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Genetic Diversity among Ten (10) Local Accessions of Tomato [Solanum Lycopersicum (Linn)] from the Southern Senatorial District of Adamawa State, Nigeria

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Abstract

Assessments of the Genetic diversity among ten local accessions of Solanum lycopersicum (Linn.) from the southern senatorial district of Adamawa State, Nigeria was carried out. The study was aimed at investigating genetic diversity and elucidating the phylogenetic relationship of the local tomato accessions. Ten SSR markers were employed to investigate the genetic diversity among the tomato accessions. Eight out of ten primers i.e. (90%) were polymorphic representing loci. 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 two and the highest number of bands across genotypes was seven. The PIC value of SSR primers obtained ranged from 0.35 to 0.77 with the average number of polymorphic bands per primer being 23.72. While clustering among local accessions of Adamawa tomato using the unweight pair group method with arithmetic mean analysis (UPGMA), three clusters (A, B and C) were observed. The clustering however was regardless of the geographical locations where seeds were collected. It is therefore suggested that tomato accession (Tt001) with large number of fruits per truss and tomato accessions (Tt009) and (Tt010) which on the other hand had divergent phenotypic characters and had clustered closely based on molecular analysis could hybridize into a robust cultivar.

Keywords: Tomato Solanum Lycopersicum, Accessions, Genetic Diversity, Molecular Markers.

Introduction

Tomato (*Solanum Lycopersicum*) originated from the Andes, in what is now called Peru, Bolivia, Chile and Ecuador where they grew wild (Peralta *et al.*, 2008; Blanca *et al.*, 2012). It belongs to the nightshade family Solanaceae together with tobacco, potato, eggplant and pepper. The importance of tomato in Nigerian diet cannot be over emphasized (Ugonna *et al.*, 2015). It can be served fresh on salads, cooked as tomato sauce and fried as tomato stew. Tomato can also be used fresh or dried (Saavedra *et al.*, 2017; Gonzalez and Torres, 2014; Foolad, 2007). 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 (Hussein *et al*., 2016). Its total carbohydrate, sugars, protein, calcium, iron and vitamin C content range from 15 to 35 mg/100 g fruit, its vitamin A is four times that of orange juice (Zhang *et al.,* 2009).

Variability is essential in any breeding program (Kadams, 2010). A major challenge to tomato breeding in Nigeria is the increased erosion of genetic resources which has led to narrow genetic variability among the cultivated species and their wild relatives, most tomato cultivars have lost variability due to excessive selection by farmers. Enhanced genetic variability through widening of the genetic resource will effectively enhance the genetic weakness of the crop (Silva and Souza, 2013; He *et al*., 2003).

Early researchers had relied on morphological variability which depends on selection of cultivars with excellent trait for improving on tomato production; however, the combination of both morphological and molecular variability to harness the much needed variation has yielded positive results (Zhou *et al*., 2015). This is partly because phenotypic variations are influenced by environmental factors (Uzun *et al*., 2021).

Molecular markers are employed today as reliable characteristic features for germplasm identification among plant genotypes. Researchers have employed genetic diversity and variability studies of plants using different types of molecular markers (Zhou *et al*., 2015). Molecular markers are an effective tool for 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 pairs repeats in DNA sequences of the various accessions of the plant under study which may have functional and/or structural properties (Saravanan *et al*., 2014). SSRs have shown higher efficiency among other markers due to its reproducibility, its co-dominance nature, ease of amplification by Polymerase Chain Reaction (PCR), and their typically high allelic diversity at different loci resulting to high polymorphism alongside the availability of powerful and automated equipment (Banhos *et al*., 2008; Saravanan *et al*., 2014). The reduction of genetic variation in tomato through domestication and breeding leading to breeding depression has resulted in the need for molecular technique in bringing out the needed polymorphism.

Materials and Methods

Table 1. Showing locations for collection of tomato cultivars

L.G.A.	Town	Cultivars Collected
Demsa	Borong	Tt002
Ganye	Ganye	Tt007
Guyuk	Prokayo	Tt001, Tt003
Jada	Jada	Tt006

Lamurde	Tingno	Tto10
Mayo-belwa	Mayo-belwa	Tto05
Numan	Ngballang	Tto08
Shelleng	Kiri	Tto09
Toungo	Toungo	Tto04

Each seed sample was planted in a pot filled with well drained top soil, labeled according to the different accessions. Each accession was sown in the already labeled pot using broadcast method. 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 at the IITA Bioscience Laboratory Ibadan, from young, fresh and healthy leaves of three weeks old plants from each of the ten tagged tomato genotypes using CTAB method with modification by Lorenz (2012). This protocol consists of:

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

Twenty milligrams (20 mg) of tender leaves of the tomato genotype leaves of each accession was weighed into 2 (ml) eppendorf tube, 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.

The protein contaminants from the cell lysis were then removed by adding equal volume (500 µl) of chloroform isoamyl-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 then centrifuged at 6000 rpm for 5 minutes. The ethanol was decanted and the DNA pellets were air-dried at room temperature (25°C) 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 5 µl of RNase.

DNA Quality and Quantity Estimation

The quality and quantity of the DNA extracted were determined using a spectrophotometer (Thermo scientific) and the absorbance reading taken at 260 nm and 280 nm levels. The diluted DNA samples were loaded to the cuvette of the spectrophotometer for estimation of the absorbance. The DNA quality was assessed using the absorbance ratio at 260 nm to that at 280 nm wavelengths (A260: A280). If the ratio was 1.8 to 2.0, the absorption was due to nucleic acids; hence it was of good quality. A ratio of less than 1.8 indicated there may be proteins and/or other UV absorbers in the sample, and a ratio higher than 2.0 indicated the samples may be contaminated with RNA or phenols. The integrity of the DNA was also assessed by running the DNA samples on 1% agarose gel.

Each well contained a mixture of 2µl of loading dye and 3µl of the genomic DNA sample. The gel was run with 1X TBE buffer from the cathode to the anode with a constant voltage of 120 V for 45 min. They were visualized after electrophoresis with a UV Trans illuminator camera. The DNA samples had no visible shearing and proceeded to PCR amplification.

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 for 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 ethylene diamine 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.

The following SSR primers were ordered from Inqaba Biotech West Africa Ltd.

Table 2. Sequences of SSR primers optimized

S/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′

Data Analysis

The fragments were scored for presence (1) or absence (0) in each accession for each of the primer. Each DNA amplicon size was considered as a unique characteristic and scored as presence (1) or absence (0) for cluster analysis. The genetic distance between each pair of the accessions was calculated using the frequency based distance. All calculations were done using the software DARwin version 6.0 (Perrier *et al.*, 2003) and Power Marker Version 3.5. Liu and Muse, (2005).

Results and Discussion

Table 3: Polymorphic information of eight SSR markers utilized in characterizing ten cultivars of tomato

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

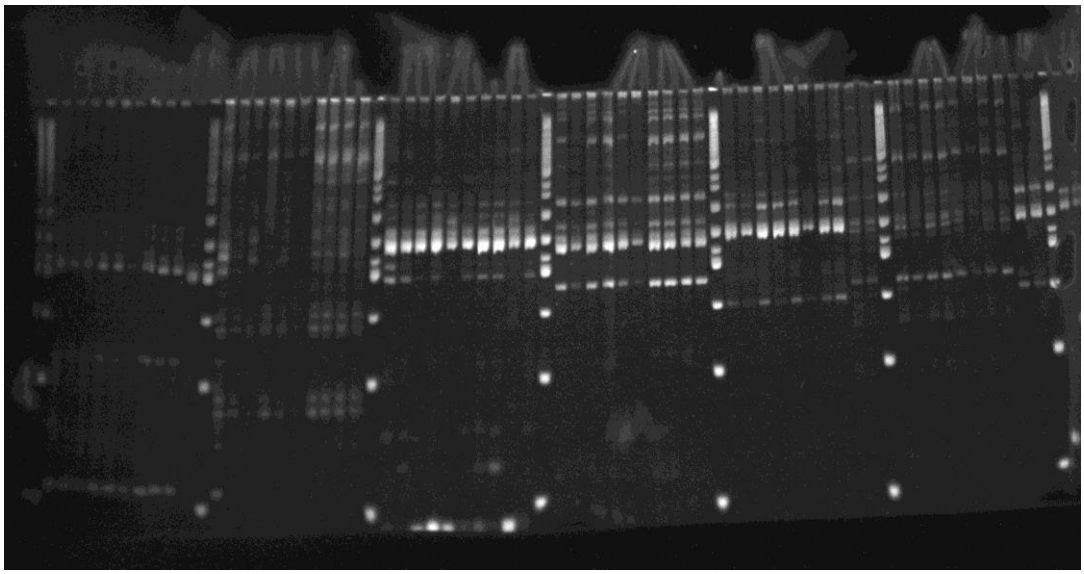

Plate 1: PCR Amplification of Ten Local Tomato accessions using six SSR primers TP 122, TP 123, TP 124, TP 125, TP126, TP127 with a (50bp ladder)

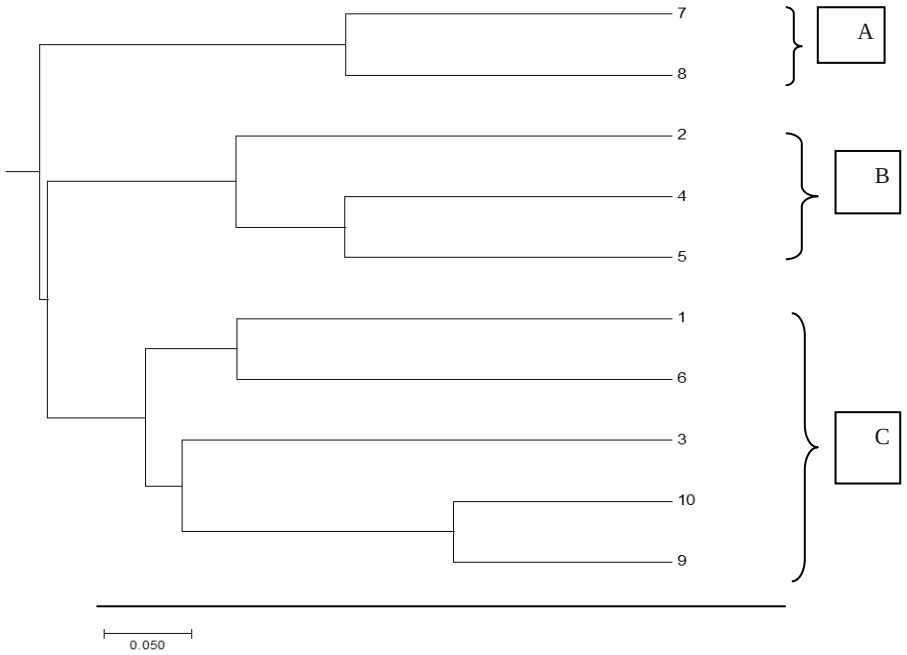

Figure 1: Dendrogram based on (8) SSR markers for 10 tomato cultivars using similarity coefficient and UPGMA clustering.

Ten SSR primers were employed to investigate the variability or otherwise among the ten local tomato accessions. Upon authenticating the polymorphism of the primers, eight SSR primers representing (90%) were found to be polymorphic among the tomato genotypes while the remaining two were discarded. These polymorphic markers generated polymorphic banding patterns, which were used in the analysis of genetic diversity.

The detection of minor and nonspecific products which could be-shadow heteroduplex or faint bands may affect the allele scoring process and increases the difficulty of legitimate allele identification. These minor bands were also considered during allele scoring as Wang *et al.*, (2003) and Rodriguez *et al.*, (2009) did and reported that the minor bands can be useful during gel scoring for genotype verification, because they are generally consistent. This polymorphic band demonstrates that SSR analysis is a robust and efficient method for detecting differences between the ten tomato genotypes.

SSR Marker-Based Similarities among Tomato Accessions

Similarity matrix computed using Pearson coefficient among ten accessions of local tomato has clustered the accessions into three distinct clusters A, B and C. Each cluster consists of tomato accessions that are closely related phylogenetically. This clustering however was

irrespective of the geographical locations from which the samples were collected. This might be owing to the fact that 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 with respect to the geographical locations from which the samples were collected. This could be likely because the samples were collected from different countries across long distances.

The dendogram in Figure 1 clustered tomato accessions Tt007 and Tt008 together in cluster (A), accessions Tt002, Tt004 and Tt005 in cluster (B), and lastly accessions Tt001, Tt006, Tt003, Tt010 and Tt009 together in cluster (C). This shows that accessions that are clustered together on the phylogenetic tree not only share a common ancestor down the genetic line but also share similar genomic constituents. Similar results were reported by other researchers: (Balada *et al*., 2021; Hamrick and Loveless 2019).

SSR Marker-Based Variability among Tomato Accessions

Average polymorphic percentage from eight polymorphic primers was utilized to analyze for genetic relationship of the taxon. Ninety seven percent (97%) genic variability was recorded for variation within individuals. Similar numbers of primers were previously 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 are on each accession. Roy *et al*., (2021) opined that a minimum of four polymorphic markers can be utilized to study genetic diversity.

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. (Wang *et al*., 2003).

In this study, the highest dissimilarity distance of 0.8750 was recorded between tomato cultivars Tt008 and Tt009, also between cultivars Tt009 and (Tt004 and Tt005). A similar high dissimilarity distance was observed between tomato cultivars Tt008 and (Tt004 and Tt005). 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, 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 the primers TP127 to 0.2688 for the primer TP123, respectively (Table 2). The lowest polymorphic bands across genotypes was 2 in TP122, TP123 and TP124. The highest number

polymorphic bands was 7 and 6 in the primers TP127 and TP129. The average number of polymorphic bands per primer was 3.875 (Table 2). 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 were ranged from 0.35 to 0.77. These results may be related to the genetic relationships of 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), 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.

SSR procedure is faster and more appropriate for thorough-put study and highly traceable (Caramante *et al*., 2009). Genetic diversity study that avoids vague genotype discrimination relies heavily on the use of molecular markers to enhance both breeding objectives and efficient germplasm conservation of tomato and other crop species (Caramante *et al*., 2009). Higher number of markers and more samples would be preferred for more diversity. Although, many of these accessions might also have shared similar genetic background since the fact that they were collected from different locations does not rule out the fact that they might be related.

Phylogenetic Relationship among Tomato Accessions

The dendogram in Figure 1 reveals the phylogenetic relationship among the ten tomato accessions under study. By truncating the dendrogram at a genic similarity distance of 0.050, three clusters (A, B and C) of tomato accessions can be observed. Cluster A comprises of two tomato accessions: Tt007 and Tt008. Cluster B is made up of tomato accessions Tt004 and Tt005 which are closely related and a distant relative Tt002. Cluster C is made up of two accessions which are the most genetically related, accessions Tt009 and Tt010, and other distant relatives: Tt003, Tt001 and Tt006. Ancestors of tomato accessions Tt007 and Tt008 in cluster A, and accessions Tt004 and Tt005 in cluster B, might have evolved separately at a genic similarity coefficient of 0.15 down the phylogenetic tree. This result is similar to results obtained by Benor *et al*., (2008) and Korir *et al*., (2014) who reported a genic similarity coefficient of 0.18 and 0.77 for accessions in the same cluster.

Conclusion and Recommendation

The genetic diversity among ten (10) local accessions of tomato: *Solanum lycopersicum* (Linn) in the Southern Senatorial District of Adamawa State, Nigeria was successfully investigated using SSR markers. The investigation revealed that there is high 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. The tomato in Adamawa South though collected from relatively close locations yet, 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 as a result of the hyper variable 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 recommended that landrace cultivar Tto01 can be crossed with other accessions like
- Tto09 Tto10 to harness its unique characters of producing high number of fruits per truss and resistance to adverse environmental conditions.

Declaration Conflict of Interest

The authors wish to declare that there was no conflict of interest.

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