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Full Length Research Paper

Genetic diversity in 14 tomato (*Lycopersicon esculentum* Mill.) varieties in Nigerian markets by RAPD-PCR technique

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A total of 226 tomato (*Lycopersicon esculentum* Mill.) fruits sold in markets within three states in Southwestern Nigeria were purchased and differentiated into 14 varietal groups based on morphological parameters. The genomic DNA from the young apical leaves of the seeded plants were extracted and analyzed by the randomly amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) technique. Seventy-four (74) amplified products were scored with 62.2% of them revealing polymorphism. The OPB-18 primer recorded the highest polymorphism (83.3%) and OPU-14 the least (44.4%). The dendrogram revealed 3 clusters. The genotypic relationships between varieties within clusters ranged from 2 in clusters 1 and 3 to 10 in cluster 2. Cluster 2 revealed 4 subgroups with two pairs of varieties (var. c and i and l and m) showing 100% similarities. The local names, place of cultivation and phenotypic characters expressed by the varieties were not suitable for varietal differentiation since phenotypically different fruits showed genotypic relatedness. The impression that tomato varieties from a particular geographical zone are genetically similar was therefore nullified.

Key words: Tomato, randomly amplified polymorphic DNA, polymerase chain reaction, molecular, genetic diversity, *Lycopersicum*.

INTRODUCTION

Tomato (*Solanum lycopersicum* Mill.), a highly perishable fruit vegetable of the family Solanaceae, has gained wide use in every Nigerian home as Nigeria ranks highest in Africa in terms of tomato production, 879,000 t/annum (Grubben and Denton, 2004). *Lycopersicon esculentum* Mill. is one of the two taxa found in West Africa (Heine, 1963). Its members are diploid (2n) with chromosome number of 24 (Grubben and Denton, 2004). Emerging molecular techniques such as the gel electrophoresis for crude leaf proteins (Azeez and Morakinyo, 2004), the randomly-amplified polymorphic DNA (RAPD) and sequence-tagged micro-satellites (SSR) have helped to trace genotypic relationships among vintage and modern accessions (Temiesak et al., 1993; Noli et al., 1999; Archak et al., 2002; Rajput et al., 2006; Singh et al., 2007). The earlier method of plant identification based on morphology has a number of limitations that resulted to the search for precise tools for identification of plant species and varieties.

The RAPD technique is a powerful tool for identification and monitoring pedigree breeding record of inbred parents or varieties, evaluation in test crosses (Baird et al., 1992; Echt et al., 1992; Struss et al., 1992) and determining genetic relationships among genotypes (Vierling and Nguyen, 1992). It is an updated plant varietal identification method that is independent of restriction sites and is employed in the detection of polymorphisms by using the polymerase chain reaction (PCR) technology (Welsh and McCleland, 1990). This technique which uses specific oligonucleotide primers is highly sensitive and avoids many of the technical limitations of the restriction

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fragment length polymorphism (RFLP) in mapping traits and fingerprinting individuals for crop improvement (Carlson et al., 1991; Klein-Lankhorst et al., 1991; Rafalsik et al., 1991; Waugh and Powell, 1992). However, the reproducibility of amplified products is usually low (Rajput et al., 2006).

Odeigah et al. (1999) reported the application of the SDS-polyacrylamide gel electrophoresis in the characterization of Nigerian varieties of pepper, *Capsicum annum* and *Capsicum frutescens*. However, to the best of our knowledge, there has been no application of RAPD-PCR in the varietal classification of Nigerian tomatoes containing local cultivars as well as the introduced types and their hybrids. Several researches on the tomato rots including physiological studies of the rot-inducing pathogens, changes in nutritional composition of the fruits during rotting and control of rot induction in the Nigerian cultivars/varieties have not considered the genetic variability (Fajola, 1978; Wokocha and Ebenebe, 1985; Okoli and Erinle, 1990; Oladiran and Iwu, 1992; Bankole, 1996). In essence, these authors have used only the traditional morphological classification scheme to separate fruits into varieties in their studies. However, estimation of genetic variability on the basis of morphological observations alone may not be reliable, since the phenotype is an expression of the genotype and environment (Falconer, 1989). Therefore, there is a need to trace the genotypic relationship of tomatoes using characters from tomato fruits available in markets across Southwestern Nigeria for breeding and crop improvement programs.

MATERIALS AND METHODS

Market survey and sample collection

Tomato fruits available in seven major market depots within three states of Southwestern Nigeria were purchased in clean zip-lock bags and taken to the laboratory immediately for further studies. The markets are located at Mushin and Mile 12 in Lagos state, Shasha and Dugbe in Oyo state and Atakumosa and Sabo in Osun state. A total of 70, 81 and 75 tomato fruits collected from each state were used for this study. As part of the market survey, the traders were asked specific questions to determine the following: Locations where the tomatoes are cultivated, where they are transported from and local names for each tomato variety.

Characterization of varieties

The sample fruits were classified morphologically and molecularly. Features including fruit shape, size, number of lobes and local names were employed in the morphological characterization. while the RAPD technology for DNA polymorphism was used in ascertaining specific genetic variation among varieties (Welsh and McCleland, 1990).

Sample preparation for RAPD characterization

All the seeds in the randomly selected fruits from the 14 groups of tomato variety (according to our morphological classification parameters) were removed from the fruits, sun-dried for 2 days and planted in triplicate rows on a shallow bed of 60 x 45 cm spacing. The bed was watered twice daily till young apical leaves were prominent for collection.

Genomic DNA extraction

Young apical tomato leaves were pooled from three plants per variety group and used in the genomic DNA extraction. Total genomic DNA extraction was done by grinding 0.5 g of the leaf samples in 1.5 ml Eppendorf tube using liquid nitrogen. The mixture was suspended in 600 µl of 2xCTAB buffer (50 mM Tris, pH 8.0; 0.7 mM NaCl; 10 mM EDTA; 2% hexadecyltrimethylammonium bromide; 0.1% 2-mercaptoethanol), and incubated at 65°C for 20 min. DNA was purified by extracting twice with phenol : chloroform : isoamyl alcohol (24:25:1) and precipitated with -20°C absolute ethanol. After washing with 70% ethanol, the DNA was air-dried and re-suspended in 200 µl of sterile distilled water. The DNA concentration was then measured using the DU-65UV spectrophotometer (Beckman Instruments Inc., Fullerto CA, USA) at 260 nm. DNA degradation and quality was also checked by electrophoresis on a 1% agarose gel in 1xTAE (45 mM Tris-acetate, 1 mM EDTA, pH 8.0).

RAPD-PCR analysis

Decamer oligonucleotide primers (Operon Technologies Inc., USA) were used for the RAPD-PCR analysis. Two concentrations of each tomato DNA (24 and 96 ng per reaction) were prepared for use to test reproducibility and eliminate sporadic amplification products from the analysis. A total of 275 arbitrary decamer oligonucleotide primers (OPM, OPY, OPX and series) were screened with the DNA of at least four tomatoes samples for their ability to amplify the tomato DNA. After the initial primers screening analyses, potentially useful primers revealing high levels of DNA polymorphism among the isolates, were used. Each of the selected primers was used to amplify the DNA on the basis of a single primer per reaction. Amplifications were performed in 25 µl reaction mixture consisting of the DNA, 1x reaction buffer (Promega, USA), 100 µM each of dATP, dCTP, dGTP and dTTP, 0.2 µM Operon random primer, 2.5 µM $MgCl_2$ and 1 U of *Taq* polymerase (Boehringer, Germany). The amplification mixtures were kept in a thermowell microtiter plate (Costa Corporation) and amplified in a PCR machine (Perkin Elmer Programmable Thermal Controller, Model 9600). The cycling programs for DNA amplification consisted of 1 cycle of 3 min at 94°C followed by 45 cycles of 20 s at 94°C for denaturation, 20 s at 37°C for primer annealing and 40 s at 72°C for extension. A final extension period was for 7 min at 72°C.

The amplification products were loaded on 1.4% agarose gel using TAE buffer (45 mM Tris-acetate, 1 mM EDTA, pH 8.0) at 100 V for 2 h. A 1 kb marker (Life Technologies, Gaithersburg, MD, USA) was included for use as molecular DNA size marker. A loading buffer was mixed with 10 µl of each PCR amplification product before it was applied on agarose gel for electrophoresis. Gels were visualized by staining with ethidium bromide solution (0.5 µg ml^{-1}). The photograph of the gel was taken over UV light using a red filter.

Cluster analysis

The positions of reproducible and consistent RAPD bands were scored and transformed into a binary character matrix (“1” for the presence and “0” for the absence of a RAPD band at a particular position). A phylogenetic tree was created by the unweighted pair-group method arithmetic (UPGMA) average cluster analysis using the output data and the graphical module of the NTSYS-pc software

Table 1. Classification of the tomato varieties based on some classical parameters.

Code	Variety local name	Place of cultivation	Fruit shape and description	[+]Mean fruit size (cm)	Number of fruit lobe
a	Yoruba	Agbara	Round	2.8 × 4.6	5
b	Hausa (Scissors)	Kano	Oblong; pointed tip	6.7 × 3.0	3
c	Hausa	Zaria	Oval; pointed tip	4.5 × 3.7	3
d	Hausa	Sokoto	Oval; shield-like	4.5 × 3.5	6
e	Beske (Ebira)	Okene	Oval; folded-in base	3.2 × 3.3	3
f	Hausa	Kano	Oval	2.8 × 2.6	2
g	Tiwantiwa	Kajola	Oval	5.9 × 3.5	2
h	Beske	Kajola	Round	3.7 × 5.5	3
i	Hausa	Sokoto	Oblong	6.1 × 3.3	2 – 3
j	Hausa	Sokoto	Oblong; banana-like	5.8 × 3.0	2 – 3
k	Hausa (Scissors)	Kano	Oblong; pointed tip	5.9 × 2.6	2 – 3
l	Yoruba	Ogbomosho	Round	4.6 × 6.2	7 – 8
m	Hausa	Zaria	Oblong	4.2 × 2.9	3 – 4
n	Beske (Ebira)	Okene	Round	4.2 × 4.3	2

[+]Mean fruit size (cm) of triplicate fruits.

(Swofford and Olsen, 1990).

RESULTS

Morphological characterization

A total of 226 tomato fruits were collected and morphologically differentiated into 14 types (a to n) based on fruit shape, size and number of lobes (Table 1). The local names and place of cultivation were also included as the latter played a key role in the assigning of local names to the varieties by the traders. The shapes (Figure 1a to n) ranged from round through oval to oblong, while the number of lobes ranged from 2 to 8. The mean length by diameter values of the fruit varieties were between 2.8 × 2.6 and 6.7 × 3.0 cm.

RAPD-PCR characterization of varieties

The initial amplification of the pooled DNA from the varieties by the decamer oligonucleotide primers (OPM, OPY, OPX and series) resulted in the use of only 10 highly amplifying primers, which showed reproducibility of results in the final amplification (Table 2). A total of 74 amplified products of sizes ranging from 200 to 3100 bp were visualized with mean amplified fragment per primer being 7.4. The OPA-15, OPG-17 and OPU-14 primers were the highest fragment amplifiers (9 fragments), while the OPC-09 was the least (2 fragments). In terms of band polymorphism, a total of 46 bands of the 74 revealed polymorphism with an average of 4.6 polymorphic bands per primer. The OPB-18 primer recorded the highest percentage polymorphism (83.3%) as it revealed 5 polymorphic bands in 6 amplified fragments, while the OPU-14 primer recorded the least percentage (44.4%) by revealing 4 polymorphic bands in 9 amplified fragments. Figures 2 and 3 shows the amplified bands of the variety DNAs using the OPB-18 and OPC-09 primers, respectively.

The phylogenetic tree (Figure 4) was created by the UPGMA average gene cluster analysis. The dendrogram revealed 3 clusters, 1 to 3, which joined at 44% level of similarity to form one major cluster. Cluster 1 is composed of accessions a and h which joined at 88% similarity level, while cluster 2 consists of ten accessions which also joined at 88% level of similarity to form one distinct cluster. Meanwhile, clusters 1 and 2 joined to form one major cluster, having 12 accessions at 77% level of similarity, whereas all the accessions formed one major cluster at 44% level of similarity. On the other hand, cluster 3 with two accessions (d and g) appears to be the most diverse of the total accessions, since both accessions jointly formed a cluster with the rest at 44% similarity level. Accessions i and c and accessions l and m were joined to each other at 100% level of similarities, respectively. Generally, there was no evidence that the local names, place of cultivation and phenotypic characters expressed by the varieties were best suitable for varietal differentiation since phenotypically different fruits showed genotypic relatedness. Varieties e and j found in cluster 2 are genotypically related but morphological characters reveal large differences.

DISCUSSION

The possibility and application of the RAPD technique in varietal identification of tomato have been well explored (Temiesak et al., 1993; Noli et al., 1999; Rajput et al.,

Figure 1. Tomato varieties available in some Nigerian markets (see Table 1 for a to n).

Table 2. List of RAPD primers that showed polymorphism.

Primer code	Sequence	Amplified fragment	Polymorphic band	Polymorphism (%)
OPA-14	5'-TCTGTGCTGG-3'	8	5	62.5
OPA-15	5'-TTCCGAACCC-3'	9	7	77.8
OPB-17	5'-AGGGAACGAG-3'	7	4	57.1
OPB-18	5'-CCACAGCAGT-3'	6	5	83.3
OPC-08	5'-TGGACCGGTG-3'	6	3	50.0
OPC-09	5'-CTCACCGTCC-3'	5	3	60.0
OPG-17	5'-ACGACCGACA-3'	9	5	55.5
OPU-03	5'-CTATGCCGAC-3'	8	5	62.5
OPU-14	5'-TGGGTCCCTC-3'	9	4	44.4
OPV-19	5'-GGGTGTGCAG-3'	7	5	71.4
Total no. of bands		74	46	---
Mean bands per primer		7.4	4.6	---
Mean (%) polymorphic bands		---	---	62.2

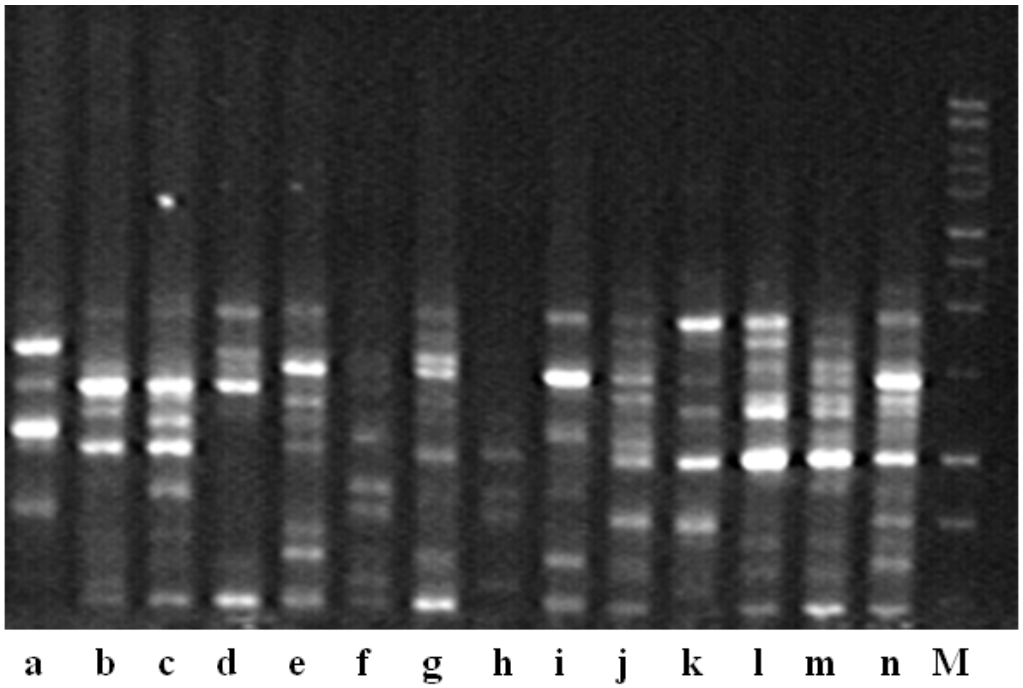

Figure 2. RAPD profiles of tomato varieties (a - n) generated using OPB-18 primer. M = marker (200 to 3100 bp) (see Table 1 for a to n).

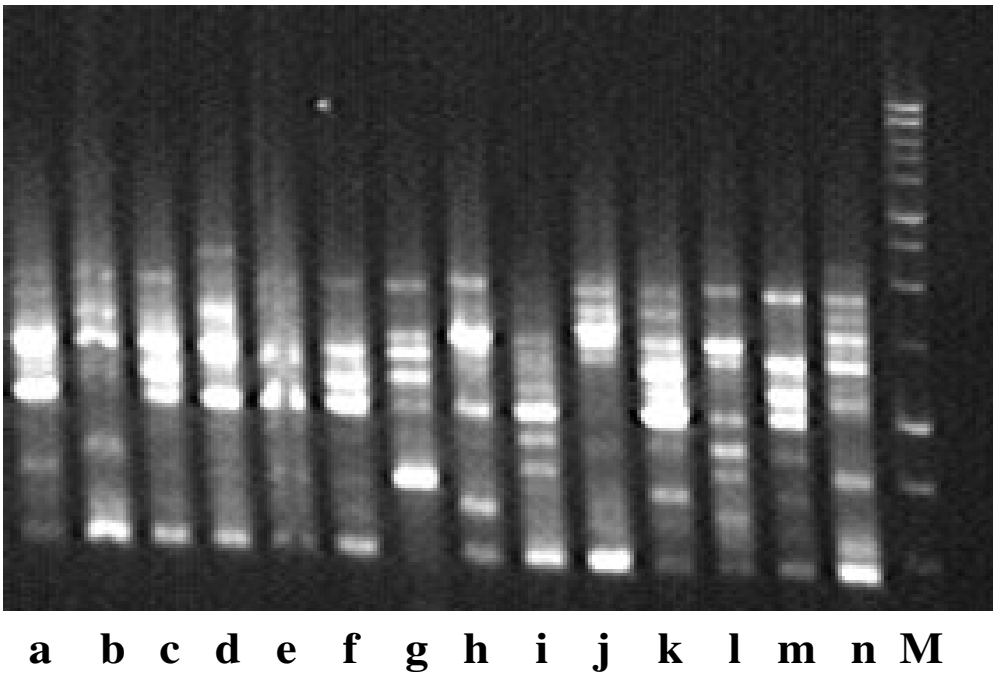

Figure 3. RAPD profiles of tomato varieties (a to n) generated using OPC-09 primer. M = marker (200 to 3100 bp) (see Table 1 for a to n).

2006; Singh et al., 2007) since the successful construction of the RAPD markers for mapping the genes of tomato by Klein-Lankhorst et al. (1991). The similarity indices of the RAPD dendrogram ranged between 44 and 100% and were averagely high enough to suggest useful variability for genetic conservation and plant breeding

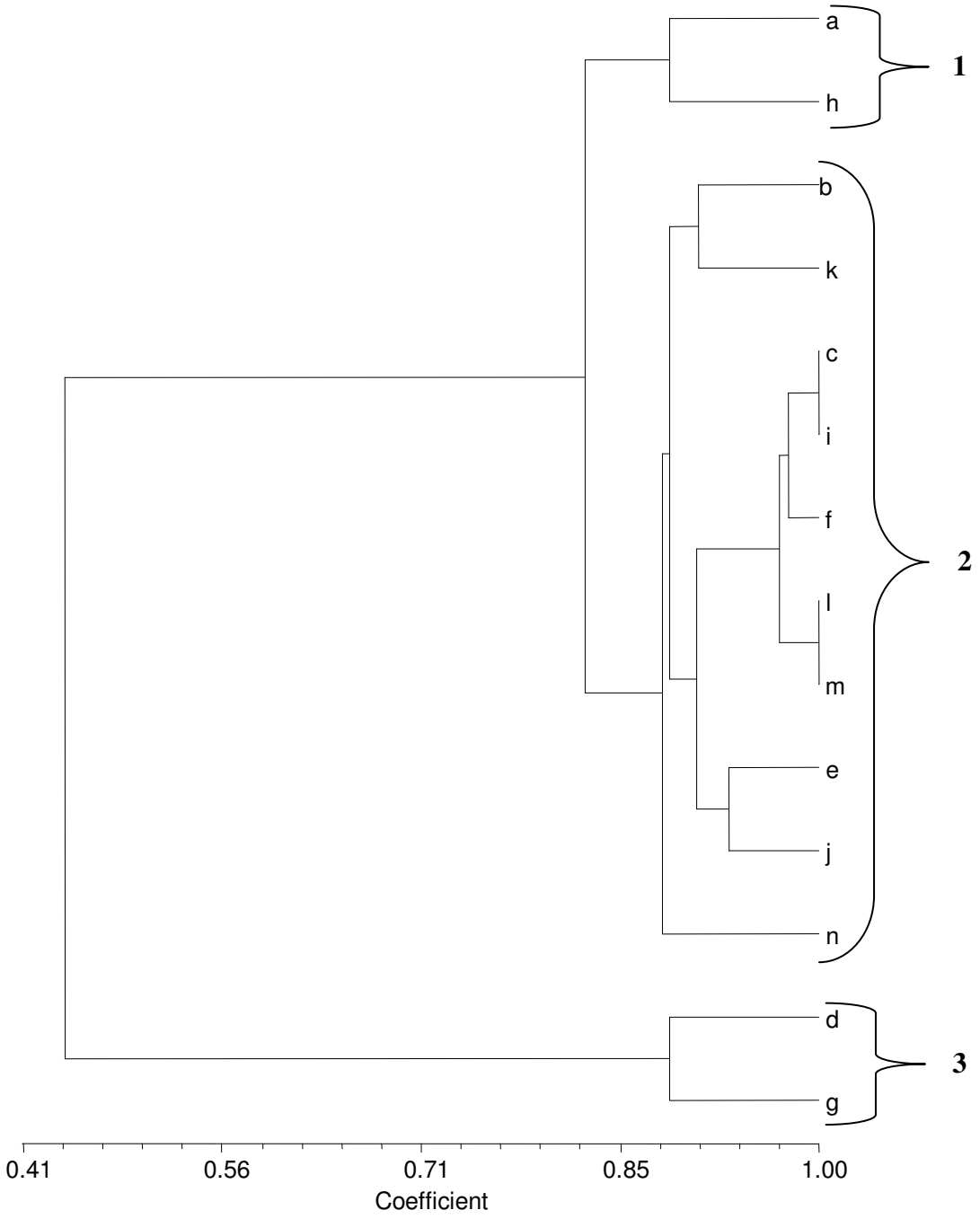

Figure 4. Dendrogram obtained from RAPD analysis using UPGMA (see Table 1 for a to n).

(Torkpo et al., 2006). Accessions i and c with 100% similarity are likely to be clones and this agrees with the same local names given to the accessions in different locality. This suggests that these two accessions must have the same center of origin. Similarly, accessions l and m which also have 100% similarity are likely to be clones, this suggests error in the classification of this two accessions based on morphological characters. This further suggests that the accessions must have been introduced from one locality to the other and assigned a new name. The impression that the varieties of tomato from a particular geographical zone are genetically similar may not be true; though, the phenotypic expressions and possible local trade names given by traders or farmers may be the same. The reverse is also true as revealed by this study. Thus, the phenotypic variations exhibited by closely-related genotypes may be attributed to response to environmental influences (Falconer, 1989).

Hence, the tomato accessions may not be varieties in the real essence as thought of by the farmers but just slight accessions, hybrids and a few clones. So, the difference could be said to be as a result of environmental interaction of the accessions in the different environments and the adaptive mechanisms developed over time to facilitate good growth in the area of cultivation at that point in time.

Conclusion

The use of RAPD was very effective in classifying more accurately, the tomato accessions genotypically. The result showed that eco-geographical difference may not necessarily determine the distribution of diversity in tomato crop in Nigeria. There is sufficient diversity in tomato crop in Nigeria. The result further indicates that accessions Hausa which is grown in Zaria and Hausa cultivated in Sokoto are indeed the same though having slightly different fruit shape and size. Similarly, there was an error in the classification of accessions l (Yoruba) and m (Hausa) as different varieties as result shows that they are indeed the same. Characterization of tomato based on a combination of molecular and morphological traits may be most reliable in determining the differences among the tomato varieties and eliminate the error of assigning different names to the varieties or vice-versa. This research provides insight for further studies on breeding programs for tomato research and improvement in Nigeria.

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