Detection of virulence-associated genes of *Fusarium oxysporum* and *Geotrichum candidum* strains in tomato cultivars from Nigeria

Olalekan Ajibolade Ogunoye*,
Ayodele Adegboyega Sobowale and
Odunayo Joseph Olawuyi

Department of Crop, Soil and Pest Management Technology,
Rufus Giwa Polytechnic,
Owo, Ondo State, Nigeria
and
Department of Botany,
University of Ibadan,
Ibadan, Oyo State, Nigeria
Email: ooda4life@rocketmail.com
Email: ogunoyeolalekan@gmail.com
Email: delesobowale@yahoo.com
Email: olawuyiodunayo@yahoo.com
*Corresponding author

Abstract: Tomato can be contaminated with fungal species, which is deadly for human and animal's consumption, as some of them produce mycotoxins. *Fusarium oxysporum* and *Geotrichum candidum* diseases still cause massive damages in greenhouses and open fields. The objectives of this study were to detect virulent genes (fmk1, endoPG {pg1}, and xyl3-genes) and ascertain pathogenic strains in *F. oxysporum* and *G. candidum* isolated from two tomato cultivars obtained from Nigeria. Isolation of the two fungal pathogens from the tomato cultivars were done on acidified Potato Dextrose Agar (APDA) plates at the Plant Pathology/Mycology laboratory, Department of Botany, University of Ibadan following standard procedures. DNA extraction from pure cultures of the pathogens was done at the Centre Laboratory of University of Ibadan. Molecular investigations of fmk1, pg1 and xyl3-coding gene in the fungal isolates were by PCR reaction. Genetic relationships among the pathogens were also estimated by constructing a Dendrogram through UPGMA using the Mega6 Software. Five strains of *F. oxysporum* and seven strains of *G. candidum* were identified. A positive amplification of the ITS region of the fungal pathogens was given by agarose gel electrophoresis on PCR reaction. 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*. Gel image indicated a negative amplification for the virulence-associated gene flk1, pg1 and xyl3-gene in all the fungal strains. More studies should be carried out to investigate the gene responsible for virulence and diverse expression of virulence genes upon infection on tomato fruit should also be investigated. This is to ascertain the possibility of more genes coding for pathogenicity in these fungi.

Keywords: *Fusarium oxysporum*; *Geotricum candidum*; pathogens; mycotoxins; virulence.

Reference to this paper should be made as follows: Ogunoye, O.A., Sobowale, A.A. and Olawuyi, O.J. (2023) 'Detection of virulence-associated genes of *Fusarium oxysporum* and *Geotrichum candidum* strains in tomato cultivars from Nigeria', *Int. J. Biotechnology*, Vol. 14, No. 4, pp.303–320.

Biographical notes: Olalekan Ajibolade Ogunoye is an Assistant Lecturer in the Department of Pest Management Technology at Rufus Giwa Polytechnic, Owo, where he has been since 2020. On 11th April 2023, he was appointed as a member of the Faculty of Agricultural Technology Seminar Committee. From 2020 to 2022, he served as a Department Project/Seminar Coordinator for ND/HND programs at the defunct Crop, Soil and Pest Management Technology. He received his BSc from University of Benin, Benin City in 2016. He obtained his MSc in Plant Pathology from the University of Ibadan in 2020. He has attended many academic conferences/seminars. He currently has 9 published articles in both local and international journals.

Ayodele Adegboyega Sobowale is a reader and researcher of Plant Pathology, Mycology and Integrated Disease Management at University of Ibadan, Ibadan, Nigeria. He earned his PhD in Botany (Plant Pathology/Mycology) from University of Ibadan, Nigeria. Previously, he was appointed as a Postgraduate Coordinator of the Department of Botany, University of Ibadan. He is a professional member of Botanical Society of Nigeria, Mycological Society of Nigeria, Nigerian Society for Molecular Biology and Biotechnology, Nigerian Society for Experimental Biology and Agricultural Society of Nigeria. He has supervised over 60 undergraduate students, more than 15 MSc and three PhD projects. He has completed three research projects. He has over 30 publications in journals contributed as author/co-author. He also attended number of conferences and seminars.

Odunayo Joseph Olawuyi is a Senior Lecturer of Genetics, Biotechnology and Molecular Biology in the Department of Botany, University of Ibadan, Ibadan, Nigeria. He earned his PhD in Botany (Genetics) from University of Ibadan, Nigeria. His area of interests are plant genetics, molecular biology, plant biotechnology, mutation genetics and cytogenetics. He currently serves as a Postgraduate Coordinator of his Department and Sub-Dean of Faculty of Sciences; and member of the Senate. His supervision experiences are 60 BSc (completed), 52 MSc (completed) and two PhD (completed). He has attended several conference and published over 150 articles both in local and international reputable journals.

1 Introduction

Tomato (*Solanum lycopersicum* L.), a crop grown in backyard garden or commercial field in several parts of Nigeria all season. It's the most widely eaten vegetable and can be affected with diseases and disorders during the growing season (Baiyeri et al., 2023). Tomato contaminated with fungal species is deadly for human and animal's consumption, since several species of them produce mycotoxins. The yield of this economically important farm product is also affected, hence lowering the production rate as a result of fungal infection (Dandago et al., 2023; Aso et al., 2022).

Fusarium oxysporum is a species complex and includes both non-pathogenic and pathogenic isolates. Non-pathogenic *F. oxysporum* isolates commonly occur in the soil as saprophytes, while some have been identified as biocontrol agents and endophytes

(Taylor et al., 2016; Uddin et al., 2023). Pathogenic *F. oxysporum* isolates are distinguished as formae speciales (f. spp.) on the basis of their host specificity and more than 120 have been identified. Recent advances in the understanding of the pathogenicity in *F. oxysporum* have been made following publication of the genome of *F. oxysporum* f. sp. *lycopersici* (FOL), which infects tomato (Taylor et al., 2016; Zuriegat et al., 2021). *Fusarium* rot on tomato fruits are often caused by *Fusarium* species and the symptoms of this disease include rots softer and spread into the centre of the fruit. The disease causes the vegetable fruits unmarketable as consumer will only choose those that are fresh and healthy (Nizamani et al., 2021; Taha et al., 2023).

Geotrichum candidum is the causal agent of sour rot of tomato fruit (*Solanum lycopersicum*) and other fresh produce (Nizamani et al., 2021). Healthy tomato fruit can be infected by *G. candidum* through micro-cracks, lesions, penetration, and contact with decaying fruit. This disease is a limiting factor for tomato production major regions of the world where tomato production is an important economic product (Thornton et al., 2013; Fiedler, 2014). According to Taha et al. (2023) and Nizamani et al. (2021), it causes losses in the field, but the primary concern is during postharvest handling. *Geotrichum candidum* infections are more common after wet harvest conditions, abrupt drops in temperature due to rainfall, and improper post-harvest handling procedures.

Fusarium oxysporum and *Geotrichum candidum* diseases still cause massive damages in greenhouses and open fields (Chehri, 2016; Ziedan et al., 2018). The objectives of this study were to detect virulent genes (fmk1, endoPG {pg1}, and xyl3-genes) and ascertain pathogenic strains in *F. oxysporum* and *G. candidum* isolated from two tomato cultivars obtained from Nigeria. The screening of virulence gene is essential for studying host-pathogen interactions and it help pathologists to manage post-harvest diseases caused by these organisms.

2 Materials and methods

Three state capitals in Nigeria (Shasha in Akure, Ondo state; Eye-nkorin in Ilorin, Kwara State and Shasha in Ibadan, Oyo State) were visited for the collection of two cultivars of tomato. The tomato fruit samples stored in local basket were collected from wholesale market at random from the location in a well-labelled brown paper envelops. Scads of tomato fruits samples from each location were collected for each cultivar and conveyed for fungal analysis at the Department of Botany (Plant Pathology/Mycology Laboratory unit), University of Ibadan [Table 1; Figures 1(a)–1(c)].

Table 1 Locations of Tomato fruit samples collected from wholesale market Akure, Ilorin and Ibadan, Nigeria

Common name of Tomato fruit samples collected	*Locations*	*Latitude*	*Longitude*
Tiwantiwa (A_1) and Hausa scissors (A_2)	Ilorin	8°29°48 N	4°32°31 E
Tiwantiwa (A_1) and Hausa scissors (A_2)	Akure	7°16°44 N	5°13°52 E
Tiwantiwa (A_1) and Hausa scissors (A_2)	Ibadan	7°29°13N	4°32°45E

Figure 1 (a) *Solanum lycopersicum* L. (Tomato) from Ilorin, Kwara state (b) *Solanum lycopersicum* L. (Tomato) from Akure, Ondo state (c) *Solanum lycopersicum* L. (Tomato) from Ibadan, Oyo state (see online version for colours)

(a) (b) (c)

Source: Ezekiel et al. (2011)

2.1 Preparation of PDA and Isolation of the fungi

Potato Dextrose Agar (PDA) were prepared according to manufacturer's specification by dissolving 39 g of P.D.A in a litre of distil water. It was allowed to soak for few minutes, thoroughly mixed, cotton plugged, covered with Aluminium foil and then sterilised in an autoclave at 121°C for 15 minutes and allowed to cool to about 45°C and poured aseptically into Petri dishes and chloramphenicol added. The fungi were isolated in Plant Pathology/Mycology Laboratory, Department of Botany, University of Ibadan using a classical method of Koch postulate as modified by Mailafia et al. (2017). A total of 15 randomly selected tomatoes were examined based on their locations of collection. The tomatoes were soaked in 0.1% hypochlorite for 30 seconds for surface sterilisation and then rinsed sterile distilled water. The tomatoes were chopped into small segments (3 mm in diameter) with a sterilised blade (surface sterilised in 0.1% hypochlorite for 30 seconds), plated on potatoes dextrose agar (PDA) aseptically and then incubated at 28°C for 5 days. A pure culture was obtained and maintained by sub-culturing each of the different colonies that emerged onto the PDA plates and incubating at 28°C for 5 days. The isolated fungi from the infected tomatoes were purified by culturing repeatedly the young active mycelia colony to obtain pure culture. The colonies that developed were sub-cultured repeatedly on PDA to obtain pure cultures Mailafia et al. (2017).

2.2 Identification of isolated fungi

The morphological characteristics and appearance of the fungi isolated were identified in accordance with Nizamani et al. (2021) and Aso et al. (2022).

2.3 Molecular identification

2.3.1 DNA extraction of fungi isolates

Dellaporta et al. (1983) method of DNA extraction as modified by Dandago et al. (2023) was used for this study at the Central Laboratory, University of Ibadan. 100 mg of fungal mycelia was grinded in a sterile mortal with 500 ml of DNA Extraction Buffer (DEB)

containing proteinase K (0.05 mg/ml) using a sterile pestle. Mixture was transferred into 1.5 ml Eppendorf tube. 50 µl of 20% Sodium Dodecyl Sulphate (SDS) was added and incubated in a water-bath at 65°C for 30 minutes. Mixture was allowed to cool to room temperature, the 100 µl 7.5 M Potassium Acetate was added and mixed briefly then centrifuged at 13,000 rpm for 10 minutes. Supernatant was transfer into new fresh autoclaved tubes then a volume of 2/3 volumes supernatant to Isopropanol was gently mixed by inverting the tubes 3–5 times followed by incubation at –20°C for 1 hour. Mixture was then centrifuged at 13,000 rpm for 10 minutes and supernatant discarded. 500 µl of 70% ethanol was added and centrifuge for 5 minutes at 13,000 rpm. Supernatant was discarded again carefully with the DNA pellet intact. Pellets were then air-dried at 37°C for 10–15 minutes to remove traces of ethanol. The pellets were re-suspend in 50 µl of Tris-EDTA (TE) buffer and store at –20°C for further laboratory analysis.

2.3.2 Polymerase chain reaction amplification

To use the ITS gene for characterisation of the fungi isolates, ITS universal primer set which flank the ITS1, 5.8S and ITS2 region was used for PCR. PCR reaction cocktail consisted of 10 µl of 5x GoTaq colourless reaction, 3 µl of $MgCl_2$, 1 µl of 10 mM of dNTPs mix, 1 µl of 10 pmol each ITS 1: 5' TCC GTA GGT GAA CCT GCG G 3'and – ITS 4: 5' TCC TCC GCT TAT TGA TAT GC 3' primers and 0.3 units of Taq DNA polymerase (Promega, USA) made up to 42 µl with sterile distilled water 8 µl DNA template. 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 (Dandago et al., 2023).

2.3.3 Purification of PCR products

After gel integrity, the amplified fragments were ethanol purified in order to remove the PCR reagents. Briefly, 7.6 µl of Na acetate 3 M and 240 µl of 95% ethanol were added to each about 40 µl PCR amplified product in a new sterile 1.5 µl tube Eppendorf, mix thoroughly by vortexing and keep at –20°C for at least 30 min. Centrifugation for 10 min at 13,000 g and 4°C followed by removal of supernatant (invert tube on trash once) after which the pellet were washed by adding 150 µl of 70% ethanol and mix then centrifuge for 15 min at 7,500 g and 4°C. Again remove all supernatant (invert tube on trash) and invert tube on paper tissue and let it dry in the fume hood at room temperature for 10–15 mins. Then re-suspend with 20 µl of sterile distilled water and kept in –20°C prior to sequencing. The purified fragment was checked on a 1.5% agarose gel ran on a voltage of 110 V for about 1hr as previous, to confirm the presence of the purified product and quantified using a nano-drop of model 2000 from thermo scientific (Pryce et al., 2003; Groenewald, 2006).

2.3.4 DNA sequencing

Sequencing of the amplified fragments were done using a Genetic Analyzer 3130xl sequencer from Applied Bio-systems as stated on manufacturers' manual while the

sequencing kit used was that of Big Dye ® terminator v3.1 cycle sequencing kit (Pryce et al., 2003; Groenewald, 2006).

Table 2 Oligonucleotide primers used for the amplification of *fmk1*, *pg1* and *xyl3* regions

Gene	*Primer name*	*Reference*	*Sequence (5'_3')*	*Tm (°C)*
fmk1	OliFmkA (forward)	Designed in this study	CAACAGCTTCATCTCTCGCA	60.40
	OliFmkB (reverse)	Designed in this study	ACCATTTGACCGCTAGACCA	60.40
Pg1	Upper PG (forward)	Posada et al. (2000)	ATCTGGCCATGTCATTGA	62.18
	Lower PG (reverse)	Posada et al. (2000)	GGTCGGCTTTCCAGTAGG	55.34
xyl3	Xyl3-sense (forward)	Ruiz-Roldán et al. (1999)	TTTTTCGGTTCTCCTCGCTCTCGC	66.28
	Xyl3-antisense (reverse)	Ruiz-Roldán et al. (1999)	ACTCTCGTGGGACCTAAACC	67.98

2.4 Molecular analysis of virulence-associated genes

2.4.1 Molecular analysis of fmk1-coding genes

Molecular investigations of *fmk1*-coding gene in the 12 fungal isolates were by simple PCR on the extracted DNA using *fmk1*-coding regions specific primers. Primer sequences and reaction profile were as earlier documented by (Groenewald, 2006). Reaction cocktail used for all PCR per primer set included (reagent volume µl) – 5X PCR SYBR green buffer (2.5), $MgCl_2$ (0.75), 10pM DNTP (0.25), 10 pM of each forward and backwards primer (0.25), 8,000 U of taq DNA polymerase (0.06) and made up to 10.5 with sterile distilled water to which 2 µl template was added. Buffer control was also added to eliminate any probability of false amplification. Table 2 shows the primer sequence and PCR profile used in amplifying each fragment. PCR was carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA) using the appropriate profile as designed for each primer pair.

2.4.2 Molecular analysis of xyl3-coding genes

Molecular investigations of xyl3-coding gene in the 12 fungal isolates were by simple PCR on the extracted DNA using xyl3-coding regions specific primers. Primer sequences and reaction profile were as earlier documented by Groenewald (2006). Reaction cocktail used for all PCR per primer set included (reagent volume µl) – 5X PCR SYBR green buffer (2.5), $MgCl_2$ (0.75), 10 pM DNTP (0.25), 10 pM of each forward and backwards primer (0.25), 8,000 U of taq DNA polymerase (0.06) and made up to 10.5 with sterile distilled water to which 2 µl template was added. Buffer control was also added to eliminate any probability of false amplification Table below shows the primer sequence and PCR profile used in amplifying each fragment. PCR was carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA) using the appropriate profile as designed for each primer pair.

2.4.3 Molecular analysis of pg1-coding genes

Molecular investigations of pg1-coding gene in the 12 fungal isolates were by simple PCR on the extracted DNA using pg1-coding regions specific primers. Primer sequences and reaction profile were as earlier documented by Groenewald (2006). Reaction cocktail used for all PCR per primer set included (reagent volume µl) – 5 X PCR SYBR green buffer (2.5), $MgCl_2$ (0.75), 10 pM DNTP (0.25), 10 pM of each forward and backwards primer (0.25), 8,000 U of taq DNA polymerase (0.06) and made up to 10.5 with sterile distilled water to which 2 µl template was added. Buffer control was also added to eliminate any probability of false amplification Table below shows the primer sequence and PCR profile used in amplifying each fragment. PCR was carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA) using the appropriate profile as designed for each primer pair (Table 2).

2.5 Data analysis

The ITS sequences were aligned and amended using ClustalW multiple sequence alignment program in MEGA v.6 Software. Sequencing results were individually keyed online into basic local alignment search tool (BLAST) in National Centre for Biotechnology Information (NCBI) nucleotide database (http://blast.ncbi.nlm.nih.gov/) to identify the isolates. Fungal haplotypes gotten in this study were used to construct a phylogenetic tree using Maximum Likelihood method in MEGA v.6 Software to analyse the genetic relationship amongst the strains.

3 Result

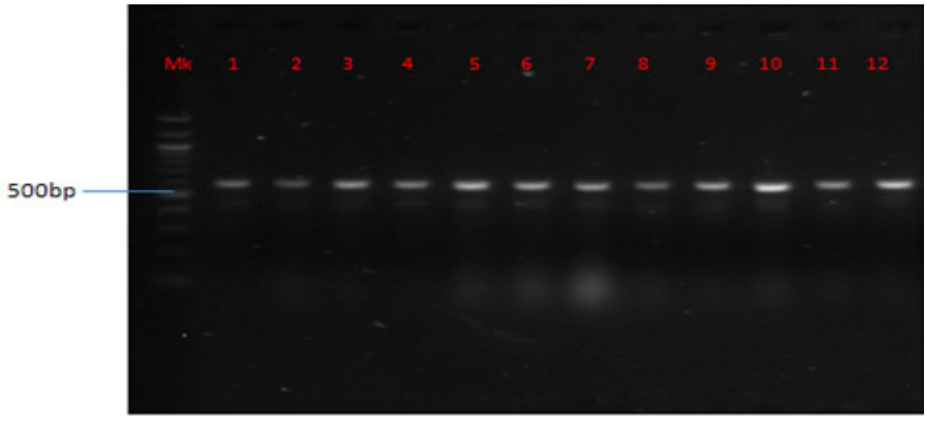
3.1 Cultural and morphological characterisation

Pure culture of the fungal isolates obtained has neat and fluffy colonies. Colony surface was white and powdery, growing rapidly, moderately and slowly; different shapes of colony and aerial mycelium (flat, dense and floccose) and white or violet white in pigmentation. Only monophialides are present; macroconidia very abundant and needle-like with thin walls; microconidia abundant, in false heads only and chlamydospores single or in chains.

3.2 Blast result and molecular epithet based on DNA ITS sequencing

The ITS PCR reactions were conducted using the ITS1 and ITS4 primers which amplify the ITS1-5.8S-ITS2 region of the ribosomal operon. Five strains of *Fusarium oxysporum* and seven strains of *Geotrichum candidum* were included. All of them produced an amplicon of approximately 550 bp (plate 14), showing positive PCR amplification was evident. Although DNA was purified, the direct PCR resulted in a single band with the expected size.

Figure 2 At band size of approximately 550bp, the agarose gel electrophoresis showed a positive amplification of the ITS region of fungal isolates (see online version for colours)



Note: 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

3.3 Blasted result showing the percentage identity of samples derived in National Center for Biotechnology Information (NCBI)

The sequences obtained were compared with sequences deposited in the GeneBank nucleotide database (NCBI, http://www.ncbi. nlm.nih.gov/) for similarity using the BLAST program (basic local alignment search tool). BLAST searches on GeneBank shown a good homology with maximum scores highest in MN650248 and lowest maximum scores MN650249 and MN650251 with E-value = 0 in all the fungal isolates as shown in Table 3.

The fungal isolates were found to have 99.63% similarity with *Fusarium oxysporum* isolate UDEAGIEM-H12 strain (accession number GenBank: MK432913.1); 99.79% similarity with *Fusarium oxysporum* isolate *Aconitum carmichaelii* Debx (accession number GenBank: MH542665.1); 100.00% similarity with *Geotrichum candidum* strain E320 (accession number GenBank: MF681547.1); 100.00% similarity with *Fusarium oxysporum* isolate CU-1 (accession number GenBank: MN219649.1); 99.24% similarity with *Geotrichum candidum* strain E320 (accession number GenBank: MF681547.1); 100.00% similarity with *Geotrichum candidum* strain E320 (accession number GenBank: MF681547.1); 99.33% similarity with *Fusarium oxysporum* isolate SMS16 (Accession Number Gen Bank: KR085974.1); 99.24% similarity with *Geotrichum candidum* strain E320 (accession number GenBank: MF681547.1); 99.17% similarity with *Fusarium oxysporum* strain ATCC 11939 (accession number GenBank: DQ452452.1); 98.48% similarity with *Geotrichum candidum* isolate f1 (Accession Number GenBank: MK559408.1); 99.43% similarity with *Geotrichum candidum* isolate f1 (accession number GenBank: MK559408.1) and 99.60% similarity with *Geotrichum candidum* isolate f1 (Accession Number GenBank: MK559408.1) as shown in Table 3.

Table 3 Blasted result showing the percentage identity of samples derived in NCBI website

	Description	*Max score*	*Total score*	*Query cover*	*E value*	*Per. identity of pathogen*	*Pathogen*
AkureA1a	*Fusarium oxysporum* isolate UDEAGIEM-H12	977	977	98%	0	99.63%	MK432913.1
AkureA1b	*Fusarium oxysporum* isolate *Aconitum carmichaelii* Debx	870	870	100%	0	99.79%	MH542665.1
AkureA2a	*Geotrichum candidum* strain E320	743	743	98%	0	100.00%	MF681547.1
AkureA2b	*Fusarium oxysporum* isolate CU-1	1,031	1031	100%	0	100.00%	MN219649.1
KwaraA1a	*Geotrichum candidum* strain E320	713	713	97%	0	99.24%	MF681547.1
KwaraA1b	*Geotrichum candidum* strain E320	734	734	100%	0	100.00%	MF681547.1
KwaraA2a	*Fusarium oxysporum* isolate SMS16	811	811	100%	0	99.33%	KR085974.1
KwaraA2b	*Geotrichum candidum* strain E320	713	713	98%	0	99.24%	MF681547.1
Ibadan_A1a	*Fusarium oxysporum* strain ATCC 11939	863	863	99%	0	99.17%	DQ452452.1
IbadanA1b	*Geotrichum candidum* isolate f1	804	1372	100%	0	98.48%	MK559408.1
IbadanA2a	*Geotrichum candidum* isolate f1	948	1570	99%	0	99.43%	MK559408.1
IbadanA2b	*Geotrichum candidum* isolate f1	922	1525	100%	0	99.60%	MK559408.1

All of the sequences generated in this study have been deposited in the GenBank under accession numbers MN650245 (*Fusarium oxysporum* strain_AkureA1a), MN650246 (*Fusarium oxysporum* strain_AkureA1b), MN650247 (*Geotrichum candidum* strain_AkureA2a), MN650248 (*Fusarium oxysporum* strain_AkureA2a), MN650249 (*Geotrichum candidum* strain_KwaraA1a), MN650250 (*Geotrichum candidum* strain_KwaraA1b), MN650251 (*Fusarium oxysporum* strain_KwaraA2a), MN650252 (*Geotrichum candidum* strain_KwaraA2b), MN650253 (*Fusarium oxysporum* strain_IbadanA1a), MN650254 (*Geotrichum candidum* strain_IbadanA1b), MN650255 (*Geotrichum candidum* strain_IbadanA2a), MN650256 (*Geotrichum candidum* strain_IbadanA2b).

Figure 3 Phylogenetic tree of *Fusarium oxysporum* and *Geotrichum candidum* isolates based on rDNA ITS sequences using maximum likelihood method in MEGA v.6 Software to analyse the genetic relationship amongst the strains (see online version for colours)

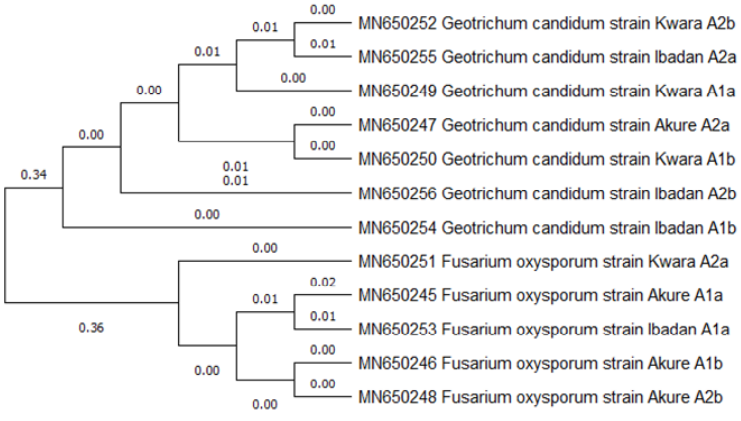
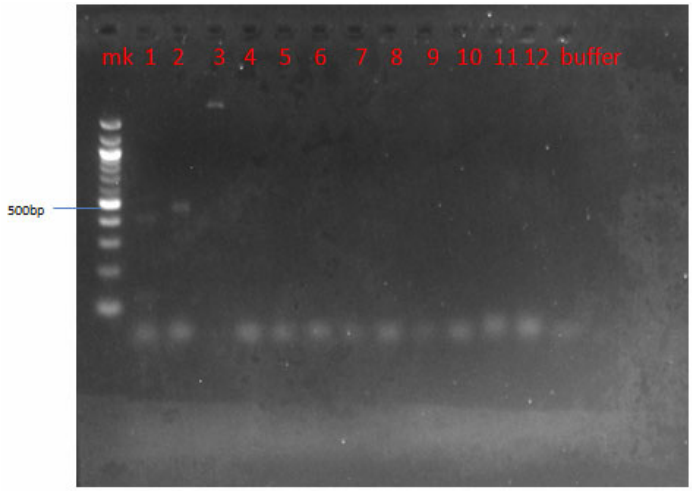


Figure 4 Agarose gel electrophoresis of the PCR products of *fmk1* gene amplified from fungal isolates using Olifmka specific primers (see online version for colours)



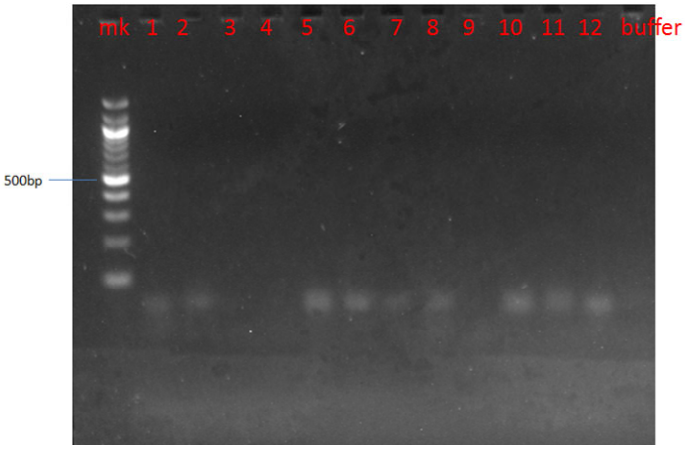
Note: Expected band size approximately 400 bp indicating *flk1* gene in *Fusarium oxysporum*.

3.4 Sequencing data analysis

The phylogenetic tree includes accession number and geographical location of *Fusarium oxysporum* and *Geotrichum candidum* strains derived from the GeneBank in NCBI website. The dendogram was separated into two major clades for *Fusarium oxysporum* and *Geotrichum candidum* strains. Each clade has sub-clades which further showed the closely related strains such as KwaraA2b –IbadanA2b and AkureA2a and KwaraA1b for *Geotrichum candidum* strains, while AkureA1a-IbadanA1a and AkureA1b-AkureA2b are closely related strains among the *Fusarium oxysporum* strains as shown in Figure 3.

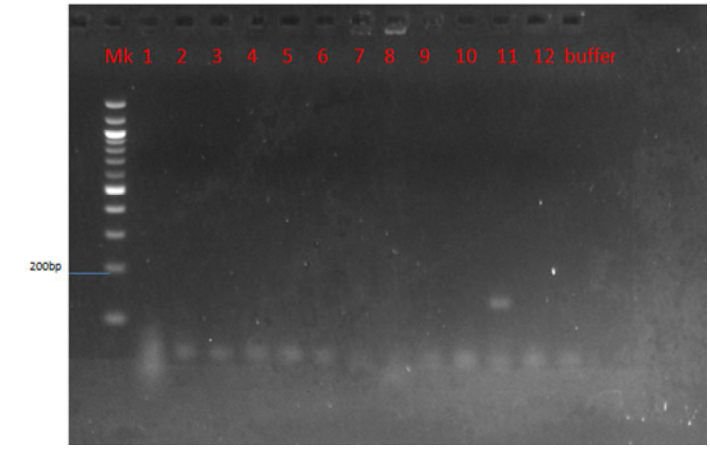
G. candidum MN650252 strain and MN650255 strain are from Eyenkorin in Ilorin, Kwara State and Shasha in Ibadan, Oyo State, Nigeria. The most distantly collected but, however similar strains are MN650247 and MN650250. As for *F. oxysporum* very similar strains were noticeable in MN650246 and MN650248, and MN650245 and MN650253.

Figure 5 Agarose gel electrophoresis of the PCR products of *pg1* gene amplified from fungal isolates using PG specific primers (see online version for colours)



Note: Expected band size approximately 740 bp indicating presence of pg1 gene in Fusarium oxysporum).

Figure 6 Agarose gel electrophoresis of the PCR products of xyl3 gene amplified from fungal isolates (see online version for colours)



Note: Expected band size approximately 260 bp indicating xyl3 gene in *Fusarium oxysporum*.

3.5 Gel image for xyl3, flk1 and pg1

Gel image indicates a negative amplification for the virulence-associated gene *flk1* in all fungal isolates except in MN650245. However, a positive amplicon at an approximately 480 bp was noticed in MN650246 as indicated in Gel image (Figure 4). Figure 5 indicates a negative amplification for *pg1* gene in all the fungal isolates. While a negative amplicon was also observed for *xyl3* gene. However, at a lower base-pair of approximately 150 bp, sample MN650255 was amplified as shown in Figure 6.

4 Discussion

The identification of the fungal isolates using cultured characteristics (such as colony growth pattern, shape of the organism, aerial mycelium) and morphological observations (chlamydospore, microconidia and macroconidia) agreed with the work of Mailafia et al. (2017) and Aso et al. (2022). The aforementioned cultural and morphological observation shows the taxonomic identity of the fungi, and the above characteristics labelled the fungus to be *Fusarium oxysporum* and *Geotrichum candidum* which were consistent with description of Tafinta et al. (2013) and Soher et al. (2015). The morphological characteristics and appearance of the *Fusarium oxysporum* and *Geotrichum candidum* isolated from the tomato cultivars used for this study were confirmed and authenticated with the help of mycological atlas of Tafinta et al. (2013).

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. (2018), Chehri (2016) and Ziedan et al. (2018). 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 Mohammed et al. (2016), Ma et al. (2017), Akbar et al. (2018) and Ziedan et al. (2018). 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. (2018), Alonzo et al. (2020) and Dandago et al. (2023). 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 Mohammed et al. (2016), Ma et al. (2017), Akbar et al. (2018) and Ziedan et al. (2018).

Recently, the all-embracing usage of DNA identification techniques has successfully remunerated for the deficiencies of morphological classification methods. rDNA-ITS is the target most generally used to classify and identify plant pathogenic fungi. The combination of morphological classification methods with molecular techniques has significantly improved the accuracy of the identification of plant pathogenic fungi (Dandago et al., 2023).

The phylogenetic tree based on the rDNA sequencing associated with the 12 fungal isolates from the two tomato varieties were separated into two major clusters. No relationship was found between the regions from which strains were isolated and clustering patterns. The same or very similar strains were isolated from widely different regions; this is similar to the work submitted by Marcellino et al. (2001). *Fusarium*

oxysporum and *Geotrichum candidum* strains from different locations revealed a common cluster which suggests that migration event is common in the population of fungal isolates as observed by Olowe et al. (2017). The phylogenetic tree based on the evolutionary relationship among fungal isolates established the interrelationship amongst the isolates from different locations in the three state capitals (Akure, Ilorin and Ibadan). The clustering of the isolates, regardless of the source denotes that the genetic distance is not correlated with the region of sample collection as similarly reported by Olowe et al. (2017). Therefore, similar or identical strains are pervasive throughout the locations considered in this study conform to the work submitted by Marcellino et al. (2001) and Olowe et al. (2017).

This finding demonstrates that the virulence-associated genes *fmk1*, *pg1* and *xyl3* genes were not present in all the fungal isolates. This study is contrary to the work submitted by Groenewald (2006) who reported that the virulence-associated genes *fmk1*, *pg1* and *xyl3* were present in all isolates of *F. oxysporum*, pathogenic and non-pathogenic to banana. However, this does not imply that the organisms are not potential pathogens since differences exist in the regulation of the gene expression patterns.

5 Conclusions and recommendation

Study on virulence-associated genes *fmk1*, *pg1* and *xyl3* genes of *F. oxysporum* and *G. candidum* can help pathologist to detect potential pathogenic strains and develop effective management strategies. More studies should be carried out to investigate the gene responsible for virulence in these pathogens and diverse expression of virulence genes upon infection on tomato fruit should also be investigated. This is to ascertain the possibility of more genes coding for pathogenicity in these fungi.

Acknowledgements

O.A.O. conceived the study, carried out the laboratory work and prepared the draft manuscript; S.A.A. sourced for literature and proofread the draft manuscript and O.O.J., Jointly supervised and proofread the study.

References

Akbar, A., Hussain, S., Ullah, K., Fahim, M. and Ali, G.S. (2018) 'Detection, virulence and genetic diversity of Fusarium species infecting tomato in Northern Pakistan', *PLoS One*, Vol. 13, No. 9, pp.1–21.

Alonzo, G., Lopes, U.P., Wang, N.Y. and Peres, N.A. (2020) 'First report of sour rot of strawberry caused by *Geotrichum candidum* in the United States', *Plant Disease*, Vol. 105, No. 1, pp.1–5.

Aso, R.E., Briska, J., Ade, T.I., Iheanacho, C.C. and Shinggu, P.P. (2022) 'Isolation and identification of fungal species associated with the deterioration of some selected fruits sold in Wukari, Taraba State, North East, Nigeria', *FUW Trends in Science and Technology Journal*, Vol. 7, No. 2, pp.1080–1083.

Baiyeri, M.R., Yusuf, K.O., Obalowu, R.O., Saad, G. and Banjoko, I.K. (2023) 'Impact of magnetization of irrigation water on growth, yield and nutritional qualities of tomato under deficit irrigation', *Notulae Scientia Biologicae*, Vol. 15, No. 1, pp.1–15.

Chehri, K. (2016) 'Molecular identification of pathogenic *Fusarium* species, the causal agents of tomato wilt in western Iran', *Journal of Plant Protection Research*, Vol. 56, No. 2, pp.143–148.

Dandago, M.A., Yunusa, A.K., Rilwan, A., Yahaya, S.M., Nassarawa, S.S. and Khaleel, A.G. (2023) 'Molecular identification of fungal species infesting tomato (*Lycopersicon esculentum*) at postharvest phase in Kwanar Gafan, Kano State', *Journal of Horticulture and Postharvest Research*, Vol. 6, No. 4, pp.349–360.

Dellaporta, S.L., Wood, J. and Hicks, J.B. (1983) 'Experimental protocols A plant DNA mini-preparation: Version II', *Plant Molecular Biology Reporter*, Vol. 1, No. 4, pp.19–21.

Ezekiel, C.N., Nwangburuka, C.C., Ajibade, O.A. and Odebode, A.C. (2011) 'Genetic diversity in 14 tomatoes (*Lycopersicon esculentum* Mill.) varieties in Nigerian markets by RAPD-PCR technique', *African Journal of Biotechnology*, Vol. 10, No. 25, pp.4961–4967.

Fiedler, K. (2014) *Integrated Approach to Understanding Tomato Sour Rot and Improving Disease Management on the Eastern Shore of Virginia*, Dissertation submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of Doctor of Philosophy In Plant Pathology, Physiology, and Weed Science, 99pp.

Groenewald, S. (2006) *Biology, Pathogenicity and Diversity of Fusarium oxysporum f.sp. cubense*. Submitted in partial fulfillment of the requirements for the degree of Magister Scientiae, In the Faculty of Natural and Agricultural Science University of Pretoria, Pretoria, 176pp.

Ma, W., Zhang, Y., Wang, C., Liu, S. and Liao, X. (2017) 'A new disease of strawberry, fruit rot, caused by Geotrichum candidum in China', *Plant Protection Science*, Vol. 54, No. 2, pp.1–9.

Mailafia, S., Okoh, G.R., Olabode, H.O.K. and Osanupin, R. (2017) 'Isolation and identification of fungi associated with spoilt fruits vended in Gwagwalada market, Abuja, Nigeria', *Veterinary World*, Vol. 10, No. 4, pp.393–397.

Marcellino, N., Beuvier, E., Grappin, R., Gueguen, M. and Benson, D.R. (2001) 'Diversity of *Geotrichum candidum* strains isolated from traditional cheesemaking fabrications in France', *Applied and Environmental Microbiology*, Vol. 67, No. 10, pp.4752–4759.

Mohammed, A.S., Kadar, N.H., Kihal, M., Henni, J.E., Sanchez, J., Gallego, E. and Garrido-Cardenas, J.A. (2016) 'Characterization of *Fusarium oxysporum* isolates from tomato plants in Algeria', *African Journal of Microbiology Research*, Vol. 10, No. 30, pp.1156–1163.

Nizamani, S., Khaskheli, A.A., Jiskani, A.M., Khaskheli, S.A., Khaskheli, A.J., Poussio, G.B., Jamro, H. and Khaskheli, M.I. (2021) 'Isolatation and identification of the fungi causing Tomato fruit rot disease in the vicinity of Tandojam, Sindh', *Agricultural Science Digest*, Vol. 41, pp.186–190, Special Issue.

Olowe, O.M., Odebode, A.C., Olawuyi, O.J. and Sobowale, A.A. (2017) 'Molecular variability of *Fusarium verticillioides* (Sacc.) in maize from three agro-ecological zones of southwest Nigeria', *American Journal of Molecular Biology*, Vol. 7, No. 1, pp.30–40.

Posada, M.L., Patino, B., De La Heras A., Mirete, S., Vázquez, C. and González-Jaén, M.T. (2000) 'Comparative analysis of endopolygalacturonase coding gene in isolates of seven *Fusarium* species', *Mycological Research*, Vol. 104, No. 11, pp.1342–1347.

Pryce, T M., Palladino, S., Kay, I.D. and Coombs, G.W. (2003) 'Rapid identification of fungi by sequencing the ITS l and ITS2 regions using an automated capillary electrophoresis system', *Medical Mycology*, Vol. 41, No. 5, pp.369–380.

Ruiz-Roldán, M.C., Di Pietro, A., Huartas-González, M.D. and Roncero, M.I.G. (1999) 'Two xylanase genes of the vascular wilt pathogen *Fusarium oxysporum* are differentially expressed during infection of tomato plants', *Molecular and General Genetics*, Vol. 261, No. 3, pp.530–536.

Soher, E.A. Amal, S.H., Nevien, A.A., Bassem, A.S. and Ahmed, F.S. (2015) 'Molecular identification and control of some pathogenic *Fusarium* species isolated from maize in Egypt', *International Journal of ChemTech Research*, Vol. 7, No. 1, pp.44–54.

Tafinta, I.Y., Shehu, K., Abdulganiyyu, H., Rabe, A.M. and Usman, A. (2013) 'Isolation and identification of fungi associated with the spoilage of sweet orange (*Citrus sinensis*) fruits in Sokoto State', *Nigerian Journal of Basic and Applied Sciences*, Vol. 21, No. 3, pp.193–196.

Taha, N.A., Elsharkawy, M.M., Shoughy, A.A., El-Kazzaz, M.K. and Khedr, A.A. (2023) 'Biological control of postharvest tomato fruit rots using *Bacillus* spp. and *Pseudomonas* spp', *Egyptian Journal of Biological Pest Control*, Vol. 33, pp.106–118.

Taylor, A., Vagany, V., Jackson, A.C., Harrison, R.J., Rainoni, A. and Clarkson, J.P. (2016) 'Identification of pathogenicity-related genes in *Fusarium oxysporum* f. sp. cepae', *Molecular Plant Pathology*, Vol. 17, No. 7, pp.1032–1047.

Thornton, C.R., Slaughter, D.C. and Davis, R.M. (2013) 'Detection of the sour-rot pathogen *Geotrichum candidum* in tomato fruit and juice by using a highly specific monoclonal antibody-based ELISA', *International Journal of Food Microbiology*, Vol. 143, No. 3, pp.166–172.

Uddin, M.J., Huang, X., Lu, X. and Li, S. (2023) 'Increased conidia production and germination in vitro correlate with virulence enhancement in *Fusarium oxysporum* f. sp. cucumerinum', *Journal of Fungi*, Vol. 9, No. 847, pp.1–11.

Valinhas, R.V., Pantoja, L.A., Maia, A.C.F., Miguel, M.G.C.P., Vanzela, A.P.F.C., Nelson, D.L. and Santos, A.S. (2018) 'Xylose fermentation to ethanol by new *Galactomyces geotrichum* and *Candida akabanensis* strains', *Peer Journal*, Vol. 6, No. e4673, pp.1–43.

Ziedan, E.H., Khattab, A.A. and Sahab, A.F. (2018) 'New fungi causing postharvest spoilage of cucumber fruits and their molecular characterization in Egypt', *Journal of Plant Protection Research*, Vol. 58, No. 4, pp.362–371.

Zuriegat, Q., Zheng, Y., Liu, H., Wang, Z. and Yun, Y. (2021) 'Current progress on pathogenicity-related transcription factors in *Fusarium oxysporum*', *Molecular Plant Pathology*, Vol. 22, pp.882–895.

Sequencing analysis

Akure

MN650245 Fusarium oxysporum strain Akure_A1a

GCAAGGTGAACTGCGGAGGGATCATTACCGAGTTTACAACTCCCAAACCCCT
GTGAACATACCACTTGTTGCCTCGGCGGATCAGCCCGCTCCCGGTAAAACGG
GACGGCCCGCCAGAGGACCCCTAAACTCTGTTTCTATATGTAACTTCTGAGTA
AAACCATAAATAAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATC
GATGAAGAACGCAGCAAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTG
AATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATG
CCTGTTCGAGCGTCATTTCAACCCTCAAGCACAGCTTGGTGTTGGGACTCGCG
TTAATTCGCGTTCCTCAAATTGATTGGCGGTCACGTCGAGCTTCCATAGCGTA
GTAGTAAAACCCTCGTTACTGGTAATCGTCGCGGCCACGCCGTTAAACCCCA
ACTTCTGAATGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCA
TATCAATAAGGGGAAAAA

MN650246 Fusarium oxysporum strain Akure_A1b

GTGAACATACCACTTGTTGCCTCGGCGGATCAGCCCGCTCCCGGTAAAACGG
GACGGCCCGCCAGAGGACCCCTAAACTCTGTTTCTATATGTAACTTCTGAGTA
AAACCATAAATAAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATC

GATGAAGAACGCAGCAAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTG
ATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGC
CTGTTCGAGCGTCATTTCAACCCTCAAGCACAGCTTGGTGTTGGGACTCGCGT
TAATTCGCGTTCCTCAAATTGATTGGCGGTCACGTCGAGCTTCCATAGCGTAG
TAGTAAAACCCTCGTTACTGGTAATCGTCGCGGCCACGCCGTTAAACCCCAA
CTTCTGAATGTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATA
TC

MN650247 Geotrichum candidum strain Akure_A2a

AACCAAAATCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTAA
TGATTTTTAATATTTGTGAATTTACTACAAACAAAAATTTAATTTTATAGTCA
AAAATAAAAAAAAAAATTAAAACTTTTAACAATGGATCTCTTGGTTCTCGTATC
GATGAAGAACGCAGCGAAACGCGATATTTCTTGTGAATTGCAGAAGTGAATC
ATCAGTTTTTCGAACGCACATTCGCACTTTCGGGGTATCCCCCAAAGTATACT
TTGTTTGAGCGTTGTTTTCTCTCTTGGAATTTGCATTTTGCTTTTTCTAAAAAA
TCGAATCAAATTTCGTTTTGAAACTTTCAATTTATCTCAACCTCAGATCAAGT
AGGATTACCCGCTGAACTTAAGCATATCAAAGGGGGGCC

MN650248 Fusarium oxysporum strain Akure_A2b

TCGTAACAAGGTCTCCGTTGGTGAACCAGCGGAGGGATCATTACCGAGTTTA
CAACTCCCAAACCCCTGTGAACATACCACTTGTTGCCTCGGCGGATCAGCCC
GCTCCCGGTAAAACGGGACGGCCCGCCAGAGGACCCCTAAACTCTGTTTCTA
TATGTAACTTCTGAGTAAAACCATAAATAAATCAAAACTTTCAACAACGGAT
CTCTTGGTTCTGGCATCGATGAAGAACGCAGCAAAATGCGATAAGTAATGTG
AATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCC
AGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCACAGC
TTGGTGTTGGGACTCGCGTTAATTCGCGTTCCTCAAATTGATTGGCGGTCACG
TCGAGCTTCCATAGCGTAGTAGTAAAACCCTCGTTACTGGTAATCGTCGCGGC
CACGCCGTTAAACCCCAACTTCTGAATGTTGACCTCGGATCAGGTAGGAATA
CCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA

Kwara

MN650249 Geotrichum candidum strain Kwara_A1a

AACAATCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTAATGA
TTTTTAATATTTGTGAATTTACTACAAACAAAAATTTAATTTTATAGTCAAAA
ATAAAAAAAAAAATTAAAACTTTTAACAATGGATCTCTTGGTTCTCGTATCGAT
GAAGAACGCAGCGAAACGCGATATTTCTTGTGAATTGCAGAAGTGAATCATC
ATTTTTCGAACGCACATTCGCACTTTCGGGGTATCCCCCAAAGTATACTTTGT
TTGCGTTGTTTTCTCTCTTGGAATTTGCATTTTGCTTTTTCTAAAAAATCGAAT
CAAATTTCGTTTTGAAACTTTCAATTTATCTCAACCTCAGATCAAGTAGGATT
ACCCGCTGAACTTAAGCATATCAAAGGCATCAC

MN650250 Geotrichum candidum strain Kwara_A1b

ATCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTAATGATTTTT
AATATTTGTGAATTTACTACAAACAAAAATTTAATTTTATAGTCAAAAATAAA
AAAAAAATTAAAACTTTTAACAATGGATCTCTTGGTTCTCGTATCGATGAAGA
ACGCAGCGAAACGCGATATTTCTTGTGAATTGCAGAAGTGAATCATCAGTTTT
TCGAACGCACATTCGCACTTTCGGGGTATCCCCCAAAGTATACTTTGTTTGAG
CGTTGTTTTCTCTCTTGGAATTTGCATTTTGCTTTTTCTAAAAAATCGAATCAA
ATTTCGTTTTGAAACTTTCAATTTATCTCAACCTCAGATCAAGTAGGATTACC
CGCTGAACTTAAGCATATCAAAGGG

MN650251 Fusarium oxysporum strain Kwara_A2a

TCTCCCTTGGTGAACCAGCGGAGGGATCATTACCGAGTTTACAACTCCCAAA
CCCCTGTGAACATACCACTTGTTGCCTCGGCGGATCAGCCCGCTCCCGGTAAA
ACGGGACGGCCCGCCAGAGGACCCCTAAACTCTGTTTCTATATGTAACTTCTG
AGTAAAACCATAAATAAATCAAAACTTTCAACAACGGATCTTTGGTTCTGGC
ATCGATGAAGAACGCAGCAAAATGCGATAAGTAATGTGAATTGCAGAATTCA
GTGAATATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGC
ATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCACAGCTTGGTGTTGGGACTC
GCGTTAATTCGCGTTCCTCAAATTGATTGGCGGTCACGTCGAGCTTCCATAGC
GTAGTAGTAAAACCCTCGTTACTGGTC

MN650252 Geotrichum candidum strain Kwara_A2b

GACAATCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTAATGA
TTTTTAATATTTGTGAATTTACTACAAACAAAAATTTAATTTTATAGTCAAAA
ATAAAAAAAAAAATTAAAACTTTTAACAATGGATCTCTTGGTTCTCGTATCGAT
GAAGAACGCAGCGAAACGCGATATTTCTTGTGAATTGCAGAAGTGAATCATC
ATTTTTCGAACGCACATTCGCACTTTCGGGGTATCCCCCAAAGTATACTTTGT
TTGCGTTGTTTTCTCTCTTGGAATTTGCATTTTGCTTTTTCTAAAAAATCGAAT
CAAATTTCGTTTTGAAACTTTCAATTTATCTCAACCTCAGATCAAGTAGGATT
ACCCGCTGAACTTAAGCATATCAAAGGCAT

Ibadan

MN650253 Fusarium oxysporum strain Ibadan_A1a

CTTAGAACCTGCGGAGGGATCATTACCGAGTTTACAACTCCCAAACCCCTGT
GAACATACCACTTGTTGCCTCGGCGGATCAGCCCGCTCCCGGTAAAACGGGA
CGGCCCGCCAGAGGACCCCTAAACTCTGTTTCTATATGTAACTTCCGAGTAAA
ACCATAAATAAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCACGAT
GAAGAACGCAGCAAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAAT
CATCGAATCTTTGACCGCACATTGCGCCCGCCAGTATTCTGGCGGGCATGCCT
GTTCGAGCGTCATTTCAACCCTCAAGCACAGCTTGGTGTTGGGACTCGCGTTA
ATTCGCGTTCCTCAAATTGATTGGCGGTCACGTCGAGCTTCCATAGCTAGTAG
TAAAACCCTCGTTACTGGTAATCGTCGCGGCCACGCCGTTAAACCCAACTTCT
GAATGTTGA

MN650254 Geotrichum candidum strain Ibadan_A1b

ACCCCCCAATCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTA
ATGATTTTTAATATTTGTGAATTTACTACAAACAAAAATTTAATTTTATAGTC
AAAAATAAAAAAAAAAATTAAAACTTTTAACAATGGATCTCTTGGTTCTCGTA
TCGATGAAGAACGCAGCGAAACGCGATATTTCTTGTGAATTGCAGAAGTGAA
TCATCATTTTTCGAACGCACATTCGCACTTTCGGGGTATCCCCCAAAGTATAC
TTTGTTTGCGTTGTTTTCTCTCTTGGAATTTGCATTTTGCTTTTTCTAAAAAATC
GAATCAAATTTCGTTTTGAAACTTTCAATTTATCTCAACCTCAGATCAAGTAG
GATTACCCGCTGAACTTAAGCATATCAAAGGCATG

MN650255 Geotrichum candidum strain Ibadan_A2a

CCATTGACAAACGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATT
AATGATTTTTAATATTTGTGAATTTACTACAAACAAAAATTTAATTTTATAGT
CAAAAATAAAAAAAAAAATTAAAACTTTTAACAATGGATCTCTTGGTTCTCGT
ATCGATGAAGAACGCAGCGAAACCCGATATTTCTTGTGAATTGCAGAAGTGA
ATCATCATTTTTCGAACGCACATTCGCACTTTCGGGGTATCCCCCAAAGTATA
CTTTGTTTGCGTTGTTTTCTCTCTTGGAATTTGCATTTTGCTTTTTCTAAAAAAT
CGAATCAAATTTCGTTTTGAAACTTTCAATTTATCTCAACCTCAGATCAAGTA
GGATTACCCGCTGAACTTAAGCATATCAAAGGCATCGT

MN650256 Geotrichum candidum strain Ibadan_A2b

ACCCCCAAAATCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATT
AATGATTTTTAATATTTGTGAATTTACTACAAACAAAAATTTAATTTTATAGT
CAAAAATAAAAAAAAAAATTAAAACTTTTAACAATGGATCTCTTGGTTCTCGT
ATCGATGAAGAACGCAGCGAAACGCGATATTTCTTGTGAATTGCAGAAGTGA
ATCATCATTTTTCGAACGCACATTCGCACTTTCGGGGTATCCCCCAAAGTATA
CTTTGTTTGCGTTGTTTTCTCTCTTGGAATTTGCATTTTGCTTTTTCTAAAAAAT
CGAATCAAATTTCGTTTTGAAACTTTTAATTTATCTCAACCTCAGATCAAGTA
GGATTACCCGCTGAACTTAAGCATATCAAAGGCATTTAAAA