Genetic Diversity of Pearl Millet (Pennisetum typhoides) Cultivars in Semi-Arid Northern Nigeria

Muhammad Nuraddeen Danjuma[1*] Salisu Mohammed[1]

1. Department of Geography, Bayero University Kano, NIGERIA

*Email of the Corresponding Author: nurdkat81@gmail.com

Abstract

Over the last two decades, several seed-related studies have been conducted in semi-arid Africa to improve farmers' access to quality seeds of dry land cereals and legumes. These have indicated that genetic diversity which is at stake is a major resource. However, there is an undeniable evidence of the erosion of crop genetic diversity. The aim of the study is to evaluate genetic variability of pearl millet cultivars obtained from four semi-arid villages of northern-eastern Nigeria namely Dagaceri and Kaska. It should be noted that all the 42 sampled respondents in all the study areas are males and heads of households. They are most active in agricultural practices and also have the final say in the activities of their household. A total of 25 pearl millet genotypes were collected based on diverse morphological data recorded on the field using Participatory Rural Appraisal. The main approach to the present study is to link the advanced biological technique (laboratory study) on genetic characteristics with social science field methodologies. The techniques used in the laboratory analysis are the Amplified Fragment Length Polymorphism (AFLP) and Multiplexed Single Oligonucleotide Amplification. Laboratory studies revealed that genetic compositions of all inventoried pearl millet are not the same. The difference within and between the landraces was estimated using molecular marker (AFLP) and from the data it was noted that farmers' husbandry practice resulted to the isolation of group ideotypes, making landrace names *quid pro quo* of genetic diversity. It was recommended that because farmers' methods of selection play an important role in genetic management and conservation, it should be linked with the formal seed system to enhance genetic management and control genetic erosion.

Key words: genetic diversity, pearl millet, amplified fragment length polymorphism, northern-eastern Nigeria

SPORE (1994): *Many plants and animals species are disappearing thereby deflating the world's genetic resource. Our heritage of biodiversity is under serious threat and one of the themes of international discussion about the environment is to devise what measure should be taken to reduce the threat.*

1.0 Introduction

That Africa is a net importer of food is puzzling but a number of complex reasons account for this (Rakotoarisoa, Lafrate, and Paschali, 2011). One of them is the stagnation of seed sector, and in some areas the actual decline, in the agricultural productivity of small-scale farmers (Virgin et al., 2007). It is against this background that it is now commonly viewed as critical and urgent to produce resilient and high yielding varieties addressing the needs of African farmers as was strongly expressed in the World Development Report 2008 (World Bank, 2007). The selection and combination of improved varieties and crop mixtures ought to improve overall robustness of farmer livelihood strategies (ASARECA, 2008). The implementation of effective and successful agricultural strategies that ensure food security in dry land Africa represents one of the most crucial issues of the 21st century.

Many communities in Africa struggle to find enough food to ensure they live active and healthy lives (Rinaudo and Sogoba, 2007). More than 30 percent of the populations of Burkina Faso, Mali, Niger and Sierra Leone suffer from food insecurity. Ten percent of Nigeria's population also remain food insufficient (IFPRI, 2004). The majority of farmers in West Africa are reliant on mono-cultural agricultural systems which, depending on the region, may be millet, sorghum, maize, or a root crop such as cassava, and herders are reliant on pasture for their livestock (Rinaudo and Sogoba, 2007). This overreliance on a narrow range of crops and pastures- in an unpredictable environment significantly increases vulnerability to food insecurity. Although rarely consumed in the West, these two staple grains (sorghum and pearl millet) feed the rural population of the dry region (Mortimore, 1992). Pearl millet has been cultivated for thousands of years and is a staple grain for much of the world's population, particularly in South Asia and East Africa. The African native variety, finger millet, likely originated in the highlands of Ethiopia and Uganda and is one of the most nutritious of the world's major cereal crops (FAO, 1996). In the semi-arid environment of Nigeria, the most important useful and sustainable crops cultivated include two principal cultivars: pearl millet (*Pennisetum typhoides*) and sorghum (*Sorghum bicolor*).

Of these two principal cultivars pearl millet is the most significant staple grain in areas where the climate is too dry for the cultivation of sorghum and it grows and thrives better in arid areas than any other crop. Pearl millet is believed to be originated in West Africa; the term millet is brooding applied over 140 species belonging to the Genus *Pennisetum* (Stoskopf, 1985). It represents a diverse group of cereal crops that typically produce small seeds. They comprise about a dozen crop species, belonging to different genera, that originated and

domesticated, and are cultivated by small farmers in Africa and Asia (De Wet, 1987). Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is in the genus *Pennisetum* of the Poaceae (grass) family. Although the current officially accepted name is *Pennisetum glaucum* (L.) R. Br. (Chase, 1921), other commonly used synonyms for pearl millet include *Pennisetum americanum* (L.) K. Schum, *Pennisetum typhoides* (Burm. F.) Stapf et Hubb., *Pennisetum typhoideum* Rich, *Pennisetum spicatum* (L.) Koern (Jauhar, 1981). Pearl millet is a diploid (2n = 14) annual species (Rau, 1929) with the haploid (1 C) DNA content of about 2.45 pg (Bennett, 1976), which is equal to about 2.26 billion bases (0.965 billion bases per pg DNA).

Genetic diversification of pearl millet cultivars has been a high priority area in pearl millet improvement research. There are a multitude of potential applications of DNA marker technologies to the improvement of pearl millet (*Pennisetum glaucum* (L.) R. Br.). While numerous studies catalogue the diversity of such markers, there are still few studies demonstrating the use of these molecular markers in pearl millet genetic diversity studies. Pearl millet exhibits a tremendous amount of diversity at both phenotypic and genotypic levels (Poncet et al., 1998). Pearl millet study shows that there is wide phenotypic variation among landraces as in Vyas (1987) who collected about 300 local landraces of pearl millet from Rajasthan (India). They were subject to series of evaluation, and a widest range of variation was observed for plant height, grain yield 1000-grain mass, head length and grain density. However genetic analysis show more complex variability in this species as no two phenotypically same samples are genotypically same. Kapoor and Sagar (1986) have used the analysis of genotype – environment interaction in a number of studies conducted at Haryana Agricultural University (HAU) India. Wilson et al. (1990) and Ovendeba et al. (1995) all indicated that genetic resource evaluation has demonstrated a high phenotypic diversity in cultivated material of pearl millet. The West African millets is a neglected crop group in biodiversity conservation and rural development programmes, yet they represent fundamental crop genetic resources for the food security, agricultural systems, and sustainable development prospects of rural communities in the dry and harsh lands of West Africa (Gari, 2001).

DNA markers are widely used in high density genetic linkage map construction because more DNA markers segregate at the same time in a population than do classical morphological markers, and DNA markers usually do not affected by environment factors. Of the commonly used are the RF technique (Liu et al., 1992) which shows that genetic polymorphism is very high in pearl millet between land races and also within land races (Pilat – Andre et al., 1992). The RAPD was used in Gupta et al., (2010) as well as the AFLP which was reported in Zabeau and Vos (1993). The more complicated distribution of diversity in pearl millet, as well as the higher degree of marker polymorphism, will make genetic diversity studies in this species (Hash and Bramel-Cox, 1999). A major milestone in applications of molecular markers in pearl millet was the creation of the first genetic map in 1993 using RFLP markers (Liu et al., 1994). The AFLP technique has many advantages, such as high reproducibility (Blears et al., 1998). Hence this study has been conceived to fill in some of the gaps related to assessment of genetic variability of pearl millet cultivar (an aspect of agro-biodiversity) in the semi-arid part of northern Nigeria and demonstrate how the farmers use genetic manipulations in dealing with dry land environmental problems.

2.0 Objective of the Study

1. To make an inventory of landraces of pearl millet cultivars in the study villages in semi-arid northern Nigeria,
2. To assess the genetic characteristics of the pearl millet cultivars collected in the study area,

3.0 The Study Area

The study area is in north eastern Nigeria. It is semi-arid area that cut across different latitudes with different levels of aridity. The first area is Dagaceri located on latitudes 12^0 45'N to 12^0 58'N and Kaska is within latitudes 13^0 20'N to 13^0 30'N. The areas display a trend of an increase in latitudinal position to Kaska and this account for pearl millet variability. Dagaceri is located at the northeastern extreme of Jigawa State. It is found some thirteen kilometers northernmost of Birniwa Local Government headquarters of Jigawa State, which are some 240 Kilometers from Kano. Kaska is located at the Northern-most part of Yobe State. It is found some 60 to 80 kilometers north of Gashua in Yusufari Local Government Area of Yobe State (about 320km from Kano). The position of the study villages in Jigawa and Yobe states of Nigeria is represented in figure 4.

Climate of the study areas have been described as 'AW' type as identified by Koppen's climatic classification. According to this classification, this climate is a tropical one with clear wet and dry season. The coolest month is normally experienced between December/January with temperature of less than 18^0C. The dominant climatic influence throughout the areas is the Inter- tropical Convergence Zone (ITCZ) also known as the Inter-tropical Discontinuity (ITD).

4.0 Methods

4.1 Field work

The first phase of the actual fieldwork started between the months of October through to December 1997. This time was the immediate period after the rains refer to as 'Kaka' in Hausa (crops harvesting period). Pearl millet inventory and samples were collected. The collected pearl millet samples were taken to the genetic laboratory at

the Cereal Department of the Cambridge Laboratory of restriction fragment length polymorphism (RFLP) Institute of plant Science Research in Norwich United Kingdom. The analysis was made in order to assess the level of variability within a variety, within ears, between ears and between fields. Simple statistics of percentages and ANOVA were used in the analysis of data collected from the field and genescan analysis was used for genetic analysis.

4.2 Laboratory Procedures

Plant Material

Pearl millet samples were collected in two villages, Dagaceri and Kaska during the harvest season of 1996. The samples designed, and stratified according to village, farmer, field and landrace. In Dagaceri, the samples were collected in the field from one stand in ten, and from one spike per stand. Three different landraces were collected from each of the different farmers' fields. Four spikes per landrace were selected for analysis of six grains from each spike. In Kaska only three farmer/landrace plots were sampled. Out of the sampled Pearl millet varieties collected for analysis, three were of Lafsir (variety) from farm B of sampled respondent named Abdullahi Kwarie. Three samples were of Badenji variety (as identified by the farmer) from Farm A of Abdullahi Kwarire of Dagaceri Village of the study area.

Genetic Material

A total 288 seeds of two landraces of pearl millet genotypes were selected based on diverse phenotypic data of the cultivars recorded in the on the field from the farmers. Six spikes from two land races from two fields of a farmer in Dagaceri were subjected to molecular analysis using the Amplified fragment length polymorphism (AFLP) technique to determine the level of genetic variability in the Cambridge Laboratory of restriction fragment length polymorphism (RFLP) and Institute of Plant Science Research in Norwich United Kingdom.

DNA Extraction

The genomic DNA was isolated as according to the method of Dellaporta et al. (1983) with slight modification. Deribonucleic Acid (DNA) was extracted from single pearl millet seeds. The 288 seeds were milled in a small mortar and pestle attached to the drill. The flour was collected in 300 μ / 'S' buffer. (100 mMTris. Hcl pH 8.5, 100mM Nacl, 50mM EDTA pH8.0 and 3% SDS) heated to 65^0C. This extraction followed a modified procedure from Sharp *et al.* (1988). Protenasek was added to a final concentration of 0.6 μ /ul and the mixtures were incubated at 65^0C for 1 hour. Following a phenoll chloroform extraction, the DNA was precipitated with isopropanol. The pellet was washed with 70% ethanol, dived and re-suspended in 300 μ L TE-buffer pH. 8.5. The DNA was treated with 10 μ g Dnase – free. FNase extracted with phenol/chloroform, ethanol precipitated and dissolved in TE-buffer to a final concentration of 100ng/ μ l in the AFLP analysis.

AFLP Analysis

The restriction enzymes used for digesting DNA are usually a combination of two enzymes, a frequent cutter such as MseI and a rare cutter such as EcoRI or PstI. The detailed procedure for AFLPs followed the protocol supplied by Perkin Elmer manufacturers of the DNA Analysis Sequencer. The optimal primer combination was established by comparing 48 EscoRI and Msel primer combinations (flowers Cent Labeled primers) and 12 combinations of PstI and MseI primers (33p Labeled primers), each with different combinations of two or three selective nucleotides. The choice of P71 (5 – GAT TGC GTA CAT GCA GGG A – 3) and M17 (5 – GAT GAG TCC TGA GTA ACG – 3) was based on the number of bands produced, homogeneity of the banding pattern over the gel (avoiding band clusters) and homogeneity of banding intensity (regular saturation signal). The 5 – Carboxi – fluorescent dye labeling of the pstI primer was carried out at the Perkin Elmer laboratories in the U.S.A. ABI sequencer in an 6% acryl/bis denaturing gel analysis was made using genescan software. The fragment size and peak intensity values were converted to binary data with the Genotyper program. The samples were doubled digested separately with two different rare cutter restriction enzymes, EscoRI and pstI, and one frequent cutter MseI. The legation of adaptors was carried out as described by Vos et al. (1995). The pre-selective and selective PCR amplifications followed the protocol supplied by Perkin Elmer. A visual check for good correspondence between binary output and Genescan electro-programs completed the analysis.

5.0 Results

5.1 Characteristics of Inventoried Landraces of Pearl Millet Cultivars in the Area

It is evident from the table 1, that twenty-five different varieties of pearl millet were inventoried. Twenty eight percent (28%) of these are early maturing (they mature between 40 to 70 days), and farmers use them because of the advantage they have over the others. Most of these varieties, in fact, over 71% are introduced. This is a clear indication that naturally or otherwise, they might have been improved genetically in order to characterize the genes responsible for maturity. If so, then such characterization has taken into cognizance the concept of environmental resiliency.

The major problem associated with varieties in this group is that they are the first to arrive at the scene where larval, pupal or even adult stages of pest and insects, as well as the birds, are waiting. Forty four percent (44%) of all varieties are the average maturing ones, and over eighty one percent (81%) of them are indigenous, and they mature between seventy days and one hundred and twenty days (70 – 120 days). Farmers plant these varieties because they are the local ones which they know for many years as a result of continuous cultivation, and they are used to their characteristics, qualities and their various adaptive measures. Twenty eight percent (28%) of all varieties are late maturing and over seventy one percent (71%) of them are indigenous. However one of their peculiar characteristic is that their continuous use is in the decline because of drought effect and other climatic and environmental uncertainties.

In terms of origin of all varieties, sixty four percent (64%) are indigenous while thirty six percent (36%) are introduced. This made it clear the fact that all the study areas are within an eco-climatic zone that favors and supports the indigenous cultivation of pearl millet varieties. In the case of grain yield of all varieties, thirty two percent (32%) are identified as high yielding, forty four percent (44%) as moderate or medium grain yielding while twenty four percent (24%) are low yielding. Generally speaking, varietal yield is attributed to various factors, which include genetic, edaphic, environmental and climatic. Varietal yield therefore, depends on one or a combination of the stated factors. The high percent of medium yielding varieties is clearly attributed to edaphic factors. Soils in the areas are sandy and lacking some nutrients that could attribute to a very high grain yield of crop. This impact is reduced to a medium grain yield as farmers in all study areas employ the application of manure, which improves soil condition and has upgraded grain yield to medium.

On seed morphology, only twelve percent (12%) of all varieties are hairy. The seeds of this varieties are covered with hairs, while eighty-eight (88%) of the seeds of all are not covered with hairs. The hairy varieties are not very popular and many farmers are not certain of their origin. Physical properties of pearl millet, such as the length of the stalk, its strength and general residue production is important and very much related to biodiversity management. Stalk length and strength have been identified to be among the qualities, which entice farmers in their selection phenomena. Comparison was made on the length of all inventories varieties, and it is noted that the stalk lengths of sixteen percent (16%) are the longest and generally over 2m in height. Sixty four percent (64%) are medium in length, of less than 2m but equal to or greater than 1.5m while the shortest varieties have stalk lengths of less than 1.5m and they carry twenty percent (20%).

5.2 Level of Genetic Variability of Landraces within a Farmer's Field

In order to acquire landraces for molecular analysis, a total of three landraces that were identified at farmers' level as phenotypically the same were selected in Dagaceri village. While in Kaska village three land races one from each farmer and physically similar to one of Dagaceri were sampled as in Table 2.

Table 2: Sampled landraces of the Study Villages

Village	Name of Farmer	H/H Code	Farmers Landraces
Dagaceri			
	Kwaire	DHH	Lafsir (MLVD5) – Fudewuwa (MLVD1) – Badenji (MLVD2)
	Mallum	DHH	Lafsir (MLVD5) – Fudewuwa (MLVD1) – Badenji (MLVD2)
	Nakuri	DHH	Lafsir (MLVD5) – Fudewuwa (MLVD1) – Badenji (MLVD2)
Kaska			
	Fujimi	KHH	Lafsir (MLVK)
	Bagari	KHH	Fudewuwa (MLVK1)
	Ba-kwato	KHH	Badenji (MLVK2)

Source: Mohammed (1998)

Practically, 3 ears x 8 seeds x 2 varieties of Lafsir MLVD5 and Badenji MLVD2 land races were analysed. The result in Figure 1 has indicated a general disparity between the DNA characters (though the pattern appears similar). Critical examinations revealed that no one of the DNA strand has similar character. However the general similarity of the pattern displayed by the DNA strands is attributed to the selection done by farmers. It is right therefore to state that selected varieties are more likely to contain similar genetic components (a phenotypic quality) than varieties, which are not selected and this has confirm a positive role of small holder farmers in maintaining diversity.

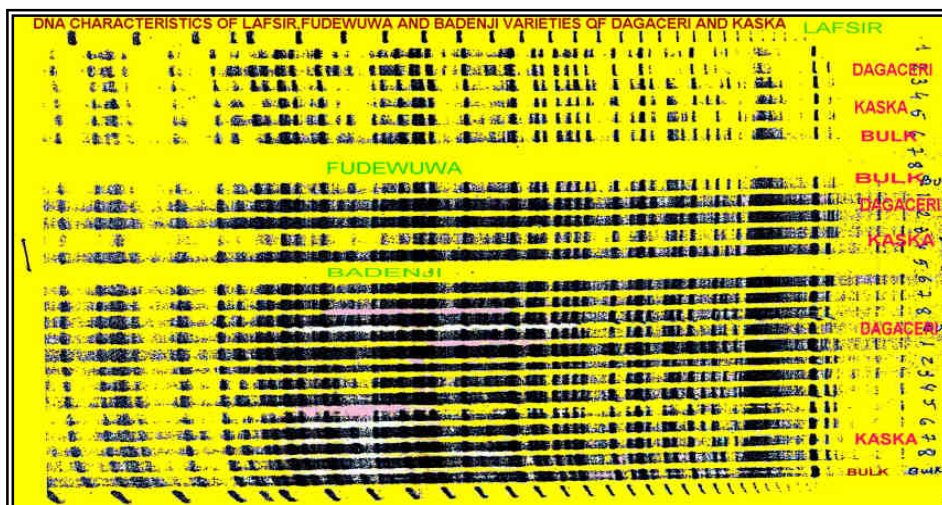**Figure 1: DNA Characteristics of Millet Varieties of Dagaceri and Kaska Villages**

Figure 1 above show banding profile of pearl millet genotypes with EscoRI and Msel primer. Polymorphic bands have been marked by named of the villages. Several studies showed that DNA markers have been used to evaluate genetic diversity in different crop species (Cooke, 1995). Various molecular markers are being used for fingerprinting such as restriction fragment length polymorphisms (RFLP) (Dubreuil and Charcosset, 1998), random amplified polymorphic DNA (RAPD) (Williams et al., 1990), micro satellites (Smith et al., 2000) and amplified fragment length polymorphism (AFLP) (Agrawal et al., 1999).

5.4 Genetic Variability of Sampled Pearl Millet Cultivars
This promise of relative initial homogeneity within landraces was plainly false. Intra-landrace differences seem to be intrinsic to pearl millet production in this area and could be found in any samples taken from the same agricultural system. The apparent genetic isolation of farmers from one another is interesting. Their agricultural practice maintains separation despite similar ideotypic selection criteria on the different farms. Based on molecular analysis, the amplified fragment length polymorphism AFLP analysis conducted has provided the key inferences from 163 independent random loci as shown in figure 2;

Figure 2: ALFP Analysis of Pearl Millet Cultivars of Farmers
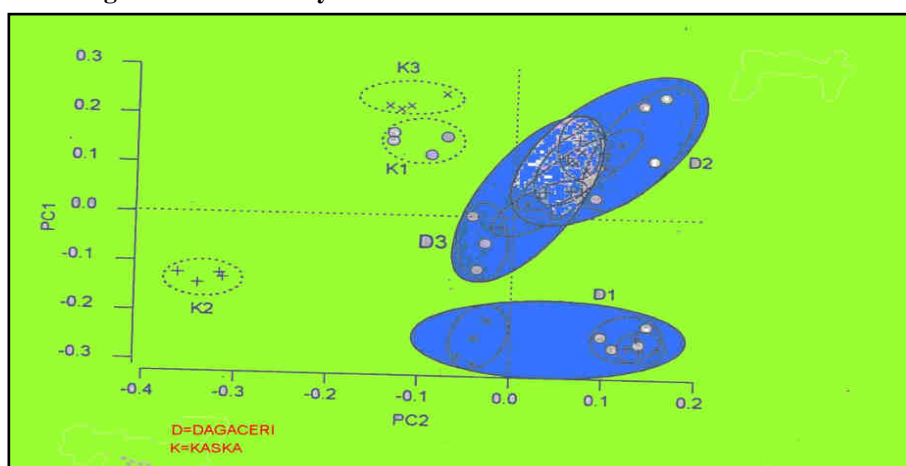
If we look at the raw binary data in figure 2, it would be seen that the data was extremely variable, both in the proportion of polymorphic AFLP band to constant bands and the non-consistent bi-allelic differences seen over the 288 DNA samples. This was reflected in the principal component analysis where the first 74 pcs (or 45% out of 163) were required to account for 90% of the variability in the data within seeds collected from any one head. It would expect only the 'mother' component to be consistent and, even then, this will be segregating for all heterozygous loci, which are expected to be a high proportion. The 'male' component, in a mixed planting regime in small fields on small farms is expected to be extremely, and randomly variable.

Results show that the most significant grouping of the material is at the level of farmer x landrace interaction. That is there are large differences between different individual farmer's stocks of landraces with the same name. Material is not by landrace, as we expected, but by farmer. Thus a group of different landraces grown by the same farmer has greater identity than the same named landrace grown by different farmers, even within the same village. Brunken (1977) suggested that pearl millet, as a whole is much more variable than its wild relatives are. Initially, it was assumed that landraces with the same 'name' would have similar genetic profiles. This led to the expectation of different degree of diversity within landraces according to the level of selection practiced over the years. Orderly differences between farmers were certainly not expected. Govindaraj et al. (2009) concluded that RAPD variations were more among the pearl millet genotypes. The genetic diversity analysis among the genotypes shows the genetic distance and similarity on the whole genome basis that is, difference in their genetic make-up throughout the genome of 20 pearl millet genotypes that were selected based on diverse morphological in Tamil Nadu India. This result is in consonance with Kale and Munjal (2005).

5.5 Genetic Variability between Areas and Land Races Using Principal Coordinates (Pc) Scores Variance
For the genetic variability between areas and collected samples, Anova was carried out for each village separately and in Dagaceri, the effects of main factors were assumed to be fixed and the factor 'Heads' (H) was used as error for the main factors (F1L1FXL). Variance components were calculated as (MS – error) /repetition. The results across pcs have shown that the factor farmer (F) and interaction farmer/landrace (FXL) were highly significant for all of the first eight pcs. The effect of landraces (L) was negligible only in the first pc. However, close observation of the Ms in each pc show that the order of importance of the values of the factors varies substantially. The comparisons of variance components across pcs showed different relations between factors. In some cases, the farmer variance could be ten times higher than interaction (pcs 1,3) and vice-versa in others but in all cases, the landrace variance was the smallest of the three-variance components. In Kaska, it was not possible to distinguish between the factor farmers, landrace or interaction. All except pc6 showed a highly significant Ms for the confounded factor.

Trace = 85.435

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	Σ
Latent Roots	9.157	5.372	5.059	3.791	3.48	2.946	2.608	2.205	
Percent of Trace	10.7	17	22.9	27.4	31.4	34.9	37.9	40.5	222.7
Individual %	10.7	6.3	5.9	4.5	4	3.5	3	2.6	

Source: Genetic Analysis (1998)

Figure 3: Principal Coordinates (Pc) Scores Variance

Variance scores show that in Dagaceri data was completely orthogonal (3 farmers x 3 landraces) whereas the more limited data for Kaska was confounded for farmers and landraces with the intention of exploiting a high level of variability. The result of the analysis indicated that the final data matrix contained binary data scores of the 288 samples with 163 molecular markers per sample, ranging in size from 50 to 495 base pairs. The studies of Johnson et al. (1989) and Skagerberg et al. (1989), indicated that 85 to 95% of the variance is accumulated by the first 30 – 40% of the total pc's calculated. In this analysis, however, the first pc contributed 10.7% and the first 45% of the 163 pc's were required to account for 90% variability. The first eight pcs as presented in Figure 2 contain 40.5% of the variability and were chosen for further analysis.

6.0 Conclusion
Based on results of this study, it could be deduced that there was considerable degree of variability present in the genotypes of the 25 pearl millet samples collected from the area. The main objectives of this study were to assess whether AFLP markers could be able to correctly disclose variability among pearl millet genotypes. Although most primers tested did not reveal any polymorphisms, 12 primers showed clear polymorphism among the pearl millet genotypes.

The results showed that AFLP is an effective tool for pearl millet germplasm management. In conclusion, AFLP variations were more among the pearl millet genotypes. The genetic diversity analysis among the genotypes shows the genetic distance and similarity on the whole genome basis that is, difference in their genetic make-up throughout the genome. If the genetic diversity in the germplasm is considerably less, measures should be taken to widen the available gene pool and germplasm and analyzed time to time to reveal genetic diversity.

Appendix

Table 1: Characteristics and Mode of Access of Pearl Millet Varieties in the Study Area

STUDY VILLAGES	INVENTORIED PEARL MILLET VARIETIES	CODE NUMBERS OF VARIETIES	MATURITY PERIODS			ORIGIN		MODE OF ACCESS				LENGTH OF STALK			
			EARLY MATURING (40–70) DAYS	AVERAGE MATURING (70-120) DAYS	LATE MATURING (120-140) DAYS	INDIGENOUS	INTRODUCED	GIFT	EXCHANGE	LOAN	SELF-CULTIVATION	LONG MORE (2m)	MEDIUM LESS THAN (2m)	SHORT LESS THAN (1.5m)	DROUGHT ESCAPING
DAGACERI	FUDEWUWA	MLVD$_1$	✓				✓	✓	✓					✓	✓
	BADENJI	MLVD$_1$		✓			✓	✓	✓				✓		
	IDON HAWAINIYA	MLVD$_1$		✓		✓		✓	✓				✓		
	CHILUM	MLVD$_1$		✓		✓		✓	✓				✓		
	GARGASA (LAFSIR)	MLVD$_1$		✓		✓		✓	✓				✓		
	ZANGO	MLVD$_1$			✓	✓		✓	✓			✓			
	MAIWA	MLVD$_1$			✓	✓		✓	✓				✓		
KASKA	FIDEWUWA	MLVK$_1$	✓				✓	✓			✓			✓	✓
	BADENJI	MLVK$_2$		✓		✓		✓			✓		✓		
	ARUM MANGA	MLVK$_3$		✓		✓		✓			✓		✓		
	ZANKO	MLVK$_4$			✓		✓	✓			✓	✓			
	MAIWA	MLVK$_5$			✓		✓	✓			✓		✓		

Source: Mohammed (1998)
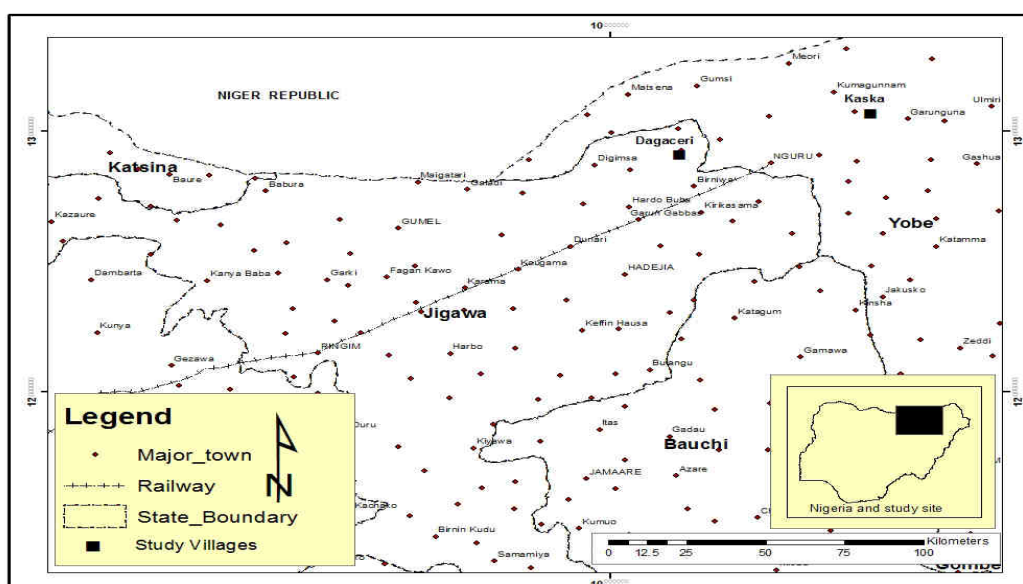
Figure 4: The Study Villages

40

Acknowledgements

References

Agrawal, R.K., Brar, D.S., Nandi, S., Huang, N., Khush, G.S. (1999). Phylogenetic relationships among *Oryza* a species revealed by AFLP markers. *Theor. Appl. Genet., 98: 1320-1328.*

Association for Strengthening Agricutural Research in Eastern and Central Africa (ASARECA), (2008). Safeguarding Biodiversity: The Convention on Biological Diversity (CBD)**.** *News Letter Vol. 11 (10).*

Bennett, M.D. 1976. DNA amount, latitude, and crop plant distribution. Environmental and Experimental Botany 16:93-108.

Blears, M.J., De Grandis, S.A., Lee, H. and Trevors, J.T. (1998). Amplified fragment length polymorphism (AFLP): A review of the procedure and its applications. Journal of Industrial Microbiology & Biotechnology 21:99-114.

Brunken, J.N. (1977). *A Systematic Study of Pennisetum Sect.* (Pennisetum Graminae)

Chase, A. (1921). The Linnaean concept of pearl millet. *American Journal of Botany 8:41-49*

Cooke, R.J. (1995). Gelelectrophoros is for the identification of plant varieties. *J. Chromatog., 698: 281-291.*

Dellaporta, S.L., Wood, J., and Hick, J.B. (1983). A plant DNA mini preparation. Version II. Plant Molecular Biology Reporter, 1:19–21.

De Wet, J.M.J. (1987). Pearl millet *{Pennisetum glaucum)* in Africa and India. In: *Proceedings of the Pearl Millet Workshop*. ICRISAT, Patancheru, India.

Dubreuil, P. and Charcosset, A. (1998). Genetic diversity within and among maize populations: a comparison between isozyme and nuclear RFLP loci. *Theor. Appl. Genet., 96: 577-587.*

FAO (1996). *The State of the World's Plant Genetic Resources for Food and Agriculture*. Information document provided for the Fourth International Technical Conference on Plant Genetic Resources, Leipzig, Germany, 17 - 23 June 1996.

IFPRI (2004). Food and Nutrition Security Policies for West Africa. Implementing Issues and Research Agenda SADAOC Regional Workshop, Bamako, Mali, January 13-15 2004. www.ifpri. org/2020Africaconverence/events/ mali.pdf

Gari, J.A. (2001). Review of the African millet diversity. Paper for the *International workshop on fonio, food security and livelihood among the rural poor in West Africa.* IPGRI / IFAD, Bamako, Mali, 19-22 November 2001.

Govindaraj, M., Selvi, B., Arun Prabhu, D. and Rajarathinam, S. (2009). Genetic diversity analysis of pearl millet (*Pennisetum glauccum* [L.] R. Br.) accessions using molecular markers. *African Journal of Biotechnology Vol. 8 (22):6046-6052*

Gupta, R., Verma, K., Joshi, D.C., Dinesh Yadav, D. and Singh, M. (2010). Assessment of Genetic Relatedness among Three Varieties of Finger Millet with Variable Seed Coat Color Using RAPD and ISSR Markers. *Genetic Engineering and Biotechnology Journal, Volume 2010: GEBJ-2*

Hash, C.T. and Bramel-Cox, P.J. (1999). Marker applications in pearl millet. In: B.I.G. Haussmann, H.H. Geiger, D.E. Hess, C.T. Hash, and P. Bramel-Cox (eds.). 2000. *Application of molecular markers in plant breeding. Training manual for a seminar held at IITA, Ibadan, Nigeria*, from 16-17 August 1999. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Andhra Pradesh, India.

Johnson, J., Erikson, L., Hellberg, S., Sjostrum, M. and Ward, S. (1989). Multivariate Parametirization of 55 coded and non-coded amino acids. *Quantitative Structure Activity Relationship, 8:204-209*

Kale, A.A. and Munjal, S.V. (2005). Molecular analysis of mitochontrial DNA of lines respresentating a specific CMS-fertility–restorer system of pearl millet (*Pennisetum glaucum* (L.) R. Br.) by RAPD Markers. *Indian J. Genet., 65(1): 1-4.*

Lieu, C.J., Witcombe, J.R., Pittaway, T.S., Nash, M., Hash, C.T., and Gale, M.S. (1992). Restriction fragment length polymorphism in pearl millet (pennisetum glaucum). In: *Complexes d'especes, flux de genes at resources genetiques des plantes.* Colloque International 8-10 Javier Paris Publication du Bureau des resources Genetiques

Liu, C.J., Witcombe, J.R., Hash, C.T., Busso, C.S., Pittaway, T.S., Nash, M., Gale, M.D. (1994). In Witcombe, J.R., Duncan, R.R. (eds): *Use of molecular marker in sorghum and pearl millet breeding for developing countries.* Overseas Development Administration: London U.K.

Mortimore, M.J. (1992). Population Growth and Land Degradation. *Workshop on Economy-ecology interaction in the Sahel*. University of Oslo, Norway. March 1992

Ovendeba, B., Ejeta, G., Hanna, W.W., and Kumar, A.K. (1995). Diversity among African Pearl Millet Landraces Populations. *Crop Science, 35:919-24*

Pilat – Andre, S., Lamy. F. and Starr, A. (1992). 'Etude de la region Adh du mil (pennisetum typhoides) par utilisation des techniques RFLP' In: *Complexes d'especes, flux de genes at resources genetiques des plantes.* Colloque International 8-10 Javier Paris Publication du Bureau des resources Genetiques

Poncet, V., Lamy, F., Enjalbert, J., Joly, H., Sarr, A., and Robert, T. (1998). Genetic analysis of the domestication syndrome in pearl millet (*Pennisetum glaucum* L, Poaceae): inheritance of the major characters. *Heredity 81: 648-658.*

Rinaudo, T. and Sogoba, J. (2007). *Greening West Africa.* The West Africa Natural Resource Management Project 2005–2007. Responses to Poverty 2007

Virgin, I., Bhagavan, M., Komen, J., Kullaya, A., Louwaars, N., Jane, E.M., Okori, P. and Persley, G. (2007). *Agricultural Biotechnology and Small-scale Farmers in Eastern and Southern Africa.* Risks, Livelihoods and Vulnerability Working Paper. Stockholm Environment Institute

Rakotoarisoa, M.A., Lafrate, M. and Paschali, M. (2011). *Why Has Africa become a Net Food Importer?.* Explaining Africa agricultural and food trade deficits, Food and Agriculture Organization of the United Nations, Rome

Skagerberg, B., Bonelli, D., Clementi, S., Criciani, G. and Ebert, C. (1989). Principal Properties for Aromatic Contituents. A Multivariate Approach for Design in QSAR. *Quatitative Structure Activity Relationship, 8:32-38*

Smith, J.S.C., Kresovich, S., Hopkins, M.S., Mitchell, S.E., Dean, R.E., Woodman, W.L., Lee, M., Porter, K. (2000). Genetic diversity among elite sorghum inbred lines assessed with simple sequence repeats. *Crop Sci., 40: 226-232*

Stoskopf, N.C. (1985). *Cereal Grain Crops.* Reston Publishing Co. Inc. Virginia. 429-443. USA, Bangalore.

SPORE (1994). Biodiversity: Our Common Heritage. *Technical Centre for Agricultural and Rural Co-operation (CTA), No. 54 (1-3) CTA,* Wageningen, the Netherlands

Vos, P., Hogers, R., Bleekers, M., Reijans, M., van de lee, T., Hormes, M., Frijers, A. Pot, J., Paleman, J., Kuiper, M., and Zabeau, M. (1995). AFLP: A new twchnique for DNA fingerprinting. *Nucleic Acid Research, 23:4407-4414*

Vyas, K.L. (1987). Genetic divergence in landraces of pearl millet in Rajasthan. p. 291. *In* J.R. Witcombe and S.R. Beckerman (ed.). *Proceedings of the International Pearl Millet Workshop,* 7–11 April, ICRISAT Centre, India, Patancheru, A.P. 502 324, India.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A., Tingey, S.V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acid Res., 18: 6531-6535.*

Wilson, J.P., Burton, G.W., Zongo, J.D., and Dicko, I.O. (1990). Diversity among Pearl Millet Landraces Collected in Central Burkina Faso. *Crop Science, 30:40-43*

World Bank (2007). *World Development Report 2008.* The World Bank, Washington D.C.

Zabeau, M., and Vos. P. (1993). *Selective restriction fragment amplification: A general method for DNA fingerprinting.*, EPO Patent No. 0534858A1.

The IISTE is a pioneer in the Open-Access hosting service and academic event management. The aim of the firm is Accelerating Global Knowledge Sharing.

More information about the firm can be found on the homepage:
http://www.iiste.org

CALL FOR JOURNAL PAPERS

There are more than 30 peer-reviewed academic journals hosted under the hosting platform.

Prospective authors of journals can find the submission instruction on the following page: http://www.iiste.org/journals/ All the journals articles are available online to the readers all over the world without financial, legal, or technical barriers other than those inseparable from gaining access to the internet itself. Paper version of the journals is also available upon request of readers and authors.

MORE RESOURCES

Book publication information: http://www.iiste.org/book/

IISTE Knowledge Sharing Partners

EBSCO, Index Copernicus, Ulrich's Periodicals Directory, JournalTOCS, PKP Open Archives Harvester, Bielefeld Academic Search Engine, Elektronische Zeitschriftenbibliothek EZB, Open J-Gate, OCLC WorldCat, Universe Digtial Library , NewJour, Google Scholar