Assessment of Genetic Diversity Among *Achishuru* Cowpea Type Land Races Using Simple Sequence Repeat Markers

Amos Cyrus[1], Mohammed F. Ishiyaku[2], Yusuf Mansir[1], US Abdullahi[1]

[1]Department of Plant Science Faculty of Agriculture, Ahmadu Bello University, Zaria. Nigeria
[2]Institute of Agricultural Research, Ahmadu Bello University, Zaria, Nigeria.
amoscyruskin@gmail.com +234-7031114820

Abstract: Most studies on cowpea in Nigeria are restricted to the mainstream cowpea germplasm with little attention in *achishuru* despite its age-long importance in the survival of over one million people of the mid central Nigeria. A total of twenty *achishuru* cowpea accessions where collected for genetic diversity studies, since little is known on the genetic architecture of the crop, six polymorphic simple sequence repeat primers, were used to ascertain the genetic diversity at the DNA level and the transferability of the primers within the collection. The twenty *achishuru* cowpea land races were discriminated into two clusters. Accessions in the first cluster comprised mostly of fourteen local beans types of the *vulgaris specie* that are characteristic kidney shape and rhomboid, the second cluster comprised mostly of the *vigna* genus types. The simple sequence repeat primer pairs of the *achishuru* cowpea accessions could successfully amplify DNA from the related wild relative in the present study. Furthermore, the six simple sequence repeat primer sets designed from the sequences of cowpea used in this study were able to amplified simple sequence repeat of beans type of the *achishuru* accessions which indicates the transferability of the primers. This study has characterized a genetic resource base from the accessions for cowpea improvement programs.

[Amos Cyrus, Mohammed F. Ishiyaku, Yusuf Mansir, US Abdullahi. **Assessment Of Genetic Diversity Among *Achishuru* Cowpea Type Land Races Using Simple Sequence Repeat Markers.** *Rep Opinion* 2017;9(1):17-22]. ISSN 1553-9873 (print); ISSN 2375-7205 (online). http://www.sciencepub.net/report. 4. doi:10.7537/marsroj090117.04.

Keyword: simple sequence repeat; *achishuru*; polymorphism; PCR; accessions

Introduction

Achishuru cowpea landraces comprised of a mixture of beans, peas, and some wild types within the fabaceae, with little attention on the *achishuru* with its high food security value occupying a small but special production niche, and its age-long importance in the survival of over one million people of the mid-central Nigeria. The land races are sown and harvested just when all other food reserves have been depleted (June-August) and so the local name *achishuru* meaning "our savior in time of hunger". As important as this *achishuru* type of cowpea, not much is known about the genetic architecture of these groups. In order to ascertain their genetic potential, there is therefore the need to access the level of diversity within this group. Intra-species genetic diversity is one of the resources enhanced in crop breeding which provide vital resources for developing new cowpea varieties with economic and agronomic attribute. Asare et al., (2010).

Materials and methods.

Germplasm collection

The experimental material used in this experiment where a collection of cowpea land races of the *achishuru* types collected from farmers, cultivated fields, market places, and two from the wild. The region of collection is the mid-central Nigeria. The collections were named based on local names, regions of collection, seed size, and time of maturity, seed shape, growing habit, and the seed colour.

***DNA Extraction**:* Plant genomic DNA was used in detecting the variable SSR region of the twenty *achishuru* cowpea accessions, extraction was done from two Weeks old.

1ul of forward and reverse primers 7.5ul of genomic DNA template, all obtained from inqaba Biotech South Africa. 7.5ul final volume with nuclease-free water (Fermentas Inc.) was added up to a final volume of 23ul. PCR amplification was performed by denaturing DNA at 94°C for 3 min which was followed by 35 cycles each consisting of 94°C for 30 s, 55°C for 30 s and 72°C for 2 min, with a final extension at 72°C for 10 min.

Separation of the Amplified SSR Fragments of the Twenty Achishuru Cowpea Accessions.

Amplified SSR loci of the twenty *achishuru* accessions were subjected to electrophoresis by melting 1.5g agarose powder in 100ml of 1xTBE buffer in a microwave for approximately 2 minutes and were allowed to cool for four minutes and 1ul of ethidium bromide was added and stirred. The gel was cast using a supplied tray and a comb, 100bp DNA ladder (New England) was used to estimate the size of

the PCR products of the twenty samples; the electric power pack of the agarose assembly was turn on at 120volt for 60 minutes. The gel was later exposed to UV light using a UV transilluminator and later photographed with a digital camera. Presence or absence of band was scored for each primer in the twenty cowpea *achishuru* accessions which generated a binary molecular data that was subjected to analysis using a DARwin® software version 6.0.13.

Result

Detection of the Amplification Products of the Achishuru SSR Loci.

To detect the SSR regions, polymerase.

Clustering of the Achishuru Accession Based on Single Sequence Repeat Data.

The clustering of the cowpea landraces of *achishuru* using average linkage algorithm and the Jaccard pair-wised coefficient separated the accessions into two groups based on dissimilarity Cluster I Comprised of fourteen *achishuru* cowpea accessions: Kaura18, jamaa09, EKL16, smallbrw19, EEDB15, wild06, sanga12, varieg11, baki10, largefl06, largerh08, mada07, zango01, Cluster II comprised of six accessions of the *achishuru* cowpea accessions: seedlings using the Gene Jet Plant Genomic DNA Purification. mini kit® obtained from Thermo Fisher Scientific USA. In order to verify that the quality of the DNA fits the desired PCR standard, 0.8% solution of agarose was prepared by melting 0.8g agarose in 100ml of 1xTBE buffer in a microwave for approximately five minutes and was allowed to cool for four minutes and 1ul of ethidium bromide was added and mix. The gel was cast using a supplied tray and a comb, a ratio of 1:5 loading dye to DNA samples was used. 1kb DNA ladder was used to know the various extracted genomic DNA sample sizes; the electric power pack of the agarose assembly was turn on at 120volt for 60 minutes. The gel was later exposed to UV light using a UV transilluminator which was photographed with a digital camera.

SSR markers and Amplification of SSR loci of the twenty Achishuru cowpea accessions.

A total of six most polymorphic simple sequence repeat primer pairs for cowpea were selected from eighty SSR markers, with the VM primer codes (Li et al., 2001, Adetiloye, 2013, F.A Badiane et al 2012). Primer names, sequences, repeat types and predicted product sizes are listed in appendix I. The DNA extracted from the twenty *achishuru* cowpea accessions were subjected to PCR which was carried out in a MJ Research PTC-200 Peltiel thermocycler DNA Engine®. Each 23 µl reaction mixture contained 12.5ul *taq* master mix with standard buffer containing 20Mm Tris-HCL (pH 8.9 @ 25^{oc}), 1.8mM $mgcl_2$, 22mM NH_4Cl, 22mM Kcl, 0.2mm dNTPs, 5% glycerol, 0.06% IGEPAL® CA chain reaction was used to investigate the level of genetic diversity among the twenty *achishuru* cowpea accessions. 100bp ladder was used to detect the product size of the SSR in accordance with their molecular sizes. Li et al (2001). A total of 38 alleles were detected by the six polymorphic SSR markers in the twenty *achishuru* cowpea accessions. (Table 1).

Table 1: Six most Polymorphic SSR Markers of Cowpea Used With their Repeat and Product Size.

primer	Repeat	No. of alleles	PIC
VM35	(AG11).(T)9	7	0.57
VM36	(CT)13	8	0.71
VM39	(AC)13.(AT)5.(TACA)4	7	0.69
VM31	(CT)16	7	0.61
VM68	(GA)15	4	0.72
VM 70	(AG)20	5	0.75

Table 3: Grouping of the *Achishuru* Accessions Based on Polymorphism of the Six SSR Markers.

Cluster	Accessions	Characteristics
I (14)	Kaura18, jamaa09, EKL16, smallbrw19, EEDB15, wild06, sanga12, varieg11, baki10, largefl06, largerh08, mada07, zango01, fariEE13	Fit into the characteristic of the Genera *phaseolus* (beans) kidney or rhomboid shape.
II (6)	Kaura20, kaura14, kaura05, jamaa04, extraE17, kaura02	Fit into the characteristic of the genus *vigna* (peas) e.g cowpea.

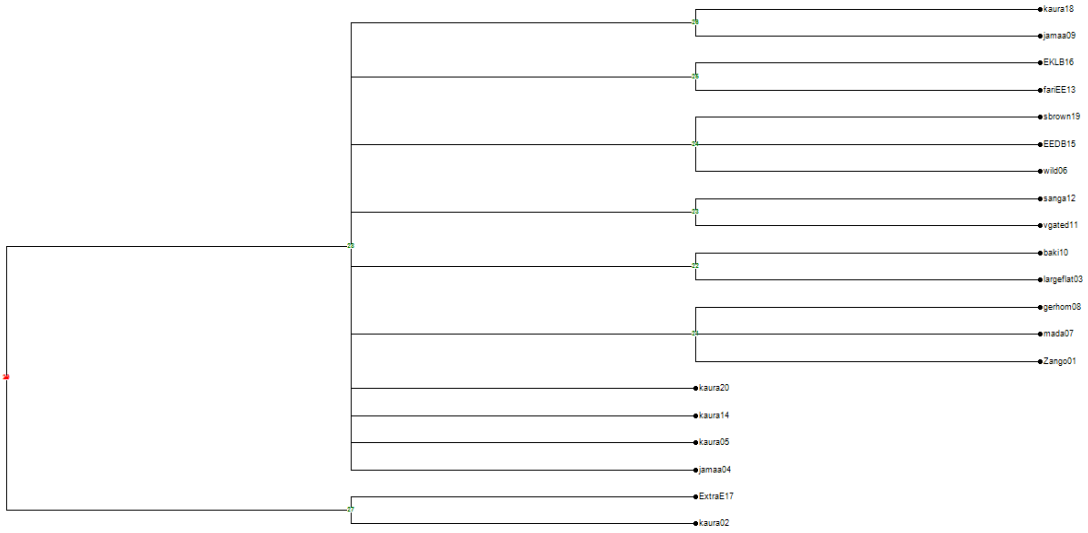

Figure 1: Dendrogram Showing the Genetic Similarity Among 20 Accessions of *Achishuru* Cowpea as Revealed by Cluster Analysis Based on SSR Marker.

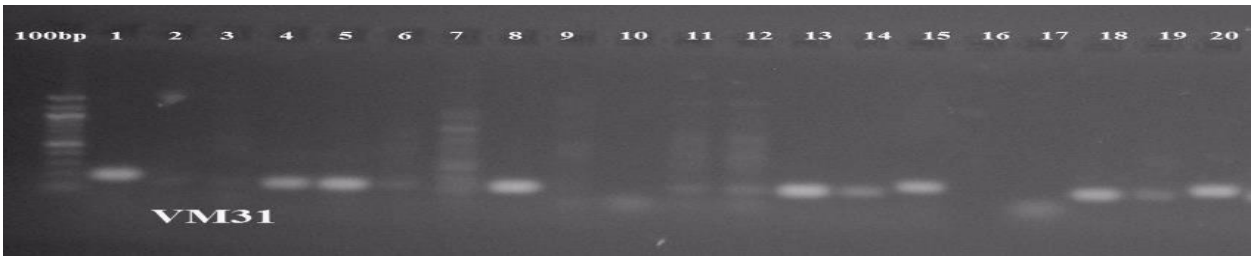

Figure 2: Polymorphism of the SSR Amplified by VM31 in 20 *Achishuru* Cowpea Land Race Accessions Shown on Ethidium Stained Agarose Gel.

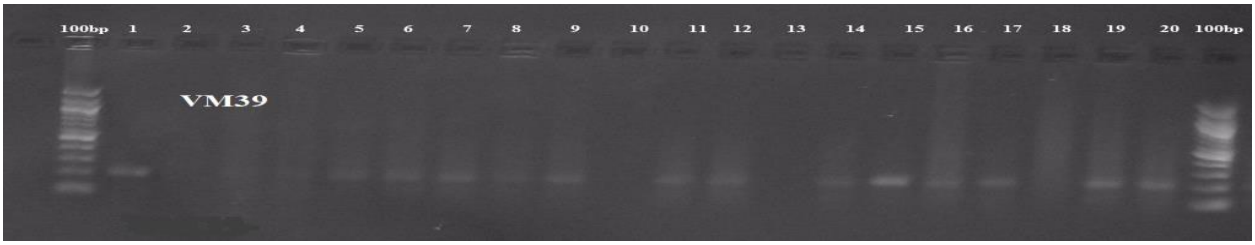

Figure 3: Polymorphism of the SSR Amplified by VM39 in 20 *Achishuru* Cowpea Land Race Accession Shown on Ethidium Bromine Stained Agarose Gel.

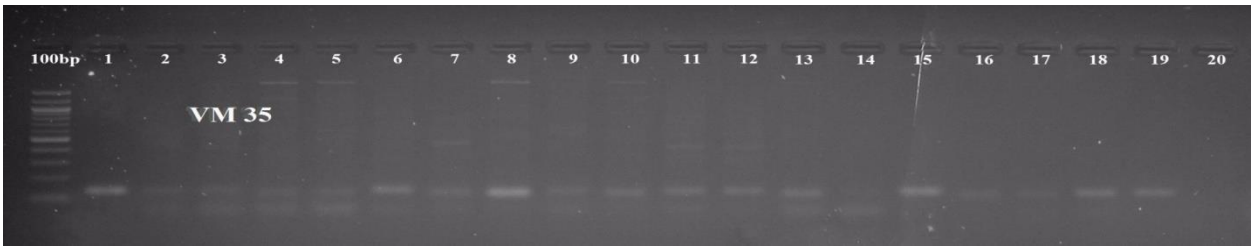

Figure 4: Polymorphism of the SSR Amplified by VM35 in 20 *Achishuru* Cowpea Land Race Accession Shown on Ethidium Bromine Stained Agarose Gel

VM31, 35, and 39 detected 7 alleles each, the highest allele was detected by the primer pair VM36 which amplified 8 alleles. VM68 amplified 4 alleles which is the lowest number of alleles, while VM70 amplified 5 alleles. The discriminatory power of the SSR makers was calculated according to Botstein et al., (1980) 0.75 is the highest polymorphic information content value (PIC) detected by VM70 while the lowest PIC was recorded by the primer pair VM35. An average polymorphic Information content of 0.67 was detected by the six SSR markers. The amplification products of VM31, VM39 and VM70 are shown in figure 2, 3 and 4. fariEE13 whose members are mostly kidney or rhomboid in shape, and characteristically fits into beans type of the *achishuru* accessions.

Kaura20, kaura14, kaura05, jamaa04, extraE17, kaura02 which are oval in shape and most of them belong to the cowpea types.

Discussion

Molecular markers have been assisting the other marker system like morphological and quantitative data trait for genetic variability studies. Simple sequence repeat markers have shown high levels of polymorphism in many important crops including rice (*Oryza sativa* L., Chen et al., (1997), barley (*Hordeum vulgare* L., Liu et al.,(1996), oat (*Avena sativa* L., Li et al., 2000), *Zea mays*, (Adeyemo et al., (2011), Sorghum, (Mace *et al*., (2009) And *Phaseolus vulgaris* Nemli et al (2014). The present study shown that the twenty *achishuru* land races were discriminated based on their *genus* into two different clusters. This agrees with Li *et al* (2001) who found out that all the simple sequence repeat for primer pairs of the cowpea successfully amplified DNA from the related wild relative. Furthermore, the six simple sequence repeat primer sets designed from the sequences of cowpea used in this study were able to amplified simple sequence repeat of beans type of the *achishuru* accessions, which indicates that the SSR design for cowpea can be used for genetic diversity studies in beans. The extent of genomic similarity between two species determines the extent of transferability and use of molecular markers from one species to the other related species. In case of SSR markers, it depends upon the extent of conserved primer binding sites flanking the SSR loci Gupta and Gopalakrishna, (2010).

Achishuru Cowpea Collections Used for This Study.

Earlier, cross-species transferability of SSR markers among legumes was studied by many workers including (Gupta and Gopalakrishana, 2009) who reported transferability of adzuki bean primers to be 100% in blackgram. High proportion of SSR markers (86-92%) were transferred in blackgram from closely related species belonging to *Vigna* genus Gupta et al (2013). The low level of polymorphism detected in this study (4-8 alleles) is in agreement with Asare et al. (2010) reported 4 to 13 alleles in cowpea collected from Ghana, while Sawadogo et al. (2010) reported 5 to 12 alleles in cowpea collected from Burkina This

result agrees with Li et al., 2001 who find out that fifteen polymorphic microsatellites were able to distinguish 88 of the 90 breeding lines of cowpea accessions based SSR markers, Sarikamis et al., (2010). Who reported that utility of SSR markers is due to their abundant distribution and high polymorphism in the whole genome and power to distinguish between closely related genotype?

This researched can be well supported by the study of Chiorato et al. (2010). They studied a set of 220 common bean accessions and reported. These accessions made two groups with 47 and 60% genetic similarity and interpreted that both molecular and morpho-agronomical data sets are equally effective to quantify and organize the genetic diversity of the common beans.

Conclusion

A low degree of genetic diversity was detected among the *achishuru* cowpea accessions using SSR markers. To the best of our knowledge, this is the first study to detect genetic diversity in this type of cowpea. Genetic diversity in the landraces based on SSR is low as compared to other crops; this may be due to inherent self pollination mechanism of the crop. Transferability of SSR markers design for cowpea has been discovered as they can be used for PCR amplification in beans. To detect higher polymorphism, higher numbers of SSRs are recommended.

Reference

1. Adetiloye IS1, Ariyo OJ, Alake CO, Oduwaye OO, Osewa SO (2013). Genetic diversity of some selected Nigerian cowpea using simple sequence repeats (SSR) marker. Afr. J. Agric. Res. 8(7):586-590. Available online at http://www.academicjournals.org/AJAR DOI:
2. Adeyemo, O., Menkir, A,. Gedil, M., & Omidiji, O. (2011). Genetic diversity assessment and relationship among tropical yellow endosperm maize inbred lines using SSR markers. Maydica, 56, 1703-1709.
3. Asare AT, Gowda BS, Galyuon IKA, Aboagye LL, Takrama JF, Timko MP (2010). Assessment of the genetic diversity in cowpea (*Vigna unguiculata L. Walp)* germplasm from Ghana using simple sequence repeat markers. Plant Genet. Res. 8: 142-150.
4. Badiane FA, Diouf D, Sané D, Diouf O, et al. (2012). Screening cowpea [*Vigna unguiculata (L.) Walp*.] varieties by inducing water deficit and RAPD analyses. Afr J. Biotechnol. 3: 174-178 varieties by inducing water deficit and RAPD analyses. Afr J. Biotechnol. 3: 174- 178.
5. Botstein, D., White, R. L., Skolnick, M., & Davis, R. W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. American Journal of Human Genetics. 32:314–331.
6. Chen X, Temnykh S, Xu Y, Cho Y, et al. (1997). Development of a microsatellite framework map providing genome- wide coverage in rice (*Oryza sativa* L.). Theor. Appl. Genet. 95: 553-567.
7. Choumane W, Winter P, Weigand F and Kahl G (2000). Conservation and variability of sequence tagged microsatellites sites (STMSs) from chickpea (*Cicer aerietinum* L.) within the genus Cicer. Theor. Appl. Genet. 101: 269-278.
8. Chiorato AF, Carbonell SAM, Benchimol LL, Chiavegato MB, Dias LAS, Colombo CA (2010). Genetic diversity in common bean accessions evaluated by means of morpho-agronomical and RAPD data. Scientia Agricola 64(3): 256-262.
9. Gupta SK, Gopalakrishna T (2013). Transferability of simple sequence repeat markers in blackgram (Vigna mungo L. Hepper) AJCS 7(3):345-353 (2013). ISSN:1835-2707.
10. Gupta SK, Gopalakrishna T (2009). Transferability of simple sequence repeat markers in blackgram (Vigna mungo L. Hepper) AJCS 7(3):345-353 (2013). ISSN:1835-2707.
11. Li C, Fatokun CA, Ubi B, Singh BB, Scoles GJ (2001). Determining genetic similarities and relationships by microsatellite markers. Crop Sci. 41: 108-117.
12. Li CD, Fatokun CA, Ubi B, Singh BB, et al. (2002). Determining genetic similarities and relationships among cowpea breeding lines and cultivars by microsatellite markers. Crop Sci. 41: 189-197.
13. Mace, E.S., Rami, J-F., Bouchet, S., Klein, P.E., Klein, R.R., Kilian, A., Wenzl, P., Xia, L., Halloran, K. and Jordan, D.R. 2009. A consensus genetic map of sorghum that integrates multiple component maps and high-throughput Diversity Array Technology (DArT) markers. BMC Plant Biology 9:13.
14. Nemli S., Kaya. H (2014) Association mapping for agronomic trait in the common Bean *Phaseolus vulgaris* J.Sc. Food Agric 94:3141-3151.
15. Sarikamis G, Yanmaz R, Ermis S, Bakir M, et al. (2010) Genetic characterization of pea (Pisum sativum) germplasm from Turkey using morphological and SSR markers. Genet. Mol. Res. 9: 591-600.
16. Sawadogo M, Ouedraogo JT, Gowda BS and Timko, Vincent Njung'e Mi (2010). Genetic diversity of cowpea [Vigna unguiculata (L.) Walp.] culivars in Burkina Faso resistance to

Striga gesnerioides. Afri. J. Biotechnol. 9: 8146-8153.
17. Upadhyaya HD, Dwivedi SL, Baum M, Varshney RK, et al. (2008). Genetic structure, diversity, and allelic richness in composite collection and reference set in chickpea (Cicer arietinum L.). BMC Plant Biol. 8: 106.

Appendix I: Six most Polymorphic SSR Markers of Cowpea Used With their Repeat and Product Size

Primer	Primer sequence	Repeat	product size (bp)
VM35	5”GGT CAA TAG AAT AAT GGA AAG TGT 3 3”ATG GCT GAA ATA GGT GTC TGA 5	(AG11).(T)9	127
VM36	5”ACT TTC TGT TTT ACT CGA CAA CTC”3 3”GTC GCT GGG GGT GGC TTA TT”5	(CT)13	160
VM39	5”GAT GGT TGT AAT GGG AGA GTC”3 3”AAA AGG ATG AAA TTA GGA GAG CA”5	(AC)13.(AT)5.(TACA)4	212
VM31	5”CGC TCT TCG TTG ATG GTT ATG”3 3”GTG TTC TAG AGG GTG TGA TGG TA”5	(CT)16	200
VM68	5'-CAA GGC ATG GAA AGA AGT AAG AT-“3 3'-TCG AAG CAA CAA ATG GTC ACA C-“5'	(GA)15	254
VM 70	5'-AAA ATC GGG GAA GGA AAC C-3' 5'-GAA GGC AAA ATA CAT GGA GTC AC-3'	(AG)20	186

1/25/2017