Agricultural and Biological Sciences Journal
Vol. 7, No. 2, 2021, pp. 46-50
http://www.aiscience.org/journal/absj
ISSN: 2381-7178 (Print); ISSN: 2381-7186 (Online)

Characterization of Some Selected Tomato (*Solanum lycorpersicum* L) Accessions Using Simple Sequence Repeat Markers

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Abstract

This study was carried out to show the usefulness of SSRs markers in characterizing tomato genotypes and to provide vital information to the geneticists for genetic improvement through development of better tomato varieties with increased fruit yield and quality. A total of 22 tomato accessions were planted in a 4 liter plastic container at University of Agriculture Makurdi screen house. 6 SSR primers were used for the characterization at the Molecular Biology Laboratory of University of Agriculture Makurdi. CTAB protocol for DNA extraction was used to obtain DNA from 23 day old leaves of accessions and amplified via PCR in a thermo-cycler. PCR products were run on 2.5% agarose gel stained with ethidium bromide and visualized under UV light. The bands formed were scored in a binary pattern for analysis. Result revealed a total of 126 bands generated across the accessions with Polymorphic Information Contents values of the SSR primers ranged from 0.703 to 0.967 with a mean value of 0.866 which shows that, they are highly informative for genetic analysis. Cluster analysis grouped the tomato accessions into three distinct clusters based on Euclidean correlation coefficient distance with cluster I, II and III having 42%, 40% and 14.0% of genetic similarity respectively. It also revealed a total of five pairs of duplicates, reducing the total number of genotypes to 5 which indicate that a very low genetic diversity existed among the genotypes obtained in these regions. The results obtained in this study will serve as a guide to agronomists and geneticists in germplasm management and improvement of tomato by intercrossing genotypes belonging to cluster III (NGB-00722 and Akeakpev) and cluster II (Roma VF, Tropimech and Mngishim) to create a suitable genotype with increased fruit yield and quality to enhance tomato production that will be helpful in the development of rural agro-industries in North central and Western Nigeria.

Keywords

Characterization, Tomato, SSR, Polymorphism and Intercrossing

Received: May 3, 2021 / Accepted: May 31, 2021 / Published online: June 29, 2021

1. Introduction

Tomato (*Solanum lycopersicum* L) is a dicot species of the family solanaceae and the genus *solanum* [16] that is consumed nearly in every household in Nigeria owing to its various uses. It is cultivated all over the world because of its high nutritive value and an excellent source of vitamin A and C [15]. According to Nkiru and Ifenkwe [17], tomato is helpful in the development of rural agro- industries. In North central and Western Nigeria, tomato production is limited to wet season with attendant shortage and high price during the

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dry season. Molecular markers can be considered as constant landmarks in the genome of an organism. They are important tool for genotype identification and studying the organization and evaluation of plant genome [18]. It characterizes variability that is not detectable by simple visual observation and the assessment of genetic diversity within and between populations at molecular level using various laboratory based techniques [14]. It define differences in nucleotide sequences which are unaffected by growth stage, season, location and agronomic practices [11]. Molecular markers have proven to be very useful in assessing genetic diversity and phylogeny, characterization of germplasm and detection of duplicates, parental verification in crosses, gene tagging in marker assisted breeding and gene cloning in genetic transformation [4, 5] and cannot be considered as a gene since they do not have any known biological function [2]. According to Solomon *et al*. [20] molecular markers can provide an effective tool for efficient selection of desired agronomic traits because they are based on the plant genotypes and thus, are independent of environmental variation.

Simple Sequence Repeats (SSRs) or microsatellites being one among the molecular markers are the most widely used types.

They are not only very common, also hyper variable for numbers of repetitive DNA motifs in the genomes of eukaryotes [21, 20], and are highly polymorphic, reproducible, co-dominant and multi-allelic types of variation [3] and are used in genome mapping, gene tagging and estimation of genetic diversity [19] They occur in chloroplast, mitochondrial as well as repetition of guanine and cytosine [7]. It is suggested that the variation or polymorphism of SSRs are a result of polymerase slippage during DNA replication or unequal crossing over [12]. They are suited to distinguish closely related genotypes; because of their high degree of variability, they are, therefore, favored in population studies and for the identification of closely related cultivars [8].

Miskoska-Milevska *et al*. [13] studied the applicability of 8 DNA microsatellite loci in genetic differentiation of 6 morphologically different tomato varieties of *Lycopersicon esculentum* Mill. Kumar *et al*. [10] studied the genetic variation of 19 genotypes of tomato (*Solanum lycopersicum* L.) with 11 polymorphic SSR markers and Nelson *et al*. [16] assessed the genetic diversity of 30 introductions of cherry tomato with 36 microsatellite molecular markers. Singh *et al.* [19] characterized 24 genotypes of tomato (*Lycopersicum esculentum* Mill) using morphological and 16 SSR primers which resulted in polymorphic, scorable and reproducible results. These necessitated this study to show the usefulness of SSRs markers in characterizing tomato genotypes, identifying duplicates accessions and to select genotypes for future hybridization program.

2. Materials and Methods

2.1. Plant Materials Location

22 tomato accessions were obtained from National Centre for Genetic Resources and Biotechnology Ibadan (8), Agrotropic Limited Jos (5) and local farmers in Benue State (9). Accessions were planted in a screen house of University of Agriculture Makurdi.

2.2. DNA Extraction

Genomic DNA was isolated according to Doyle and Doyle [6] CTAB DNA Extraction Protocol with slight modifications: 23 day old leaves of various accessions were collected and preserved in a plastic containers containing silica gel for 7 days, about 0.1g of the collected samples were ground into a fined powder using mortar and pestle and immediately transferred into 2ml tube where 800µl CTAB extraction buffer was added and the pellets of the various samples were air dried for 24 hours at room temperature. DNA yield and quality were assessed by gel electrophoresis using standard DNA marker DL 50kb.

2.3. SSR Markers

6 SSR primers were selected among the more polymorphic published study of Singh *et al.* [19]

2.4. DNA Amplification

20µl of water was added to each PCR Premix tube and each was reduced to half into a new PCR tube. 2µl of the liquid template DNA, 1.5µl of both the forward and reverse Primers were added to the 10µl of the premix, given a grand total of 15µl which were spun and loaded in the Bio-Rad thermo cycler for 35 cycles with the following thermal conditions: initial denaturation of 94°C for 5min, final denaturation of 94°C, 30Sec, primer annealing at temperature according to the primer sequence for 30Sec, extension of 72°C, 1min and final extension of 72°C, 4min and holding temperature of 4°C, ɑ (infinity)

2.5. DNA Separation on Agarose Gel

The PCR product was resolved on 2.5% agarose gel stained with 3 µl of 10 mg/ml Ethidium bromide (EtBr) [1] and allowed to cool to about 40°C. The conical flask was covered with white cork layer to prevent inhalation of the hazardous Ethidium Bromide. The gel plate was set at 25 combs with each well designate for a particular sample; they were set at 1.5 mm apart and then placed in the plate. The gel was poured and allowed to solidify for about 30 minutes.

Thereafter, 6µl of PCR products including 50kb DNA ladder loaded both ends of the well in 1X TBE and electrophoresis was performed for 2 hours at 100 volt.

2.6. DNA Visualization

The banding pattern of the samples resolved on agarose gel was viewed on a UV Bench top trans-illuminator and the gel image was captured using a camera according to a similar technique used by Kadry and Gamal [9] for band scoring and only distinct bands were score as present 1 or absent 0.

2.7. Molecular Data Analysis

Polymorphism Information Content (PIC) for each SSR was calculated according to the formula

$$PiCi = 1 - \sum pi^2$$

Where *pi* is the frequency of the *i* th allele for each SSR marker *i* th summed across all alleles for the locus in the set of 22 accessions to be investigated [22, 20].

Cluster analysis was carried out using Unweighted Pair Group Method and Arithmetic Average (UPGMA) using a Minitab 17.1 software.

3. Results and Discussion

The sums of polymorphic and monomorphic bands produced by the primers are 46 and 80 respectively, making a total of 126 bands produced across the Agarose gel with genotypes NGB-00711 showing no band for all the 6 SSR primers as shown in Figures 1, 2, 3, 4, 5 and 6. The Polymorphic Information Contents (PIC) result as revealed in Table 1 shows that, all the six SSR markers used on the genotypes were polymorphic and scorable, thus, the above observation is in tandem with the report by Sign *et al.* [19] who showed that these primers are polymorphic and scorable. The PIC values ranged from 0.967 to 0.703 with a mean diversity of 0.866. Primer T-7 produced the highest percentage polymorphism (26.087%) and all the primers evaluated obtained PIC values greater than 0.5 (Table 1). The primers evaluated with their level of informative in ascending order are; T-7, T-38, T-31, T-45, T-34 and T-43 with PIC values of 0.707, 0.833, 0.868, 0.899, 0.925 and 0.967 respectively. According to Nelson *et al*. [16], PIC values of 0.5, 0.4 and 0.2 are highly informative, moderately informative and little informative respectively. This shows that, all the primers used in this study are highly informative. This suggests that, SSR markers are effective tool for characterizing tomatoes as they are highly informative in differentiating and reviewing genetic diversity among tomato accessions.

Cluster analysis on genetic similarities among the genotypes based on SSR primers as revealed in figure 7 grouped the genotypes into three clusters with genetic similarity values for all genotypes ranging from 42% (cluster I) to 14% (cluster III). Cluster 1(NGB-00711, NGB-00721, Atumba, Dereka, NGB-00724, and Ishase) had 42%, cluster 2 (NGB-00713, UC82-B, Rio-grande, Cerel, Mngishim, Kal, NGB-00725, NGB-00726, NGB-00732, Roma VF, Roma and Tropimech) 40% and Cluster 3(NGB-00722, Apaa, Akeakpev and Gambo) 14% of genetic similarity. The lowest similarity 14% shown by NGB-00722, Apaa, Akeakpev and Gambo in genotype analysis may probably be hybrids.

The dendrogram also classified the genotypes into 5 groups namely; group 1 (NGB-00711, NGB-00721, Atumba and Dereka), group 2 (NGB-00724 and Ishase), group 3 (NGB-00713, UC82-B, Rio-grande, Cerel, Mngishim, and Kal), group 4 (NGB-00725, NGB-00726, NGB-00732, Roma VF, Roman, and Tropimech) and group 5 (NGB-00722, Apaa, Akeakpev and Gambo). This reduces the total number of genotypes to 5 based on their genetic similarities and suggests phylogenetic relationships among the accessions. This clearly shows that, molecular markers are effective tool for characterizing the variability that is not detectable by simple visual observation and assessment of genetic diversity within and between populations [14] and characterization of germplasm and detection of duplicates.

Table 1. Primer code, Sequence, Annealing temperature,%Polymorphism and PIC values for 6 SSRs used on 22 Tomato Genotypes.

Primer code	Sequence	Tm (°C)	Polymorphic bands	%Polymorphism	PIC
T-7	GTTATGGATTCACTTACCGCAAGTT (*F*)	51.0	12	26.087	0.703
	CATTCGTGGCATGAGATCAA (*R*)	51.0			
T-31	GGTAATCAATTTTGAAGCTAAAAGC (*F*)	51.0	8	17.391	0.868
	TGGGAAGAACTCAAGTCAAAAA (*R*)	51.0			
T-34	GTCAACTAGCGCTCCAATCT (*F*)	51.0	6	13.043	0.925
	AAAGGGTTGTGGGAATTGTG (*R*)	51.0			
T-38	GAATTAGAGGGTTTGTGATACCG (*F*)	55.0	9	19.565	0.833
	AAAAAGCTTCCTGGCTAAGAAAT (*R*)	55.0			
T-43	GAGAGAGAGTATGTATGTGCATTTCC	55.0	4	8.696	0.967
	TGAAAATTTGTGGTGTTGACG (*R*)				
T-45	GTGAGGGAGTGGGATTCAAAC (*F*)	53.1	7	15.217	0.899
	AATTAGGGGATACGGGATCG (*R*)	53.1			
Total			46		5.195
Mean			7.667	16.667	0.866

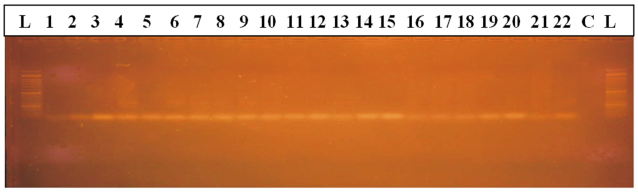

Figure 1. Primer T-7 Profile.

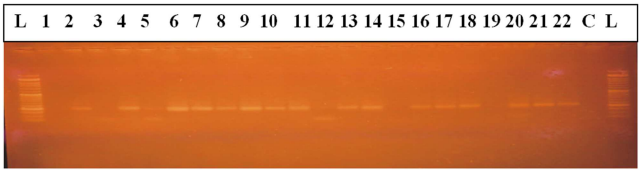

Figure 2. Primer T-31 Profile.

Figure 3. Primer T-34 Profile.

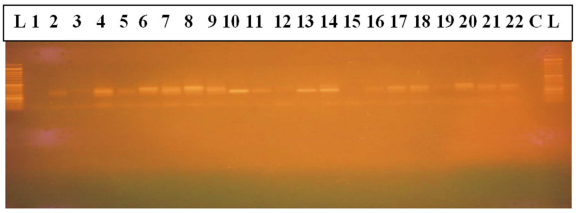

Figure 4. Primer T-38 Profile.

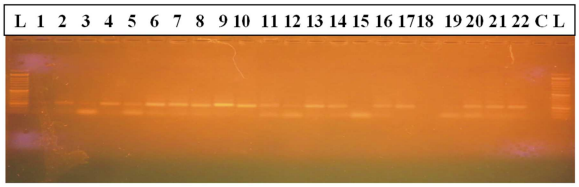

Figure 5. Primer T-43 Profile.

Figure 6. Primer T-45 Profile.

legend: 1=NGB-00711, 2=NGB-00713, 3=NGB-00721, 4=NGB-00722, 5=NGB-00724, 6=NGB-00725, 7=NGB-00726, 8=NGB-00731, 9=Roma VF, 10= UC82-B, 11=Rio-Grande, 12=Atumba, 13=Roma-Savanna, 14= Tropimech, 15=Dereka, 16=Cerel, 17=Mngishim, 18=Apaa, 19=Ishase, 20= Akeakpev, 21=Kal and 22=Gambo, L=Ladder and C= control

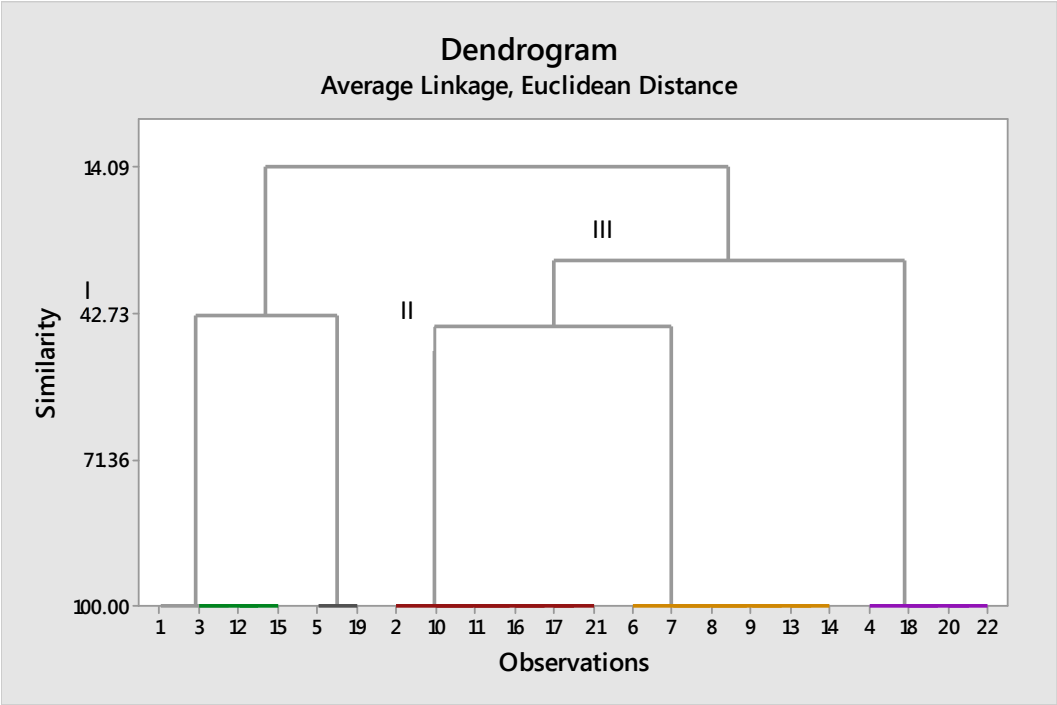

Figure 7. Dendrogram showing Genetic Similarities among the Genotypes based on SSR Primers.

Legend: 1=NGB-00711, 2=NGB-00713, 3=NGB-00721, 4=NGB-00722, 5=NGB-00724, 6=NGB-00725, 7=NGB-00726, 8=NGB-00731, 9=Roma VF, 10= UC82-B, 11=Rio-Grande, 12=Atumba, 13=Roma-Savanna, 14= Tropimech, 15=Dereka, 16=Cerel, 17=Mngishim, 18=Apaa, 19=Ishase, 20= Akeakpev, 21=Kal and 22=Gambo

4. Conclusion

This study shows the usefulness of these primers: T-7, T-31, T-38, T-43 and T-45 for effective characterization of tomatoes as they are highly informative in differentiating and reviewing genetic diversity and identifying duplicates among tomato accessions obtained in these regions, as it reduces the total number of accessions to 5. It also provide useful information to agronomists and geneticists in germplasm management and improvement of tomato by inter-crossing genotypes belonging to cluster III (NGB-00722 and Akeakpev) and cluster II (Roma VF, Tropimech and Mngishim) as they are diverse and possesses productive traits such as plant size and fruit size that can be combined to create a suitable genotype with increased fruit yield and quality to enhance tomato production that will be helpful in the development of rural agro-industries in North central and Western Nigeria.

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