Assessment of genetic diversity in accessions of pearl millet (*Pennisetum glaucum*) and napier grass (*Pennisetum purpureum*) using microsatellite (ISSR) markers

David A. Animasaun[1*], Joseph A. Morakinyo[1], Oba T. Mustapha[1], Ramar Krishnamurthy[2]

[1]Department of Plant Biology, Faculty of Life Sciences, University of Ilorin, P. M. B. 1515, Ilorin -240003, Kwara State, Nigeria.
[2]C. G. Bhakta Institute of Biotechnology, Uka Tarsadia University, Bardoli, Surat -394 350, Gujarat, India.
**Correspondening author, E-mail: biostanleydayor@yahoo.com.au.Tel: +2348060264429.*

Received: 10 Jun 2015; Accepted: 4 May 2016.

Abstract
Pearl millet (*Pennisetum glaucum* (L.) R. Br.) and napier grass (*Pennisetum purpureum* Schumach) are the most economically important species in the genus *Pennisetum.* Knowledge of genetic diversity of these two species would enhance their potentials for utilization. The present work assessed the genetic diversity and phylogenetic relationship among Nigerian and Indian accessions of pearl millet and napier grass using microsatellite markers. We extracted genomic DNA from each accession and carried out Polymerase Chain Reaction using Inter-Simple Sequence Repeat markers. Data obtained were analyzed for genetic diversity using MEGA 4.0 software. A total of 48 loci consisting of 410 bands were generated with 56.25% polymorphism. Principal Coordinates analysis revealed three principal axis contributed significantly (70.20%) to the observed variations. Accessions of napier grass from Nigeria and India were plotted on a coordinate plane while pearl millets from both countries co-existed on different quadrants. Cluster analysis, showed that Nigeria and India accessions of napier grass were similar. Consistent link of COM-CO-3 with Nigeria napier grass and millet accessions suggested a common progenitor. The polymorphism obtained in this study showed that ISSR is an effective marker for assessment of genetic diversity and the connection between most accessions indicated that they have common progenitor.

Key words: ISSR markers, Molecular genotyping, Napier grass, Pearl millet, Phylogenetic study.

INTRODUCTION

Plants are essential components of the world's biodiversity and are indispensable for the sustenance of human life. In the wakes of climatic change with its impending danger of food scarcity and insecurity, nearly a billion poor people of dry land regions of Africa and Asia lives depend on cereals such as millet, sorghum and other dry land crops that can be grown on drought and water stressed environments. The poor rural people whose main occupation is farming still awaits their salvaging crops whose genetics is well understood to develop hybrids of improved yield and stable crops for such condition. Among group of plants that could fit into these requirements are grain cereals and forage crops of the genus *Pennisetum.*

The plant genus *Pennisetum* belongs to the family Poaceae and its most economically important species are pearl millet (*Pennisetum glaucum* (L.) R. Br.) and napier grass (*Pennisetum purpureum* Schumach). These two species are of significant importance in the tropical agriculture and the understanding of their genetics could open a window of greater potentials of sustainable grain production to boost world's food security. Pearl millet is a warm season crop that can be

grown in a vast range of environmental conditions including those characterized by frequent drought events and poor soil fertility (FAO and ICRISAT, 1996). It has the highest level of tolerance to heat and drought among tropical cereals and is grown on more than 26 million hectares in the arid and semi-arid tropical regions of Asia and Africa where it is one of the most important staple foods (Khairwal *et al*., 2007; Rai *et al*., 2009). Several other economic importance and utilization of pearl millet have been reported (Poncet*et. al*., 1998). Pearl millet displays tremendous phenotypical variability for traits such as flowering time, panicle length, stover yield, biotic and abiotic tolerance as well as nutritional value (Stich *et al.,* 2010). Efficient and systematic exploitation of these diversities is the key to its improvement. Due to its small genome size, rich genetic diversity, the crop promises to be an important model for comparative genomics and functional gene studies. Also pearl millet is evolutionarily close to important biofuel grasses, such as the switch grasses and napier grass.

Napier grass on the other hand is a forage crop belonging to the secondary gene pool of the *Pennisetum* (Morakinyo and Adebola, 1991; Barbosa *et al.,* 2003). It is a tall evasive perennial species which is native throughout humid, tropical mainland Africa and the Island of Boiko (Burkill, 1994). Though it grows in the wild mostly, it has been cultivated for forage in many tropical areas of Asia, America's and the Oceania (Farrell *et al*., 2002). They are basically of two variants of pale green or purple stem and foliar colouration (Morakinyo and Adebola, 1991). However, unlike pearl millet, napier grass is an inconsistent flower producer and rarely develop seeds, when seeds are produced they are often less viable (Langeland *et al*., 2008). Thus clones and cuttings are the major means of propagation. Economic importance and usefulness of napier grass have been elucidated by other researchers (Skerman and Riveros, 1990; Tchamba and Seme, 1993**).**

In West Africa where millet and napier grass are widely distributed, the genetic relationship between the two species have not been well investigated. However, reports are available on characterization using agronomic traits and a few studies on their cytogenetic (Morakinyo and Adebola, 1991; Barbosa *et al*., 2003; Techio *et al*., 2006). Assessment of diversity and relatedness in crop species is important as it facilitates the establishment of conservation strategies, use of genetic resources and study of evolution. Also, it helps in the selection of traits with low heritability, resistance or tolerance for biotic or abiotic stresses and estimation of genetic relatedness among crop accessions faster than the conventional methods (Semagn *et al.,* 2006). At present, full genome sequences are not available for many species in particular for "orphan" and forage crops such as millet and napier grass. Researchers therefore relied on markers technologies for the analysis of genetic variation, development of improved cultivars and establishment of linkage maps that allow breeders to identify, characterize and use genetic variability in economically important plants (Varshney *et al.,* 2006).

Several protocols and cutting edge technologies have been developed to study genotypes and DNA fingerprinting of agricultural crops with various degrees of efficiency. Polymerase Chain Reaction (PCR) and markers technologies have been used often for this purpose (Nybom, 1990; Ogunkanmi *et al*., 2009). In comparison with cereals such as rice, wheat, maize, and barley, less research on application of molecular tools for diversity study of pearl millet and napier grass have been documented (Bhattacharjee *et al.,* 2002). Nevertheless, markers application in the study of millet have been reported, RFLPs (Liu *et al.,* 1994), STSs (Gale *et al*., 2001), AFLPs (vom-Brocke *et al*., 2003), SSRs (Kannan *et al.,* 2014) and DArT (Supriya *et al.,* 2010). In addition, RAPD has been utilized in diversity studies of finger millet (Kumari and Pande, 2010) and isozymes (Hilu, 1995) have been successfully used in genetic diversity analysis. Hash *et al*. (2003) reported quantitative trait loci (QTL) mapping and marker-assisted selection (MAS) for stover yield, foliar disease resistance, and *in vitro* estimates of the nutritive value of various stover fractions for ruminants in pearl millet and sorghum.

Molecular marker studies reported for *Pennisetum* are mainly on pearl millet while very scanty information is available on napier grass in particular on its genetic diversity. Considering the potentials of the DNA-based marker in genetic diversity analysis, the present study assessed the genetic diversity between pearl millet and napier grass using ISSR marker with the view of generating information which can be harnessed and utilized for breeding programmes.

MATERIALS AND METHODS

Plant materials

A total of 12 accessions comprising of 9 pearl millet and 3 napier grass were used for the study. Pearl millet seeds were collected from National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan, Oyo State, Nigeria and from International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India. In addition, some accessions were obtained from farmer's

Table 1: The accessions number, common name, source of collection and the status of the twelve accessions used for the ISSR diversity studies.

No.	Accession	Common Name	Source of Collection	Accession Status
1	IP3616	Pearl millet	ICRISAT	Natural
2	NGB00463	Pearl millet	NACGRAB	Natural
3	BALKUVE	Pearl millet	GUJARAT	Natural
4	NGB00616	Pearl millet	NACGRAB	Natural
5	IP4133	Pearl millet	ICRISAT	Hybrid
6	NGB00476	Pearl millet	NACGRAB	Natural
7	JALGONE	Pearl millet	NACGRAB	Natural
8	IP3122	Pearl millet	NACGRAB	Natural
9	NGB00537	Pearl millet	NACGRAB	Natural
10	COM-CO-3	Napier grass	TNAU	Hybrid
11	OMUO-GREEN	Napier grass	EKITI, NIGERIA	Natural
12	OMUO-PURPLE	Napier grass	EKITI, NIGERIA	Natural

NACGRAB: National Centre for Genetic Resources and Biotechnology, Ibadan, Nigeria.
ICRISAT: International Crops Research Institute for the Semi-Arid Tropics, Andhra Pradesh, India.

field at Bardoli, Gujarat state of India. Napier grass clones and stem cuttings of two varieties were collected from wild populations in Omuo-Ekiti, Ekiti State of Nigeria while a hybrid line was collected from the Department of Forage Crop, Tamil Nadu University of Agriculture, Coimbatore, India (Table 1).

DNA isolation and quantification

DNA samples were extracted from all 12 accessions of pearl millet and napier grass used for this study. About of 300 mg of fresh young leaves for each of the sample was ground in liquid nitrogen using pre-chilled mortar and pestle, the powder was transferred into a 2-ml tube. DNA extraction was carried out using Cetyltrimethylammonium bromide (CTAB) procedure described by Cota-Sanchez *et al*. (1995) with modifications. In addition of all required extraction buffers and centrifuge, the precipitated nucleic acid was collected in a 1.5-ml tube and washed twice with buffer (75% ethanol, 3 M sodium acetate, TE). The resultant DNA pellet was washed thrice with 70% ethanol and air dried until the ethanol evaporated completely. The pellet was re-suspended in 100 µl of TE buffer and 2 µl of RNase added. The tube was incubated at 37°C for 30 min to dissolve the genomic DNA. The quality of the gDNA was checked on 1% (w/v) agarose gels and the DNA concentrations estimated using a Nanodrop 8000 spectrophotometer (Nanodrop Technologies, Wilmington, Delaware, USA).

Amplification of DNA with ISSR primers

A total of 8 ISSR primers were used for the Polymerase Chain Reaction (PCR) procedure. The PCR was performed with a final reaction volume of 25 µl in 200 µl capacity thin wall PCR tube in a Eppendorf Thermal Cycler (Eppendorf, USA). Composition of each reaction mixture for PCR was 2.0 µl (80 ng) of gDNA, 1.0 µl of primer (10 pmol/ml), 12.5 µl of 2× PCR Master mix (Xcelris Genomics Labs, Ahmedabad, India) and 9.5 µl nuclease free water. The PCR tubes containing the mixture were spun briefly at 8,000 rpm and placed in the thermal cycler. The PCR conditions began with the initial denaturation at 94°C for 2 minutes followed by 35 touchdown decrement cycles. The final denaturation was at 94°C for 30 seconds, appropriate annealing temperature for each of the primers for 50 seconds. The extension was carried out at 72°C for 90 seconds and ended with an elongation stage at 72°C for 10 minutes. The final hold for the PCR procedure was at 4°C for 10 minutes. The PCR products were visualized to confirm the targeted PCR amplification by mixing 5 µl of PCR product from each tube with 1 µl of 6× gel loading dye and were electrophoresed on 1.5% (w/v) agarose gel containing ethidium bromide (1 percent solution of 10 µl/100 ml) at constant 5V/cm for 40 min in 1× TAE buffer. The amplified product was visualized as a single compact band of expected size under UV light and documented by gel documentation system (Biorad, USA; Gel Doc. EZ Imager).

Data analysis

Clear and distinct bands amplified by ISSR primers were scored for presence and absence of the corresponding band among the accessions, the scores '1' and '0' indicates the presence or absence of bands respectively. The data were entered into a binary matrix

Table 2: Amplification information of eight ISSR markers used in the diversity study of accessions of pearl millet (*Pennisetum glaucum*) and napier grass (*P. purpureum*).

SN	Marker Code	Sequence (5'---3')	TNA	TNL	NML	NPL	P (%)
1	HB10	GAGAGAGAGAGACC	51	6.00	3.00	3.00	50.00
2	844A	CTCTCTCTCTCTCTCTAC	51	7.00	3.00	4.00	57.14
3	17899A	CACACACACACAAG	56	5.00	2.00	3.00	60.00
4	ISSR17	CAGCACACACACACACACA	37	5.00	2.00	3.00	60.00
5	HB11	GTGTGTGTGTGTCC	47	6.00	1.00	5.00	83.33
6	844B	CTCTCTCTCTCTCTCTGC	52	6.00	4.00	2.00	33.33
7	HB14	CTCCTCCTCGC	59	7.00	3.00	4.00	57.14
8	HB8	GAGAGAGAGAGAGG	57	6.00	3.00	3.00	50.00

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

Table 3. Principal coordinates of ISSR loci and alleles data for accessions of pearl millet and napier grass.

Principal Coordinates	Percentage of variation	
	Individual (%)	Cumulative (%)
Axis 1	32.40	32.40
Axis 2	26.30	58.70
Axis 3	12.02	70.20

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 and dendogram was constructed using MEGA 4.0 software. Relationships between the genotypes were graphically represented in form of dendogram, neighbour joining and phylogram.

RESULTS

The concentrations of gDNA extracted from the 12 accessions of pearl millet and napier grass, ranged from 123.8-199.2 ng/µl and the OD (Optical Density) 260/280 ranged from 1.4-1.9. The results of electrophoresis of the amplification of eight ISSR primers used for the present study as resolved on 1.5% (w/v) agarose gel is presented in Figure 1. A total of 48 loci were generated by the primers with the twelve accessions having a cumulative amplification of 410 alleles. Out of the 48 loci amplified, 27 (56.25%) were polymorphic while 21 (43.75%) were monomorphic (Table 2). The allelic richness ranged from 2 -7 alleles per locus with a mean of 4.28, which indicates allelic variation in a locus. Primers 844A and HB14 produced the maximum amplification of 7 loci, while maximum of 5 loci were detected by primers 17899A and ISSR17, respectively. The loci amplification, the total number of alleles, number of monomorphic and polymorphic alleles as well as the percentage polymorphism expressed by each of the primers is presented in Table 2. The allelic amplification frequency of the eight ISSR primer is shown in Figure 2. The frequency of null allele was high for primers ISSR7 and 844B.

The principal coordinate (PCo) analysis of the loci and allelic data showed that three coordinates were important and accounted for 70.72% of the genetic variation (Table 3). The PCoA plots of PCoA1 against PCoA2 showed the accessions of napier grass occupied quadrants I and II, the natural napier grass accessions Omuo green and purple were in quadrant II while COM-CO-3 was located alone in quadrant I (Figure 3A). Quadrant III and IV had accessions of millet, but mostly in quadrant III and fewer (3 accession) in the fourth quadrant. However, in the plot of PCoA2 versus PCoA3, Napier grass Omuo green and Omuo purple occurred in the same quadrant (I) with pearl millet;

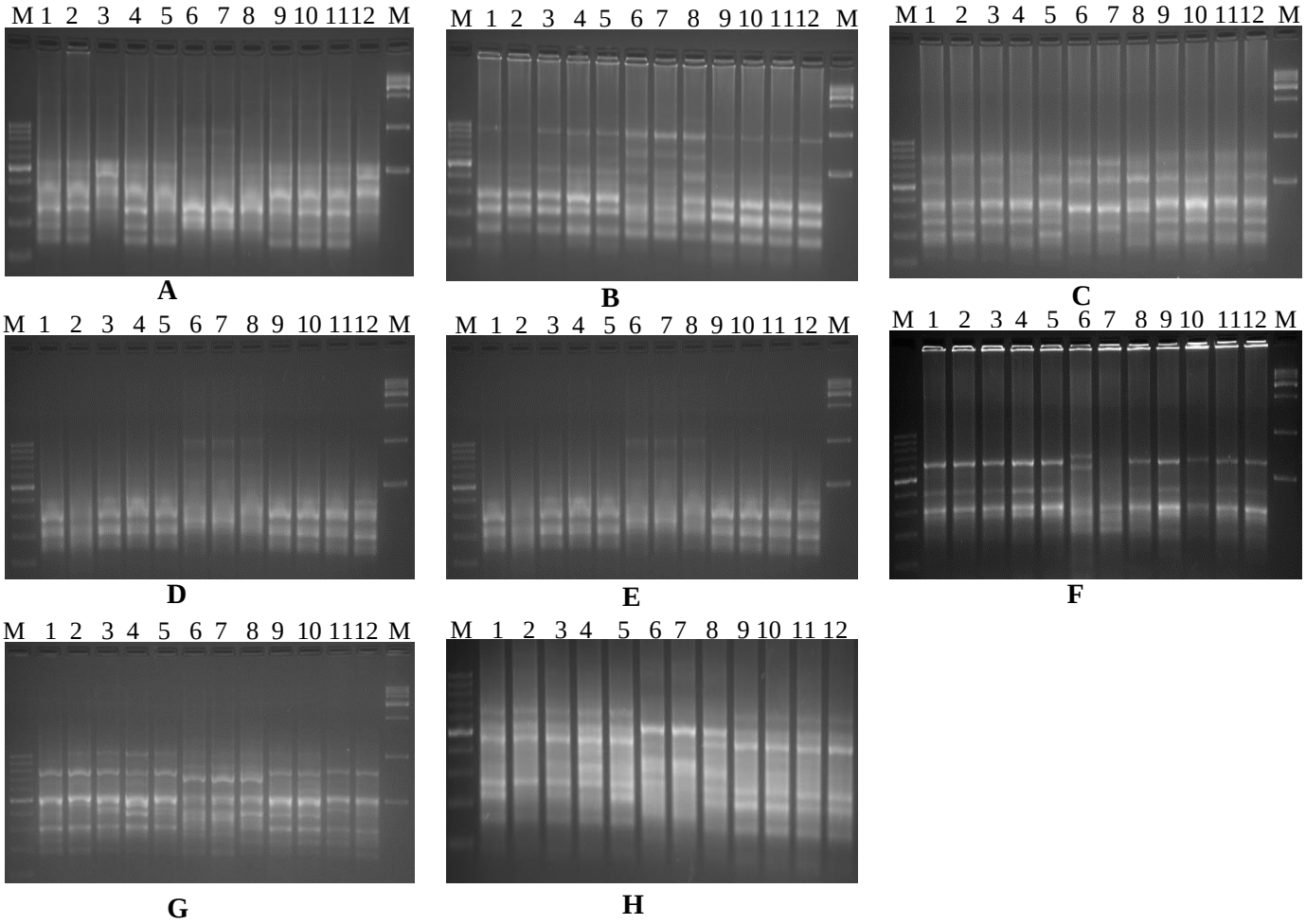

Figure 1. A-H: Amplification of eight ISSR primers used for the diversity study of 12 accessions of pearl millet and napier grass as resolved on 1.5% agarose gel.
Key: **A:** HB10, **B:** 844A, **C:** 17899A, **D:** ISSR17, **E:** HB11, **F:** 844B, **G:** HB14, **H:** HB8; M = 100Kb ladder, 1 = IP3616, 2 = NGB00463, 3 = BALKUVE, 4 = NGB00616, 5 = IP4133, 6 = OMUO-GREEN, 7 = OMUO-PURPLE, 8 = COM-CO-3, 9 = NGB00476, 10 = JALGONE, 11= IP3122, 12 = NGB00537.

IP4133, NGB00616 and BALKUVE. Quadrant II consisted of two ICRISAT and a NACGRAB accession. Only COM-CO-3 occupied quadrant III while two accessions of each from ICRISAT and NIGERIA were found in quadrant IV (Figure 3B).

The genetic similarity among the twelve accessions of pearl millet and napier grass was scaled between 0.15 and 1.0. The UPGMA dendogram as defined by the eight ISSR markers revealed two major groups (Figure 4A).The first group (Group A) consisted of two accessions of napier grass (Omuo green and Omuo purple)which had similarity index above 90%.The second group (Group B) includedan accession of napier grass; COM-CO-3 and all accessions of pearl millet. Group B was further sub-divided into subgroups (B1 and B2). The napier grass COM-CO-3 solely formed a cluster B2 separated from the pearl millet. While accessions of pearl millet fell into other subgroups BI(i) and BI(ii) (Figure 4A). The pearl millet accessions were grouped in three distinct clusters, the largest cluster (BI(ii)) consisting of four accessions (NGB00476, JALGONE IP3122 and NGB00537), second cluster; BI(i) made of three members (BALKUVE NGB00616, IP4133) and the third cluster consisted of two accessions (IP3616 and NGB00463). Accessions IP3122 and NGB00537; NGB00616 and IP4133; NGB00463 and IP3616 were similar at 98, 96 and 92% similarity scales, respectively. The neighbor joining (Figure 4B**)** showed that COM-CO-3 was a napier grass and a neighbour to the pearl millet accession

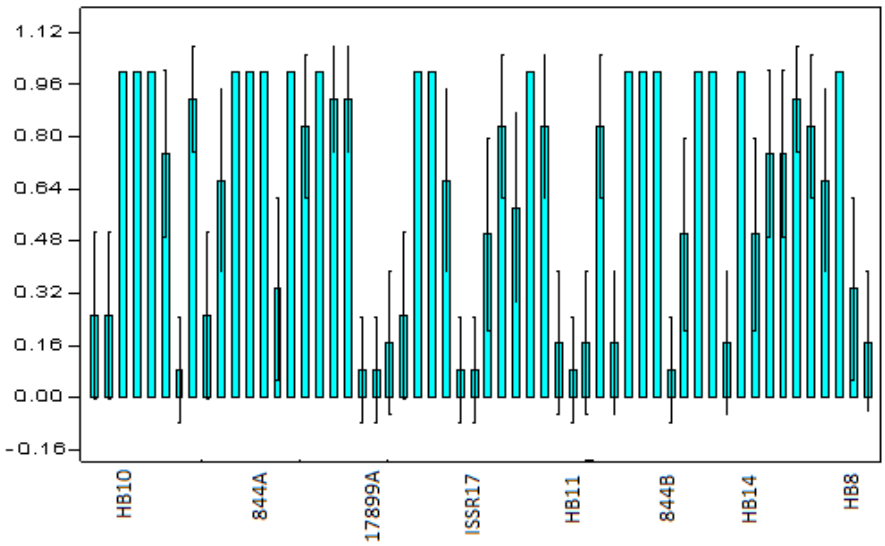

Figure 2. Allelic amplification frequencies of ISSR primers on twelve accessions of pearl millet and napier grass. Key: Y-axis: the allelic frequency; X-axis: the ISSR markers used for the diversity study.

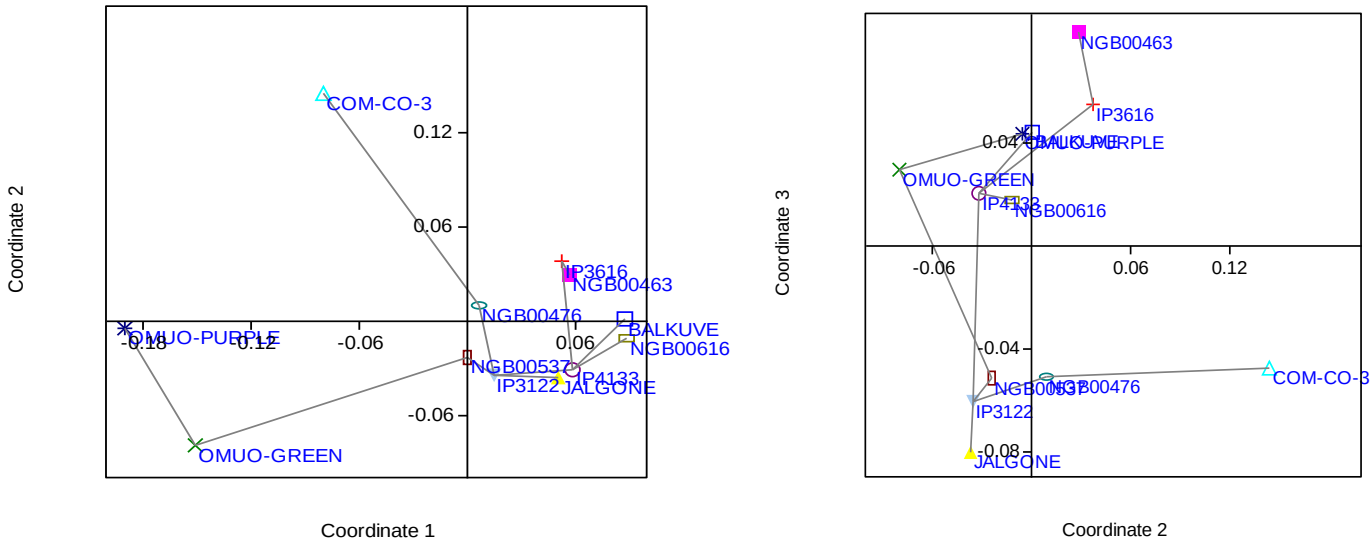

Figure 3. A: Principal coordinates Axis 1 versus PCoA2 of ISSR allelic data for twelve accessions of pearl millet and napier grass; **B:** PCoA2 versus PCoA3 of ISSR allelic data for twelve accessions of pearl millet and napier grass.

NGB00476. In addition, some ICRISAT and NACGRAB accessions were joined together as neighbours. For instance, IP3122 (ICRISAT) was the closest neighbour to NGB00537 (NACGRAB). Also, JALGONE (a natural accession obtained from farmers farm in India) was a close neighbour to Nigerian (NACGRAB) NGB00476. Based on Wanger-Phylogram analysis, all napier grass accessions were on the same branch of the tree alongside pearl millet accession NGB00476 (Figure 5). However, the phylogenic distance among the napier grass was shorter than millet. Similarly, JALGONE, NGB00537 and

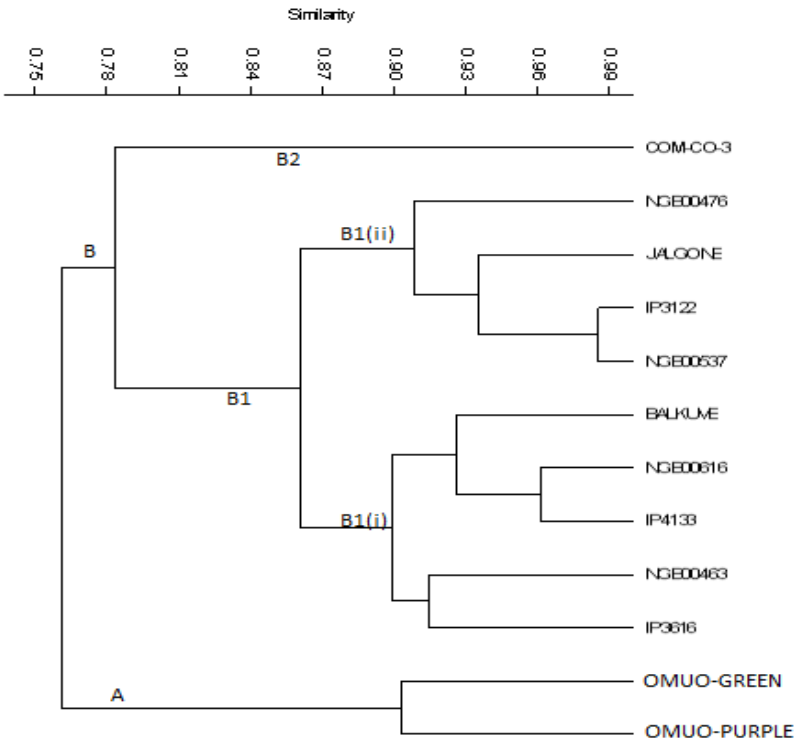

Figure 4 A. Genetic relatedness of the accession of napier grass and pearl millet based on UPGMA cluster analysis.

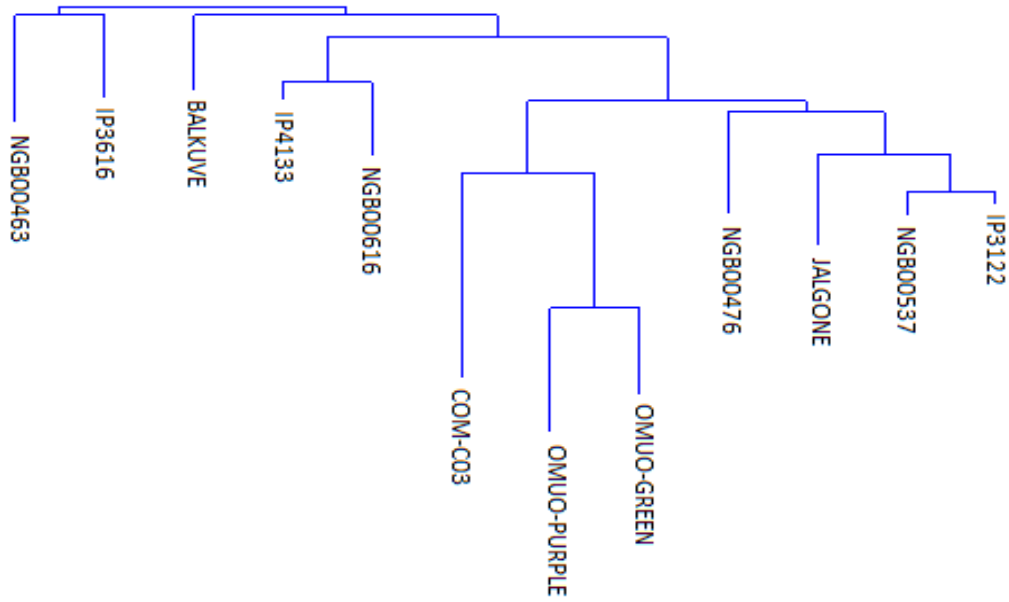

Figure 4 B. Neighbor joining diagram of accessions of pearl millet and napier grass based on ISSR marker analysis.

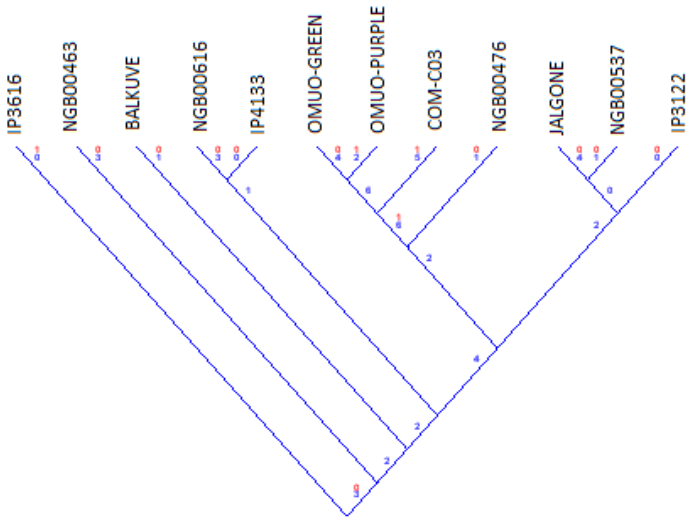

Figure 5. Wanger-Phylogram of the accession of pearl millet and napier grass based on ISSR maker.

IP3122 were on the same branch of the tree. Accessions BALKUVE, NGB00463 and IP3616 maintained separate branches on the tress.

DISCUSSION

The present study revealed genetic diversity and relatedness among the accessions of pearl millet and napier grass collected from Nigeria and India using ISSR marker. Inter and intra- specific genetic variability assessment of germplasm is of interest in conservation of genetic resources and prediction of combining ability of breeding materials. The PCR amplification of DNA depends on the quality of the gDNA among other factors, DNA quality is usually measured by Optical Density (OD) or Absorbance (A) reading at 260 nm and 280 nm. A ratio of OD_{260}/OD_{280} between 1.8 and 2.0 implies good DNA quality. Values below this range indicate protein presence while values above this range reveal RNA contamination. The OD values obtained in this study ranged from 1.4-1.9. Although genomic DNA extracted from four of the samples had OD values less than 1.8, nevertheless, good results were obtained from ISSR analysis due to the ability of the PCR technique to amplify DNA with relatively small impurity. Successful use of genomic DNA with OD values range 1.02-1.4 for SSR genetic analysis was reported by Otoo *et al*. (2009). In addition, Scotti *et al.* (1999) remarked that impure genomic DNA with low OD values could give a good PCR product, however, this statement is relative and did not distinctively elucidate the degree of impurity which PCR technique could accommodate.

A total of 48 loci were detected by eight ISSR primers with 27 (56.25%) polymorphic loci and mean allelic richness of 4.28 showed the markers are effective for napier grass and pearl millet diversity study, while comparing the allelic loci divergence among the genus *Pennisetum*, Oumar *et al.* (2008) observed significantly lower numbers of alleles and lower gene diversity in cultivated pearl millet accessions than in wild accessions using SSRs. Ajibade *et al.* (2000), generated ISSR fragments ranging from 4 to 12 markers in *Vigna* and distribution of 8 markers in *Phaseolus vulgaris* was reported by Galvan *et al.* (2003). In the present study**,** out of 48 loci identified, 5 were found in napier grass alone, 12 occurred in pearl millet and 31 in both pearl millet and napier grass. This is at variance with the report of Azevedo *et al.* (2012) who reported amplification of more loci in napier grass than in millet or both combined. Thus, the accessions showed considerable heterologous amplification of the alleles where about 43% were monomorphic and over 56% were polymorphic.

A higher number of alleles and high polymorphism are very important for correct estimation of genetic diversity of a germplasm. Microsattelite profile in diversity studies are commonly interpreted in terms of allele phenotypes (Esslink *et al.*, 2004), and the degree of polymorphism showed the extent of diversity and effectiveness of the markers (Pfeifer *et al.*, 2011). Consequently, polymorphic information is related to expected heterozyosity and is usually determined from allele frequency. Though, all primers used were polymorphic, ability of primers 844A and HB14 to

produce higher allele frequencies and polymorphic loci in this study indicated that the two primers are most informative and suitable for diversity study in accessions of pearl millet and napier grass. Marker effectiveness for assessment of genetic diversity and validation of hybrids between napier grass and pearl millet have been demonstrated previously (Dowling *et al*., 2014).

The principal coordinates analysis revealed three important axis for the observed variation. The occurrence of napier grass COM-CO-3 solely in one quadrant but with link to other napier grass accessions showed their relatedness. Omuo-green and Omuo purple were closely related and linked to pearl millet accessions (NGB000476) and napier grass COM-CO-3. This suggests that COM-CO-3 is likely a hybrid or having a common origin with Omuo-green and NGB00476. Successful hybridization between pearl millet and napier grass has earlier been reported (Jauhar, 1981; Dowling *et al*., 2014). Occurrence and close linkage of JALGONE an accession obtained from farmer's field, with ICRISAT accessions IP4133, IP3122 and NACGRAB's NGB00537 also indicated that IP4133 is possibly an hybrid form Nigerian (NGB00537) and Indian (JALGONE) accessions. Plotting of millet accessions together in a quadrant indicated that they have a common ancestor. This is possible because pearl millet was believed to be originated in the Sahel zone of west Africa and was domesticated in Africa and India more than 3,000 years ago (Allchin, 1969; Brunken, 1977).

The PCoA2 against PCoA3 revealed that NACGRAB accessions are related to ICRISAT accessions. Similar results were obtained by Mignouna *et al.* (2003) who argued that there is no geographical segregation in clustering of yam germplasm collected from eight west African countries. Presence of NACGRAB and ICRISAT accessions on the same plane further gave an insight and supported the West Africa origin of pearl millet. The consistent connection between COM-CO-3, NGB00476 and Omuo green justifies our advancement that COM-CO-3 is a hybrid of Nigerian pearl millet and wild napier grass. Non linkage between Omuo purple and any other accessions except Omuo green suggests that probably they were later evolved from the former.

The result of cluster analysis revealed that napier grass accessions Omuo-green and Omuo-purple are similar at similarity scale above 0.9. The similarity in these two accessions was earlier reported by Morakinyo and Adebola (1991), although their summation was based on morphological and cytogenetic studies, utilization of ISSR in this study agreed with their report. Furthermore, at similarity scale of 0.78, COM-CO-3 was connected to other napier grass as well as pearl millet accessions and the neighbour joining result placed Omuo-green and NGB00476 as immediate neighbours of COM-CO-3. Since both pearl millet and napier grass are predominantly cross-pollinated species due to their protogymous flowering behaviour which results in high heterozygosity, extensive genetic diversity is available for hybridization. In corroboration of this finding, Mariac *et al.* (2006) reported that there is possibility of hybridization of pearl millet with its close relatives including napier grass. Consequently, hybrids have been developed from these two species. It could therefore be deduced from the phylogenic results that COM-CO-3 is a hybrid of Omuo-green and NGB00476 which are both interestingly Nigerian accessions. Likewise, similarity between IP3122 and NGB00537, IP4133 and NGB00616 at above 0.95 scale suggests that ICRISAT accessions originated from Africa. Consequently, they belong to the same branch in the phylogram with short distance length. The occurrence of Nigerian and Indian accessions of pearl millet in close link above 0.97 similarity scale revealed those accessions are genetically similar. Though BALKUVE, NGB000616 and IP4133 are related at similarity scale of 0.9, however, they are distinctively located on separate branches on the phylogram. This implies that accessions may be related based on basic genetic composition, nevertheless, some variations are possible. Local cultivation of BALKUVE (unimproved) by farmers explains its distant relatedness to other India accessions from ICRISAT. The observed relatedness of the napier grass and pearl millet in this study corroborates that napier grass belongs to the secondary gene pool of the pearl millet (Martel *et al.*, 1997).

CONCLUSION

Accurate assessment of genetic diversity is important in crop breeding and adequate genetic information would identify potential parental combinations to create segregating progenies with maximum genetic variability for further selection. Findings from the present study showed that ISSR markers are valuable assets for estimating genetic diversity. This will help in identification of genotypes as potentially sources of alleles for enhancing important characteristics, analyzing the evolutionary and historical development of cultivars at the genomic level in hybridization programmes for *Pennisetum*. The ISSR markers used were polymorphic and able to reveal the diversity among accessions of pearl millet and napier grass from Nigeria and India. The markers distinctively grouped

napier grass together, however, some Nigerian and India millet accessions were clustered together indicating their relatedness and suggesting their common origin. It is recommended that more accessions from diverse sources be used in further finger printing and phylogeny as well as evolutionary studies of pearl millet and napier grass which will be of significant importance in breeding and germplasm conservation.

ACKNOWLEDGMENTS

The first author is thankful to Management of University of Ilorin, Ilorin Nigeria and C. G. Bhakta Institute of Biotechnology, UkaTarsadia University, Bardoli, India for granting Staff Research Exchange to carry out this study.

CONFLICT OF INTERESTS

We declare there are no conflict of interests in whatsoever form between the authors in research design, execution, finance, manuscript preparation and choice of journal.

REFERENCES

Ajibade S. R., Weeden N. F., and Michite S. (2000). Inter simple sequence repeat analysis of genetic relationships in the genus *Vigna*. *Eupytical*, 111: 47-55.

Allchin F. R. (1969). Early cultivated plants in India and Pakistan. In: P. J. Ucko and G. W. Dimbleby (eds). The domestication and exploitation of plants and animals, London. G. Duckworth and Co. 323-329.

Azevedo A. L., Costa P. P., Machado J. C., Machado M. A., Pereira A.V., and da SilavaLedo F. J. (2012). Cross species amplification of *Pennisetum glaucum* Microsatellite markers in *Pennisetum purpureum* and genetic diversity of Napier grass accessions. *Crop Science*, 52: 1776-1785.

Barbosa S., Davide L. C., and Pereira A. V. (2003). Cytogenetics of *Pennisetum purpureum* Schumack × *Pennisetum glaucum* L. Hybrids and their Parents. *Ciência Agrotecnologia*, 27: 26-35.

Bhattacharjee R., Bramel P. J., Hash C. T., Kolesnikova-Allen M. A. and Khairwal I.S. (2002). Assessment of genetic diversity within and between pearl millet landraces. *Theoretical Applied Genetics*, 105: 666-673.

Brunken J.N. (1977). Systematic study of *Pennisetum*-sect - *Pennisetum* (*gramineae*). *American Journal of Botany*, 64: 161-176.

Burkill H.M. (1994). *The useful plants of West Tropical Africa*. Royal Butanic Gardens. Kew, UK p. 636

Cota-Stanchez J.R. Remarchuk K and Ubayasena K. (2006). Ready to use DNA extracted with CTAB method adopted for Herbarium specimensand mucilaginous plant tissue. *Plant Molecular Biology Reports*, 24: 161-167.

Davies O. (1968). The origins of agriculture in West Africa. *Current Anthropology*, 9: 479-482.

Dowling C. D., Burson B. L., and Jessup R. W. (2014). Marker-assisted verification of Kinggrass (*Pennisetum purpureum* Schumch. ×*Pennisetum glaucum* [L.] R. Br.). *Plant Omics Journal*, 7: 72-79

Esselink G. D., Nybom H., and Vosman B. (2004). Assignment of allelic configuration in polyploids suing the MAC-PR (Microsatellite DNA allele counting-peak ratios) method. *Theoretical Applied Genetics*, 109: 402-408.

Farrell G., Simons S. A., and Hillocks R. J. (2002). Pests, diseases and weeds of Napier grass, *Pennisetum purpureum*; a review. *International Journal of Pest Management*, 48: 39-48.

FAO and ICRISAT (1996). *The world Sorghum and millet economics; facts, trends and outlook*. Food and Agricultural Organization of the United Nations: Rome, Italy and International Crops Research Institute for the Semi-Arid Tropics: Patanchera 502 324, Andhra Pradesh, India p. 23-26.

Gale M. D., Devos K. M., Zhu J. H., Allouis S., Couchman M. S., Liu H., Pittaway T. S., Qi X. Q., Kolesnikova-Allen M., and Hash C.T. (2001). New molecular marker technologies for pearl millet improvement. *International Sorghum and Millets Newsletter*, 42: 16-22

Galvan M. Z., Bornet B., Balatti P. A., and Branchard M. (2003). Inter sequence repeat (ISSR) markers as a tool for assessment of both genetic diversity and gene pool origin in common bean (*Phaseolus vulgaris* L.). *Euphytical*, 132: 297-301.

Hanna W. W., Baltensperger D. D., and Seetharam A. (2004). Pearl millet and millets. In: Moser (ed.) *Warm season (C4) grasses*. Agron. Monogr. 45. ASA, CSSA, and SSSA, Madison, W1.

Hash C. T., Bhasker Raj A.G., Lindup S., Sharma A., Beniwal C. R., Folkertsma R.T., Mahalakshmi V., Zerbini E. and Blümmel M. (2003). Opportunities for marker-assisted selection (MAS) to improve the feed quality of crop residues in pearl millet and sorghum. *Field Crops Research*, 84: 79-88.

HiluK.W. (1995). Evolution of finger millet: evidence from random amplified polymorphic DNA. *Genome*, 38: 232-238.

JauharP. P. (1998). *Cytogenetics and breeding of pearl millet and related species*. AR Liss, New York.

Kannan B., Sanapathy S., Raj A. G. B., Chandra S., Muthiah A., Dhanapal A. P., and Hash C. T. (2014). Association analysis of SSR markers with phenology, grain and stover-yield realted traits in pearl millet (*Pennisetum glaucum* (L.) R. Br.). *The Scientific World Journal*. http://dx.doi.org/10.1155/2014/562327

Khairwal I. S., Rai K. N., Diwakar B., Sharma Y. K., Rajpurohit B. S., Nirwan B., and Bhattacharjee R. (2007). *Pearl millet: Crop management and seed production manual*.Patancheru, Andhra Pradesh, India: ICRISAT. 108 pp.

Kumari K., and Pande A. (2010). Study of genetic diversity in finger millet (*Eleusinecoracana*L. Gaertn) using RAPD markers. *African Journal of Biotechnology*, 9: 4542-4549.

Langeland K. A., Ferrell J. A., Sellers B., MacDonald G. E., and Stocker R. K. (2008). *Identification and biology of non-native plants in Florida's natural areas*. IFAS. Sp.

Island Press, Washington, DC. pp 21-37

Liu C. J., Witcombe J. R., Pittaway T. S., Nash M., Hash C. T., Busso C. S., and Gale M. D. (1994). An RFLP-based genetic map of pearl millet (*Pennisetum glaucum*). *Theoretical Applied Genetics*, 89*:* 481-487.

Mariac C., Luong V., Kapran I., Mamadou A., Sagnard M., Deu M., Chantereau J., Gerard B., Ndjeunga J., Bezancon G., Pham J., and Vigouroux Y. (2006). Diversity of wild and cultivated pearl millet accessions (*Pennisetum glaucum* [L.] R. Br.) in Niger assessed by microsatellite markers. *Theoretical Applied Genetics*, 114: 49-58.

Martel E., De Nay D., Siljak-Yakovlev S., Brown S., and Sarr A. (1997). Genome size variation of basic chromosome number in pearl millet and fourteen related Pennisetum species. *The Journal of Heredity*, 88: 139-143.

Mignouna H. D., Abang M. M., and Fagbemi S. A. (2003). A comparative assessment of molecular markers assay (AFLP, RAPD and SSR) for white yam (*Dioscorearotundata*) germplasm characterization. *Annals of Applied Biology*, 142: 269-276.

Morakinyo J. A., and Adebola P. O. (1991). Karyotype analysis and meiotic chromosome behaviour in *Pennisetum americanum, P. purpureum and P. pedicellatum*. *Nigerian Journal of Botany*, 4: 127-134

Nybom H. (1990). Application of DNA fingerprinting in plant breeding. *Proceedings of International DNA Fingerprinting Sympossium.* Bern. pp 32-45

Ogunkanmi L. A.,Oboh B., Williams O., Monu G., and Ogundipe T. O. (2009). Phylogenetic and genomic relationships in the genus Malus based on RAPD'S. *Journal of Biotechnology*, 8: 3387-3391

Otoo E., Akromah R., Kolesnikova-Allen M., and Asiedu R. (2009). Delineation of pona complex of yam in Ghana using SSR markers. *International Journal of Genetics and Molecular Biology*, 1: 006-016

Oumar I., Mariac C., Pham J. L., and Vigouroux Y. (2008). Phylogeny and origin of pearl millet (Pennisetum glaucum [L.] R. Br) as revealed by microsatellite loci. *Theoretical Applied Genetics*, 117: 489- 497.

Pfeiffer T., Roschanski A. M., PannellKorbecka G., and Schnitter M. (2011). Characterization of microsatellite loci and reliable genotyping in a polyploidy plant, *Mercurialisperennis*(Euphorbiaceae). *The Journal of Heredity*, 102: 479-488.

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. *Hered*, 81: 648-658.

Rai K. N., Gupta S. K., Bhattacharjee R., Kulkarni V. N. M., Singh A. K., and Rao A. S. (2009). Morphological characteristics of ICRISAT-bred pearl millet hybrid seed parent. *Journal of SAT Agriculture Research*, 7: 56-71

Scotti I., Paglia G., Magni F., and Morgante M. (1999). Microsatellite markers as a tool for detection of intra- and interpopulational genetic structure. In: E. D. Gillet (ed.) *Which DNA marker for which purpose? Final Compendium of the Research Project Development, Oprtimization and validation of molecular tools for assessment of biodiversity in forest trees in the European Union DGXII Biotechnology*. FW IV Research Programme Molecular Tools for Biodiversity. http://webdoc.sub.gwdg.de/ebook/1999/whichmarker/index.htm

Semagn K., Bjornstad A., and Ndjiondjop M. N. (2006). An overview of molecular marker methods for plants. *African Journal of Biotechnologh*, 5: 2540-2568.

Skerman P. J., and Rivers F. (1990). *Tropical grasses:* FAO plant production and protection series 23. Food and Agriculture Organization of the United Nations. Rome 832 p.

Stich B., Haussmann B. I. G., Pasam R., Bhosale S., Hash C. T., Melchinger E., and Parzies H. K. (2010). Patterns of molecular and phenotypic diversity in Pearl millet (*Pennisetum glaucum* (L.) R. Br.) from West and Central Africa and their relation to geographical and environmental parameters.*BMC Plant Biology*,10: 216

SupriyaS. S., Nepolean T, Hash C. T., Rajaram V., Eshwar K., Sharma R., Thakur R. P., Pandurangarao V., and Yadav R. C. (2010). Identification of quantitative trait loci associated with rust resistance in pearl millet using DArT and SSR based linkage map. National Symposium on Genomics and Crop Improvement: Relevance and Conservations, February 25-27, 2010, Acharya N.G. Ranga Agricultural University, Rajendranagar, Hyderabad.

Tchamba M. N., and Seme P. M. (1993). Diet and feeding behaviour of the forest elephant in the Santchon Reserve, Cameroon. *African Journal of Ecology* 31: 165-171

Techio V. H., Davide L. C., and Pereira A. V. (2006). Meiosis in elephant grass (*Pennisetum purpureum*) (Poaceae, Poales) and their interspecific hybrids. *Genetics and Molecular Biology*, 29: *415-423*

Varshney R. K., Hoisington D. A., and Tyagi A. K. (2006). Advances in cereal genomics and applications in crop breeding. *Trends in Biotechnology*, 24: 490-499.

vom Brocke K., Christinck A., Weltzien E. R., Presterl T. and Geiger H. H. (2003). Farmer's seed systems and management practices determine pearl millet genetic diversity patterns in semi-arid regions of India. *Crop Science*, 43: 1680-1689.