Genetic Diversity Analysis of Pearl Millet (*Pennisetum Glaucum* [L. R. Rr.]) Accessions from Northwestern Nigeria

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ABSTRACT

Pearl millet, a critically drought-tolerant cereal supporting millions in Northern Nigeria, possesses significant unexplored genetic diversity. This study addressed the research gap in north-western Nigeria by analysing the genetic diversity and evolutionary relationships of 69 pearl millet accessions using 8 polymorphic Simple Sequence Repeat (SSR) markers. The analysis confirmed substantial molecular diversity, revealing a total of 53 alleles with an average of 6.875 per locus, ranging from 3 (PSMP2248) to 9 (PSMP2080) alleles. Both factorial and phylogenetic cluster analyses demonstrated that genetic grouping was based on molecular similarity rather than geographical origin. The resulting clusters were small and exhibited high internal similarity, suggesting a history of seed exchange or shared ancestry among accessions. These findings confirm the rich genetic diversity present within the region's pearl millet populations. The study recommends further investigation using additional molecular markers to provide a more comprehensive understanding of the genetic resources available for this vital, under-researched crop.

Keywords: pearl millet, genetic diversity, simple sequence repeats (SSR)

INTRODUCTION

Pearl millet is an annual crop and can be grown in the drier areas where rainfall is insufficient for other tropical cereal crops (Bashir, *et al*., 2014; Abdulhakeem, *et al*., 2019; Meena *et al* 2025). It serves as a major source of food for more than 40 million smallholder farmers living in the marginal agricultural lands of Northern Nigeria, as well as many other Sahelian regions of West Africa (Abubakar, *et al*., 2021). Among all cereals, it is the cheapest source of energy, protein, iron and zinc to poor and vulnerable households (Vanisha *et al.*, 2011; Jukanti *et al.*, 2016; Kumar, *et al*., 2025). In Nigeria, pearl millet exhibits a tremendous amount of diversity at both phenotypic and genotypic levels (Abdulhakeem, *et al.*, 2019. The genetic diversity of pearl millet in the dryland area of Northwestern Nigeria is inadequately understood due to the relatively little research attention compared with other crops (Ajeigbe, *et al*., 2020; Abubakar, *et al*., 2021).

Various works on Pearl millet in Nigeria and other parts of the world were conducted using applied diagnostic technologies (Danjuma, *et al*., 2018). Some works that have been conducted include (Muhammad, 2018) who assessed the genetic diversity of the inventoried cultivars using Amplified Fragment Length Polymorphism technique (AFLF) in Semi-arid of North-eastern Nigeria. Govindaraj *et al*. (2009) evaluated the genetic diversity in pearl millet genotypes for drought tolerance using the Random Amplified Polymorphic DNA (RAPD) technique. Also, Jika *et al*. (2017) used similar method, and inventoried pearl millet cultivars of Lake Chad Basin using RAPD markers.

Arguably, no sufficient work has investigated the genetic diversity of pearl millet accessions in North-western Nigeria. Hence, the present study was undertaken with the aim to analyse the genetic diversity of the pearl millet accessions and determine the extent of evolutionary relationship among pearl millet accessions at a molecular level using SSR markers in North-western Nigeria.

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MATERIALS AND METHODS

Study Area and Plant Material

The study was conducted in the dry region of North-western Nigeria approximately 226,662 km^2 located between latitudes 12°–14° N and longitudes 4°–10° E, characterized by a by a tropical wet and dry climate ('Aw' Köppen) (Danjuma, &Abubakar, 2017). A total of 69 pearl millet (*Pennisetum glaucum*) accessions were collected from ten rural areas across the region (Figure 1) between August and November 2023. Accessions were categorized by maturity period: early (27), moderate (14) and late (28). Seedlings were raised using collected seeds in a greenhouse at the Department of Agriculture, Cape Peninsula University of Technology, South Africa.

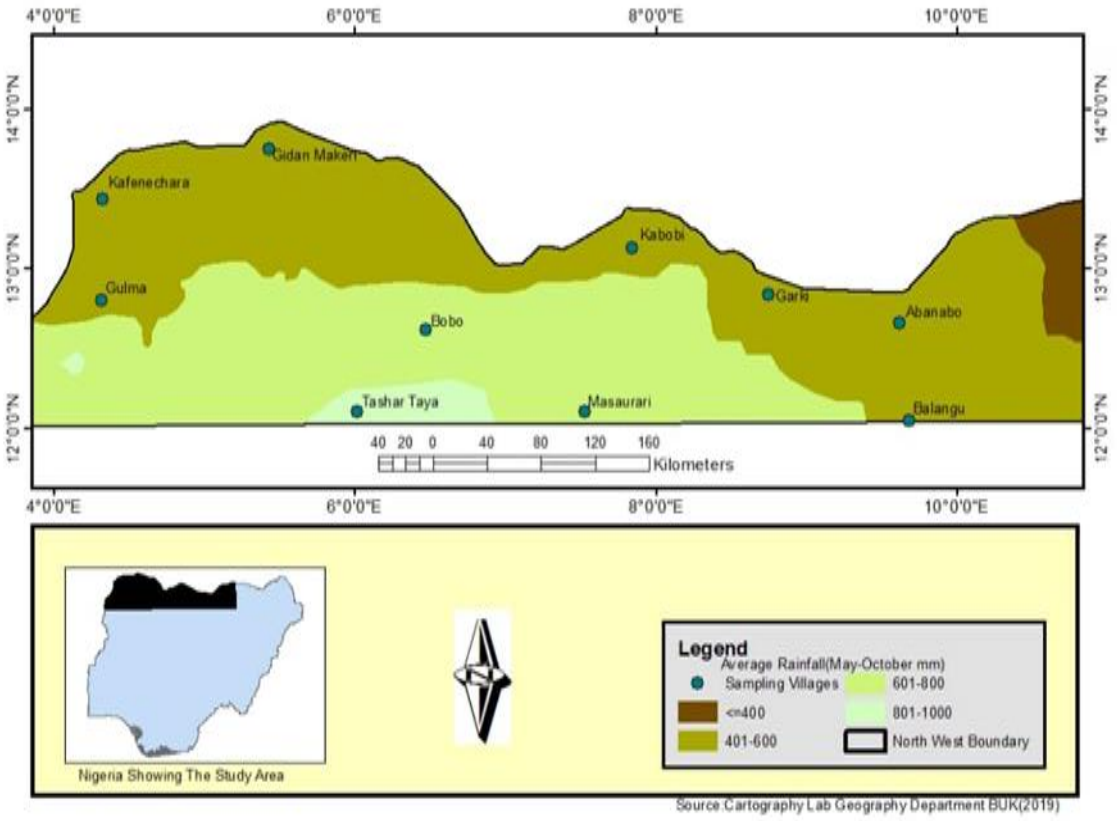

Figure 1: Map of the Dryland of North-western Nigeria showing Sampled Villages

2.2 DNA Extraction and Quality Assessment

Genomic DNA was extracted from young fresh leaves of 2-week-old seedlings, using a protocol adapted from (Doyle & Doyle, 1990). Briefly, leaf tissue was homogenized using Tissuelyser (Qiagen) in CTAB extraction buffer, incubated at 65°C, and purified through chloroform-isoamyl alcohol extraction followed by isopropanol and ethanol precipitation. DNA pellets were suspended in TE buffer. DNA concentration and purity were assessed using a Nano Drop spectrophotometer (A260/A280 and A260/A230 ratios) and by electrophoresis on 0.8% agarose gels. All samples were normalized to 10 ng/µL for PCR.

SSR Marker Amplification and Electrophoresis

Eight polymorphic SSR primer pairs (Table 1) were used to genotype the 69 accessions. PCR was performed in 10 µL reactions containing 10 ng genomic DNA, 1× PCR buffer, 1.75 mM $MgCl_2$, 0.2 mM dNTPs, 0.25 U GoTaq DNA polymerase, and 10 pmol of each primer. Amplification was carried out in a Veriti™ 96-well Thermal cycler with the following touchdown program: initial denaturation at 95°C for 2 min; 31 cycles of 95°C for 1 min, annealing at 72°C for 1 min (decreasing by 0.5°C per cycle), and 72°C for 1 min; final extension at 72°C for 5 min. PCR products were separated on 2% agarose gels in 0.5× TBE buffer, stained with ethidium

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bromide, visualized under UV light, and photographed. DNA was quantified through spectrophotometric absorbance (A) readings at wavelengths (λ) of 260 and 280 nm and 260/230, using a Nanodrop spectrophotometer.

Table 1: List of microsatellites markers used for diversity study

Marker Name	Primer_Sequences_Forward	Primer_Sequences_Reverse
PSMP2090	AGCAGCCCAGTAATACCTCAGCTC	AGCCCTAGCGCACAACACAAACTC
PSMP2080	CAGAATCCCCACATCTGCAT	TGCAACTGAGCGAAGATCAA
PSMP2085	GCACATCATCTCTATAGTATGCAG	GCATCCGTCATCAGGAAATAA
PSMP2237	TGGCCTTGGCCTTTCCACGCTT	CAATCAGTCCGTAGTCCACACCCCA
PSMP2001	CATGAAGCCAATTAGGTCTC	ACCATCTGACTTGTTCTTATCC
PSMP2008	GATCATGTTGTCATGAATCACC	ACACTACACCTACATACGCTCC
PSMP2275	CCAGTGCCTGCATTCTTGGC	GCATCGAATACTTCATCTCA
PSMP2248	TCTGTTTGTTTGGGTCAGGTCCTTC	CGAATACGTATGGAGAACTGCGCATC

Date Analysis

Genetic Diversity parameters, including allele number, polymorphic information content (PIC) and genetic diversity were calculated using PowerMarker v5.0. A dissimilarity matrix and dendrogram were generated using DARwin v4.0 to assess genetic relationships among accessions.

RESULTS AND DISCUSSION

Gel images, quality, and quantity of extracted genomic DNA by Agarose gel electrophoresis

The Genomic DNA was extracted from sixty-nine pearl millet accessions. The quality of the extracted DNA was evaluated by agarose gel electrophoresis. For the first extraction in (figure 2), the quality of the DNA was good but some were not cleared until for the second extraction in (Figure 3). The bands on the electrophoresis were distinct and sharp.

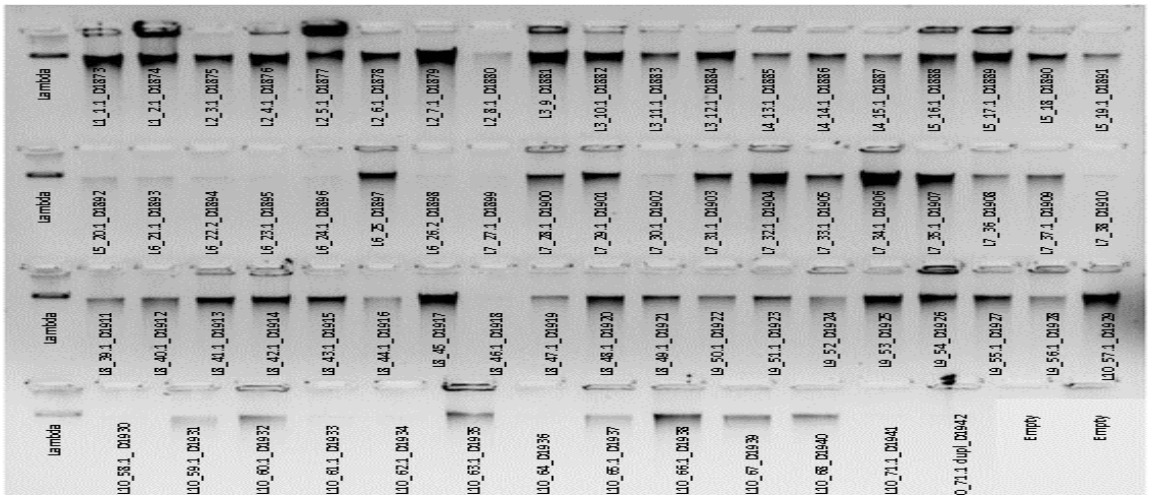

Figure 2: First Extraction (CTAB - Doyle & Doyle., 1990).

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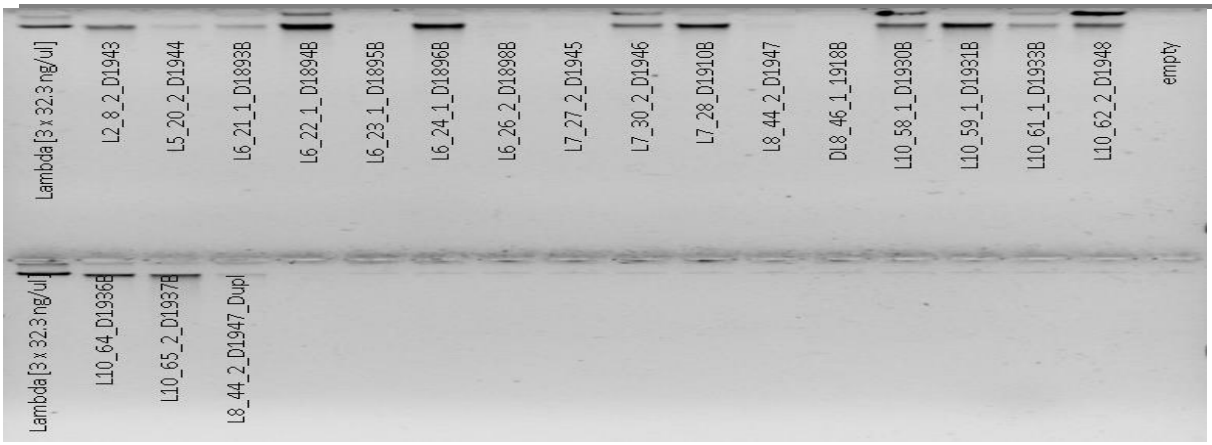

Figure 3: Re-extracted samples (CTAB Doyle & Doyle., 1990)

Combine gene diversity analysis

The analysis of the 69 accessions genotyped (Table 2) revealed a total of 53 alleles recorded with 8 microsatellites with an average of 6.875 per microsatellite. The range was from 3 to 9 alleles in PSMP2248 and PSMP2080, respectively. Several earlier studies Hu, *et al* (2009); Lin *et al* (2012); Choi *et al* (2018) reported an average of 2.4, 3.83 and 0.355 alleles per locus in foxtail millet, rice and foxtail millet [*Setaria italica* (L.) P. Beauv.] respectively. The recent similar study conducted with Algerian pearl millet revealed a total of 87 alleles were detected across 24 SSR markers, resulting in a low average of 3.62 alleles per locus compared with this study (Kirouani *et al.,* 2025)

Table 2 Genetic diversity analysis using 8 polymorphic SSR primers in 69 pearl millet accessions

Marker	**Major. Allele Frequency**	**NGT**	**SS**	**NOB**	**NA**	**PIC**	**Gene Diversity**	**HT**
PSMP2090	0.53623188	8	69	69	8	0.598598	0.6410418	0
PSMP2080	0.34782609	9	69	69	9	0.765699	0.79185045	0
PSMP2085	0.46376812	5	69	69	5	0.556984	0.6284394	0
PSMP2237	0.86956522	8	69	69	8	0.235935	0.24070573	0
PSMP2001	0.65217391	8	69	69	8	0.525242	0.54820416	0
PSMP2008	0.33333333	8	69	69	8	0.780123	0.80403277	0
PSMP2275	0.63768116	6	69	69	6	0.463817	0.52215921	0
PSMP2248	0.91304348	3	69	69	3	0.151783	0.16089057	0
Mean	**0.5942029**	**6.875**	**69**	**69**	**6.875**	**0.509773**	**0.54216551**	

NGT: Genotype No, **SS**: Sample Size, **NOB**: No. of observances, **NA**: No. Allele, **PIC**: polymorphic information content, **GD** Gene Diversity, Ho: Heterozygosity.

The results obtained in this study showed that PIC range from 0.151783 to 0.780123 for PSMP2248 and PSMP2008 respectively, with a mean of 0.509773. Among the 8 microsatellites used 6 (75%) have the highest PIC values, while the 2 lowest (25%) were recorded among PSMP2248 and PSMP2237. The range values obtained in this study were compared positively with those reported by Anderson *et al.* (1993) and Kapila *et al.* (2007) of 0.613 and 0.58 for Pearl millet respectively. The average PIC obtained in the present study was closely

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similar with that obtained from diversity of 17 Indian pearl millet produced from 74SSR markers with ranged from 0.10 - 0.89 and mean average of 0.55 as reported by (Kumar *et al.* 2020).

Phylogeny Tree

The results of the present study also confirmed diversity among the pearl millet accessions in the drylands of Northwestern Nigeria, that the diversity was not clustered based on geographical pattern but rather on similarities. This is in agreement with Adeoti *et al*. (2017) who reported that genetic diversity among the cultivated pearl millet in Benin Republic was not according to geographical regions. A total of eleven clusters were identified in this study and consisted different number of accessions in each cluster, the number of accessions per cluster varied from 2 to 45 compared with 15 to 94 (Deu *et al*., 2010). The first cluster was bulk with 45 genotypes (65%), two moderate clusters were generated with a total of 4 accessions each and the lowest 8 clusters possessed two accessions each (Figure 4). The results indicated more closer similarities among clusters with very few numbers of accessions such as in cluster two *L10-58-Maiwa Bahausa* and *L7-36-Maiwa Gagera* obtained from Gulma in Kebbi State and Bobo in Zamfara State respectively.

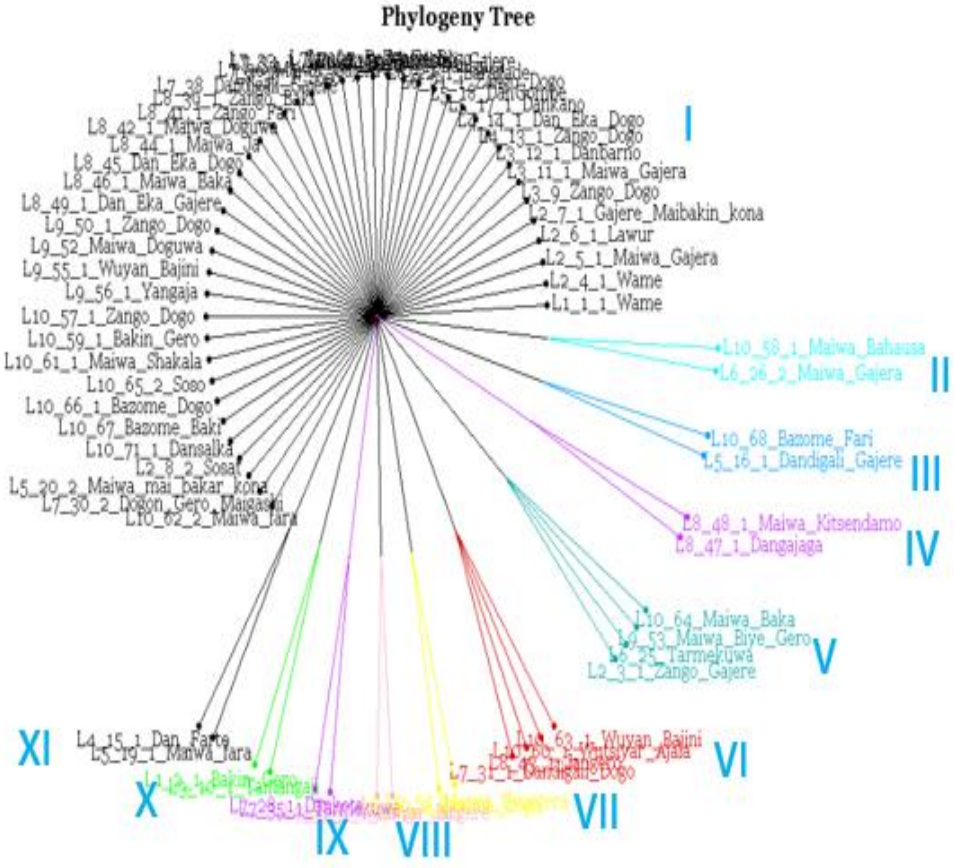

Figure 4: Dendrogram showing genetic diversity and relationship of 69 accessions of Pearl Millet

Factorial Analysis

The scattered plot generated four clusters of interrelationships within accessions from different study locations of 7,6,8 and 8 as cluster I, II, III and IV respectively (Figure 5). It shows that these genotypes in each cluster belong to the same ancestor. The results obtained in the present study grouped 7 *Maiwa* accessions of *L5-19 Maiwa Fara, L5-20 Maiwa Mai bakar Kona, L6-26 Maiwa Jagera, L8-42 Maiwa Doguwa, L8-44 Maiwa Ja, L10-58 Maiwa Bahausa, L10-61 Maiwa Shakala* and *L10-64 Maiwa Baka* in cluster IV having the same evolutionary origin.

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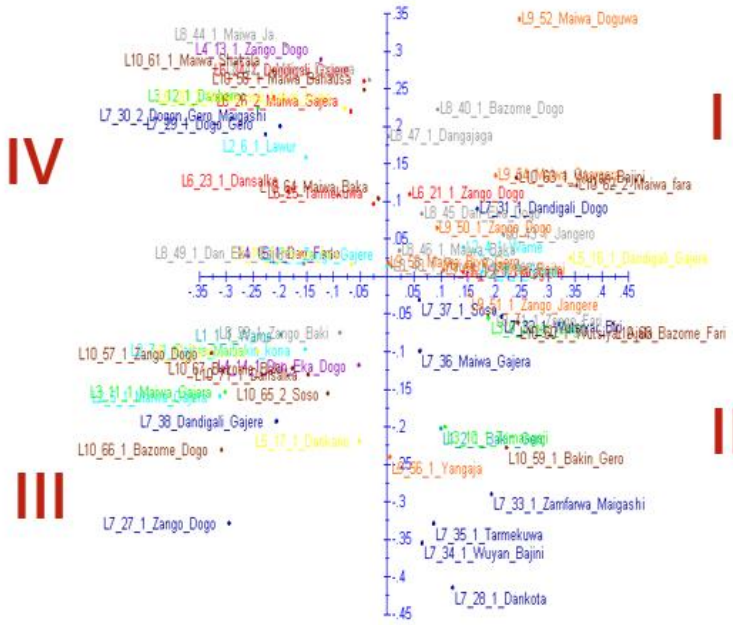

Figure 5: The scattered plot generated four clusters of interrelationships among accessions

Dissimilarity Values

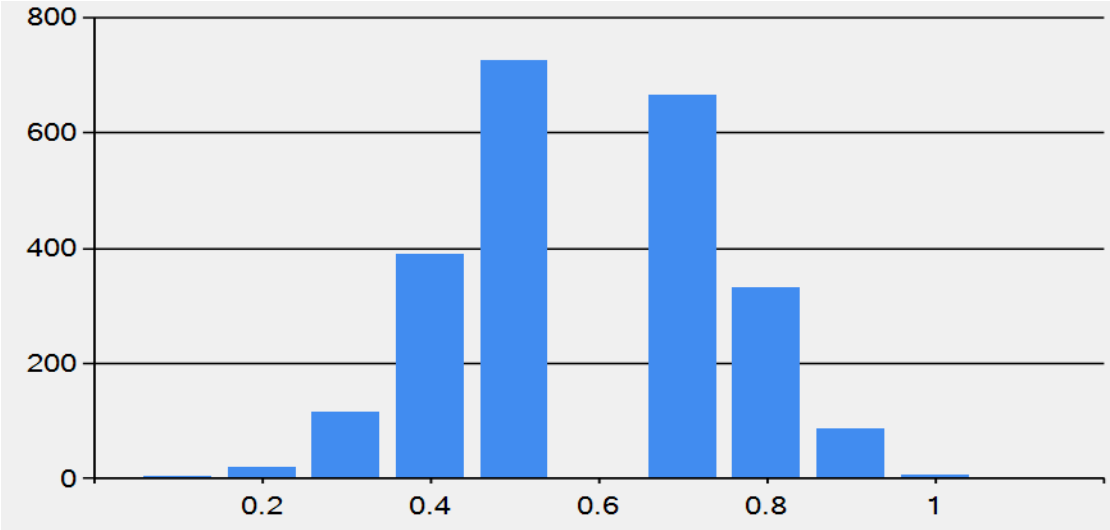

Figure.6 Dissimilarity values of 69 Pearl millet accessions

Present study exhibited three different hierarchies of dissimilarity among the 69 accessions with a total of 2346 dissimilarity values (Figure 6). The maximum dissimilarity value of 1 was among accessions *L10-62 Maiwa Fara* dissimilar with the *L1-1- Wame* while the minimum value of 0.2 was documented from accession *L1-1-1-Wame* and *L2-4-Wame* from Abonabo and Bulangu in Jigawa State respectively. The result was greater than those documented by other studies Jika *et al*. (2017) whom conducted research on unexpected pattern of pearl millet genetic diversity among ethno-linguistic groups in the Lake Chad Basin. They reported a maximum dissimilarity value of 0.62 between accessions *G202* and *G214* and the minimum dissimilarity value of 0.20 between accessions *G213* and *G 214***.**

CONCLUSION AND RECOMMENDATION

The present study therefore revealed that genetic relationship and diversity among cultivated pearl millet accession existed in the drylands of Northwestern Nigeria. Molecular markers revealed a maximum similarity

among clusters with few numbers of accessions. SSR marker was considered effective in investigating the genetic diversity of pearl millet accessions at the DNA level and can be used to cluster accessions based on certain genetic similarities such as high polymorphic information content, a total number of alleles and heterozygosity or homozygosity among accessions. Relative information of each marker can be evaluated on the basis of its PIC value. Other molecular marker methods were recommended to be tested in the same study area to measure their level of relative informativeness of each marker.

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Declaration of conflicting interests

No conflict of interest declared among the authors

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