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COAST
JOURNAL OF THE FACULTY OF SCIENCE
Volume 2, Issue 1. June, 2020 ISSN: 2714-3716
Coast, J. Fac. Sci. 2 (1): 326 - 335

MOLECULAR CHARACTERIZATION OF TOMATO (*Solanum* lycopersicum L.) VARIETIES USING SIMPLE SEQUENCE REPEATS (SSRs) MARKERS.

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Abstract

Genetic diversity is a crucial factor in breeding crop plants. Molecular characterization of tomato (*Solanum lycopersicum* L.) varieties using Simple Sequence Repeats markers was conducted with a view to selecting the best among the varieties. Eleven accessions were sourced from Akure, Ikare and Ibadan, sown in germination trays, transplanted to the field and was laid out in Randomized Complete Block Design (RCBD). Data on plant height, number of branches/plant, days to first flowering, days to first fruit set, number of fruits/plant, number of locules/fruit, number of flowers/plant and number of clusters/plant as well as pericarp thickness, fruit weight, average fruit weight and fruit yield/plant were collected and analyzed using SPSS version 20. Analysis of variance revealed significant variation ($P \leq 0.05$) among varieties for all studied characters. Morphological relationships among varieties were observed using Cluster Analysis which classified genotypes into five divergent groups. Thirteen polymorphic Expressed Sequence Tag-derived (EST) SSRs primers amplified 143 DNA fragments and detected 61 alleles. The number of alleles per locus ranged from 5 to 7 with a mean of 4.69 per primer. The Polymorphic Information Content (PIC) also ranged from 40% to 84%. Considerable genetic diversity among studied varieties was observed at morphological and molecular levels indicating valuable tomato germplasms which could be used for further genetic studies.

Keywords: EST-SSRs, Polymorphic Information Content (PIC), Solanum lycopersicum

Introduction

Tomato (*Solanum lycopersicum Linn.*) plays an important role in human nutrition which is mainly attributed to its high content of vitamin C and carotenoids constituents, particularly lycopene and β-carotene, accumulated in plasma and tissues (Dorais *et al.,* 2008; Gerster 1997; Clinton *et al.,*1996). Tomato is one of the most extensively studied cultivated plant species grown all over the world, occupying globally a cultivation area of approximately 4.338.834 million hectares (FAOSTAT, 2010). With the rapid increase in the size of human population, the world faces a greater demand for agricultural products than at any time in history bringing the growth of the world population well ahead of crop production. According to FAO, the production of agricultural products on a global scale must be increased by 70% in the year 2050 (Pardey, 2011). In order to achieve high levels of increase in food production, the contribution of plant biotechnology innovations will have to be greater than in the past. Traditional protocols of plant genetics and breeding, which are based on phenotypic characterization, selection and progeny testing, have been very effective in improving crop productivity and quality during the past several decades (Warren, 1998), but are often time consuming and not without inherent difficulties thus, more efficient breeding strategies are needed to ensure the achievement of the expected increase in food production. An alternative approach to improving selection efficiency in plants is the use of markers and maps which can facilitate determination of the number, chromosomal location, and individual and interactive effects of genes or QTLs affecting desirable traits (Tanksley, 1993).

Molecular markers are powerful tools for analyzing genome diversity within a species, and to evaluate genetic relationships between individuals and populations. They have been used for mapping of genomic regions containing genes of agricultural interest (Charcosset and Moreau, 2004). As yield of tomato fruit is a polygenic character, it is associated with many agronomic, morphological and physiological traits, so direct selection for yields may be often misleading. Microsatellites or SSRs are one of the most indicated molecular markers used in studying polymorphism between DNA sequences. Smulders *et al.,* (1997); Bredemeijer *et al.,* (2002); He *et al.,* (2003) confirmed a significant role of SSR markers in survey of genetic diversity and variability of genus *Solanum*, as well as for tomato variety identification. Tomato, no doubt is a popular fruit vegetable, however, its production in Nigeria is low compared to its demand which resulted from lack of high yielding varieties thereby posing a threat to food security. Hence, the need to use molecular markers to genetically characterize selected varieties of tomato and to correlate their morphological traits with genetic information with a view to selecting elite genotypes for crop breeding in Nigeria so as to help curb food insecurity.

Materials and Method

A total of eleven tomato accessions namely NG/AKR/MAY/10/001, NG/AA/SEP/09/042, NG/SA/01/10/002, NG/IKR/JUN/01/002, NGHB/01362, NG/IKR/JUN/01/001, NGHB/09/113, NG/MR/JAN/01/001, NG/AA/SEP/09/053, NGHB/01649 and LO0169 represented by T1, T3, T4, T5, T6, T7, T11, T12, T13, T14 and T15 respectively were sourced from Oja Oba, Akure, Oja Oba, Ikare and National Centre for Genetic Resources And Biotechnology (NACGRAB), Ibadan. Selected tomato accessions were sown in nursery trays using top soil and seedlings were raised using regular nursery practices. The experimental plots were ploughed and divided into fifteen (15) blocks each of 1.5m by 1.5m per plot with an alley of 1.5m by 1m and was pegged. At four weeks after planting (WAP), nine (9) seedlings were transplanted on each block and watered regularly. Three replicates were laid out in a Randomized Complete Block Design (RCBD). Five seedlings were randomly selected and tagged for data collection. Data obtained from morphological characterization were subjected to Analysis of Variance and Significance means were separated using Duncan Multiple Range Test (DMRT) at 5% significance. DNA was isolated and extracted from seed samples using Zymos Research Kit and molecular analysis was done using 13 Expressed Sequence Tag (EST) derived SSRs. Polymerase Chain Reaction was carried out using Multigene Optimal PCR machine and amplification was done separately for each primer following specific amplification conditions. Agarose gel was prepared and stained with ethidium bromide for 10-15mins and the quality of the

DNA was viewed under UV light. The DNA bands were scored as positive (+) or negative (-) for the presence or absence of polymorphism respectively. Genetic parameters and Polymorphic Information Content (PIC) were calculated.

Table 1: Names and sequence of primers used

PRIMER	SEQUENCE	TM
EST-SSR1F	ACCTACCTGTCTCCGCCTCT	55
EST-SSR1R	TGACAAGGTAAAGCCAACCC	55
EST-SSR2F	CTTATGTGAAAACACCTCGCTC	54
EST-SSR2R	TTCAAAATTCCCCAAAGACG	54
EST-SSR7F	GAAGAAGATGGTGGGGATGA	54
EST-SSR7R	CTTGCAACAATCGTGAATGC	54
EST-SSR19F	ACCTGCACACACCACACACT	53
EST-SSR19R	GATCAAAGAAGCGGGATGAT	53
EST-SSR23F	TAGACTGGGCCTGTGGTGTCTT	52
EST-SSR23R	TGGTGAATCAATTTTGGGT	52
EST-SSR25F	ATTGGGGAATGGGTTTTCTC	54
EST-SSR25R	AAACGAAGGCAACAACGAAG	54
EST-SSR26F	TCAAATGGCTTCTCTTGTTCTTT	54
EST-SSR26R	TTGTTGGAAACTCCTTTGGC	54
EST-SSR35F	CATAAGAAGAAAGGTGTGAATGAGA	52
EST-SSR35R	GTTGCTTTGTCTTTGTCGCC	52
EST-SSR42F	CCAAAAGAAGTGGGTCCAAA	54
EST-SSR42R	AAACTAGCGACAAATAAAAGCAGA	54
EST-SSR67F	ATCTCGATTTGCTGCTCCAT	54
EST-SSR67R	CAAGTTCACACCATTTTCTCTCC	54
EST-SSR71F	GGACCAAGCGAAGTTGGATA	54

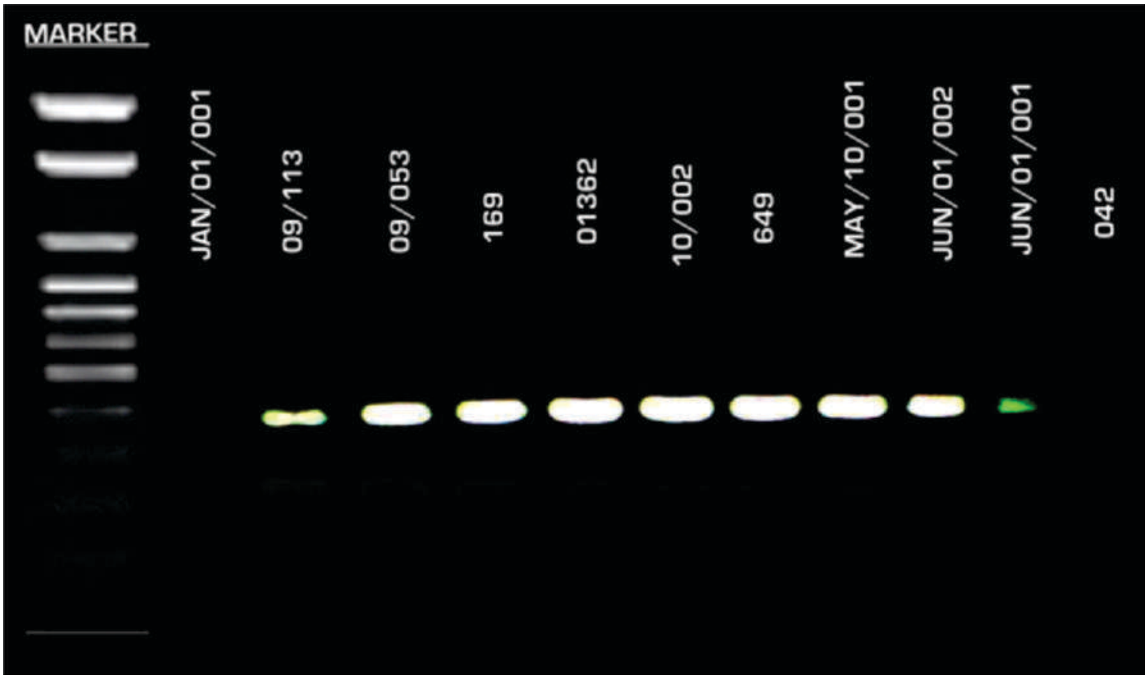

Figure 1: SSR profiles of tomato varieties generated using SSRs markers

Results

Observations for all characters were highly significant. Tomato varieties varied in plant heights across all experimental weeks. Flowering appeared as early as 64days after planting in NG/AA/SEP/09/042 as it had the lowest mean value, while NG/AA/SEP/09/053 flowered 80 days after planting. NGHB/09/113 was the tallest variety while NG/MR/JAN/10/001 was the shortest variety and the lowest fruit yielder. NG/AA/SEP/09/042 were the earliest to set fruits at 74 days after planting while NG/01649 fruited last among the varieties studied at 88 days after planting. Poor number of branches per plant were observed in NG/01649. The highest average fruit weight of the varieties was obtained in NG/AKR/MAY/10/001, followed by NG/AA/SEP/09/042 while LO0169 was the variety with the lowest average fruit weight. NG/AA/SEP/09/053 was the highest fruit yielder while the lowest fruit yielder of the studied varieties was NG/MR/JAN/10/001. Fruit yield indicated positive correlation with all characters except days to first flowering, days to first fruit set and number of locules per plant.

Morphological and SSRs Marker Cluster Analyses

Morphological cluster analysis placed varieties into two divergent groups- Group A and Group B (Figure. 2). Group A subdivided into subgroup A1 consisted of 3 accessions namely NG/AKR/MAY/10/001, NG/AA/SEP/09/042 and NGHB/09/113 while subgroup A2 had NG/AA/SEP/09/053 as the only accession. Accessions in group B subgroup B1were NGB/01362, NG/01649, LO0169 and NG/MR/JAN/10/001 while subgroup B2 consisted of NG/IKR/JUN/01/002, NG/SA/01/10/002 and NG/IKR/JUN/01/001 accessions.

Molecularly, UPGMA dendogram grouped all DNA samples into three major clusters

(Figure III). Group I had only one accession (NG/MR/JAN/10/001), group II (NG/SA/01/10/002), while group III had two subclusters. Subcluster I had 3 accessions (NG/01649, LO0169 and NG/AA/SEP/09/042) while subcluster II had 6 varieties namely; NG/IKR/JUN/01/001, NG/IKR/JUN/01/002, NG/AKR/MAY/10/001, NGB/01362, NGHB/09/113 and NG/AA/SEP/09/053.

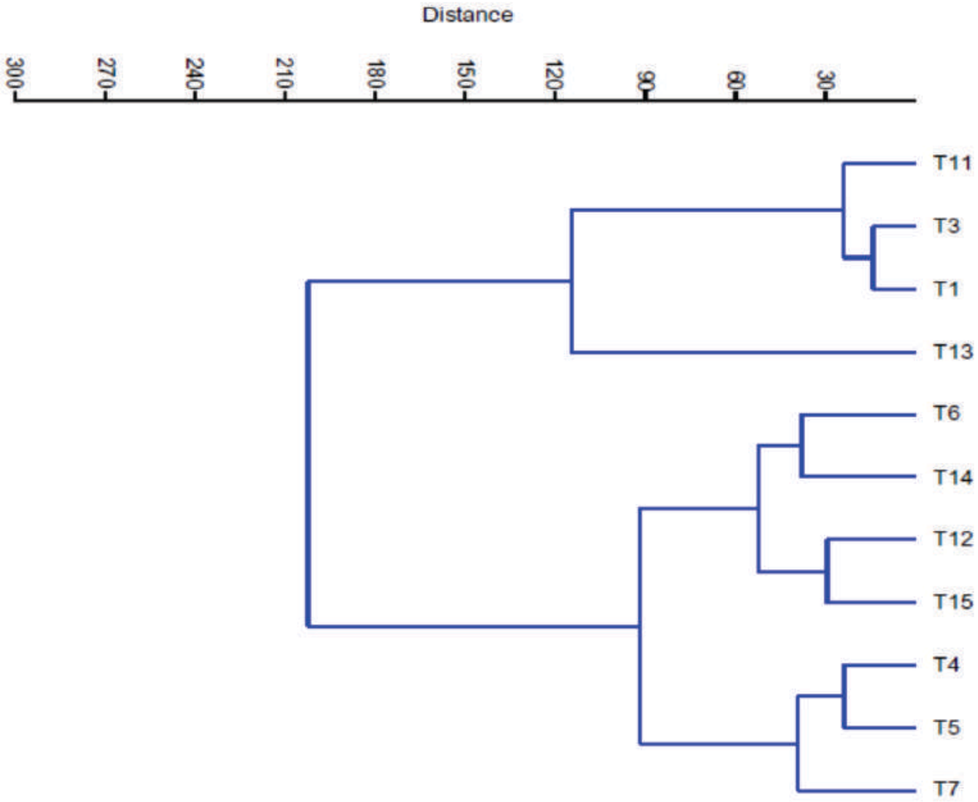

Figure 2: Morphologial Cluster Analysis

Key: T1= NG/AKR/MAY/10/001; T3= NG/AA/SEP/09/042; T4= NG/SA/01/10/002; T5= NG/IKR/JUN/01/002; T6= NGHB/01362; T7= NG/IKR/JUN/01/001; T11= NGHB/09/113; T12= NG/MR/JAN/01/001; T13= NG/AA/SEP/09/053; T14= NGHB/01649; T15= LO0169.

Allelic Variation Based on SSRs Markers

The number of clear fragments detected by 13 polymorphic EST-SSR in the studied tomato accessions was 61 out of the 143 DNA fragments amplified. The number of alleles per locus ranged from 5 to 7 with a mean of 4.69 per primer. DNA fingerprinting of the EST-SSR primers used is shown in Figure 1. The mean Polymorphic Information Content (PIC) for EST-SSR loci ranged from 0.40 to 0.84.

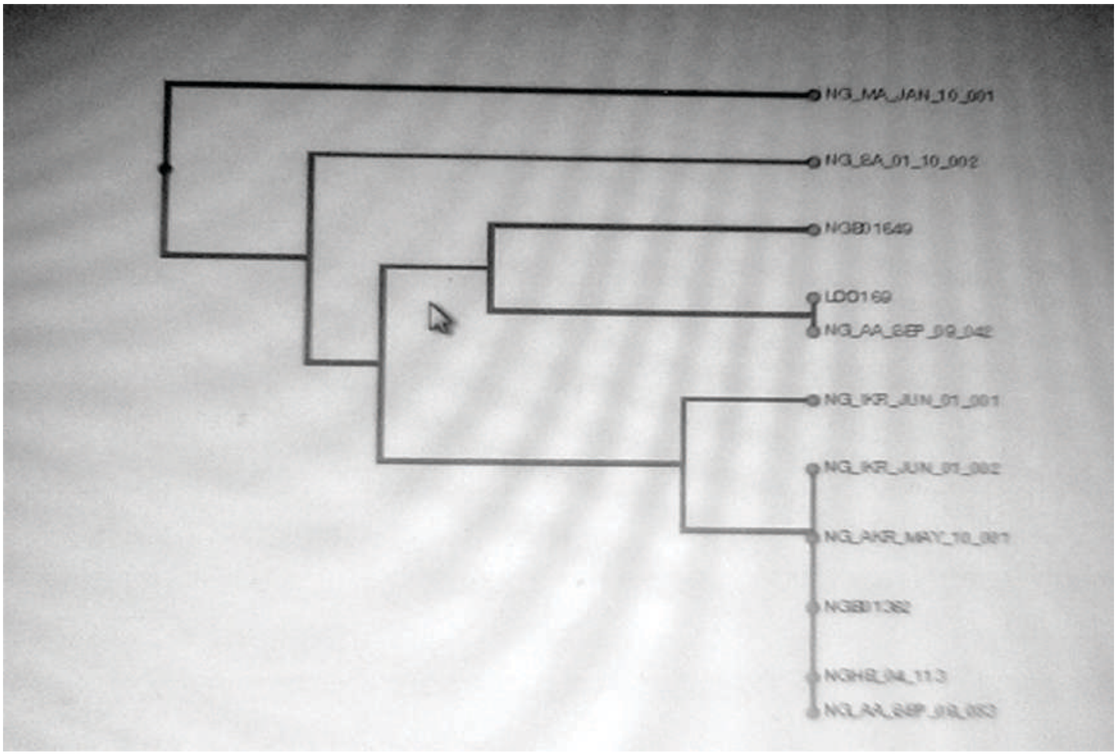

Figure 3: Dendrogram of tomato accessions developed from SSRs data using Unweighted Pair Group of Arithmetic Means (UPGMA)

Discussion

The identification of variability among accessions is pivotal to the maintenance and utilization of germplasm resources (Mwirigi *et al.*, 2009). Systematic study and evaluation of germplasm is of great importance for current and future agronomic and genetic improvement of the crop (Reddy *et al.*, 2013). To identify and estimate the genetic diversity of plants, various methods can be used including morphological, biochemical and molecular markers. The variability of eleven varieties of tomato was investigated using morphological traits and molecular markers to study the extent of genetic variability and to select elite varieties for future breeding programmes. The result of this study showed that there were significant differences ($P \leq 0.05$) in all characters which are due to differences in genetic and environmental conditions. This was anticipated since different genotypes perform differently in the same environment Blay (1999). Tomato accessions varied in plant height across all experimental weeks. This was in agreement with Messian (1992), who indicated that tomato plant height may vary up to 2m. NGHB/09/113 which had the highest height was seconded to having the highest number of branches and fruit yield per plant. This variety (NGHB/09/113), if planted, will be very tall, more branched and it will produce more fruits. The shortest variety had the lowest fruit yield per plant. This is because the fruits were hanging low on the plant as a result of its shortness, thereby touching the ground making the fruits susceptible to fungal diseases such as fruit rot resulting to low fruit yield. The high positive correlation between plant height and number of branches across all weeks indicated that as plants grow taller, they produce more flowers and become more branched. This is in accordance with Trites and Bidwell, (1987), who asserted that increase in height of plant gives the plant an advantage in competing with other plants for light and that the formation of new branches, more efficient and better positioned leaves at the top enhances photosynthesis. There was also positive correlation between plant height and fruit yield per plant which indicates that the taller the plant, the more flowers are produced and more leaves are formed to photosynthesize and provide assimulates to the flowers and fruits. Therefore, fruit yield can be improved by increasing plant height.

Environmental and genetic variations during this study, provided evidence for disparity in the number of flowers per cluster and number of fruits per cluster. Variety (LO0169) with the highest number of flowers per cluster, however, is a short plant, had an average number of branches and very low fruit yield per plant, while variety (NG/AA/SEP/09/053) seconded to it, is very tall, had high number of branches per plant, had the highest number of fruits per cluster and consequently it was the highest fruit yielder. Although NG/AA/SEP/09/053 flowered and fruited late, it could be crossed with LO0169 to produce plants with early flowering and fruiting. No significant correlation between number of flowers per cluster and fruit yield per plant but there was a positive correlation between number of fruits per cluster and fruit yield per plant indicating that fruit yield can be increased by improving upon number of fruits per cluster. Positive correlation between number of fruits per plant and fruit yield per plant is in accordance with the submission of Aravindakumar and Malge, (2002), who noticed positive correlation of number of fruits per plant with fruit yield per plant.

John *et al.,* (2005), justified that varieties have inherent potential for production of cluster and consequently produces more fruits. Variety with the highest number of clusters per plant was the tallest of all the studied varieties, had the second highest number of branches per plant, second highest number of flowers per cluster and second highest fruit yield per plant. This variety is suitable for selection based on these characters. The variety (NG/AKR/MAY/10/001) with the highest fruit weight is a tall plant, had high number of branches per plant and high fruit yield per plant. It also flowered early and fruited 8 days after flowering. This variety can be selected as a result of its fruit shape and size and can be crossed with variety NG/AA/SEP/09/053 to obtain desirable variety with the highest fruit yield in future breeding programmes. Olaniyi and Fagbayide, (1999) submitted that variations in yield may be due to genetic differences among the varieties since they were grown under the same environmental conditions. Raviv*et al.,* (2004), reported that responses to low yield may also be attributed to high or low rainfall, high day and night temperatures during the experimental period. Morphologically, the only accession (NG/AA/SEP/09/053) in subgroup A2 had fruit yield per plant and number of branches at week 4 higher than other varieties. In subgroup A1, varieties (NG/AKR/MAY/10/001, NG/AA/SEP/09/042 and NGHB/09/113) were very tall and they also have high average fruit weight except NGHB/09/113. Accessions in subgroup B1 (NGB/01362, NG/01649, LO0169 and NG/MR/JAN/10/001) flowered early, fruited late and also had low fruit yield. Characters like average fruit weight, number of fruits per cluster, number of locules per plant, number of flowers per cluster, days to first flowering and days to first fruit set were viewed for these accessions without significant differences (P≥0.05). Number of flowers per cluster, number of fruits per cluster and number of fruits per plant of the accessions in subgroup B2were low compared to other varieties and all fruited at the same time. On the other hand, UPGMA dendogram grouped the tomato accessions used for molecular analysis into three major clusters. LO0169 and NG/AA/SEP/09/042 with 100% similarity are likely to be clones because they are genetically closely-related than NGB/01649 found in the same cluster with them. NG/MR/JAN/10/001 and NG/SA/01/10/002 were distinct genetically as they stood out as the only accession in separate clusters. NG/IKR/JUN/01/002, NG/AKR/MAY/10/001, NGB/01362, NGHB/04/113 and NG/AA/SEP/09/053 have the same genetic background but they exhibit differences in their response to environmental variation. Thus, the phenotypic variations exhibited by closely-related genotypes may be attributed to response to environmental influences Falconer (1989).Of all the studied tomato varieties, only two accessions (NG/AKR/MAY/10/001 and NGHB/09/113) were morphologically and genetically related as they were seen clustered together on both analyses.LO0169 and NG/AA/SEP/09/042 exhibited different phenotypic features but had similar genetic background. While some of these varieties exhibited similar phenotypic characters but were genetically different, some were morphologically different and were genetically distinct from each other.

Conclusively, it is evident that considerable genotypic variation existed among the studied genotypes indicating greater potentiality for their exploitation to improve yield and its components. There was a good scope for selection also. The overall performance in relation to fruit yield and number of fruits per plant was best in accession NG/AA/SEP/09/053while the promising genotypes were NG/AKR/MAY/10/001 and NG/AA/SEP/09/042. However, further work is warranted in this regard.

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