Assessment of Genetic Diversity among Selected Tomato (*Solanum lycopersicum* L.) Lines Based on SSR Markers

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Abstract
Genetic diversity in eight Nigeria, two Benin Republic and five United Kingdom cultivated tomato lines was assessed using simple sequence repeat (SSR). Genotyping was carried out with 15 SSRs. Among the markers used, 11 SSR markers were polymorphic. For the SSR analysis, the total number of polymorphic alleles was 33, with a mean of 3.00 and the average polymorphic information content (PIC) was 0.36. The genetic diversity within the lines was considerably moderate (0.31). The Estimates of Rogers' distance varied from 0.03 to 0.66. The 15 lines were clustered into two major groups and a singleton based on unweighted pair-group method with arithmetic mean (UPGMA) cluster analysis of the SSR-based genetic distance (GD) estimates. Group I included 10 lines from all the geographical regions evaluated, and the group II consists of 4 lines which are essentially from Nigeria. TM09 (UK line) grouped separately forming a singleton. Understanding the genetic diversity among the tomato lines is a good starting point for establishing and maintaining collections, germplasm banks and proper characterisation of Nigerian tomatoes in order to preserve the genetic variability.

Keywords: Tomato, Genetic diversity, molecular markers, genetic distance

Introduction

The cultivated tomato (*Solanum lycopersicum* L.) is grown in different parts of the world, primarily in tropical and temperate zones but has its origin in Chile, Boliva, Ecuador, Colombia and Peru (Peralta and Spooner, 2001; Robertson and Labate, 2007; FAOSTAT, 2011). The time and place of domestication of tomato are not known with certainty but since the 20th century, morphologically and physiologically different cultivars have been developed and the cultivars available today show a wide variety of fruit traits (size, shape, colour, seed-weight and morphology) (Doganlar *et al*., 2000; Frary and Doganlar, 2003; Tanksley, 2004).

Selection for varied fruit shapes, which is a distinctive feature of the tomato history and their local adaptation to local environments started tomato domestication (Bauchet and Causse, 2012). Breeding research has shown that cultivated tomato has suffered several bottlenecks, and when compared with its wild relatives; the amount of genetic variation of the cultivated tomato is considered very limited (Miller and Tanksley, 1990). In order to achieve some breeding goals like stress resistance and nutritional value, the genetic diversity among lines must be analysed (Bauchet and Causse, 2012). Furthermore, the assessment of the genetic diversity among cultivated tomato lines will be useful for understanding the existing genetic relationships among them, characterisation of accessions and for managing and conservation of germplasm, thus allowing the expansion of the genetic basis of tomato breeding programmes.

The genetic diversity in plants has been conventionally assessed using morphological or biochemical traits and recently with molecular markers. Molecular markers allow the direct assessment of diversity in genotypes at DNA level generating information not affected by the influence of environment on gene expression (Larry and Joanne, 2007). Several markers have been used in genetic analysis of tomato such as restriction fragment length polymorphisms (RFLP), random amplification of polymorphic DNA (RAPD), inter simple sequence repeats (ISSRs), amplified fragment length polymorphisms (AFLPs) and Simple Sequence Repeats (SSRs) (Kidwell *et al*., 1994; Stevens *et al*., 1995; Vos *et al*., 1995; Bredemeijer *et al*., 2002; Park *et al*., 2004; Carelli *et al*., 2006).

The use of molecular marker such as SSRs are effective and reliable for measuring genetic diversity among crop germplasm (Mengoni *et al*., 2000; Tam *et al*., 2005; Behera *et al*., 2008; Chen *et al*., 2009; Korir *et al*., 2014). The application of the SSR markers in varietal identification of tomato have also been well explored (Hokanson *et al*., 1998; Smulders *et al*., 1997; He *et al*., 2003; Rajput *et al*., 2006; Benor *et al*., 2008; Pritesh *et al*., 2010). They are co-dominant markers and are very useful for a number of plant species (Al-quadumii *et al*., 2012).

Genetic diversity studies in tomato species have been

carried out using lines collected from diverse geographical origins, as most of the cultivated tomato lines of the same geographical location have been shown to have low genetic variability (He *et al*., 2003; Frary *et al*., 2005; Benor *et al.,* 2008). This may have been due to the narrow genetic base and the self-pollination method in cultivated tomato (Alvarez *et al*., 2001; Wang *et al*., 2006; Yi *et al.,* 2008). To date, some tomato lines from some diverse geographical origins have not been characterised. Therefore, in this present study, we characterise tomato lines based on DNA marker (SSR) to assess the genetic diversity in cultivated tomato lines grown in Nigeria, Benin Republic and United Kingdom. The molecular data will effectively contribute to understanding the level of genetic variability necessary for proposing collection and maintenance in germplasm banks of Nigerian tomato lines.

Materials and Methods

Plant Material and Genomic DNA Extraction

The 15 cultivated tomato lines (*S. lycopersicum*) used in this study are listed in Table 1. The lines included 8 lines from Nigeria (collected from 6 states), 2 lines originated from Republic of Benin (collected from Cotonou) and 5 lines from United Kingdom (seeds were purchased from Seed Parade, UK). DNA was extracted from fresh leaf tissue of 2-3 plants as described by Dellaporta *et al*. (1983). The DNA precipitates were air dried, suspended in 20 µL of 1X TE buffer and quantified by spectrophotometer (NanoDrop Technologies, Wilmington, Delaware, USA).

SSR Analysis

The set of 15 SSR primers used for genotyping were selected from Benor *et al*. (2008) and the primers were synthesised by Thermo Scientific, Hatfield, South Africa. The polymorphic primers are listed in Table 2. Amplification reaction by PCR assay was carried out in a final reaction volume of 10 µL, containing 2.9 µL distilled water, 1.0 µL of 10 X PCR buffer with 1.0 µL of 25 mM $MgCl_2$, 2.0 µL of 25 ng/µL template DNA, 0.5 µL each of 5 pMol forward and reverse primers, 1.0 µL DMSO (dimethyl sulphoxide), 0.8 µL of (2.5 mM of each dNTPs) and 0.3 µL (1u/L) Taq DNA polymerase. The PCR profile starts with initial denaturation at 94 °C for 5 min, followed by denaturation for 30 s at 94 °C, 65 °C for 30 s, 70 °C for 30 s, then back to 9 cycles of 30 s at 94 °C, followed by 93°C for 15 s, 55 °C for 20 s, extension at 72 °C for 30 s, then back to 34 cycles of 93 °C for 15 s, followed by a final extension at 72 °C for 5 min. Then PCR reaction was allowed to hold at a temperature of 10 °C till needed.

The PCR products were visualised through electrophoresis in non-denaturing 6% (m/v) polyacrylamide gel run in 0.5 X TBE at 80 V, 300 mA 60 Watt for 1.5 h. The gels were stained with ethidium bromide solution for 5 min, and visualised under UV trans-illuminator using a gel photo documentation system attached to a monitor. The allele sizes of amplicons were determined using a 50-bp DNA ladder (Invitrogen). Polymorphic alleles were scored for presence and absence as 1 and 0, respectively.

Table 1: Geographic Origin of the Tomato Lines Used in this Study

Code	Origin and Place of Cultivation
TM01	Nigeria (Ilorin East, Kwara State)
TM02	Nigeria (Osin Village, Osun State)
TM03	Nigeria (Oyi Village, Osun State)
TM04	Benin Republic (Cotonou I)
TM05	Benin Republic (Cotonou II)
TM06	Nigeria (Ogbomoso, Oyo State)
TM07	United Kingdom (Marmande)[a]
TM08	United Kingdom (Subarctic Planty)[a]
TM09	United Kingdom (Golden Sunrise)[a]
TM10	United Kingdom (Alberga)[a]
TM11	United Kingdom (Yellow Pear)[a]
TM12	Nigeria (Kano, Kano State)
TM13	Nigeria (Sokoto, Sokoto State)
TM14	Nigeria (Ilorin West, Kwara State)
TM15	Nigeria (Oyo, Oyo State)

[a]Common Name

Data Analysis

The number of alleles per locus, total number of alleles of the polymorphic SSR loci amplified in all 15 lines and the mean number of alleles were reported. The polymorphic information content (PIC) of each locus was calculated using the formula of Smith *et al.* (1997) in Microsoft Excel (2010) and the mean PIC was also included. Diversity estimates were performed for the SSR data based on the modified Rogers' distance (Rogers, 1972) using Winboot software. Cluster was graphically represented as dendrogram using unweighted pair-group method with arithmetic mean (UPGMA) clustering algorithm, which was done with NTSYS-pc package (Rohlf, 1997; Exeter Software. Setauket, USA). The principal component analysis (PCA) was also performed. The efficiencies of the cluster matrices were evaluated through bootsrap analysis (1,000 resampling value) and the correlation between matrices was verified using Mantel test (with 10,000 simulations) according to Mantel (1967).

Results

Genetic Diversity and Grouping of Tomato Lines Based on SSR Markers

Among the 15 SSR loci amplified to evaluate genetic

diversity in 15 lines, 11 markers were polymorphic and 3 markers were monomorphic. In addition, one SSR marker in a total of the 15 SSR used in this study did not amplified in the 15 lines. 33 alleles were detected among 15 tomato lines, the number varied for each SSR locus from 2 to 5 with a mean of 3.00 alleles per locus for the 11 loci (Table 2). The PIC mean is 0.36 (ranging from 0.12 to 0.76). Estimates of Rogers' distance varied from 0.03 to 0.66, with a mean value of 0.31 among the pairs of lines evaluated. The genetic distances observed between pairs for each of the estimation were used for the construction of dendrogram (Figure 1). The genetic distance (GD) between TM09 and TM03 is 0.66 while TM04 and TM13 pair has 0.23. The 15 lines were grouped into two major groups and a singleton based on UPGMA cluster analysis of SSR-based GD estimates (Figure 1). Group I included 10 lines from all the geographical regions evaluated, UK (TM07, TM08, TM10 and TM11), Nigeria (TM01, TM02, TM06 and TM15) and Benin Republic (TM04 and TM05), which represents the major group. Additionally, in Group I was divided into two clear subgroups, each subgroup includes representative(s) of the tomato lines from the 3 geographical regions sampled. 2 lines (TM10 and TM11) are observed to have a level of similarity based on the present SSR data and both are from the UK. The group II consists of 4 lines, which are essentially from Nigeria (TM03, TM12, TM13 & TM14). TM09 (UK line) is distinct; it separated from other lines forming a singleton. The Mantel correlation coefficient between the dendrogram constructed using UPGMA and the calculated GD (r = 0.85), indicated that the clusters accurately represented the GD estimates. Principal component analysis (PCA) result of SSR-based GD estimates clearly separated the lines into two main groups which is consistent with cluster analysis (Figure 2).

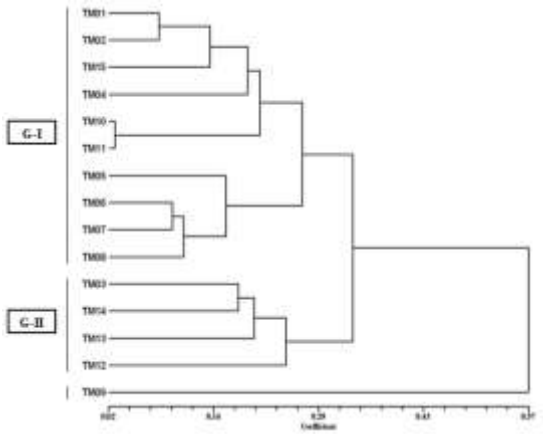

Figure 1: **Dendrogram Obtained through UPGMA Based on the Modified Rogers' Distance for SSR Data**

Table 2: Characteristics of Polymorphic SSR Markers used in the Genotyping of Fifteen (15) Tomato Lines from Nigeria, Benin Republic and the United Kingdom

S/No	SSR Name	Forward Primer and Reverse Primer Sequences	Core Motif	No of Alleles	PIC Values
1	AI773078	F: gat gga cac cct tca att tat ggt R: tcc aag tat cag gca cac cag c	(aat) 14	5	0.70
2	AI778183	F: gcg aag aag atg agt cta gag cat ag R: ctc tct ccc atg agt tct cct ctt c	(aat) 12	3	0.54
3	AW037347	F: gcc acg tag tca tga tat aca tag R: gcc tcg gac aat gaa ttg	(aat) 12	3	0.23
4	AI491065	F: act gca ttt cag gta cat act ctc R: ata aac tcg tag acc ata ccc tc	(at) 9	2	0.41
5	Y09371	F: tga gaa caa cgt tta gag gag ctg R: cgg gca gaa tct cga act c	(at) 12	2	0.50
6	AI780156	F: tcc aat ttc agt aag gac ccc tc R: ccg aaa acc ttt gct aca gag tag a	(ct) 12	3	0.57
7	X90937	F: tgc cca tga cgt tcc atc R: gac aga cag aga gac aga ctt aga g	(ctat) 8	5	0.76
8	TMS33	F: agc atg gga aga aga cac gt R: ttg agc aaa aca tcg caa tc	(ga) 26 imperfect	2	0.12
9	TMS37	F: cct tgc agt tga ggt gaa tt R: tca agc acc tac aat caa tca	(ga)21,(ta)20	3	0.37
10	TMS9	F: ttg gta att tat gtt cgg ga R: ttg agc caa ttg att aat aag tt	(gata) 26	3	0.37
11	SSR50	F: ccg tga ccc tct tta caa gc R: ttg ctt tct tct tcg cca tt	(tc) 6, (ccttc) 2	2	0.47
	Total Number of Alleles			**33**	
	Mean			**3.00**	**0.36**

PIC- polymorphic information content

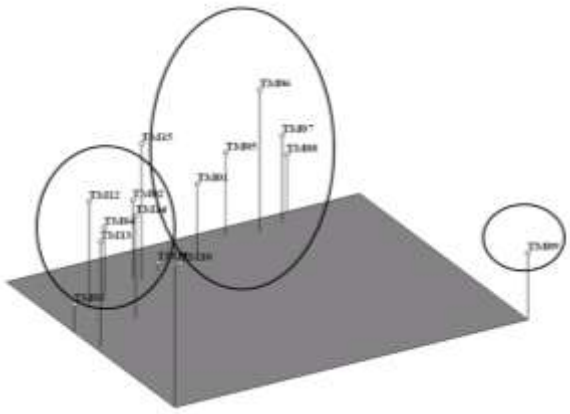

Figure 2: 3D Plot of 15 Tomato Lines Determined on the Basis of Principal Component Analysis Based on SSR Data

Discussion

The SSR markers employed in this study discriminated the cultivated tomato lines. DNA polymorphisms among diverse cultivated tomato lines have been identified at the molecular level (Labate and Roberts, 2002; Park *et al*., 2004; Tam *et al*., 2005). It is estimated that the genomes of tomato cultivars contain, 5% of the genetic variation of their wild relatives (Miller and Tanksley, 1990). The PIC mean (0.36) found in this study was low but higher than the average PIC (0.31) among 39 determinate and indeterminate tomato inbred lines collected from China, Japan, S. Korea, and USA using 35 SSR polymorphic markers reported by Benor *et al*. (2008). However, they detected a mean number of alleles per locus (4.3) which was higher than the mean number of allele (3.00) observed in this study. This might be as a result of differences in the composition of lines evaluated in the two studies. In another study, an average of 2.7 alleles per locus was found among 17 varieties and 2 parental lines when assessed with 65 polymorphic SSR loci (He *et al*., 2003).

Furthermore, little allelic variation was observed among 216 tomatoes from four breeding centres in China genotypes using 12 SSR and 35 SNP markers (Chen *et al*., 2009). Estimates of Rogers' distance observed in this study varied from 0.03 to 0.66, with a mean value of 0.31 among the pairs of lines evaluated. This also shows considerably moderate level of variation was evidenced among the tomato lines we analysed.

In the cluster analysis based on SSR data, the tomato inbred lines were distinguished despite the number of loci considered. The majority of the lines which constituted group I and its subgroups are from the three geographical regions, which indicated that the lines have some degree of relatedness and possibility of the common genetic background. Furthermore, our study confirmed that cultivated tomato has suffered different bottlenecks, and the moderate amount of genetic variation is considered very limited when compared to its wild relatives (Miller and Tanksley, 1990). Also, the further sub-grouping of lines reflects certain amount of genetic variation. The distant separation of TM09 (UK line) from the other 14 lines appears that its high genetic variation was easily detected by the SSR markers. It shows its genetic variability from those that have their origins in West Africa (Nigeria and Benin republic). In addition, the genetic similarity of two UK lines was revealed and the placement of the 4 Nigerian lines into group II in this study provides insights into detection of geographical origins of tomato.

SSR markers have the ability to discriminate and assign individuals into groups. Although, in a previous work (unpublished data), some SSR markers used to assess genetic diversity of some Nigerian tomatoes could not detect genetic variation as a result of few alleles and very low polymorphism. Genetic variability among tomato varieties is limited, which may be due to domestication and selection processes. Carelli *et al*. (2006) was able to detect significant variation between Brazilian landraces and commercial cultivars using RAPD markers.

This present study reveals the genetic diversity within selected tomato lines of different geographical regions. It is well noted that our results showed that SSR markers are efficient for assessment of genetic variation and are informative for detecting genetic diversity of geographical locations in tomato. Understanding the genetic diversity among the tomato lines grown in Nigeria, UK and Benin Republic is a good starting point for establishing and maintaining collections, germplasm banks and proper characterisation of Nigerian tomatoes in order to preserve the natural diversity.

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