

Genetic Diversity of *Capsicum* L. Accessions from South West Nigeria using Simple Sequence Repeats (SSR) Markers

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ABSTRACT

Pepper (*Capsicum* L.) is a widely consumed vegetable in South West Nigeria because of its nutritional and medicinal potentials. This study is aimed at evaluating genetic diversity among 30 pepper accessions collected from different pepper-growing areas in South West Nigeria using SSR markers. Amplification potentials and bands clarity were considered for selecting 17 among 29 SSR markers screened. Genetic diversity was evaluated using principal coordinates analysis (PCoA), cluster analysis (CA), and analysis of molecular variance (AMOVA). The sum of 208 alleles was detected with an average value of 12.24 alleles per locus for each accession. Genetic diversity was high in all loci with the mean value ranging from 0.23 to 0.77. The result of AMOVA showed that 2 % of the genetic diversity was due to interspecific variations while 98 % of the differences were due to intraspecific variations among accessions. The results of cluster analysis showed clearly high genetic similarity coefficient at > 71 %. The intraspecific and interspecific genetic relationships observed could be an integral part of the useful tools for genetic improvement of the genus *Capsicum*

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through breeding purposes especially the wild varieties.

Keywords: *Capsicum*, genetic diversity, Nigeria, South West, SSR

INTRODUCTION

Capsicum (L.) pepper fruit is a vegetable with colour range from red, purple to yellow when mature (Oni, 2011). The pungency properties in pepper fruit, resulting from their high concentration of capsaicinoid make them important ingredients in people's diet all over the world (Germplasm Resources Information of Network [GRIN], 2009). This characteristic makes Nigeria pepper to be on high demand. Pepper production is an important agribusiness worldwide and one of the revenue sources in South West, Nigeria (Showemimo & Olanrawaju, 2000). Food and Agriculture Organization [FAO] (2010) reported that Nigeria produced 695,000 metric tons of pepper from a total area of 77,000 ha accounting for 50 % total production in Africa. Today, pepper has become widely exploited in tropical and temperate regions because of its nutritional contents, antioxidants properties and high health-protecting factors (Christine et al., 2014; Mady et al., 2005). Abdullahi et al. (2003) reported the potentials of pepper in African medicine as sore throat treatment.

Genetic diversity is a possible guarantee in reviving some of the economic plant species that near extinction. Research on genetic resources and plant breeding is

one innovational activity most relevant for agriculture sustainability (Almeida et al., 2005). In response to the report of Almeida et al. (2005), the significant efforts with collection, characterization, and conservation could help to conserve pepper for various genetic breeding programmes. In Nigeria, most past research effort on pepper was focused mainly on food (Ado, 1999; Falusi & Morakinyo, 2001; Gill, 1992; Mady et al., 2005), and taxonomic classification using anatomical structures (Nwachukwu et al., 2007) with little or no effort on genetic improvement using SSR markers. However, an effort made by Falusi (2006) on genetic diversity of *Capsicum* in Nigeria using morphological markers was not comprehensive because he used few morphological characters. This scientific gap could be filled through proper diversity study of collected accessions at the molecular level to examine the level of genetic relatedness. Molecular markers will be much more appropriate in evaluating genetic relationships among the collected accessions. Previous workers had reported number of chromosomes of $2n=24$ ($x=12$) for the genus *Capsicum* (Morakinyo & Falusi, 1992; Nwakiti, 1981; Stebbins, 1971). High chromosome number suggests higher productivity and wide genetic base for the genus *Capsicum* (National Research Council [NCR], 2006; Silva et al. 2011). Information on the genetic diversity in the population of *Capsicum* varieties in South West, Nigeria is scanty. Hence, there is a need to investigate the genetic relationships

among collected pepper accessions for breeding purposes and genetic improvement of the genus *Capsicum*.

Anu and Peter (2003) as well as Odeigah et al. (1999) had reported the use of biochemical and molecular markers to characterize some *Capsicum* accessions in Nigeria, but both of them used SDS-polyacrylamide gel electrophoresis of seed proteins markers. However, review of pieces of literature revealed no reports on the use of simple sequence repeats (SSR) makers in evaluating the genetic diversity populations of pepper in Nigeria. Some studies on *Capsicum* using SSR markers were reported in China, Russia, and Japan with the highest degree of genetic diversity recorded compared to other molecular markers (Chen et al., 2006; Luo et al., 2006; Zhou et al., 2009). They all reported SSR makers to be most suitable in evaluating genetic diversity because of its hypervariable allelic variations attribute.

Therefore, this study focused on the use of SSR to examine genetic diversity among 30 *Capsicum* accessions collected from different pepper growing areas within South West Nigeria. This is with a view to provide scientific information for genetic improvement of the genus through breeding purposes especially the wild *Capsicum* varieties.

MATERIALS AND METHODS

Capsicum Accessions and Areas of Collection

Thirty (30) accessions of *Capsicum* were collected from rural farmers within the

South West, Nigeria and the gene bank of National Centre for Genetic Resources and Biotechnology (NACGRAB) in Nigeria between December 2014 and December 2015. The sources, dates and other collection data of the accessions studied, and a map of collection areas are detailed in Table 1 and Figure 1 respectively.

Sample Preparation

Young fresh leaves of studied accessions were plucked and freeze-dried for three days and thereafter stored at -20°C in Dr. Wang Lihao's laboratory at the Institute of Vegetables and Flowers (IVR), Chinese Academy of Agricultural Sciences (CAAS), Beijing, China.

DNA Extraction and Quantification

A modified mini-preparation CTAB protocol was employed for DNA extraction while DNA quantification was conducted using NanoDrop Spectrophotometer at 260 nm (Fulton et al., 1995).

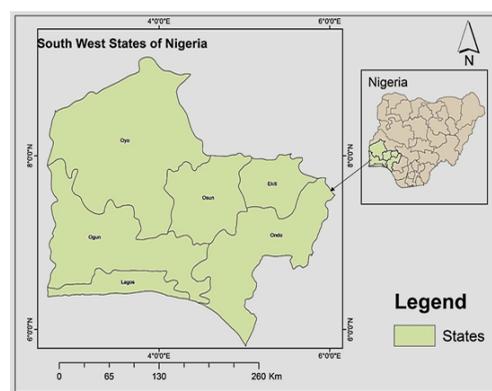


Figure 1. Map of South West Nigeria showing the study area and the vegetational zones. Scale in KM (Source: Agboola, 1979)

Table 1
Accessions number, local name, area of collection, states within South West, Nigeria and latitude and longitude where samples were collected

S/N	Accession Number	Local name	Taxonomic information	Area of collection	State	Latitude	Longitude
1	Og001	Rodo Hausa	<i>Capsicum chinense</i> Jacq.	Osiele	Ogun	7.19171	3.44524
2	Og002	Rodo Yoruba	-	Ago-Iwoye	Ogun	6.93456	3.89995
3	Og004	Rodo Hausa (yellow fruit)	<i>C. chinense</i> Jacq.	Odogbolu	Ogun	6.90826	3.66554
4	Og007	Rodo Hausa	<i>Capsicum annuum</i> L.	Ijebu-Ode	Ogun	6.82378	3.91793
5	Og010	Rodo Hausa	<i>C. annuum</i> L.+ <i>C. chinense</i> Jacq.	Iperu	Ogun	6.90826	3.66554
6	Oy018	Rodo Hausa	<i>C. chinense</i> Jacq.	Lanlate	Oyo	7.67845	3.44516
7	Ek021	Tiny rodo	<i>C. chinense</i> Jacq.	Ikole-Ekiti	Ekiti	7.78366	5.52441
8	La026	Shombo	<i>C. annuum</i> L.	Badagry	Lagos	6.43766	2.87833
9	Oy032	Rodo Yoruba	<i>C. chinense</i> Jacq.	Ijio	Oyo	7.93333	2.96670
10	On029	Green pepper tatashe	<i>C. annuum</i> L.	Igbokoda	Ondo	6.34956	4.80245
11	Oy031	Tatashe	<i>C. annuum</i> L.	Iganna	Oyo	7.97608	3.24667
12	Og006	Ijosi (original)	<i>C. chinense</i> Jacq.	Ayetro	Ogun	7.24227	3.02362
13	Og009	Ijosi	<i>Capsicum frutescens</i> L.	Orile-Ilugun	Ogun	7.36658	3.66848
14	Oy030	Ijosi	<i>C. frutescens</i> L.	Gaa Fulani	Oyo	7.85613	3.90959
15	La011	Round shape (Ijosi)	<i>C. frutescens</i> L.	Epe	Lagos	6.58433	3.97733
16	Os016	Atawere funfun	<i>C. frutescens</i> L.	Yakoyo	Osun	7.49613	4.44109
17	On028	Atawere funfun	<i>C. frutescens</i> L.	Ondo town	Ondo	7.11111	4.85427
18	Os013	Small bawa	<i>C. annuum</i> L.	Bode-osi	Osun	7.63312	4.21323
19	On019	Big bawa	<i>C. annuum</i> L.	Owena Alade	Ondo	7.19441	5.01983

Table 1 (Continued)

S/N	Accession Number	Local name	Taxonomic information	Area of collection	State	Latitude	Longitude
20	Ek024	Long Bawa	<i>C. annuum</i> L.	Iye-Ekiti	Ekiti	7.98655	5.22046
21	On027	Medium size Bawa	<i>C. annuum</i> L.	Oka-Akoko	Ondo	7.46214	5.83462
22	Os033	Bawa	<i>C. annuum</i> L.	Ita osa	Osun	7.43701	4.56132
23	Oy034	Atawere	<i>C. chinense</i> Jacq.	Ipapo	Oyo	8.13008	3.50983
24	Og003	Long shombo	<i>C. annuum</i> L.	Iyana -Agbede	Ogun	Unknown	Unknown
25	NGB01010	Unknown	<i>C. chinense</i> Jacq.	NACGRAB	Unknown	Unknown	Unknown
26	NGB01066	Unknown	<i>C. chinense</i> Jacq.	NACGRAB	Unknown	Unknown	Unknown
27	NGB01240	Unknown	<i>C. chinense</i> Jacq.	NACGRAB	Unknown	Unknown	Unknown
28	NGB01017	Unknown	-	NACGRAB	Unknown	Unknown	Unknown
29	NGB01022	Unknown	<i>C. chinense</i> Jacq.	NACGRAB	Unknown	Unknown	Unknown
30	NGB01282	Unknown	-	NACGRAB	Unknown	Unknown	Unknown

Source of SSR Primers

Twenty-nine (29) SSR polymorphic microsatellite markers specific to *Capsicum* publicly available from Nicolai et al. (2013) were adopted and used for this study.

PCR Amplification Reaction

This was done using 10 µL volumes with 2 µL of 25 ng/ µL genomic DNA as a template, 5 µL of 2 × GoTaq® Green Master Mix polymerase, 0.25 µL each of both primers, and 2.5 µL of sterilized ddH₂O. The amplification reaction was performed with initial denaturation at 94 °C for 2 mins, 35 cycles of 94 °C for 3 secs, 55.0 °C for 20 secs, 72 °C for 30 secs and 72 °C for 7 mins (Sun et al., 1993).

SSR PAGE Analysis

The products were evaluated on 6 % (w/v) polyacrylamide gel electrophoresis (PAGE) for 1.5 hr in 1 X Tris/borate/EDTA buffer with 7.5 M urea at constant voltage using the manufacturer's protocol. The gels were washed in water and stained with 2 g/ mL of silver nitrate (AgNO₃). The size of individual DNA band was determined using DNA ladder. Bands were developed by dissolving 15 g of NaOH in 1 L of distilled H₂O and added 3 ml of formaldehyde. The gels were allowed to dry before spreading the gels on trans-illuminator and gel images were taken using a digital camera.

Statistical Analysis

After scoring of all SSR fragments, gene diversity was determined using PowerMarker software program (Liu &

Muse, 2005). Shannon information index was used to determined polymorphic information content and the number of alleles (Shannon & Weaver, 1949). Popgene software version 1.31 (Yeh et al., 1999) was used to analyze genetic similarity, genetic distance, allele number, number of alleles with a frequency of greater than 5 % and less than 50 %. GenAlEx 6.501 software was used to estimate analysis of molecular variance (AMOVA), mean diversity, expected and unbiased heterozygosity (Peakall & Smouse, 2006).

Principal coordinates analysis (PCoA) was constructed using Minitab software. NTSYSpc v. 2.20 software was used to determine a genetic similarity between accessions while Jaccard's similarity coefficient of accessions was employed to construct UPGMA dendrograms (Rohlf, 2005).

RESULTS

SSR Primers Genetic Information/ Polymorphisms

Twenty-nine (29) primers were used in determining the genetic diversity between 30 *Capsicum* accessions studied. Twelve (12) SSR primers did not clearly exhibit polymorphism, thus, they were not included in the analysis. Table 2 shows the SSR primers sequences used.

Genetic Parameters Estimates of the SSR Primers

The primers generated polymorphic bands, with size ranging from 50bp to 350bp. Two hundred and eight (208) alleles were

Table 2

The sequences of the primers used for this study

Primer / Locus name	Forward Sequence (5'– 3') Reverse (5'– 3')
Epms-350	TGGGAAGAGAAATTGTGAAAGC AGGAAACATGGTTCAATGCC
Hpms1-214	AAGCTTATCCCTTCAAATATAA ATATCTCACGTATTGCGGATTCTT
HpmsCaSIG19	TGGCCAGCTTCACACAGAGGTA TGTCACAATATTGGAGGCCAGAA
Hpms1-5	CCAAACGAACCGATGAACACTC GACAATGTTGAAAAAGGTGGAAGAC
Epms-725	CGCTCGCTACCCTTTCATTA AATTCGGAAGGGCAAAGAT
Gpms-169	TCGAACAAATGGGTCATGTG GATGAGGGTCCTGTGCTACC
Gpms-100	TCCATACGGTTGGAGGAGAG ACTATGCTCTGCTGTGCCCT
HpmsE064	CCCTCCTTTTACCTCGTCAAAAA ATGCCAAGGAGCAATGAGAACC
Gpms-104	GCAGAGAAAATAAAATTCTCGG CAATGGAAATTTTCATCGACG
HpmsE013	GCGCCAAGTGAGTTGAATTGAT CACCAATCCGCTTGCTGTTGTA
Gpms-29	CAGGCAATACGGAGCATC TGTGTTGCTTCTTGGACGAC
HpmsE008	CCCCTTAACTTTAATTCTAGATCTGC TCGTTGTTCCCTCCATCACC TCA
HpmsAT2	TGGATCCCAAAAGACTCAGAACA TATTTCCCTCAGTCGAGGTCGT
Gpms-101	CCTATCACCCCTCTTTGAGCC TAAAGACCAGCCCTGGATGA
Epms-391	TTTCTTCTCTGGCCCTTTTG ACGCCTATTGCGAATTCAG
Hpms2-24	TCGTATTGGCTTGTGATTTACCG TTGAATCGAATACCCGCAGGAG
Epms-397	GCACCCTCCCAATACAAATC GATCACGGAGAAAGCAAAGG

Source: Nicolai et al. (2013).

recorded in all 30 accessions with the mean value of 12.24 alleles per locus (Table 3).

The allele number per locus was 6 in loci H1-214 and E-725, while locus HE013 had 18. Minor allele frequencies ranged from 0.13 in locus E-350 and locus G-101 to 0.87 for locus HE008, with an average of 0.37. There was high gene diversity in all loci except HE008 (0.231). Gene diversity ranged from 0.23 in HE008 to 0.94 in G-101 with an average of 0.77. The polymorphic information content (PIC) ranged from

0.20 in HE008 to 0.93 in G-101 with a mean value of 0.75 (Table 3). The most informative markers were primers E-350, H1-214, HCaSIG19, G-169, G-100, G-104, HE013, G-29, G-101, E-391 and E-397 with PIC values of 0.91, 0.77, 0.71, 0.92, 0.91, 0.84, 0.82, 0.79, 0.93 and 0.92 respectively (Table 3).

The Allelic Pattern Across Sub-groups among Accessions

This was performed to determine allele’s variability among the accessions according

Table 3
Summary of genetic parameters estimates of the SSR markers

Locus	MAF	NA	NPB	GD	PIC
Epms-350	0.13	14	7	0.92	0.91
Hpms1-214	0.37	6	3	0.79	0.77
HpmsCaSIG19	0.50	12	6	0.72	0.71
Hpms1-5	0.63	16	8	0.58	0.56
Epms-725	0.40	6	3	0.72	0.68
Gpms-169	0.17	16	8	0.92	0.92
Gpms-100	0.17	14	7	0.91	0.91
HpmsE064	0.53	12	6	0.65	0.62
Gpms-104	0.27	14	7	0.86	0.84
HpmsE013	0.37	18	9	0.83	0.82
Gpms-29	0.30	8	4	0.81	0.79
HpmsE008	0.87	8	4	0.23	0.20
HpmsAT2	0.57	8	4	0.65	0.63
Gpms-101	0.13	16	8	0.94	0.93
Epms-391	0.17	16	8	0.92	0.92
Hpms2-24	0.53	10	5	0.64	0.60
Epms-397	0.17	14	7	0.92	0.91
Total	6.27	208	104	13.04	12.73
Mean	0.37	12.24	6.12	0.77	0.75

Keys. MAF = Minor allele frequency; NA = Number of alleles per locus; NPB = Number of polymorphic bands; GD = Gene diversity; PIC = Polymorphic information content

to their sources. The numbers of different alleles (N_a) ranged from 1.15 in Lagos collection to 1.79 in NACGRAB and Ogun subgroups (Table 3). The analysis showed that NACGRAB and Ogun accessions produced a higher number of different alleles ($N_a = 1.79$), and effective alleles ($N_e = 1.57$ and 1.54) respectively (Table 4 and Figure 2).

Lagos had the lowest Shannon information index (I) value of 0.11 and the highest value of 0.48 was recorded for NACGRAB. Result showed that allelic

pattern according to the source of accessions at $\leq 50\%$ was one (1) in NACGRAB, Ogun, and Oyo respectively. The diversity (H) was low across the board, with values ranging from 0.08 in Lagos to 0.33 in NACGRAB. The unbiased diversity (uh) values ranged from 0.15 in Lagos to 0.39 in NACGRAB. This shows that intraspecific diversity is low among the sub-groups. The percentage of polymorphic loci ranged from 15.38 % in Lagos to 80.77 % in NACGRAB (Table 4 and Figure 2).

Table 4

Alleles/Bands pattern according to the source of accessions within groups

Population	Ekiti	Lagos	NAGRAB	Ogun	Ondo	Osun	Oyo
No. of accessions per population	2	2	6	8	4	3	5
N_a	1.20	1.15	1.79	1.79	1.50	1.45	1.63
No. alleles	97	90	102	104	100	96	104
No. alleles ($\geq 5\%$)	97	90	102	104	100	96	104
N_e	1.14	1.02	1.57	1.54	1.39	1.42	1.42
I	0.14	0.11	0.48	0.46	0.32	0.34	0.36
No. LComm alleles ($\leq 50\%$)	0	0	1	1	0	0	1
H	0.10	0.08	0.33	0.31	0.22	0.24	0.24
Uh	0.20	0.15	0.39	0.35	0.30	0.35	0.30
% of polymorphic loci	20.19 %	15.38 %	80.77 %	79.81 %	53.85 %	52.88 %	62.50 %

Keys. N_a = Number of different alleles; No. alleles = Number of private alleles; Number alleles frequency $> 5\%$ = Number of different alleles with a frequency $> 5\%$; N_e = Effective alleles; I = Shannon's information index; No. LComm alleles ($< 50\%$) = Number of locally common alleles (frequency $> 5\%$) found in 50 %; H = Diversity; Uh = Unbiased diversity

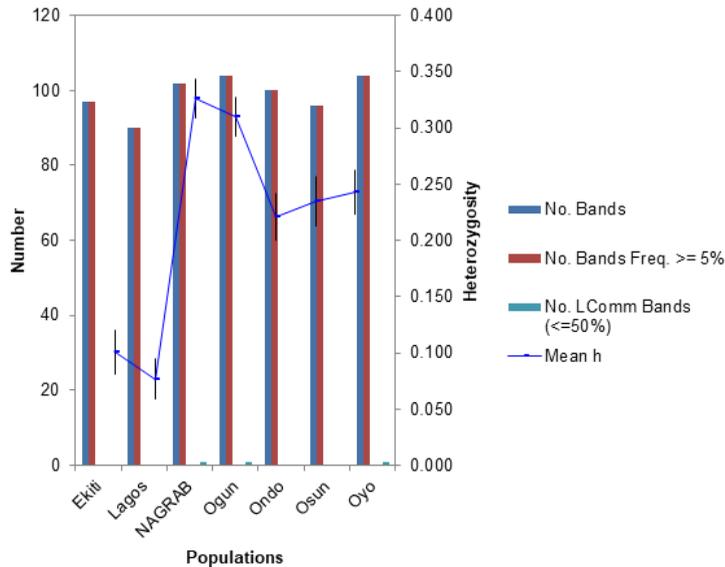


Figure 2. Allelic patterns across the seven populations

Keys. No. Bands = Number of different alleles; No. Bands Freq. > 5 % = Number of different alleles with a frequency > 5 %; No. LComm Bands (< 50 %) = Number of locally common alleles; Mean h = Mean diversity

Percentages of Molecular Variance of Accessions (AMOVA)

Table 5 shows that 2 % of the genetic diversity was due to interspecific variations while 98 % of the differences were due to intraspecific variations among accessions. This indicates higher intraspecific diversity within the accessions and less interspecific diversity among the accessions.

Principal Coordinate Analysis of Accessions of *Capsicum* based on Areas of Collection

Principal coordinate analysis of 30 *Capsicum* accessions was constructed using the SSR data matrix. Three major cluster groups were generated from the scattered plot of the PCoA from the 30 accessions. Cluster group A comprised a total of 10 accessions

from (Ogun, Lagos, Osun, Ondo, Oyo, and NACGRAB) subgroups while cluster group B composed of 18 accessions from all subgroups. Cluster group C comprised 2 accessions from NACGRAB (Figure 3).

Cluster Analysis of Accessions of *Capsicum*

A dendrogram was constructed from the raw data of the seventeen (17) SSR markers. The similarity coefficient (SC) delineated the 30 accessions into two (2) main clusters A and B at SC = 0.64. However, at a similarity coefficient level of 0.88 all accessions are separated (Figure 4).

However, at similarity coefficient (SC) of 0.71, cluster group A was also segregated into six sub-cluster groups A1, A2, A3, A4, A5, and A6. The sub-cluster A1 consisted of 2 accessions (NGB01066

and NGB01012) while A2 had an isolated accession of (Og001). The sub-cluster A3 had 2 accessions (Og002 and On027) while A4 consisted of 10 accessions (Og003, Ek024, Os013, On019, Oy031, On29, Oy018, Os033, Ek021, and Og007). The sub-cluster A5 had 2 accessions (Og004 and Oy032) while A6 consisted of 3 accessions (NGB01010, NGB01017, and NGB01240). Cluster group B was further segregated into

2 sub-cluster groups B1 and B2. The sub-cluster B1 contained 7 accessions (Og006, La011, La026, NGB01282, Oy034, On028, and Os016) while sub-cluster group B2 contained 3 accessions (Og009, Oy030, and Og010) (Figure 4). In all 30 accessions investigated, eight sub-cluster groups were identified.

Table 5

AMOVA among and within accessions variations

Source	Df	SS	MS	Est. Var.	TV %	<i>p</i> -value*
Among Accns	6	114.233	19.039	0.433	2 %	< 0.001
Within Accns	23	396.867	17.255	17.255	98 %	< 0.001
Total	29	511.100		17.688	100 %	

Keys. Accns = Accessions; Df = Degree of freedom; SS = Sum of square; MS = Mean square; EV = Estimated variation; TV = Total variation; * = After 999 random permutations

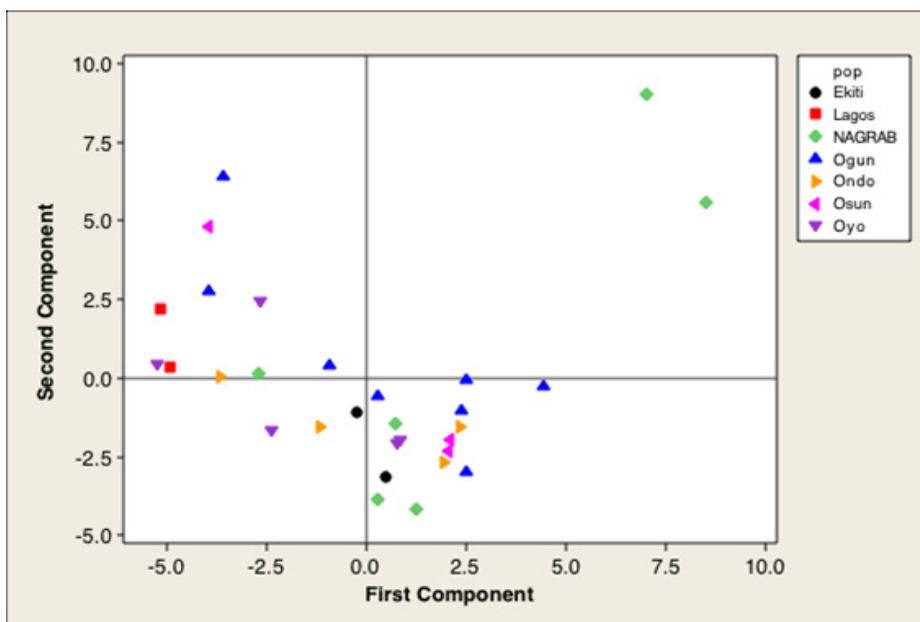


Figure 3. Scatter plot of 30 *Capsicum* accessions based on first and second components of principal coordinate analysis using SSR data on areas of collection

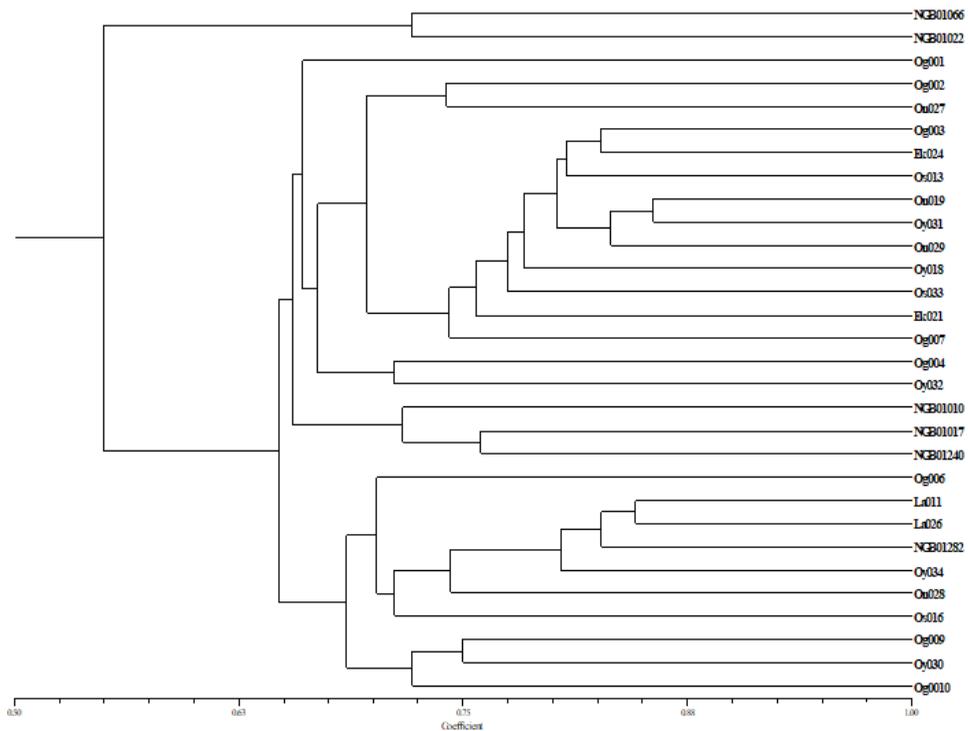


Figure 4. Dendrogram generated from SSR markers used for the 30 accessions of *Capsicum* species
 Keys. A and B = Major cluster groups; A1 to A6 = Sub cluster groups of A; B1 and B2 = Sub cluster of B

DISCUSSION

Seventeen SSR markers employed in this study were very effective. This is because they segregated the accessions into varieties; based on genetic similarities. These markers effectively distinguished the accessions.

The mean observed N_a per locus (12.24) detected was similar to 13.79 alleles/SSR primer reported by Zhang et al. (2016). Alleles ranges per primer (6 to 18) recorded corroborated the work of Zhang et al. (2016). They reported range of 6 to 29. However, numbers of alleles per locus recorded in three markers E725, HE013 and HE008 with (6, 8 and 10 respectively) were very close to the reported values of alleles

per locus (7, 6 and 11) for exact markers by Zhang et al. (2016). This finding agreed to the reports of several authors on the varying range of allele's number per locus (Zhang et al. 2010;2016).

The mean of PIC obtained is close to the range of the previous studies by Zhang et al., (2010, 2016). Zhang et al. (2016) reported a 60 % PIC value on *Capsicum* germplasm from China, United States, Brazil, Bulgaria and Japan. The high level of polymorphisms obtained for these *Capsicum* accessions could be traced to their cultivation in pepper growing regions in South West, Nigeria. The average mean values of genetic diversity (0.77) recorded agreed to the findings of Zhang et al. (2016). These findings

suggest the effectiveness of SSR markers in the genetic intraspecific and interspecific diversity of pepper.

From this study accessions collected from the same location were grouped together, with those belonging to the same species showing closer relationships at the molecular level. This study also showed higher intraspecific diversity within the accessions and less interspecific diversity among the *Capsicum* accessions. These findings corroborate the reports of Nikolai et al. (2013). These authors worked on 46 accessions of *Capsicum* and reported high genetic diversity similar to the values observed in this study among accessions of *C. annuum*.

Furthermore, high values recorded for all the measured genetic diversity suggest allelic richness among the accessions collected from South West, Nigeria. This could be relied on in evaluating diversity for genetic improvement of pepper. However, the negligible number of private bands recorded in growing areas showed the genetic similarities of accessions and that these bands insignificantly contributed to the overall diversity of the accessions studied.

The mean allelic pattern divided all accessions into sub-groups based on the collection areas (Ekiti, Lagos, NACGRAB, Ogun, Ondo, Osun, and Oyo) with narrow or no diversity. Five out of the seven areas of collection displayed similarity in effective alleles (N_e) and private alleles (unique) while Ekiti and Lagos areas of the collection displayed lower values and unique alleles. This trend also reflected in the percentage

polymorphic loci indicating high allelic diversity among accessions except for Ekiti and Lagos accessions.

The allelic pattern plot across accessions showed a gradual increase in allelic richness from Lagos to NACGRAB accessions down to Ogun, though with no sharp demarcation among Ondo, Osun and Oyo accessions. The gradual increase in allelic richness suggests strong correlation among the accessions due to similarity of alleles and exchange of a number of alleles at a particular locus. However, Ekiti accessions were isolated. This suggests weak connectivity between Ekiti accessions and other accessions and this may be due to differences in alleles and no exchange of a number of alleles. The results on genetically homogenous nature of the accessions as a result of alleles exchange agreed with the report of Balloux and Lugon-Moulin (2002) as well as NRC (2006). They reported that genetic structures reflecting the allele number exchanged between populations.

With respect to the allelic patterns again, there is a slight increase in connectivity from Lagos accessions to other areas, while there is a slight reduction from NACGRAB to other areas. However, there is a stable connection from Ondo via Osun en route Oyo accessions. This allelic connectivity finding is interesting and suggests a genetic link among accessions irrespective of an area of the collection in South West, Nigeria. This study provides additional genetic information between landraces and exotic hybridized pepper species in South West, Nigeria.

There was a very high level of diversity within accessions (98 %) and relatively low diversity among accessions (2 %). The natural interbreeding is perhaps responsible for the higher diversity within accessions compared to less genetic differences among accessions while low genetic diversity indicates low gene flow/genetic differentiation among accessions. This result corroborated the earlier findings on consistency of high genetic diversity within populations than between populations (Ganesan et al., 2014; Yang et al., 2016) on genetic diversity among *M. oleifera* and woody species respectively.

The results of CA and PCoA revealed a high degree of similarity among accessions particularly at > 71 % genetic similarity level and segregated 30 accessions into eight groups. The widely distribution of *Capsicum* accessions particularly in cluster groups A and B showed their adaptability to different areas in South West, Nigeria.

The findings in this study have enriched the understanding of the level of genetic relationships among populations of pepper in South West, Nigeria for breeding programs, thus, some wild varieties in South West, Nigeria that are underexploited but with valuable agronomic characters could be genetically improved upon for utilization as food, medicine, and also for other product development.

CONCLUSION

The intraspecific and interspecific genetic relationships observed could be an integral part of the useful tools for genetic

improvement of the genus *Capsicum* through breeding purposes especially the wild varieties.

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REFERENCES

- Abdullahi, M., Muhammad, G., & Abdulkadir, N. U. (2003). *Medicinal and economic plants of Nupe land*. Bida, Nigeria: Jube-Evans.
- Ado, S. G. (1999). *Potentials of native and exotic pepper germplasm in Nigeria: An exploitable resource in the next millennium*. Lafia, Nigeria: Genetic Society of Nigeria Publishing.
- Agboola, S. A. (1979). *An agricultural atlas of Nigeria* (1st ed.). Oxford, United Kingdom: Oxford University Press.
- Almedia, C. M. C. V., Dias, L., Okabe, E. T., & Medeiros, J. R. P. (2005). Variability in genetic resources of cacao in Rondonia, Brazil. *Crop Breeding and Applied Biotechnology*, 5(1), 318-324.
- Anu, A., & Peter, K. V. (2003). Analysis of seed protein of 29 lines of *Capsicum annum* L. by polyacrylamide gel electrophoresis. *Genetic Resources and Crop Evolution*, 50(1), 239-243.

- Balloux, F., & Lugon-Moulin, N. (2002). The estimation of population differentiation with microsatellite markers. *Molecular Ecology*, *11*(1), 155-165.
- Chen, X. J., Chen, J. F., Di, H. & Lou, Q. F. (2006). RAPD diversity analysis of 5 domesticated species. *Acta Horticulturae Sinica*, *33*(1), 751–756.
- Christine, E., Peters, H., & Orim, A. O. (2014). Comparative evaluation of the nutritional, phytochemical and microbiological quality of three pepper varieties. *Journal of Food and Nutrition Sciences*, *2*(3), 74-80.
- Falusi, O. A. (2006). Interchromosomal connections and metaphase I clumping in meiosis of two *Capsicum* Linn. species in Nigeria. *African Journal of Biotechnology*, *5*(22), 2066-2068.
- Falusi, O. A., & Morakinyo, J. A. (2001). Pollen and hybridization studies in some Nigerian species of peppers. *Nigerian Journal of Technology*, *1*(2), 40-43.
- Food and Agriculture Organization. (2010). *Food composition table for use in Africa, FAO and US Department of Health, Education and Welfare*. Rome, Italy: FAO.
- Fulton, T. M., Chunwongse, J., & Tanksley, S. D. (1995). Microprep protocol for extraction of DNA from tomato and other herbaceous plants. *Plant Molecular Biology Reporter*, *13*(1), 207–209.
- Ganesan, S. K., Singh, R., Roy-Choudhury, D., Bharadwaj, J., Gupta, V., & Singode, A. (2014). Genetic diversity and population structure study of drumstick (*Moringa oleifera* Lam.) using morphological and SSR markers. *Industrial Crop and Product*, *60*(1), 316–325.
- Germplasm Resources Information of Network. (2009). *Capsicum L. germplasm*. Retrieved November 18, 2018, from <http://www.ars.grin.gov/cgi>
- Gill, L. S. (1992). *Ethno medicinal uses of plants in Nigeria*. Benin City, Nigeria: UNIBEN Publishing.
- Liu, K., & Muse, S. V. (2005). Power marker: Integrated analysis environment for genetic marker data. *Bioinformatics*, *21*(1), 2128-2129.
- Luo, Y. D., Li, J. G., & Li, M. F. (2006). Analysis of genetic diversity of *Capsicum* germplasm resources by using SSR markers. *Biotechnology Bulletin*, *51*(1), 337–341.
- Mady, E. A., Uguru, M. I., & Ugwoke, K. I. (2005). Interrelations of growth and disease expression in pepper using principal component analysis. In *Proceeding of 30th Annual National Conference of Genetic Society of Nigeria* (pp. 1-5). Nsukka, Nigeria: Genetic Society of Nigeria Publishing.
- Morakinyo, J. A., & Falusi, O. A. (1992). Chromosome behaviour in *Capsicum annum* and *C. frutescens* and their intra and inter specific hybrids. *Nigerian Journal of Botany*, *14*(1), 135-143.
- National Research Council. (2006). *Lost crops of Africa: Volume II: Vegetables, development, security and cooperation*. Washington, USA: NRC.
- Nicolai, M., Cantet, M., Lefebvre, V., Sage-Palloix, A. M., & Palloix, A. (2013). Genotyping a large collection of pepper (*Capsicum* spp.) with SSR loci brings new evidence for the wild origin of cultivated *C. annum* and the structuring of genetic diversity by human selection of cultivar types. *Genetic Resources and Crop Evolution*, *60*(1), 2375-2390.
- Nwachukwu, C. U., Mbagwu, F. N., & Onyeji, A. N. (2007). Morphological and leaf epidermal features of *Capsicum annum* and *Capsicum frutescens* Solanaceae. *Nature and Science*, *5*(3), 54-60.
- Nwakiti, O. C. (1981). Sterility intraspecific hybrids of *Capsicum*. *Indian Journal of Genetics*, *41*(1), 200-204.

- Odeigah, P. G., Oboh, B. O., & Aghalokpe, I. O. (1999). The characterization of Nigerian varieties of pepper, *Capsicum annuum* and *Capsicum frutescens* by SDS-polyacrylamide gel electrophoresis of seed proteins. *Genetic Resources and Crop Evolution*, 46(1), 127-131.
- Oni, M. O. (2011). Evaluation of seed and fruit powders of *Capsicum annuum* and *Capsicum frutescens* for control of *Callosobruchus maculatus* (F.) *International Journal of Biology*, 3(2), 185-188.
- Peakall, R., & Smouse, P. E. (2006). GENALEX 6: Genetic analysis in excel: Population genetic software for teaching and research. *Molecular Ecology*, 6(1) 288–295.
- Rohlf, F. J. (2005). *Numerical taxonomy and multivariate analysis system, version 2.2. Exeter software*. New York, NY: Applied Biostatistics Publishing.
- Shannon, C. E., & Weaver, W. (1949). *The mathematical theory of communication*. Chicago, USA: Urbana Press.
- Showemimo, F. A., & Olanrewaju, J. O. (2000). Yield performance heritability and interrelations in some quantitative traits of “Tatase” pepper (*Capsicum annum* L.). *Journal of Horticultural Science*, 6(1), 25-30.
- Silva, N., Mendes-Bonato, A. B., Sales, J. G. C., & Pagliarini, M. S. (2011). Meiotic behavior and pollen viability in *Moringa oleifera* (Moringaceae) cultivated in Southern Brazil. *Genetics and Molecular Research*, 10(3), 1728-1732.
- Stebbins, G. L. (1971). *Processes of organic evolution* (2th ed.). Upper Saddle River, USA: Pearson Prentice-Hall Press.
- Sun, Y., Hegamyer, G., & Colburn, N. (1993). PCR-direct sequencing of a GC-rich region by inclusion of 10 % DMSO: Application to mouse c-jun. *Biotechniques*, 15(3), 372-374.
- Yang, H., Li, X., Liu, D., Chen, X., Li, F., Qi, X., ... Wang, C. (2016). Genetic diversity and population structure of the endangered medicinal plant *Phellodendron amurense* in China revealed by SSR markers. *Biochemical Systematics and Ecology*, 66(1), 286-292.
- Yeh, F. C., Boyle, R., Yang, R. C., Ye, Z., Mao, J. X., & Yeh, D. (1999). *The user-friendly freeware for population genetic analysis version 1.31*. Edmonton, Canada: University of Alberta Press.
- Zhang, B. X., Wang, L. H., Mao, S. L., & Zhang, Z. H. (2010). Research progress on pepper breeding and genetic during China’s eleventh five-year plan. *China Vegetables*, 24(1), 1-9.
- Zhang, X., Zhang, Z., Gu, X., Mao, S., Li, X., Joel, C., ... Zhang, B. (2016). Genetic diversity of pepper (*Capsicum* spp.) germplasm resources in China reflects selection for cultivar types and spatial distribution. *Journal of Integrative Agriculture*, 15(9), 1991-2001.
- Zhou, J., Shen, H. L., Yang, W. C., Tan, F., Wang, Y. L., & Guo, S. (2009). Analysis of genetic diversity of *capsicum* germplasm by using SSR markers. *Acta Agriculturae Boreali-Sinica*, 24(1), 62-67.