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IDENTIFICATION AND GENETIC DIVERSITY OF TOMATO (*Solanum lycopersicum* L.) GERMPLASMS IN IBADAN METROPOLIS, NIGERIA

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ABSTRACT

The adoption of invasive tomato cultivars by peasant farmers has led to a decline in the cultivation of landraces. Presently, a huge number of tomatoes in Ibadan, Oyo State, Nigeria are either hybridized or foreign cultivars which are difficult to identify and classify. It is likely that new varieties may have arisen through anthropogenic influences and natural hybridization. This negligence of landraces has contributed to the limitation on the extent of their genetic diversity for effective breeding and conservation for present and future uses. Consequently, six tomato germplasms were collected from local farmers in Ibadan and had their genetic diversity assessed for improvement, identification, and documentation purposes. The germplasms had their young leaves subjected to DNA extraction, PCR analysis, Sanger sequencing at Inqaba Biotec West Africa, Ibadan. Basic Local Alignment Search Tool (BLAST) was used to confirm the identity of the landraces on the NCBI Genbank. The nucleotide sequences submitted to GenBank were assigned accession numbers: OR809185.1, OR809186.1, OR809187.1, OR809188.1, OR809189.1, and OR809190.1. The Bootstrap phylogenetic tree revealed genetic and evolutionary variation among accessions. The result of BLAST confirmed the identities of the germplasms with accessions in the Genbank as Grape tomato, Heirloom tomato, Red Brandywine, Red Beefsteak, and Cherry tomato for OR809185.1, OR809186.1, OR809187.1, OR809188.1, OR809189.1, and OR809190.1, respectively. The finding showed that variation by phylogenetic tree could be a potential source of genetic diversity for tomato crop improvement. It also documented Grape tomato, Grape tomato, Heirloom tomato, Red Brandywine, Red Beefsteak, and Cherry tomato as tomato varieties available in Ibadan metropolis.

Keywords: Tomato, landrace, classification, molecular biology, crop improvement

INTRODUCTION

Solanum lycopersicum L. (Tomato) is a tropical and herbaceous vegetable plant that belongs to the family Solanaceae with about 7500 varieties worldwide (Olayemi, 2000). It originated from South America and is cultivated in temperate and tropical regions of the world (Peralta and Spooner, 2005; Labate and Robertson, 2012). *Solanum lycopersicum* L.

is derived from two wild ancestor species, *Solanum pimpinellifolium* and *S. cerasiforme*. It has 12 wild relatives, all natives to western South America. These wild members demonstrate a high level of phenotypic and genetic variation, which could be useful for breeding disease resistance, colour improvement and desirable quality traits (Terzopoulos and Bebeli, 2010). Virtually all cultivated tomato fruits are red, but some cultivars produce fruits in other colours, such as green, yellow, orange, pink, black, brown, ivory, white, and purple (Adedeji *et al.*, 2006).

Tomato production accounts for approximately 14% of vegetable production worldwide (Osei *et al.*, 2014). The fruits are consumed whole, in salads, as juice, paste, and as the main ingredient in hundreds of dishes worldwide (Adedeji *et al.*, 2006; Saeed-Awan *et al.*, 2012; Qumer *et al.*, 2014). It is a regular ingredient of African meals and accounts for about 18% of the average daily consumption of vegetables in Nigeria (Ebimieowei and Ebideseghabofa, 2013). Tomato fruit is an excellent source of essential amino acids, vitamins, iron, fiber, mineral nutrients, sugars, and bioactive compounds, commonly known as secondary metabolites. Daily intake of tomatoes has been reported to provide the human body with essential nutrients like carotene, vitamins, and lycopene which lower the risk of cancer and cardiovascular diseases (Onifade *et al.*, 2013).

The adoption of invasive tomato cultivars by peasant farmers has led to a decline in the cultivation of landraces and this requires urgent evaluation for genetic improvement through breeding, conservation and ensures tomato wide gene pool. Considering the different types of tomato fruits available nowadays at the markets in Ibadan metropolis of Oyo State, it is obvious that invasive species capable of higher yields and more consistent fruit quality, are rapidly replacing landraces and this results in decline in cultivation of tomato landraces by farmers. The negligence of these landraces has contributed to the limitation on the extent of their genetic diversity for effective breeding and conservation for present and future uses.

The level of damages that the introduction of invasive species has done to the populations of tomato is unknown. At present, a huge number of tomato cultivars in Ibadan, Oyo State, Nigeria are either hybridized or foreign cultivars which are difficult to identify and classify. A large number of these landraces are known to have disappeared completely. This rate of disappearance needs to be checked to ensure wide genetic base for tomato improvement in the future.

Identification and estimation of the genetic diversity of plants is usually done using morphological and molecular markers (Osei *et al.*, 2014). Despite the fact that several morphological identification of tomato species have been done, the true identity of some members are still being contested. One of the challenges of tomato species identification in Nigeria is that there is paucity of information on the identity of these plants on molecular basis. However, several studies have reported the limitation on the use of morphological characters due to the interference of the environmental factors (Agudelo *et al.*, 2011; Bello *et al.*, 2013; Oyelakin *et al.*, 2021). Also, many authors had reported the use of molecular markers such as Simple Sequences Repeats (SSR) to unraveling the genetic diversity in tomato germplasm (Song *et al.*, 2006; Mazzucato *et al.*, 2008; Al-Aysh *et al.*, 2012). However, the information on the use of Internal Transcribed Spacer (ITS) marker for tomato germplasms from Ibadan in Oyo

state, Nigeria is scanty.

Therefore, this present study was initiated to assess the genetic diversity among tomato landrace germplasms collected from Ibadan metropolis for genetic improvement, identification and documentation for conservation purposes using the Internal Transcribed Spacer (ITS) marker.

MATERIALS AND METHODS

Sample Collection and Preparation

Six (6) accessions of *S. lycopersicum* were collected from local farmers within Ibadan metropolis in Oyo State, Nigeria (Plate 1). The fruits were identified as *S. lycopersicum* in the Herbarium of the Department of Pure and Applied Botany at the Federal University of Agriculture Abeokuta, Ogun State, Nigeria (Table 1). The fruits were extracted to obtain the seeds. Ten seeds of each accession were sown in perforated 5-liter buckets containing topsoil and were arranged in a screen house at the Department of Pure and Applied Botany, Federal University of Agriculture, Abeokuta, Nigeria. Pots were irrigated once every 2 days with water. No pesticides or fertilizers were applied. Two weeks after emergence, young fresh leaves were detached from each accession, preserved in Ziploc bags, and transported to Inqaba Biotec West Africa, Ibadan for genomic DNA extraction and molecular studies.

Genomic DNA Extraction, Quantification, Amplification, and Sequencing

A modified SDS-based DNA extraction method was employed on the young fresh leaves for DNA extraction, while DNA quantification was conducted using a NanoDrop Spectrophotometer at 260 nm. Amplification was carried out using PCR system thermal cycler (Applied Biosystem Inc., USA) with PCR profile of an initial denaturation, 94°C for 5 minutes; 35 cycles of 94°C for 30s, 55°C for 30s and 72°C for 1 minute 30 seconds; and a final extension at 72°C for 10 minutes after which the purified PCR products were subjected to Sanger Sequencing and the sequence chromatogram analysis was performed using BioEdit analysis software.

Table 1: **Genbank accession number, collection number, passport data, location coordinates and altitude of *Solanum lycopersicum* accessions studied**

S/N	GenBank Accession No	Collection number	Area of Collection	Local Government Area	State	Latitude NS	Longitude EW
1	OR809185	SpSl001	Ikereku	Oluyole	Oyo	7.6319 ° N	3.9753 ° E
2	OR809186	SpSl002	Moniya	Akinyele	Oyo	7.5249 ° N	3.9152 ° E
3	OR809187	OsSl003	Lagun village	Lagelu	Oyo	7.5399 ° N	4.0908° E
4	OR809188	MoSl004	Igbooloyin	Akinyele	Oyo	7.5332 ° N	3.9091 ° E
5	OR809189	MoSl005	Wofun village	Egbeda	Oyo	7.5332 ° N	3.9091 ° E
6	OR809190	MoSl006	Elebu village	Ido	Oyo	7.5332 ° N	3.9091 ° E

Key: NE= NorthEast , EW= EastWest

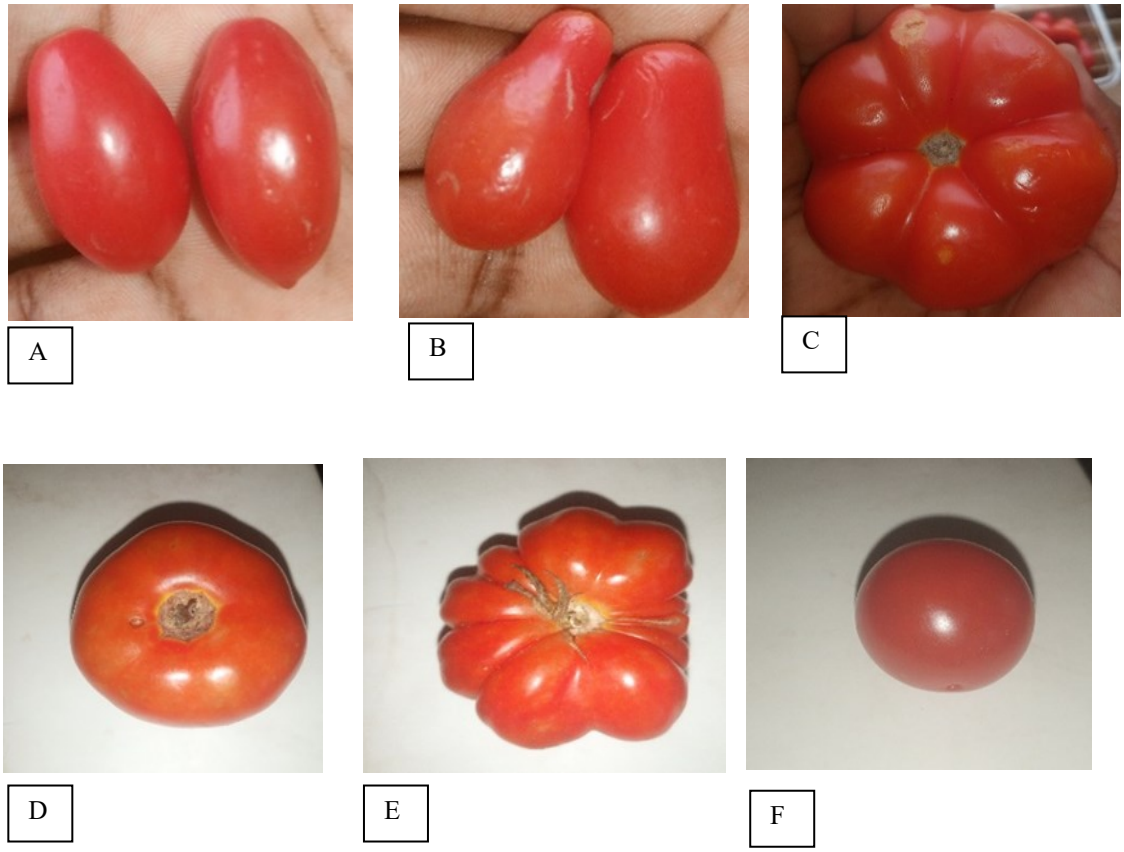

Plate 1: Tomato fruits collected from the local farmers

A: SpSl001, B: SpSl002, C: OsSl003, D: MoSl004, E: MoSl005, F: MoSl006

Data analysis

The Sanger sequences were aligned using ClustalW on BioEdit (ver. 7.2.5). The sequences were submitted to the NCBI GenBank and accession numbers were assigned. The codon usage indices and amino acid residues of tomato accessions were estimated using CodonW. The phylogenetic tree was constructed using the UPGMA method (Kumar *et al.*, 2018).

RESULTS

The results from the gel electrophoresis showed a clean DNA of 6 accessions (Plate 2). The Consensus nucleotide sequences are presented in the appendix.

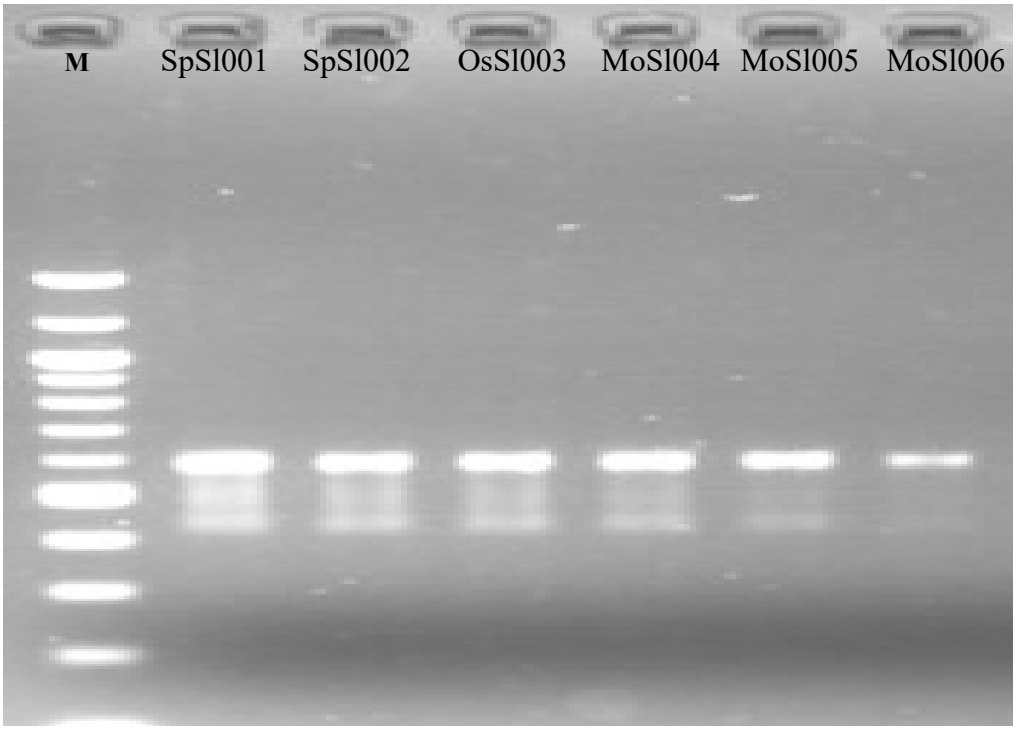

Plate 2. PCR products amplified using ITS1F and ITS4R primers and resolved on 2% Agarose gel electrophoresis M-100bp DNA ladder, A-F samples

Genbank Accession number, percentage nucleotide content of the ITS gene of *S. lycopersicum*

The submitted sequences of the collected accessions had 100% identity similarity with the matched organisms in NCBI Genbank with varied sequence length (bp) of 544 in *OR809185 to 660 in* OR809190 (Table 2). The percentage nucleotide contents of the 6 accessions for % GC ranged from 64.49 in OR809190 to 65.40 in OR809186 while % T (U) ranged from 14.88 in OR809185 to 16.50 in OR809186 and OR809190. The % C varied from 33.00 in OR809186 to 35.11 in OR809186 while % A ranged from 18.10 in OR809186 to 20.60 in OR809185. The % G ranged from 29.41 in OR809185 to 32.40 in OR809186 (Table 3). DNA base composition represented as G + C content, where G + C percentage in the six accessions ranged from 64.52% in OR809185 to 65.40% in OR809186 with an average of 65% in all. GC (guanine-cytosine) was dominant over AT (adenine-thymine) in the composition of nucleotides (Table 3).

Table 2: Summary of the deposited sequences, Genbank accession numbers, matched organism, and sequence length and BLAST result

Sequence Name	GenBank Accession No	Matched Organism	Matched variety	% Identity SL	Sequence length
Solanum lycopersicum breed Grape Tomato internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	*OR809185.1*	*Solanum lycopersicum*	grape tomato	100%	544
Solanum lycopersicum breed Grape Tomato internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	OR809186.1	*Solanum lycopersicum*	Grape tomato	100%	547
Solanum lycopersicum breed Heirloom tomato internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	OR809187.1	*Solanum lycopersicum*	Heirloom tomato	100%	654
Solanum lycopersicum breed Red Brandywine internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	OR809188.1	*Solanum lycopersicum*	Red brandywine	100%	657
Solanum lycopersicum breed Red beefsteak internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	OR809189.1	*Solanum lycopersicum*	Red beefsteak	100%	657
Solanum lycopersicum breed Cherry tomato internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	OR809190.1	*Solanum lycopersicum*	Cherry tomato	100%	660

Key: SL= *Solanum lycopersicum*

Table 3: Genbank Accession number, percentage nucleotide content of the ITS gene of *S. lycopersicum*

S/N	GenBank Accession Number	% GC	T%	%C	%A	%G	% AT
1	OR809185	64.52	14.88	35.11	20.60	29.41	35.48
2	OR809186	65.40	16.50	33.00	18.10	32.40	34.60
3	OR809187	64.66	16.20	34.86	19.11	29.80	35.31
4	OR809188	65.00	16.00	35.00	19.00	30.00	35.00
5	OR809189	65.00	16.00	35.00	19.00	30.00	35.00
6	OR809190	64.49	16.50	34.80	18.90	29.69	35.40
	Average	65.00	16.01	34.63	19.11	30.21	35.13

Key: GC= (Guanine-Cytosine), T= Thymine, C= Cytosine, A= Adenine, G= Guanine, AT= (Adenine-Thymine)

Amino Acid Composition of six Accessions of S. lycopersicum

All the accessions had identical alanine content of 13 with the exception of OR809186.1 which has the highest alanine content of 16. The glycine content ranged from 8 to 12, with most accessions having a value of 8. Accession OR809186.1 had the lowest proline content of 9 while OR809185.1 had highest proline content of 12. All the accessions had identical arginine content of 17 except OR809186.1 with the lowest arginine content of 13. The serine content of the six accessions ranged from 10 to 11, with most accessions having a value of 10. The phenylalanine, histidine, lycine, tryptophan, and tyrosine contents of the all accessions was low, ranging from 0 to 1, but most accessions had value of 1. However, none of the accessions had methionine content, with all having 0 values (Table 4).

Table 4: Amino Acid molecular weight profile of the *Solanum lycopersicum* sequences

Accession No	Ala	Cys	Asp	Glu	Phe	Gly	His	Ile	Lys	Leu	Met	Asn	Pro	Gln	Arg	Ser	Thr	Val	Trp	Tyr	Total
OR809185.1	13	2	3	4	1	9	2	5	1	7	0	4	12	3	14	11	5	2	1	0	161
OR809186.1	16	2	2	2	1	12	1	4	1	8	0	4	9	3	13	10	6	4	1	0	164
OR809187.1	13	3	2	2	1	8	1	4	1	7	0	4	10	4	17	10	6	4	1	1	206
OR809188.1	13	3	2	2	1	8	1	4	1	6	0	4	10	4	17	10	6	4	1	1	207
OR809189.1	13	3	2	2	1	8	1	4	1	6	0	4	11	4	17	10	6	3	1	1	206
OR809190.1	13	3	2	2	1	8	1	4	1	6	0	4	10	4	17	11	6	4	1	1	208
Average	13	3	2	3	1	9	1	4	1	7	0	4	10	4	16	10	6	4	1	1	192

Key: Ala- Alanine, Cys- Cysteine, Asp- Aspartate, Glu- Glutamine, Phe- Phenylalanine, Gly- Glycine, His-Histidine, Ile- Isoleucine, Lys-Lysine, Leu- Leucine, Met- Methionine, Asn- Asparagine, Pro- Proline, Gln- Glutamine, Arg- Arganine, Ser- Serine, Thr- Threonine, Val- Valine, Trp- Tryptophan, Tyr- Tyrosine

Codon Usage Bias of six Accessions of *S. lycopersicum*

The number of times each codon is used to encode each amino acid, as well as the Relative Synonymous Codon Usage (RSCU) value for each codon. The RSCU value is a measure of how frequently a codon is used relative to the most frequently used codon for the same amino acid. A value of 1 indicates that the codon is used as frequently as expected by chance, while a value greater than 1 indicates that the codon is used more frequently than expected. RSCU values falls between 0.19 and 2.92 (Table 5). It reveals some interesting patterns of codon usage in the gene. For example, the codon AUG, which codes for the amino acid M (methionine), is the start codon. It is the only codon that can initiate translation, and it has an RSCU value of 1, indicating that it is used as expected by chance.

Table 5: Codon usage bias: Relative Synonymous Codon Usage (RSCU) and count for *Solanum lycopersicum* ITS nucleotides

Codon	Count	RSCU	Codon	Count	RSCU	Codon	Count	RSCU	Codon	Count	RSCU
UUU(F)	1.7	1.82	UCU(S)	5.7	1.71	UAU(Y)	0.7	1	UGU(C)	1.7	0.63
UUC(F)	0.2	0.18	UCC(S)	1.3	0.4	UAC(Y)	0.7	1	UGC(C)	3.7	1.38
UUA(L)	0.3	0.15	UCA(S)	2.5	0.76	UAA(*)	3.2	1.3	UGA(*)	3.2	1.3
UUG(L)	2.7	1.23	UCG(S)	5.8	1.76	UAG(*)	1	0.41	UGG(W)	2.3	1
CUU(L)	2	0.92	CCU(P)	2.3	0.48	CAU(H)	2	1.41	CGU(R)	2.8	0.55
CUC(L)	6.3	2.92	CCC(P)	9.7	1.98	CAC(H)	0.8	0.59	CGC(R)	10.3	2
CUA(L)	1.2	0.54	CCA(P)	0.5	0.1	CAA(Q)	6.2	1.76	CGA(R)	5.8	1.13
CUG(L)	0.5	0.23	CCG(P)	7	1.44	CAG(Q)	0.8	0.24	CGG(R)	8.7	1.68
AUU(I)	0.7	0.26	ACU(T)	1.7	0.6	AAU(N)	2	0.53	AGU(S)	1	0.3
AUC(I)	4.2	1.6	ACC(T)	3.5	1.25	AAC(N)	5.5	1.47	AGC(S)	3.5	1.06
AUA(I)	3	1.15	ACA(T)	2.8	1.01	AAA(K)	0	0	AGA(R)	1.8	0.35
AUG(M)	0.7	1	ACG(T)	3.2	1.13	AAG(K)	1.7	2	AGG(R)	1.5	0.29
GUU(V)	1.7	0.95	GCU(A)	2.8	0.44	GAU(D)	0.3	0.16	GGU(G)	1.2	0.27
GUC(V)	3	1.71	GCC(A)	6.8	1.06	GAC(D)	3.8	1.84	GGC(G)	6.7	1.55
GUA(V)	0.3	0.19	GCA(A)	2.3	0.36	GAA(E)	3.3	1.33	GGA(G)	4.7	1.09
GUG(V)	2	1.14	GCG(A)	13.8	2.14	GAG(E)	1.7	0.67	GGG(G)	4.7	1.09
Average# codons=19											

Bootstrap Phylogenetic tree of six Accessions of *S. lycopersicum*

Aligned sequences were used to create phylogenetic trees using the maximum composite likelihood model and bootstrap resampling. Gene sequences from the six accessions were used to create the phylogenetic tree. The numbers at branch nodes are bootstrap values, which show the percentage of bootstrap iterations that support the tree at that specific point of divergence for each phylogenetic tree node. The more the topology of the phylogenetic tree is supported, the higher the bootstrap value (Figure 1).

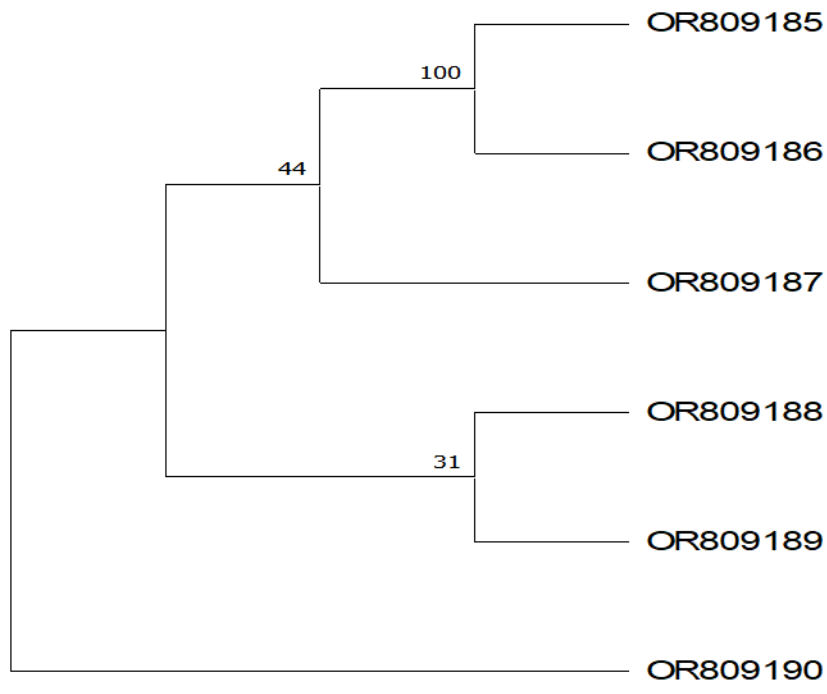

Figure 1: Bootstrap Phylogenetic tree showing evolutionary relationship existing among tomato germplasms using the Neighbor-Joining method

DISCUSSION

Identification of plant species using a molecular approach is not a new concept and has been adopted by many researchers for delimitation, hybridization, classification, breeding and genetic improvements of crops (Zang *et al.,* 2016; Oyelakin *et al.*, 2019; Popoola *et al.*, 2023). The current research demonstrates that DNA identification approach effectively distinguishes all samples at the species level, emphasizing the reliability of Internal Transcribed Spacer (ITS) marker as identification marker. This marker proves accurate for amplification and sequencing, underscoring its crucial role in identification of plants at species level. Analyzing nucleotide quantities and genetic variation among germplasms sheds light on the uniqueness of individual in the population.

In this study, the DNA identification approach differentiated the six accessions up to species level. This result corresponds to the report of Naim and Mahboob (2020) on the

reliability of DNA approach in species identification. Oyelakin *et al.* (2019) reported DNA identification approach as one of the accurate methods to amplify and sequence, for tracing evolutionary relationships and identifying species in plants. The result of this study clearly distinguished individual accession, with no overlap. This result is in line with the report of Chen *et al.* (2021).

The higher amino acid contents of alanine, glycine, proline, arginine, and serine in the six accessions is an indication that the landraces contain useful traits which would make them suitable to be improved upon and subsequently recommended for cultivation to both peasant and commercial farmers.

The stop codons UAA(*), UAG(*) and UGA(*) signal the termination of translation as recorded in this study means that UAA and UGA are used more often as stop codons, and that UAG is rarely or never used. The codon CUC, which codes for leucine with the highest RSCU value of 2.92, indicates a strong codon bias. This suggests that CUC is favoured over other leucine codons, such as CUG, CUA, and CUU, which have RSCU values of 0.23, 0.54, and 0.92, respectively. The RSCU values of between 0 and 2.92 recorded in this study indicates some degree of codon usage bias in the gene, and reflects the influence of factors such as mutation, selection, and gene expression level. This study corroborates the works of Popoola *et al.* (2023).

In the phylogenetic tree, accession OR809185 and OR809186 are closely linked and demonstrated to be closely related with a bootstrap support value of 100 (cluster 1), indicating that it is strongly supported, whereas OR809188, and OR809189 grouped together (cluster 2) with a bootstrap value of 31, shows that these two accessions are distantly related and there is high genetic variation between the accessions. An isolated OR809189 indicates high genetic diversity with other accessions. Accessions OR809185 and OR809186 are closely linked with OR809187 with a moderate bootstrap support rating of 41 (cluster 3). This indicates high genetic diversity among the accessions.

The result of BLAST with 100% sequence matching showed that six tomato accessions from different areas within Ibadan metropolis belong to the same species of *S. lycopersicum*. This implies that some landraces of *S. lycopersicum* in Ibadan with certain changes in morphology which may not be morphologically evident but exist at genetic level due to natural hybridization and cross pollination has been addressed by the findings from this study.

In Ibadan, there is no scientific document to show the available tomato varieties at molecular level. A few attempts have been on the basis of morphology without any molecular basis ever utilized to authenticate the morphological markers for proper identification of these plants.

Conclusion

The phylogenetic trees that resulted from this study provided strong evidence of phylogenetic relationships among the accessions investigated. The samples investigated formed a monophyletic clade with very strong support, suggesting that the investigated samples evolved from a common ancestor of *S. lycopersicum*. The result of BLAST confirmed the identities of the collected germplasms with the Genbank accessions as grape tomato, grape tomato, heirloom tomato, red brandywine, red beefsteak, and cherry

tomato for OR809185.1, OR809186.1, OR809187.1, OR809188.1, OR809189.1, and OR809190.1, respectively and therefore resolved identical topologies. This present study has shown grape tomato, heirloom tomato, red brandywine, red beefsteak, and cherry tomato as tomato varieties available in Ibadan metropolis of Oyo State, Nigeria.

RECOMMENDATION

The genetic diversity among the collected landrace germplasm in this study could be a potential source of improvement for tomato landraces. These tomato landraces are hereby recommended to breeders for improvement.

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