanum tuberosum L.), cassava (*Manihot esculenta Crantz*) and sweet

potato (*Ipomoea batatas* (L.) pori.) (Tamiru, 2006; Loko et al., 2013). West Africa is the predominant yam producing sub-region (FAOSTAT, 2006; FAO, 2010) contributing 95% of the world's yam production (Hamadina et al., 2009; Dansi et al., 2013).

In Ethiopia, yam is mainly used as a source of human food, medicine and cash income source through the sale of storage tubers (Hildebrand, 2003; Tamiru et al., 2007). Ethiopia is believed to be the centers of origin and diversity of yam (Rehim and Espig, 1991; Tamiru et al., 2011). Eleven species of yams have been described in Ethiopia (Miege and Demessew, 1997). Some of the species have both cultivated and wild forms in South, Southwestern and Western parts of the country. Edwards, (1991) reported that *Dioscorea* species are widely adapted in Ethiopia as cultivated and wild relatives. Further, Hildebrand, (2003) and Terauchi et al., (1992) reported that *D. abyssinica* is native to Ethiopia and grown over a wide range of agro-ecologies in Ethiopia.

Comprehensive phenotypic and genetic diversity analyses of the crop need to be undertaken to understand the population dynamics of yam landraces across the major growing regions in Ethiopia. Tamiru et al., (2011) sampled 84 yam accessions collected from Southern region of Ethiopia and reported the existence of a high level of phenotypic variation in accessions from the region of collections. Mulualem, (2016) also found morphological variation among yam landraces collected from Southwest region. Abebe, (2008), evaluated the genetic diversity of some 40 yam accessions collected from South and Southwest regions of Ethiopia.

Morphological characterization of germplasm is essential for crop improvement programs and conservation of existed genetic resources (Arnau et al., 2009; Alina et al., 2014). Germplasm characterization can be achieved using morphological traits and molecular markers (Paterne et al., 2019). Qualitative and quantitative traits are important agronomical traits that measured directly from the population (Mulualem et al., 2020). Nevertheless, this method is time consuming, requires phenotyping skills, multi-locations and multi-years experimentation, to account for environmental and genotype by environment effects (Spooner et al., 2005; Arnau et al., 2009). In yam, SSR (Simple Sequence Repeats) markers have been widely applied for the assessment of genetic diversity and characterization of germplasm and estimation of genetic distances between and within populations (Tamiru et al., 2011). SSR markers are currently the marker of choice for diversity analysis due to their ability to provide information on multi-allelic loci and greater genotypic differentiation (Abebe, 2008; Mulualem et al., 2018). The landraces used in this study were mainly collected from southwestern parts of

the country where the largest genetic diversity of yam is present (Hildebrand, 2003). In the region farmers developed and maintained large number of yam landraces for centuries based on their traditional knowledge (Demissew et al., 2003). Many authors indicated the presence of a wide morpho-agronomical variation in the Ethiopian yam landrace collections. However, most of the germplasm characterization in yam using phenotypic or limited molecular markers did not cover yam landraces from different growing areas of Ethiopia. The objective of this study was to assess genetic diversity present in yam landraces collections from southwest Ethiopia using morphological traits and SSR markers.

Materials and Methods

Study site, plant materials and experimental design

A total of 36 yam landraces collected across a wide altitudinal range (1171-1940 m.a.s.l from the southwestern parts of Ethiopia were used for this study (Table 1). The landraces were planted at Jimma Agricultural Research Center using a 6 x 6 simple lattice design with two replications with inter- and intra- row spacing of 1m and 1m, respectively. Tubers of the same size which started sprouting were used as planting material. All other agronomical practices were followed according to the recommendations and farmers practices of the areas. Each yam plant was tended using dried coffee sticks of 3.5-4.5 m long to provide support and induce good canopy and vine development. Five middle plants within a row were sampled and tagged for data collection and final harvest.

Five plants from each landrace were selected and tagged individually before sampling for DNA extraction. Genomic DNA samples were collected by using Whatman Flinders Technology Associates (FTA^{TM}) cards three weeks after planting. The FTA cards were labeled prior to sampling. Individual leaf was excised from the plant, wrapped round the FTA paper strip, and leaf sample extract were pressed onto the FTA paper until the FTA card was soaked with leaf sap. To prevent cross contamination in between samples, 70% of ethanol was used for cleaning materials.

Table 1. Names of the 36 yam landraces used for the study with collection zones, districts, geographical coordinates and altitude

Serial. No	Name of landraces	Zone	District	Latitude	Longitude	Altitude (m)
1	59/02	Jimma	Mana	07^{0}40'37N	036^{0}49'10E	1718
2	68/01	Jimma	Dedo	07^{0}30'63N	036^{0}53'45E	1784
3	6/02	Bench Maji	Sheko	06^{0}59'66N	035^{0}34'11E	1728
4	75/02	Jimma	Kersa	07^{0}40'43N	036^{0}48'76E	1734
5	3/87	Jimma	Manna	07^{0}40'58N	036^{0}48'75E	1731
6	56/76	Jimma	Manna	07^{0}41'89N	036^{0}48'06E	1837
7	54/02	Bench Maji	Sheko	07^{0}02'03N	035^{0}32'77E	1892
8	46/83	Jimma	Dedo	07^{0}31'28N	036^{0}53'59E	1771
9	08/02	Jimma	Kersa	07^{0}40'46N	036^{0}48'79E	1740
10	116	Jimma	Dedo	07^{0}31'28N	036^{0}53'63E	1683
11	01/75	Sheka	Yeki	07^{0}11'30N	035^{0}26'22E	1171
12	06/83	Jimma	Dedo	07^{0}31'32N	036^{0}53'64E	1692
13	17/02	Sheka	Yeki	07^{0}11'27N	035^{0}26'26E	1176
14	07/03	Jimma	Dedo	07^{0}31'50N	036^{0}53'60E	1733
15	45/03	Jimma	Mana	07^{0}41'86N	036^{0}48'08E	1810
16	27/02	Jimma	SekaChekorsa	07^{0}35'06N	036^{0}41'91E	1877
17	37/87	Jimma	Mana	07^{0}41'87N	036^{0}48'13E	1940
18	10/002	Bench Maji	Sheko	07^{0}02'91N	035^{0}29'76E	1668
19	76/02	Jimma	Kersa	07^{0}40'64N	036^{0}48'84E	1728
20	06/2000	Jimma	SekaChekorsa	07^{0}35'43N	036^{0}41'86E	1850
21	7/83	Jimma	Sekachekorsa	07^{0}35'06N	036^{0}41'91E	1898
22	58/02	Sheka	Yeki	07^{0}11'22N	035^{0}26'25E	1192
23	39/87	Jimma	SekaChekorsa	07^{0}35'42N	036^{0}42'94E	1885
24	32/83	Jimma	ShebeSombo	07^{0}26'74N	036^{0}24'01E	1372
25	24/02	Jimma	ShebeSombo	07^{0}26'75N	036^{0}24'07E	1379
26	2/87	Jimma	ShebeSombo	07^{0}26'76N	036^{0}24'12E	1365
27	60/87	Sheka	Yeki	07^{0}11'72N	035^{0}26'48E	1199
28	15/2000	Bench Maji	Sheko	07^{0}04'13N	035^{0}37'74E	1320
29	34/87	Jimma	Dedo	07^{0}31'37N	036^{0}53'44E	1911
30	21/02	Jimma	SekaChekorsa	07^{0}36'48N	036^{0}45'09E	1785
31	57/76	Bench Maji	Sheko	07^{0}02'88N	035^{0}29'74E	1654
32	0001/07	Jimma	ShebeSombo	07^{0}26'74N	036^{0}24'12E	1367
33	0004/07	Jimma	Kersa	07^{0}40'55N	036^{0}48'75E	1741
34	7/84	Bench Maji	Sheko	07^{0}02'88N	035^{0}29'74E	1661
35	7/85	Sheka	Yeki	07^{0}14'30N	035^{0}26'17E	1173
36	06/2001	Bench Maji	Sheko	06^{0}59'69N	035^{0}34'09E	1387

Phenotyping and data analysis

Thirteen phenotypic characters, the standard yam descriptor for characterization, were used for this study (IPGRI, 1999). Shannon-Weaver index (H') was computed for each phenotypic trait from frequency distributions observed in the different classes (Hennink and Zevan, 1991; Perry and McIntosh, 1991) as follows:

$$H' = -\sum_{i=1}^{n} pi\ loge\ pi$$

Where H' = Shannon diversity Index; pi= the proportion of landraces in the i th class of an n–class character; n = the

number of phenotypic classes of traits. Each diversity index value was divided by its maximum value ($\log_e n$) and normalized to keep the values between 0 and 1. The diversity index for each character was computed from the complete data set while the average diversity index was computed for each character.

In addition, the data was further subjected to principal component (PCA) and cluster analysis procedures using Genres (Genres, 2008) and SAS (SAS, 2000) statistical soft wares. Principal components (PC's) with eigen values > 1.0 were selected and morphological traits with load coefficient values > 0.5 were considered highly relevant to that PC (Morimoto et al., 2005).

Genotyping and data analysis

For molecular diversity assessment, a set of 10 microsatellite markers were used (Table 2). The markers used in this study were selected based on their polymorphic information content and diagnostic when used in yam (Tamiru et al., 2015). Genotyping was conducted at Incotec Biotechnology Laboratory, South Africa. All samples were used in bulk amplification, using DNA from five individual plants. A single punch of each card per submission was taken and homogenized in the Finnzymes dilution buffer. Two, micro-liters of each bulked sample were used in the polymerase chain reaction (PCR). Of the total of 36 yam landraces collected, three landraces namely, 59/02, 68/01 and 0001/07 were excluded from final analysis due to poor amplification. PCR amplification reaction contained 20 µl of PCR mix (1XPCR buffer, 3 mM MgCl, 1.25 U Taq polymerase, 0.2 mM dNTPs, 4pM each primer) and 2 FTA disc or 5 µl of CTAB extracted g DNA. A PCR profile of initial denaturation for 2 min at 94 ^{o}C, and 33 cycles of denaturation for 1 min at 94 ^{o}C, annealing temperature of 63 ^{o}C for 2 min, extension for 2 min at 72 ^{o}C was used. The PCR products were fluorescently labeled and separated by capillary electrophoresis on an ABI 3013 automatic sequencer (Applied Biosystems, Johannesburg, South Africa); analysis was performed using Gene Mapper 4.1. Product size was scored in base pairs based on the relative migration of the internal size standard. Information generated from the GeneMapper software was then used to determine the diversity parameters.

Table 2. Selected SSR primers for yam genetic diversity study

Locus	Repeat motif	Primers (5´to 3´) Forward	Primers (3´to 5´) Reverse	Tm ^{0}C		**GC (%)**		Product Size
				F	R	F	R	
YM02	$(AAG)_6$	TAGATTTCGCTTTTCCACTAGC	CCTAATCATCATCATCGTCATC	58	57	41	41	263
YM03	(GAT)6	TCACTCAAACAATGAGCGTAG	GATGGCTGCTGCATGACTG	60	60	58	58	202
YM05	(AAG)8	AGGATTATCACTGAAAGGGCT	CCTTCCAATTACTCTCCAAGA	57	56	43	43	140
YM09	(CTT)12	AGGAACATTCCCACTCAGTTA	ATTGGGCAAGTGTGGTGTG	59	59	43	53	193
YM12	AAC)8	TGAGCATTCTTGTTTTGCCG	CTTTCAGGGCGTGCATGG	58	60	45	61	215
YM13	(CTT)8	CCAATCACATCACGTCTAGTC	GACAATAGAAACTTCGAGACC	57	57	45	45	328
YM15	(CTT)7	CCATCTCCTCCCTTATCTACAC	GGGATTGAAGTTCCAGAGACT	57	57	50	45	485
YM17	(AC)8	TCCCTCAATTAAAGCATAGCC	AGCCACCAAACATCTTGCTC	59	60	43	50	181
YM18	(GT)19	GACATTGGGGATCTCTTATCA	TAGCAGCAGTAACGTTAAGGA	57	57	41	41	266
YM21	(GAT)5	AATGATGCATCTGAGGATAGT	GATGCTATTACGACAACCTTG	57	57	41	41	340

Genotypic data were subjected to analysis with various measure of genetic diversity within and among genotypes using GenAlEx software version 6.5 (Peakall and Smouse, 2012). Genetic diversity parameters such as total number of alleles per locus (N_a), observed fragment size (OFS) number of effective alleles per locus (N_e), observed heterozygosity (Ho), unbiased expected heterozygosity (gene diversity) (H_e) and polymorphic information content (PIC) were determined using the protocol of Nei and Li (1979). Further, phenotypic and genotypic relationships among yam landraces were determined by using neighbor-joining algorithm using the unweighted pair group method (UWPGM) in DARwin 5.0 software (Perrier and Jacquemoud Collet, 2006). A dendrogram was then generated on the dissimilarity matrix. Bootstrap analysis was performed for node construction using 10000 bootstrap values.

Results

Phenotypic diversity

From all traits considered, 20 (55.55%) and 28 (77.78%) of landraces exhibited medium and high leaf density, respectively (Table 3). Ten landraces (27.78%) produce spine on their tuber surfaces with variable shape; 22.22% had curved and 77.78% (28 landraces) had straight shape, and highly associated with the wildness of the landraces. In wild type landraces spines distributed all over the surface of vine and tuber in different amount with variable sizes. Tuber shape of the landraces varied from irregular (36.11%) to oval (8.33%). The following landraces have cylindrical tuber shape 68/01, 75/02, 3/87, 17/02, 07/03, 27/02, 10/002, 06/2000, 58/02, 24/02, 34/87, 21/02 and 7/85. The predominant tuber flesh colour was white with purple (25.0%) followed by purple (19.44%), purple with white (13.89%) and outer purple/inner white (11.11%), with dominant light and dark brown tuber skin colour. Most of the landraces (47.22%), considered in this study exhibited branched tuber with rough (77.78%) and smooth (22.22%) surfaces. The predominant tuber flesh colour at central transverse cross section was white (38.89%). Landraces such as 59/02, 75/02, 56/76, 46/83, 06/83, 27/02, 76/02, 06/2000, 58/02, 2/87, 34/87, 0001/07 and 7/85 recorded white fleshed colour. Other flesh colours observed include white with purple displayed by 19.44% and 13.89% of landraces producing similar colour, for example, light purple, purple and purple with white flesh colour.

All the phenotypic characters evaluated were highly polymorphic, with the maximum and minimum diversity index scores of 0.53 and 1.50 represented by leaf density, tuber surface texture and hairiness of tuber surface and flesh colour at central transverse, respectively (Table 3).

Table 3: Phenotypic variation and Shannon Weaver diversity indices of 36 yam landraces from Southwest Ethiopia

Landraces	Lsi	LD	Sp	SSh	CSpb	SVB	TS	TFC	TSC	TBr	TStex	HOTSu	F CC
59/02	Large	Medium	Absent	Straight	Present	None	Irregular	Purple with white	Light brown	Slightly branched	Rough	Small	White
68/01	Small	High	Absent	Curved	Absent	None	Cylindrical	White with purple	Dark brown	Branched	Smooth	Medium	Purple with white
6/02	Small	High	Absent	Straight	Present	Many	Irregular	White	Light brown	Highly branched	Rough	Medium	Purple with white
75/02	Small	High	Absent	Straight	Present	None	Cylindrical	White	Light brown	Highly branched	Rough	Medium	White
3/87	Medium	High	Present	Straight	Absent	Many	Cylindrical	Purple	Light brown	Highly branched	Rough	Small	Purple
56/76	Small	High	Absent	Straight	Present	Few	Oval	White	Dark brown	Highly branched	Rough	Medium	White
54/02	Small	High	Present	Curved	Absent	Many	Oval-oblong	Purple	Light brown	Branched	Smooth	Medium	Purple with white
46/83	Large	Medium	Absent	Straight	Present	None	Oval-oblong	Outer purple inner white	Light brown	None	Rough	Medium	White
08/02	Small	High	Absent	Straight	Present	None	Irregular	White	Dark brown	Branched	Rough	Small	White with purple
116	Small	High	Absent	Straight	Present	None	Flattened	Outer purple inner white	Dark brown	Branched	Rough	Small	Purple with white
01/75	Small	High	Absent	Straight	Present	None	Irregular	Purple	Dark brown	Branched	Smooth	Medium	Purple with white
06/83	Large	Medium	Present	Straight	Absent	Few	Oval-oblong	White	Light brown	Highly branched	Rough	Medium	White
17/02	Medium	High	Absent	Straight	Present	None	Cylindrical	Purple with white	Light brown	None	Rough	Small	White with purple
07/03	Medium	High	Absent	Straight	Present	Few	Cylindrical	White with purple	Light brown	Highly branched	Rough	Small	White with purple
45/03	Medium	High	Absent	Straight	Present	Few	Irregular	White with purple	Light brown	Branched	Rough	Medium	White with purple
27/02	Medium	High	Absent	Straight	Present	None	Cylindrical	White	Dark brown	Branched	Rough	Medium	White
37/87	Medium	Medium	Absent	Straight	Present	None	Irregular	Outer purple inner white	Dark brown	Highly branched	Rough	Medium	white with purple
10/002	Medium	High	Present	Curved	Absent	Few	Cylindrical	Purple with white	Light brown	Branched	Rough	Medium	purple with white
76/02	Medium	High	Absent	Straight	Present	None	Irregular	White	Light brown	Branched	Rough	Medium	White
06/2000	Medium	High	Absent	Straight	Present	None	Cylindrical	White with purple	Dark brown	Branched	Smooth	Medium	White
7/83	Medium	High	Absent	Straight	Present	None	Irregular	white	Light brown	None	Rough	Medium	Light purple
58/02	Medium	Medium	Absent	Straight	Present	None	cylindrical	White with purple	Dark brown	Branched	Rough	Medium	White
39/87	Large	High	Present	Curved	Absent	Few	Oval-oblong	White with purple	Dark brown	Branched	Rough	Medium	Purple
32/83	Medium	High	Present	Curved	Absent	Few	Irregular	Purple	Dark brown	Branched	Smooth	Medium	Purple
24/02	Large	Medium	Present	Straight	Absent	Few	Cylindrical	Purple	Dark brown	Highly branched	Rough	Small	White with purple
2/87	Medium	High	Absent	Straight	Present	None	Irregular	White	Light brown	Branched	Rough	Medium	White
60/87	Large	High	Present	Curved	Present	Few	Flattened	Purple	Dark brown	Highly branched	Rough	Medium	Light purple
15/2000	Medium	High	Present	Curved	Absent	Many	Oval	White with purple	Light brown	Branched	Rough	Medium	White with purple
34/87	Medium	High	Absent	Straight	Present	None	Cylindrical	White	Light brown	Branched	Rough	Medium	White
21/02	Small	High	Absent	Straight	Present	None	Cylindrical	Purple with white	Light brown	None	Rough	Medium	Light purple
57/76	Medium	High	Present	Curved	Absent	Many	Irregular	Purple	Light brown	Branched	Smooth	Medium	Purple
0001/07	Large	Medium	Absent	Straight	Absent	None	Oval	Outer purple inner white	Light brown	Highly branched	Rough	Small	White
0004/07	Medium	High	Absent	Straight	Present	None	Irregular	White with purple	Dark brown	Highly branched	Rough	Small	Light purple
7/84	Medium	High	Absent	Straight	Present	Many	Irregular	Purple	Dark brown	Slightly branched	Rough	Medium	Purple
7/85	Medium	Medium	Absent	Straight	Present	None	Cylindrical	White with purple	Dark brown	Highly branched	Smooth	Medium	White
06/2001	Medium	High	Absent	Straight	Present	None	Flattened	Purple with white	Dark brown	None	Rough	Medium	Light purple
H'	**0.99**	**0.53**	**0.61**	**0.79**	**0.61**	**0.96**	**1.39**	**1.59**	**0.69**	**1.26**	**0.53**	**0.53**	**1.50**

LSi=Leaf size, LD=Leaf density, Sp=, Spine on tuber surface, SSh= Spine shape, CSpb= colour at spine base, SVB=spine on vine base, TS=Tuber shape, TFC=Tuber flesh colour, TSC=Tuber skin colour, TBr= tuber branching, TStex= tuber surface texture, HOTs= hair on tuber surface, FCCS= flesh colour at central transverse. H'= Shannon-Weaver diversity index.

The mean phenotypic diversity index of genotypes was 0.985, showing high variability with respect to all phenotypic character classes.

The first seven PCAs, each with eigenvalues greater than one explained 88.4% of the total variation among the studied landraces for all morphological characters (Table 4). About 32.5% of the total variation was accounted for by PC1 which positively correlated to flesh colour at central transverse, spine on vine base and tuber flesh colour. Spines on vine base and tuber flesh colour had the highest loadings on PC2 and accounted 22.8% of the total variation, while PC3 associated with the spine on vine base and flesh colour at central transverse, explained 12.9% of the total variation. The remaining PCs accounted 20.2% of the total variation, which was mainly associated with spine on vine base, tuber shape, flesh colour at central transverse and tuber branching.

Table 4. Eigen values, proportion, cumulative variance and component scores of the first seven principal components for

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Leaf size	-0.003	-0.056	0.077	0.057	-0.091	0.128	-0.084
Leaf density	0.021	0.057	-0.034	-0.041	0.005	-0.023	-0.006
Spines on tuber surface	0.070	0.039	0.084	-0.008	0.081	0.108	-0.031
Spine shape	0.131	0.054	0.137	-0.016	0.124	0.164	-0.089
Colour at spine base	0.062	0.022	0.078	0.027	0.123	0.100	-0.024
Spine on vine base	**0.482**	**0.498**	**0.428**	**0.410**	-0.165	-0.144	0.076
Tuber shape	0.008	0.062	-0.255	-0.269	**-0.578**	**-0.458**	0.338
Tuber flesh colour	**0.461**	**-0.812**	0.258	0.094	-0.114	-0.103	0.083
Tuber skin colour	0.020	-0.078	-0.051	-0.090	0.238	-0.071	0.119
Tuber branching	0.010	0.067	0.052	0.159	0.207	0.087	**0.669**
Tuber surface texture	-0.036	0.008	-0.042	0.010	-0.024	-0.013	-0.051
Hair on tuber surface	0.026	0.080	0.091	-0.040	-0.031	-0.213	-0.075
Flesh colour at central transverse	**0.717**	0.155	**-0.496**	-0.317	0.125	0.157	-0.061
Eigen value	12.925	9.095	5.120	3.695	1.752	1.487	1.113
% total variance	32.50	22.80	12.90	9.30	4.40	3.70	2.80
% cumulative variance	32.50	55.30	68.20	77.50	81.90	85.60	88.40

13 qualitative traits in 36 yam collections.

SSR polymorphism

The 33 yam landraces evaluated in this study were differentiated uniquely, using 10 SSR markers (Table 5). A total of 30 putative alleles were detected from the population sampled. The observed fragment size (OFS) ranged from 155 to 495 nucleotides. The total number of polymorphic alleles per locus (N) varied from 1 (YM13 and YM18) to 5 (YM09) with a mean of 3.0. The number of effective alleles per locus (Ne) ranged from 1.00 to 3.57 and markers YM18 and YM09 had the lowest and highest numbers of effective alleles. This indicated the presence of genetic diversity among yam landraces from southwest Ethiopia.

Phenotypic and genetic relationships

Genetic relationships among the yam genotypes were studied using the Neighbour-joining dendogram constructed using unweighted pair group method of arithmetic means (UPGMA) algorithm based on morphological and SSR markers, which classified the landraces into four and three clusters, respectively (Figures 1 and Figure 2). Cluster analysis based on phenotypic traits grouped 24 (66.67%), 8(22.22%), 2(5.55%) and 2(5.55%) of landraces in Clusters I, II, III and IV, respectively (Figure 1). Cluster analysis based on SSR markers classified 14 (42.42%), 12 (36.36%) and 7 (21.21%) of the landraces into Clusters I, II and III, respectively (Figure 2). Phenotypic traits and SSR markers showed similar clustering patterns of yam landraces except some discrepancies.

Table 5. Genetic diversity within and among 33yam landraces based on 10 SSR markers

Locus	N_a	OFS (bp)	K	Ne	Ho	He	F_{IS}	PIC
YM02	3.0	237 - 242	0.471	2.22	0.71	0.47	-0.29	0.55
YM03	4.0	214 - 235	0.735	1.13	0.03	0.74	0.74	0.12
YM05	2.0	155 - 158	0.735	1.10	0.10	0.74	0.05	0.09
YM09	5.0	201 - 225	0.645	3.57	0.68	0.65	-0.06	0.72
YM12	4.0	221 - 232	0.657	2.34	0.70	0.66	-0.22	0.57
YM13	1.0	319	0.116	1.00	0.00	0.00	0.00	0.00
YM15	3.0	491 - 495	0.793	1.19	0.03	0.79	0.78	0.16
YM17	4.0	192 - 211	0.802	1.41	0.30	0.80	0.05	0.29
YM18	1.0	256	0.280	1.00	0.00	0.00	0.00	0.00
YM21	3.0	368 - 373	0.403	2.14	0.89	0.40	0.67	0.53
Mean	3.0	-	5.637	1.71	0.34	0.53	0.24	0.30
SE	0.42	3.47	0.567	0.27	0.11	0.10	0.12	0.08

Where, N_a= Total number of alleles per locus, OFS= Observed fragment size, K= Expected heterozygosity, Ne= Number of effective alleles per locus, Ho= Observed gene diversity within landraces, He= Average gene diversity within landraces, F_{IS}=Inbreeding coefficient, PIC= Polymorphic information content and SE= Standard error.

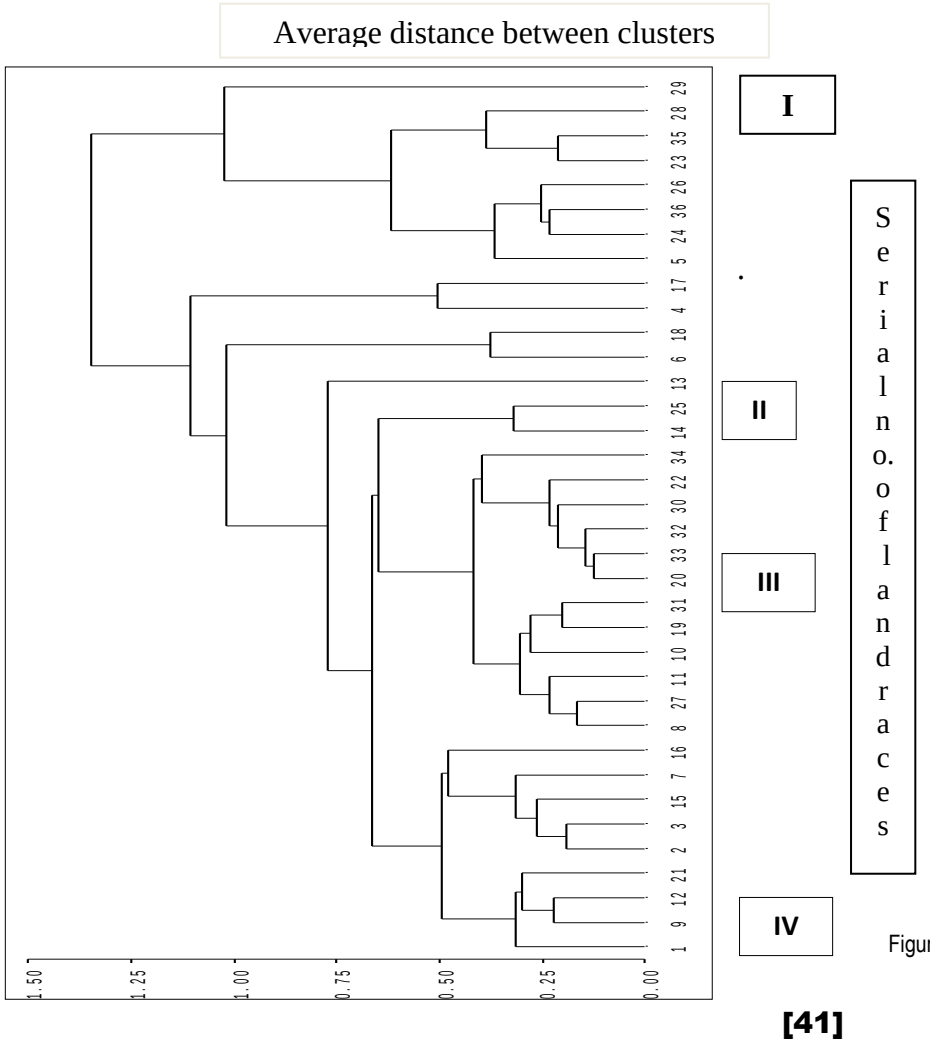

Figure 1. Neighbour-joining dendogram constructed using the unweighted pair group method of arithmetic means (UPGMA) algorithm depicting genetic relationship among 33 yam landraces based on phenotypic traits.

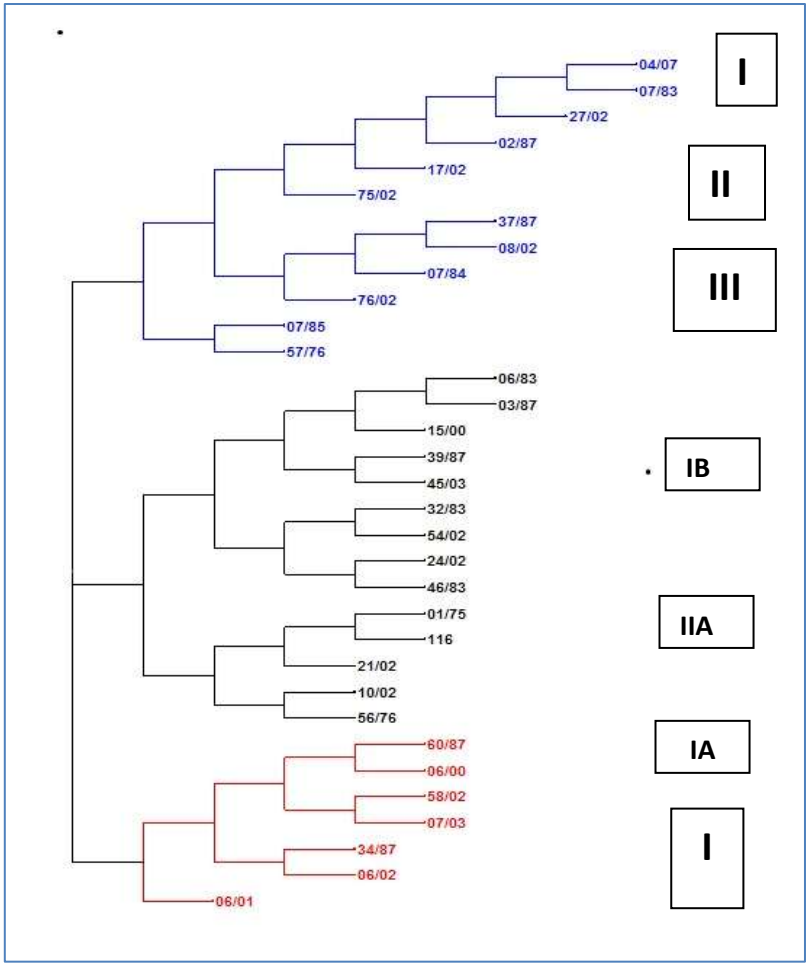

Figure 2. Neighbour-joining dendogram constructed using the unweighted pair group method of arithmetic means (UPGMA) algorithm depicting genetic relationship among 33 yam landraces based on SSR markers

Discussion

The present study analyzed genetic diversity of yam landraces collected from Southwest Ethiopia on the basis of phenotypic and SSR markers. Most phenotypic traits showed high genetic diversity on tuber characteristics such as: tuber colour, tuber flesh colour, tuber shape, tuber surface texture, tuber branching, tuber skin colour, hairiness of tuber surface and flesh colour at central transverse cross section. The present results are in agreement with the results of different reports who reported that yam exhibits significant variation on tuber characteristics (Obidiegwu et al., 2009; Dansi et al., 2013). The observed variation in tuber characteristics among the yam landraces could be partly due to the result of long-term selection by growers, the farming system, environmental effects and the mating system of the crop (Koffi et al., 2009; Mashilo et al., 2015). Probably farmers may have selected various unique tuber shapes for different uses (Loko et al., 2013). Yam is a dioecious plant and has high probability for cross-pollination, which result in considerable variation affecting the genetic identity of populations. Similar result was reported by Mulualem and Mohammed, (2013) on aerial yam (Dioscorea bulbifera).

The result of 'H' value for all observed phenotypic characters ranged from 0.53 for leaf density, tuber surface texture and hairiness of tuber surface to 1.50 for flesh colour at central transverse with the overall mean of 0.985. This result is in accordance with the report of Silvia and Gustavo, (2006) who found an average level of diversity in Colombian collections of water yam (Dioscorea alata) and Tamiru et al., (2011) in yams from South Ethiopia. High 'H' value indicates relatively high level of diversity and evenly distribution of landraces (Hennink and Zevan, 1991; Abebe, 2008). The low level diversity may also indicate the narrow genetic base of the plant and the lower probability of sexual reproduction in yam.

The number of alleles ranged from 1 to 5 with a mean of 3.0 per locus (Table 5). This result was lower than the mean alleles of 7.3 reported by Tostain et al., (2006) on yam, who used 17 polymorphic SSR loci. Zhigang et al., (2014) reported number of alleles of 6.09 per locus in 37 yam entries by using 7 polymorphic SSR loci, which is greater than the present findings. Further, Silva and Gustavo, (2006) reported the number of alleles per locus varying from 1.0 to 2.0 (mean 2.8), suggesting low allelic richness. In this study, the number of effective alleles per locus ranged from 1.0 to 3.57 with a mean of 1.71. Expected heterozygosity values in this study ranged from 0.116 to 0.80 with a mean of 0.567. The mean heterozygosity value observed in this study was quite smaller than the report of Obidiegwu et al., (2009) who

reported a mean value of 0.67 in Côte d'Ivoire using 13 SSR markers and comparing 89 water yam (Dioscorea alata L.) accessions from West African countries. The high level of heterozygosity observed among genotypes signified that landraces used in this study were collected from wide range of geographic areas with different levels of selection pressure and will enhance selection efficiency.

In the present study the PIC value ranged from 0.0 to 0.72 with a mean of 0.3. This value is similar with those reported by Emmanuel et al., (2015) who reported PIC values of 0.86 to 0.94; Marcos et al. (2011) with 0.39 to 0.78 (mean 0.4) and Obidiegwu et al., (2009) with 0.30 to 0.82. The PIC defines a relative measure of the in formativeness of a marker or discriminatory power of a polymorphic marker, which depends on the number of alleles and relative frequency of an allele in the population (Gaikward et al., 2008; Bekele, 2014). Four markers (YM02, YM09, YM12 and YM21) in this study had PIC values > 0.5, suggesting high discriminatory ability for classifying the landraces. The dendogram based on phenotypic traits and SSR markers classified the yam landraces into four and three main clusters, respectively. This indicated both SSR and phenotypic traits showed similar trend for clustering of studied landraces in southwest Ethiopia.

Conclusion

The phenotypic and SSR markers revealed high genetic diversity among yam landraces collections of south western Ethiopia. This variation would be attributed to the result of long-term selection and management of the yam by growers, the exchange of landraces between farmers and traders and environmental effects and the mating system of the crop. The variation obtained among the collection has good possibility to make selections for any of the traits in south west Ethiopia, assuming that a significant portion of the phenotypic variation is genetic. It was also found that the landraces showed a wide range of variation for tuber colour, tuber flesh colour, tuber shape, tuber surface texture, tuber branching, tuber skin colour, hairiness of tuber surface and flesh colour at central transverse cross section are useful morphological parameters for genetic analysis in yam. The detection of a significant number alleles that could be attributed to the high genetic diversity in the yam landrace. This conforms that Ethiopia was one of the primary centers for the domestication of yam. The diversity available in the studied landrace would allow for future breeding programs in the country.

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References

Abebe Wendawek. 2008. Morphological and molecular characterization of cultivated Guinea yam accessions and wild relatives (Dioscorea cayenensis Lam complex from south and southwest Ethiopia. Ph.D. Thesis, Addis Ababa University, Ethiopia.

Alina, M., Hussein, S., Pangirayi, T. and Mark, L. 2014. A genetic diversity analysis of South African sorghum genotypes using SSR markers. South African Journal of Plant and Soil, 31(3):145–152.

Arnau, G, Nemorin, A., Maledon, E., and Abraham, K. 2009. Revision of ploidy status of Dioscorea alata L. (Dioscoreaceae) by cytogenetic and microsatellite segregation analysis. Theor Appl Genet. 118:1239–1249.

Bekele Atnafua. 2014. Studies on molecular genetic diversity and useful genomic traits of yam (Dioscorea spp.) germplasm collections from Ethiopia. Ph.D. Thesis, Addis Ababa University, Ethiopia.

Dansi, A., Dantsey, M., Barry, H., and Vodouhè, R. 2013. Production constraints and farmers' cultivar preference criteria of cultivated yams (Dioscorea cayenensis-Dioscorea rotundata complex) in Togo. International Journal of Biology, 4:191-199.

Demissew Sebsebea., Nordal, I. and Stabbetorp, P.O.E. 2003. Flowers of Ethiopia and Eritrea: Aloes and other Lilies, 1st edn. Shama Books. Addis Ababa, Ethiopia.

Edwards, S.B. 1991. Crops with wild relatives found in Ethiopia. In: Engles, J.M.M, Hawkes, J.G and Worede Meleaku.(eds) Plant genetic resources of Ethiopia. Cambridge University Press, UK.

Emmanuel, O., Michael A., Asare PA. and Tetteh, JP. 2015. Molecular categorization of some water yam (Dioscorea alata L.) germplasm in Ghana using microsatellite (SSR) markers. Journal of Agricultural Science, 7(10): 225-234.

FAOSTAT. 2006 Food and Agriculture Organization of the United Nations, statistical data base, http//faostat.fao.org/faostat/collections?Subset=agriculture.

FAO. 2010. Food and Agricultural Organization of the United Nations, statistical database,

Gaikward, AB., Singh, AK., Chandel, D., Karihaloo, JL., and Staub, JE. 2008. Amplified fragment length polymorphism analysis provides strategies of bitter gourd (Momordica charantia L.). Hort Science, 43: 127–133.

Genres. 2008. Genres statistical software user's guide version 16, Genres Inc. USA.

Hamadina, E.I., Craufurd, P.Q. and Asiedu, R. 2009. Flowering intensity in white yam (Dioscorea rotundata). J. Agric Sci., 147(4):469–477.

Hennink , S. and Zeven, AC. 1991. The interpretation of Nei and Shannon-Weaver within population variation indices, Euphytica, 51: 235-240.

Hildebrand, E.A. 2003. Motives and opportunities for domestication: an ethno-archaeological study in southwest Ethiopia. Journal of Anthropological Archeology, 22:358-369.

IPGRI/IITA. 1999. Descriptors for yam (Dioscorea spp.). International Institute for Tropical Agriculture, Ibadan, Nigeria / International Plant Genetic Resources Institute, Rome, Italy, 66.

Koffi KK., Anzara, GK., Malice, M., Djè ,Y., Bertin, P., Baudoin, J-P. and Zoro, Bi IA. 2009. Morphological and allozyme variation in a collection of Lagenaria

siceraria (Molina) Standl. from Côte d'Ivoire. Biotechnologies, Agronomie, Société et Environment, 13: 257–270.

Loko, L.Y., Dansi, A., Linsoussi, C., Vodouhè, R., Akoegninou, A. and Sanni, A. 2013. Current status and spatial analysis of Guinea yam (Dioscorea cayenensis Lam. Dioscorea rotundata Poir. complex) diversity in Benin. Genetic Resources and Crop evolution, 5(4):223-239

Loko, Y.L, Adjatin, A., Dansi, A., Vodouhe, R. and Sanni, A**. 2015. Participatory evaluation of Guinea yam (**Dioscorea **cayenensis** Lam.**D. rotundata** Poir. complex) landraces from Benin and agro-morphological characterization of cultivars tolerant to drought, high soil moisture and chips storage insects. Genet Resour Crop Evol., 62:1181–1192.

Marcos, V., Siqueira, B., Thiago, M., Marconi, G., Maria. L. Maria, B., Zucchi, I., and Elizabeth, A. 2011. New microsatellite loci for water yam (Dioscorea alata) and cross amplification for other Dioscorea species. American Journal of Botany, 144-146.

Mashilo, J., Shimelis, H. and Odindo, A. 2015. Genetic diversity of bottle gourd (Lagenaria siceraria (Molina) Standl.) landraces of South Africa assessed by morphological traits and simple sequence repeat markers. South African Journal of Plant and Soil, 1–12.

Miege, J. and Demissew, S. 1997. Dioscoreaca. In: Edwards Demissew S., Hedberg S, I (eds.) Flora of Ethiopia and Eriteria, Vol 6, Hydrocharitance to Aracea. The national herbarium, Addis Abeba, Ethiopia/The department of systematic botany, Uppsala, Sweden.

Mignouna, H.D, Dansi, A. and Zok, S. 2002. Morphological and isozymic diversity of the cultivated yams (Dioscorea cayenensis/ Dioscorea rotundata complex) of Cameroon. Genetic Resources and Crop Evolution, 49: 21-29.

Morimoto, Y., Maundu, P., Fujimaki, H., and Morishima, H. 2005. Diversity of landraces of the white-flowered gourd (Lagenaria siceraria) and its wild relatives in Kenya: fruit and seed morphology. Genetic Resources and Crop Evolution, 52: 737–747

Mulualem Tewodros and Mohammed Hussein. 2013. Genetic diversity of aerial yam (Dioscorea bulbifera L.) accessions in Ethiopia based on agronomic traits. Journal of Science Publishing group, Agriculture, Forestry and Fishery, 2(2):67-71.

Mulualem Tewodros. 2016. Genetic diversity, path coefficient analysis, classification and evaluation of yams (Dioscorea spp.) in southwest Ethiopia. Ph.D. Thesis, Haramaya University. Ethiopia.

Mulualem Tewodros, Firew Mekbib, Shimelis Hussein. Endale Gebre and Amelewerk Bekele. 2020. Genetic diversity of yam (*Dioscorea* spp.) landrace collections from Ethiopia using simple sequence repeat markers. Australian Journal of Crop Science, 12(08):1223-1230

Mulualem Tewodros, Firew Mekbib, Shimelis Hussein and Endale Gebre. 2020. Effect of harvest stages on yield and yield components of yams (*Dioscorea* spp.) in Southwest Ethiopia. J. Biol. Chem. Research. 37 (1): 113-119.

Nei ,M .and Li .1979. Mathematical method for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences, 76: 5256–5273.

Obidiegwu, J. E., Kolesnikova, A., Eneobong, E.E and Muoneke, C.O. 2009. SSR markers reveal diversity in Guinea yam (Dioscorea cayenensis/D. rotundata) core set. African Journal of Biotechnology, 8(12):2730-2739.

Paterne, A., Flora, Kwabena D., Alex, E., Guillaume, B., Robert, A., Patrick, A. and Asrat, A. 2019.

Phenotypic and molecular assessment of genetic structure and diversity in a panel of winged

yam (Dioscorea alata) clones and cultivars. Scientific Reports, 9:18221.

Peakall, R. and Smouse, P.E. 2012. Genetic analysis in excel. Population genetic

software for teaching and research. Bioinformatics, 28: 2537-2539.

Perrier, X, Jacquemoud-Collet J.P. 2006. DARwin software. Available at http://www. darwin. cirad.fr/darwin.htm

Perry, M.C, and McIntosh, M.S. 1991. Geographic patterns of variation in the USDA soybean germplasm collection: I. Morphological traits. Crop Sci. 31: 1350-1355.

Rehm, S. and Espig, G. 1991. The cultivated plants of the tropics and sub-tropics: cultivation, economic value and utilization. Verlag Josef Margraf scientific Books, Weikersheim, Germany.

SAS, Institute. 2000. Statistical Analytical Systems SAS / STAT user's guide version 8(2): cary NC: SAS institute Inc.

Sesay, L., Norman, P.E., Massaquoi, A., Gboku, M.L. and Fomba, S.N. 2013. Assessment of farmers' indigenous knowledge and selection criteria of yam in Sierra Leone. Sky Journal of Agricultural Research, 2(1):1–6.

Silva, B. and Gustavo, B. 2006. Molecular characterization of Colombian yam germplasm by selective Amplification of Microsatellite Polymorphic Loci. Rev. Colombian Biotechnology, 8(2):60-66.

Spooner, D, van Treuren, R., and de Vicente, M.C. 2005. Molecular markers for gene bank management. IPGRI Technical Bulletin No. 10, International Plant Genetic Resources Institute, Rome, Italy.

Tamiru, M. 2006. Assessing diversity in yam (Dioscorea spp.) from Ethiopia based on morphology, AFLP marker and tuber quality, and farmers' management of landraces. Ph.D. Thesis, George August University, Germany.

Tamiru, M., Heiko, C., Becker, B. and Maass, .L. 2007. Genetic diversity in yam germplasm from Ethiopia and their relatedness to the main cultivated Dioscorea species assessed by AFLP markers. Journal of Crop Science, 47:1744–1753.

Tamiru, M., Heiko, C., Becker, B. and Maass, .L. 2011. Comparative analysis of morphological and farmers' cognitive diversity in yam landraces (Dioscorea spp.) from Southern Ethiopia, Tropical Agriculture Development, 55 (1):28–43.

Tamiru, M., Shinsuke, Y., Chikako, M., Pachakkil, B., Hiroko, T., Antonio, L., Alieu, S., Robert A., and Ryohei, T. 2015. Development of genomic simple sequence repeats markers for yam. Crop science, 55:2191–2200.

Terauchi, R., Chikaleke, V., Thottappilly, A. and Hahn, S.K. 1992. Origin and phylogeny of Guinea yams as revealed by RFLP analysis of chloroplast DNA and nuclear ribosomal DNA. Theor. Appl. Genet. 83:743-751.

Tostain, S, Scarcelli, N, Brottier, P, Marchand, J.L, Pham, J.L, and Noyer, J.L. 2006. Development of DNA microsatellite markers in tropical yam (Dioscorea sp.). Mol Ecol Notes, 6:173–175.

Zhigang, W., Wu, J., Xiao, X., Zheng, M., Chuan, Y., Xin, C. and Nitin, M. 2014. Genetic diversity analysis of yams (Dioscorea spp.) cultivated in China using ISSR and SRAP markers. Genet Resource Crop Evol., 61:639–650.