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Molecular Diversity of *Fusarium oxysporum* and *Geotrichum candidum* Isolated from Two Tomato Varieties Commonly Consumed in Some Southwestern Town in Nigeria

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Abstract

Fusarium oxysporum and *Geotrichum candidum*, which are among important pathogens of *Solanum lycopersicum* L. (Tomato), are sometimes misidentified during morphological misidentification. The study was carried out to evaluate molecular diversity of *F. oxysporum* and *G. candidum* isolated from two tomato varieties obtained from Akure, Ilorin and Ibadan, Nigeria. The tomato samples were collected and brought back to the laboratory for fungal isolation. Isolation of the pathogens were done following standard procedures. DNA extraction from pure cultures of the pathogens was done at the Centre Laboratory of University of Ibadan. Genetic relationships among the organisms were also estimated by constructing a Dendrogram through UPGMA using the Mega6 Software and genetic distance was computed also using the Mega6 Software. Five strains of *F. oxysporum* and seven strains of *G. candidum* were identified. Percentage similarity of the pathogens with those in GenBank was 99.17% - 100.00% for *F. oxysporum* and 98.48% - 100.00% for *G. candidum*. The T-01 marker showed the lowest major allele frequency of 0.0833, while T-10 marker has the highest value for major allele frequency of 0.6667 and an average value of 0.3958. Evolutionary relationship showed that the two strains of *G. candidum* (MN650247 and MN650250) were similar. The three strains of *F. oxysporum* (MN650246 and MN650248, MN650245 and MN650253) were also similar. Genetic distances among pairs of the fungal strains ranged from 0.12 to 6.30 in pairwise fashion, with an average of 1.32. Evolutionary relationship or closeness among strains of a fungal species can thus be said not to depend on location.

Keywords

Fusarium oxysporum, *Geotricum candidum*, Genetic Distances, Evolutionary Relationship and Genes

1. Introduction

Solanum lycopersicum L. (Tomato) is a red fruit with high economic value worldwide and is one of the major crops involved in North-South trade in Nigeria. Tomato is grown as either a small scale in backyard garden or commercial field in several parts of Nigeria, both as dry or wet season crops. It is regarded as one of the most important vegetables worldwide because of its high consumption, almost year round availability and large content of health related components. It can be affected by fungi such as *Fusarium oxysporum* and *Geotrichum candidum* [1].

Fusarium oxysporum is a soil-borne fungus that affects a several of host crops, including onion, lettuce, tomato, cucurbits, peas, watermelon, peppers, spinach, beans and banana, and also important non-food crops, such as carnation and narcissus; causing crown and root rots as well as vascular wilts [2]. Due to its varied host range in addition to its economic and scientific impact, means that *F. oxysporum* was recently identified as the fifth most important plant pathogenic fungus. *F. oxysporum* is a pervasive soil-born fungus, having a high genetic and ecological diversity with latent to cause diseases of many crop species such as tomatoes [2].

Geotrichum candidum is a filamentous "yeast-like" fungus. It's a ubiquitous organism as it's found in a wide range of habitats including air, water, silage and soil. *Geotrichum candidum* is the causal agent of sour rot of tomato fruit (*Solanum lycopersicum*) and other fresh produce [3].

F. oxysporum and *G. candidum* have an inherent morphological instability, which enables the organism to occupy a wide range of ecological niche, thereby making its taxonomy complicated and controversial [4] [5] [6]. Morphologically identification of *F. oxysporum and G. candidum* sometimes suffers errors and misidentification, leaving a need for molecular tools for strain identification. Characterization by molecular techniques using polymerase chain reaction (PCR) to amplify the internal transcribed sequences (ITS) and random amplified polymorphic DNA (RAPD) marker allows identifying organisms that cannot be distinguished morphologically; and can also help to comprehend the mechanisms of pathogenic variation [7]. The study was carried out to evaluate molecular diversity of *F. oxysporum* and *G. candidum* isolated from two tomato varieties obtained from Akure, Ilorin and Ibadan, Nigeria.

2. Methodology

2.1. Sample Materials and Collections

Two varieties of tomato samples were collected randomly in a well-labeled

brown paper envelop from three state capitals in Nigeria (Shasha in Akure, Ondo state; Eye-nkorin in Ilorin, Kwara State and Shasha in Ibadan, Oyo State). From each location, and for each variety, as many as twenty samples were collected (Figures 1-3).

Figure 1. *Solanum lycopersicum* L. (Tomato) from Ilorin, Kwara state. A1: Tiwantiwa, A2: Hausa scissors.

Figure 2. *Solanum lycopersicum* L. (Tomato) from Akure, Ondo state. A1: Tiwantiwa, A2: Hausa scissors.

Figure 3. *Solanum lycopersicum* L. (Tomato) from Ibadan, Oyo state. A1: Tiwantiwa, A2: Hausa scissors [10].

2.2. Isolation and Identification of the Fungi

The fungi were isolated in Plant Pathology/Mycology laboratory, Department of Botany, University of Ibadan, Nigeria, using a classical method of Koch postulate as modified by Mailafia *et al.* [8]. Fungal isolates were identified using cultural and morphological characteristics in accordance with Tafinta *et al.* [9] and Mailafia *et al.* [8].

2.3. DNA Extraction

DNA extraction was done according to the method of Dellaporta *et al.* [11] as modified by [12].

2.4. Molecular Identification of Fungi Isolates

To use the ITS gene for characterization of the fungi isolates, ITS1 and ITS4 primers set which flank the ITS1, 5.8S and ITS2 region was used for PCR. PCR carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA); PCR conditions included an initial denaturation at 94˚C for 5 min, followed by 35 cycles of each cycle comprised of 30 seconds denaturation at 94˚C, 30 seconds. Annealing of primer at 55˚C, 1.5 min extension at 72˚C and a final extension for 7 min at 72˚C [13].

2.5. DNA Integrity and Sequencing

The integrity of the amplified about 550 Mb gene fragment was checked using the method of Pryce *et al.* [13] and Groenewald [14] on a 1.5% Agarose gel ran to confirm amplification. The sizes of the PCR products were estimated by comparison with the mobility of a 100 bp molecular weight ladder that was ran alongside experimental samples in the gel.3.5.3.

The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer from Applied Bio-systems using manufacturers' manual while the sequencing kit used was that of Big Dye® terminator v3.1 cycle sequencing kit [13] [14].

2.6. Molecular Characterization of Fungal Isolates Using RAPD Markers

PCR Protocol and Bands Separation

Polymorphic four (4) RAPD universal markers were used for genotyping the entire genome of the fungal isolates. The separation of bands as produced by each primer was done on a 1.5% Agarose gel (**Table 1**).

Table 1. RAPD universal markers for genotyping the entire fungal genome.

Primer name	Primer sequence 5'→3'
OPT-01	GGGCCACTCA
OPT-02	GGAGAGACTC
OPT-10	CCTTCGGAAG
OPB-08	GTCCACACGG

2.7. Data Analysis

Data matrix was analyzed using the Power-marker V2.35 software. Genetic diversity parameters such as major allele frequency, gene diversity and polymorphic information content were then generated using the power marker software. The genetic relationship among samples were also estimated by constructing a Phylogenetic tree through Unweighed Pair Group Method with Arithmetic Means [UPGMA] using the Mega6 Software and genetic distance where computed also using the Mega6 Software.

3. Results

3.1. Molecular Characterization and Blast Result Based on DNA ITS Sequencing

Figure 4 shows the agarose gel electrophoresis wherein an amplicon of approximately 550 bp was produced, showing positive PCR amplification. Although DNA was purified, the direct PCR resulted in a single band with the expected size.

Table 2 shows the quality of RAPD primers used in screening fungal isolates. The study showed the same numbers of observation for all the RAPD primers. T-01 marker showed the lowest major allele frequency of 0.0833, while T-10 marker has the highest value for major allele frequency of 0.6667 and an average value of 0.3958. The highest allele number was observed on T-01 RAPD marker (12) with the least value (5) obtained on T-10 and a mean value of 8.250. T-01 marker showed maximum value (0.9167) of genetic diversity, while RAPD marker T-10 had minimum value (0.5278) of genetic diversity. Primer T-01 gave a maximum value of PIC with 0.9103 (91.03%), whereas T-10 primer gave minimum

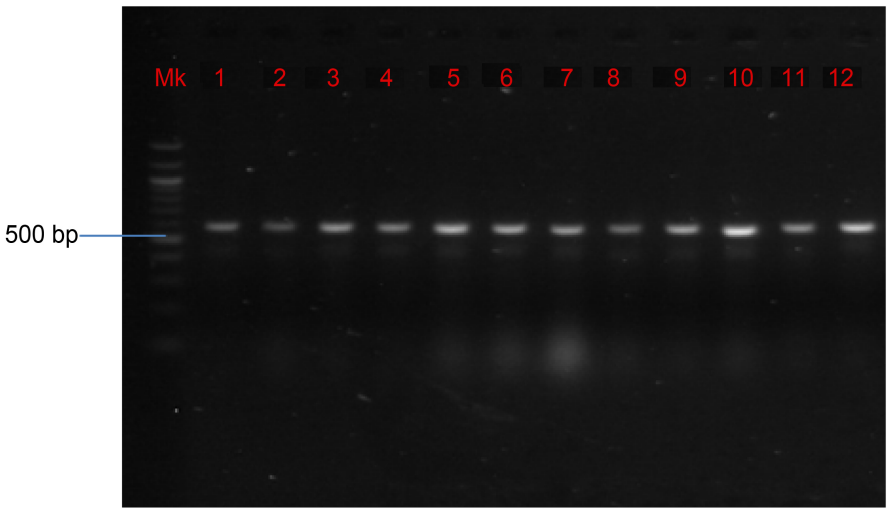

Figure 4. Agarose gel electrophoresis indicating a positive amplification of the ITS region of fungal isolates. A band size of approximately 550 bp indicates a positive amplification. Loading arrangement Mk, 1 - 9 indicates molecular marker, AkureA1a, AkureA1b, AkureA2a, AkureA2b, KwaraA1a, KwaraA1b, KwaraA2a, KwaraA2b, Ibadan A1a, Ibadan A1b, Ibadan A2a, Ibadan A2b and respectively.

PIC value of 0.5025 (50.25% polymorphism). The table shows the mean value of polymorphic information content value of the RAPD markers in this study is 0.7293 (72.93% polymorphism). The mean number of polymorphic information content value of the RAPD markers in this study is 0.7293 (72.93% polymorphism).

Table 3 gives the Agarose Gel matrix derived by scoring the presence or absence of amplification as produced by the individual RAPD primers. The study shows that all the RAPD markers produce a scoreable polymorphic band. The highest number of scoring was observed on T01 primer and the lowest number of scoring was noticed on T10 primer. Each primer sets was scored using 1 (presence of positive amplification at a particular band size) and 0 (absence of positive amplification at a particular band size).

Table 2. Quality of RAPD primers used in screening fungal isolates.

Marker	Major Allele Frequency	Sample Size	No. of obs.	Allele No	Availability	Gene Diversity	PIC
T01	0.0833	12.0000	12.0000	12.0000	1.0000	0.9167	0.9103
T10	0.6667	12.0000	12.0000	5.0000	1.0000	0.5278	0.5025
B08	0.5000	12.0000	12.0000	7.0000	1.0000	0.7083	0.6861
T02	0.3333	12.0000	12.0000	9.0000	1.0000	0.8333	0.8183
Mean	0.3958	12.0000	12.0000	8.2500	1.0000	0.7465	0.7293

PIC—Polymorphism Information Content.

Table 3. Agarose Gel matrix derived by scoring the presence or absence of amplification as produced by the individual RAPD primers.

Level-1	T01	T10	B08	T02
AkureA1a	0/1/1/0/0/0/0/0/0/0/1/0	1/1/1/1/1	0/0/0/1/0/0	0/1/0/0/1/0
AkureA1b	0/0/0/0/0/0/0/0/0/0/1/1	0/0/0/0/0	1/0/0/0/0/0	1/1/1/0/0/1
AkureA2a	0/1/0/0/1/0/0/0/0/0/1/0	1/1/0/0/0	0/0/0/1/0/0	0/1/0/0/1/1
AkureA2b	1/0/1/0/0/0/0/0/0/0/1/0	1/1/0/0/0	0/1/0/0/0/0	0/0/0/0/0/0
KwaraA1a	1/0/0/0/0/0/0/0/0/0/0/0	1/1/0/0/0	0/0/0/1/0/0	0/0/0/0/0/0
KwaraA1b	0/0/0/0/1/0/0/0/0/0/0/0	1/1/0/0/0	0/0/0/0/1/0	0/0/1/0/0/0
KwaraA2a	0/0/0/0/1/1/1/1/1/1/1/1	1/1/0/1/0	0/0/0/0/0/0	0/0/1/1/0/0
KwaraA2b	0/0/0/0/0/0/0/0/0/0/0/0	1/1/1/0/0	0/0/1/0/1/0	0/0/0/0/0/0
IbadanA1a	0/0/0/0/1/0/0/0/0/0/1/0	1/1/0/0/0	0/0/0/1/0/0	0/1/0/1/1/0
IbadanA1b	0/0/0/0/0/0/0/0/1/0/0/0	1/1/0/0/0	0/0/0/1/0/0	1/0/0/0/1/0
IbadanA2a	0/0/0/0/1/1/1/1/1/0/0/1	1/1/0/0/0	0/0/0/0/0/1	0/0/0/0/0/1
IbadanA2b	0/0/0/0/0/1/0/0/0/0/1/0	1/1/0/0/0	0/0/0/1/0/0	0/0/0/0/0/0

3.2. Sequencing Data Analysis

Figure 5 gives the phylogenetic tree of isolates based on their evolutionary relationship. The phylogenetic tree showed the evolutionary relationships among the fungal isolates were separated into two different clusters. The first cluster was further separated into three sub-clusters and the other group into a sub-cluster. The tree includes reference strains of *Fusarium oxysporum* and *Geotrichum candidum*.

Table 4 gives the genetic distance between the 12 fungal isolates in pairwise

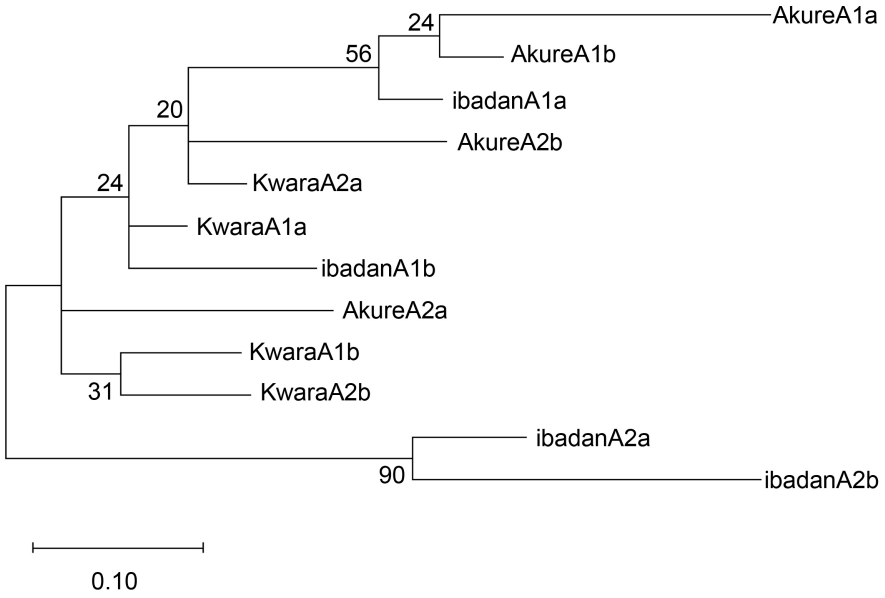

Figure 5. Phylogenetic tree of isolates based on their evolutionary relationship constructed through Unweighed Pair Group Method with Arithmetic Means [UPGMA] using the Mega6 Software.

Table 4. Genetic distance between the 12 fungal isolates in pairwise fashion.

	AkureA1a	AkureA1b	AkureA2a	AkureA2b	KwaraA1a	KwaraA1b	KwaraA2a	KwaraA2b	IbadanA1a	IbadanA1b	IbadanA2a	IbadanA2b
AkureA1a	0											
AkureA1b	1.51	0										
AkureA2a	0.30	0.58	0									
AkureA2b	0.65	0.44	0.65	0								
KwaraA1a	0.65	0.25	0.39	0.17	0							
KwaraA1b	3.37	0.25	0.50	0.39	0.23	0						
KwaraA2a	5.97	4.27	5.24	3.67	3.67	0.93	0					
KwaraA2b	0.93	0.25	0.93	0.39	0.23	0.17	5.24	0				
IbadanA1a	0.39	0.34	0.12	0.50	0.30	0.39	3.85	0.65	0			
IbadanA1b	0.65	0.34	0.39	0.50	0.17	0.39	3.67	0.39	0.30	0		
IbadanA2a	6.30	3.64	3.85	3.37	0.93	0.65	0.39	3.85	3.37	0.93	0	
IbadanA2b	0.50	0.44	0.30	0.23	0.12	0.30	0.93	0.30	0.23	0.23	0.65	0

fashion. Genetic distances (GD) among pairs of fungi isolate strains ranged from 0.12 to 6.30, for the 12 fungal isolates in pairwise fashion, with an average of 1.32 as shown in Table 4. The smallest genetic distance obtained was observed between isolates from IbadanA1a and AkureA1a; and IbadanA2a and KwaraA1a, while the greatest distance was between isolates from AkureA1a and IbadanA2a.

4. Discussion

The cultural and morphological characteristics of isolates from two tomato varieties were confirmed and authenticated as *F. oxysporum* and *G. candidum* with the help of Mycological Atlas. All the 12 fungal isolates identified using DNA sequences of ITS and the targeted regions which were successfully amplified from all the fungal isolates was similar to the work of Akbar *et al.* [15], Thornton *et al.* [3] and Ziedan *et al.* [6]. The non-significant difference in the magnitude of amplified sequences of the fungal isolates compared with that from the GenBank after alignment agreed with the submissions of Si Mohammed *et al.* [16], Akbar *et al.* [15] and Ziedan *et al.* [6]. Moreover, according to ITS sequences alignment conducted on BLAST using NCBI (http://www.ncbi.nlm.nih.gov/), the fungal isolates revealed a 99% - 100% similarity for *Fusarium oxysporum* and 98% - 100% similarity for *Geotrichum candidum* with the related fungi documented in the GenBank which agrees with the work reported by Valinhas *et al.* [17]. Therefore, the name consigned to the species was in accordance with the closest match with identified species. Based on ITS sequences, the NCBI tool confirms the identity of our isolates as *F. oxysporum* and *Geotrichum candidum*, which corroborated the report of Si Mohammed *et al.* [16], Akbar *et al.* [15] and Ziedan *et al.* [6].

The PIC value indicates that the fungal isolates had high degree of genetic diversity. Polymorphism has been detected in other fungi as a reliable record of genetic evolution [15] [18] [19]. The same or very similar strains were isolated from widely different regions; this is similar to the work submitted by Marcelino *et al.* [18] and Olowe *et al.* [20]. Genetic distances among the strains range from 0.12 to 6.30 for the 12 fungal isolates in pairwise fashion, with an average of 1.32. Souza *et al.* [21] and Bruel *et al.* [22] obtained a mean genetic divergence of 0.65 and 0.51 between seven and sixteen commercial maize hybrids respectively. Study on molecular of *F. oxysporum* and *G. candidum* strains shows that evolutionary relationship among the strains was not determined by their location.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Appendices

Fusarium oxysporum strain Akure_A1a

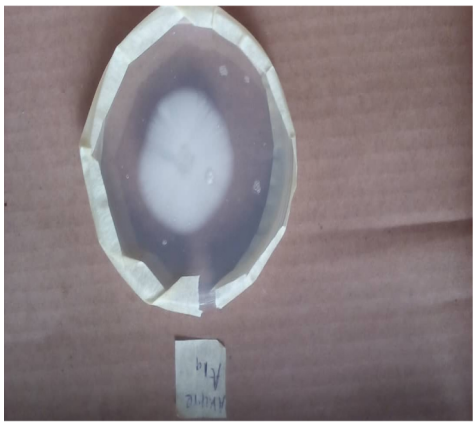

Plate 1. Culture plate of *Fusarium oxysporum* strain.

Fusarium oxysporum strain Akure_A1b

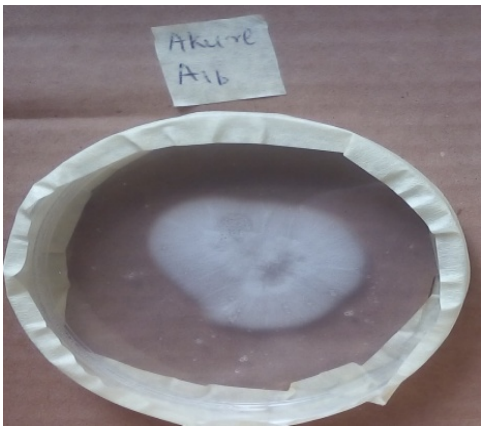

Plate 2. Culture plate of *Fusarium oxysporum* strain (A).

Geotrichum candidum strain Akure_A2a

Plate 3. Culture plate of *Geotrichum candidum* strain.

Geotrichum candidum strain Ilorin_A1a

Plate 4. Culture plate of *Geotrichum candidum* strain.

Geotrichum candidum strain Ilorin_A1b

Plate 5. Culture plate of *Geotrichum candidum* strain.

Fusarium oxysporum strain Ilorin_A2a

Plate 6. Culture plate of *Fusarium oxysporum* strain.

Geotrichum candidum strain Ilorin_A2b

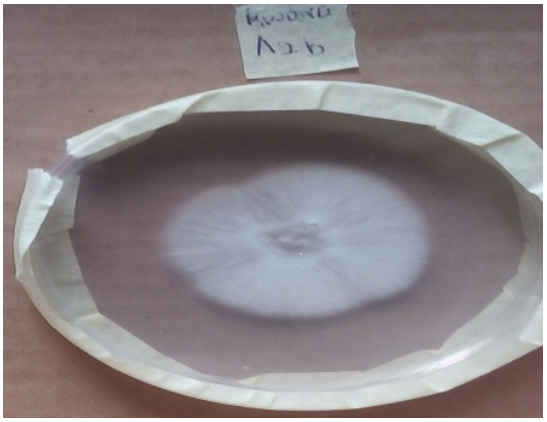

Plate 7. Culture plate of *Geotrichum candidum* strain.

Fusarium oxysporum strain Ibadan_A1a

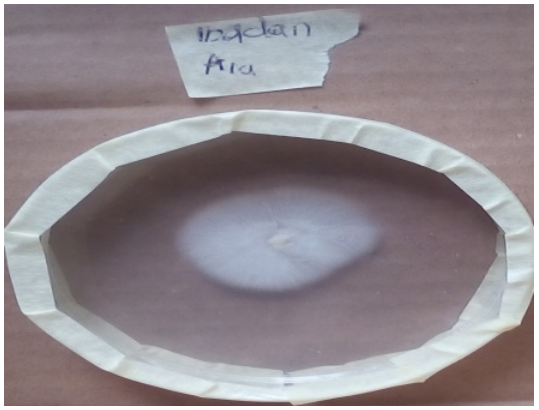

Plate 8. Culture plate of *Fusarium oxysporum* strain.

Geotrichum candidum strain Ibadan_A1b

Plate 9. Culture plate of *Geotrichum candidum* strain.

Geotrichum candidum **strain Ibadan_A2b**

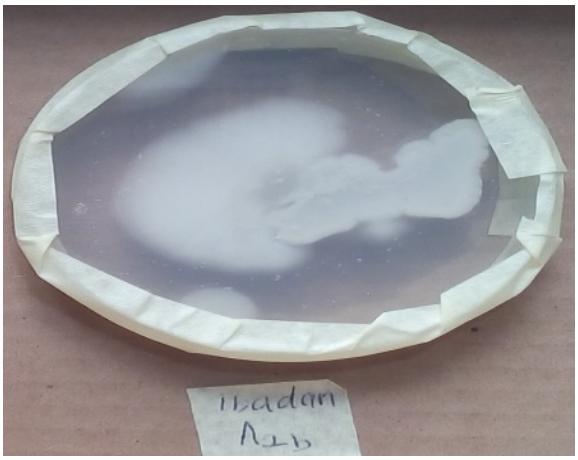

Plate 10. Culture plate of *Geotrichum candidum* strain.

Sequencing Analysis

Akure

MN650245 ***Fusarium oxysporum*** **strain Akure_A1a**

GCAAGGTGAACTGCGGAGGGATCATTACCGAGTTTACAACTCCCAAACCCCTGTGAACATACCACTTGTT
GCCTCGGCGGATCAGCCCGCTCCCGGTAAAACGGGACGGCCCGCCAGAGGACCCCTAAACTCTGTTTCTA
TATGTAACTTCTGAGTAAAACCATAAATAAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGA
TGAAGAACGCAGCAAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACG
CACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCACAGCTT
GGTGTTGGGACTCGCGTTAATTCGCGTTCCTCAAATTGATTGGCGGTCACGTCGAGCTTCCATAGCGTAG
TAGTAAAACCCTCGTTACTGGTAATCGTCGCGGCCACGCCGTTAAACCCCAACTTCTGAATGTTGACCTC
GGATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAATAAGGGGAAAAA

MN650246 ***Fusarium oxysporum*** **strain Akure_A1b**

GTGAACATACCACTTGTTGCCTCGGCGGATCAGCCCGCTCCCGGTAAAACGGGACGGCCCGCCAGAGGA
CCCCTAAACTCTGTTTCTATATGTAACTTCTGAGTAAAACCATAAATAAATCAAAACTTTCAACAACGGAT
CTCTTGGTTCTGGCATCGATGAAGAACGCAGCAAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGA
TCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCA
ACCCTCAAGCACAGCTTGGTGTTGGGACTCGCGTTAATTCGCGTTCCTCAAATTGATTGGCGGTCACGTC
GAGCTTCCATAGCGTAGTAGTAAAACCCTCGTTACTGGTAATCGTCGCGGCCACGCCGTTAAACCCCAAC
TTCTGAATGTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATATC

MN650247 ***Geotrichum candidum*** **strain Akure_A2a**

AACCAAAATCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTAATGATTTTTAATATTTGTG
AATTTACTACAAACAAAAATTTAATTTTATAGTCAAAAATAAAAAAAAAATTAAAACTTTTAACAATGGAT
CTCTTGGTTCTCGTATCGATGAAGAACGCAGCGAAACGCGATATTTCTTGTGAATTGCAGAAGTGAATCA
TCAGTTTTTCGAACGCACATTCGCACTTTCGGGGTATCCCCCAAAGTATACTTTGTTTGAGCGTTGTTTTC
TCTCTTGGAATTTGCATTTTGCTTTTTCTAAAAAATCGAATCAAATTTCGTTTTGAAACTTTCAATTTATCT
CAACCTCAGATCAAGTAGGATTACCCGCTGAACTTAAGCATATCAAAGGGGGGCC

MN650248 ***Fusarium oxysporum*** **strain Akure_A2b**

TCGTAACAAGGTCTCCGTTGGTGAACCAGCGGAGGGATCATTACCGAGTTTACAACTCCCAAACCCCTGT

GAACATACCACTTGTTGCCTCGGCGGATCAGCCCGCTCCCGGTAAAACGGGACGGCCCGCCAGAGGACCC
CTAAACTCTGTTTCTATATGTAACTTCTGAGTAAAACCATAAATAAATCAAAACTTTCAACAACGGATCTC
TTGGTTCTGGCATCGATGAAGAACGCAGCAAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATC
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CCCTCAAGCACAGCTTGGTGTTGGGACTCGCGTTAATTCGCGTTCCTCAAATTGATTGGCGGTCACGTCG
AGCTTCCATAGCGTAGTAGTAAAACCCTCGTTACTGGTAATCGTCGCGGCCACGCCGTTAAACCCCAACT
TCTGAATGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA

Kwara

MN650249 *Geotrichum candidum* strain Kwara_A1a

AACAATCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTAATGATTTTTAATATTTGTGAATT
TACTACAAACAAAAATTTAATTTTATAGTCAAAAATAAAAAAAAAATTAAAACTTTTAACAATGGATCTCT
TGGTTCTCGTATCGATGAAGAACGCAGCGAAACGCGATATTTCTTGTGAATTGCAGAAGTGAATCATCAT
TTTTCGAACGCACATTCGCACTTTCGGGGTATCCCCCAAAGTATACTTTGTTTGCGTTGTTTTCTCTCTTG
GAATTTGCATTTTGCTTTTTCTAAAAAATCGAATCAAATTTCGTTTTGAAACTTTCAATTTATCTCAACCTC
AGATCAAGTAGGATTACCCGCTGAACTTAAGCATATCAAAGGCATCAC

MN650250 *Geotrichum candidum* strain Kwara_A1b

ATCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTAATGATTTTTAATATTTGTGAATTTACT
ACAAACAAAAATTTAATTTTATAGTCAAAAATAAAAAAAAAATTAAAACTTTTAACAATGGATCTCTTGGT
TCTCGTATCGATGAAGAACGCAGCGAAACGCGATATTTCTTGTGAATTGCAGAAGTGAATCATCAGTTTT
TCGAACGCACATTCGCACTTTCGGGGTATCCCCCAAAGTATACTTTGTTTGAGCGTTGTTTTCTCTCTTGG
AATTTGCATTTTGCTTTTTCTAAAAAATCGAATCAAATTTCGTTTTGAAACTTTCAATTTATCTCAACCTCA
GATCAAGTAGGATTACCCGCTGAACTTAAGCATATCAAAGGG

MN650251 *Fusarium oxysporum* strain Kwara_A2a

TCTCCCTTGGTGAACCAGCGGAGGGATCATTACCGAGTTTACAACTCCCAAACCCCTGTGAACATACCAC
TTGTTGCCTCGGCGGATCAGCCCGCTCCCGGTAAAACGGGACGGCCCGCCAGAGGACCCCTAAACTCTGT
TTCTATATGTAACTTCTGAGTAAAACCATAAATAAATCAAAACTTTCAACAACGGATCTTTGGTTCTGGCA
TCGATGAAGAACGCAGCAAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATATCGAATCTTTGA
ACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCACAG
CTTGGTGTTGGGACTCGCGTTAATTCGCGTTCCTCAAATTGATTGGCGGTCACGTCGAGCTTCCATAGCGT
AGTAGTAAAACCCTCGTTACTGGTC

MN650252 *Geotrichum candidum* strain Kwara_A2b

GACAATCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTAATGATTTTTAATATTTGTGAATT
TACTACAAACAAAAATTTAATTTTATAGTCAAAAATAAAAAAAAAATTAAAACTTTTAACAATGGATCTCT
TGGTTCTCGTATCGATGAAGAACGCAGCGAAACGCGATATTTCTTGTGAATTGCAGAAGTGAATCATCAT
TTTTCGAACGCACATTCGCACTTTCGGGGTATCCCCCAAAGTATACTTTGTTTGCGTTGTTTTCTCTCTTG
GAATTTGCATTTTGCTTTTTCTAAAAAATCGAATCAAATTTCGTTTTGAAACTTTCAATTTATCTCAACCTC
AGATCAAGTAGGATTACCCGCTGAACTTAAGCATATCAAAGGCAT

Ibadan

MN650253 *Fusarium oxysporum* strain Ibadan_A1a

CTTAGAACCTGCGGAGGGATCATTACCGAGTTTACAACTCCCAAACCCCTGTGAACATACCACTTGTTGC
CTCGGCGGATCAGCCCGCTCCCGGTAAAACGGGACGGCCCGCCAGAGGACCCCTAAACTCTGTTTCTATA
TGTAACTTCCGAGTAAAACCATAAATAAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCACGATG
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CATTGCGCCCGCCAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCACAGCTTGG
TGTTGGGACTCGCGTTAATTCGCGTTCCTCAAATTGATTGGCGGTCACGTCGAGCTTCCATAGCTAGTAGT
AAAACCCTCGTTACTGGTAATCGTCGCGGCCACGCCGTTAAACCCAACTTCTGAATGTTGA

MN650254 *Geotrichum candidum* strain Ibadan_A1b

ACCCCCCAATCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTAATGATTTTTAATATTTGTG
AATTTACTACAAACAAAAATTTAATTTTATAGTCAAAAATAAAAAAAAAATTAAAACTTTTAACAATGGAT
CTCTTGGTTCTCGTATCGATGAAGAACGCAGCGAAACGCGATATTTCTTGTGAATTGCAGAAGTGAATCA
TCATTTTTCGAACGCACATTCGCACTTTCGGGGTATCCCCCAAAGTATACTTTGTTTGCGTTGTTTTCTCTC
TTGGAATTTGCATTTTGCTTTTTCTAAAAAATCGAATCAAATTTCGTTTTGAAACTTTCAATTTATCTCAAC
CTCAGATCAAGTAGGATTACCCGCTGAACTTAAGCATATCAAAGGCATG

MN650255 *Geotrichum candidum* strain Ibadan_A2a

CCATTGACAAACGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTAATGATTTTTAATATTTGT
GAATTTACTACAAACAAAAATTTAATTTTATAGTCAAAAATAAAAAAAAAATTAAAACTTTTAACAATGG
ATCTCTTGGTTCTCGTATCGATGAAGAACGCAGCGAAACCCGATATTTCTTGTGAATTGCAGAAGTGAAT
CATCATTTTTCGAACGCACATTCGCACTTTCGGGGTATCCCCCAAAGTATACTTTGTTTGCGTTGTTTTCT
CTCTTGGAATTTGCATTTTGCTTTTTCTAAAAAATCGAATCAAATTTCGTTTTGAAACTTTCAATTTATCTC
AACCTCAGATCAAGTAGGATTACCCGCTGAACTTAAGCATATCAAAGGCATCGT

MN650256 *Geotrichum candidum* strain Ibadan_A2b

ACCCCCAAAATCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTAATGATTTTTAATATTTGT
GAATTTACTACAAACAAAAATTTAATTTTATAGTCAAAAATAAAAAAAAAATTAAAACTTTTAACAATGG
ATCTCTTGGTTCTCGTATCGATGAAGAACGCAGCGAAACGCGATATTTCTTGTGAATTGCAGAAGTGAAT
CATCATTTTTCGAACGCACATTCGCACTTTCGGGGTATCCCCCAAAGTATACTTTGTTTGCGTTGTTTTCT
CTCTTGGAATTTGCATTTTGCTTTTTCTAAAAAATCGAATCAAATTTCGTTTTGAAACTTTTAATTTATCTC
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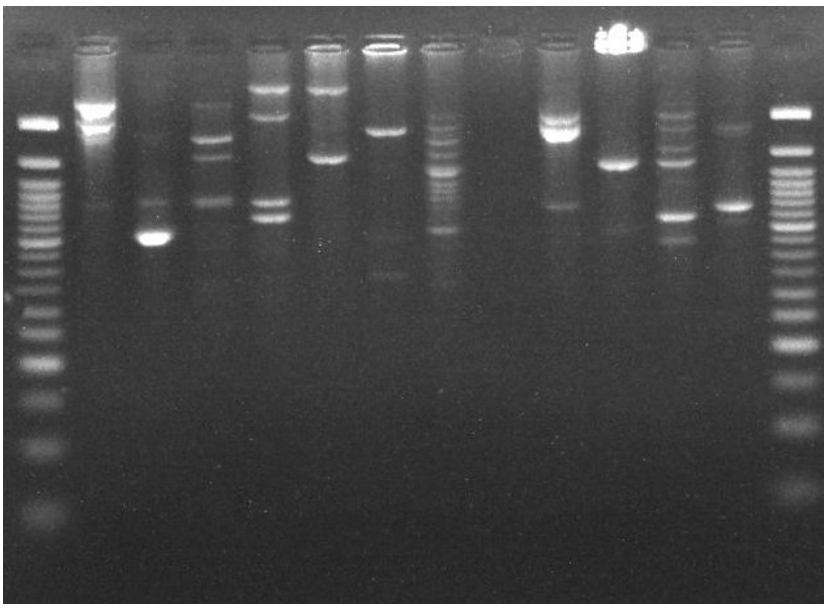

Plate 11. 12 SAMPLES (P-Z, Z1) RAPD_T01. RAPD profiles of the twelve fungi isolated from two tomato accessions collected from different locations as detected by RAPD primers OPT-01 (5'-GGGCCACTCA-3'), M = 100 bp marker. M is molecular marker of low range DNA ladder.

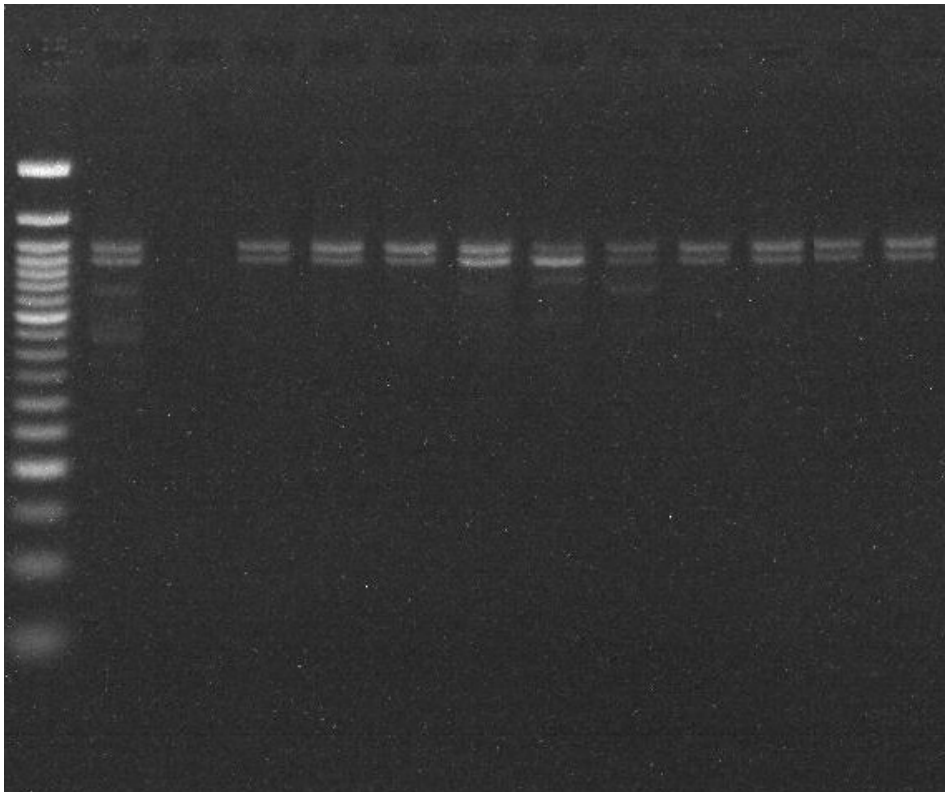

Plate 12. 12 SAMPLES (P-Z, Z1) RAPD_T10. RAPD profiles of the twelve fungi isolated from two tomato accessions collected from different locations as detected by RAPD primers OPT-10 (5'-CCTTCGGAAG-3'), M = 100 bp marker.

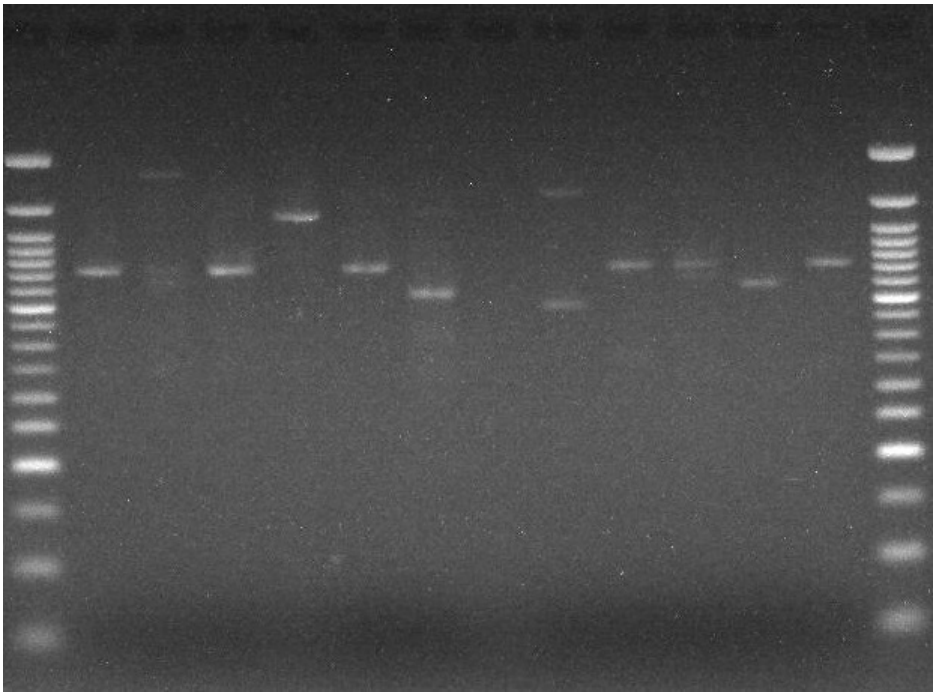

Plate 13. 12 SAMPLES (P-Z, Z1) RAPD_B08. RAPD profiles of the twelve fungi isolated from two tomato accessions collected from different locations as detected by RAPD primers OPB-08 (5'-GTCCACACGG-3'), M = 100 bp marker.

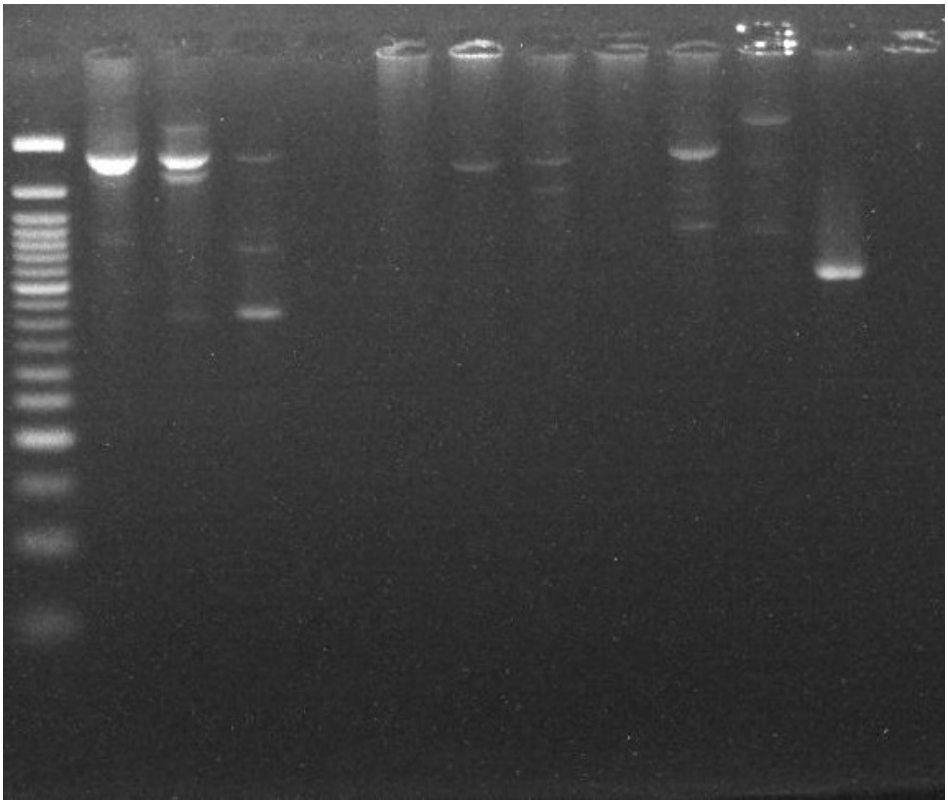

Plate 14. 12 SAMPLES (P-Z, Z1) RAPD_T02. RAPD profiles of the twelve fungi isolated from two tomato accessions collected from different locations as detected by RAPD primers OPT-02 (5'-GGAGAGACTC-3'), M = 100 bp marker.