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Assessment of Variability in Morphological and Genetic Traits Among Nine Local Cultivars and One Wild Accession of Tomato (*Solanum Lycopersicum* L.) Using SSR Markars in Adamawa State, Nigeria

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ABSTRACT: *Variability is necessary in any crop breeding program. Morphological and genetic variability among ten local cultivars of Solanum lycopersicum (Linn.) in Adamawa State Nigeria was studied. The study was aimed at assessing the morphological and genetic variability and smilarities and elucidating on phylogenetic relationship of these local tomato cultivars. Two morphological traits, fruit size and fruit weight were utilized followed by ten SSR marker analysis to investigate their genetic diversity. Eight out of the ten primers representing (90 %) were polymorphic representing loci. The most variable mean across the morphological traits was obtained for fruit weight with a mean square value of 7122.88, while the least variable mean was recorded for fruit size with a mean square value of 321.66. There was significant positive correlation between fruit size and fruit weight per cultivar and fruit size positively correlated with fruit weight per plant and across the ten cultivars (r = 0.92). Total of (31) alleles were recorded and the mean number of alleles per locus was 0.2580. The lowest number of bands across genotype was three and the highest number of bands across genotypes was seven. The PIC value of SSR markers obtained ranged from 0.35 to 0.77 with the average number of polymorphic bands per primer being 23.72. While clustering among local cultivars of Adamawa tomato using the unweighted pair group method with arithmetic mean analysis (UPGMA), the clustering was not related to the geographical locations where seeds were collected. It can therefore be inferred that tomato accession (Tt001) with large number of fruits per truss having small sized fruits and tomato cultivars (Tt009 and Tt010) which on the other hand recorded high figures with regards to morphological traits, and clustered closely based on molecular analysis may hybridize into a better cultivar.*

KEYWORDS: morphological parameters, alleles, molecular markers, *Solanum lycopersicum*.

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INTRODUCTION

Tomato (*Solanum lycopersicum)* L. is an important vegetable crop. It is reported to have originated from the Andes, cultivated among the Aztecs and Incas (Saavedra *et al*., 2017). It is the world's largest vegetable crop after potato and it tops the list of canned vegetables. In Nigeria, tomato is regarded as the most important vegetable after onions and pepper (Ugonna *et al.,* 2015). World tomato production is on the increase, it ranks first in the world for vegetables, accounting for 14% of world vegetable production (over 100 million metric tons/year at $ 1.6 billion, FAO, 2010). In 2013, tomato production was estimated to be about 161.8 million tons in the world (FAOSTAT, 2014). It is recorded that, China produces about one-quarter of the world's tomatoes, which makes it the most prominent producer followed by India, the United States, and Turkey. The major contributions of tomatoes to the health and welfare of humankind cannot be over-emphasized. "Hidden hunger" or micronutrients and vitamin insufficiency is a serious problem in the third world that is caused by a deficiency of vitamins and minerals such as vitamin A, iodine, and iron in the human diet. It hurts billions of people in the developing world and increases the risk of disease or death from contagious diseases causing children not to grow to their full physical and mental potential (Dias *et al*., 2005). Tomato is a rich source of antioxidants (mainly lycopene and *β*-carotene), Vitamin A, Vitamin C, and minerals like Ca, P, and Fe in diet (Salim *et al.,* 2018). Lycopene is an antioxidant that reduces the risk of prostate cancer. It supplies sugar, ascorbic acids, carotenoid, and vitamins (Theeranat (2018). Tomato's total carbohydrate, sugars, protein, calcium, iron, and vitamin C content range from 15 to 35 mg/100 g fruit and its vitamin A is four times that of orange juice (Zhang *et al.,* 2009).

One of the major challenges to tomato improvement in Nigeria is the increased erosion of genetic resources, this has led to narrow genetic variability among the cultivated species and their wild relatives. Enhanced genetic variability through the widening of the genetic resource will effectively enhance the genetic weakness of the crop (Silva and Souza, 2013). Inter and intra-genetic differences in the population of tomato genotypes have been exploited by employing techniques such as morphological markers, and biochemical and molecular markers (Ozturk, 2022). Morphological characterization of the species seems to be the most common tool employed for its improvement over the years since it allows selection to be based on the excelled performance of individuals in terms of desirable traits. Nevertheless, the selection of improved genotypes based on phenotypic traits may be heavily influenced by the environment thereby obstructing the estimate of the genetic diversity of the crop (Uzun *et al*., 2021).

Molecular markers are also employed today as reliable characteristic features for germplasm identification among plant genotypes. Genetic diversity and variability studies of plants have been carried out using different types of molecular markers (Zhou *et al*., 2015). Molecular markers can provide an effective tool for the efficient selection of desired agronomic traits because they are

based on genotypes and are thus independent of the effects of environmental factors. One of which is Simple Sequence Repeats (SSR) also referred to as Microsatellites, these are composed of ubiquitous tandem iterations of short oligonucleotide units of 1-6 base pair DNA sequences of the various accessions of the plant under study which may have functional and/or structural properties. SSRs have shown higher efficiency among other markers due to their reproducibility, co-dominance nature, ease of amplification by Polymerase Chain Reaction (PCR), and their typically high allelic diversity at different loci resulting in high polymorphism alongside the availability of powerful and automated equipment (Banhos *et al*., 2008; Saravanan *et al*., 2014). The reduction of genetic variation in tomatoes through domestication and breeding has resulted in the need for molecular techniques in bringing out the needed polymorphism.

Simple sequence repeats (SSRs) often tend to modify genes with which they are associated. The influence of SSRs on gene regulation, transcription, and protein function typically depends on the number of repeats, while mutations that add or subtract repeat units are both frequent and reversible. SSRs thus provide a prolific source of quantitative and qualitative variation. Over some years now researchers have found that this spontaneous variation has been tapped by natural and artificial selection to adjust almost every aspect of gene function. The hypothesis that SSRs, by their special mutational and functional qualities, have a major role in generating the genetic variation underlying adaptive evolution is confirmed by other researchers (El-Mansy *et al*., 2021).

MATERIALS AND METHODS

Fruit Identification and Labeling

Ripe fruits collected from different locations in the southern zone of Adamawa State were identified in the Department of Plant Science, Modibbo Adama University, Yola. The identified fruits were snapped using a digital camera, and the pictures printed were labeled as Tt001 (wild accession), Tt002, Tt003, Tt004, Tt005, Tt006, Tt007, Tt008, Tt009 and Tt010 (as cultivated varieties). Taking each cultivar of the labeled ripe tomato fruit at a time, the fruit size and weight were measured and recorded accordingly. The fruit was then cut transversely using a clean scalpel or knife and the seeds were squeezed out completely into a labeled container. After washing thoroughly with clean water, the seeds were shade dried, labeled and stored in a dry place.

Each seed sample was later planted using the broadcast method in a pot filled with well-drained topsoil, and labeled according to the different accessions. The seedlings were watered daily using a watering can for one week after which the seeds germinated. Three weeks after germination fresh young leaves were collected and labeled accordingly for DNA extraction.

DNA extraction

Total genomic DNA was isolated from young, fresh and healthy leaves of plants from each of the ten tagged tomato genotypes at the Bioscience laboratory of the International Institute for

Tropical Agriculture (IITA) Ibadan, Nigeria using CTAB method by Lorenz (2012) with little modification. This protocol consists of:

1. Cell lysis.
2. Separation of nucleic acid from other contaminants.
3. DNA precipitation and purification.

Tender tomato leaves of each genotype was weighed into (20 mg) and ground to fine powder in liquid nitrogen using Teflon pestle. To each tube, 800 µl of lysis buffer containing 2 (g) CTAB, 2 (g) PVP, 28 (ml) $NaCl_2$, 4 (ml) EDTA (pH 8.0), 10 (ml) Tris–HCl (pH 8.0), and 0.1 (ml) Beta–mercapto ethanol was added under lamina flow and shaken several times until a homogenous mixture was obtained to lyse the nuclear membranes. The mixture was incubated at 65 ˚C for 30 min with intermittent vortexing by hand three times at ten minutes intervals to ensure uniform temperature within the tube. The samples were cooled at room temperature. Protein contaminants from the cell lysate were then removed by adding equal volume (500 µl) of chloroform iso amyl-alcohol (24:1) and mixed gently by inversion of the tube. The samples were centrifuged at 10000 rpm for 10 min using centrifuge and the upper phase containing aqueous phase transferred into clean 1.5 ml tubes without disturbing the tube. The same volume (500 µl) of chloroform isoamyl-alcohol (24:1) was added again to ensure total removal of the protein contaminants as possible.

The nucleic acids were precipitated by adding two-thirds volume of ice-cold isopropanol (500 µl) and the tube gently inverted ten times. The precipitation was enhanced by storing the samples at -20 ˚C in a refrigerator for one hour. The samples were centrifuged again at 10000 rpm for 10 min to pelletize the nucleic acid and the isopropanol (supernatant) was decanted and discarded. The DNA pellet was washed with 500 µl of 70% ethanol and then centrifuged at 6000rpmfor 5 minutes. The ethanol was decanted and the DNA pellets were air-dried at room temperature (25ºC) on the laboratory bench for 10 min when the smell of the ethanol was no longer detectable. The DNA pellets were suspended in 95µl of 1X Low salt TE buffer (Tris-ethylene diamine tetracetic acid) and 5µl of RNase.

Amplification of SSR Markers

Amplifications were carried out in Applied biosystem thermal cycler GeneAmp PCR System 9700 (Applied Biosystems, USA). The DNA from the 10 tagged tomato samples were finger-printed using SSR markers in a 25µl reaction volume of master mix containing, 2.5µl of 10X Reaction Buffer, 1µl of 50mM $MgCl_2$, 2µl of 10 mM of dNTPs (Deoxynucleotide Triphosphates), 0.6 µM each of forward and reverse primer and 0.1µl of Taq polymerase. Water was added to make the final volume.

Reactions were conducted at an initial denaturation step at 94 ˚C for 5 min. The annealing step was 55 ˚C for 30 s reducing at -1 ˚C per cycle 10 times, followed by 94 ˚C for 30 s, 45 ˚C for 30 s; 72 ˚C for 1 min for 30 cycles, and a final extension/elongation step at 72 ˚C for 10 min and then held at 4 ˚C. The amplified products were stored at -20 ˚C until required to run gels.

Agarose Gel Electrophoresis

The PCR products were separated using Agarose gel electrophoresis (AGE). The amplified DNA fragments were separated on 2 % Agarose gel at 100 V for 45 min–1 h in 1X TBE (Tris-boric ethylenediamine tetraacetic acid) (1X) using a gel electrophoretic apparatus (MS Major Science, UK) and BIO-RAD (Criterion TM cassettes), where 1 X DNA loading dye was added to the PCR products for visual tracking of DNA migration during electrophoresis. A 50bp DNA marker (gene rule) was used as a reference to estimate the size of specific DNA bands in the PCR-amplified products visualized on a UV trans illuminator and photographed using a Gel Documentation System.

Table 1: Sequences of SSR primers optimized

Serial No.	Primer Code & Status	Primer Sequence
1.	TP 121	F: 5’- GTGGATTCACTTACCGTTACAAGTT - 3’
		R: 5’- CATTCGTGGCATGAGATCAA -3’
2.	TP 122	F: 5’- GTGGACCATTTCAAGTTCAACA -3’
		R: 5’- TGAATGACATCCATCCATGA -3’
3.	TP 123	F: 5’- GTGACCACATGAGATATCCAGA -3’
		R: 5’- CAGTTGTCCATATTGTGTGGC -3’
4.	TP 124	F: 5’- AACATGCGGAGAAAAATT -3’
		R: 5’- GGAACACGTCCCAAAAATGT - 3’
5.	TP 125	F: 5’- GCACAAATAATTTTTCAAGACCAA -3’
		R: 5’- AAAAACGGACATAGCTTTGTACT -3’
6.	TP 126	F: 5’- ACTGAAACTTCTTTGCACTT -3’
		R: 5’- GTTATAAAATTTGCGATAAATT -3’
7.	TP 127	F: 5’- AAACACAATGTTTGAACCGA -3’
		R: 5’- TGGGACTAATGAAGCTAACC -3’
8.	TP 128	F: 5’- ACTGATTTACCTTTCACCAC -3’
		R: 5’- GGGAAAGAAACAAAAGTACA -3’
9.	TP 129	F: 5’-AAGTGTCTAATAGTAAGAGTCTCAG -3’
		R: 5’- TGATGATCAGATTGAGAAGA -3’
10.	TP 130	F: 5’- GCTATCTTTTATCCAAGAGA -3’
		R: 5’- GTTGATTATTTTATATTAAAAAGT -3’

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RESULTS AND DISCUSSION

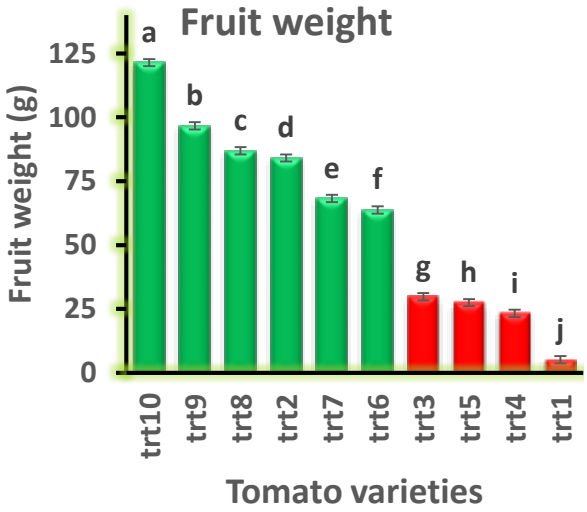

Figure1**:** Bar chart showing fruit weight of the different tomato accessions. (Green and Red bars are above and below mean weight respectively).

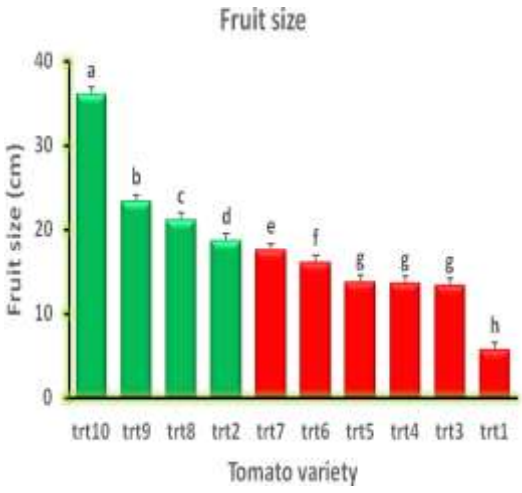

Figure 2**:** Bar chart showing fruit sizes of different tomato accessions (Green and Red bars are above and below mean size respectively).

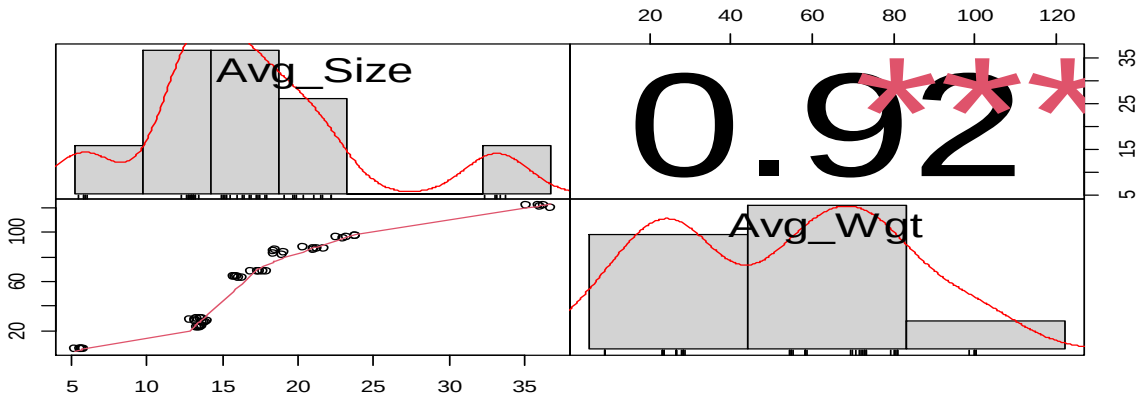

Figure 3: Histogram showing correlation between fruit size on fruit weight of tomato accessions.

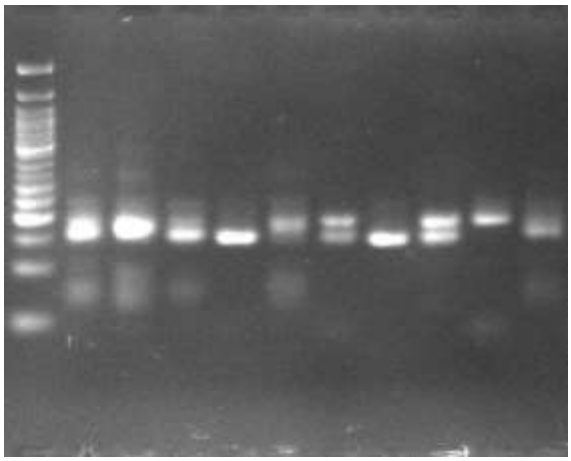

Plate 1

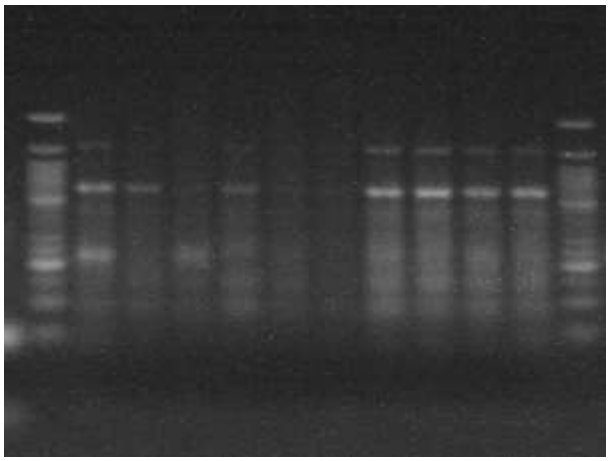

Plate 2

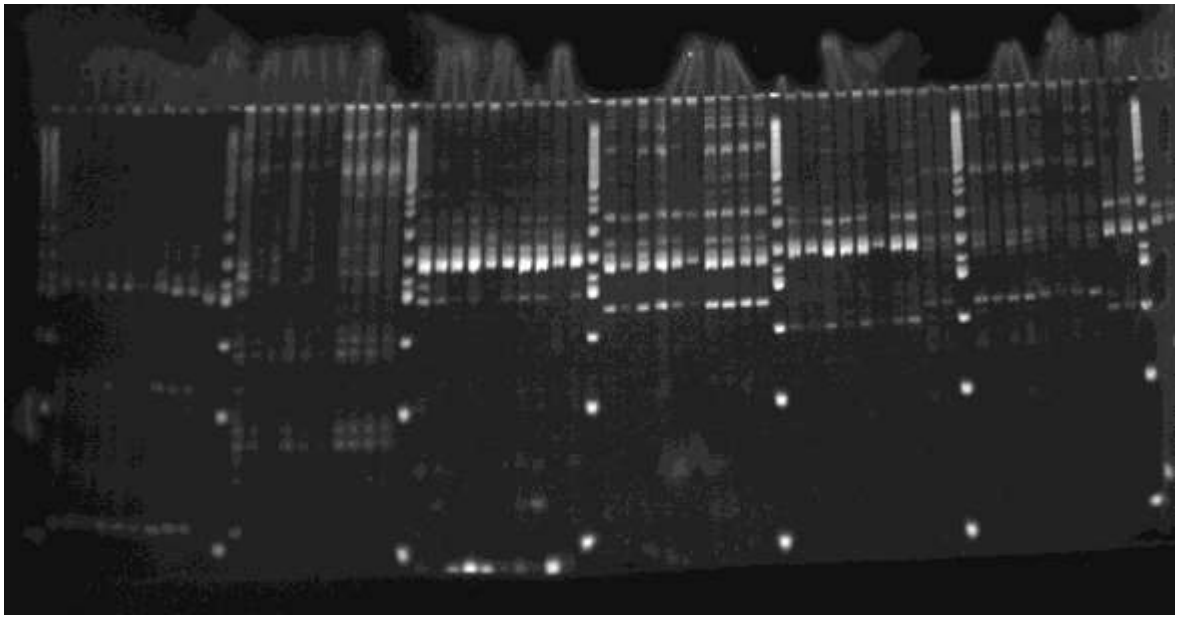

Plate 3.

Plate 1: PCR Amplification of Ten Tomato accession using primer TP 122 on a (50bp ladder) on Agarose gel.

Plate 2: PCR Amplification of Ten Tomato accession using primer TP 123 with a (50bp ladder) on Agarose gel.

Plate 3: PCR Amplification of Ten Tomato accessions using six SSR primers TP 122, TP 123, TP 124, TP 125, TP126, TP127 with a (50bp ladder) on PAGE

Table 2: Polymorphic information of eight SSR markers utilized in characterizing ten cultivars of tomato

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Marker	Sample size	Major Allele Freq.	Allele No.	Heterozygosity	Gene Diversity	PIC
TP122	10	0.6000	2.0000	1.0000	0.4800	0.3648
TP123	10	0.8000	2.0000	1.0000	0.3200	0.2688
TP124	10	0.7000	2.0000	1.0000	0.4200	0.3318
TP125	10	0.4000	6.0000	1.0000	0.7600	0.7300
TP126	10	0.6000	3.0000	1.0000	0.5400	0.4662
TP127	10	0.2000	7.0000	0.5620	0.8400	0.8196
TP128	10	0.3000	6.0000	1.0000	0.8000	0.7716
TP130	10	0.5000	3.0000	1.0000	0.5800	0.4918
Mean	**10**	**0.5125**	**3.8750**	**0.94525**	**0.5925**	**0.5306**

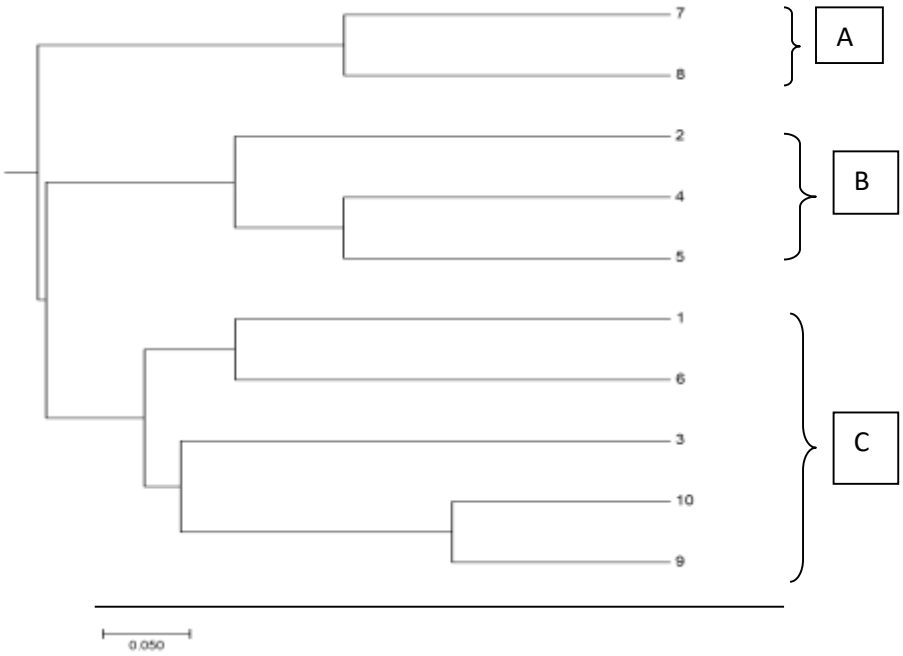

Figure 4: A Dendrogram based on (8) SSR markers for 10 tomato cultivars using similarity coefficient and UPGMA clustering.

Assessment of variability in fruit size and fruit weight of ten tomato cultivars was carried out. Significant positive correlation of 0.92 was recorded between the traits investigated. Similar results were reported by Salim *et al*., (2018); Yaman and Uzun, (2021) who reported a positive correlation of 0.71 and 0.87 between tomato weight on one hand and fruit width and fruit length on the other hand respectively. Fruit descriptors in tomatoes are more promising markers for morphological differentiation of tomato genotype than leaf size or number of leaves. This was similarly reported

by Sharifova *et al*., (2017); and Kiani and Siahchehreh, (2018). This is also in concordance with Yildiz *et al*., (2021) for fruit size, Kumar *et al*., (2006) and Singh (2009) for fruit weight, and Reddy *et al*., (2015) for both Fruit size and Fruit weight. Tomato cultivar Tt010 (locally called: Kafar Giwa) performed outstandingly well in terms of fruit size, and fruit weight. The high positive correlation recorded between fruit size and fruit weight could be a result of the high quantity of water and other nutritive components in the large-sized cultivars.

While tomato accession Tt001 recorded the lowest average fruit size of 5.63cm on the contrary cultivar Tt010 recorded the highest average fruit weight of 36.0g. In the same vein, accession Tt001 recorded the lowest average fruit weight of 5.1g while Tt010 recorded the highest average fruit weight of 121.4g. This result is similar to the research findings reported by Yesmin *et al*., (2014), where they reported fruit length ranging from 3.24 to 5.48 cm in tomato genotypes. This result is also similar to earlier studies published by Patwary *et al*., (2013) and Vishwanath *et al*., (2014). These higher levels of variations may be used as pointers to select materials in a genetic enhancement program. These are in agreement with the findings of Mazzucato *et al*., (2010), and Henareh *et al*., (2016). Morphological traits have also been found useful in discriminating genotypes of tomatoes by Caramante *et al*., (2009), who utilized fifteen morphological traits on four tomato accessions.

SSR Marker-based Similarities Among Tomato Cultivars

Out of the ten SSR markers amplified, eight were found to be polymorphic representing 90 % and the others (TP121 and TP 129) were discarded. Similarity matrix computed using the Pearson coefficient among ten cultivars of local tomato revealed three distinct clusters A, B, and C (Figure 4). Each cluster consists of tomato cultivars that are closely related phylogenetically. This clustering however was irrespective of the geographical locations from which the samples were collected. This might be because the samples were collected from relatively close locations and might have shared some common ancestry down the genetic tree. This result disagrees with results obtained by Benor *et al.,* (2008), who reported that tomato accessions were clustered in relation to the geographical locations from which the samples were collected. This could be likely because those samples were collected from different countries across long distances. The dendrogram clustered tomato cultivars Tt007 and Tt008 together in cluster (A), cultivars Tt002, Tt004, and Tt005 in the cluster (B), and lastly cultivars Tt001, Tt006, Tt003, Tt010, and Tt009 together in cluster (C).This indicates that accessions that are clustered together on the phylogenetic tree not only share common ancestry down the genetic line but also share similar genomic constituents. Tomato accessions Tt004 and Tt005 were not only clustered phylogenetically in the same group but also shared closely related morphological data in fruit size of 13.54cm and 13.73cm respectively and fruit weight of 23.31g and 27.50g respectively. This has further proven the reliability of SSR markers in revealing the phylogeny of different plant materials. Similar results were reported by other researchers: (Balada *et al*., 2021; Hamrick and Loveless, 2019).

SSR Marker-based Variation Among Tomato Cultivars:

The average polymorphic percentage from eight polymorphic primers was utilized to analyze the genetic relationship of the taxon. Ninety-seven percent (97 %) genic variability was recorded for variation within individuals. Similar numbers of primers were used by other researchers to determine the genetic diversity of wheat cultivars which generated 80.2% polymorphism (Najaphy *et al*., 2011). While six SSR primers were used to detect polymorphism of (20) *Lilium species* (Srisamoot and Padsri, 2018), the results indicated that the average polymorphic percentage does not rely on the number of primers used but on how polymorphic the primers were on each cultivar. Roy *et al*., (2021) opined that a minimum of four polymorphic markers can be utilized to study genetic diversity. Besides morphologic traits, molecular markers are a powerful tool in discriminating for genetic diversity in crops, especially the microsatellite's ability to discriminate between homozygous and heterozygous loci.

In this study, the highest dissimilarity distance of 0.8750 was recorded between tomato cultivars Tt008 and Tt009, and also between cultivars Tt009 and (Tt004 and Tt005). A similar high dissimilarity distance was observed between tomato cultivars Tt008 and (Tt004 and Tt005). A similar high genetic distance was reported by Korir *et al*., (2014) who recorded a genic distance of 0.77 as the highest dissimilarity distance. The dissimilarity distance is a measure of the genic dissimilarity between two or more tomato cultivars where the distance 0.0000 indicates a match of genotype between tomato cultivars and genetic variation among individuals of the same geographical location was 3%.

The mean PIC value of the eight SSR markers was 0.5306, with values ranging from 0.8196 for tomato primer TP127 to 0.2688 for tomato primer TP123, respectively. The lowest polymorphic bands across genotypes were 2 in TP122, TP123, and TP124. The highest number of polymorphic bands was 7 and 6 in the primers TP127 and TP129. The average number of polymorphic bands per primer was 3.875. Similar work was also done by several scientists viz: He *et al*., (2003); Bredemeijel *et al*., (2002), and Garcia-Martinez *et al*., (2006). The PIC value of SSR primers obtained in the present study was lower than the PIC values for previous tomato SSR studies reported by these scientists which ranged from 0.35 to 0.77. These results may be related to the relationship between tomato genotypes chosen for this study which were of local origin. The average number of polymorphic bands per primer i.e. 23.72 is much higher as compared to that reported previously by Salunke *et al*., (2012), and Ezekiel *et al*., (2011), who reported an average of 7.09, and 4.6 polymorphic bands, respectively. The polymorphism obtained in the present investigation indicates a high discrimination capacity of SSR markers.

Analyses that combine morphological traits with molecular markers have been found to provide better information in genetic diversity assessments (Zhou *et al*., 2015). Nevertheless, morphological diversity is not always revealed at the molecular level (Hu *et al*., 2012). The worth of SSR markers and their relationships to morphology in tomato accessions have been documented by Caramante *et al*., (2009). A higher number of markers and more samples would be preferred

for more diversity. Although, many of these cultivars might also have shared similar genetic backgrounds since the fact that they were collected from different locations does not rule out the possibility that they might be related.

Phylogenetic and Cytotaxonomic Relationship Among Tomato Accessions

Genic data was utilized to construct a phylogenetic tree that relates the phylogenetic relationship among the ten tomato cultivars under study. Unlike chromatin length which was reported by Egbucha (2011) to be vital in establishing cytotaxonomic and phylogenetic relationships, where a decrease in chromatin length is considered to be a major factor in the evolutionary history of plants. Genomic data obtained using SSR markers to construct a dendrogram shows that cultivars with short similarity coefficients e.g. Tt003 on the phylogenetic tree are ancestral while accessions like Tt009 and Tt010 are considered to have evolved lately on the phylogenetic tree.

Garcia-Martinez *et al*., (2006) reported a low level of polymorphism among tomato cultivars, pointing out a significantly lower genetic diversity than other self-pollinating species. However, when wild accessions were included, high numbers of alleles per SSR locus were reported (Alvarez *et al*., 2001). They also reported that large phenotypic differences may occur as a result of a minor genotypic difference.

While Tabatabaei *et al*., (2011) is of the opinion that the convergent evolution and complex genetic structure of traits that result in environmental effects on trait expression are the main causes of the limited linkage of molecular markers with morphological traits. This study suggests that there is a strong correlation between the results of morphological study and analysis using SSR markers, as reported also by Khan *et al*., (2020). This may however be because the morphological traits studied in this research were both quantitative characters. This, therefore, suggests a high correlation between quantitative morphological data and genetic data investigated using SSR markers. Researchers had earlier established that quantitative characters are governed by polygenic or quantitative pattern of inheritance (controlled by many loci in an additive manner since each allele adds a certain amount to the phenotype) compared to qualitative characters on the other hand which are governed by a qualitative pattern of inheritance and controlled by single or few gene loci.

CONCLUSION AND RECOMMENDATION

The different accessions studied though collected from closely related geographical locations, showed that they were not all from the same ancestral decent while others were closely related cultivars. The tomato accessions also showed hybridization potentials and the possibility of producing better cultivars that can meet the demand of farmers and consumers. There is high morphological and genic variation in the accessions studied. This, therefore, opens the door for crop improvement, since variability in plants is a prerequisite for any breeding program. SSR marker analysis revealed a high level of variability among the tomato accessions, this has once

again proven the reliability of SSR markers in revealing variation even among closely related accessions that have undergone intensive selection. This is a result of the hypervariable nature of SSR markers to produce very high allelic variations even among very closely related varieties, their co-dominance, reliability, and high reproducibility.

Recommendations: It is necessary to collect and preserve different tomato germplasm in order to conserve the fast eroding variability in tomato cultivars due to excessive selection by farmers. This will go a long way in conserving the potentials in tomato landraces such as high reproduction potentials and resistance to adverse environmental conditions.

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