

Molecular Characterization of *Trichosanthes cucumerina* Using SSR Markers

¹A.N. Osuagwu, ²C.U. Aguoru, ³L.O. Omoigui, ²J.O. Olasan and ⁴O.I. Onen

¹Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike, Nigeria

²Department of Botany and Environmental Science, University of Agriculture, Makurdi, Nigeria

³Department of Plant Breeding and Genetics, University of Agriculture, Makurdi, Nigeria

⁴Department of Plant Science and Biotechnology, University of Cross River State, Calabar, Nigeria

ABSTRACT

Background and Objective: *Trichosanthes cucumerina* is a neglected and underutilized vegetable valued by traditional communities for food, yet its genetic diversity remains underexplored in Nigeria. This study aimed to assess the genetic diversity of *T. cucumerina* accessions from four geopolitical zones in Nigeria using simple sequence repeat (SSR) markers, to generate data useful for gene identification, mapping, and molecular breeding to enhance food security. **Materials and Methods:** Fourteen-day-old seedlings from fourteen accessions collected across four geopolitical zones were analyzed. Genomic DNA was extracted using the SDS method, and seventeen SSR primers were used for amplification. Banding patterns were scored into a binary matrix and analyzed in Minitab 17. Genetic diversity indices, phylogenetic clustering, Principal Component Analysis (PCA), and Analysis of Molecular Variance (AMOVA) were performed. **Results:** Major allele frequency ranged from 0.3571–0.8571, allele number from 2.000–4.000, gene diversity from 0.2800–0.7245, and polymorphic information content (PIC) from 0.2800–0.6740. A total of 25 alleles were amplified, averaging two per locus. Three primers (CMTM 34, CMTM 9, LSR 015) showed the highest PIC values. Dendrogram analysis grouped the accessions into three clusters, with PCA explaining 80.61% (PC1) and 7.38% (PC2) of the variation. The AMOVA revealed significant genetic differentiation ($p < 0.001$), with 91% of the variation occurring within accessions and 9% among clusters. **Conclusion:** The study reveals considerable genetic diversity among *T. cucumerina* accessions in Nigeria, highlighting specific SSR markers with high discriminatory power. These findings provide essential baseline data for molecular breeding and conservation, contributing to the crop's potential role in enhancing food security.

KEYWORDS

Trichosanthes cucumerina, genetic diversity, SSR markers, polymorphic information content, molecular characterization, Nigeria

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INTRODUCTION

Indigenous people in Nigeria have long used a wide range of plants for food and medicine. About 130 genera and 900 species make up the Cucurbitaceae family, which is known for its great genetic variety¹.

The majority of these species are located in tropical and subtropical regions. The plant family is considered economically significant since many of its species generate edible commodities and useful fibers for human consumption².



Fruit shape and related features exhibit great genetic variety among members of this plant group.

Their distinctive aboveground growth pattern, which encourages a wide range of phenotypic expression². The snake tomato (*Trichosanthes cucumerina*), also known as viper gourd or long tomato, is an underutilized crop whose fruit is primarily eaten as a vegetable^{3,4}. In rural Nigerian communities, it is valued for its sweet flavor and deep red endocarp pulp, commonly used in stews when mature. Underutilized plants are species that are not widely available, seldom used, and typically limited to a certain region³. Underutilized crops or plant species are defined as those traditionally cultivated by various communities for purposes such as food, fibre, animal fodder, oil, or medicine⁵. These species possess significant, yet unexploited, potential for wider applications. Furthermore, recent proposals indicate that numerous species, their varieties, and the associated traditional cultivation knowledge are declining rapidly⁶.

Morphological studies have been useful in getting information on genetic studies, but this method has limitations because it measures phenotypic characters, which are affected by environmental and climatic factors. Genetic diversity found in species showed a valuable genetic resource for breeding and genetic^{7,8}. Living germplasm collections are important resources for showcasing and conserving genetic and phenotypic diversity and provide novel material for breeding efforts⁹. Genetic diversity studies using molecular markers are distinguished from morphological analysis because it is not affected by environmental factors. Molecular markers have shown themselves as a powerful tool in the assessment of genetic variation and in pointing out genetic relationships within and among species. Simple sequence repeat, also known as microsatellite in this study, is preferable because it is reproducible, highly polymorphic, codominant, multiallelic, and amplified by PCR. Simple sequence repeats (SSR), which are short, tandemly repeated DNA sequences, are commonly applied in studying genetic relationships among closely related species, as well as in evaluating their cross-species transferability across different genera within the species Cucurbitaceae family¹⁰⁻¹³.

Research conducted on *Cucurbita pepo* landraces in KwaZulu-Natal, South Africa, used RAPD and SSR markers. The results showed that the genetic differentiation coefficient between populations ranged from 0.0022 to 0.0100 using the RAPD marker and from 0 to 0.0076 using the SSR marker. Both markers discriminated the landraces well. However, only the RAPD marker could discriminate between landraces based on fruit colour, changes at maturity, and agro-ecological origins. All genetic parameters indicated high genetic diversity in *C. pepo* landrace¹⁴.

Genetic diversity is essential for plant survival and crop improvement, as demonstrated by the application of simple sequence repeat (SSR) markers in genetic studies. For instance, investigations of *Cucumis melo* landraces in the Xinjiang Uygur Autonomous Region and Zhang *et al.*¹⁵ and Yook *et al.*¹⁶ assessed 69 *Miscanthus* species accessions using 31 SSR primers, identified genetic similarity coefficients between 0.34 and 0.86. These findings indicate substantial intraspecific variability. Such broad genetic diversity underpins both adaptation to natural environments and the success of crop enhancement initiatives¹⁷. Despite widespread acknowledgment of its significance, the genetic resources of this species in Nigeria remain limited. This limitation is attributed to the absence of a national research program and persistent institutional and policy barriers, as reported by Roberts *et al.*⁸. Molecular characterization of cucurbits has been carried out on different genera of Cucurbitaceae^{1,8} using RAPD and SSR. Thus, there is a need to study the diversity of *Trichosanthes cucumerina* and var *anguina* in Nigeria. This study aims to characterize landraces of *T. cucumerina* in Nigeria using simple sequence repeats.

MATERIALS AND METHODS

Viable seeds of snake tomato (*Trichosanthes cucumerina* L.) ecotypes were sourced and collected from the following locations for the laboratory study as follows (Table 1).

Table 1: Sources, ecotypes, and designations of the 14 accessions collected from different locations in Nigeria

Sr/N	Source location	Ecotype	Designation
1	Benin, Edo State	Rainforest	V1
2	Nasarawa State	Derived savannah	V2
3	Oye-Ekiti, Ekiti State	Rainforest	V3
4	Ilorin, Kwara State	Guinea savannah	V4
5	NHST 0588	Guinea savannah	V5
6	Makurdi, Benue State	Derived savannah	V6
7	Rumibekwe, Rivers State	Rainforest	V7
8	Ikom, Cross River State	Rainforest	V8
9	Oshogbo Osun State	Rainforest	V9
10	Ikwuano, Abia State	Rainforest	V10
11	Elelenwo-Rivers State	Rainforest	V11
12	NAGRAB-00753	Guinea savannah	V12
13	Ukwa, Abia State	Rainforest	V13
14	Iberenta, Abia State	Rainforest	V14
Total			14

Table 2: Primer sequences (5-3', forward/reverse) of the 17 SSR markers used in PCR reactions with genomic DNA of 14 *T. cucumerina* L. ecotypes

Marker name	Forward primer	Reverse primer	References
LSR015	CTTACCTTCACAAAACCCCATC	ACTCTGTTTCGACTCTGCTTCC	Xu <i>et al.</i> ¹⁹
LSR040	TTCCATCCAGACCAAACCTATC	CAAAGGCCATAGACAAACACAA	Xu <i>et al.</i> ¹⁹
LSR056	TAATAATGCCACTGCACATGGT	AGATGAATCCCAATATCCCAGA	Xu <i>et al.</i> ¹⁹
LSR063	AAGAGAGGGGCGGAAGTAAAT	AGAAAACACACAGTACGCCTCC	Xu <i>et al.</i> ¹⁹
LSR077	GACAGATCCTTCTGGGACTTTT	TTCTGCAATAGAGTACGTTGGC	Xu <i>et al.</i> ¹⁹
LSR112	CTCTCTATATGTCTAATTCCTCGCC	CAAATTCACAGTTGTTGTCACG	Xu <i>et al.</i> ¹⁹
CUTC017708	TGTGATTGTTGGGCTCTCTGT	ACATGGAAATCCACCTCTTCGT	Gong <i>et al.</i> ²⁰
CUTC002749	GTGGGCTAAGTTCAAATCGTTC	GAACCAATCTCTCATTTCCTCA	Gong <i>et al.</i> ²⁰
CUTC046645	CCCTCGGTAGCAATTTGTAGT	TTTGGTGGGAGTGATACGATG	Gong <i>et al.</i> ²⁰
CUTC009607	CAAACTGCCCAGTACCACTAC	CTTTCTACCCCAACCCACAT	Gong <i>et al.</i> ²⁰
CMTP46	TTCCCTTCTGCAGAGATGCT	CCATGCGCATAATTGTATCG	Blanca <i>et al.</i> ²¹
CMTP142	TCAACCAAGTGCCAATCTCA	ACTGATCCACCGACTGATACG	Blanca <i>et al.</i> ²¹
CMTM 34	TGAACTACAACATGACCTTGG	TGGGTTGGTAGACTGTAGTTGA	
CMTM9	GCCCAGAAGACAAAAGTTTCG	TTTTTGTGTGCGTGTGTGG	
CMTM54	GTGTGGATGCAATGGTGAG	GGGAATCGAGGGTTTGAAT	
CMTM172	CATTGGAGTGCCTGAAATTG	GTGAGTGGTCAAAGGCATT	
CMTP66	ACGACATGAGGGAAGATTCTG	TTCAATGCCATTGCGCTAC	

The study was carried out on the 8th June, 2021-3rd March, 2022 in the Molecular Biology Laboratory of Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. Old modified method of the SDS DNA extraction was used¹⁸. The sampled tubes were centrifuged at 1000 rpm for 10 min, and transferred supernatant was transferred into freshly labelled tubes. About 2/3 volume of ice-cold Isopropanol was added, mixed gently, and incubated in -80°C for 15 min to precipitate the DNA. The tubes were centrifuged at 1000 rpm for 10 min. The supernatant was decanted until the last drop 400 µL of 70% ethanol was added to wash the DNA pellet. The sampled tubes were centrifuged at 1000 rpm for 10 min. The supernatant was decanted until the last drop, and air-dried the pellet. About 60 µL of ultra pure water or low salt TE to re-suspend the DNA, 2 µL of RNase was added and incubated in 37°C for 30-40 min.

Agarose gel electrophoresis: The diversity analysis was carried out using 17 SSR primers from Inqaba Biotec West Africa Ltd., in South Africa synthesized the primers (Table 2). For successful amplification, PCR products were first run on a 2% agarose gel and visualized with ethidium bromide. Non-resolved products were then resolved on a 6% non-denaturing polyacrylamide gel (29:1, acrylamide: bis), visualized under UV lights in the presence of the Safe View TM DNA stains (Applied Biological Materials Inc., Richmond, BC, Canada), and images were captured.

Data analysis: For data analysis, the amplified products in the gel pictures were manually scored in a binary format, with '1' and '0' representing the presence and absence of the amplicons, respectively, for all accessions.

Statistical analysis: Power Marker version 3.25 was used to calculate the number of alleles per marker, the major allele frequency, gene diversity, and the polymorphic information content (PIC) value for each locus)²². The genetic analysis was done with GenAEx²³.

RESULTS

The gel image presented in Fig. 1 illustrates the integrity and band clarity of genomic DNA extracted from the *Trichosanthes cucumerina* snake gourd samples. The distinct and well-defined bands observed confirm that the extraction procedure yielded high-quality DNA with minimal degradation or smearing. This level of band resolution is indicative of sufficient purity and concentration, confirming the suitability of the DNA samples for subsequent molecular analyses, including PCR amplification and marker-based characterization.

The nanodrop readings from the PCR gel images. The nucleic acid images varied from -1884.1-8419. 5 ng/μL at 260/280 bp from 1.91-1.97 in Table 3.

In Table 4 marker major allele frequency ranges from 0.3571-0.8571 with a mean of 0.611. The allele number of the marker was within the range of 2.0000-4.0000 with a mean of 2.4444. The availability of the marker was 1.000. The gene diversity of the marker ranges was 0.2449-0.7245 with a mean of 0.4819.

Table 3: UV absorbance-based quantification and purity assessment of DNA using nanodrops

Sr/N	Sample ID	Nucleic acid	Unit	260/280
1	Benin Edo State	2272	ng/μL	1.91
2	Nassarawa State	6248.5	ng/μL	1.97
3	Oye Ekiti, Ekiti State	8122	ng/μL	1.95
4	Ilorin Kwara	8419.5	ng/μL	1.97
5	NHST 0588	5639	ng/μL	1.95
6	Makurdi Benue State	4503.8	ng/μL	1.94
7	Rumibekwe River State	8513.7	ng/μL	1.94
8	Ikom Cross River	-1844.1	ng/μL	1.94
9	Oshogbo Osun State	6202.5	ng/μL	1.91
10	Ikwuano Abia State	7241.4	ng/μL	1.96
11	Elelenwo River State	5546.9	ng/μL	1.95
12	NAGRAB 00753	7723.6	ng/μL	1.96
13	Ukwa Abia State	6509.7	ng/μL	1.94
14	Iberenta Abia	6315.4	ng/μL	1.95

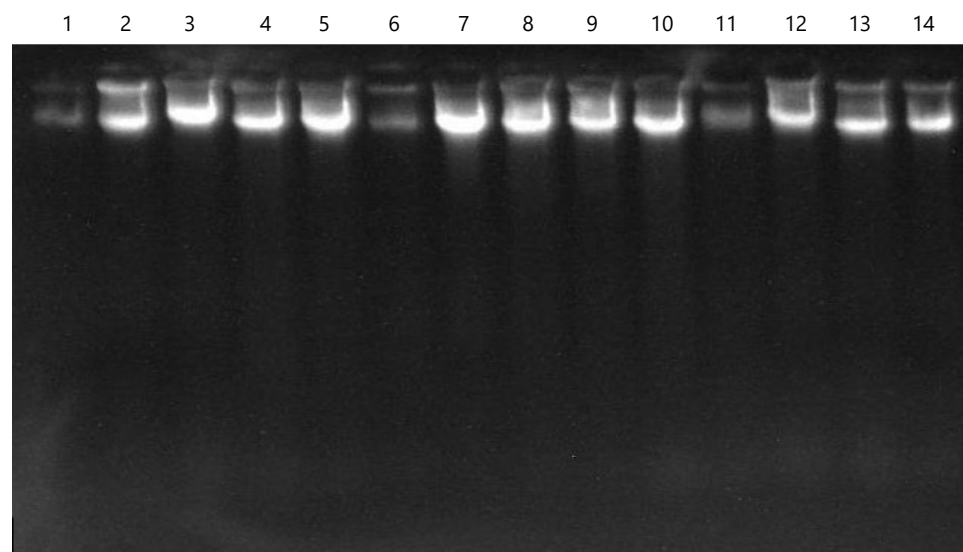


Fig. 1: Gel image showing Genomic DNA extracted from the *Trichosanthes cucumerina* (snake gourd) samples

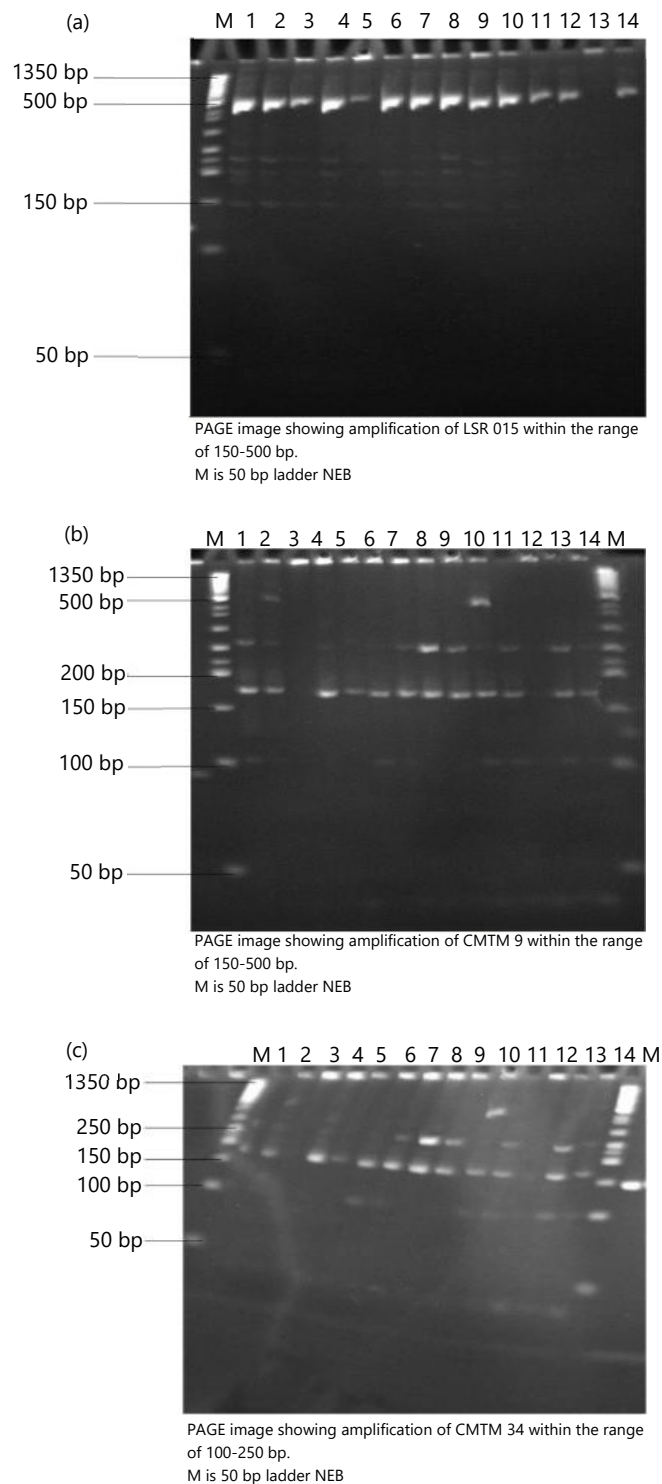


Fig. 2(a-c): Page gel electrophoresis of (a) *T. cucumerina* of genomic DNA amplified with CMTM 9 within the range of 100-500 kb and 50 kb as the molecular ladder, (b) Molecular ladder at 50 kb. CMTM 34 is one of the primer used for amplifying genomic DNA, which ranges from 100-250kb and (c) *T. cucumerina* genomic DNA as amplified by the three best primers

Polymorphism information centre (PIC) ranges from (0.2149-0.6740), and the mean was 0.3987. A total of 25 alleles were amplified, with an average of 2 markers per allele. The average gene diversity is 0.48, while the gene diversity ranges from 0.24 to 0.72. The average polymorphic information content (PIC) value for each locus was 0.40 and varied from 0.21 to 0.67, indicating the level of allele variation among the species. Primer CMTM 34 has the highest allele number and the highest PIC value among the SSRs.

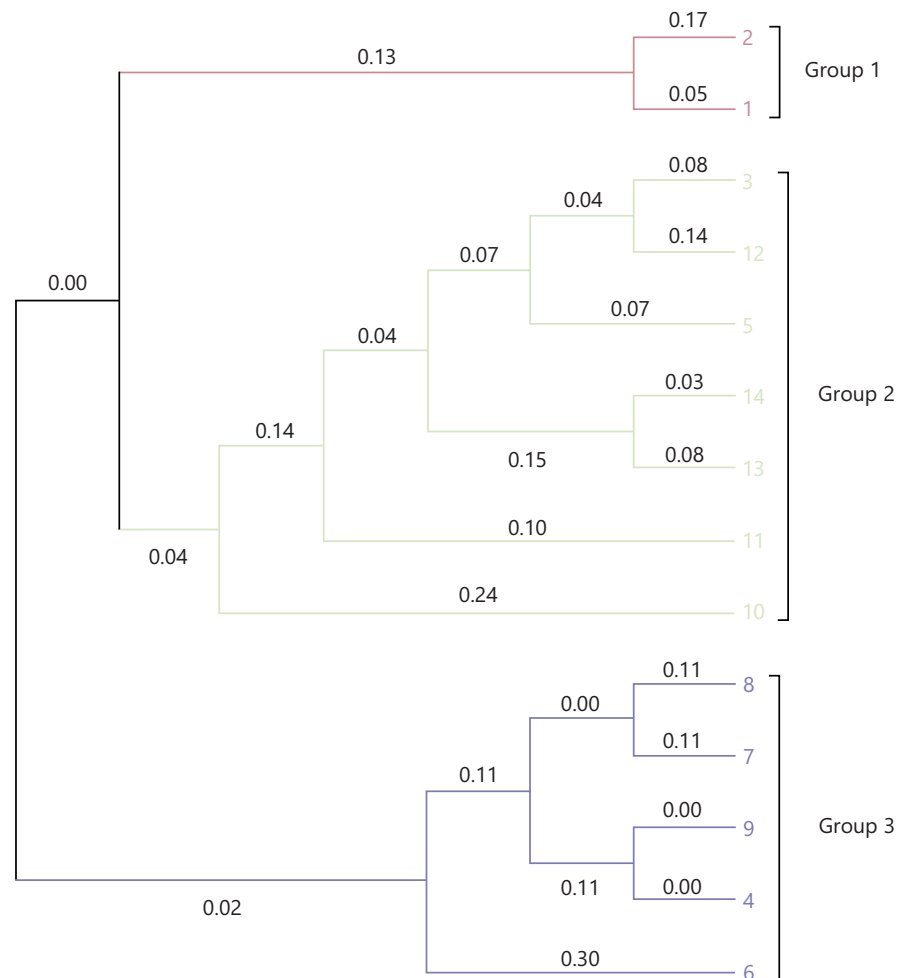


Fig. 3: Dendrogram of simple sequence repeats which characterized the fourteen ecotypes

Table 4: Markers, major allele frequency, allele number, gene diversity, polymorphisms information centre (PIC)

Marker	Major allele frequency	Allele no.	Gene diversity	PIC
CMTM 54	0.6429	2.0000	0.4592	0.3538
CMTM 34	0.3571	4.0000	0.7245	0.6740
CMTM 46	0.6429	2.0000	0.4592	0.3538
LSR015	0.5000	3.0000	0.5612	0.4650
CMTM 9	0.5000	3.0000	0.6020	0.5229
CMTM 172	0.7857	2.0000	0.3367	0.2800
CMTM 142	0.6429	2.0000	0.4592	0.3538
CUTCO02749	0.8571	2.0000	0.2449	0.2149
CUTCO46645	0.5714	2.0000	0.4898	0.3698
Mean	0.6111	2.4444	0.4819	0.3987

Polyacrylamide gel electrophoresis (Page) of *T. cucumerina* genomic DNA amplified with LSR015 primer showing molecular weight marker at 50 bp. The LSR015 is within the range of 150-500 bp.

The selected gel made from the study is shown in Fig. 2a-c. All the primers give good DNA amplification of fourteen accessions of *T. cucumerina*. The result depends on the number of bands on the SSR primer and the *T. cucumerina* location. The following primers were determined as follows CMTM 34 (Kadarko, Nassarawa), CMTM 9 (Ikom, Cross River), and LSR 015 (Rumibekwe, Rivers). The polymorphic information content revealed that the primer with the highest PIC was CMTM 34(0.6740) followed by CMTM 9 (0.5229) and LSR 015 (0.4650).

Eigen values by axis and sample eigen vectors							
Axis no.	1	2	3	4	5	6	7
Eigen value	3.121	0.286	0.231	0.105	0.069	0.054	0.006

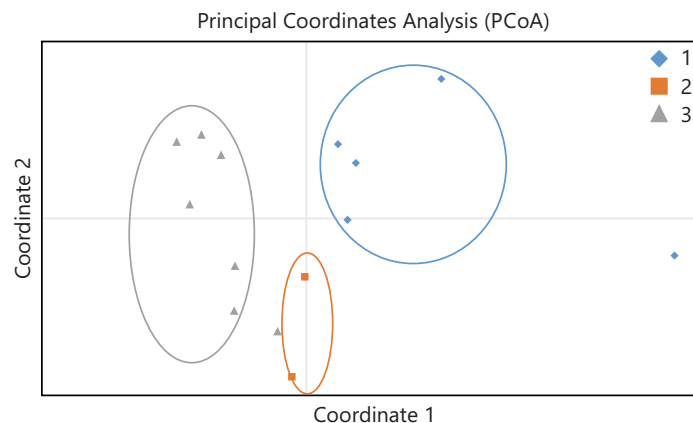


Fig. 4: AMOVA showed significant genetic differentiation within individuals ($p < 0.001$), 91% of the total genetic variations were due to Differences within the lines, while the remaining 9% was observed among clusters

Phylogenetic assessment: The dendrogram was constructed using Neighbour-joining. The analysis divided the accessions into three major groups, with group two having the highest number of accessions. This was followed by group three, while group one had the lowest number of accessions grouped together (Fig. 3). There were three cluster groups in the sequence dendrogram. Benin and Kadarko accessions were closely related to each other.

Principal component analysis: The Principal Component Analysis (PCA) clustered the lines into three subgroups. The first principal component (PC1) explained 80.61% of the variation, and PC2 explained 7.38%. Figure 4: AMOVA showed significant genetic differentiation within individuals ($p < 0.001$), 91% of the total genetic variations were due to differences within the lines, while the remaining 9% was observed among clusters. The eigenvalue was found to be more than one in eigenvalue 1, while eigenvalue 2-7 was less than less. This implies all the ecotypes are positively related to one another.

DISCUSSION

The marker major allele frequency ranges from 0.3571-0.8571 with a mean of 0.611. The allele number of the marker was within the range of 2.000-4.000 with a mean range of 2.444. The availability of the marker was 1.000. The gene diversity of the marker ranged from 0.2449-0.7245, with a mean of 0.4189. Polymorphism information centre (PIC) ranged from (0.2149-0.6740) with a mean of 0.3987. This observation is consistent with the findings of an old study of Merheb *et al.*²⁴, which characterized snake melon using 10 SSR markers and reported fifty-six distinct alleles, with an average of 5.6 alleles per locus. The polymorphism information content (PIC) of simple sequence repeat (SSR) markers ranged from 0.06-0.84, with an average of 0.38. Consistent allele richness across related studies demonstrates the effectiveness of SSR markers in detecting genetic variation within *Cucurbitaceae* species and supports their reliability for assessing genetic diversity in snake gourd populations. Similarly, a study of Dar *et al.*¹³ reported on the genetic diversity and population structure of *Cucumis sativus* L. using SSR markers, where allele frequencies ranged from 0.215 to 0.561 with an average of 0.403, and PIC values varied from 0.158 to 0.4985 with a mean of 0.333. In a related study of Liu *et al.*²⁵ investigated genetic diversity and relationships among 38 Chinese and 28 Russian accessions of *Cucurbita* and 10 Chinese accessions of *Curcubita pepo* with thirty-five simple sequence repeats (SSR) markers. A total of 85 DNA fragments were

amplified, of which 85.89% were polymorphic. This result indicates a high level of genetic diversity within the population. These findings are consistent with those of a previous study that evaluated the genetic diversity and phylogenetic relationships of Iranian cucurbits using inter-sample sequence repeat (ISSR) markers. In that study, 17 primers were tested, and 11 produced scorable bands, resulting in 263 polymorphic bands. The average polymorphism information content (PIC) value was 0.327, further confirming significant genetic variability within cucurbit species.

Also Esmailnia *et al.*²⁶ the genetic diversity of *Brassica oleracea* using simple sequence repeat (SSR) from twenty-five accessions and found 47 alleles; the observed heterozygosity (0.6991, average value of fixation indices (F) was negative, which showed excess of heterozygotes, and polymorphic information content (PIC) values of SSR loci ranged from 0.27-0.66, with an average of 0.571.

Phylogenetic analysis based on the dendrogram divided the accessions into three major clusters, with group two containing the highest number of accessions, followed by group three, while group one had the least. This clustering pattern reflects relatively low genetic diversity among the analyzed *Trichosanthes cucumerina* landraces. Comparable patterns have been reported in previous studies of Peakall and Smouse²³, such as those involving *Cucumis melo* subsp. *melo* L. var. *flexuosus*, where accessions were categorized into five distinct clusters, indicating high genetic variability. Similarly, research utilizing simple sequence repeat (SSR) markers in sweet potatoes showed moderate genetic diversity, with a similarity coefficient of 0.49²⁷. On the other hand, a study on Chinese watermelon ecotypes and germplasm from other countries grouped all cultigens into six clusters, with genetic similarity coefficients among Chinese ecotypes ranging from 0.37 to 0.99. Except a wild relative, most of the watermelon ecotypes exhibited close genetic relationships²⁸. Apart from a wild watermelon relative, the majority of ecotypes were genetically similar, while the genetic distance among non-Chinese cultigens varied between 0.67 and 0.91.

The principal component analysis (PCA) clustered the lines into three subgroups. This is in agreement with the previous work of Liu *et al.*²⁵ reported the least distance was observed between two species of *Cucumis* L. genus with a genetic similarity value of 64.34%, while the highest genetic distances were observed between *Cucumis* species L. and *Cucurbita* species L. with a genetic similarity value of 20.71%. This tends to agree with the work of Gong *et al.*¹¹, who reported genetic relationship and evolution in *Cucurbita pepo* using simple sequence repeat (SSR) results obtained, showing three clusters distinguishing *pepo*, *texana*, and *fraterna*. The accessions indicated there was no crossing between subspecies. The genetic diversity and population structure of cucumber (*Cucumis sativus* L.) and observed the separation of accessions into three groups using Principal Component Analysis (PCA) and a phylogenetic tree, which corroborates this work of Lv *et al.*²⁹. Reports of Forrester *et al.*³⁰ on the genetic diversity of Bambara groundnut (*Vigna subterranea* (L) verdc) landraces in Kenya using microsatellite markers and observed 24 alleles, which were revealed with a mean of 2, and gene diversity values averaged 0.28 and 0.35, respectively, indicating low genetic diversity. Jaccard's similarity coefficient from the simple sequence repeat (SSR) ranged from 0.08-1.16 to indicate the genetic distance among the landraces. The phylogenetic tree of 105 accessions of Bambara groundnut revealed three clusters. The Principal Coordinate Analysis (PCoA) revealed that the first three axes accounted for 84.3% of the total variation, with each axis explaining 63.58, 12.21, and 8.24% variation in that order. This is in agreement with the findings of this work.

This study assessed the genetic diversity of *Trichosanthes cucumerina* in Nigeria using SSR markers; however, its scope was constrained by a limited number of accessions, restricted geographical coverage, and a relatively small set of SSR loci. Potential sampling bias and the absence of phenotypic data limited the capacity to link molecular variation to agronomic traits. Additionally, the work reflects a single collection period, omitting temporary variability.

Future research should expand germplasm sampling to cover all agro-ecological zones, employ a larger and more diverse marker (including SNP), and integrate morphological and agronomic evaluations to identify marker-trait associations useful for breeding programs. Longitudinal studies capturing seasonal and multi-year variation would provide deeper insights. Comparative genetic analysis between Nigerian germplasm and populations from other parts of Africa and Asia could further elucidate evolutionary relationships and domestication pathways.

CONCLUSION

Seventeen primers were used for this study; three primers distinguished themselves. They are CMTM 34 (Kadarko, Nassarawa), CMTM 9 (Ikom, Cross River) and LSR 015 (Rumibekwe, Rivers) respectively. The polymorphic information content revealed that the primer with the highest PIC was CMTM 34 (0.6740) followed by CMTM 9 (0.5229) and LSR 015 (0.4650). CMTM 34 can be recommended as a primer for snake gourd studies using a simple sequence repeat marker. The present study can be useful for gene identification, gene mapping, and molecular breeding of *T. cucumerina* from four geopolitical zones in Nigeria for food security.

SIGNIFICANCE STATEMENT

This study discovered the considerable genetic diversity of *Trichosanthes cucumerina* accessions across Nigeria, which can be beneficial for gene identification, mapping, and molecular breeding to improve the crop's productivity and adaptability. The identification of SSR markers with high discriminatory power provides a reliable tool for assessing genetic relationships and guiding conservation strategies. By revealing the extent of variation within and among accessions, the study highlights the untapped potential of this neglected and underutilized vegetable for food and nutritional security. Importantly, this study will help researchers to uncover the critical areas of genetic diversity and molecular breeding that many researchers were not able to explore. Thus, a new theory on the crop's improvement and utilization may be arrived at.

REFERENCES

1. Adeyemo, O.A., S.R. Adegoke, D.J. Oladapo, C.C. Amaghereonu and A.E. Thomas *et al.*, 2020. Transferability of SSR markers used for assessment of genetic relationship in five species/genera in cucurbitaceae. *Egypt. J. Bot.*, 60: 275-286.
2. Bisognin, D.A., 2002. Origin and evolution of cultivated cucurbits. *Cienc. Rural*, 32: 715-723.
3. Liyanage, R., H. Nadeeshani, C. Jayathilake, R. Visvanathan and S. Wimalasiri, 2016. Comparative analysis of nutritional and bioactive properties of aerial parts of snake gourd (*Trichosanthes cucumerina* Linn.). *Int. J. Food Sci.*, Vol. 2016. 10.1155/2016/8501637.
4. Badejo, A.A., A.P. Adebawale and V.N. Enujiugha, 2016. Changes in nutrient composition, antioxidant properties, and enzymes activities of snake tomato (*Trichosanthes cucumerina*) during ripening. *Prev. Nutr. Food Sci.*, 21: 90-96.
5. Arora, R.K., 2014. Diversity in Underutilized Plant Species-An Asia-Pacific Perspective 1938. Bioversity International, New Delhi, India, ISBN: 978-92-9255-007-3, Pages: 234.
6. Padulosi, S., J. Thompson and P. Rudebjer, 2013. Fighting Poverty, Hunger and Malnutrition with Neglected and Underutilized Species: Needs, Challenges and the Way Forward. Bioversity International, Rome, Italy, ISBN: 978-92-9043-941-7, Pages: 60.
7. Klich, M.A., 2002. Identification of Common *Aspergillus* Species. ASM Press, Washington, D.C., USA, ISBN: 9789070351465, Pages: 116.
8. Roberts, E.M.I., I.O. Agbagwa and B.E. Okoli, 2018. Genetic diversity and RAPD-based DNA fingerprinting of some members of the Cucurbitaceae in Nigeria. *J. Adv. Biol. Biotechnol.*, Vol. 17. 10.9734/JABB/2018/40081.
9. Migicovsky, Z., E. Warschefsky, L.L. Klein and A.J. Miller, 2019. Using living germplasm collections to characterize, improve, and conserve woody perennials. *Crop Sci.*, 59: 2365-2380.

10. Ajenifujah-Solebo, S.O.A., I. Ingelbrecht, N.A. Isu, O. Olorode, G.I.B. Obioh and S. Nnadi, 2018. Molecular characterization of three cultivars of tomato (*Lycopersicon esculentum* L.) in South-West Nigeria using SSR markers. *Int. J. Environ. Agric. Biotechnol.*, 3: 1401-1408.
11. Gong, L., H.S. Paris, M.H. Nee, G. Stift, M. Pachner, J. Vollmann and T. Lelley, 2012. Genetic relationships and evolution in *Cucurbita pepo* (pumpkin, squash, gourd) as revealed by simple sequence repeat polymorphisms. *Theor. Appl. Genet.*, 124: 875-891.
12. Sheng, Y., F. Luan, F. Zhang and A.R. Davis, 2012. Genetic diversity within Chinese watermelon ecotypes compared with germplasm from other countries. *J. Am. Soc. Hortic. Sci.*, 137: 144-151.
13. Dar, A.A., R. Mahajan, P. Lay and S. Sharma, 2017. Genetic diversity and population structure of *Cucumis sativus* L. by using SSR markers. *3 Biotech*, Vol. 7. 10.1007/s13205-017-0944-x.
14. Ntuli, N.R., P.B. Tongoona and A.M. Zobolo, 2015. Genetic diversity in *Cucurbita pepo* landraces revealed by RAPD and SSR markers. *Sci. Hortic.*, 189: 192-200.
15. Zhang, Y., X. Fan, Y. Aierken, X. Ma, H. Yi and M. Wu, 2017. Genetic diversity of melon landraces (*Cucumis melo* L.) in the Xinjiang Uygur Autonomous Region on the basis of simple sequence repeat markers. *Genet. Resour. Crop Evol.*, 64: 1023-1035.
16. Yook, M.J., S.H. Lim, J.S. Song, J.W. Kim and C.J. Zhang *et al.*, 2014. Assessment of genetic diversity of Korean *Miscanthus* using morphological traits and SSR markers. *Biomass Bioenergy*, 66: 81-92.
17. Bhandari, H.R., A.N. Bhanu, K. Srivastava, M.N. Singh, Shreya and A. Hemantaranjan, 2017. Assessment of genetic diversity in crop plants-An overview. *Adv. Plants Agric. Res.*, 7: 279-286.
18. Dellaporta, S.L., J. Wood and J.B. Hicks, 1983. A plant DNA miniprep: Version II. *Plant Mol. Biol. Rep.*, 1: 19-21.
19. Xu, P., X. Wu, J. Luo, B. Wang and Y. Liu *et al.*, 2011. Partial sequencing of the bottle gourd genome reveals markers useful for phylogenetic analysis and breeding. *BMC Genomics*, Vol. 12. 10.1186/1471-2164-12-467.
20. Gong, L., G. Stift, R. Kofler, M. Pachner and T. Lelley, 2008. Microsatellites for the genus *Cucurbita* and an SSR-based genetic linkage map of *Cucurbita pepo* L. *Theor. Appl. Genet.*, 117: 37-48.
21. Blanca, J., J. Cañizares, C. Roig, P. Zarsolo, F. Nuez and B. Picó, 2011. Transcriptome characterization and high throughput SSRs and SNPs discovery in *Cucurbita pepo* (Cucurbitaceae). *BMC Genomics*, Vol. 12. 10.1186/1471-2164-12-104.
22. Liu, K. and S.V. Muse, 2005. PowerMarker: An integrated analysis environment for genetic marker analysis. *Bioinformatics*, 21: 2128-2129.
23. Peakall, R. and P.E. Smouse, 2012. GenAEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics*, 28: 2537-2539.
24. Merheb, J., M. Pawełkiewicz, F. Branca, H. Bolibok-Brągoszewska, A. Skarzyńska, W. Pląder and L. Chalak, 2020. Characterization of Lebanese germplasm of snake melon (*Cucumis melo* subsp. *melo* var. *flexuosus*) using morphological traits and SSR markers. *Agronomy*, Vol. 10. 10.3390/agronomy10091293.
25. Liu, C., Y. Ge, D.J. Wang, X. Li, X.X. Yang, C.S. Cui and S.P. Qu, 2013. Morphological and molecular diversity in a germplasm collection of seed pumpkin. *Sci. Hortic.*, 154: 8-16.
26. Esmailnia, E., M. Arefrad, S. Shabani, M. Karimi, F. Vafadar and A. Dehestani, 2015. Genetic diversity and phylogenetic relationship of Iranian indigenous cucurbits investigated by Inter Simple Sequence Repeat (ISSR) markers. *Biharean Biologist*, 9: 47-54.
27. El-Esawi, M.A., K. Germaine, P. Bourke and R. Malone, 2016. Genetic diversity and population structure of *Brassica oleracea* germplasm in Ireland using SSR markers. *C.R. Biol.*, 339: 133-140.
28. Koussao, S., V. Gracen, I. Asante, E.Y. Danquah and J.T. Ouedraogo *et al.*, 2014. Diversity analysis of sweet potato (*Ipomoea batatas* [L.] Lam) germplasm from Burkina Faso using morphological and simple sequence repeats markers. *Afr. J. Biotechnol.*, 13: 729-742.
29. Lv, J., J. Qi, Q. Shi, D. Shen and S. Zhang *et al.*, 2012. Genetic diversity and population structure of cucumber (*Cucumis sativus* L.). *PLoS ONE*, Vol. 7. 10.1371/journal.pone.0046919.
30. Forrester, O.O., E.O. Maurice, W. Victor, O.O. James, K. Laura and K. Paul, 2015. Genetic diversity of Bambara groundnut (*Vigna subterranea* (L.) verdc.) landraces in Kenya using microsatellite markers. *Afr. J. Biotechnol.*, 14: 283-291.