RAPD PCR GENOTYPING OF TEN ACCESSIONS OF TOMATO (*Lycopersicon esculentum* Con. [Mill.] Syn. *Solanum lycopersicon* [Linn.])

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ABSTRACT

Tomato (Lycopersicon esculentum Con. [Mill.] Syn. Solanum lycopersicon [Linn.]) belongs to the family Solanaceae and order Solanales. The plant originated from South America but has been domesticated worldwide. This study investigated the variation in Tomato germplasm using RAPD PCR genotyping technique. Seven accessions; NGB01254, NGB01250, NGB01302, NG/DE/MAR/09/019, NGB01301, NGB01255 and NGB 01237 were collected from Gene Bank while three accessions were obtained from home gardens including DE/IK-L-001, DE/IK-L-002 and ED/EG-L-001. The samples were cultivated in a Screen House in the University of Benin, Benin City, Nigeria. Standard procedures were used for DNA extraction and purification while the commercial primer OPA-09 (5^1–GGG TAA CGC C-3^1) was employed for genotyping. Each DNA fragment generated from RAPD PCR genotyping was treated as a separate character and scored as a discrete variable, which was used to distinguish the accessions. Results suggest the primer was able to amplify less than 50 % of the samples studied but was able to amplify more than 50 % of the collections from home gardens. Three genetic types were observed, which may be related to nutrients, growth condition and species of Tomatoes studied. Of the ten samples studied, 4 (40.0 %) were amplified and typeable with the OPA 09 commercial primer while the remaining 6 (60.0 %) were not typeable with this primer. Although this study have further enumerated the advantages of molecular markers in plant characterization, it is important that a combination of molecular and morphological traits be considered for effective characterization of plant germplasm.

Keywords: Vegetable, DNA, Genotyping, Plant Characterization and Conservation

INTRODUCTION

Tomato (*Lycopersicon esculentum* Con. [Mill.] Syn. *Solanum lycopersicon* [Lin.]) most likely originated in western part of South America as the origin of domestication is still an issue of debate. Two hypotheses based on botanical evidence and molecular studies have been postulated for the origin of domestication suggesting Peru but there is no archeological proof to support the evidence (de Candolle 1882; Miller, 1940; Luckwill, 1943; McMeckin, 1992; Nesbitt and Tansley, 2002). Another hypothesis is based on the fact that there is no evidence of pre-Colombian cultivation of Tomato in Mexico (Jenkins, 1948). However, the domestication of Tomato remains unresolved since evidence given are not conclusive enough to show origin of domestication (Peralta and Spooner, 2007). The Tomato plant was introduced into various parts of Africa by early European visitors and is widely cultivated and used.

Tomato belongs to the family Solanaceae also called nightshade family. Carolus Linnaeus placed it in the genus *Solanum* as *Solanum lycopersicon*. In 1768, Miller placed it in a new genus *Lycopersicum* as *Lycopersicum esculentum*. This naming was based on morphological analysis (Luckwill, 1943; Child, 1990). Genetic analysis later confirmed that Linnaeus classification was correct and has recently been reintegrated into the genus *Lycopersicon* (Bohs and Olmstead, 1997; Peralta and Spooner, 2001).

The characterization of Tomato is important particularly in crop improvement and breeding. Characterization is the description of plant germplasm to provide information on the traits of accessions. It employs markers to facilitate easy and fast description of phenotype, grouping of accessions, understanding of species adaptation as well as retrieval of valuable germplasm for breeding programs. More so, it promotes a better understanding about composition of the collections, their genetic diversity and aid documentation of the genetic variability in a population. Recently, several studies have been carried out with the aim of evaluating plant characteristics including Osawaru *et al.* (2012); Mezette *et al.* (2013); Osei *et al*. (2014). However, a more accurate approach using molecular marker technique is

required to establish this objective (O'Donnell *et al.,* 2008). Molecular markers refer to assays that allow the detection of specific sequences between two or more individuals and are important for studying the organization of plant genome (Modini *et al*., 2009). Molecular markers are very important tools for identification of genotypes in studying the organization and evaluation of plant genome. With developments in molecular technique, large number of DNA markers such as RFLP, SSR, CAP, EST and RAPD markers have been developed and used for the identification of genetic polymorphism (Singh *et al.,* 2007).

The characterization of plant germplasm with molecular markers has many advantages; they are less affected by environment, subjected to rapid detection and are not pleiotropic. Of all genetic molecular marker developed so far, RAPD is the most widely used, for identification of relationships amongst species and between cultivars (Singh and Pal, 2013; Sharifova, 2013). Stand out advantages of using RAPD is that it requires small amount of DNA, short primers of arbitrary sequence, easy, fast and highly cost effective (Welsh and McClelland, 1990).

Hence, this study aims to investigate the variation and diversity in Tomato germplasm obtained from gene bank and locally using RAPD genotyping technique. This will aid an understanding of the relevant methods of conservation and seed storage for this essential crop and contribute to Tomato database in Nigeria.

MATERIALS AND METHODS

STUDY AREA: The samples were planted in the Screen House, Department of Plant Biology and Biotechnology, University of Benin, Benin City [6.20 °N and 5.37 °E] located within the tropical rain forest zone.

SOURCE OF SEEDS: Seven accessions; NGB01254, NGB01250, NGB01302, NG/DE/MAR/09/019, NGB01301, NGB01255 and NGB01237 were collected from the Gene Bank of the National Center for Genetic Resources and Biotechnology, Ibadan, Nigeria. Three local accessions were obtained from home gardens including DE/IK-L-001 and DE/IK-L-002 from Agbor, Delta State and ED/EG-L-001 Uselu, Edo State.

PLANTING: Prior to planting, soil samples were collected, air dried and filtered to remove soil particles and debris. Nursery pots were slightly perforated to allow drainage. 0.75 kg of soil samples were weighed into bowls. Six seeds per hole were sown at a depth of 2 cm into the soil and later thinned to three. Plants were watered every day. Fresh leaves of Tomato were harvested 16 weeks after sowing and taken to the laboratory for DNA extraction and RAPD PCR genotyping.

DNA EXTRACTION

DNA extraction and purification was done using ZR PLANT SEED DNA Miniprep™50 Preps model D6020 (Zymo Research, California, USA). 150 mg of the Tomato was transferred to ZR BashingBead™ Lysis tube. 750 µL lysis solution was added to the tube. The bead containing the solution was secured in a bead beater fitted with a 2 ml tube assembly and processed at maximum for 5 minutes. The ZR Bashing Bead™ Lysis tube was centrifuged in a micro centrifuge at 10,000 x g for 1 min 400 µL of the supernatant was pipetted into a Zymo-Spin™ IV Spin Filter in a collection Tube and centrifuged at 7000 x g for 1 min. This was followed by the addition of 1,300 µL of seed/plant DNA Binding Buffer into filtrate in the Collection Tube. Afterwards, 800 µL of the mixture was transferred into a Zymo-Spin™ IIC Column in a collection Tube and centrifuged at 10,000 x g for 1 min. The flow through was discarded from the Collection Tube and the process was repeated to obtain the remaining products. The 200 µL DNA Pre Wash Buffer was added into the Zymo-Spin™ IIC Column DNA Pre-Wash Buffer was added into the Zymo-Spin™ IIC Column in a new collection tube and centrifuged at 10,000 x g for 1 min. This was followed by the addition of 500 µL. Plant DNA Wash Buffer into the Zymo-Spin™ IIC Column and centrifuged at 10,000 x g for 1 min. The zymo-Spin™ IIC Column was transferred into a clean 1.5 ml microcentrifuge tube and 100 µL of DNA Elution Buffer was then added directly to the Column matrix. The DNA obtained was used as a template during the assay.

DNA AMPLIFICATION USING RAPD-PCR AND ELECTROPHORESIS

Randomly amplified polymorphic DNA (RAPD PCR) was performed according to the method of (Hsueh *et al.,* 2002) DNA amplification was carried out by using 20 μL reaction mixture containing 16.5 μL sterile ultra-pure water and bead that contains 2.5 uL of IX PCR Buffer, 1.5 μL $MgCl_2$ (50 mmol/L), 1.0 μL dNTP (0.2 mmol/L), 0.5 μL OPA- 09 primer (0.4 μmol/L), 0.25 μL Platinum Taq DNA polymerase (0.5U) (Promage) and 3 ng DNA. The commercial primer OPA-09 (5^1 –GGG TAA CGC C-3^1) was used for genotyping based on the better discriminatory power observed in previous studies. Amplification was performed in a DNA thermocycler (a & e laboratory model 005 gradient 48 well PCR) programmed for initial denaturing at 94 ºC for 1 minute, annealing at 42 ºC for 2 mins, at 72 ºC for 10 mins for final extension. The number of cycles repeated was 30. A negative control without a DNA template was included in each RAPD- PCR run. Amplified products were separated using 1.5 % agarose gel electrophoresis in TAE buffer (40 Mm Tris- acetate, 2 Mm EDTA [pH 8.3] Performed at 70 V for 2.5 hrs. Gels were stained with 0.5 μg/ml of ethidium bromide and then visualized. The electrophilic profiles were observed visually. Only the major amplicons and consistent minor bands were considered in the analysis. Stained gels were examined under ultra-violet trans illuminator in a photo documentation system. A DNA ladder digest of 1 kb (Fermenters USA) was used as molecular weight marker.

DATA ANALYSIS

Each DNA fragment generated from PCR genotyping was treated as a separate character and scored as a discrete variable using 1 to indicate presence and 0 for absence of band. Similarities between similar specifics were scored based on number of bands and band sizes.

RESULTS

The results of PCR genotyping are presented in Plates 1a, 1b, 2a, 2b and Tables 1 and 2

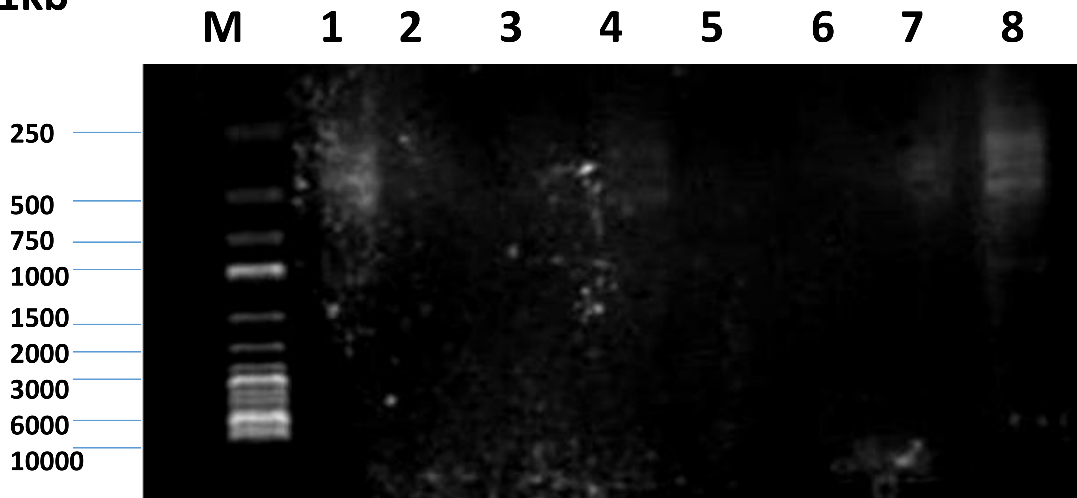

Plate 1a: Amplification pattern for the leaf samples.
M is 1kb DNA Marker.

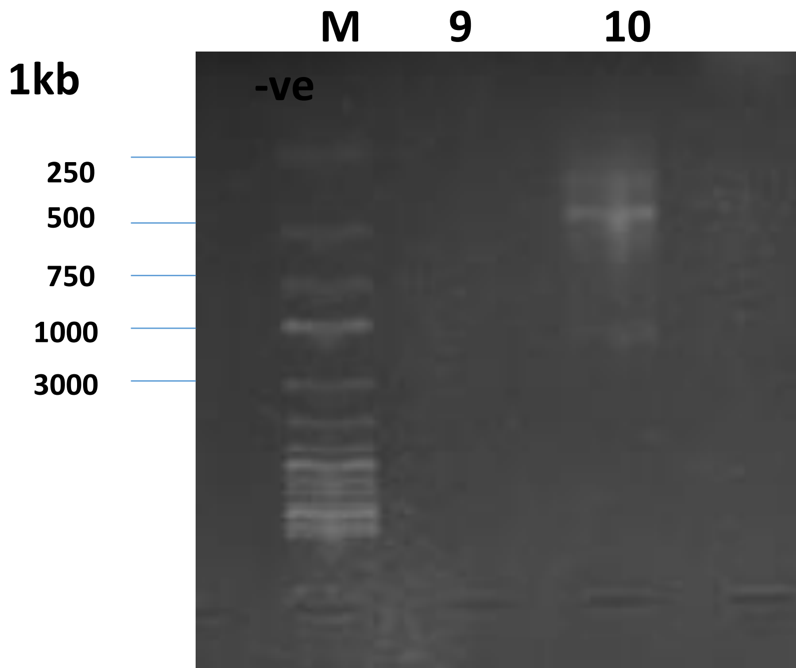

Plate 1b: Amplification pattern for the leaf samples.
M is 1kb DNA Marker.

Key:
1 = NGB01254, 2 = NGB01250, 3 = NGB01302, 4 = NG/DE/MAR/09/019, 5 = NGB01301,
6 = NGB01255, 7 = NGB01237, 8 = DE/IK-L-001, 9 = DE/IK-L-002 and 10 = ED/EG-L-001

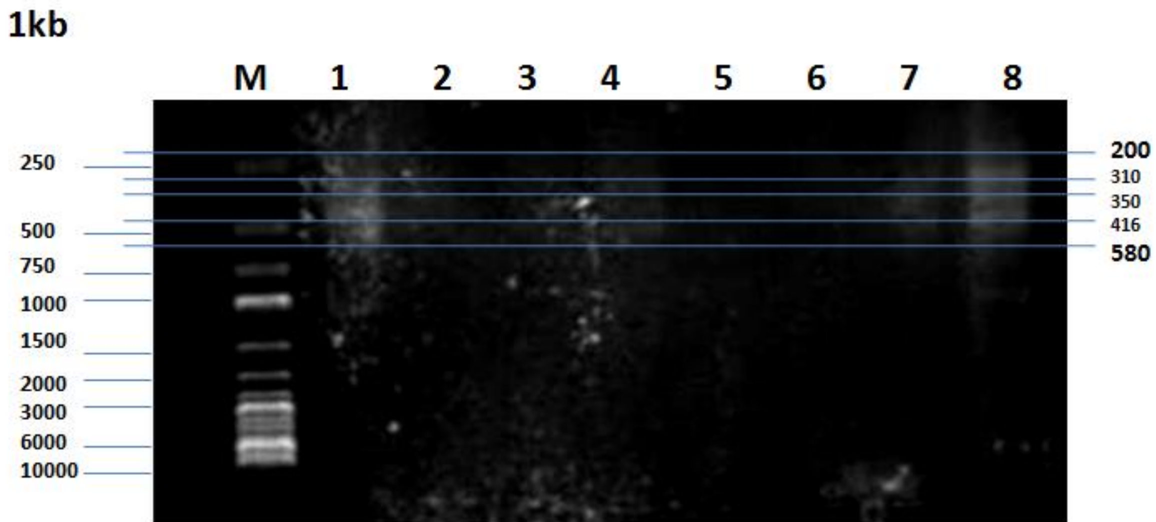

Plate 2a: Calculation of the amplified band sizes with corresponding DNA marker.

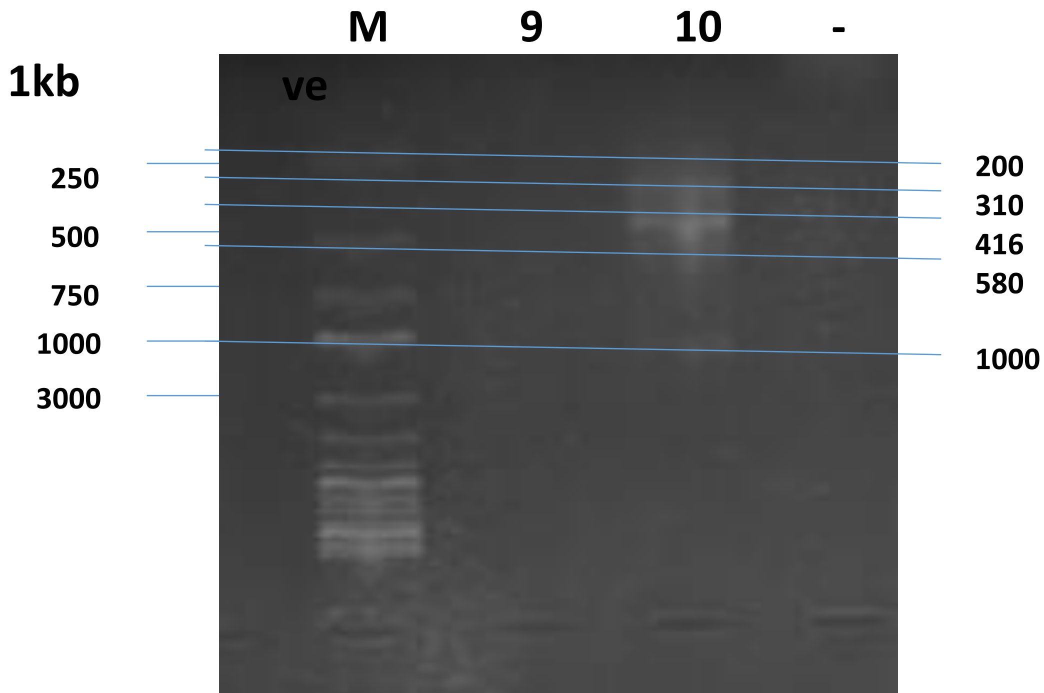

Plate 2b: Calculation of the amplified band sizes with corresponding DNA marker.

Key:
1 = NGB01254, 2 = NGB01250, 3 = NGB01302, 4 = NG/DE/MAR/09/019, 5 = NGB01301,
6 = NGB01255, 7 = NGB01237, 8 = DE/IK-L-001, 9 = DE/IK-L-002 and 10 = ED/EG-L-001

Table 1: Detection of amplification (genetic) band on leaves of Tomatoes accessions after RAPD-PCR amplification with OPA 09 Primer

		Samples			
DNA Marker (Major bands)	**Detected band levels**	**1**	**4**	**8**	**10**
10,0000	**-**	**-**	**-**	**-**	**-**
6,000	**-**	**-**	**-**	**-**	**-**
3,000	**-**	**-**	**-**	**-**	**-**
1000	**1000**	**0**	**0**	**0**	+
750		**-**	**-**	**-**	**-**
	580	**0**	**0**	+	+
500	**-**	**-**	**-**	**-**	**-**
	416	+	+	+	+
	350	+	+	+	+
	310	+	+	+	+
250					
	200	+	+	+	+

Key:

1 = NGB01254, 4 = NG/DE/MAR/09/019, 8 = DE/IK-L-001, 10 = ED/EG-L-001

\+ = Presence - = Absence

Table 2: Occurrence of bands and frequency

Samples Numbers N = 4	Number of bands	Samples N = 10	Number of bands observed	**Frequency**	**Percent (%)**
1	4				
4	4		4	2	50
8	5		5	1	25
10	6		6	1	25
		Total Number of samples typeable= 4 (40.0%)	Number of genetic type =3		
		Total			**100 %**

Key:
1 = NGB01254, 4 = NG/DE/MAR/09/019, 8 = DE/IK-L-001, 10 = ED/EG-L-001

DISCUSSION

Tomato genotyping have been done in this study using RAPD technique. RAPD markers have been applied in gene mapping, population genetics, molecular evolutionary genetics and plant breeding because they are simple, cheap, fast and effective. This study was done to determine the genetic fingerprints of Tomato accessions sampled from gene bank and home gardens. Results suggest adequate protection is necessary for accessions sampled from home gardens in Edo and Delta states as well as the plant breeders' rights. Molecular fingerprinting of plant variety is importance for protecting plant breeders' rights as well as for the identification of hybrids from their respective parents (Pal and Singh, 2013).

RAPD-PCR method provides a direct analysis of the genome not possible with morphological methods and therefore, serves as a powerful tool for biosystematics studies (Taylor-Grant and Soliman, 1999). The technique does not require previous knowledge of the target genome and is relatively simple and rapid to carry out, hence, applicable in population genetics, analyses of biodiversity and studies of relationships among species (Ayten *et al*., 2009).

Commercial OPA -09 primer was used due to the high discrimination power in genotyping Tomatoes in previous studies (Pal and Singh, 2013). The OPA 09 primer showed bands which can be used to distinguish between the plant hybrids. This is similar to previous works (Schnell *et al.,* 1999; Pal and Singh, 2013). The primer was able to amplify less than 50 % of the samples studied (NGB01254, NG/DE/MAR/09/019, DE/IK-L-001 and ED/EG-L-001) suggesting that more commercial OPA primers should be tested on the leaf samples. This will highlight the most appropriate primer for genotyping tomatoes accessions. Interestingly, in this study, the primer was able to amplify more than 50 % of the collections from home gardens including DE/IK-L-001 and ED/EG-L-001. Three genetic types were observed, variation may be related to nutrients, growth condition and species of tomatoes studied. Of the nine samples studied, 4 (44.4 %) were amplified and typeable with the OPA 09 commercial primer. OPA-9 was a convenient polymorphic marker in the present study, which was discernible with the appearance and disappearance of DNA bands suggesting a high degree of potential variation.

The remaining 5 (55.5 %) were not typeable with this primer. Of the four samples that showed amplifications, two had four bands occurring between 200 - 416 bp showing that they most likely possess and/or belong to the same genotype. One had five bands ranging between 200 – 580 bp belonging to another genotype. Another sample had six bands ranging from 200 – 1000 bp showing that they two may also belong to another different genotype. In all three different genotypes were observed. In addition, the leaf samples were harvested and kept in a polythene sack for two days and then stored in a -30 ºC freezer prior to DNA extraction, which may have affected the yield of DNA obtained from the various leaves. More so, it was difficult to macerate the leaves after it had absorbed liquid. In addition, DNA extraction was done by a commercial Kit. There is a need to compare yield with other extraction method like boiling or Phenol: chloroform methods.

Ayten *et al.* (2009) opined that species identification should not rely only on RAPD because it may not be a reliable method of identification alone, rather it works well in combination with other strong co-dominant methods. Furthermore, the relationships established by electrophoretic profile of RAPD do not match those established relationships based on morphological characters as RAPD seems to show more variation than morphological analysis, which can be explained by considering the advantage of representing full genome of a species unlike morphological differences which are more dependent on environmental conditions (Singh *et al*., 1994). The study of Ezekiel *et al.* (2011) suggests the use of RAPD was very effective in classifying tomato accessions genotypically and that eco-geographical differences may not necessarily determine the distribution and diversity in Tomato crop in Nigeria. Hence, they suggested that characterization of Tomato be based on a combination of molecular and morphological traits to reliably determine the differences among the Tomato varieties and eliminate the error of assigning different names to the varieties or vice-versa.

In conclusion, advancement in the field of systematics have promulgated the use of molecular techniques to classify, identify, describe and name plant species against the use of changeable characters. This study

has contributed to the knowledge base of Tomato in Nigeria and suggests the use of more samples and additional RAPD-PCR primers as well as the different types of molecular markers techniques with more reliability than RAPD-PCR technique. This is necessary because RAPD-PCR can give inconsistent results (Karp *et al.* 1997), do not detect co-dominance and therefore cannot measure allelic frequency and results are not always comparable between laboratories and sometimes even between experiments (Hodgkins, 2001).

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