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Genetic Diversity Analysis of Ethiopian Yam (*Dioscorea* spp) from Different Geographic Origin Using Simple Sequence Repeat

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Abstract: Yam (*Dioscorea* spp L.) is one of root and tuber crops grown in Ethiopia as source of food and income. It is cultivated mainly in south, southwest, and western part of the country. Two hundred ten yam accessions from ten different geographic origins of major growing areas of the country were used in this study. The sprouted tubers of some accessions were received from research centers where others were directly collected from farmers' fields during early March, 2010. The collected yam genotypes were planted in complete randomized block design at two research sites (namely at Hawasa and Wonago) which are found under South Agricultural Research Institute in the end of April 2010. All important cultural practices such as staking, weeding and irrigation were done starting from planting till harvesting. Yam leaf samples with of different origin were observed separately using twenty SSR markers. A similarity matrix derived UPGMA cluster analysis based on geographical sites showed nine distinct clustering groups which indicated presence of relationship between genetic distances to that of geographical distance in most of clustering groups. These cluster grouping is supported by Analysis of Molecular Variance (AMOVA) that indicated presence of significance genetic variation of 210 yam accessions within and among ten collection sites.

Keywords: Yam, Genetic Diversity, Geographic Distance, SSR

1. Introduction

Ethiopia has diverse biological wealth that includes plants, animals, and microbial species. The crop diversity of the country is very high due to the existence of diverse farming systems, socioeconomics, cultures and agro-ecologies. There are several indigenous cultivated or semi-cultivated root and tuber crops in Ethiopia. These crops have an important place in the diet of the population [1]. Yam is among the most important tuber crops in the country, especially in humid areas where there is heavy year round precipitation [2]. It is cultivated in South, South Eastern and South Western parts of the country across a range of agro-ecologies as the main co-stable food stuffs. It is known by different vernacular names in different locations such as Boye, by Dauro people; Kocho or Wocheno, Oromo people; Bohe, Wolayita people; Kuso, Yem and Sidama people: Boina, [3].

It is a preferred food and plays a major role in food security. It has high price at market compared to other root and tuber crops produced in the country. Hence, it is a good source of income for producing farmers.

Several species of *Dioscorea* grow in different parts of Ethiopia but not all are edible. Eleven described *Dioscorea* species, (both wild and cultivated), are found in the country indicating presence of wide diversity of the species. The species *D. alata* L is not indigenous to Ethiopia and its center of origin is Southeast Asia. In Ethiopia, *D. alata* it is sterile and grows only in cultivation. Predominantly six yam species are grown by farmers in the country. These *are D. rotundata D. bulbifera, D. abyssinica, D. cayenensis, D. praehensilis,* and *D. alata* [4].

The present study is conducted with more yam accessions from Gamogofa and Wolita regions based on earlier recommendations and tried to cover more geographic regions

of major growing areas of the country (Table 3). It evaluates the genetic diversity and relationships of 210 yam accessions across in ten geographic area of Ethiopia using Simple Sequence Repeat Poly Acrylamaide Gel Electrophoresis based analysis.

2. Objective of the Study

The major objectives of this research is to determine the degree of genetic diversity and the relationship among and within Ethiopian yam germplasms collected from ten major growing areas of the country.

3. Methodologies Used in the Study

3.1. DNA Extraction

Young and clean leaves ***were*** collected and dried in silca-gel. These dried samples were grounded with sterile pestle and mortar with addition of liquid nitrogen. 0.2 g of pulverized leaf sample was used to isolate genomic DNA using modified triple Cetyl Trimethyl Ammonium Bromide (CTAB) extraction technique [5]. The quality and yield of DNA was carried out by running 3µl of freshly extracted genomic DNA samples on 1% agarose gel stained with 3µl of ethidium bromide, and visualized under an ultraviolet transiluminator in Genetic Research Laboratory of Addis Ababa University, Addis Ababa, Ethiopia (Figure 1). The quality and concentration of all DNA samples were determined using NanoDrop spectrophoto meter (NanoDrop Technologies, Wilming Ton, DE) at the International Institute of Tropical Agriculture (IITA), Ibadan. The NanoDrop uses 2 µl of diluted DNA samples (100 µl TE) with a sensitivity range from 5ng to 3700 ng. Absorbance at 260 nm (A260) is measured for each DNA samples to determine the DNA quantity. Nucleic acids and proteins have absorabance maxima at 260 and 280nm respectively. A260 measurments are quantitatitaive for relatively pure DNA preparations in microgram quanities. However the absorbance wave length ratio (260/280) was used to determine the quality of DNA. The ratio of 260 and 280 nm used as an indicator of DNA purity. The absorbance wave length ratio (260/280) of the genomic DNA was between 1.8 to 2.0.

3.2. PCR Amplification

All the DNA samples were diluted to a standard concentration of 25 ng/µl with ultra pure water for PCR amplification. Polymerase chain reactions (PCRs) were carried out in a total volume of 10 µl, containing 2µl of 25 ng of genomic DNA, 1µl of 5mM forward primer, 1µl of 5mM of reverse primer, 1µl 10X NH4 reaction buffer, 0.4 µl of 50 mM $MgCl_2$, 0.4 µl of 5 mM $dNTP_S$, and 0.06µl (5u/µl, Bioline) Biotaq ^{TM} DNA polymerase. Touchdown PCR was performed using Applied Biosystems verti 96 well thermal cycler. In this program the anealing temprature was gradually decreased by 1^0 C during the cycling program in order to favour only specific product amplification. The PCR cycle consisted of initial denaturation at 95°C for 2 minute followed by 35 cycles for 65^0 C anealing for 20 seconds then with 1°C reduction in temprature per cycle for 10 cycles and the last extension step was 72°C for 5 minute. The PCR products were storred at 4°C until used. Touchdown PCR is a modification of PCR in which the intial annealing temprature is higher than the optimal and is gradually reduced over subsequent cycles until the Tm temprature or "touchdown temprature" is reached. The annealing temprature is decreased 1^0 C every other cycle to approx 10^0 C below the calculated Tm to permit exponential amplification [6].

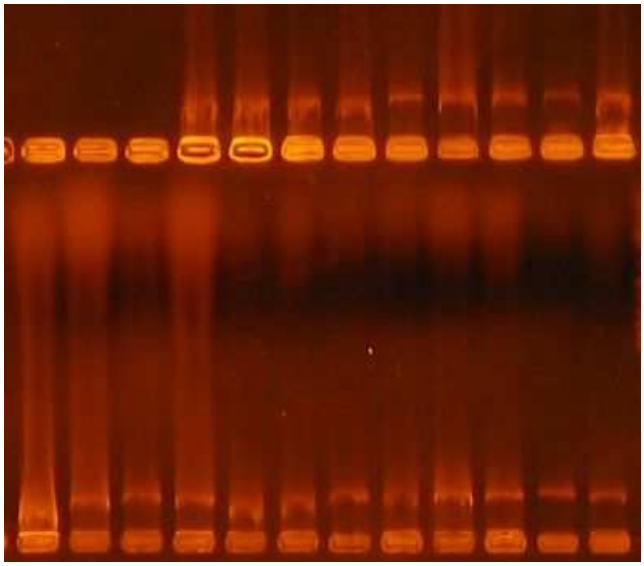

Figure 1. *Test gel of some diluted DNA samples extracted from Ethiopian Yam (Dioscorea spp) collections.*

3.3. Microsatellite Markers and Detection of PCR Products

In this procedure, two cleaned balanced plates were taken and at the edges of the plates, polyacrylamide gel was poured immediately. After the gel become polymerized, the plates were fixed to the tanker and 1x TBE was added at its upper side. The tanker was connected to a circute and left until it become warm. The denatured PCR products through formamide dye were loded in to gel approximetly with amount of 2.5 ul inside each well. This was allowed to run at 80 Watt for one and half hours to two and half hours according to the molecular weight of the primers. Then after, the gel was subjected to fixing solution for 20-30 minutes, staining solution for 30 minutes, distilled water for seconds, developing solution and it was shaked by hand until the bands were visable. At end the bands were fixed using fixing solution.

3.4. Details of Germplasm Studied and Micro Satellite Primers

Two hundred fifty eight yam accessions which were collected from ten major growing areas of Ethiopia were considered for this study. A total of 20 (thirteen DNA microsatellite markers and seven EST derived) markers were used in this study, of which 14 (eleven DNA microsatellite

markers and three EST derived) microsatellite markers resulted in allelic bands (Table 1). However, among these twelve (ten DNA microsatellite markers and two EST derived) microsatelite markers were gave clear bands for all studied germplasms and these SSR markers were taken for diversity analysis study (Table 2).

Loading of the DNA samples on Polyacrylamide Gel were carried in two different phases. First 96 denatured DNA samples were loaded on plate 1 and depending on the expected size of the PCR products with 20-30 minutes gap the second denatured DNA samples were loaded on plate 2. The rest 66 samples were loaded on other third plate.

Table 1. *The list of microsatellite primers observed in this study.*

No.	SSR Primers	Plate1	Plate2	Plate3	No.	SSR Primers	Plate1	Plate2	Plate3
1	YM13	*	*	*	11	D9	*	*	*
2	Da1C12	*	*	*	12	Dpr3F12	*	*	X
3	Da1A01	*	*	*	13	Dpr3B12	*	*	*
4	Dpr3D06	*	*	*	14	D95	X	X	*
5	Da1F08	*	*	*	15	D58	X	X	*
6	DA1D08	*	*	*	16	D25	X	X	*
7	Dab2C12	*	*	*	17	YM26	X	X	X
8	Ym30	*	*	*	18	DPR3F04	*	*	*
9	D83	*	*	*	19	D91	*	*	X
10	D55	*	*	*	20	Dab2C05	*	*	*

Note:-*= SSR primers showed amplified products, x = SSR primer did not work, Plate 1 & Plate 2 = Each 96 samples were loaded Plate 3= 66 DNA samples were loaded

Table 2. *Primer sequences (forward and reserve) used in the SSR analyses and their respective repeat motif, annealing temperature (Ta), Size (bp) and number of alleles per locus (A).*

Locus	Primer sequence (5'-3')	Repeat Motif	Size (bp)	Ta (ºC)	A
DalC12	F:GCCTTTGTGCGTATCT R:AATCGGCTACACTCATCT	(GT)9	163		5
Ym30	F:GGTCCTCTTCTATCCCAACAA R:CACGTATTAACTCCATCATCC				4
Da1Do8	F:GATGCTATGAACACAACTAA R:TTTGACAGTGAGAATGGA	(CA)8	335	51	4
Dpr3Fo4	F:AGACTCTTGCTCATGT R:GCCTTGTTACTTTATTC	(AG)15	128	51	10
D55	F:TGGACTAACGTGGTGTAGG R:CTAACAACACACACACGGG				2
D83	F:AGCTGAGATGGGAGGATCAA R:AGGAGGAGGTGGAGGACTTC				7
Da1Ao1	F:TATAATCGGCCAGAGG R:TGTTGGAAGCATAGAGAA	GT)8	204	51	6
dab2C12	F:GCCTTTGTGCGTATCT R:AATCGGCTACACTCATCT	(AC)8	163		4
Da1Fo8	F:AATGCTTCGTAATCCAAC R:CTATAAGGAATTGGTGCC	(TG)13	177	51	6
YM13	F:TTCCCTAATTGTTCCTCTTGTT R:GTCCTCGTTTTCCCTCTGTGT			58	5
Dpr3D06	F:ATAGGAAGGCAATCAGG R:ACCCATCGTCTTACCC	(GA)15	151	51	6
Dpr3B12	F:CATCAATCTTTCTCTGCTT R:CCATCACACAATCCATC	(TG)8	135	51	8

Note:- F=forward primer sequence R= reverse primer sequence

3.5. Data Scoring and Statistical Data Analysis

Microsatelites (SSR) scores were done for all 258 DNA samples. The score is converted into binary data by presence (1) or absence (0) of the selected fragment (respective allele in the case of SSR loci).

Among 258 yam accession, 48 accessions were duplicates which were identical at all SSR loci. During the analysis one acession was selected from each duplicates to represent the genotype and hence 210 yam land races were considered for the statistical anlysis (Table 3)

Analysis of Molecular Variance was computed following detection of PCR products and data scoring using the computer program Gen Alex [7].

The POPGENE 1.32 program [8] was used to calculate the genetic diversity parameters: allele frequencies at each locus the number of observed alleles, the number of effective alleles, Shannon information index (I) and gene diversity (H). The distance matrixes and the un-weighted Pair Group Method with arithmetic mean (UPGMA) [8]

were generated using Free Tree 0.9.1.50 Software and the distance tree was then viewed using TREEVIEW 0.9.1.50 Software of Tree un-rooted and slanted methods for species based and site based populations respectively [9].

The patterns of variation among yam population on 2D, a principal coordinated analysis (PCA) was examined using NTSYS-pc version 2.2 based on Nei's unbiased genetic distance [10 and 11].

Table 3. *List of Ethiopian yam collection from 10 different major growing areas of the country.*

SN	Accession number	Zone	Location	Altitude(Local name)
1	Si1	Gamogofa	Kucha	1500 (Bunne3)
2	Si2	Sidama	Dalle	1940 (Gellawcho-1)
3	Si3	Sidama	Dalle	1940 (Gelawcho-2)
4	Si4	Sidama	Dalle	1940 (Midasho-1)
5	Si5	Sidama	Dalle	1940 (Midasho-2)
6	Si6	Sidama	Dalle	1940 (Ouwisho-1)
7	Si7	Sidama	Dalle	1940 (Ouwisho-2)
8	Si8	Sidama	Dalle	1940 (Ouwisho-3)
9	Si9	Sidama	Dalle	1940 (Wendu-1)
10	Si10	Sidama	Dalle	1940 (Adameado-1)
11	Si11	Sidama	Dalle	1940 (Adameado-2)
12	Si12	Sidama	Dalle	1940 (Wendu-2)
13	Si13	Sidama	Dalle	1940 (Wendu-3)
14	Si14	Sidama	Dalle	1940 (Wendu-4)
15	Si15	Sidama	Dalle	1940 (Genticha-1)
16	Si16	Sidama	Dalle	1940
17	Si17	Sidama	Dalle	1940 (Genticha-2)
18	Si18	Sidama	Dalle	1940 (Genticha-3)
19	Si19	Sidama	Dalle	1940 (Genticha-4)
20	Si20	Sidama	Dalle	1940
21	Si21	Sidama	Dalle	1940 (Gellawcho-1)
22	Si22	Sidama	Dalle	1940
23	Si23	Sidama	Dalle	1940 (Adameado)
24	Si24	Sidama	Dalle	1940 (Gellawcho-2)
25	Si25	Sidama	Dalle	1940 (Genticha-5)
26	Si26	Sidama	Dalle	1940 (Genticha-6)
27	Si27	Sidama	Dalle	1940 (Midasho)
28	Si28	Sidama	Dalle	1940 (Genticha-5)
29	Si29	Sidama	Dalle	1940 (Wendu-5)
30	Si30	Sidama	Dalle	1940 (Wendu-6)
31	Si31	Sidama		
32	Wo1	Wolita	KedidaGamela	1970
33	Wo2	Wolita	KedidaGamela	1970
34	Wo3	Wolita	Damotwoyde	1780 (Oha-1)
35	Wo4	Wolita	Wolita	
36	Wo5	Wolita	Wolita	
37	Wo6	Wolita	Wolita	
38	Wo7	Wolita	Wolita	
39	Wo8	Wolita	Wolita	
40	Wo9	Wolita	Wolita	
41	Wo10	Wolita	Wolita	
42	Wo11	Wolita	Damotgalle	1950 (Gassa)
43	Wo12	Wolita	Sodozuria	1780 (Oha2)
44	Wo13	Wolita	Sodozuria	1780 (Oha3)
45	Wo14	Wolita	Sodozuria	1780 (Oha4)
46	Wo15	Wolita	Sodozuria	1850 (Chocha-1)
47	Wo16	Wolita	Sodozuria	1850 (Chocha-2)
48	Wo17	Wolita	Wolita	
49	Wo18	Wolita	Wolita	
50	Wo19	Wolita	KedidaGamela	1970
51	Wo20	Wolita	KedidaGamela	1970
52	Wo21	Wolita	KedidaGamela	1970

SN	Accession number	Zone	Location	Altitude(Local name)
53	Wo22	Wolita	Alabanatembar	1630
54	Wo23	Wolita	Alabanatembar	1630
55	Wo24	Wolita	Alabanatembar	1630
56	Wo25	Wolita	Wolita	
57	Wo26	Wolita	Wolita	
58	Wo27	Wolita	Damotwoyde	1780 (Wiyacha-1)
59	Wo28	Wolita	Damotwoyde	1780 (Wiyacha-2)
60	Wo29	Wolita	Wolita	
61	Wo30	Wolita	Damotwoyde	1780 (Oha-5)
62	Wo31	Wolita	Damotwoyde	1780 (Oha-6)
63	Wo32	Wolita	Wolita	
64	Wo33	Wolita	Kokate	Chaye Woncharo
65	Wo34	Wolita	Damotgalle	2220 (Arkiya)
66	Wo35	Wolita	Areka	1870 (Gaffella-1)
67	Wo36	Wolita	Areka	1870 (Gaffella-2)
68	Wo37	Wolita	Areka	1870 (Gaffella-3)
69	Wo38	Wolita	Wolita	
70	Wo39	Wolita	Wolita	
71	Wo40	Wolita	Areka	1870 (Gaffela-4)
72	Wo41	Wolita	Areka	1870 (Gaffela-5)
73	Wo42	Wolita	Wolita	
74	Wo43	Wolita	Wolita	(Wancharo-1)
75	Wo44	Wolita	Wolita	(Wancharo-2)
76	Wo45	Wolita	Wolita	(Wancharo-3)
77	Wo46	Wolita	Wolita	-
78	Wo47	Wolita	Damotgalle	2220 (Arkiya-1)
79	Wo48	Wolita	Damotgalle	2220 (Arkiya-2)
80	Wo49	Wolita	Wolita	-
81	Wo50	Wolita	Damotwoyde	2220 (Wiyacha-1)
82	Wo51	Wolita	Damotwoyde	2220 (Wiyacha-2)
83	Wo52	Wolita	Damotwoyde	2220 (Wiyacha-3)
84	Wo53	Wolita	Wolita	-
85	Eg1	EastWolega	Diga	1650 (Lalo-1)
86	Eg2	EastWolega	Diga	1650 (Lalo-2)
87	Eg3	EastWolega	Diga	1650 (Lalo-3)
88	Eg4	EastWolega	Diga	1650 (Msreta-1)
89	Eg5	EastWolega	Diga	1650 (Msreta-2)
90	Eg6	EastWolega	Diga	1650 (Msereta-3)
91	Eg7	EastWolega	Sasiga	1750 (Sasiga-1)
92	Eg8	EastWolega	Sasiga	1750 (Sasiga-2)
93	Eg9	EastWolega	Sasiga	1750 (Sasiga-3)
94	Eg10	EastWolega	Diga	1650 (Dhoknuma-1)
95	Eg11	EastWolega	Diga	1650 (Dhoknuma-2)
96	Eg12	EastWolega	Diga	1650 (Dhokuma-3)
97	Ar1	ArekaRC	ARC	1780
98	Ar2	ArekaRC	ARC	1780
99	Ar3	ArekaRC	ARC	1780
100	Ar4	ArekaRC	ARC	1780
101	Ar5	ArekaRC	ARC	1780
102	Ar6	ArekaRC	ARC	1780
103	Ar7	ArekaRC	ARC	1780
104	Ar8	ArekaRC	ARC	1780
105	Ar9	ArekaRC	ARC	1780
106	Ar10	ArekaRC	ARC	1780
107	Ar11	ArekaRC	ARC	1780
108	Ar12	ArekaRC	ARC	1780
109	Ar13	ArekaRC	ARC	1780
110	Ar14	ArekaRC	ARC	1780
111	Ar15	ArekaRC	ARC	1780

SN	Accession number	Zone	Location	Altitude(Local name)
112	Ar16	ArekaRC	ARC	1780
113	Ar17	ArekaRC	ARC	1780
114	Ar18	ArekaRC	ARC	1780
115	Ar19	ArekaRC	ARC	1780
116	Ar20	ArekaRC	ARC	1780
117	Ar21	ArekaRC	ARC	1780
118	Ar22	ArekaRC	ARC	1780
119	Ar23	ArekaRC	ARC	1780
120	Ar24	ArekaRC	ARC	1780
121	Ar25	ArekaRC	ARC	1780
122	Ar26	ArekaRC	ARC	1780
123	Ar27	ArekaRC	ARC	1780
124	Ar28	ArekaRC	ARC	1780
125	Ar29	ArekaRC	ARC	1780
126	Ar30	ArekaRC	ARC	1780
127	Ar31	ArekaRC	ARC	1780
128	Ar32	ArekaRC	ARC	1780
129	Ar33	ArekaRC	ARC	1780
130	Ar34	ArekaRC	ARC	1780
131	Ga1	GamoGofa	Gofa	1340
132	Ga2	GamoGofa	Kucha	1500 (Bunne-3)
133	Ga3	GamoGofa	Bonke	2070 (Kemba-1)
134	Ga4	GamoGofa	Bonke	2070 (Kemba-2)
135	Ga5	GamoGofa	Bonke	2070 (Kemba-3)
136	Ga6	GamoGofa	Arbaminch	1140 (Hatiya-2)
137	Ga7	GamoGofa	Breda	1655 (Bunne-2)
138	Ga8	GamoGofa	Gofa	1340 (Tolla-1)
139	Ga9	GamoGofa	Gofa	1340 (Tolla-2)
140	Ga10	GamoGofa	Gofa	1340 (Tolla-3)
141	Ga11	GamoGofa	Gofa	1340 (Tolla-3)
142	Ga12	GamoGofa	Gofa	1340 (Bunne-1)
143	Ga13	GamoGofa	Gofa	1340 (Bunne-2)
144	Ga14	GamoGofa	Gofa	1340
145	Ga15	GamoGofa	Gofa	1340
146	Ga16	GamoGofa	Gofa	1340
147	Ga17	GamoGofa	Gofa	1340
148	Ga18	GamoGofa	Gofa	1340
149	Ga19	GamoGofa	Gofa	1340
150	Ga20	GamoGofa	Gofa	1340
151	Ga21	GamoGofa	Bonke	1540 (Arfa-1)
152	Ga22	GamoGofa	Bonke	1540 (Arfa-2)
153	Ga23	GamoGofa	Bonke	1540 (Arfa-3)
154	Ga24	GamoGofa	Bonke	1540 (Arfa-4)
155	Ga25	GamoGofa	Gofa	1340
156	Ga26	GamoGofa	Gofa	1340
157	Ga27	GamoGofa	Kucha	1500 (Bunne-3)
158	Ga28	GamoGofa	Gofa	1340 (Tolla)
159	Ga29	GamoGofa	Arbaminch	1140 (Bunne-3)
160	Ga30	GamoGofa	Arbaminch	1140 (Bunne-4)
161	Ga31	GamoGofa	Arbaminch	1140 (Bunne-5)
162	Ga32	GamoGofa	Arbaminch	1140 (Bunne-6)
163	Ga33	GamoGofa	Bonke	1540 (Kemba-1)
164	Ga34	GamoGofa	Bonke	1540 (Kemba-2)
165	Ga35	GamoGofa	Bonke	1540 (Kemba-3)
166	Ga36	GamoGofa	Gofa	1340
167	Gd1	Gedio	Gedio	1590
168	Gd2	Gedio	Gedio	1590
169	Gd3	Gedio	Gedio	1590
170	Gd4	Gedio	Gedio	1590

SN	Accession number	Zone	Location	Altitude(Local name)
171	Gd5	Gedio	Gedio	1590
172	Gd6	Gedio	Gedio	1770 (Ganticho1)
173	Gd7	Gedio	Gedio	1770 (Ganticho2)
174	Gd8	Gedio	Wonago	1770
175	Gd9	Gedio	Wonago	1770
176	Gd10	Gedio	Wonago	1770
177	Gd11	Gedio	Wonago	1770
178	Gd12	Gedio	Wonago	1770
179	Ib1	Kefa	Kefa	1600
180	Ib2	Kefa	Kefa	1600
181	Ib3	Kefa	Kefa	1600
182	Ib4	Kefa	Kefa	1600
183	Ib5	Kefa	Kefa	1600
184	Ib6	Kefa	Kefa	1600
185	K1	Kembata	Hadero	1140
186	K2	Kembata	Hadero	1140 (Makawa-1
187	K3	Kembata	Hadero	1140 (Makawa-2)
188	J1	JRC	JRC	1780
189	J2	JRC	JRC	1780
190	J3	JRC	JRC	1780
191	J4	JRC	JRC	1780
192	J5	JRC	JRC	1780
193	J6	JRC	JRC	1780
194	J7	JRC	JRC	1780
195	J8	JRC	JRC	1780
196	J9	JRC	JRC	1780
197	J10	JRC	JRC	1780
198	J11	JRC	JRC	1780
199	J12	JRC	JRC	1780
200	J13	JRC	JRC	1780
201	J14	JRC	JRC	1780
202	Da1	Dauro	Konta	1900 (Gebiche-1)
203	Da2	Dauro	Konta	1900 (Gebiche-2)
204	Da3	Dauro	Konta	1900 (Gebiche-3)
205	Da4	Dauro	Konta	1900 (Gebiche-4)
206	Da5	Dauro	Mareka	1580 (Dorsita-1)
207	Da6	Dauro	Mareka	1580 (Dorsita-2)
208	Da7	Dauro	Mareka	1580 (Dorsita-3)
209	Da8	Dauro	Mareka	1580 (Dorsita-4)
210	Da9	Dauro	Mareka	1580 (Dorsita-5)

4. Result and Discussion

Analysis of Molecular Variance revealed a significant difference among population based on their geographical location (Table 4).

Table 4. *Analysis of Molecular Variance (AMOVA) showing the distribution of genetic diversity within and among 210 yam land races.*

Source	Df	SS	MS	Est. Var.	%	P-Value
Among Pops	9	79.163	8.796	0.022	1%	0.004
Among Indiv	200	1586.602	7.933	2.487	45%	0.457
Within Indiv	210	621.500	2.960	2.960	54%	0.459
Total	419	2287.264		5.468	100%	

4.1. Yam (Dioscorea Species) Diversity Within Geographical Locations

Genetic diversity was estimated for location using Jaccard's similarity coefficient, Neis' Gene diversity, Polymorphism percentage and Shanon Weaver diversity index (Table 5). The total percentage of polymorphic loci ranged from 98.53% (Wolita) to 94.14% (Gamogofa). Gene

diversity was highest (0.38) within accessions collected from Gedio followed by (0.35) with in accessions collected from Jima and Gamogofa. Accessions from Wolega and Kembata with (0.29) revealed lower Gene diversity. Shannon's information index (I) also showed the existence of high genetic variation within the accessions of Gedio, Wolita and Gamogofa. The mean of Jacard's similarity coefficient for comparisons among accessions within locations was 0.18 (Table 5).

4.2. Yam (Dioscorea Species) Diversity Among Geographical Locations

The genetic distance was computed based on 10 yam geographical location where originally the germplasms were collected. The highest genetic distance was encountered between Gamogofa and Wolita (0.98), followed by Gagomofa and Sidama (0.96) and Gamogofa and ARC (0.96). Generally lower genetic distances were observed between Kembata to all other locations (Table 6).

Table 5. *Summary of Gene Diversity Parameters with in different geographical locations of 210 yam (Dioscorea species) landraces from Ethiopia.*

Zone	No of entries	P%	Jaccard'sCoefficient	Nei's geneDiversity (h)	Shannon's InformationIndex (*I*)
Sidama	31	89.71	0.14	0.28	0.427±0.026
Wolita	53	98.53	0.20	0.30	0.458±0.020
Wolega	12	82.35	0.17	0.29	0.399±0.028
ARC	34	92.65	0.19	0.32	0.459±0.025
Gamogofa	36	94.12	0.24	0.35	0.481±0.022
Gedio	12	82.35	0.20	0.38	0.457±0.036
Kefa	6	75.00	0.09	0.34	0.417±0.31
Kembata	6	47.06	0.21	0.29	0.295±0.039
Jima	11	80.88	0.17	0.35	0.444±0.030
Dauro	9	79.41	0.16	0.31	0.414±0.029
Mean		82.21	0.18	0.32	0.414±0.029
SD				0.13	0.16
SE	4.56				
Total Number Polymorphic Loci	208				
Total% of Polymorphic Loci	99.05				

Table 6. *Neis' genetic distance measure of Ethiopian yam (Dioscorea species) on the bases of 10 geographical sites.*

	Sidama	Wolita	Wolega	ARC	Gamogofa	Gedio	kefa	Kembata	Jima
Sidama									
Wolita	0.95								
Wolega	0.92	0.91							
ARC	0.95	0.97	0.91						
Gamogofa	0.96	0.98	0.92	0.96					
Gedio	0.92	0.91	0.91	0.91	0.92				
Kefa	0.86	0.86	0.82	0.84	0.89	0.82			
Kembata	0.68	0.66	0.70	0.67	0.68	0.70	0.67		
Jima	0.93	0.90	0.88	0.92	0.92	0.90	0.83	0.71	
Dauro	0.92	0.89	0.91	0.91	0.92	0.93	0.82	0.69	0.88

4.3. Cluster Analysis

A dendrogram was constructed with a UPGMA cluster analysis based on Nei's genetic distance among 10 sites. The dendrogram based on SSR markers distinguished 210 yam landraces in to eight distinctly cluster groups (Figure 2).

Comparison of yam landraces within the same geographical zone revealed that most of yam population from the same origin falls in one or two cluster groups and had the range of similarity coefficient from 0.09 to 0. 24 (Table 5). This indicates that the presence of associations between geographical locations and genetic distance.

The second larger cluster group (Cluster 2) consisted of 40, 10, 7, 6, 4, 6 and 11 yam landraces from Gamogofa, Gedio, Jima, Kefa, Kembata, Wolita zones and Areka Research Center. Group 1 is the smallest whereas cluster 6 is the largest group consists of 1 and 52 yam landraces respectively from Wolita zone. Group 3 and 5 is composed of 14 and 10 yam land races from Sidama Zone. Group 4 is made of 11 and 3 yam accessions from Sidama and Dauro zones respectively. Group 8 is composed of 18 yam accessions of Areka Research Center and Group 9 is the smaller group next to cluster 1 which composed of 5 yam accessions where each 2 and 1 accession from Jima and Dauro and, Sidama Zones respectively.

A three dimensional PCO was done using Nei's genetic distances among 10 geographical sites (Figure 3). The first three principal coordinates accounted for 29% of the total variation among geographical sites which PCO-1 accounts for 14%, PCO-2 for 8% and PCO-3 for 7%. As it is observed from the 3D graph (Figure 3), yam germplasms from Gamogofa zone isolated from most of the yam germplasm of other localities.

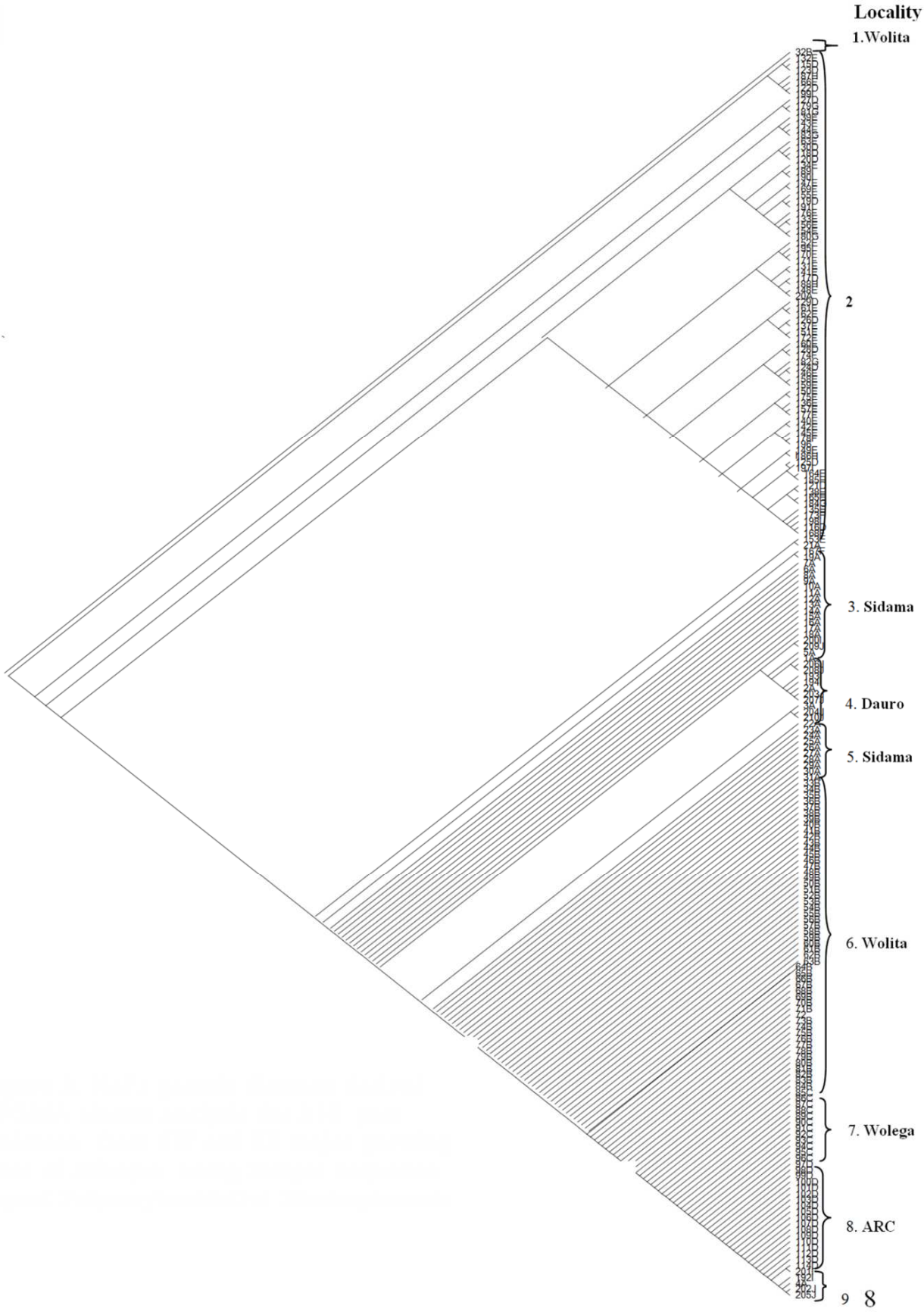

Figure 2. *Nei's genetic distance derived UPGMA cluster analysis for 210 yam landraces from SW and SE major growing areas of Ethiopia using Simple Sequence Repeat Polyacrylamide Gel-Electrophoresis.*

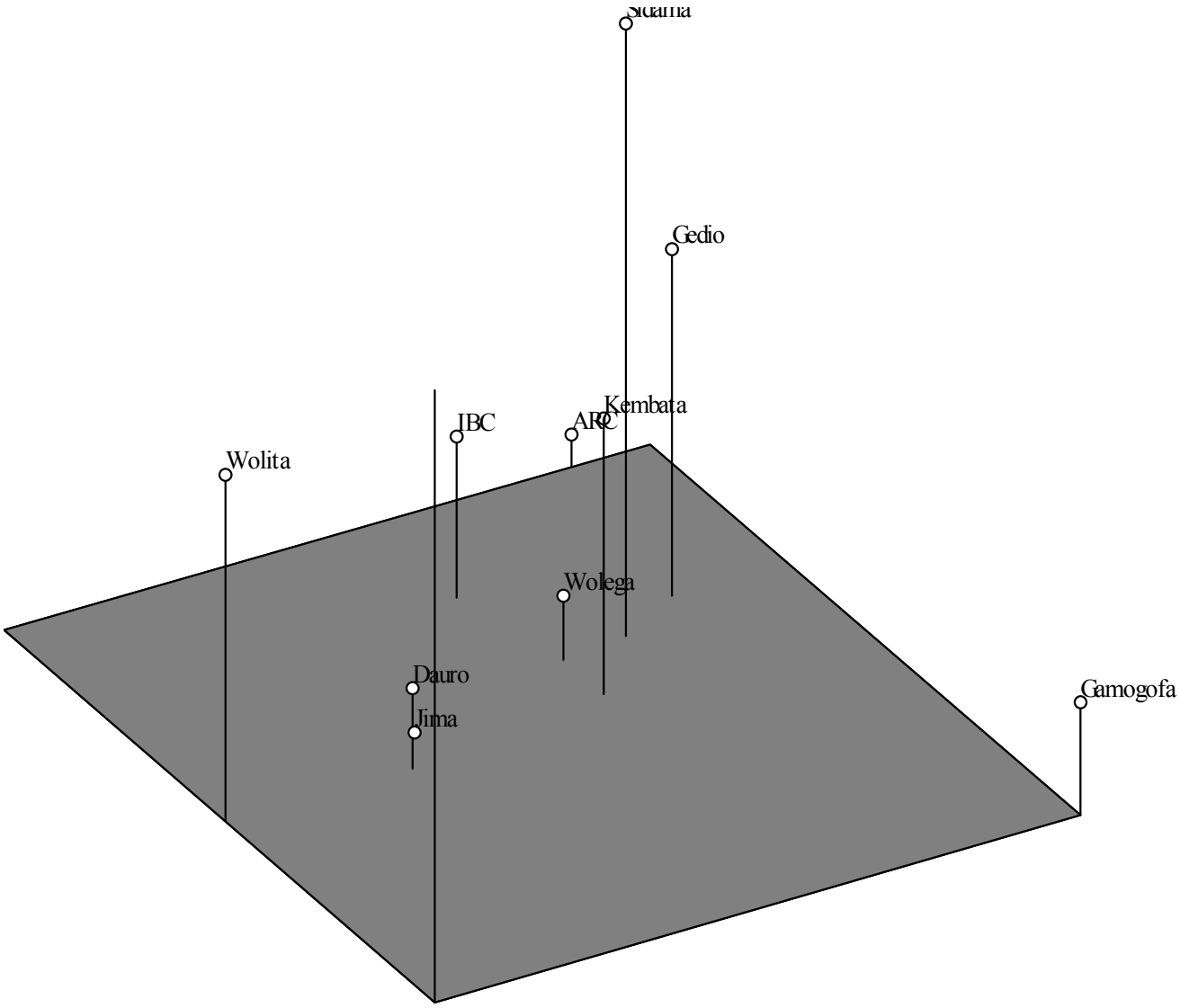

Figure 3. *Three diamentional plot for two hundred ten yam landraces of 10 major growing areas of SW and SE Ethiopia using Simple Sequence Repeat Polyacrylamide Gel Electrophoresis.*

5. Discussions

Understanding the molecular basis of essential biological phenomena in plants is crucial for effective conservation, management, and efficient utilization of plant genetic resources (Linda, *et al*, 2009). A research of published DNA sequences reveals that Microsatellites (SSRs) are shorter (1-6 base long) which are highly abundant and randomly distributed throughout the eukaryotic genomes [12] and [13]. They are the preferred molecular marker system for analysis in plant genetics and ecology [14].

The present study showed highest polymorphism among Ethiopian yam landraces. The polymorphism percentage among populations of different sites ranged from 98.53% (Wolita) to 47.06% (Kembata) with a mean value of 82.21. This highest level of polymorphism of SSR techniques indicates that this molecular marker is very useful for yam molecular characterization studies.

The un-weighed neighbor joining tree obtained using UPGMA analysis revealed nine distinct clustering groups (Figure 2). It is recommended that geographic diversity may be used as an index of genetic diversity in parental selection [15]. This shows that the power of microsatellite markers for genotyping yam (*Dioscorea* spp) populations. Microsatellite markers used in this study indicated presence of geographical pattern of diversity within Ethiopian yam landraces apart from cluster group 2. Cluster 2 grouping is in line with the genetic variation result of AMOVA which was very low among geographical sites (Table 4). Earlier, it is also reported similar genetic variation results of AMOVA [16]. An additional reason for cluster 2 grouping may be due to yam germplasms distribution across different locations through market. There is an agreement of this result which stated that yam accessions from different countries were grouped together to one cluster without having any relationship and relatedness of these accessions and their geographical origin [17].

Ethiopian yam genotyping based on this molecular marker showed, landraces of Gamogofa exhibits high genetic distance to the majority of yam landraces of other localities and similarly yam germplasms of wolita gave high genetic distance to Wolega, Gamogofa and Areka research center. So it indicates importance of giving consideration to these distantly related populations of Gamogofa, Wolita and Wolega during improvement of the crop through breeding Whereas Kembata landraces revealed less genetic distance towards most of other location. Additionally, high genetic variability was obtained with in Jima and Gedio yam landraces. Accordingly, it is vital to observe these germplasms during selection activities to increase yam production and productivity.

A three diamentional plots (Figure 3) shows the first three principal component scores cumulatively explains 39% of the total variation which indicated that the genetic variability of Ethiopian yam landraces. This result is comparable to 34% of total variation research report on the characterization of West Africa yam (*Dioscorea alata*) using SSR [18].

6. Conclusion and Recommendations

The existence of genetic variability is the bases of any plant breeding and improvement activities. The present study showed highest polymorphism 99.05% among Ethiopian yam landraces. The obtained highest level of polymorphism with SSR techniques indicates that, this molecular marker is vital and powerful for yam molecular characterization studies.

There was clear relationship of genetic distance to that of geographical distance to most of clustering groups. Hence, it is possible to use yam landraces with heritable and desirable characters from distantly related localities as a source of parental material in yam breeding program.

The landraces of Gamogofa exhibits high genetic distance to the majority of yam landraces of other localities and similarly yam germplasms of wolita gave high genetic distance to Wolega, and Gamogofa, so it will be important to consider these distantly related populations of Gamogofa, Wolita, and Wolega in yam breeding for its' genetic gain. Kembata land races revealed less genetic distance towards other location.

High genetic variability was obtained with in Gamogofa, Wolita, Jima and Gedio yam landraces. Accordingly, it is vital to observe these locations during collection and selection of elite clones in yam improvement programs.

References

[1] IBC, (2008). Ethiopia; Second Country Report on the State of PGRFA to FAO Addis Ababa, Ethiopia.

[2] Kay, D. (1987). Root Crops Tropical Development and Research Institute. London.

[3] Edossa Etissa (1998). Yams: exploration, collection and evaluation. News letter of Ethiopia Agricultural Research Organization. Vol. 13, No. 2.

[4] Miège, J., and Demissew Sebsebe (1997). *Dioscoreaceae*. In: Flora of Ethiopia an Eriterea (Edwards, S., Sebsebe Demissew and Hedberg, I., eds.). A joint publication of the National Herbarium, Biology Department, AAU, Ethiopia and the depar-tment of Systematic Botany, Uppsala University, Sweden.

[5] Borsch, T., Hilu, K. W., Quandt, D., Wilde, V., Neinhuis, C. and Barthlott, W. (2003). Noncoding plastid trnT-trnF sequences reveal a well resolved phylogeny of basal angiosperms. J. Evol. Biol. 16: 558-576.

[6] Rous, K. (1995). Optimization and troubleshooting in PCR, in PCR primer (Diefferbach, C. W. and Dveksler, G. S eds.), CSH press, New York.

[7] Peakall, R., and Smouse, P. (2006) GenAlEx 6: Genetic analysis in Excel Population genetic software Mol. Ecol. Notes 6, 288-295.

[8] Yeh, F., Boyle, T. (1997). Population genetic analysis of co-dominant markers and quantitative traits. Belgian Journal of Botany, 129: 157.

[9] Pavlicek, A., Hrda, S. and Flegr, J. (1999). Free tree free ware program for construction of phylogenetic trees on the basis of distance data and bootstrap/Jack Knife analysis of the tree robustness. Application in the RAPD analysis of genus Frenkelia. Folia. Biologica. 45: 97-99.

[10] Sneath, P, and Sokal, R. (1973). Principles of Numerical Taxonomy Freeman San Francisco.

[11] Pavlicek Nei's, M. (1978). Estimation of average heterozygosis and genetic distance from a small number of individuals. Genetics, 3 (89). 583–590.

[12] Linda, M., Arshiya, N., and Mario, A. (2009). Assessing Plant Genetic Diversity by Molecular Tools. Department of Aerobiology and Agro chemistry, Tuscia University, Italy.

[13] Morgante, M., and Olivier, A. (1993). PCR-amplified microsatellites as markers in plant genetics. Plant J. 3, 175–182.

[14] Morgante, M., Hanafey, H., and Powell, W. (2002). Microsatellites are preferentially associated with non repetitive DNA in plant genome. Nature Genet 30: 194-200 Tropical Agriculture (Trinidad) 54: 1–13.

[15] Singh, B. D. (2000). Plant Breeding: Principles and Methods. Kalyani Publishers, New Delhi-Ludhiana.

[16] Muluneh, Tamru. (2006). Assessing Diversity in Yams (*Dioscorea* spp.) from Ethiopia based on, Morphology, AFLP Markers and Tuber Quality, and Farmers management of landraces.

[17] Obidiegwu, J., Kolesnikova-Allen M., Ene-obong, E., Muoneke, C., and Asiedu, A., (2009). SSR markers reveal diversity in Guinea yam (D. cayenensis/D. rotundata) core set. African Journal of Biotechnology Vol. 8 (12), pp. 2730-2739.

[18] Obidiegwu, E., Asiedu, R., Ene-Obong, E., Muoneke, C, and Kolesnikova, M, (2009). Genetic characterization of some water yam (*Dioscorea alata* L.) accessions in West Africa with simple sequenc repeats. Journal of Food & Agriculture.