Molecular Characterization and Genetic Diversity of Pennisetum Spp

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Abstract: The genus Pennisetum is one of the exotic grass family with species endowned with exquisite characters that are of great economic importance to man and animals. Despite the fodder and forage quality, drought tolerance, high quantity of protein, medicinal values, aesthetic values and other unique characteristics its species possess, there has been great negligence on some of its species which may be useful in hybridization programme and crop improvement and hence, there is a need to exploit genetic diversity of this genus. Genetic diversity of plant species now serves as a panacea to the changing environmental conditions affecting plants growth and is the basis for survival and adaptation; making it possible to continue advancing adaptive processes on which evolutionary success and to some extent human survival depends. Genetic diversity of eleven Pennisetum genotypes was carried out on the extracted DNA using polymerase chain reaction amplification with fourteen different RAPD primers to assess the degree of polymorphisms. The DNA fragments that were amplified showed reproducibly polymorphism among the species ranging from 55.60% - 100% showing that the markers used are effective. The dendrogram and neighbor-joining diagram obtained demonstrate clearly that genetic diversity exists among the eleven species studied and revealed the possibilities of a hybrid from *P.pupureum* var. 11. Furthermore, this study demonstrated that in most instances similarity in the RAPD-PCR banding patterns reflected relationship due to the origin.
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Key words*:* Molecular Characterization, genetic diversity, RAPD markers, Pennisetum Spp

Introduction

The genus Pennisetum belongs to the grasses, which are a large, diverse and successful group of family Poaceae of monocotyledonous plants. The genus includes cultivated species such as pearl millet as well as many other grasses of high fodder value that includes species such as pearl millet as well as many other grasses of high fodder value that includes species such as P. pedicellatum, P.purpureum and P. orientale. The basic chromosome number in the genus varies (x = 5, 7, 8 or 9) as well as the ploidy (from 2n to 3n). Almost 76% of Pennisetum species are polyploids. Pennisetum is an economically important genus as it comprises many grain crops such as Pennisetum glaucum an important member of the genus which is highly recongnized for both food and fodder and it is so far the most drought tolerant major cereal. Pennisetum purpureum and some other species are also notable for their fodder quality. Some species are of medicinal importance such as Pennisetum divisum (Sujatha et al., 1989) and others like Pennisetum alopecuroides from the tertiary gene pool have been discovered and used as ornamentals as a result of their notable inflorescence.

One unique characteristics of this genus is the difference in their chromosome numbers of which several researches has been carried out in understanding their ploidy and due to this; there has been segregation of its gene pool into primary, secondary and tertiary. This can be relatively represented cytogenetically as (2n=2x=14), (2n=4x=28) and (2n=18, 27, 36, 45, 54) which shows the number of chromosomes and their level of ploidy. Further study exposed strong reproductive barriers that disrupt natural gene flow and occurrence of hybrids between the members of the tertiary gene pool and the forms belonging to primary and secondary gene pool of Pennisetum and this has been a significant challenge in relation to improvement of some species of this genus through interspecific hybridization.

Although significant progress has been made in developing improved cultivars of P. glaucum which is known for its high-quality forage (Hanna et al. 1988) by successfully crossing it with P. purpureum to produce high quality and high yielding perennial interspecific (PMN) forage hybrids (Hanna and Monson 1980, Muldoon and Pearson 1979), species like P. polystachion and P. pedicellatum that have been overlooked for forage production and are small seeded apomicts, not winter hardy but combine excellent dry matter yield with good quality and palatability (Hanna et al. 1989) have been neglected in improvement of some species this genus. Despite the reproductive barrier among some species of this genus, further research has not been carried out on several other wild species of pennisetum that belong to the tertiary gene pool (P.flaccidum Griseb., P. massiacum Stapf, P. mezianum Leeke, and P. orientale L.C. Rich) which are adapted to drier climates and are valuable forage grasses in the semi-arid regions of the

world. There are many desirable characters that are present in the wild species like stress tolerance, apomixes, pereniality, high tillering etc., if transferred to the some cultivated species of Pennisetum, can make them more valuable than ever.

The effort of interspecific hybridization with wild relatives has not yet resulted in commercially viable germplasm except few cases and one of the major hinderence in getting successful interspecific hybrids is the ploidy barrier of the cultivated and the wild species. Various techniques like the use of colchicine for the induction of polyploidy in this genus have been used for the enhancement of ploidy in cultivated specie that are diploid (2n =2x = 14) and researches has been done using different tools like isozyme, AFLPs, RAPDs in improving the cultivated specie of this genus. With recent trend, analysis of genetic diversity is now been done with the use of molecular markers. Though this technique has been employed in various researches involving P. glaucum and P.purpureum, little or no research has been done on the entire genus i.e selected species apart from P. glaucum and P.purpureum using molecular marker.

Characterization of diversity has long been based mainly on morphological traits (Somasundaram and Kalaiselvam, 2011). However, morphological variability is often restricted i.e (Characters may not be obvious at all stages of the plant development and appearance may be affected by the environment) (Somasundaram and Kalaiselvam, 2011). Nowadays, a variety of different genetic markers have been proposed to assess genetic variability as a complementary strategy to more traditional approaches in genetic resources management. Molecular tools provide valuable data on diversity through their ability to detect variation at the DNA level and for evaluation of species diversity, it is essential for accurate classification of individuals. (Somasundaram and Kalaiselvam, 2011). Among the various molecular markers, one of the most used is the microsatellite marker which are advantageous in terms of their locus specificity, codominant nature, high polymorphism, and reproducibility (Powell et al. 1996). In addition their detection can be easily automated (Hernandez et al. 2002) and they do not require prior information of DNA sequences (Zietkiewicz et al. 1994). Peakall et al. (1998) discovered 65% marker transferability when using microsatellite markers among different species within the genus Glycine, although the efficiency dropped to 13% when analysis were carried out on species within different genera. Transfer rates among species are variable and Azevedo et al. 2012 confirmed the cross species amplification of microsatellite markers in P. glaucum and P. purpureum to be approximately 50% indicating the efficiency of using microsatellites as a tool for molecular characterization.

In this study, RAPD markers were used to evaluate the genetic diversity and phylogenetic relationships among selected species of genus Pennisetum. Furthermore, these orthologous RAPD markers helped in revealing a high number of polymorphic fragments.

Materials And Method

Plant Materials

The eleven different Pennisetum species viz P. spatiolatum, P. pedicellatum, P. alopecuroides, P.villosum, P.orientale, P.polystachion, P. macrorum, varieties of P. glaucum, and varieties of P. purpureum comprise of cereal and grass; Pennisetum having divergent geographical origin were used in the study. Some of these plant materials were gotten from Botanical garden of University of Ilorin, two varieties of pearl millet from NCRI and two varieties of napier grass (stem cutting) were sourced from wild population in Ilorin Kwara State. (Table 1)

S/N	NAME OF SPECIES	LOCATION GOTTEN FROM
1	*Pennisetum spatioladum*	UNIVERSITY OF ILORIN
2	*Pennisetum pedicellatum*	UNIVERSITY OF ILORIN
3	*Pennisetum alopecuroides*	UNIVERSITY OF ILORIN
4	*Pennisetum polystachion*	UNIVERSITY OF ILORIN
5	*Pennisetum villosum*	UNIVERSITY OF ILORIN
6	*Pennisetum orientale*	UNIVERSITY OF ILORIN
7	*Pennisetum macroum*	UNIVERSITY OF ILORIN
8	*Pennisetum purpureum* (green)	ILORIN, KWARA STATE
9	*Pennisetum purpureum* (white)	ILORIN, KWARA STATE
10	*Pennisetum glaucum* var 1	NCRI
11	*Pennisetum glaucum* var 2	NCRI

NCRI: NATIONAL CEREAL RESEARCH INSTITUTE

Dna Isolation And Quantification

DNA samples were extracted from 9 species of pennisetum with (*Pennisetum purpureum* and *Pennisetum glaucum* having two varieties each) used for this study. 0.1 g of fresh young leaves for each of the sample was ground in liquid nitrogen using pre-chilled mortar and pestle and the powder tissue content was transferred into a 2-ml eppendorf tube. DNA extraction was carried out using DNA Extraction Kit which is a recent technology applicable and useful in the isolation of DNA from plants. It employs Anion-exchange chromatography and the use of spin columns, which contain a silica-gel base membrane that binds the DNA. The DNA while bound to the gel membrane can be washed from contaminants and then eluted from the column (membrane) using distilled water. The DNA obtained is usually more purer and cleaner than DNA extracted by other methods; this apart from other attributes of this technology like simplicity, rapidness, harmful chemicals (phenol or chloroform) free and minimal handling as accrued this process many advantages over C-TAB and other methods although it is more expensive. The genomic DNA obtained from extraction process was quantified and quality checked on 1% (w/v) agarose gel and the purity of the gDNA samples was determined by UV-Visible spectrometer (Varian, Australia).

Amplification of DNA using RAPD markers

A total of fourteen RAPD random primers (Decamers) of 10bp were selected from list of operon developed by University of British Columbia, Canada and sequences which ran from 5' to 3' coded names were sent to GeNei, Banalore, India for synthesis. RAPD markers amplification was performed as described by Sharma *et al.* (2008) using 14 decamer random primers (GeNei, Bangalor, India). The PCR reaction was carried out with 25µl volume in 200 µl capacity thin wall eppendorf tube containing 12.5 µl reaction mixture whose composition is (1 xreaction buffer with 2.0 mM MgCl2, 200 µM each of deoxynucleotides (dNTPs), 0.5 µl of *Taq* polymerase), 1 µl of 10 pMole primer and 1µl of 50 ng genomic DNA. The reaction tubes were spun briefly at 8,000 rpm and placed in the Eppendorf gradient Mastercycler (Eppendorf, USA). The PCR protocol was run with a cycle of initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 27°C (depending on annealing temperature of each primer) for 50 sec and extension at 72°C for 1 min with a final extension at 72°C for 10 min. The reaction was put on hold for 10 min at 4°C. The PCR products were visualized to confirm the targeted RAPD PCR amplification by mixing 5 µl of PCR product with 1 µl of 6X gel (Bromophenol blue) loading dye. It was electrophoresed on 1.5% agarose gel containing 5µl of ethidium bromide (1 per cent solution of 10 µl/100 ml) at constant 5V/cm for 40 min in 1X TAE buffer. Fragment sizes of the amplification products obtained from the markers were obtained from the gel by comparison with standard molecular weight marker ladder-low range DNA Ruler Plus (3000 bp to 100 bp; Genei, Bangalore, India). The amplified product was visualized as a single compact band of expected size under UV light and documented by gel documentation system (Bio-Rad, USA).

Data Analysis

Table 4: Amplification information of 14 RAPD markers used in the diversity study of some species of Pennisetum

SN	Marker Code	Sequence (5'-3')	TNA	TNL	NML	NPL	P (%)
1	OPD-8	GTGTGCCCCA	58	7	3	4	57.10
2	OPH-19	CTGACCAGCC	64	7	2	5	71.40
3	OPK-19	CACAGGCGGA	40	6	1	5	85.70
4	OPH-20	GGGAGACATC	40	8	1	7	87.50
5	ADG-4	CCCGCCGTTG	20	11	1	10	90.90
6	OPB-1	GTTTCGCTCC	22	9	0	9	100
7	LC-71	TGCCGAGCTG	44	11	1	9	90.90
8	RAPD-010	CCGCCACTGT	39	8	1	7	87.50
9	RAPD-012	CGGCCACTGT	19	9	0	9	100
10	RAPD-015	CGGCCCCGGC	30	7	0	7	100
11	RAPD-04	CGGCGCGCGA	74	9	4	5	55.60
12	RAPD-09	GAAGAACCGC	68	8	3	5	62.50
13	RAPD-036	GAAGAACCGC	63	7	3	4	57.10
14	RAPD-021	GACGGATCAG	66	6	6	0	0.00

TNA = total number of allele, TNL = total number of loci, NML =number of monomorphic loci, NPL = number of polymorphic loci, %P =Percentage of polymorphism

The PCR fragments were scored for the presence (1) or absence (0) of equally sized bands and two matrices of the different RAPD phenotypes were assembled and used in the statistical analysis. The

fragments were only considered on ability to detect clearly resolved and polymorphic amplified loci among the accessions studied and fourteen RAPD primers were selected for analysis. The data were entered in to binary matrix for analysis. Principal Coordinate (PCO) analysis and diversity indices were conducted using PAST (Paleontological Statistics), version 3.5. Cluster analysis was performed by agglomerative technique using the Un-weighted Pair Group Method with Arithmetic Mean (UPGMA) method Dendrogram was constructed using the UPGMA algorithms in the MEGA 4.0 software. Relationships between the genotypes were graphically represented in form of dendrogram, neighbour joining and phylogram.

Table 3: Principal coordinates of RAPD loci and alleles data for some species of Pennisetum

Principal Cordinate Axis	Percentage Variation	
	Individual %	Cumulative %
Axis 1	20.233	20.233
Axis 2	13.398	33.631
Axis 3	11.222	44.853
Axis 4	10.634	55.487
Axis 5	9.1852	64.672
Axis 6	8.9549	73.6271
Axis 7	7.9993	81.6264
Axis 8	7.6147	89.2411
Axis 9	6.6073	95.8484
Axis 10	4.1615	100.0099

Results

RAPD analysis of diversity study of the 11 species of Pennisetum showed high degree of polymorphism. The amplified PCR product of the 14 selected markers resolved on agarose (1.5% Agarose gel electrophoresis) and viewed under imaging and gel documentation system (BioRad) showed arrays of monomorphic and polymorphic band (Fig 1) using DNA ruler as calibrator (Appendix III). A total of 113 reproducible bands (loci), ranging from 100bp (primer OPD-8,) to 250bp (primer RAPD-021) were generated from the amplification profiles of the 11 species with 14 random primers having a cumulative amplification of 647 alleles (Table 2). The number of bands detected per primer ranged from 6 (RAPD-021, OPK-19) – 11 (ADG-4, LC-71) with an average of 8 bands per primer. 87 loci (77.70%) out of the 113 loci amplified were polymorphic showing an average of 6.28 polymorphic markers per primer ranging from (OPD-8, RAPD-036) to 10 (ADG-4, LC-71) while 26 loci (23.00%) were monomorphic (Table 2). The percentage polymorphism of primers ranged from 55.60% - 100% for first 14 primers while the last primer recorded 100% monomorphism. The primers that showed the highest polymorphism were RAPD-012, RAPD-015 and OPB-1 with 100% respectively (Table 2). The allelic richness ranged from 1.72-6.72 alleles per locus with a mean of 4.19 indicating allelic variation in a locus. Maximum amplification of 11 loci was produced by Primers LC-71 and ADG-4 while minimum amplification of 6 loci was produced by OPK-19 and RAPD-021 respectively. The total number of allele's i.e number of monomorphic and polymorphic alleles, the number of loci amplified and the percentage polymorphism expressed by each of the primers is represented in Table 2.

The allelic frequencies for RAPD markers amplification is shown in Figure 4. Frequencies of loci amplification was highest with RAPD-09 (above 0.96 scale) and other markers like OPK-8, OPH-19, RAPD-021, and RAPD-036 had high frequencies of allelic amplification on the species. The graph made some clear cut differences in relation to percentage polymorphism and loci amplification. The marker ADG-4 and LC-71 which amplified maximum of 11 loci had low amplification frequency while primer RAPD-015 which recorded highest percentage polymorphism conversely had low frequency of amplification (at $p<0.05$) among the species. Although RAPD-15 and OPB-1 inclusive has less occurrence of DNA fragment amplification among the species, however, other markers produced remarkably high DNA fragment amplification.

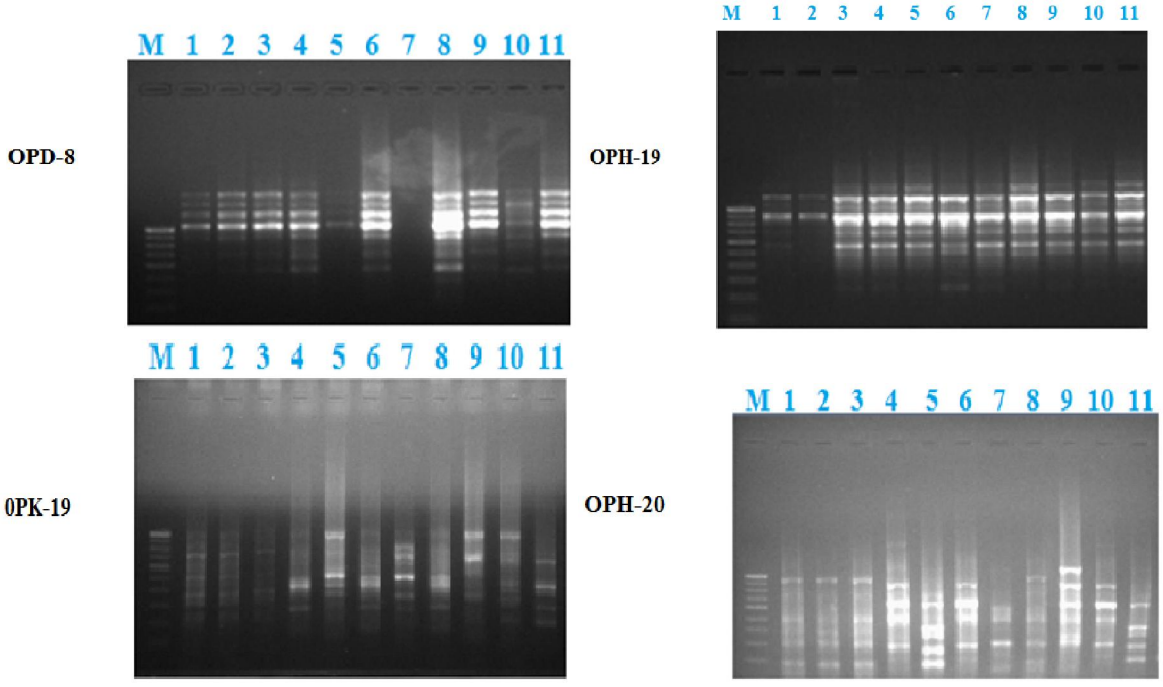
The principal coordinate (PCo) analysis of the loci and allelic data showed ten coordinates were important and accounted for100.001% of the genetic variation (Table 3). PCoA1 plotted against PCoA2 in PCo analysis biplots showed three species of Pennisetum occupied quadrant I, five species of Pennisetum occupied quadrant II, none in quadrant III and three species of Pennisetum in quadrant IV. *P. glaucum* var 1, *P. glaucum* var 2 and *P.villosum* were located in quadrant I with *P.glaucum* (var 1 and var 2) closest while *P. alopecuroides, P. pedicellatum* and *P.*

spatioladum were located in quadrant IV. Quadrant II accounted for the highest number of Pennisetum species which are; *P. purpureum* (11), *P. macroum, P. polystachion, P.purpureum* (10) and *P. orientale* (Figure 5). Pennisetum species *P. spatioladum* and *P. pedicellatum* were found in far end of quadrant IV with significant distance from *P. alopecuroides* which is close the centroid. It is also observed that Pennisetum species in the quadrant II are distributed in a random pattern.

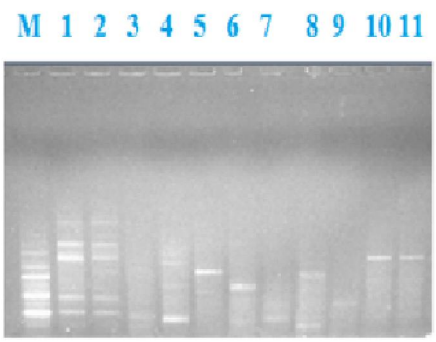
Cluster analysis generated from matrix similarity analysis of eleven speeeceies of Pennisetum based on fourteen RAPD markers by Wards method revealed two major groups; Group A and Group B at genetic distance less than 1.6 as shown in figure 4. The genetic similarity was scaled between 0.5 and 1.0 according to Jaccard similarity co-efficient.

Group A as defined consists of two Pennisetum species *P. spatioladum* and *P. pedicellatum*) which had diversity distance above 1.6. On the other hand, Group B at diversity distance 11.2 sub-divided into B1 and B2. Pennisetum species *P.glaucum* var 1 and *P. glaucum* var 2 formed a cluster B1 while the remaining species formed clusters at B2. At similarity distance above 9.6, cluster B2 segregated with *P. purpureum* (10) on a sole divide as outgroup B2 (i) and two distinct clusters B2 (ii). the first cluster consists *P. macroum* and *P. villosum* while the second cluster which is the largest consists *P. alopecuroides, P. orientale, P. polystachion* and *P.purpureum* (11). Pennisetum species *P.glaucum* var 1 and *P. glaucum* var 2, *P. macroum* and *P. villosum* and *P.polystachion and P.purpureum* (11) were similar at 3.2, 4 and 3 similarity scales respectively.

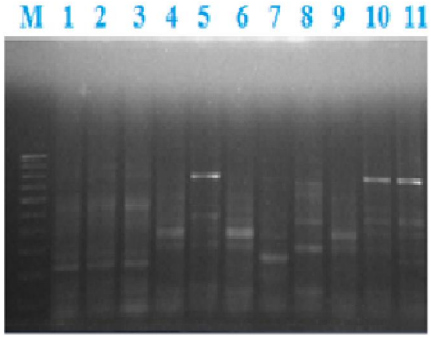
The Neighbour – Joining diagram of eleven species of Pennisetum (figure 5) showed two Pennisetum species with distinct positioning i.e *P. purpureum* (10) and *P. macroum. P. purpureum* (10) at 32 evolved from *P. purpureum* (11) and is the only Pennisetum specie with the highest tendency to evolve a new breed in the nearest future whereas *P.macroum* at 14 is the ancestor from which other Pennisetum species evolved. Among the species studied, *P.spatioladum* and *P. pedicellatum* have stopped evolving, are the most stable and are closest neighbours to each other. Although *P.macroum* is a main ancestor of the Pennisetum species as shown, *P. glaucum* var 1 and *P.glaucum* var 2 have a co-ancestor *P. villosum* which they evolved from and they are relatively close neighbours.



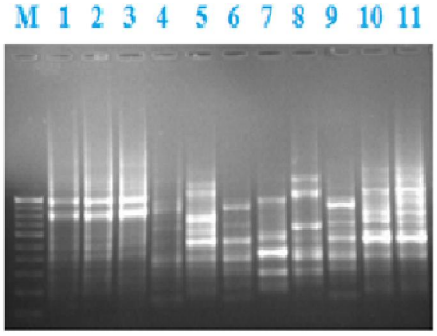
ADG-4



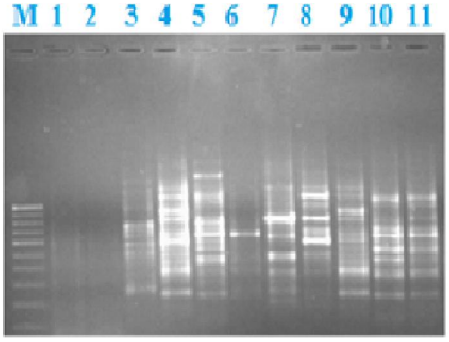
OPB-1



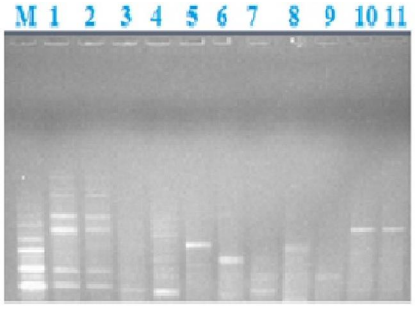
LC-71



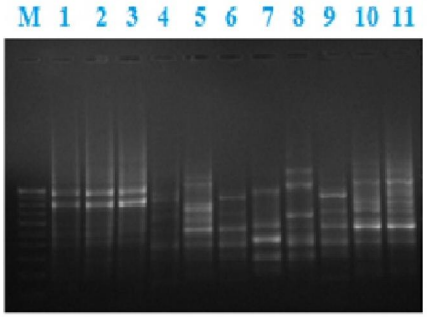
RAPD-010



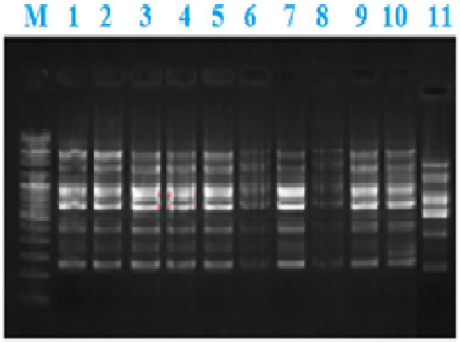
RAPD-012



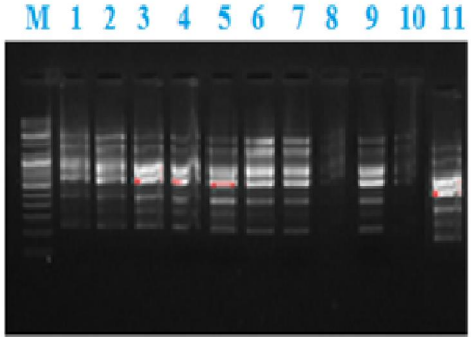
RAPD-015



RAPD-04



RAPD-09



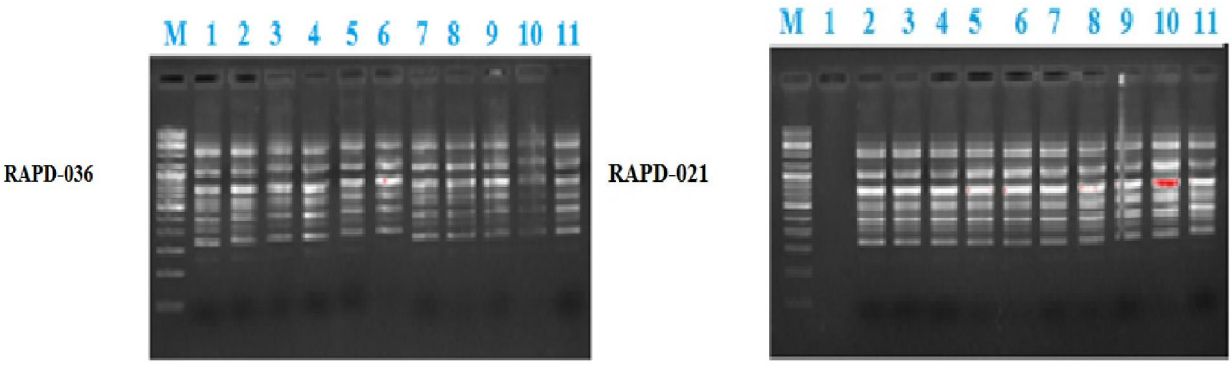


Figure 1. OPD-8 – RAPD-021; Amplification of RAPD primers used for the diversity study of 11 species of Pennisetum as resolved on 1.5% agarose gel.
Key: M= 100Kb ladder, 1= *P.spatioladum*, 2 = *P. pedicellatum*, 3 = *P. alopecuroides*, 4 = *P.polystachion*, 5 = *P.villosum*, 6 = *P. orientale*, 7 = *P. macroum*, 8 = *P. purpureum* (10), 9 = *P.purpureum* (11), 10 = *P. glaucum* var 1, 11 = *P. glaucum* var 2.

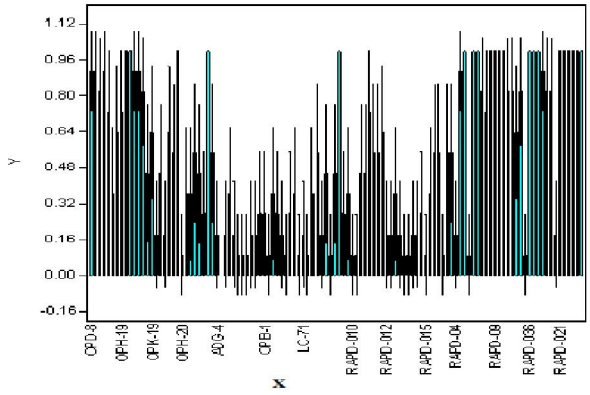


Figure 2. Bar chart showing allele amplification frequency by RAPD markers used for diversity study among eleven species of Pennisetum with standard errors of the mean (SEM) at $p<0.05$ based in amplification intensity of the loci.
Key: Y-axis: the allelic frequency; X-axis: the RAPD markers used for diversity studies.

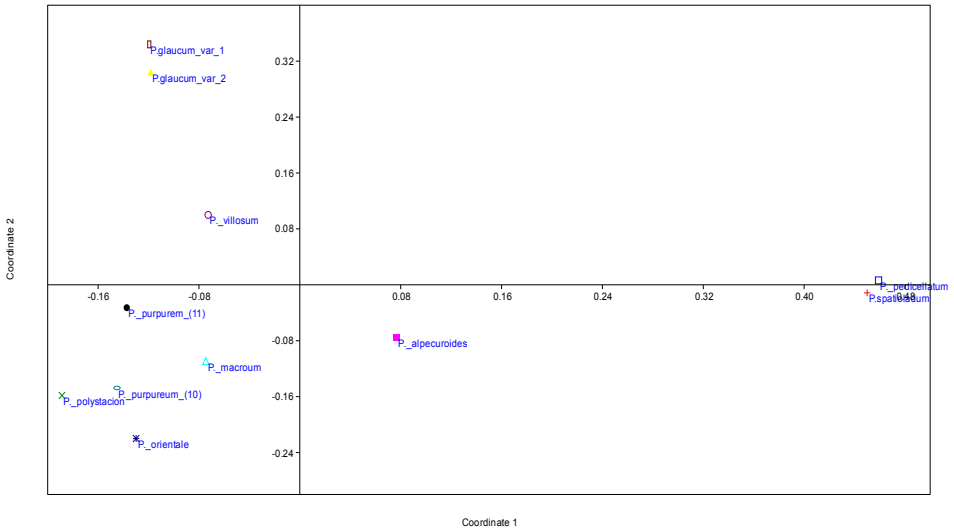


Figure 3. Principal coordinate Axis 1 versus Principal coordinate Axis 2 of RAPD allelic data for eleven species of Pennisetum

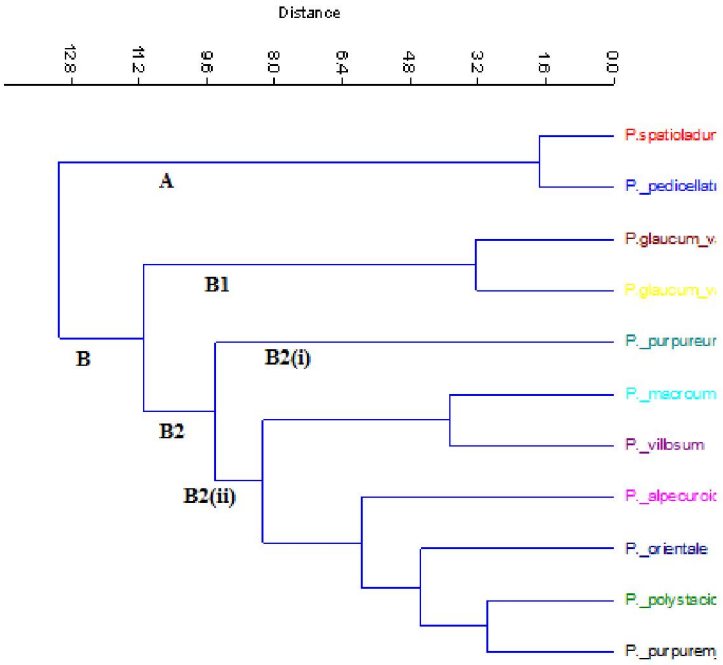


Figure 4. Genetic relatedness of the species of Pennisetum based on UPGMA cluster analysis.

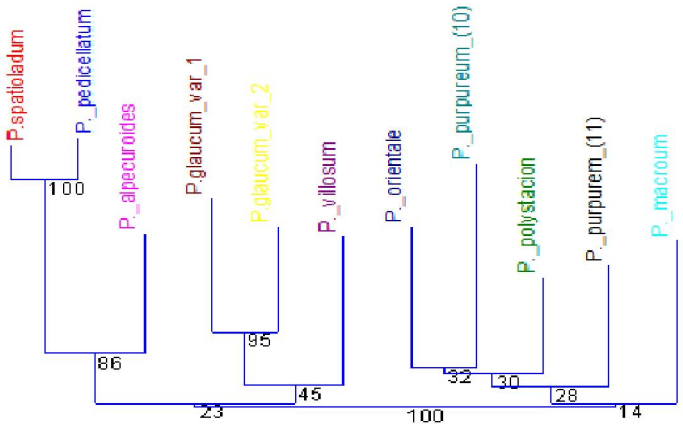


Figure 5. Neighbour-Joining diagram of eleven species of pennisetum based on fourteen RAPD markers

Discussion

Diversity studies in the Pennisetum germplasm offer possibilities for their use in the improvement of the species. The efforts for the rapid assessment of genetic relationships require effective DNA marker-based fingerprinting strategies (Langridge, 2005; Varshney and Tuberoda, 2007). The study carried out on some species of Pennisetum in Nigeria revealed genetic diversity and relatedness using RAPD markers.

The first step for all genetic diversity studies is extraction of DNA template from sample used. Extraction of genomic DNA from species of Pennisetum using QIAGEN DNA Extraction Kit was simple, easy and precise. In contrast to other extraction methods, it is not time consuming and the amount of impurities is minimal. Fresh young leaf tissues (Doyle and Doyle, 1990; Zidani *et al*., 2005; Ogunkanmi, *et al*., 2010; Azevedo; 2012), and dried root tissue (Kumar *et al,* 2003) were used in this method because nucleic acid extracted has lesser contamination by plant metabolites that interfere with solubilisation (Puchaa, 2004). For RAPDs, little amount of DNA template is prepared since, theoretically, a single DNA molecule may be amplified by PCR (Wang et al; 1998, Talebi, 2008). DNA purity has been emphasized as one of the most important factors in RAPD reproducibility. In the

extraction method employed, washing of DNA template with a short run centrifugation was done for purification and removal of endogenous nucleases or other proteins which aligns with the findings of Zidani et al., (2005). The DNA quality is measured by Absorbance (A) or Optical Density (OD) which starts reading at 260nm and 280 nm. The purity of DNA obtained in this study varied from 1.46OD- 1.92 OD which shows that some of the DNA extracted is of high quality further corresponding with the isolation of high quality DNA by Das et al., (2009). Despite DNA template extracted from some of the species having O.D values below 1.8 which indicates presence of proteins, appreciable results were obtained from RAPD analysis based on the ability of PCR to amplify genomic DNA with impurities. Otoo et al. (2009) reported successful use of genomic DNA with low OD values range 1.02-1.4 for SSR genetic analysis.

High yield of DNA extracted is another key factor required (Prasad and Padmalatha, 2006). The DNA yield using DNA Extraction Kit ranged from 126.43-196.83 per extraction signifying that the DNA has high yield for some samples (Maniatis et al., 1982, Guliar et al; 2010). The DNA isolated in this study showed suitability for RAPD-PCR with its characteristically strong and reliable amplified products.

Ambiguous genetic polymorphisms among eleven species of Pennisetum were revealed by the RAPD-PCR analysis carried out in this study. RAPD is been used for detection of genetic variability in plants due to its rapidity, simplicity and lack of need for any genetic information about the plant. Welsh and McClelland, 1990; Micheli et al., 1994 reported consistency in RAPD patterns irrespective of the plant source and age. The existence of genetic variability between the species was evident in the analysis of the RAPD profiles. From the eleven species genotyped, a total of 647 alleles were recorded with 113 reproducible loci and an average allelic richness of 4.19 alleles per locus which showed the markers were effective for pennisetum diversity study. Oumar et al. (2008) observed lower number of alleles and lower genetic diversity in cultivated varieties of pearl millet while comparing allelic divergence among the genus pennisetum using SSRs. Paular Martins-lopes et al. (2004) generated RAPD amplification profiles from fifteen markers in *Olea europaea* (Olive tree) and five markers in plant species of medicinal value from arid environment was reported by I. A. Arif (2010). 77.70% i.e 87 out of 113 loci produced were polymorphic which correlates with the report of high level of polymorphism by Kale and Munjal (2005) and Jaya Prakash et al., (2006). The percentage of polymorphism of primers ranged from 55.60% - 100% signifying effectiveness in the study which is in line with Pfeifer et al., 2011 assertion that high degree of polymorphism showed the extent of diversity and effectiveness of markers.

Estimation of large portion of sampling variance of diversity is due to the variation of diversity levels among loci across the genome (Nei, 1987; Weir, 1990). The eveness of allele frequency is accounted for by the measures of average observed heterozygosity, expected heterozygosity and effective number of alleles. Interpretation of microsatellite profile is therefore in terms of allele phenotypes (Esslink et al., 2004) and degree of polymorphism is determined from allele frequency. Although majority of the primers used were polymorphic except for RAPD-021, distinct allelic frequency was observed in RAPD-04 and ADG-4 in this study indicating that this two primers are most informative for the diversity study in Pennisetum.

The principal coordinate analysis revealed ten important axes which contributed to the observed variation. The occurrence of *P.glaucum* var 1, *P.glaucum* var 2 and *P.villosum* in quadrant I showed they are closely related and neighbor joining diagram further revealed *P.glaucum* var 1 and *P.glaucum* var 2 are hybrids of *P.villosum.* Occurrence of *P. pedicellatum* and *P. spatioladum* at the farther end of quadrant IV revealed uttermost genetic variation and distance between them and the other Pennisetum species. The random patterning of Pennisetum species in quadrant II indicated closeness amongst them and further revealed that they have a common origin.

The interpretation of cluster analysis of diversity study of Pennisetum species showed that *P.pedicellatum* and *P.spatioladum* at genetic distance above 1.6 were similar but at genetic distance above 12.8, there is a linkage to other specie which signifies a vast difference in genotype and showed they are distant neighbours as seen in neighbour joining diagram. Three outgroup *P.pupureum* (11), *P. alopecuroides* and *P. orientale* were observed at genetic distance below 9.6 and below 4.8 for the other two respectively and six other species (*P.glaucum* var 1 and *P.glaucum* var 2), *P.macroum* and *P.villosum and P. purpureum* (10) and *P. polystachion* were similar at 3.2, 4 and 3 genetic distance respectively. Overall, a very high level of similarity was revealed within genotypes that clustered together and moderate level of dissimilarity was observed among the accessions which indicate better opportunities for genetic improvement of the crop through selection and cross breeding (Fikreselassie, 2012).

Analysis diversity study on Pennisetum species with neighbour joining diagram confirmed earlier speculations on the diversity and closeness amongst the species. *P. macroum* was discovered to be the common ancestor to the remaining ten species from

which they evolved. It was further revealed that *P.purpureum* (11) is the only specie actively evolving with great possibilities of producing hybrid and that *P. glaucum* var 1is an hybrid of *P.glaucum* var 2. In future perspective, the neighbor joining diagram revealed an opportunity of influencing evolving species.

Conclusion

Molecular methods have become an essential part of most studies on genetic diversity and distribution and in the analyses of breeding system, bottlenecks and other key features affecting genetic diversity patterns. Microsatellites have obvious advantages for crop species (e. g. ease of use, high levels of simply inherited variation) although they remain expensive and time consuming to develop for each and every crop and generic markers have not been identified but the wealth of information gotten from studies will help to select the markers which are well distributed throughout the genome. (Govindaraj et al., 2009) reported that this can be exploited, not only to drive current advances in agriculture, but also to be used to get an insight into the genetic resources that can be employed in future hybridization programmes. RAPD is an effective tool for pennisetum germplasm management. This study has shown that RAPD is quite efficient in diagnosing genetic diversity in Pennisetum species at DNA level. Presently about 100% variation has been observed among eleven species of Pennisetum and RAPD markers used were polymorphic and revealed diversity among the species. Pennisetum specie was grouped distinctively in a cluster by the markers as observed in the dendogram and closeness and distance was revealed through the neighbor joining diagram.

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