Journal of Underutilized Legumes
Vol. 1 (1): 20 - 33

Society for Underutilized Legumes
https://sulegumes.org/
e-ISSN: 2705-3776

Genetic Diversity of some African Yam Bean Accessions in Ebonyi State Assessed using Inter-Simple Sequence Repeat (ISSR) markers.

[1]Nnamani CV, [2]Afiukwa CA,[3]Oselebe HO, [2]Igwe DO, [1]Uhuo CA, [2]Idika KO, [1]Ezigbo E, Oketa CN, [3]Nwankwo VO, [1]Ukwueze CK and [1]Nwaojiji CO

[1]Department of Applied Biology, Ebonyi State University, Abakaliki, Nigeria.
[2]Department of Biotechnology, Ebonyi State University, Abakaliki, Nigeria.
[3]Department of Crop Science and Land Scape management, Ebonyi State University, Abakaliki, Nigeria.

E-mail: afiukwa@yahoo.com

Received November 25, 2018
Accepted for publication June 21, 2019
Published July 7, 2019

Abstract

The genetic variability of 17 accessions of African yam bean [*Sphenostylis stenocarpa* (Hochst ex. A. Rich) Harms] collected from eight different local government areas of Ebonyi State, Nigeria, was evaluated using Inter-Simple Sequence Repeat (ISSR) markers. A total of 18 primers were used to access the degree of polymorphism in the ISSR loci out of which 14 primers produced clear amplification bands that were used for the diversity analysis. The 14 markers amplified a total of 107 alleles ranging from 3 in ISSR 825 to 11 in ISSR 811and ISSR 826 with a mean of 8.27 alleles per primer. The polymorphic information content (PIC) values ranged from 0.38 to 0.88 with a mean value of 0.74. Twelve (12) out of the 14 ISSR primers (including ISSRs 811, 901, 835, 814, 818, 889, 826, 816, 890, 858, 827 and 825) demonstrated high potentials to discriminate among the African yam bean accessions by yielding PIC values as high as 0.66 to 0.88. A dendrogram of the ISSR data by Unweighted Pair Group Mean with Arithmetic (UPGMA) procedure clustered the 17 accessions into four major groups showing the genetic relatedness of the accessions and germplasm migrations among the producing LGAs of the State. The clustering pattern indicates that accessions from Abakaliki and Afikpo North LGAs of the State are more closely related, and so also are accessions from Ikwo and Afikpo South. The study revealed wide variation among the African yam bean accessions in Ebonyi State indicating ample opportunity for genetic improvement of the species.

Keywords: African yam bean, underutilized crop, genetic diversity, ISSR marker, Ebonyi State.

Introduction

African Yam Bean (*Sphenostylis stenocarpa ex*. A. Rich Harms) is a neglected and underutilized leguminous food crop belonging to the Fabaceae Family, sub Family Papilionoideae, tribe Phaseoleae and sub-tribe Phaseolinae (Azeke *et al.,* 2005; Moyib *et al.,* 2008) and is the most economically important species in the genus *Sphenostylis* (Machuka, 2001). It was formally grouped with the leguminous taxa within the genus *Dolichos* and *Vigna*, but later found to be the closest relation of genus *Nesphostylis* based on sheared morphological features (Milne-Redhead and Polhill, 1971; Potter and Doyle, 1994). It is an important grain legume in tropical Africa especially in Nigeria, Ivory Coast, Ghana, Togo, Gabon, Congo, Ethiopia and some parts of East Africa, where it is used as food or food components (Olasoji *et al.,* 2011). The crop produces both aerial beans in pods and underground tuber, a reason it is called a “yam bean”. Nigeria is very significant in the production of this crop, where extensive cultivation had been reported in the Eastern, Western and Southern parts of the country (Saka *et al.,* 2004).

African yam bean (AYB) has immense nutritional values derived from both the seed and tuber. It has been found to contain about twice the protein content of other African root crops such as yam and sweet potato and has almost ten times the protein value of cassava with the quality of its protein very similar to that of soybeans **(**Norman and Cunningham, 2006). Total protein content up to 28%, total carbohydrates (78%), fat (2.5%), ash (2.8%) and crude fibre (12%) have been reported in the raw seeds of African yam bean (Kay, 1987; Edem *et al*., 1990; Ameh, 2007), while Duke (1981) reported about 0.6% protein, 85.3% total carbohydrates, 1.1% fibre and 2.2% ash in the root. The amino acid (lysine and methionine) values in AYB seeds are found to be higher than those of pigeon pea, cowpea, and bambara groundnut (Uguru and Madukaife, 2001), while the amino acid profile were comparable with that of whole chicken egg and can meet the daily requirement for protein according to Food and Agriculture Organization (FAO) and World Health Organization (WHO) (Ekpo, 2006). Saxon (1981) asserted that AYB is the most nutritionally rich out of the seventeen (17) tuberous legumes of international significance. The crop is cultivated for both the tuber that looks like elongated sweet potato but tastes more like Irish potato, and good yields of edible seeds above ground which are highly proteinous (Moyib *et al*,. 2008).

This important food crop is rapidly going into extinction with declining yields perhaps owing to changing climate as one major factor. The adverse impact of climate change on crop yields requires that species with capacity to yield satisfactorily under unfavourable climate be developed. However, adequate understanding of genetic diversity and relatedness among varieties in a species is fundamental to successful genetic improvement (Perera, 2000; Zeng *et al*., 2004). There is therefore a need to properly characterize and identify this important food resource to allow development of appropriate breeding programme for improving the crop for adaptation to the changing climate.

Nevertheless, the conventional identification and selection techniques in the area based on seed coat features (colour and texture) and other phenotypic characteristics would not be efficient for proper selection of parents for successful improvement of the existing accessions. Effective genetic improvement relies on crosses between genetically diverse genotypes (Perera, 2000). Phenotypic trait expression is largely dependent on environmental conditions (Blum, 1988). So, choice based on phenotypic traits alone would not be efficient for selecting suitable divergent genotypes for meaningful breeding (Adewale *et al.,* 2010). Molecular markers have proven to be highly effective tools for revealing variations within and among species (Matin *et al.,* 2012; Causse *et al.,* 1994).

Molecular markers are inheritable and detected DNA sequences at specific locations of the genome that can be used to identify specific genotypes (Ayres *et al.,* 1997; Senior *et al.,* 1998; Gurta *et al.,* 1999; Kelly *et al.,* 2003). There are various types of molecular markers such as Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Single Nucleotid Polymorphism (SNP), Directed Amplified Minisatellite DNA (DAMD) and Start Codon Targeted (SCoT) markers (Botstein *et al*., 1980; Heath *et al.* 1993; Amadou *et al*., 2001; Somers and Demmon, 2002; Ntundu *et al.,* 2003; Collard and Mackill, 2009; Ho *et al.,* 2017) but simple sequence repeats (SSRs) are the most frequently used in genetic diversity studies owing to its high efficiency and cost effectiveness (Matin *et al*., 2012). SSR makers and its variant Inter-Simple Sequence Repeat (ISSR) markers stand out in genotypic discrimination (high degree of polymorphism), co-dominance, reproducibility, abundance and wide distribution in the genome (Panaud *et al*., 1996; McCouch *et al*., 1997; Temnykh *et al*., 2000; Ni *et al.*, 2002) which make it ideal for genetic diversity studies (Cho *et al*., 2000).

Simple sequence repeats also called microsatellites are short DNA sequences, usually 1 - 5 nucleotides long and repeated a variable number of times in tandem (Scribner and Pearce, 2000; Mburu and Hanotte, 2005). The SSR method of assessing genetic variation utilizes the high degree of sequence length variation resulting from certain nucleotides repeats in the genome (Ubi, 2008). Microsatellite markers have shown high levels of polymorphism in many important crops including rice (Chen *et al*., 1997), wheat (Devos *et al*., 1995), barley (Liu *et al*., 1996), maize (Senior *et al*., 1998), sorghum (Brown *et al*., 1996), soybean (Akkaya *et al*., 1992) and beans (Yu *et al*., 1999). Information on the genetic characterization of AYB using molecular markers is scanty. However, Shitta *et al.* (2015) has successfully applied SSR while Ojuederie *et al.* (2014) used AFLP, while Moyib *et al.* (2008) employed RAPD markers to characterize genetic diversity in Afican yam bean.

Inter-Simple Sequence Repeat (ISSR) marker system was first describes by Zietkiewics *et al*. (1994) and Kantety *et al*. (1995). The ISSR analysis involves the PCR amplification of DNA regions between adjacent, inversely oriented microsatellites using a single simple sequence repeat (SSR)-containing primers. The technique can be applied for any species that contains a sufficient number and distribution of SSR motifs and has the advantage of not requiring genomic sequence data for its development (Gupta *et al*., 1994; Goodwin *et al*., 1997). Blair *et al*. (1999) opined that the primers used in ISSR can be based on any di-, tri-, tetra, or penta-nucleotide SSR motifs found at SSR loci, which confers a wide range of possible amplification products on ISSR markers. This marker type does not only generate larger numbers of polymorphisms per primer owing to its ability to target variable regions in the genome, but is found to be more consistent than RAPD (Hantula *et al*., 1996). An ISSR marker produces a number of amplification bands that reflects the relative occurrence of a given SSR motif in the genome and therefore provides an estimate of the SSR abundance in the genome (Blair *et al*., 1999). The ISSR technique has been used to evaluate genetic diversity in maize (Kantety, *et al*., 1995), to successfully study the frequency and level of polymorphism of different SSR loci and fingerprinting in rice and potato (Blair, *et al*., 1999; McGregor *et al*., 2000).

This study was intended to reveal the genetic differences and relatedness among AYB accessions cultivated in Ebonyi State and to associate the result with seed coat features. This is the first report of molecular characterization of this crop species in the South East of Nigeria. The result could be a useful tool for developing suitable breeding program for AYB improvement in the region.

Materials and Methods

Seed collection and seed coat features

A total of seventeen (17) AYB accessions were collected from farmers in eight (8) Local Government Areas (L.G.A) of Ebonyi State for the study. Their source L.G.A. and code numbers are presented in **Table 1**. The code numbers are 6 alphabets with the first 2 letters (EB) indicating Ebonyi State, the next 2 letters reflect the L.G.A. while the last lowercase 2 letters denote the exact community within the L.G.A. where the seeds were collected.

DNA Extraction

Genomic DNA was extracted from approximately 100 mg of the powdered AYB seed endosperm using Zymo Research plant/seed DNA isolation kit (Zymo Research Corporation, USA), following the manufacturer's instructions.

Polymerase Chain Reaction (PCR) and agarose gel electrophoresis

The PCR amplification was carried out in a total reaction volume of 25µL consisting of 100ng of genomic DNA, 2.5µl of 10x Taq Buffer, 1.5µl of 50mM $MgCl_2$, 2.0µl of 2.5mM dNTPs, and 0.2µl 500U *Taq* DNA polymerase (all Bioline), 1.0µl of 10µM of each ISSR primer and made up to 25µl with diethylpyrocarbonate (DEPC)-treated water (Invitrogen Corporation). The list of ISSR primers, their sequences, GC content and annealing temperatures are presented in **Table 2**. The PCR cycling profile comprised of an initial denaturation at 94°C for 5 min., followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55^{o}C, primer extension at 72°C for 1min, and a final extension at 72°C for 10 min. The PCR products (6 µl each) were resolved in a 1.5 % agarose gel containing 0.5 mg/ml ethidium bromide and photographed on Transilluminator UV light (Fotodyne Incorporated, Analyst Express, USA).

Data analyses

The gel results were scored for presence (1) or absence (0) of specific bands or allele to generate binary data matrix of the ISSR markers. The resulting data matrix was used for phylogenetic reconstruction using Unweighted Pair Group Mean with Arithmetic (UPGMA) and analysed for dissimilarity index using DARwin software version 5.0 (Perrier *et al*., 2006). The data matrix was also subjected to Principal component analysis (PCA) using GenAlex 6.41 software (Peakell *et al*., 2006) to cluster the African yam bean accessions according to their genetic similarities. Furthermore, genetic diversity parameters including total number of alleles, allele frequency, major allele frequency (i.e. allele with the highest occurrence), accession-specific alleles, gene diversity and polymorphism information content (PIC) were equality computed using PowerMarker version 3.25 (Liu and Muse, 2005).

Results and Discussion

A total of 14 ISSR primers were used to access the degree of genetic diversity in 17 accessions of African yam bean [*Sphenostylis stenocarp*a (Hochst ex. A. Rich) Harms] out of which 14 primers that produced scorable amplification bands were selected for the analyses (Fig. 1). The 14 markers amplified a total of 107 alleles ranging from 3 in ISSR 825 to 11 in ISSR 811 and ISSR 826 with a mean of 8.27 alleles per primer. The polymorphic information content (PIC) values ranged from 0.38 to 0.88 with a mean value of 0.74. Twelve (12) out of the 14 ISSR primers (including 811, 901, 835, 814, 818, 889, 826, 816, 890, 858, 827 and 825) yielded PIC values as high as between 0.66 to 0.88. The obtained gene diversity values ranged from 0.44 to 0.89 with a mean value of 0.77 while major allele frequency was in the range of 0.18 to 0.71 with a mean value of 0.36 (Table 3). The allele count spanned between 1 and 12 while its frequency ranged from 0.0588 and 0.7059. Of the 14 markers, ISSR 811, ISSR 826, UBC 814 and ISSR 818 showed higher values of diversity indices while ISSR 856 followed by ISSR 888 were the poores**t (**Table 3**).**

A dendrogram of the ISSR data using UPGMA procedure clustered the 17 accessions into four major groups at the genetic distances of 20.00, 29.00, 30.00 and 31.00, respectively (**Fig. 2**). Group I that was resolved at a genetic distance of 31.00 consisted of accessions from Abakaliki and Afikpo North LGAs of the state; Group II (at a distance of 30.00) contained a mixture of accessions from four LGAs (Izzi, Ishielu, Onicha and Ezza South); Group III having a genetic distance of 29.00, comprised of only accessions from Ikwo and Afikpo South; while Group IV at a distance of 20.00 was found composing of accessions from Ishielu and Ikwo.

Principal component analysis of the ISSR data also clustered the 17 accessions into four related groups different from the pattern revealed by the dendrogram. Cluster I had 4 accessions from 3 LGAs (Ishielu, Izzi and Onicha); Cluster II comprised of 3 accessions each from a different LGA (Ezza South, Abakalki and Afikpo North); Cluster III contained 5 accessions from 3 LGAs (Ishielu, Ikwo and Afikpo South) including all accessions from Ikwo; while Cluster IV was made up of 4 accessions all from Afikpo (**Fig. 3)**.

Estimation of the degree of genetic diversity in populations of crop species using molecular markers has become fundamental in plant breeding, identification and conservation of superior genotypes (Saeed *et al*., 2011; Upadhyaya *et al*., 2008). Inter-simple sequence repeat (ISSR) markers are highly reproducible and were found to provide highly polymorphic fingerprints (Zietkiewicz *et al*., 1994; Kojima *et al*., 1998; Bornet and Branchard, 2001). This marker system has been successfully used for the assessment of genetic diversity in corn (Kantety *et al.,* 1995), for cultivar identification in oilseed rape and potatoes (Charters *et al.*, 1996, Bornet *et al.*, 2002), for chromosomal mapping (Kojima *et al*., 1998) and for analysis of linkage to a specific gene (Akagi *et al*., 1996a).

In this study, polymorphic ISSR markers were used to assess the level of genetic divergence among 17 African yam bean (AYB) accessions collected from around Ebonyi State of Nigeria and to identify markers appropriate for differentiating and possible fingerprinting of the accessions. The ISSR markers demonstrated high efficacy for discriminating the African yam bean genotypes, which is important for any successful improvement breeding programme on the species (Kronstad, 1986; Govindaraj *et al.,* 2015; Thakur *et al*., 2016). Polymorphism information (PIC) value denotes the relative informativeness and discriminatory capacity of a marker (Nachimuthu *et al*., 2015). In this study, the values ranged from 0.38 to 0.88 of which as high as 10 of the 14 markers (about 71%) yielded PIC values above 0.70 out of a maximum value of 1.00. The markers UBC 826 (PIC, 0.875) was the most discriminating of the 14 ISSR markers, followed by ISSR 811 (PIC, 0.860) and UBC 814 (PIC, 0.837), while ISSR 856 was the least discriminating (PIC, 0.384) followed by ISSR 888 (PIC, 0.529). The Ebonyi AYB collection appears to have a very broad diversity. Ojuederie *et al.* (2014) had earlier reported higher values of PIC (0.945 to 0.963) from 40 AYB accessions assessed with just 4 primer combinations of Amplified Fragment Length Polymorphism (AFLP) markers. The higher values of PIC reported by these authors may partly be a reflection of the superiority of AFLP markers over SSR markers (Saker *et al.*, 2005). A similar high degree of diversity in AYB was reported by Shitta *et al*. (2015) with PIC values ranging from 0.6691 to 0.7791. With the high capacity of the evaluated markers to amplify polymorphic loci in African yam bean, ISSR markers may be usefully exploited for genetic fingerprinting, gene mapping and development of marker-assisted selection (MAS) technology for improvement and germplasm conservation of species (Shete *et al.,* 2000; Hadi *et al*., 2014).

The wide variation revealed among the African yam bean accessions in Ebonyi State in the present study indicates ample opportunity for genetic improvement of the species (Ojuederie *et al*., 2014). The UPGMA genetic relationship tree clustered the 17 AYB accessions into four distinct groups which did not strictly reflect the geographic origin of the accessions. In the dendrogram, all the accessions from Afikpo North LGA (EBANmg, EBANao, EBANaa and EBANea) and Abakaliki LGA (EBABam and EBABac) appeared more closely related and were cluster together in Group I. It is noteworthy here that none of the accessions from these two LGAs appeared in any other group. Two out of the 3 accessions from Ikwo LGA (EBIKok and EBIKno) were found to be genetically close to the accessions from Afikpo South LGA (EBASoe and EBASem) and were clustered together in Group III, while the remaining one accession from Ikwo (EBIKea) is associated with one of the two accessions from Ishielu LGA (EBISlo) in Group IV. Accessions from Afikpo South were found only in Group III. Group II comprised of accessions from diverse locations (four LGAs). Two of the 5 accessions in this cluster are from Izzi LGA (EBIZib and EBIZwa) while each of the remaining 3 accessions were from Ishielu (EBISla), Onicha (EBONos) and Ezza South (EBESom) suggesting that the accessions in these areas may have a common origin (Rungnoi *et al.*, 2012; Ojuederie *et al.,* 2014). This study indicated that African yam bean may have been introduced several times in Ebonyi from different places. According to the genetic relationship structure in the dendrogram, accessions grown in some Local Government Areas are distinct from those cultivated in other LGAs of the same State. The study did therefore reveal a specific centre of divergence of the species in the state.

Although the Principal Component Analysis revealed somewhat a different pattern of genetic relatedness among the Ebonyi accessions of African yam bean, it also supported that the accessions are highly divergent with no specific centre of divergence within Ebonyi. Therefore, the species may have been introduced in Ebonyi from different places more than one time.

Conclusion

This study revealed wide variation African yam bean accessions cultivated in Ebonyi State of Nigeria and showed that the crop could have been introduced into Ebonyi more than once and from different regions. We report here that ISSR markers are highly efficient in characterizing the genetic differences among AYB accessions and could be exploited for possible germplasm fingerprinting and marker-assisted selection of useful genes in the species.

Acknowledgements

The authors are grateful to the Nigeria's Tertiary Education Trust Fund (TETFund) for funding this research. We are also thankful to the Ebonyi State University Biotechnology Research and Development Centre (EBSU-BRDC) for technical support and provision of the laboratory facilities.

Conflict of Interests

There is no conflict of interest among the authors.

Tables, Figures and Charts

Table 1: List of African Yam Bean Accessions Evaluated for Genetic Diversity, their source L.G.A.

S/N	State	Source Local L.G.A.	Community	Code
1	Ebonyi	Abakaliki	Achinwamgboko	EBABac
2	Ebonyi	Abakaliki	Amachi	EBABam
3	Ebonyi	Afikpo North	Amogu Akpoha	EBANaa
4	Ebonyi	Afikpo North	Akpoha	EBANao
5	Ebonyi	Afikpo North	Ezimba Akpoha	EBANea
6	Ebonyi	Afikpo North	Mgbom	EBANmg
7	Ebonyi	Afikpo South	Edah	EBASem
8	Ebonyi	Afikpo South	Owutu Edah	EBASoe
9	Ebonyi	Ezza South	Onueke	EBESom
10	Ebonyi	Ikwo	Eka-Awoke	EBIKea
11	Ebonyi	Ikwo	Nsuba-Ettam Okpuitumo	EBIKno
12	Ebonyi	Ikwo	Okpuitumo	EBIKok
13	Ebonyi	Ishielu	Labassa	EBISIa
14	Ebonyi	Ishielu	Labassa Okpoto	EBISIo
15	Ebonyi	Izzi	Iboko	EBIZib
16	Ebonyi	Izzi	Waka	EBIZwa
17	Ebonyi	Onicha	Oshiri	EBONos

Table 2: List and Sequences of ISSR Primers used to Study Genetic Diversity of African Yam Bean (AYB) Accessions from Ebonyi State, Nigeria

S/N	Primer name	Primer sequence (5'-3')
1	ISSR811	ACACACACACACACT
2	ISSR901	AGAGAGAGAGAGAGAGYC
3	UBC835	CTCTCTCTCTCTCTCAT
4	UBC814	ACACACACACACACACC
5	ISSR818	ACACACACACACACACG
6	ISSR889	GAGAGAGAGAGAGAGATT
7	UBC826	AGAGAGAGAGAGAGAGC
8	UBC816	GAGAGAGAGAGAGAGC
9	ISSR 890	GAAGAAGAAGAAGAAGAA
10	ISSR 858	CACACACACACACACARY
11	ISSR827	CAACAATGGCTACCACCC
12	ISSR825	ACGACATGGCGACCATCG
13	ISSR856	ACCATGGCTACCACCGAC
14	ISSR 888	ACCATGGCTACCACCGCG

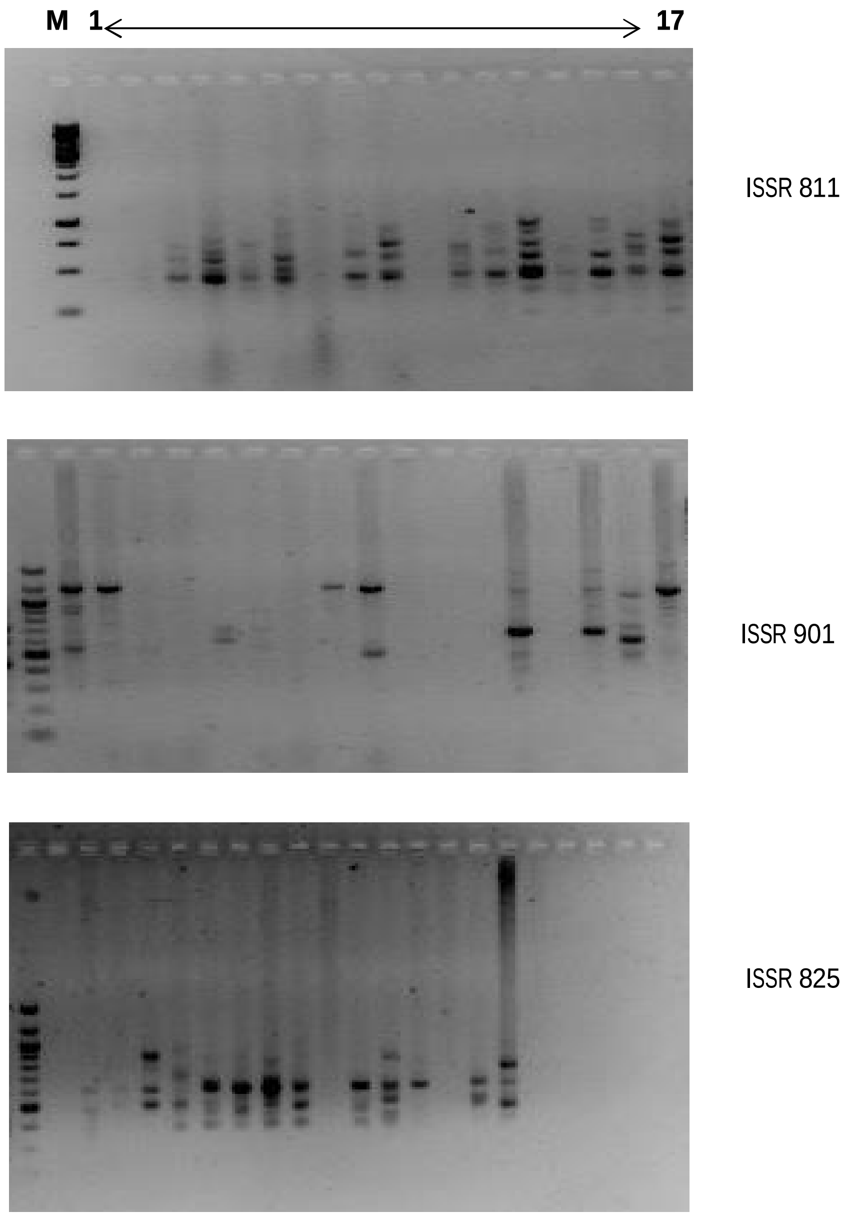

Fig. 1: A representative amplification profiles of African yam bean DNA with ISSR markers. Lane M = 100 bp DNA ladder. 1 to 17 = the 17 African yam bean accessions

Table 3: Genetic diversity parameter values generated with ISSR markers in African yam bean accessions from Ebonyi State

Marker	Major allele frequency	Allele No	Gene diversity	PIC
ISSR811	0.2353	11	0.8720	0.8599
ISSR901	0.5294	9	0.6920	0.6759
UBC835	0.3529	7	0.7958	0.7713
UBC814	0.2941	10	0.8512	0.8372
ISSR818	0.4118	10	0.7889	0.7734
ISSR889	0.2941	9	0.8235	0.8032
UBC826	0.1765	11	0.8858	0.8752
UBC816	0.3529	7	0.7612	0.7275
ISSR890	0.3529	9	0.8097	0.7902
ISSR858	0.3529	6	0.7474	0.7076
ISSR827	0.4706	5	0.6990	0.6595
ISSR825	0.2941	6	0.7958	0.7661
ISSR856	0.7059	3	0.4429	0.3839
ISSR888	0.5882	4	0.5813	0.5290
Total		**107**		
Mean	**0.3647**	**8.27**	**0.7659**	**0.7399**

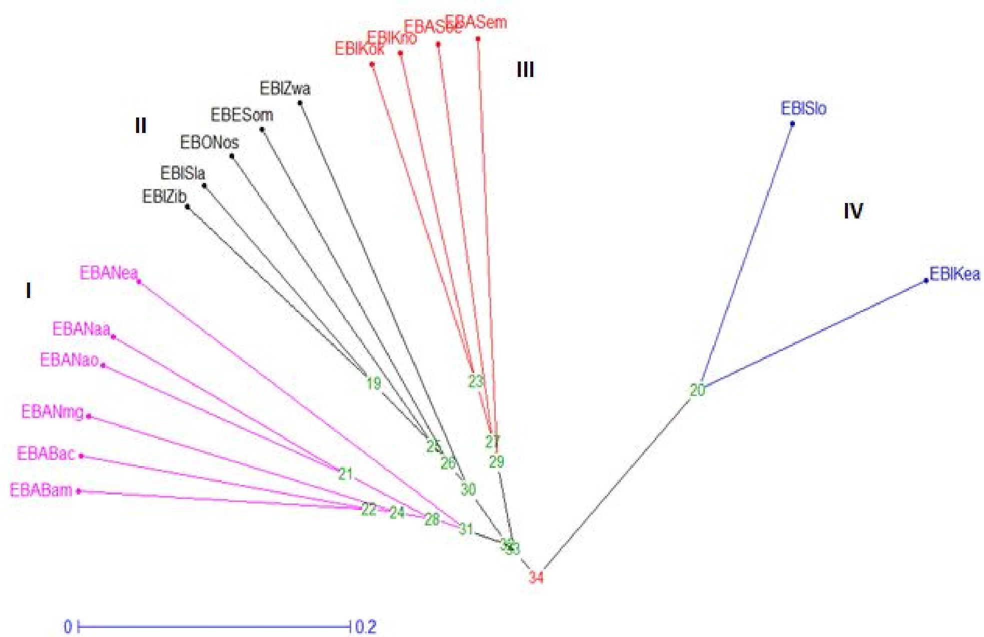

Fig. 2: Dendrogram of African yam bean accessions from Ebonyi State generated with 14 ISSR markers

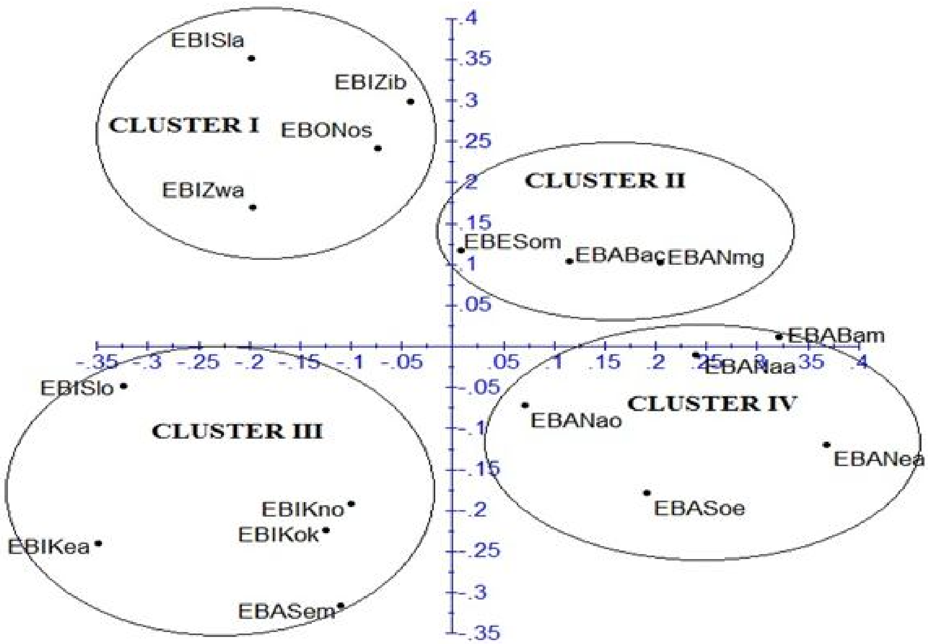

Fig. 3: Principal component analysis of African yam bean accessions from Ebonyi State generated with 14 ISSR markers

References

Adewale B. D. and Odoh N. C. (2013). A Review on Genetic Resources, Diversity and Agronomy of African Yam Bean (*Sphenostylis stenocarpa* (Hochst. Ex A. Rich.) Harms): A Potential Future Food Crop. *Sustainable Agriculture Research* 2(1): 32-43.

Adewale D. (2010). African yam bean: a food security crop? 31 March, 22, 035 View 22 Comment.

Akagi H., Yokozeki Y., Inagaki A., Nakamura A. and Fujimura T. (1996). A co-dominant DNA marker closely linked to the rice nuclear restorer gene, Rf-1, identified with inter-SSR fingerprinting. *Genome* 39: 1205–1209.

Akkaya M.S., Bhagwat A.A. and Cregan P.B. (1992). Length polymorphisms of simple sequence repeat DNA in soybean. *Genetics* 132:1131-1139.

Amadou H.I., Bebeli P.J., Kaltsikes P.J. (2001). Genetic diversity in bambara groundnut (*Vigna subterranean* (L.) Verdc) germplasm revealed by RAPD markers. *Genome* 44: 995-999.

Ameh G. I. and Okezie C. E. A. (2005). Pests and diseases of African yam bean, *Sphenostylis stenocarpa* (Hoechst. ex A. Rich) harms. *Bio-Research* 3:14-20.

Ayres N. M., McClung A. M., Larkin P. D., Bligh H. F. J., Jones C. A. and Park W. D. (1997). Microsatellites and a single-nucleotide polymorphism differentiate apparent amylose classes in an extended pedigree of U.S. rice germ plasm. *Theoretical and Applied Genetics* 94:773-781.

Azeke M. A., Barara F., Han B. P., Wilhelm H. and Thomas B. (2005). Nutritional value of African yam bean (*Sphenostylis stenocarpa*): Improvement by lactic acid fermentation. *Journal of Science and Food Agriculture* 85:963-970.

Blair M.W., Panaud O. and McCouch S.R. (1999). Inter-simple sequence repeat (ISSR) amplification for analysis of microsatellite motif frequency and fingerprinting in rice (*Oryza sativa* L.) *Theoretical and Applied Genetics* 98:780-792.

Blum A. (1988). Genetic and physiological relationships in plant breeding for droughtresistance. *Agriculture and Water Management* 7:195-205.

Bornet B. and Branchard M. (2001). Nonanchored inter-simple sequence repeat (ISSR) markers: reproducible and specific tools for genome fingerprinting. *Plant Molecular Biology Reporter* 19: 209-215.

Bornet B., Muller C., Paulus F. and Branchard M. (2002). High informative nature of inter simple sequence repeat (ISSR) sequences amplified with tri- and tetra-nucleotide primers from cauliflower (*Brassica oleracea* var. botrytis L.) DNA. *Genome* 45: 890-896.

Botstein D., White R.L., Skolnick M.H. and Davis R.W. (1980). Construction of a genetic map in man using restriction fragment length polymorphisms. *American Journal of Human Geneti,* 32:314 – 331.

Brown S.M., Hopkins M.S., Mitchell S.E., Senior M.L., Wang T.Y., Duncan R.R., Gonzalez-Candelas F. and Kresovich S. (1996). Multiple methods for the identification of polymorphic simple sequence repeats (SSRs) in sorghum [*Sorghum bicolor* (L.) Moench]. *Theoretical and Applied Genetics* 93:190-198.

Causse M.A., Fulton T.M., Cho Y.G., Ahn S.N., Chunwongse J., Wu K., Xiao J., Yu Z., Ronald P.C., Harrington S.E., Second G., McCouch S.R. and Tanksley S.D. (1994). Saturated molecular map of the rice genome based on an interspecific backcross population. *Genetics* 138:1251–1274.

Chen X., Temnykh S., Xu Y., Cho Y.G., McCouch S.R. (1997). Development of a microsatellite framework map providing genome-wide coverage in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics* 95:553-567.

Cho Y.G., Ishii T., Temnykh S., Chen X., Lipovich L., Park W.D., Ayres N., Cartinhour S., and McCouch S.R. (2000). Diversity of microsatellites derived from genomic libraries and GenBank sequences in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics* 100:713–722.

Collard B.C.Y and Mackill D. J. (2009). Start codon targeted (SCoT) polymorphism: a simple novel DNA marker technique for generating gene-targeted markers in plants. *Plant Molecular Biology Reports* 27: 86-93.

Devos K.M., Bryan G.J., Collins A.J., Stephenson P. and Gale M.D. (1995). Application of two microsatellite sequences in wheat storage proteins as molecular markers. *Theoretical and Applied Genetics* 90:247-252.

Dukes J. A. (1981). *Handbook of legumes of world economic importance*. Plenum Press, New York. pp. 220-222.

Edem D. O., Amugo C. I. and Eka O. U. (1990). Chemical composition of yam beans (*Sphenostylis stenocarpa*).*Tropical Science* 30:59-63.

Ekpo A. S. (2006). Changes in amino acid composition of african yam beans (*Sphenostylis stenocarpa*) and African locust beans (*Parkia filicoida*) on cooking. *Pakistan Journal of Nutrition* 5:254-256.

Goodwin I.D., Aiktken E.A.B. and Smith L.W. (1997). Application of inter simple sequence repeat (ISSR) markers to plant genetics. *Electrophoresis* 18:1524-1528.

Govindaraj M., Vetriventhan M. Srinivasan M. (2015). Importance of Genetic Diversity Assessment in Crop Plants and Its Recent Advances: An Overview of Its Analytical Perspectives. *Genetics Research International* 2015:1-14.

Gupta M., Chyi Y.S., Romero-Severson J. and Owen J.L. (1994). Amplification of DNA markers from evolutionarily diverse genome using single primers of simple-sequence repeats. *Theoretical and Applied Genetics* 89:998-1006.

Gurta P.K., Varshney R.K., Sharm P.C. and Ramesh B. (1999). Molecular markers and their application in wheat breeding. *Plant Breeding* 118: 369 –390.

Hadi I., Abdullah M., Jaber A. and Yoke C. (2014). Genetic variation of twenty autosomal STR loci and evaluate the importance of these loci for forensic genetic purposes. *African Journal of Biotechnology* 13(11):1210-1218.

Hantula J., Dusabenygasani M. and Hamelin R.C. (1996). Random amplified microsatellites (RAMS) - a novel method for characterizing genetic variation within fungi. European Journal of *Plant Pathology* 26:15-166.

Heath D. D., Iwama G. K. and Devlin R. H. (1993). PCR primed with VNTR core sequences yields species specific patterns and hypervariable probes. *Nucleic Acids Research* 21:5782–5785.

Ho W. K., Chai H. H., Kendabie P., Ahmad N. S., Jani J., Massawe F., Kilian A. and Mayes S. (2017). Integrating genetic maps in bambara groundnut [*Vigna subterranea* (L.) Verdc.] and their syntenic relationships among closely related legumes. *BioMed Central* 18: 192.

Kantety R.V., Zeng X., Bebbetzen J.L. and Zehr B.E. (1995). Assessment of genetic diversity in dent and popcorn (*Zea mays* L.) inbreed lines using inter-simple sequence repeat (ISSR) amplification. *Molecular Breeding* 1:36-373.

Kay D. E. (1987). *Crop and product digest No. 2 – Root Crops*. Tropical Development and Research Institute, London.

Kelly J.D., Gepts P,; Miklas P.N. and Coyne D.P. (2003).Tagging and mapping genes and QTL and molecular marker-assisted selection of traits of economic importance in bean and cowpea. *Field Crops Research* 82:135 - 154.

KojimaT., Nagaoka T., Noda K. and Ogihara Y. (1998). Genetic link-age map of ISSR and RAPD markers in Einkorn wheat in relation to that of RFLP markers. *Theoretical and Applied Genetics* 96: 37–45.

Kronstad W. E. (1986). *Genetic diversity and plant improvement*. New Zealand Agronomy Society Special Publication No. 5, paper 3. DSIR Plant Breeding Symposium, pp 16-20.

Liu K. and Muse S.V. (2005) PowerMarker: an integrated analysis environment for genetic marker analysis. *Bioinformatics* 21(9):2128–2129.

Liu Z.W., Biyashev R.M. and Saghai-Maroof M.A. (1996). Development of simple sequence repeat markers and their integration into a barley linkage map. *Theoretical and Applied Genetics* 93:869-876.

Lu H., Redus M.A., Coburn J.R., Rutger J.N., McCouch S.R. and Tai T.H. (2005). Population structure and breeding patterns of 145 US rice cultivars based on SSR marker analysis. *Crop Science* 45(1):66–76.

Machuka J., and Okeola O. G. (2000). One- and two-dimensional gel electrophoresic identification of African yam bean seed proteins. *Journal of Agriculture and Food Chemistry* 48(6):2296–2299.

Mburu D. and Hanotte O. (2005). *A practical approach to microsatellite genotyping with special reference to livestock population genetics*. *A manual prepared for the IAEA/ILRI training course on molecular characterisation of small ruminant genetic resources of Asia*, ILRI, Nairobi, Kenya, pp 6-12.

McCouch S.R., Chen X., Panaud O., Temnykh S., Xu Y., Cho Y.G., Huang N., Ishii T. and Blair M. (1997). Microsatellite marker development, mapping and applications in rice genetics and breeding. *Plant Molecular Biology* 35(1-2):89–99.

McGregor C.E., Lambert C.A., Greyling M.M., Louw J.H. and Warnich L.A. (2000). Comparative assessment of DNA fingerprinting techniques (RAPD, ISSR, AFLP and SSR) in tetraploid potato (*Solanum tuberosum* L.). *Euphytica* 113:135-144.

Milne-Redhead E. and Polhill R. M. (1971). *Flora of Tropical East Africa.* Millbank, London. pp. 670-674.

Moyib O. K., Gbadegesin M. A., Aina O. O. and Odunola O. A. (2008). Genetic variation within a collection of Nigerian accessions of African yam bean *(Sphenostylis stenocarpa)* revealed by RAPD primers. *African Journal* of *Biotechnology* 7: 1839-1846.

Nachimuthu V.V., Muthurajan R, Duraialaguraja S., Sivakami R., Pandian B. A., Ponniah G., Gunasekaran K., Swaminathan M., Suji K. K. and Sabariappan R. (2015). Analysis of population structure and genetic diversity in rice germplasm using SSR markers: an initiative towards association mapping of agronomic traits in *Oryza sativa*. *Rice* 8:30-54.

Ni J., Colowit P.M. and Mackill D.J. (2002). Evaluation of genetic diversity in rice subspecies using microsatellite markers. *Crop Science* 42(2):601–607.

Norman B. and Cunningham A. (2006). *Lost crops of Africa Vol. II: Vegetables development, security, and Cooperation Policy and Global Affairs.* National Academics Press, Washington, D.C., p. 354.

Ntundu W. H., Bach I. C., Christiansen J. L. and Andersen S. B. (2003). Analysis of genetic diversity in bambara groundnut [*Vigna subterranea* (L.) Verdc] landraces using amplified fragment length polymorphism (AFLP) markers. *African Journal of Biotechnology* 3 (4): 220-225.

Ojuederie B. O., Igwe D. O., Okuofu S. I. and Faloye B. (2013). Assessment of genetic diversity in some *Moringa oleifera* Lam. landraces from Western Nigeria using RAPD markers. *African Journal of Plant Science and Biotechnology* 7 (1): 15-20.

Ojuederie O. B., Balogun M. O., Fawole I., Igwe D. O. and Olowolafe M. O. (2014). Assessment of genetic diversity of African yam bean (*Sphenostylis stenocarpa* Hochst ex. A Rich Harms) accessions using amplified fragment length polymorphism (AFLP) markers. *African Journal of Biotechnology* 13(8): 850 – 858.

Olasoji J.O., Akande S. R. and Owolade O. F. (2011). Genetic variability in seed quality of African yam beans (*Sphenostylis stenocarpa* Hochst. Ex. A. Rich Harms). *African Journal of Agricultural Research* 27:5848-5853.

Panaud O., Chen X., and McCouch S.R. (1996). Development of microsatellite markers and characterization of simple sequence length polymorphism (SSLP) in rice (*Oryza sativa* L.). *Molecular and General Genetics* 252:597–607.

Peakall R. and Smouse P.E. (2012). GenAlEx 6.5: genetic analysis in excel. Population genetic software for teaching and research - an update. *Bioinformatics* 28(19):2537–2539.

Perera L., Russell J.R., Provan J. and Powell W. (2000). Use of microsatellite DNAmarkers to investigate the level of genetic diversity and population genetic structure of coconut (*Cocos nucifera* L.). *Genome* 43: 15–21.

Perrier X. and Jacquemoud-Collet J.P. (2006). DARwin software. http://darwin.cirad.fr/darwin.

Potter D. and Doyle J. J. (1992). Origin of African yam bean (*Sphenostylis stenocarpa*, Leguminosae): evidence from morphology, isozymes, chloroplast DNA and linguistics. *Economic Botany* 46:276-292.

Rungnoi O., Suwanprasert J., Somta P. and Srinives P. (2012). Molecular genetic diversity of bambara groundnut (*Vigna subterranea* L. Verdc.) revealed by RAPD and ISSR marker analysis. *SABRAO Journal of Breeding and Genetics* 44(1): 87-101

Sadia M., Ashrafuzzaman M., Islam Md. M., Sikdar S. U. Zobayer N. (2012). Molecular marker based (SSR) genetic diversity analysis in deepwater rice germplasms of Bangladesh. *International Journal of Biosciences* 2(10/2): 64-72.

Saeed A., Hovsepyan H., Darvishzadeh R., Imtiaz M., Panguluri S. K. and Nazaryan R. (2011). Genetic diversity of Iranian accessions, improved lines of chickpea (*Cicer arietinum* L.) and their wild relatives by using simple sequence repeats. *Plant Molecular Biology Reports* 29: 848–858.

Saka J. O., Ajibade S. R., Adeniyan O. N., Olowoyo R. B. and Ogunbode B. A. (2004). Survey of underutilized grain systems in the Southwest Agricultural Zone of Nigeria. *Journal* of *Agricultural and Food Information* 6: 93-107.

Saker M. M., Youssef S.S, Abdallah N.A., Bashandy H.S. and El-Sharkawy A. M. (2005). Genetic analysis of some Egyptian rice genotypes using RAPD, SSR and AFLP. *African Journal of Biotechnology* 4(9):882-890.

Saxon E. C. (1981). Tuberous legumes: preliminary evaluation of tropical Australian and introduced species as fuel crops. *Economic Botany* 35:163-173.

Scribner K. T. and Pearce J.M. (2000). Microsatellites: evolutionary and methodological background and empirical applications at individual, population, and phylogenetic levels. *In*: A. Baker (ed.) *Molecular Methods in Ecology*. Blackwell Science Limited, London, England, pp 235-271.

Senior M.L., Murphy J.P., Goodman M.M., Stuber C.W. (1998). Utility of SSRs for determining genetic similarities and relationships in maize using an agarose gel system. *Crop Science* 38:1088-1098.

Shete S., Tiwari H. and Elston R. C. (2000). On Estimating the heterozygosity and polymorphism information content value. *Theoretical Population Biology* 57(3): 265-271.

Shitta N. S., Abberton M. T., Adesoye A. I. and Adewale D. B. (2015). Analysis of genetic diversity of African yam bean using SSR markers derived from cowpea. *Plant Genetic Resources* 1-7.

Somers D. J. and Demmon G. (2002). Identification of repetitive, genome-specific probes in crucifer oilseed species. *Genome* 45:485–492.

Temnykh S., Park W.D., Ayers N., Cartinhour S., Hauck N., Lipovich L., Cho Y.G., Ishii T., and McCouch S.R. (2000). Mapping and genome organization of microsatellite sequences in rice (*Oryza sativa* L.) *Theoretical and Applied Genetics* 100: 697–712.

Thakur A., Barthwal S. and Ginwal H.S. (2016). *Genetic diversity in bamboos: Conservation and improvement for productivity*. Kaushik, S., Singh, Y. P., kumar, D., Thapaliyal, M. and Barthwal, S. (Eds.) ENVIS Centre on Forestry, Dehradun, India. pp.131-146.

Ubi B.E. (2008). Genetic markers and their utility in plant breeding. *Journal of Agriculture, Biotechnology and Ecology*. Monograph Series (No. 1), pp 3-31.

Uguru M. I. and Madukaife S. O. (2001). Studies on the variability in agronomic and nutritive characteristics in African yam bean *(Sphenostylis stenocarpa)* Hochst Ex. A. Rich Harms). *Plant Production and Research Journal* 6: 10-19.

Upadhyaya H. D., Dwivedi S. L., Baum M., Varshney R. K., Udupa S. M. and Gowda C. L. L. (2008). Genetic structure, diversity, and allelic richness in composite collection and reference set in chickpea (*Cicer arietinum* L.). *BMC Plant Biology* 8:106 -

Yu K., Park S.J. and Poysa V. (1999). Abundance and variation of microsatellite DNA sequences in beans (*Phaseolus* and *Vigna*). *Genome* 42:27-34.

Zietkiewics E., Rafalski A. and Labua D. (1994). Genome fingerprint by sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics* 20:176-183.