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Evaluation of Genetic Diversity of Local and Exotic Tomatoes using Morphological Traits and Simple Sequence Repeat Molecular Markers

Omoyeni, D. I., Fayeun, L. S., Odiyi,, A. C., Mogaji, B. O., and Adegbaju, M. S.

Department of Crop, Soil and Pest Management, The Federal University of Technology, Akure, Nigeria.

Correspondence: ibukunomoyeni3@gmail.com

ABSTRACT

Tomato being a crop of global importance and the necessity of germplasm knowledge for breeding and conservation lead to this study which investigated the genetic diversity among local, exotic tomato varieties, and their progenies. The research employed both morphological traits and simple sequence repeat molecular markers. Means for the morphological traits studied; including growth and yield traits were calculated and subjected to Principal Component Analysis and Single Linkage Cluster Analysis. Molecular data were organized into a binary data matrix, inputed into Numerical Taxonomy and Multivariate Analysis System Software to generate the similarity matrix and dendrogram using the Unweighted Pair Group Method with Arithmetic Mean. Results from this study revealed significant genetic diversity among the tomato genotypes. However, differences were observed in genotype clustering when comparing morphological and molecular characterization, although some genotypes showed consistent grouping across both methods. The study concludes by recommending the combined application of morphological traits and molecular markers for comprehensive diversity studies, leveraging their complementary effects for more robust insights into tomato germplasm.

Keywords: Diversity; Molecular; Morphological; Genotypes; Markers.

Introduction

Tomato (*Solanum lycopersicum* Mill.) is known to be the second-most significant vegetable crop after potato and it is a member of the *Solanaceae* family (FAOSTAT, 2019). Several countries in the world cultivate it since it can adapt to different soil and climatic conditions. On the average, 376 ton of tomatoes are grown per hectare all over the world (FAOSTAT, 2019). It supplies low calorie and it is likewise a great source of fiber, phenols, minerals, vitamins A, C, and E; including lycopene and β-carotene which are antioxidants; this makes it a great "functional food" that supplies nutrients that are essential in human's body (Giovannetti *et al.*, 2012; Saleem *et al.*, 2013). Zhu *et al.* (2018) and Verma *et al.* (2020) reported that a number of factors influence tomato fruits' quality, these include appearance, color intensity, size, shape, flavor, nutritional content, and texture of the tomato's pericarp, which determines a consumer's acceptability of it. Knowledge of germplasm diversity is vital to achieve conservation and plant breeding aims, by creating variations and increasing output and productivity, (Poczai *et al.*, 2011). Nikoumanesh *et al.* (2011) mentioned that an approach that is mostly used to quantify genetic differences and for genetic diversity analysis is the morphological description and classification. Most often, principal component analysis (PCA) and single linkage cluster analysis (SLCA) are multivariate statistical techniques employed by researchers to

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measure crop genetic diversity for morphological characters (Odiyi *et al.*, 2014; Fayeun *et al.*, 2018). Molecular markers are known to be a reliable method for the identification of plant genotypes (Cao *et al.*, 2015). Simple Sequence Repeat (SSR) molecular markers are one of the most widely used because of their co-dominance, polymorphism and reproducibility attributes (Powell *et al.*, 1996).

In Nigeria, there are limited literatures on tomato breeding programs that focus on both morphological and molecular attributes (Ibitoye, 2020). Molecular markers provide an effective approach to efficiently select desired agronomic traits because the assessment is based on the plant genotypes and are not affected by variation in the environment. Although, a high level of accuracy is not assured on morphological data due to the stressful nature of collection (Fiorani and Schurr, 2013), they cannot be neglected because they are still needed for assessing the genetic diversity and breeding value of crops (Salim *et al.*, 2020). Thus, using a combination of morphological and molecular markers to identify variation in plant is gaining popularity (Naz *et al.*, 2013, Zhou *et al.*, 2015; Herison *et al.*, 2018) and a greater level of research accuracy can be assured. Thus, provision of information on the level of genetic diversity that exists among the tomato genotypes studied using both morphological and molecular markers are necessary. This study aimed to investigate the magnitude of genetic diversity in some local and exotic tomato varieties and their progenies using morphological traits and SSR molecular markers.

Materials and Methods

Study location

The location for this experiment is the screen house situated at the Teaching and Research Farm, Federal University of Technology, Akure (FUTA), Ondo State, Nigeria (7º16'N, 05º12'E). The experiment was carried out from September, 2021 to July 2023.

Collection of tomato genotypes

Fifteen tomato genotypes were selected at random from different groupings based on the fruit shape. This is because fruit shape was reported to be an important morphological feature that distinguishes tomato varieties (Adeniji *et al.*, 2020). The tomato genotypes used are nine (9) parent materials, including exotic and local varieties which were sourced from different locations in Nigeria, and six progenies generated from cross fertilization. The procedures highlighted for cross-pollination of tomato plants by Chetelat and Peacock (2013) was followed. The parent materials and their progenies, source of collection and acronyms are presented in Table 1. Completely Randomized Design (CRD) was used as the experimental design.

Experimental procedures

Twenty kilogramme of sterilized topsoil were put in bags and organized in three replications inside the screen house. The tomato seeds were planted in the nursery and nurtured for five weeks. Seedlings of the genotypes were transplanted into the appropriate bags at two seedlings per bag. Four bags were used for each of the fifteen genotypes in each replication. The descriptors used for the plants and fruits, and the method of assessment of the morphological traits studied in this research were those highlighted by the International Plant Genetic Resource Institute (IPGRI, 1996).

Agronomic data that were collected were number of fruits per plant, leaf length, number of days to 50% flowering, number of days to maturity, plant height at harvest, leaf width, plant height at flowering, fruit size, fruit weight, fruit length, thickness of fruit wall and fruit width. To collect data on leaf length and leaf width; ten leaves were randomly selected on each genotype in each of the three replications and measured. Similarly, for the fruit size, fruit weight, fruit length, thickness of fruit wall and fruit width; ten physiologically matured tomato fruits were randomly selected from each genotype, harvested and measured. But for the plant height, all the eight tomato plants for each genotype in the three replications were measured. A micro weighing scale was used to measure the weight of the tomatoes. A measuring tape was used to measure the plant height, leaf length and leaf width. A vernier caliper was used to measure

Table 1: Tomato Parent Materials, their Progenies, Source of Collection and Code

S/N	Genotype	Source of Collection	Code
1	Alausa	Ondo	ALS
2	Ibadan local	Ibadan	IBL
3	Ikaram 1	Ikaram	IKR 1
4	Ikaram 2	Ikaram	IKR 2
5	Igedegede	Igedegede-Akoko	IGED
6	UC82B	NIHORT	UC82B
7	Padma	Commercial seed store	PADM
8	Platinum	Commercial seed store	PLA
9	Diva F1	Commercial seed store	DIV
	Progenies		
10	Ibadan local × Diva F1	Crop Soil and Pest Management (CSP Department, FUTA	IBL×DIV
11	Ikaram 1 × Igedegede	CSP Department, FUTA	IKR1×IGED
12	Ikaram 1× Roma VF	CSP Department, FUTA	IKR1×RMVF
13	Diva F1 × Ibadan local	CSP Department, FUTA	DIV×IBL
14	Ibadan local × Beske	CSP Department, FUTA	IBL×BSK
15	Igedegede × Padma	CSP Department, FUTA	IGED×PAD

the thickness of the tomato fruit wall. Mean data for both growth and yield traits were standardized and subjected to multivariate analysis, and single linkage cluster analysis were performed using PAST, Version 3.25.

Molecular characterization involved deoxy-ribose nucleic acid (DNA) extraction, polymerase chain reaction (PCR) and gel electrophoresis. Extraction of DNA was carried out at Biosafety Research Laboratory, FUTA, Ondo State, Nigeria. Checking of the DNA quality and PCR was done at Drosophila laboratory, FUTA. Nanodrop spectrophotometer was used in checking the DNA quality. Concentration and absorbance of the DNA samples at 260 and 280 wave length was also recorded and presented in Appendix. Molecular analysis was carried out using 4 polymorphic simple sequence repeat (SSR) markers that were selected and their characteristics are presented in Table 2. Sequence database have generated many molecular markers that have been applied for identification and analysis of the genetic diversity among tomato cultivars successfully (Korir *et al.*, 2014).

The PCR forward and reverse primers were designed in three steps. Firstly, literature search was done on variability studies in tomato using SSR molecular markers. Out of the four primers that were synthesized, three among them are primers that housed quantitative trait loci (QTLs) in their regions that have been reported to affect fruit shape and/or size as revealed by the findings of Mazzucato *et al.*, (2008). The fourth primer was selected from a variability study on tomato by Solomon *et al.*, 2008, where they used SSR markers for differentiation of tomato genotypes. Secondly, primers that were highly polymorphic were selected. Thirdly, the sequences of the polymorphic markers were copied and forwarded to Inqaba Biotech, South Africa for synthesis of the primers. These markers were used to assess the genetic variability of the tomato genotypes. Gel electrophoresis of the extracted DNA was done at Molecular Laboratory, School of Life Sciences,

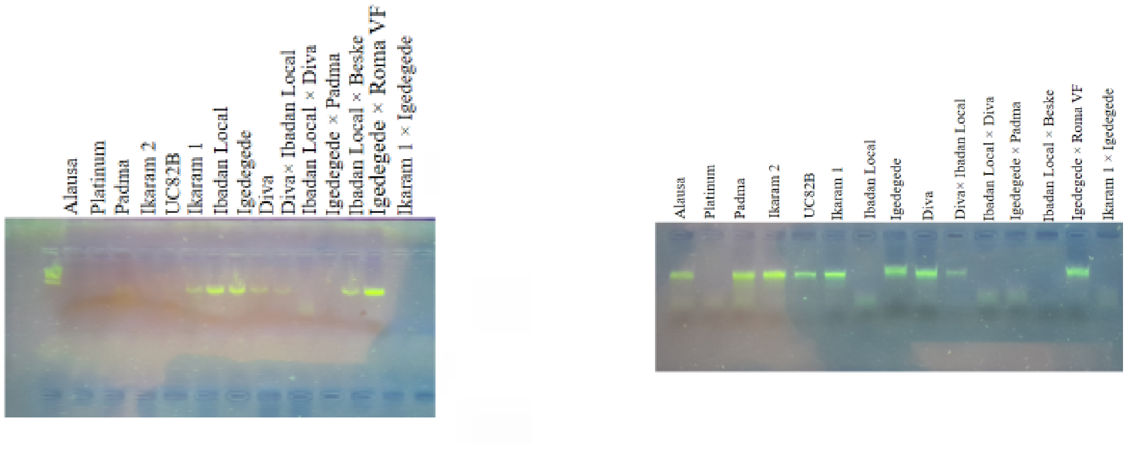
Table 2: Characteristics of the Polymorphic SSR Markers Used

S/N	Name of Primers	Annealing Temperature	Sequence	Linkage to reported QTLs controlling fruit shape	Allele No
1	EST253712 (Areshchenkova and Ganal, 2002)	55°C	Forward Primer: GAA ATG AAG CTC TGA CAT CAA A Reverse Primer: TCA TTG CTT GCA TAT GTT CAT G	*MF6a* (1)	4
2	Tom 236-237 (Suliman-Pollatschek *et al.*, 2002)	47°C	Forward Primer: GTT TTT TCA ACA TCA AAG AGC T Reverse Primer: GGA TAG GTT TCG TTA GTG AAC T	*nsf9.1*, *nX9.3* (12)	9-3/9-3-2
3	TMS60 (Areshchenkova and Ganal, 2002)	60°C	Forward Primer: GCA GGT ACG CAC GCA TAT AT Reverse Primer: GCT CCG TCA GGA ATT CTC TC	*fw1.1*(3), *sblk1.1, hrt1.1*, *nsf1.1*, *nX1.1* (12)	3
4	SSR 139 (Solomon Benor *et al.*, 2008)	–	Forward Primer: TGG GTA TGG GAT TTA CAC CAA Reverse Primer: AAA CGA AGG CAA CAA CGA AG	Not linked to fruit shape	8

FUTA. The gel images are shown in Figure 1. The band images from gel electrophoresis were scored. The cells where band is present is scored 1 while cells where band is absent is scored 0.

Data analysis

The means of the growth and yield parameters were calculated and principal component analysis and single linkage cluster analysis were performed using the PAST package. A software called "Numerical Taxonomy and Multivariate Analysis System for personal computer" (Ntsys-pc), version 2.1e was used for analyzing the molecular data. Before using the software, the raw data was firstly organized into a binary data matrix where each row represents a sample (each tomato variety) and each column represents a

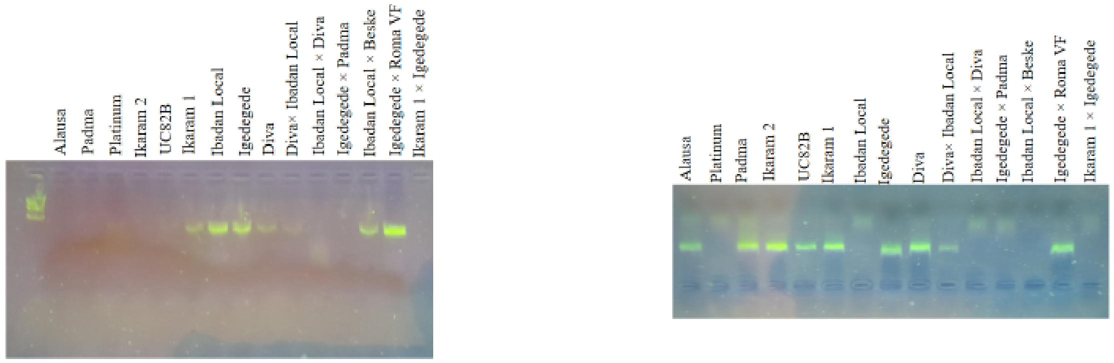


SSR 139

i

Tom 236-237

ii



EST253712

iii

TMS60

iv

Figure 1: Gel Images for the Four SSR Molecular Markers

scored band/locus. The data was inputed in the correct NTSYS format and the analysis was executed to obtain a similarity matrix. Afterwards a dendrogram was also generated using the Unweighted Pair Group Method with Arithmetic Mean by the same software.

Results and Discussion

Means of quantitative traits in the fifteen selected tomato genotypes were shown in Table 3 and Table 4.

Morphological Characterization

The dendrogram in Figure 2 drawn from SLCA grouped the fifteen genotypes into three distinct clusters based on their level of similarity. The genotypes: IBL×DIV, and IKR 1×IGD and IKR 1×RM VF were grouped in the same cluster with only their female parents. The reciprocal crosses

Table 3: Means of Growth Traits for the Fifteen Tomato Genotypes

Genotype	Plant Height at Harvest (cm)	Plant Height at Flowering (cm)	Days to 50% flowering	Days to Flowering	Leaf Width (cm)	Leaf Length (cm)
DIV	120.49d-g	112.19b-e	83.50ab	82.00ab	19.23ab	30.92ab
IBL	126.83c-g	102.13c-e	79.50ab	78.00b	6.36f	9.8g
IKR 1	105.28fg	98.88de	81.50ab	79.00ab	15.92a-e	25.24a-f
IKR 2	127.64c-g	107.88b-e	80.50ab	80.00ab	15.50a-e	25.44a-f
IGED	153.16bc	132.63ab	81.50ab	78.50b	12.71a-f	21.03b-g
UC82B	112.05b-e	102.13c-e	85.00ab	82.50ab	16.48a-e	25.69a-f
PADM	126.88c-g	112.53a-e	83.50ab	82.00ab	17.51a-e	29.14a-d
PLA	120.91d-g	109.58b-e	80.00ab	78.00b	17.68 a-d	30.17a-c
ALS	125.48c-g	108.25b-e	78.50b	78.00b	11.33b-f	19.47b-g
IBL X DIV	142.92b-e	115.33a-e	78.00b	78.00b	10.28c-f	16.88c-g
IKR 1 X IGED	128.00c-g	118.38a-e	79.50ab	78.50b	12.03af	20.51b-g
DIV X IBL	165.35ab	129.75a-c	78.50b	78.00b	13.75a-f	24.72a-f
IBL X BSK	151.50b-d	133.38ab	80.00ab	79.00ab	13.44a-f	22.47a-g
IGED X PADM	121.33d-g	93.00e	82.00ab	83.00ab	18.57a-c	27.67a-e
IKR1×RMVF	127.50c-g	107.96b-e	78.00b	78.00b	11.82a-f	19.96b-g

The values above represent the means of three replicates. Means with different letters in a column are significantly different at a 5% probability level, based on the Tukey HSD test.

IBL×DIV and DIV×IBL were in different clusters for morphological characterization (Figure 2). Also, Genotypes DIV and PLA were in the same cluster in the dendrogram for morphological traits (Figure 2).

Some studies on morphological variation reported that the grouping of tomato accessions into clusters by the dendrogram might be due to both geographic and genetic factors (Herison *et al.*, 2018). Each cluster contains tomato genotypes that share

Table 4: Means of Yield Traits for the Fifteen Tomato Genotypes

Genotype	Fruit Width (cm)	Fruit Length (cm)	Fruit Weight (g)	Number of fruits harvested	Thickness of fruit wall (mm)	Days to Maturity
DIV	4.70a	4.92a-c	72.05a	28.00bc	0.38a	89.50a
IBL	4.36a-c	2.96a-c	44.09ab	15.00fg	0.27a-d	91.00a
IKR 1	2.61d	4.03a-c	20.85b	22.50cd	0.18a-d	86.00a
IKR 2	2.83cd	4.26a-c	24.85b	7.50h-k	0.34ab	91.50a
IGED	3.66a-d	2.99a-c	30.10b	22.50cd	0.26a-d	89.50a
UC82B	2.77d	4.16a-c	38.38b	3.50k	0.24a-d	93.00a
PADM	3.81a-d	4.69a-c	41.55ab	21.50de	0.28a-d	87.00a
PLA	3.46a-d	4.05a-c	48.80ab	41.00a	0.23a-d	86.00a
ALS	2.88cd	5.32a	27.15b	30.00b	0.27a-d	86.50a
IBL X DIV	4.59ab	3.09a- c	41.50ab	11.00g-j	0.30a-c	85.00a
IKR 1 X IGED	3.82a-d	2.96a-c	26.91b	4.50k	0.23a-d	88.50a
DIV X IBL	3.78a-d	3.61a-c	36.10b	7.00i-k	0.35ab	89.50a
IBL X BSK	3.71a-d	2.86b-c	26.35b	12.00g-i	0.28a-d	84.50a
IGED X PADM	3.11b-d	3.07a-c	28.30b	5.50jk	0.27a-d	89.00a
IKR1×RMVF	3.14a-d	3.44a-c	21.65b	11.00g-j	0.12cd	84.00a

The values above represent the means of three replicates. Means with different letters in a column are significantly different at a 5% probability level, based on the Tukey HSD test.

common attributes and that are genetically similar. The grouping of the cross IBL×DIV and IKR 1×RM VF in the same cluster with only its female parent and the lack of similarity between the reciprocal crosses could be due to maternal factor (Figure 2). Grigorcea (2014) reported that bio-morphological characters of fruit and productivity elements by the regulation actions of gene is controlled by maternal factors. Another study by Liu *et al.* (2024) on genetic regulation of fruit shape in horticultural crops revealed that the inheritance of biomorphological character like fruit shape is determined by a combination of genetic factors, hormonal regulation, gene regulatory networks, and environmental influences. It was

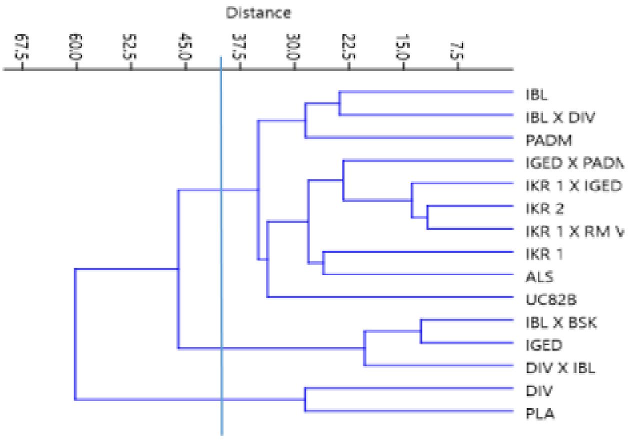


Figure 2: Cluster Analysis of Fifteen Tomato Genotypes Using Morphological Markers

reported that maternal factor influences characters like pericarp thickness, fruit length, fruit weight, number of fruits per plant, index fruit, and number of seminal lodges (Lupaşcu *et al.*, 2015). Maternal inheritance has also been reported as a factor for influencing morphological characters in other plants. In another study by Muojiama *et al.* (2024), it was revealed that maternal inheritance significantly influences fruit setting in cassava varieties.

Molecular Characterization

In Figure 3, the dendrogram grouped the genotypes into 5 clusters at a distance of 0.72 based on molecular markers. PLA was distinct from other genotypes. Genotypes IKR 1 and IKR 2 were grouped in the same cluster. This could have influenced their performances in terms of morphological traits like fruit weight, plant height, leaf width and leaf length (Table 3 and 4). The reciprocal crosses IBL×DIV and DIV×IBL were in the same cluster but occupied a distinct group from their parents; IBL and DIV. PLA which was distinct from other genotypes suggests that it was not genetically similar to any of the genotypes studied. Genotypes IKR 1 and IKR 2 which were grouped in the same cluster (Figure 3) are genetically similar. This could be because the two genotypes were sourced from the same location. Similar study was carried out by Ruiz and Garcia-Martinez (2005) where they examined the similarity between some Spanish traditional cultivars and reported that genotypes from the same localities were more similar to each other than those from different locations. The genotypes; PLA and DIV are in different cluster groups (Figure 3). The reciprocal crosses; IBL×DIV and DIV×IBL were in the same cluster but occupied a distinct cluster from their parents, this implies that they are similar based on molecular characterization but dissimilar from their parents in morphological traits.

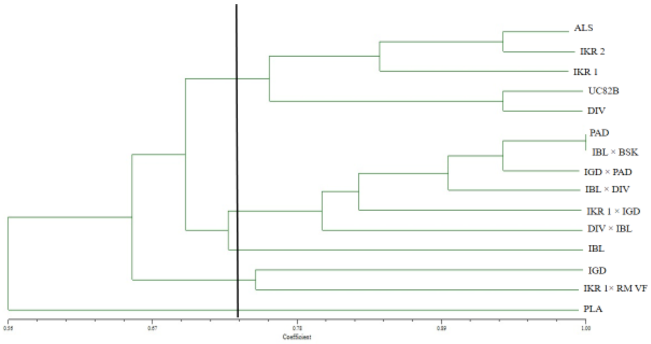


Figure 3: Cluster Analysis of Fifteen Tomato Genotypes on the Basis of Similarity Index Using SSR markers

Summary of Findings

The grouping of the genotypes into clusters based on morphological traits and molecular markers using the dendrogram were not the same for all the tomato genotypes studied. The reciprocal crosses; IBL×DIV and DIV×IBL, were seen in the same cluster for molecular characterization, but occupied different clusters in morphological characterization. Likewise, the genotypes; PLA and DIV which occupied the same group in the cluster generated by morphological traits (Figure 2) were distributed into different cluster groups for the molecular characterization (Figure 3). The genotypes IKR 1 and IKR 2 and also, ALS, IKR 1, IKR 2 and UC82B; PADM, IBL×DIV, IGED×PADM and IKR 1×IGED were grouped into the same cluster for both characterizations.

The genotypes found in the same cluster for morphological characterization but in different cluster for molecular characterization only have similar morphological attributes but are not genetically similar, while those found in the same cluster for molecular characterization but in different cluster for morphological characterization are genetically similar. The three genotypes, which are progenies; IBL×DIV, IKR 1×RMVF and IKR 1×IGD which were in the same cluster with their female parents in the dendrogram generated by morphological traits are in different clusters in the one generated for molecular characterization. This indicates that though their phenotype is similar with that of their female parent, their genotypes are not similar. This could be attributed to the fact that the genetic composition of the crosses has been altered due to introgression of genes during crossing and hybridization. Hybridization is an important means of genetic improvement and has been widely used in field and vegetable crops (Liu *et al.*, 2019).

Lack of uniformity in the groupings of the various genotypes when comparison is made between the dendrograms generated by morphological traits and molecular markers is an indication that one of the methods is more reliable in identifying differences among genotypes than the other. According to Zhou *et al.* (2015), characterization of genotypes using morphological traits is not reliable because it is easily influenced by environmental factors, however, since characterization of genotypes using molecular markers depends more on the genetic composition of the various genotypes and are not affected by the environmental factors, it is more reliable in measuring differences among cultivars. Thus, the use of molecular markers in combination with morphological markers for variability studies helps to give more reliable and accurate result.

Conclusion

Morphological traits and molecular characterization revealed that the genotypes: ALS, IKR 1, IKR 2, UC82B, PADM, IBL×DIV, IGED×PADM, IKR 1×IGED are genetically similar and different from IBL, IGED, PLA, DIV. Depending on the goal of a plant breeding exercise, any of the parent genotypes: ALS, IKR 1, IKR 2, UC82B, PADM can be crossed with any of these: IBL, IGED, PLA, DIV; for improved productivity and desirable output. The use of both morphological traits and molecular markers for diversity studies in tomato is therefore, recommended because both will complement each other to give reliable and accurate results.

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Appendix

DNA Quality, Absorbance and Concentration in nanometer (nm) of Fifteen Tomato Genotypes

S/N	Genotypes	Concentration (nm)	A260	A280	Ra550
1	Alausa	2417.97	48.26	29.95	1.61
2	Padma	2390.72	47.81	35.94	1.33
3	Platinum	1730.51	34.61	18.84	1.84
4	Ikaram 2	621.80	12.44	7.30	1.7
5	UC82B	1303.25	26.07	13.73	1.9
6	Ikaram 1	1312.25	26.25	14.33	1.83
7	Ibadan Local	1727.41	34.55	27.46	1.26
8	Igedegede	8377.76	16.76	8.81	1.9
9	Diva F1	1638.51	32.77	24.14	1.38
10	Diva F1 × Ibadan Local	556.29	11.13	10.57	1.05
11	Ibadan Local × Diva F1	1778.31	35.59	41.94	0.85
12	Igedegede × Padma	1345.47	26.91	25.99	1.04
13	Ibadan Local × Beske	2726.81	54.53	43.43	1.26
14	Ikaram 1 × Roma VF	479.56	95.91	59.26	1.62
15	Ikaram 1 × Igedegede	1111.91	22.24	19.50	1.14

Jaccard Coefficient of Similarity between 15 Tomato Genotypes

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	1.00														
2	0.75	1.00													
3	0.75	0.63	1.00												
4	0.94	0.81	0.69	1.00											
5	0.75	0.75	0.5	0.81	1.00										
6	0.81	0.69	0.56	0.88	0.81	1.00									
7	0.69	0.81	0.56	0.75	0.69	0.75	1.00								
8	0.56	0.69	0.44	0.63	0.69	0.75	0.75	1.00							
9	0.69	0.69	0.44	0.75	0.94	0.75	0.63	0.63	1.00						
10	0.75	0.88	0.50	0.81	0.75	0.69	0.69	0.56	0.81	1.00					
11	0.75	0.88	0.63	0.69	0.63	0.56	0.69	0.56	0.56	0.75	1.00				
12	0.69	0.94	0.56	0.75	0.69	0.63	0.75	0.63	0.63	0.81	0.94	1.00			
13	0.75	1.00	0.63	0.81	0.75	0.69	0.81	0.69	0.69	0.88	0.88	0.94	1.00		
14	0.69	0.69	0.56	0.75	0.69	0.75	0.63	0.75	0.63	0.56	0.56	0.63	0.69	1.00	
15	0.56	0.81	0.44	0.63	0.69	0.63	0.63	0.63	0.63	0.69	0.81	0.88	0.81	0.75	1.00

Numbers 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15 represent ALS, PAD, PLA, IKR 2, UC82B, IKR 1, IBL, IGD, DIV, DIV×IBL, IBL×DIV, IGD×PAD, IBL×BSK, IKR 1×RMVF, IKR 1×IGD respectively.