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GENETIC DIVERSITY OF TOMATO *(SOLANUM LYCOPERSICUM)* ACCESSIONS USING CHLOROPLAST DNA AND RANDOM AMPLIFIED POLYMORPHIC DNA MARKERS

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ABSTRACT

Information regarding genetic diversity and genetic relationships among different genotypes is invaluable in crop improvement of which its success is largely dependent on genetic variability. As molecular markers continue to be an effective tool for localization of a gene to improvement of plant varieties, the need to establish phylogenetic relationships becomes extremely important for the process of breeding new cultivars. This study reports genetic diversity for Tomato accessions across Nigeria (Six geo-Political Zones) as revealed by chloroplast DNA (cpDNA) and Random Amplified Polymorphic DNA (RAPD) markers. cpDNA data showed a Nucleotide diversity for all accessions of Tomato at 0.302 while the number of segregated sites as well as parsimony informative sites to be 4.0. Data from five Random Amplified Polymorphic DNA (RAPD) primers showed a low Gene diversity (h) which ranged from 0.10 to 0.28, Percentage polymorphism (Pp) ranged from 20 % to 84%. The Unweighted Pair Group Method with Arithmetic Mean (UPGMA) dendrogram grouped the accessions into two groups at similarity coefficient of 72%, with a value of r = 0.98, showing an excellent correlation between the accessions and the values of the initial distances (similarity matrix). Similar results were seen with the dendrogram constructed for genetic relationships of Tomato accessions using RAPD or cpDNA (you have mentioned RAPD up) markers. Split tree analysis equally revealed a 2 structured gene pool for Tomato gene pool in Nigeria. The high homogeneity of species observed in the study signifies a low genetic diversity and limited variability in tomato species in Nigeria. Amongst the consequences of low genetic diversity is the absence of unique variants that can possibly combat disease conditions or adapt to unfavourable environmental changes. The use of both cpDNA and RAPD markers in this study has efficiently shown that continuous cycling/shuffling of species within the narrow gene pool is expected to lead to a continuous reduction in genetic variability. No addition of new variant into the gene pool and in turn leads to inbreeding depression, thus suggesting the need to breed with known wild cultivars to increase genetic variability.

Key words: CpDNA, Gene pool, Genetic Diversity, Inbreeding depression, RAPD marker, Tomatoes breeding

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INTRODUCTION

Modern plant breeding has succeeded spectacularly in raising crop productivity in line with the rising human population. Practicably, efficient breeding depends mainly on the available genetic diversity and trait inheritance [1]. The modern trend of breeding for a particular specific trait/gene has brought considerable genetic uniformity among recent cultivars despite the use of different parent lines. This breeding strategy gradually continues to reduce the genetic variability or the amount of new diversity introduced into the breeding gene pool since only favourable alleles will continue to be selected and fixed. The breeders' desire to develop better and higher-yielding varieties unwittingly causes the loss of genetic diversity and in turn, hampers broad genetic diversity, which is the primary basis for successful plant breeding and the successful development of adaptations to environmental conditions [2, 3].

The success of most crop improvement programs is largely dependent upon the genetic variability and the heritability of desirable traits [4, 5]. The presence of genetic variability among crop genotypes depicts the richness of the gene pool and assures plant breeders of the possibilities of combating subsequent food security crisis for the crop. Genetic diversity exposes the genetic variability in different populations and rationalises introgression and ideotype breeding programmes to enhance crop performance [6]. Sufficient genetic variation, or diversity, in target traits (resistance to diseases and pests, stress conditions such as coldness, drought, salinization, and enhancing quality) is a requirement for progress in plant breeding [7].

Molecular markers have been used as application tools ranging from localization of a gene to improvement of plant varieties by marker-assisted selection [8]. They have also become extremely popular markers for phylogenetic analysis adding new dimensions to the evolutionary theories. Hence, establishing phylogenetic relationships is extremely important for the process of breeding new cultivars, which can be enriched with functional traits derived [9].

In the last two decades, they have been improved to provide easy, fast and automated assistance to scientists and breeders. Genome analysis based on molecular markers has generated a vast amount of information and a number of databases are being generated to preserve and popularize it [10].

In angiosperms, the Chloroplast DNA (cpDNA) is inherited through the maternal lineage, has a low mutation rate, and is rarely subjected to recombination [11,12,13]. According to Guo *et al.* [14], cpDNA sequences can also be used to determine the estimates of genetic diversity which provide inferences on the

evolutionary history of plant species. Equally, Qiong *et al.* [15] also reports the possibility of identifying recolonization routes, diversification events, gene flow, ecologically important species, such as those with a high degree of endemism [16], medicinal plants [17] especially, those with commercial potential [18]. Chloroplast DNA have become very useful tools for determining phylogenetic relationships and studying plant populations [9,19, 20]. Techniques such as the Restriction Fragment Length Polymorphism (RFLP) [21]; Amplified Fragment Length polymorphism (AFLP) [22]; Random Amplified Polymorphic DNA (RAPD) [23]; [24] and Simple Sequence Repeat (SSR) [25]. As well as a combination of markers (AFLP/cpDNA) have been employed to evaluate genetic diversity in plants [26].

Tomato (*Solanum lycopersicum L.),* is one of the world's most important food crops largely consumed and grown on wide environmental conditions [27]. According to the Food and Agricultural Organization, the annual global tomato production is approximately 161 million tons produced on about 4.8 million hectares [28]. It is a single cultivated species with a number of wild relatives that have vast morphological variability and diverse environmental adaptability [29, 30]. Currently, it has become one of the most famous and widely consumed vegetable cultivated on adaptable environmental conditions including field, green houses and plastic tunnels. It is versatile in nature as fresh and/or processed and is a globally adapted food commodity [27] and as a result, has been of keen interest to plant breeders and biotechnologists [31, 32, 33]. Tomato provides an excellent model system to study genetic diversity due to its high self-fertility and homozygosity [34]. The ease of controlled pollination and hybridization, diploid species (small genome: 950 Mbp), has equally been identified and reported [30]. The lack of gene duplication, the ability to develop haploids, and availability of a wide array of mutants and genetic stocks (including wild species) are important characteristics of tomatoes which makes the plant an important model for genetic diversity studies [30].

Morphological markers such as size, form and colour of fruit and plant size have been used to identify and collect different genotypes of tomatoes, however, limitations greatly influenced by environmental factors at developmental stages of the plant has hinder the accurate use of this markers [35]. In recent years, there has been a significant increase in the application of molecular genetic methods for assessing diversity, conservation and use of plant genetic resources. Therefore, applying the Chloroplast DNA (cpDNA) and Random Amplified Polymorphic DNA (RAPD) markers to assess genetic diversity for Tomato accessions across Nigeria provides opportunities to increase the understanding of the distribution and extent of genetic variation within and between tomatoes accessions.

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MATERIALS AND METHODS

Seed collection and Tomato Nursery Development

Thirty-Five accessions of tomatoes including germplasm accessions used for this study were obtained from different major markets in at least 1 State representing the six agro-ecological zones in Nigeria. Two exotic lines (Asia) was also obtained from a supermarket in Abuja making a total of Thirty-seven tomatoes samples (Table 1). In order to obtain leaf materials for DNA extraction, all the genotypes were planted in the screen house at the Biotechnology Advanced Research Centre, Sheda Science and Technology Complex, Abuja in two replications (Figure 1). Four to six seeds were planted in each nursery bag and later thinned to two seedlings per bag after germination. At three weeks old, two young leaves of each replicate were harvested, put in sample papers and preserved at -20 °C prior to DNA extraction.

Plate 1A-D Accessions of Tomatoes planted in the screen house

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DNA isolation

Total genomic DNA was extracted from fresh leaves using CTAB method [36] with the following modifications: 700 µl of CTAB buffer were used for initial incubation, 500 µl of isopropanol were used for DNA precipitation with two subsequent washing steps using 100 µl of 70% ethanol each. DNA was then dissolved in 200 µl 1xTris EDTA including 2µl RNase (10mg/mL-1) and stored in a -20 ^{o}C for further analysis.

Chloroplast DNA (CpDNA) Sequencing

The cpDNA trnH-psbA intergenic spacer was amplified using the primers trnH (gug) 5'- CGC GCA TGG TGG ATT CAC AAT CC-3' and psbA 5'-GTT ATG CAT GAA CGT AAT GCTC-3' [37]. The reaction mix of 25 µl contained 21.9 µl 1× MM OneTaq PCR Master Mix (New England BioLabs), 0.5 µl bovine serum albumin (10 mgml-1) (New England Biolabs, USA), 1 µl dimethyl sulfoxide (DMSO; Carl Roth, Karlsruhe, Germany),1 µl of template DNA, and 0.3 µl of each primer (10 µM). PCR reactions carried-out with 37 DNA samples was performed on a Master-Cycler X50a (Eppendorf, USA) with initial denaturation of 2 min at 95 ^{o}C followed by 35 cycles of denaturation at 95 ^{o}C for 1 min, annealing at 53 ^{o}C for 1 min and extension at 72 ^{o}C for 1 min, followed by a final extension step for 10 min at 72 ^{o}C [37]. PCR products were cleaned using the NucleoSpin1Extract II Kit (Macherey-Nagel, DuÈren, Germany), or the QIAquick1Gel Extraction Kit (Qiagen, Hilden, Germany). Sequencing was accomplished for both strands using 3730 DNA analyzer (Applied Biosystems, Foster City, USA) by the Eurofins Commercial laboratory with the primers used for PCR. Sequences were manually edited for bad quality bases and assembled in contigs using Geneious Pro v9.1.1 (Biomatters, Auckland, New Zealand). Sequences were aligned using the pairwise alignment algorithm implemented in Geneious Pro v9.1.1 1 (Biomatters, Auckland, New Zealand) and the alignments were manually refined [37].

Random Amplified Polymorphic DNA (RAPD)

In order to select the best RAPD markers analysis, twenty-two (22) RAPD primers (Eurofins MWG Operon, Germany) were pre-screened in three replicates and RAPD band reproducibility and scoring error were evaluated using the methods as described by [38] to select the best makers for further analysis of tomato DNA samples. The final Polymerase chain reaction (PCR) protocol for the tomato samples was carried out in a volume of 28 µl containing 20 µl 1× MM OneTaq master mix (New England BioLabs) containing 10x buffer [10 Mm TrisHCl], 2 mM $MgCl_2$, 500 µM deoxynucleotide triphosphates (dNTPs), 0.5 units of Taq DNA polymerase, 1 µl bovine serum albumin (BSA) (Thermo Scientific, Lithuania). 2µl primer and 5µl of total genomic DNA was also added to the PCR cocktail. This

screening was repeated three times to check reproducibility of the primer sets. Modifying the methods of Qadir *et al.* [39], PCR reactions was performed on Master-Cycler X50a (Eppendorf, USA), with initial denaturation of 3 min at 94 ^{o}C followed by 40 cycles of denaturation at 94 ^{o}C for 1min, annealing at 40 ^{o}C for 1 min and extension at 72 ^{o}C for 1 min, followed by a final extension for 10 min at 72 The amplified products were separated in 1% agarose gel electrophoresis using 1X TBE buffer stained with GR Green and Visualized on gel documentation system (Flour Shot LAB-KITS, Hong Kong). The molecular size of the amplification products was estimated using 1kb DNA ladder (Biolabs, New England). The number of monomorphic bands, number of polymorphic bands and intensity of bands were recorded.

Data Analysis

Indels from 37 cpDNA sequences were manually coded for presence and absence using the approach described by Simmons and Ochoterena [40] and treated as single polymorphic sites. A statistical parsimony network among cpDNA haplotypes was reconstructed using TCS v1.2 [41] with a default connection limit of 95%. The distance tree using clustering with the Maximum likelihood was constructed while the Nucleotide diversity, Number of segregating sites, Number of parsimony-informative sites were also calculated [42].

To create a binary matrix, amplified fragments of 500-3000bp were scored visually as having present (1), or absent (0) peaks in the output traces. Only distinct peaks were scored as present, and the manual scoring procedure was repeated many times on separate occasions to reduce scoring errors. The similarity index was calculated from the data using the Nei similarity index coefficient [42]. Taking the Hardy-Weinberg (HW) principle to be at equilibrium, all genetic diversity parameters were calculated using the POPGENE v1.32 software [43].

RESULTS AND DISCUSSION

Indices of haplotypic (cpDNA) diversity of Tomato *(Solanum lycopersicum)* accessions

The length of the analyzed trnH-psbA fragments ranged from 600 to 756 bp. Eight (8) nucleotide substitutions was detected. The length of the alignment was 756 bp and was reduced to 640 bp after manual editing sequences. Four parsimony-informative sites were shown. Newly generated sequences have been deposited in the GenBank and awaits GenBank Numbers (www.ncbi.nlm.nih.gov/genbank/). Two (2) haplotypes were identified, and the unrooted statistical parsimony haplotype network revealed two informal groups of haplotypes (Figure 3),

separated from each other by four mutations. cpDNA data also showed a Nucleotide diversity for all accessions of Tomato at 0.302 (Table 2).

RAPD Marker Selection

Reactions were carried out in triplicate using genomic DNA to test the reproducibility by checking the pattern of RAPD profiles of each sample. Five primers, OPA 2, OPD 1, OPJ 1, OPJ 2 and OPL 1 (Table 3) produced clear and reproducible polymorphic fragments in all samples, and all of the primers detected significant polymorphisms in the genomic DNA analysis. Equally, Primers with 40 – 60 % GC content ensure stable binding of primer and template. When the primer GC content is between 50 – 60 %, favorable amplification performance was obtained experimentally. According to Williams and St. Clair [23], RAPD primers should contain 40% G + C bases (usually containing 50 – 80 % G + C) or greater to generate detectable levels of amplification products.

Diversity estimates

Nei's genetic variation statistics for all loci within population is represented in Table 4. Gene diversity (h) varied from 0.10 to 0.28. Number of Polymorphic loci ranges from 4 to 21 while Percentage polymorphism (Pp) ranged from 24% to 84%.

The Cluster analysis

The Maximum likelihood average genetic clustering analysis of tomato sequences grouped the accessions into two groups at similarity level of 72%, with a value of r = 0.98, showing an excellent correlation between the accessions and the values of the initial distances (similarity matrix) (Figure 1). Two main Clusters was identified with Cluster 1 having Samples T5 (South West), T6 (South West), T 23 (South West) and T 26 (North West) clustering on different clades while T1 (South South) and T4 (South West), T16 (South West), T30 (South East) were grouped on the same clade. The rest samples were all grouped in Cluster 2 (Figure 1).

Similar results were seen with the dendrogram constructed for genetic relationships of Tomato accessions using RAPD markers (Figure 2). Dendrogram from RAPD makers showed Cluster A comprised of 1 sub-cluster with three accessions; South South: Panther (1), South East: NHGB/09/114 (30) and South West: NG/AA/SEP/09/051(5). The sub-cluster in this group one comprised of five accessions North West: Bull-Nose (26), South West: NG/AA/SEP/09/044 (23), South West: PHE-5 (Estelo) (29), South West: NG/AA/SEP/09/037 (27) and South West – NGBO 1250 (18) (Figure 2). Cluster B comprised of six sub-clusters; (i, ii, iii, iv, v, vi and vii) (Table 2). Sub-cluster i consists of 2 accessions North West: Bull Nose 1(2) and South West: NG/AA/SEP/09/042 (6). Sub-cluster ii comprised of 4

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accessions (North Central: Roma VF (3), South West: NG/AA/SEP/09/043 (35), South East: NHGB/09/113 (24), South West: NGBO 1665(7). Sub-cluster iii comprised of 10 accessions (South East: F1Thoral (37), South West: NG/AA/SEP/09/045 (16), North East: Rio Grande (17), South West: NGBO 1362 (20), South West: NG/AA/SEP/09/053(21), South West: NG/MR/MAY/09/005 (22), South South: Jos (Better Boy) (19), North West: NG/RM/JAN/10/001 (36), South West: NGBO 1301 (14), South West: NG/AA/SEP/09/040 (15). Sub-cluster iv. Consists of 3 accessions L00170 (10), South West: NG/OE/MAY/09/019 (11), South West: NG/MR/MAY/09/006 (12). Sub-cluster v comprised of 4 accessions South South: Benue (Sausage) (25), North West: NGHB/09/120 (34), South West – NG/AA/SEP/09/050 (33), North Central: UTC Variety (9). Sub-cluster vi comprised of five accessions South West: NGBO 1302 (4), South West: NG/SA/01/10/002 (8), L00169 (28), South South: NGBO 1649 (31) and North Central: Tropimech (32). Split tree analysis equally revealed a 2 structured gene pool for tomatoes in Nigeria (Figure 3).

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Figure 1: Maximum likelihood cluster of cpDNA Sequences of Tomato *(Solanum lycopersicum)* accessions

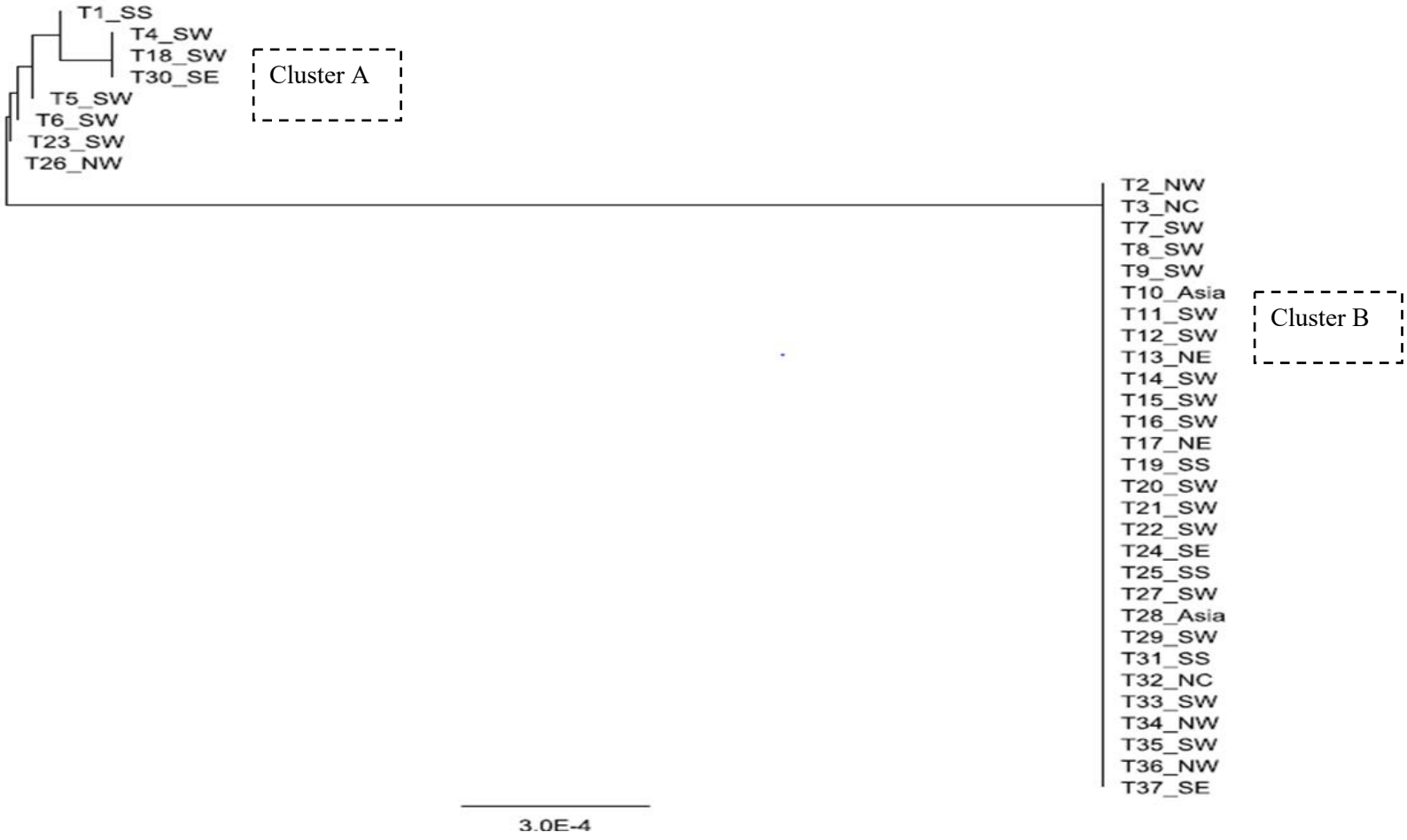

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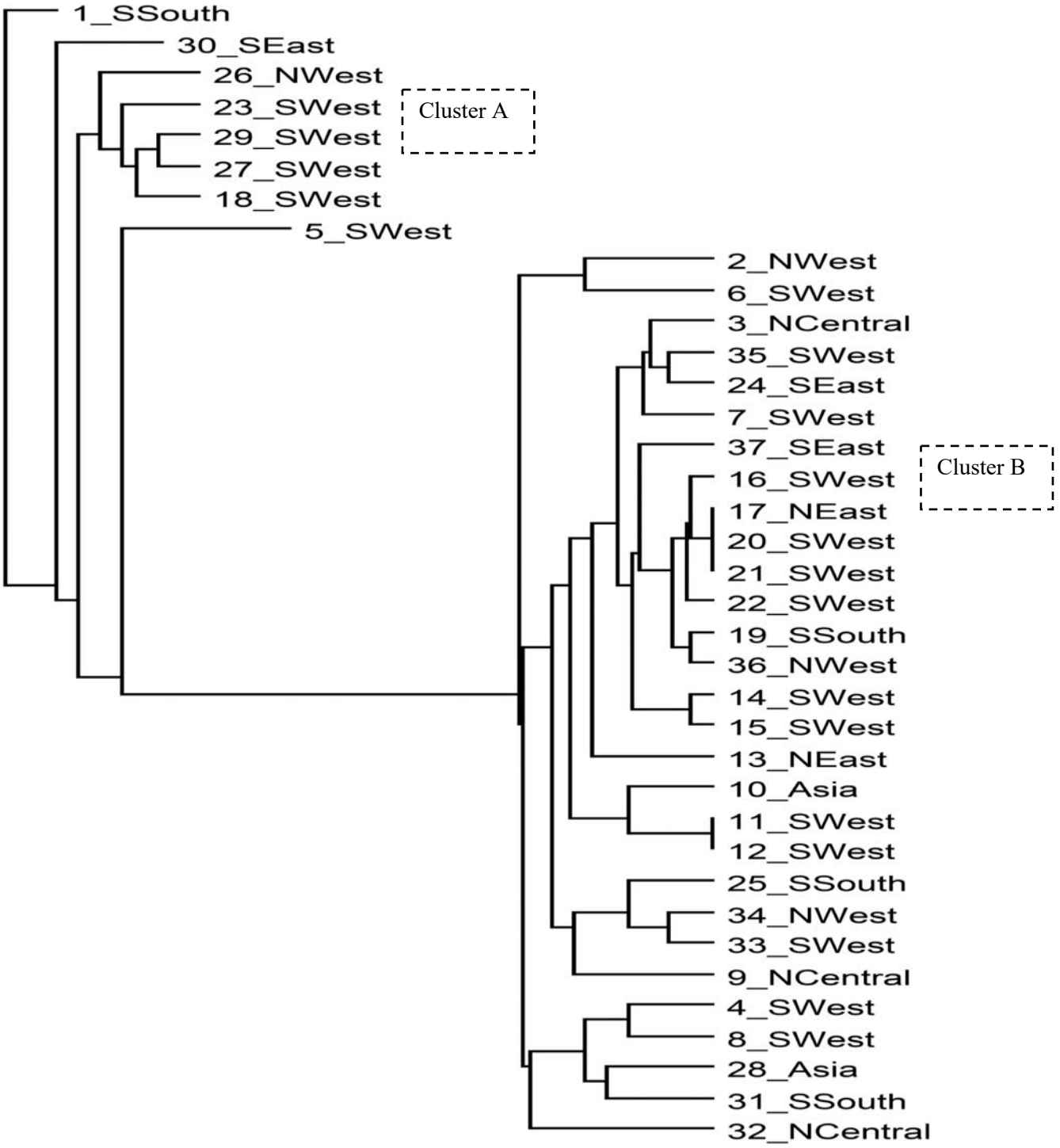
Figure 2: A UPGMA Dendrogram of genetic relationships of Tomato (*Solanum lycopersicum)* accessions using RAPD markers



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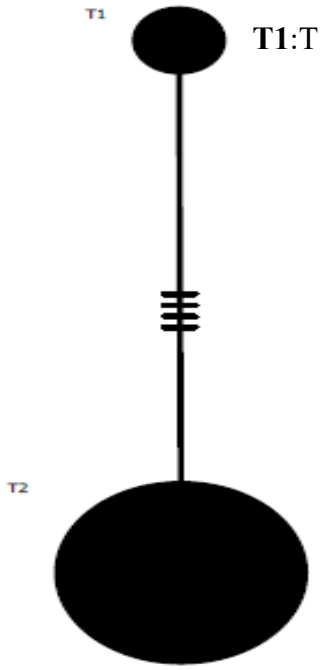

T1:T1, T4, T5, T6, T18, T23, T26, T30

T2: T2, T3, T7, T8, T9, T10, T11, T12, T13, T14, T15, T16, T17, T19, T20, T21, T22, T24, T25, T27, T28, T29, T31, T32, T33, T34, T35, T36, T37.

Figure 3: Statistical parsimony network based on trnH-psbA cpDNA sequences of tomato accessions

Genetic diversity of plant species reflects their breeding systems (Hamrick and Godt [44]. One of the major contributory factors to the low degree of polymorphism observed from tomato samples in this study may be on account of its evolutionary status as a selfing (in-breeding) angiosperm. This is in line with Achigan-Dako *et al.* [45] that immediate degree of polymorphism seen in some plants might be as a result of some level of autogamous breeding system. Generally, asexually reproducing species exhibit low levels of population diversity whereas sexually reproducing species show high levels of genetic variability [46]. Variation in genetic diversity within species according to Loveless [47] is usually related with geographic range, mode of reproduction mating systems, seed dispersal and reproduction rate as seen in this study.

Genetic diversity in wild tomatoes is said to be high [48] as seen especially within the self-incompatible species like *Solanum chilense* and *S. peruvianum* [48]. Sacks *et al.* [49], Villand *et al.* [50] and Egashira *et al.* [51] have all reported high variation using molecular markers and have all observed that these genetic variations were within a single accession of the self-incompatible species than in all accessions of any of the self-compatible species. Compared with the rich reservoir in wild species, the cultivated tomato is genetically poor [21]. It is estimated that the genomes of tomato cultivars contain, 5 % of the genetic variation of their wild relatives [21]. It has also

been reported that genetic diversity of cultivated tomatoes is extremely poor perhaps due to severe genetic bottlenecks suffered during its transportation from its centre of origin and on its path of domestication through Central America to Europe [52].

The dendrograms shown in figures 1 and 2also demonstrated that the tomato accessions were grouped into two gene-pools on the Neighbour-joining (Nexus) indicating a relatively low level of genetic variation. This result was consistent with that of Jemelková *et al.* [53] where (more than 80 %) Jordanian and Israeli samples were grouped into one compact cluster in on the Neighbour network in *Latuca aculeate* [54] where nearly 75 % of samples formed a compact cluster and showed a similar genetic background. Although modern tomato shows higher phenotypic diversity, there has been a loss of genetic diversity in modern cultivars as compared to the wild or landraces, low genetic diversity in cultivated tomatoes lines have equally been reported [55, 56].

Similar results were also found by de-Freitas *et al.* [57] who estimated the genetic distances among 14 genotypes of Brazilian wheat and a dendrogram and reported that despite the low variability found, two groups of genotypes could be identified, which probably reflected their pedigree. This could be explained by the fact that though most of these accessions were cultivated types from different geographic regions in Nigeria, they are of close originality. Irrespective that the two exotic line were from Asia, the lines also clustered with the Nigerian samples showing a likelihood of very narrow genetic diversity, just like when cultivated hybrids from the same parental lineage are compared [57].

This study shows that insufficient diversity exists in tomato crop in Nigeria signified by the low genetic diversity seen in the results. This is also consistent previous studies from [58, 59, 60, 61]. The high homogeneity of species observed in the study signifies a low genetic diversity and limited variability in tomato species in Nigeria. Interestingly too, Zhou *et al.* [62] observed a high variability amongst 29 cultivated, 14 wild tomatoes and seven introgression tomato lines in China. The disparity could be as a result of specific oligonucleotide primers used and geographical area of study in the case of Ezekiel *et al.* [35] while the wild species present in Zhou *et al.* [62] which are storage for many valuable genes, enlarge gene pool of cultivated species and sources of genetic variability have the tendency of introducing variability into populations respectively. Amongst the consequences of low genetic diversity is the absence of unique variants that can combat disease conditions or adapt to unfavourable environmental changes. According to Evans, [63], the breeding of crops over millennia for yield and productivity has led to reduced genetic diversity. As a result, valuable traits of wild species, such as disease resistance and stress tolerance, have been lost [64]. A very good example was the near-total eradication of tomato in Nigeria between

2016 and 2018 as a result of an outbreak of *Tuta absoluta* (tomato leaf-miner/tomato borer) that devastated the tomato crops in the field. The results of this study has informed the possibility that suitability to diseases in plants could be an attributed low genetic variability.

CONCLUSION

The use of both cpDNA and RAPD markers in this study has efficiently classified the selected tomato accessions according to their genetic make-up. Migration of species or artificial introduction of varieties from one point to the other did not played very significant role in determining variation in tomato thus the location of species did not determine their originality/ pedigree or genetic make-up. The continuous cycling/shuffling of species within the narrow gene pool is expected to lead to a continuous reduction in genetic variability, zero addition of new variability into the gene pool and in turn leads to inbreeding depression thus this study suggests the need to breed with known wild cultivars to increase genetic variability.

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Table 1: Sampling localities of Tomatoes Accessions studied

S/N	TOMATO ACCESSION	LOCATION	PLACE
T1.	Panther 17 F1	South South	Market in Asaba, Delta State
T2.	Bull Nose (Dan Eka)	North West	Market in Yola, Adamawa State
T3.	Roma VF	North Central	Market in Jos, Plateau State
T4.	NGBO 1202	South West	Gene bank, NACGRAB
T5.	NG/AA/SEP/09/15	South West	Gene bank, NACGRAB
T6.	NG/AA/SEP/09/041	South West	Gene bank, NACGRAB
T7.	NGBO 1665	South West	Gene bank, NACGRAB
T8.	NG/SA/01/10/002	South West	Gene bank, NACGRAB
T9.	UTC	South West	Gene bank, NACGRAB
T10.	L00169	Asia	Gene bank, NACGRAB
T11.	NG/OE/MAY/09/019	South West	Gene bank, NACGRAB
T12.	NG/MR/MAY/09/006	South West	Gene bank, NACGRAB
T13.	NG/AA/SEP/09/042	North East	Gene bank, NACGRAB
T14.	NGB01301	South West	Gene bank, NACGRAB
T15.	NG/AA/SEP/09/040	South West	Gene bank, NACGRAB
T16.	NG/AA/SEP/09/047	South West	Gene bank, NACGRAB
T17.	Rio-Grande	North East	Market in Bauchi, Bauchi State
T18.	NGBO 1250	South West	Market in Ibadan, Oyo State
T19.	Jos Better Boy	South South	Market in Uyo, Akwa-Ibom State
T20.	NG/AA/SEP/09/053	South West	Gene bank, NACGRAB
T21.	NG/AA/SEP/09/045	South West	Gene bank, NACGRAB
T22.	NG/MY/MAY/09/005	South West	Gene bank, NACGRAB
T23.	NG/AA/SEP/09/044	South West	Gene bank, NACGRAB
T24.	NHGB/09/113	South East	Gene bank, NACGRAB
T25.	Benue Sausage	South South	Market in Calabar, Cross Rivers State
T26.	Bullnose	North West	Market in Sokoto, Sokoto State
T27.	NG/AA/SEP/09/037	South West	Gene bank, NACGRAB
T28.	L00170	Asia	Gene bank, NACGRAB
T29.	PHE -5 (Estelo)	South West	Market in Lagos, Lagos State
T30.	NHGB/09/114	South East	Gene bank, NACGRAB
T31.	NGBO 1649	South South	Market in PH, Rivers State
T32.	Tropimech	North Central	Market in the FCT
T33.	NG/AA/SEP/09/050	South West	Gene bank, NACGRAB
T34.	NHGB/09/120	North West	Gene bank, NACGRAB
T35.	NG/AA/SEP/09/043	South West	Gene bank, NACGRAB
T36.	NG/RM/JAN/10/001	North West	Gene bank, NACGRAB
T37.	FI Thoral	South East	Market in Enugu, Enugu State

Key
T1-T37: Experimental Tags given to the samples
NACGRAB: National Centre for Genetic Resources and Biotechnology

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Table 2: Indices of haplotypic (cpDNA) diversity of Tomato (*Solanum lycopersicum*)-accessions

s/n	Item	Diversity Indices.
1.	Nucleotide diversity	0.302
2.	Number of segregating sites	4
3.	Number of parsimony-informative sites	4

Table 3: List of RAPD Decamer primers and their base sequences

S/N	Primer	Nucleotide sequence of Bases (5^1 - 3^1)	G + C content (%)
1.	OPA 2	TGCCGAGCTG	70%
2.	OPD 1	ACCGCGAAGG	70%
3.	OPJ 1	CCCGGCATAA	60%
4.	OPJ 2	CCCGTTGGGA	70%
5.	OPL 1	GGCATGACCT	60%

Table 4: Nei's Genetic Diversity statistics for population of tomato accessions

S/N	Geopolitical Zone	Sample size	h	PmL	Pp
1.	South South	4	0.260 ± 0.22	15	60%
2.	North West	6	0.220 ± 0.22	13	52%
3.	North East	2	0.120 ± 0.22	6	24%
4.	South West	17	0. 282 ± 0.20	21	84%
5.	South East	3	0.249 ± 0.23	4	56%
6.	North Central	2	0.249 ± 0.23	14	56%
7.	Exotic	2	0.200 ± 0.23	4	56%

h = Nei's genetic diversity, PmL = No. of Polymorphic loci, Pp = % polymorphism

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