CHARACTERISATION OF *FUSARIUM OXYSPORUM* SPECIES COMPLEX ASSOCIATED WITH FUSARIUM WILT OF SWEET POTATO IN SOUTH AFRICA

by

BRIGHTNESS ZAMA NKOSI

submitted in accordance with the requirements

for the degree of

MASTER OF SCIENCE

in the

COLLEGE OF AGRICULTURE AND ENVIRONMENTAL SCIENCES

DEPARTMENT OF LIFE AND CONSUMER SCIENCE

at the

UNIVERSITY OF SOUTH AFRICA

FLORIDA CAMPUS

SUPERVISOR: DR M CLOETE

CO-SUPERVISOR: DR A JACOBS-VENTER

JUNE 2020

DECLARATION

Name: **Brightness Zama Nkosi**

Student number: **56600194**

Degree: **MSC IN LIFE SCIENCES**

Exact wording of the title of the dissertation as appearing on the copies submitted for examination:

CHARACTERISATION OF *FUSARIUM OXYSPORUM* SPECIES COMPLEX

ASSOCIATED WITH *FUSARIUM* WILT OF SWEET POTATO IN SOUTH AFRICA

I declare that the above dissertation is my own work and that all the sources that I have used or quoted have been indicated and acknowledged by means of complete references.

_____ 05 June 2020

SIGNATURE DATE

Abstract

Sweet potato is a popular food security crop in South Africa and has a considerable commercial value. *Fusarium* wilt (FW), caused by the fungal pathogen *Fusarium oxysporum formae speciales (f. sp.) batatas,* has been reported worldwide and is widespread in sweet potato production areas in South Africa. Preliminary molecular identification of South African isolates from diseased sweet potato plants indicated that there are other *formae speciales* besides *F. oxysporum f. sp. batatas* associated with FW. The objectives of the study were to conduct a field survey and to characterise the isolates of the *Fusarium oxysporum* species complex (FOSC) using phylogenetic analyses, morphological characterisation and DNA barcoding. Phylogenetic analyses revealed two other *formae speciales*, namely *F. oxysporum f. sp. tuberosi* and *F. oxysporum f. sp. vanillae that* were associated with FW. This study has contributed in understanding and knowledge of FOSC associated with FW of sweet potato in South Africa.

KEY TERMS:

Beta-tubulin, DNA barcoding, *formae speciales*, *Ipomoea batatas*, ITS, morphological characterisation, phylogenetic analysis, RPB2, South Africa, TEF-1α

Acknowledgements

I would like to thank God for the blessings, protection, life and strength to complete my work.

I would like to express my sincere gratitude to my co-supervisor Dr. Adriaana Jacobs-Venter for her understanding, patience, encouragement and infinite support and motivation over the last few years. I would also like to thank you for teaching me the analysis and write-up direction.

To my supervisor Dr. Michele Cloete, I am grateful for your supervision, support, advices, constructive criticism and for creating a good working environment.

To Dr. Mariette Truter, I am forever grateful for the microbiology skills you taught me from day one, as well as technical skills and analysis. I will also like to thank you for introducing me to mycology and always assisting me with my lab work, organisation and theory.

To Dr. Sonja Venter and Dr. Dean Oelofse, I am thankful for the support, patience and advise provided.

My colleagues at Agricultural Research Council (ARC) Crop Protection, in particular the Plant Pathology group, thanks for creating a conducive environment and Ms Kate Phetla for assisting me with field trips.

Grace Kwinda, I am grateful for providing me with preserved fungal cultures, motivation and sharing a word of God with me.

Karabo Mashiloane, thank you for assisting me with the preservation of fungal cultures.

I would like to thank my siblings and my grandmother for encouragement throughout my study.

I would like to thank the University of South Africa, Department of Life and Consumer Sciences and the ARC for providing me with the services and finance necessary to successfully finish this research.

The research was partially funded by the National Research Foundation-Research and Technology Fund (NRF-RTF), grand no. 98653 and the National Research Foundation-Technology and Human Resources in Industry Partnership (NRF-THRIP), grand no. 96369.

Dedication

To my daughter Thandolwethu Nkosi, who changed my life for the better and taught me to love infinitely.

Table of contents

Abbreviations

Acl1	ATP (adenosine triphosphate) citrate lyase gene
AE	Elution buffer
AP1	Lysis buffer
ARS	Agricultural Research Service
ATP	Adenosine triphosphate
AW1	Wash buffer 1
AW2	Wash buffer 2
ARC-VOP	Agricultural Research Council-Vegetable and Ornamental Plants
β-tubulin	Beta-tubulin gene
CAL	Calmodulin gene
CBS	Centraalbureau voor Schimmelcultures
CI	Consistency index
CIP	International Potato Center
CLA	Carnation leaf agar
COI	Cytochrome c oxidase I gene
DAFF	Department of Agriculture, Forest and Fisheries
EDTA	Ethylene diamine tetraacetic acid
DNA	Deoxyribonucleic acid
dNTPs	Deoxy-nucleotide triphosphates
FAO	Food and Agricultural Organization
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
FDSC	*Fusarium dimerum species* complex
FCSC	*Fusarium chlamydosporum* species complex
$FeSO_4$.7H2O	Ferrous sulfate heptahydrate
FIESC	*Fusarium incarnatum-equiseti* species complex
FFSC	*Fusarium fujikuroi* species complex
FGSC	*Fusarium graminearum* species complex
FRSC	*Fusarium redolens* species complex
FOSC	*Fusarium oxysporum* species complex
FSAMSC	*Fusarium sambucinum* species complex
FSSC	*Fusarium solani* species complex

FW	*Fusarium* wilt
f. sp.	*Formae speciales*
G-C	Guanine-cytosine
GCPSR	Genealogical Concordance Phylogenetic Species Recognition
g/l	Gram per litre
GPS	Geographic positioning system
ICN	International Code of Nomenclature
ID	Identification
IGS	Intergenic spacer region
ITS	Internal transcribed spacer gene region
KCl	Potassium chloride
$KHPO_4$	Potassium dihydrogen phosphate
KNO_3	Potassium Nitrate
LSU	Large subunit of the Rdna operon
MAFFT	Multiple Alignment using Fast Fourier Transform
MEGA	Molecular Evolutionary Genetics Analysis software
$MgSO_4$.7H2O	Magnesium sulfate heptahydrate
Mm	Millimolar
ML	Maximum Likelihood
ml	Millilitre
MLST	Multilocus DNA Sequence Typing
MP	Maximum Parsimony
MtSSU	Mitochondrial small subunit gene
$NANO_3$	Sodium nitrate
nBLAST	Nucleotide Basic Local Alignment Search Tool
NCBI	National Centre for Biotechnology Information
NCF	National Collection of Fungi (South Africa)
NIR	Nitrate reductase
nm	Nanometre
NRF-RTF	National Research Foundation-Research and Technology Fund
NRF-THRIP	National Research Foundation-Technology and Human Resources in Industry Partnership

NRRL	Agricultural Research Service Culture Collection (United States of America
PAUP	Phylogenetic Analysis Using Parsimony software
PCNB	Pentachloronitrobenzene
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
PHO	Phosphate permase
PhyML	Phylogeny based on Maximum Likelihood
P3	Neutralising buffer
PDA	Potato Dextrose Agar
PHP	Plant Health and Protection
PS	Phylogenetic species
rDNA	Ribosomal deoxyribonucleic acid gene region
RI	Retention index
RNA	Ribonucleic acid
RNase A	Enzyme for removal of RNA
RPB1	RNA (Ribonucleic Acid) Polymerase II small subunit
RPB2	RNA (Ribonucleic Acid) Polymerase II large subunit
rpm	Revolutions per minute
s	Seconds
SADC	Southern African Development Community
SDS	Sudden death syndrome
SIX	Secreted in xylem
SNA	Synthetic nutrient agar
STs	Sequence types
SSU	Small subunit of the rDNA operon
sp.	Species
spp.	Several species
TAE	Tris base, acetic acid and EDTA
TBR	Tree bisection reconnection
TEF-1α	Translation elongation factor 1-alpha
TFC	Terminal *Fusarium* clade
U	Units

USA	United States of America
UV	Ultra violet
µl	Microlitre
µm	Micrometre
µM	Micromolar
WA	Water agar

List of figures

List of tables

List of appendices

List of buffer and media composition

TAE Buffer Composition

242 g tris, 57.1 ml acetic acid, 100 ml 0.5 M ethylene diamine tetraacetic acid (EDTA).

***Fusarium* selective media**

200 g/l glucose (dextrose), 5 g/l potassium hydrogen phosphate, 20 g/l sodium nitrate, 5 g/l magnesium sulphate heptahydrate, 10 g/l yeast extract, 1 ml of 1% ferrous sulphate heptahydrate, 200 g/l agar, and 10 g/l pentachloronitrobenzene.

Synthetic Nutrient Agar

10 g/l potassium hydrogen phosphate, 10 g/l potassium nitrate, 5 g/l magnesium sulphate heptahydrate, 5 g/l potassium chloride, 2 g/l sucrose, and 200 g/l agar.

Potato Dextrose Agar

4.0 g potato extract, 20 g dextrose, and 15 g agar.

Water agar

15 g agar.

CHAPTER 1

INTRODUCTION

Sweet potato (*Ipomoea batatas* L. Lam.) is the seventh main food crop produced globally and it is an essential source of beta-carotene carbohydrates, fibre, iron, potassium and proteins (Ssali *et al.*, 2019). Apart from being an important food security crop, orange-fleshed sweet potato cultivars are vital for addressing vitamin A shortage as these cultivars are rich sources of provitamin A (Laurie *et al.*, 2015b; Laurie *et al.*, 2017; Mulabisana *et al.*, 2019). Vitamin A shortage in South Africa is a nation wide communal health problem with 43.6% of children under five years lacking vitamin A (Laurie *et al.*, 2015a; Laurie *et al.*, 2018). Sweet potato is recognised for its contribution to food and nutrition security and has the potential for being processed into various products, such as biscuits, doughnuts, juice and chips that can be processed in household kitchens (Laurie *et al.*, 2015b; Laurie *et al.*, 2017).

Sweet potato is a vital resourceful food crop, which is adaptable to diverse soil and climatic conditions (Jaganathan *et al.*, 2019). Globally, China is one of the largest cultivators of sweet potato, producing about 80% of the world supply, while in Africa the two countries include Nigeria with 3.3% and Uganda with 2.7% (Adejuwon *et al.*, 2019). South Africa produced around 83 000 tons of sweet potato annually and the average price sold on the major fresh produce markets was R3 920 per ton (Department of Agriculture, Fisheries and Forestry (DAFF), 2019).

Sweet potato is an important food source to many rural families, mainly ensuring food security for the poor and as a cash crop in most parts of the world, including South Africa (Laurie, 2010; Laurie *et al.*, 2012; Laurie *et al.*, 2015b). These attributes formed the basis for the successful introduction of sweet potato in more than 166 countries worldwide, including 17 Sub-Saharan African countries (Vimala *et al.*, 2011). In South Africa, sweet potato is mostly cultivated in the Eastern Cape, Free State, Gauteng, Limpopo, Northern Cape and the Western Cape Province (Laurie *et al.*, 2018).

Sweet potato production can be severly limited by several fungal diseases (Hedge *et al.*, 2012) of which FW is one of the economically important fungal diseases worldwide (Clark, 2013). *Fusarium* wilt is caused by *F. oxysporum f. sp. batatas*

(Wollenw.) W.C. Snyder & H.N. Hansen (Clark, 2013; Gerlach and Nirenberg, 1982). In South Africa, FW on sweet potato was first reported by Thompson (2004). The disease can be locally damaging on semi-commercial and commercially produced sweet potato plants. *Fusarium* wilt prevalence was between 37.5%-66.7%, as found in 14 of the 31 fields sampled in Mpumalanga, Eastern Cape, Limpopo, and Western Cape provinces of South Africa during the 2006-2008 survey (Thompson *et al.*, 2011).

Fusarium is generally regarded as one of the most destructive plant pathogens (Yadav *et al.*, 2017). The genus contains more than 200 species (Al-Hatmi *et al.*, 2016). *Fusarium oxysporum* is a species complex that contains strains that can cause similar disease symptoms on different hosts. Vascular wilt diseases are usually caused by members of the FOSC and are recognised for their ability to cause disease in specific host plants (Summerell and Leslie, 2011). Members of the FOSC, both saprophytic and pathogenic, are commonly found worldwide in soil, infecting plants at the root and crown levels and spread steadily through the vascular system (Koyyappurath *et al*., 2016). The plant pathogenic strains infect their host by penetrating the roots, causing severe damage and yield losses on many economically important plant species (Fourie *et al*., 2011). Species boundaries and limits of genetic exchange within FOSC are not properly demarcated, due to the absence of a sexual state and the lack of morphological characteristics (Laurence *et al.*, 2014).

Phylogenetic analysis has become a vital method in the characterisation of *F. oxysporum* and can use one or multiple gene markers (O'Donnell *et al.*, 2004; Stewart *et al.*, 2006; Laurence *et al.*, 2012; Laurence *et al.*, 2014). Currently, *Fusarium* species are identified by a combination of phylogenetic and morphological characterisation approaches (Bushula, 2008; Jacobs *et al.*, 2010; Hafizi *et al.*, 2013; Jacobs *et al.*, 2013; Al-Hatmi *et al.*, 2016; Laurence *et al.*, 2016; Mojela, 2017; Jacobs *et al.*, 2018; Sandoval-Denis *et al.*, 2018). Morphological characterisation is laborious, time consuming and the results can lack clear morphological characteristics separating species, leading to species descriptions that are too wide and taxonomic classifications that poorly represent species diversity (Geiser *et al.*, 2004). Therefore, these approaches are now being gradually substituted by culture-independent phylogenetic analyses methods, which are much quicker, more precise and sensitive

(Saikia and Kadoo, 2010). Morphological characterisation of *F. oxysporum* is generally based on observable morphological characteristics like the shape and size of macroconidia and microconidia, presence or absence of chlamydospores and culture colour (Leslie and Summerell, 2006). Summerell *et al.* (2003), offered useful methodology on the *Fusarium* integrated approach followed to identify a *Fusarium* species.

The main objective of the study was to identify *F. oxysporum formae speciales* within the FOSC associated with FW of sweet potato in South Africa using phylogenetic analyses. The second objective was to characterise the representative isolates using morphological characterisation. A third objective was to generate DNA barcodes of the internal transcribed spacer (ITS) region for *F. oxysporum* isolates within the FOSC associated with FW of sweet potato in South Africa. The characterisation of *F. oxysporum* isolates within the FOSC were assessed by means of a combined approach using both phylogenetic analyses and morphological characterisation. Phylogenetic analyses were performed on strains obtained from diseased sweet potato plants using the translation elongation factor-1alpha (TEF-1α), ribonucleic acid (RNA) polymerase II second largest subunit (RPB2), beta-tubulin (β-tubulin) and ITS gene regions. In addition, phylogenetic analyses were performed on strains obtained from soil collected in diseased sweet potato fields using the TEF-1α gene region. Morphological characterisation was observed on representative *F. oxysporum* isolates within the FOSC and on other representative *Fusarium* isolates that were recovered from diseased sweet potato and soil. Morphological characterisation was done to confirm the molecular results and to provide an indication of morphological characteristics presented by *Fusarium* species obtained in this study. DNA barcoding through ITS gene region was performed to enhance the possible discrimination of *F. oxysporum* within the FOSC by determining the presence or absence of distinct single nucleotide polymorphisms.

Data generated in this study will provide information regarding the genetic diversity of strains in the FOSC associated with FW of sweet potato in South Africa. Furthermore, knowledge and understanding FOSC can assist sweet potato breeders with informed choices on which *F. oxysporum formae speciales* associated with FW

of sweet potato to use when screening for resistance against FW. Therefore, a follow up on this study can focus on implementing effective control measures of FW such as the use of resistant cultivars (Fravel *et al.*, 2003; Pietro *et al.*, 2003; Hedge *et al.*, 2012). Farmers can be assisted by being aware of FW disease by identifying the symptoms on sweet potato fields and educate them on better control measures.

CHAPTER 2

LITERATURE REVIEW

2.1 Origin and production of sweet potato

Ipomoea batatas belongs to the *Convolvulaceae* family (Purseglove, 1968). This family contains about 55 genera (Kreuze, 2002). The genus *Ipomoea* comprise of approximately 600 to 700 species (Veasey *et al.*, 2008). The series *batatas* is made up of 13 species closely related to cultivated sweet potato (Orjeda *et al.*, 1990; Nimmakayala *et al.*, 2011).

Ipomoea batatas and the wild *Ipomoea s*pecies originated from Central or South America in the area between the Yucatan Peninsula of Mexico and the Orinoco River in Venezuela, about 8000-6000 Before Christ (BC), where *Ipomoea trifida* and *Ipomoea triloba* might have crossed to produce the wild ancestor of *Ipomoea batatas* (Austin, 1988; Gichuki *et al.*, 2003). Although the crop originated in Central America, its wide adaptation has led to its successful introduction and production in more than 166 countries worldwide (Vimala *et al.*, 2011). The secondary centres of diversity are found in Guatemala, Colombia, Ecuador and Peru (Austin, 1983).

2.2 Importance of sweet potato

Sweet potato is extensively grown in tropical and sub-tropical regions of the world, characterised by sub-optimal levels of vital nutrients (Minemba *et al.*, 2019). The crop is a commodity in African countries, like Kenya, Uganda, Rwanda and Tanzania. Tanzania and Uganda are amongst the top ten main producers of sweet potato globally (Kagimbo *et al.*, 2018). Sweet potato is vital in addressing food insecurity in rural households and is grown by small-scale and commercial farmers in seven provinces of South Africa (DAFF, 2019; Mulabisana *et al.*, 2019). Sweet potato is an important crop to small-scale farmers as it is drought and heat tolerant, crowds out weeds and easy to grow (Nhlapho *et al.*, 2018). Sweet potato is used for human feeding as well as livestock feed (Ssali *et al.*, 2019).

2.3 *Fusarium* wilt disease

Fusarium is a genus of filamentous fungi that contains many agronomical significant plant pathogens, mycotoxin producers, and opportunistic animal and human pathogens. Collectively, *Fusarium* diseases include wilts, blights, rots, and cankers of many horticultural field, ornamental, and forest crops in both agricultural and natural ecosystems (Ma *et al.*, 2013). Fusaria also produce an array of toxic secondary metabolites known as mycotoxins, including trichothecenes and fumonisins that can contaminate agricultural products, making them unsuitable for use as food or feed (Ilgen *et al.*, 2008). *Fusarium* wilt is also known commonly as stem rot, or less commonly as vine wilt, blue stem, yellow blight, or yellows (Clark and Moyer, 1988). *Fusarium* wilt has been reported in most areas of the world where sweet potato is grown including, Australia, the United States of America (USA), China, India, Japan and Oceania (Brayford, 1992; Clark, 2013).

Fusarium oxysporum can cause FW in over 100 plant species, and these crops are mostly economically significant, including banana, bulb flowers, cucumber, cutting flowers, date palm, melon, tomatoes, potatoes, legumes, cloves, wheat, barley, oats, maize, sugarcane, cotton, and sweet potato (Burgess *et al.*, 1994; Gordon and Martyn, 1997; Lievens *et al.*, 2008). The pathogenic strains are highly host-specific and are divided into 150 *formae speciales* based on the host they infect (Gordon and Martyn, 1997; Fourie *et al*., 2009; Bertoldo *et al*., 2015).

The pathogen usually infects through vascular wounds that are caused by plant cuttings for planting. The optimal development of FW infection is at a temperature of about 30ºC, although it can still develop at lower temperatures. The infection can occur from the stems where the leaves have detached or through contaminated seeds, infested soil or compost (Di Primo *et al.*, 2001). Irrigation water, human movement and the use of farm tools previously used on an infected crop may also spread the disease. Plants can also be affected by the growth of the pathogen mycelia into the vascular tissues causing blockage of the xylem tissues and damaging the vascular system therefore stopping the flow of water from the roots to the upper plant, which results in plant wilting and eventually the plant dies from insufficient water

and nutrient uptake due to the loss of root tissues (Summerell and Leslie, 2011; Koyyappurath *et al*., 2016).

The symptoms on the older leaves display initial yellowing and wilting, resulting in stunting of vine growth and abscise as indicated in Figure 2.1. In cases of severe infection, the stem may turn tan to light brown, the pith within the stem may decay as indicated in Figure 2.2 and the plant may die (Clark, 2013). Discoloration of the vascular tissues of the stem is an early diagnostic symptom and may be accompanied by rupturing of the cortex of the stem. Therefore, if the stem is dissected longitudinally, the xylem shows a dark reddish brown discoloration. Commonly, the symptoms are one-sided, with only a portion of the vascular ring discolored. The surface of vine stems killed by FW often may have a pinkish exterior growth, consisting of numerous macroconidia and microconidia of the pathogen (Clark, 2013).

Figure 2.1: Symptoms of *Fusarium* wilt on sweet potato plant showing yellowing and browning of leaves.

Figure 2.2: Symptoms of *Fusarium* wilt of sweet potato plant showing browning of vascular tissues in a stem.

Generally, effective control prior to infection include the use of resistant cultivars (Fravel *et al.*, 2003; Pietro *et al.*, 2003; Hedge *et al.*, 2012), soil fumigation and disinfestation of plant material (Lievens *et al.*, 2008). Jackson *et al.* (2010) identified sweet potato cultivar Charleston Scarlet that was extremely resistant to insects and nematodes and moderately resistant to FW. Jackson *et al.* (2011) identified sweet potato cultivar Liberty that was extremely resistant to nematodes and a low level of resistance to FW. Lee *et al.* (2018) identified sweet potato cultivar Yeseumi that was resistant to FW.

Chemical control of FW is difficult because *F. oxysporum* produces chlamydospores that remain infectious in the soil for many years (Martyn, 1998). Methyl bromide was used as a chemical control as it was cost-effective but has subsequently been withdrawn from the market (Wechter *et al.*, 2012). Prochloraz and bromuconazole fungicides are recognised as the most effective fungicides against FW (Amini and Sidovich, 2010). Fungicides like benomyl, carbendazim, thiabendazole are effective in reducing FW incidence (Maitlo *et al.*, 2014; Maurya *et al.*, 2019).

Crop rotation with plants like broccoli, cabbage and squash (Wright *et al.*, 2017) that are a non-host of the pathogen can minimise the pathogens in soil (Hedge *et al.*, 2012). Disease management by biological control has been investigated by using a non-pathogenic *F. oxysporum* and *Pseudomonas fluorescens* strains against the

pathogen within the soil and the results showed that they were effective in suppressing the activities of *F. oxysporum f. sp. vanillae* W.L. Gordon 1965 (Tombe and Liew, 2010). Non-pathogenic *Fusarium* strains have been reported to control FW on various crops (Alabouvette *et al.*, 1998), such as cucumber (Mandeel and Baker, 1991), sweet potato (Ogawa *et al.*, 1996), watermelon (Robert *et al.*, 1996), carnation (Minuto *et al.*, 1995), pea (Benhamou and Grand, 2001) and tomato (Silva and Bettiol, 2005).

2.4 The taxonomy of *Fusarium oxysporum*

Link (1809) originally described this genus of ascomycetous fungi as *Fusisporium*. Fries (1821) authorised the name *Fusarium* and during the next 110 years many novel species were described in the genus. However, many of them were not based on host plant correlation. The genus *Fusarium* belongs to the kingdom Fungi, phylum Ascomycota, class Sordariomycetes, subclass Hypocreomycetidae, order Hypocreales and family Nectriaceae (Leslie and Summerell, 2006). Wollenweber and Reinking (1935) revised the *Fusarium* taxonomy to include 16 Sections, namely, *Eupionnotes*, *Macroconia*, *Spicarioides*, *Submicrocera*, *Pseudomicrocera*, *Arachnites*, *Sporotrichiella*, *Roseum*, *Arthrosporiella*, *Gibbosum*, *Discolor*, *Lateritium*, *Liseola*, *Elegans*, *Martiella*, and *Ventricosum*. These sections contained 65 species, 55 varieties, and 22 forms at that time. Snyder and Hansen (1940), reduced the numbers of species within the *Fusarium* to nine. The nine species were *F. oxysporum*, *F. solani* (Mart.) Appel and Wollenw. emend. W.C. Snyder and H.N. Hansen, *F. moniliforme*, *F. roseum*, *F. lateritium*, *F. tricinctum*, *F. nivale*, *F. rigidiuscula*, and *F. episphaeria* (Snyder and Hansen, 1940; Nelson *et al.*, 1983). Booth (1971) documented 44 species based on morphological characterisation including using microconidia as a distinguishing characteristic and the sexual reproductive structures for differentiating species. Moreover, Gerlach and Nirenberg (1982) documented more than 90 species. Nelson *et al.* (1983) recognised 41 species with additional 16 species that was inadequately documented. During middle 1980s, three species concepts (morphological, biological and phylogenetic) was used to define and differentiate *Fusarium* species therefore, species concepts have been explained in more details (Leslie *et al.*, 2001). Nirenberg and O'Donnell (1998) used morphological

and phylogenetic species concept to describe *Fusarium* species. Species complex specifies a clustering of species with shared morphological characteristics and phylogenetic markers and offer a system to aid in the demarcation of species (Summerell, 2019). Currently, there are 23 defined species complexes (O'Donnell *et al.*, 2013; Laurence *et al.*, 2015; Sandoval-Denis *et al.*, 2018).

Sanger sequence-based phylogenetic analyses placed the FOSC unambiguously in the *Gibberella* clade, close to the *F. fujikuroi* species complex (FFSC) (O'Donnell *et al.*, 1998a). Morphologically and phylogenetic related species include *F. nisikadoi* (Nirenberg and Aoki, 1997), *F. miscanthi* (Gams *et al.*, 1999), and *F. redolens* (Baayen *et al.*, 2001). Historically, molecular techniques have proven that these morphologically related fungi have multiple phylogenetic origins (Baayen *et al.*, 2000).

Formae speciales in *Fusarium* was accepted to offer a way of classifying pathogenic strains of the *F. oxysporum* that cause vascular wilt disease on a variety of host plants (Armstrong and Armstrong, 1981). Different *F. oxysporum formae speciales* are morphologically indistinguishable (Lievens *et al.*, 2008). However, with the advancement of molecular marker technology, *Fusarium* isolates within *formae speciales* can be distinguished by using DNA markers such as microsatellites (Bogale *et al.*, 2005). Some *formae speciales* are further divided into races based on virulence to a group of different cultivars within the same plant type for an example*, F. oxysporum f. sp. lycopersici* W.C. Snyder & H.N. Hansen has been separated into three races based on pathogenicity difference to tomato cultivars containing race specific, dominant resistance genes (Mes *et al.*, 1999). When *formae speciales* were tested with molecular makers, strains in the same *formae speciales* were often found to be related (Baayen *et al.*, 2000; Lievens *et al.*, 2008).

Some specific strains, indicated as 'radicis', do not spread through the vascular system towards the aerial parts of the plant, but are involved in the rotting of the root and crown tissues. These include *F. oxysporum f. sp. radicis-cucumerinum* Vakal. 1996, *F. oxysporum f. sp. radicis-lycopersici* Jarvis & Shoemaker 1978 and *F. oxysporum f. sp. radicis-vanillae* that can cause root and stem rot on different plants (Rowe, 1980; Menzies *et al.*, 1990; Koyyappurath *et al.*, 2016).

Fusarium oxysporum species complex have been investigated with multilocus DNA sequence data (Baayen *et al*., 2000; Skovgaard *et al*., 2001). O'Donnell *et al*. (1998b) determined that FOSC consisted of three major clades that potentially represented several morphologically cryptic species. These clades were designated as Clades 1-3. The inclusion of clinical isolates in 2004 identified a fourth clade within the FOSC (O'Donnell *et al.*, 2004).

'*The International Code of Nomenclature (ICN) for algae, fungi and plants* states that "…for a taxon of non-lichen-forming *Ascomycota* and *Basidiomycota*… [all names] compete for priority", regardless of their particular morph (Article 59.1, McNeill *et al*. 2012). This stipulates that only one scientific name be used for each species of fungi, contrary to previous editions of the *International Code of Botanical Nomenclature* and its predecessors. The preceding Code "…provided for separate names for mitotic asexual morphs (anamorphs) of certain pleomorphic fungi …" (Note 2. McNeill *et al*. 2006, McNeill *et al*. 2012; Norvell, 2011). As a result, the nomenclature of fungi must now conform to the principle of priority that applies to other groups of organisms governed by this Code. This change came into effect on 30 July 2011, when the decisions of the Nomenclature Section were ratified by the plenary session of the Melbourne Congress, although the application of some aspects was delayed until 1 January 2013' (Rossman *et al.*, 2013, p 42).

Fusarium is well characterised phylogenetically and can be considered as one large genus (Geiser *et al*., 2012). Even though views vary on how to limit the genus *Fusarium*, there was a universal agreement that the asexual morph-typified generic name *Fusarium* should be used instead of the sexual morph-typified *Gibberella* (Geiser *et al.*, 2013; Rossman *et al.*, 2013).

In the past, identification and naming of observed diversity in the FOSC was complicated by numerous subspecific taxonomy systems and the lack of living ex-type material to function as basic reference for phylogenetic interpretation. Therefore, to alleviate the taxonomic position of *F. oxysporum* and allow naming of the numerous *F. oxysporum* in the FOSC, an epitype was designated for *F.*

oxysporum. Lombard *et al.* (2019) resolved 15 taxa and described these as species using a multi-locus phylogenetic interpretation and a refined morphological differences with the newly recognised epitype of *F. oxysporum* as reference point. Naming *F. oxysporum formae speciales* are not subject to the ICN for algae, fungi, and plants (Article 59.1, McNeill *et al.*, 2012; Turland *et al.*, 2018), and therefore no diagnosis nor the deposit of type material in a recognised repository is required. This decision was made due to the difficulty in accepting *formae speciales* within the Code (Lombard *et al.*, 2019).

Lombard *et al.*, 2019 reported that the *forma specialis* name can be connected to the lineage specific chromosome as this chromosome was discovered in *F. oxysporum f. sp. lycopersici* by Ma *et al.* (2010). The question was raised whether *F. oxysporum* accurately denotes a species as these classification systems applied to *F. oxysporum* taxonomy and nomenclature is unclear (Lombard *et al.*, 2019). Therefore, to accurately place the taxonomic and nomenclatural position of *F. oxysporum* and allow naming of the unclear species in the FOSC, Lombard *et al.* (2019) collected *Fusarium* isolates from the type locality and the type substrate and used phylogenetic analysis and morphological characterisation resulting in the designation of an epitype for *F. oxysporum* from the collected *Fusarium* isolates. Therefore, epitypification of *F. oxysporum* resulted in the recognition of 21 phylogenetic species and 15 were provided with the names (Lombard *et al.*, 2019).

2.5 History of *Fusarium oxysporum f. sp. batatas*

Fusarium oxysporum formae speciales batatas was first observed by Halsted and called the pathogen *Nectria ipomoea* based on stem rot or wilt on sweet potato (Halsted, 1890). Harter and Field (1913) reported *Fusarium* as the true causal agent of stem rot on sweet potato, followed by Harter and Field (1914) identifying *F. batatatis* Wr. and *F. hyperoxysporum* Wr. as the two causal agent species of *Fusarium* causing stem rot on sweet potato. A monographic study of the sweet potato disease including the fungal pathogen using morphological characterisation, showed that the conidia of *F. batatatis* and *F. hyperoxysporum* were usually three septate, seldom four septate, while those of *N. ipomoea* were usually five septate and they

were much larger and different in shape (Harter and Weimer, 1929). Snyder and Hansen (1940) suggested that *F. batatatis* and *F. hyperoxysporum* should be named as *F. oxysporum* Sachlecht. *f. batatas* (Wr.) Snyder and Hansen.

2.6 Historical relationship of *Fusarium oxysporum f. sp. batatas* and *F. oxyporum f. sp. vanillae*

A study by Tucker (1927) explains that a fungal culture isolated from a vanilla plant was sent to C. D. Sherbakoff for the pathogen description. The isolate from vanilla was identified as closely related to *F. batatatis* Wollenw (Wollenweber, 1914), but the inoculations of sweet potato plants with the strain did not produce infection. In addition, the *F. batatatis* culture that was received from L. L. Harter also did not cause infection of vanilla. The two strains, *F. batatatis* and the strain from vanilla, resemble each other relatively closely morphologically but differed primarily in the type of sclerotia produced. The vanilla *Fusarium* isolate produced sclerotia much less abundantly than *F. batatatis*. Therefore, the vanilla fungus was considered to be *F. batatatis* var. *vanillae* (Tucker, 1927). The morphological characteristics of *F. batatatis* var. *vanillae* included macroconidia that were usually 3-septate, sometimes 1 to 2 septate and seldom 4 to 5 septate. The 3 septate macroconidia were 23-45 X 2.6-4 µm with average of 34.2 X 3.6 µm. Macroconidia were curved, pedicellate and hyaline. The apical cells were slightly tempered. Microconidia were hyaline and oval-elongate. Microconidia were 4.5 - 7 X 2.2 - 3.6 µm. Chlamydospores were present and were thick-walled, single or in short chains. Chlamydospores were 6.5 to 10 µm. Reddish purple sporodochia were produced on Potato Dextrose Agar (PDA) (Tucker, 1927).

Fusarium batatatis var. *vanillae* is non-pathogenic to sweet potato, pathogenic to vanilla and parasitic on roots of *Vanilla vanilla* (L.) Br. in Porto Rico (Tucker, 1927). In addition, *F. oxysporum f. sp. vanillae* has been reported as the causative agent of root and stem rot on *Vanilla planifolia* (Tucker, 1927) in production areas like Indonesia, Seychelles, India, Thailand, Tonga and China (Tombe and Liew, 2010). Therefore, *F. oxysporum f. sp. vanillae* and *F. oxysporum f. sp. batatas* should not be confused as they have a close genetic relationship. Pinaria *et al.* (2015) studied the

origin of *F. oxysporum f. sp. vanillae* in Indonesia using a multigene phylogenetic approach and the results suggested that the vanilla stem rot pathogen in Indonesia has a complex origin. Furthermore, a study by Koyyappurath *et al.* (2016) suggested that the causal agent of vanilla root and stem rot should be named *F. oxysporum f. sp. radicis-vanillae* instead of *F. oxysporum f. sp. vanilla*e and the results was based on the pathogenicity and histopathological data and because there was no progression of hyphae within the vascular tissues of either vanilla species tested, limiting the rot only to the emerging roots (Koyyappurath *et al.*, 2016).

2.7 Molecular techniques used for the identification of *Fusarium oxysporum*

In general, molecular methods are faster, more precise, sensitive and accurate than the traditional morphological approaches. Currently, morphological approaches and phylogenetic analyses techniques are in use to define species and to discover previously undescribed species. After a species is defined, DNA barcoding approaches can be used to identify species by the presence or absence of discrete nucleotide characters (Al-hatmi *et al*., 2016).

Genealogical Concordance Phylogenetic Species Recognition (GCPSR) approach was constructed on the knowledge that recombination within a lineage will generate a conflict amongst gene trees, with the change from conflict to congruence representing the species limit (Taylor *et al.*, 2000). Study by Laurence *et al.* (2014) identified two phylogenetic species (PS) within the FOSC based on the GCPSR using nine protein-coding loci namely TEF-1α, mitochondrial small subunit (mtSSU), RPB1, RPB2, nitrate reductase (*NIR*), phosphate permase (*PHO*), calmodulin (*CAL*) and the large subunit of the ATP citrate lyase (*acl1*). These molecular markers were selected on the basis of their capability to determine both deep and shallow nodes within the FOSC in previous studies (Laurence *et al.*, 2014). The GCPSR firstly identified seventeen independent evolutionary lineages, which were then collapsed into two PS (Laurence *et al.*, 2014). The PS 1 corresponded to the established Clade 1 and PS 2 corresponded to Clades 2, 3, and 4 (O'Donnell *et al.*, 1998b; O'Donnell *et al.*, 2004). The resulted three clades from O'Donnell *et al.* (1998b) was based on Maximum Parsimony (MP) of the combined TEF-1α and mtSSU rDNA sequence data.

Translation elongation factor-1 alpha has high phylogenetic usefulness because it is extremely informative at the species level in the genus *Fusarium*, non-orthologous duplicates of the TEF-1α gene have not been identified across the *Fusarium* genus and the universal primers have been generated that undertake the phylogenetic range of the genus (Geiser *et al.*, 2004). The generated primers were used for fungi to study the phylogenetic analyses of the FOSC (O'Donnell *et al.*, 1998b). Sequences of the TEF-1α and the mtSSU ribosomal RNA genes have been useful in differentiating *Fusarium* species (O'Donnell *et al.*, 1998b; Baayen *et al.*, 2000; O'Donnell *et al.*, 2004). The mtSSU gene region is highly conserved and is very effective at determining deeper nodes within species complexes of *Fusarium* (Laurence *et al.*, 2014).

Largest and second largest subunits of RNA polymerase II (RPB1 and RPB2) have been used previously to determine deep level *Fusarium* phylogeny (O'Donnell *et al.*, 2007; Grafenhan *et al.,* 2011; Laurence *et al.*, 2011). The RPB1 and RPB2 have been noted for their informativeness in analyses of diverse fungi, including *Fusarium* (O'Donnell *et al.*, 2010; O'Donnell *et al.*, 2013; Lombard *et al.*, 2015). The RPB2 provides good phylogenetic resolution of Ascomycota and has a modest rate of evolutionary change (Liu *et al.*, 1999). Maximum likelihood (ML), MP and Bayesian (B) analyses on RPB1 and RPB2 of 93 fusaria was conducted to gather the inintial inclusive and significantly supported phylogenetic analyses. Their analyses revealed that *Cylindrocarpon* formed a basal monophyletic sister to a terminal *Fusarium* clade (TFC) including 20 significally supported species complexes and nine monotypic lineages, which were recognised as *Fusarium* (O'Donnell *et al.*, 2013). In conclusion, the RPB1 and RPB2 phylogenetic analyses has provided the first strong genus-wide framework for evaluating whether the traditional morphology-based sectional grouping (Gerlach and Nirenberg, 1982) precisely reflects evolutionary relationships within *Fusarium* (O'Donnell *et al.*, 2013). The three molecular makers namely, *NIR*, *PHO* and *CAL* have been used for intra-*formae speciales* resolution in the FOSC (Jimenez-Gasco *et al.*, 2002; Skovgaard *et al.*, 2002; Kim *et al.*, 2005). In addition, the *CAL* gene showed to be consistent when it was used for the phylogenetic analyses of the *F. fujikuroi* related species and *Fusarium* related species (O'Donnell

et al., 2000). The *NIR* and *PHO* genes were used in the phylogenetic analyses of the FOSC (Laurence *et al.*, 2014).

The larger subunit of ATP citrate lyase marker has been used to determine genera closely related to *Fusarium* (Grafenhan *et al*., 2011). In addition, species determination is best made with the combined phylogeny of protein-coding genes such as TEF-1α, RPB2, β-tubulin (O'Donnell *et al*., 2012) and ATP citrate lyase (Grafenhan *et al*., 2011).

The β-tubulin gene provides a strong support for a fully resolved phylogeny of all biological and morphological species (O'Donnell and Cigelnik, 1997). Studies have demonstrated the phylogenetic utility of the β-tubulin (Schardl *et al.*, 1994; Tsai *et al.*, 1994) at the interspecific level in fungi. A phylogenetic diversity study using TEF-1α, Histone 3 and β-tubulin gene regions was done to distinguish between *Fusarium* spp. in sugar beet from different geographic locations of Egypt. All three genes were able to separate the recovered isolates to five *Fusarium* species namely *F. oxysporum, F. solani, F. proliferatum, F. equiseti* and *F. veticillioides* (Taha *et al.*, 2016). However, TEF-1α gene region revealed the highly resolution compared to β- tubulin gene region (Taha *et al.*, 2016).

The locus intergenic spacer (IGS) gene region was not included in Laurence *et al.* (2014) however, it is one of the significant makers in *F. oxysporum.* The IGS gene region divides the ribosomal DNA (rDNA) repeat units and is convenient to study the composition of genetic populations of *F. oxysporum* (Kawabe *et al.*, 2005). A study by Srinivasan *et al.* (2010) using a phylogenetic analysis based on IGS sequences of *F. oxysporum formae speciales* discovered a close association between genetic phylogeny and pathogenicity, furthermore, non pathogenic isolates differed genetically from pathogenic isolates. Phylogenetic analyses of TEF-1α, mtSSU and IGS gene regions have assisted to discover the genetic and evolutionary relaionships within and between *F. oxysporum formae speciales* (Lievens *et al.*, 2008). The ITS gene region is one of the most used molecular marker in fungi (Martin and Rygiewicz, 2005). The advantages of ITS includes having a huge number of reference sequences accessible in GenBank (Schoch *et al.*, 2012). Detection of the *formae*

speciales within the FOSC is usually done by testing the fungus for pathogenicity on several plant species, while races are determined by pathogenicity assays on different cultivars of a single plant species (Correll, 1991; Cai *et al*., 2003; Lievens *et al.*, 2008). Molecular identification of pathogenic strains is based on diagnostic characters that are directly connected to pathogenicity (Lievens and Thomma, 2005).

The effective molecular approach based on the websites that facilitates the identification of fusaria by comparison with databases that conducts nucleotide BLAST (nBLAST™) requests of the dedicated DNA sequences by means of the internet. *Fusarium* Multilocus DNA sequence typing (MLST) (http://www.cbs.knaw.nl/fusarium) (O'Donnell *et al.*, 2012) and *Fusarium*-ID (http://isolate.fusariumdb.org) (Geiser *et al.*, 2004) are the two websites that can be used for BLAST analyses of any *Fusarium* sequences. Multilocus DNA sequence typing indicates a significant approach for the characterisation of the genetic diversity of FOSC (O'Donnell *et al.*, 2009a). In addition, another website that can be used for BLAST analyses of *Fusarium* sequences is National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov) as it a GenBank that provides a wide variety of biomedical and genomic information for all species (Geiser *et al.*, 2004; O'Donnell *et al.*, 2012). According to Geiser *et al.* (2004), the GenBank has a potential for misidentified accessions concerning precise identification of sequences therefore, it was recommended that *Fusarium*-ID should be used as it contained voucher and accurate sequences that correspond to publicly available cultures that can be used for confirmation. However, *Fusarium*-ID can be used in combination with GenBank (Geiser *et al.*, 2004).

2.8 DNA barcoding of fungi

The concept of DNA barcoding is a worldwide fast identification of organisms at the species level and has a big impact on normalising identification of eukaryotes. Futhermore, Hebert *et al.* (2003) proposed the first marker as a barcode, the mitochondrial COI gene for the species identification in the animal kingdom. The COI region have been accepted for barcoding animals because of its generally conserved priming sites and third position nucleotides with a greater incidence of base

substitutions than the other mitochondrial genes. The easily, short amplified regions of DNA, based on firmly identified vouchers, resulted to a strong identification process (Riaz *et al*., 2011). The DNA target should be same among the entities of the same species but different between species with highly conserved priming sites, trustworthy DNA amplifications and sequencing, phylogenetically informative and short enough to have lower processing prices and allow amplification of degraded DNA (Valentini *et al.*, 2009). The perfect DNA target region is not available and more than one DNA barcode have been proposed. Min and Hickey (2007) assessed the effect of varying sequence length of DNA barcodes for the grouping of unknown specimens at the species level as well as for phylogenetic reconstruction in fungi. They found that decreasing the length of the barcode had an insightful effect on the correctness of resulting phylogenetic trees. The short barcode sequences (~600 bp) were appropriate for species identification but not for inferring accurate phylogenetic relationships among the fungi. It is possible that the standard DNA barcoding might accurately distinguish different *Fusarium* spp., however, longer barcodes would be essential to accurately identify different *formae speciales* and races of the FOSC. Internal transcribed spacer region (Figure 2.3) was selected for fungi for DNA barcoding (Kelly *et al.*, 2011; Schoch *et al.*, 2012). The RPB1 and RPB2 regions are promising for phylogeny and barcoding in *Fusarium* (O'Donnell *et al.*, 2013). The RPB2 gene (Figure 2.4) have been used to delineate *Fusarium* phylogenetic resolution (O'Donnell *et al.*, 2007; O'Donnell *et al.*, 2010; Laurence *et al.*, 2011; O'Donnell *et al.*, 2012; O'Donnell *et al.*, 2013;) and is also used to define deep level of fungal phylogenies (Lutzoni *et al.*, 2004; James *et al.*, 2006). Phylogenetic resolution within *Fusarium* species complexes is determined by using TEF-1α gene region (Figure 2.5) (Geiser *et al.*, 2004). The β-tubulin gene region (Figure 2.6) has proven to be phylogenetic useful at the interspecific level in fungi (Schardl *et al.*, 1994; Tsai *et al.*, 1994).

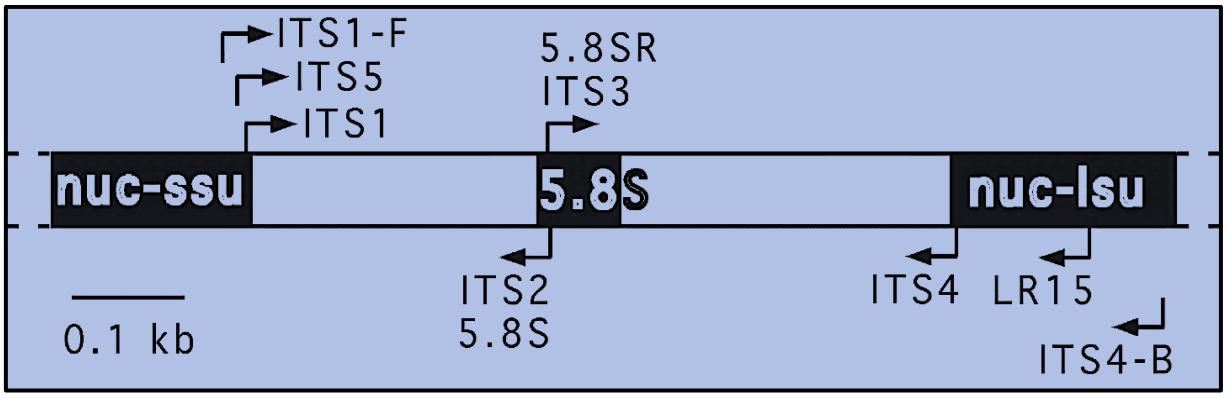


Figure 2.3: Map of the ITS gene region. Coding regions and ribosomal RNA regions are shown as boxes/ractangles, and introns and spacers as lines. Labelled arrows indicate the primers used for polymerase chain reaction (PCR) amplification and sequencing. The ITS1 and ITS4 amplify the highly variable ITS1 and ITS2 sequences surrounding the 5.8S-coding sequence. This gene region is situated between the SSU and the LSU of the ribosomal operon. Reviewed from White *et al.* (1990).

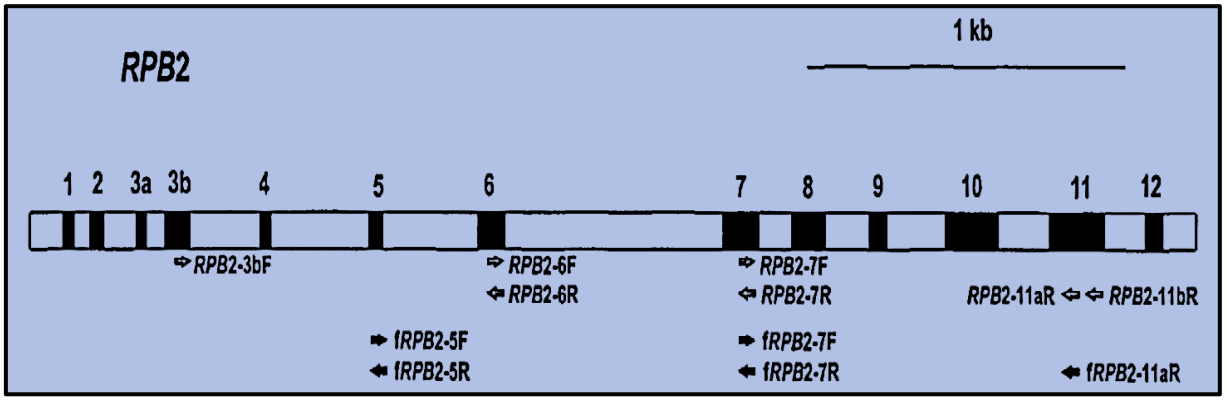


Figure 2.4: Map of the RPB2 gene region. The black boxes and lines indicate RPB2 exons, introns are indicated by light blue boxes/rectangles. Labelled arrows indicate the primers used for PCR amplification and sequencing. The RPB2 encodes the second largest subunit of RNA polymerase II. This gene is the central component of the basal RNA polymerase II transcription machinery. Reviewed from Liu *et al.* (1999).

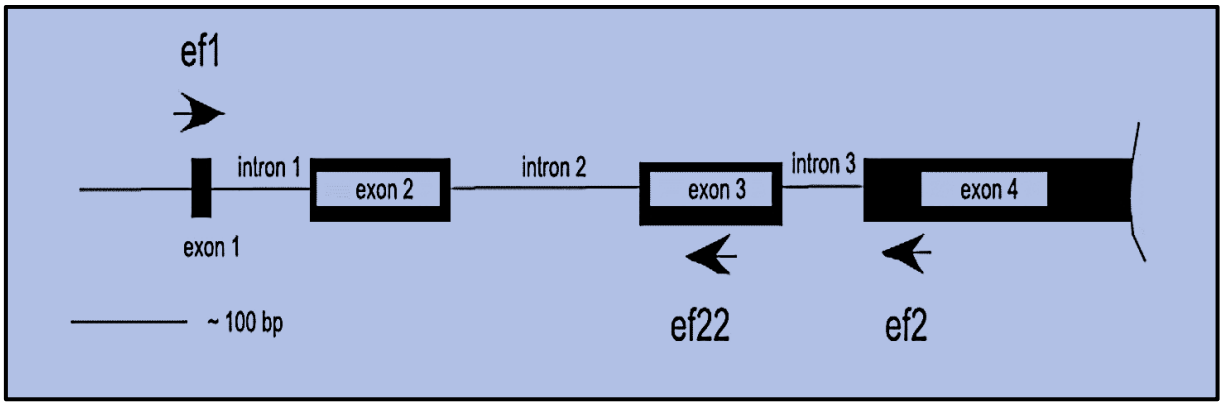


Figure 2.5: Map of the TEF-1α gene region. The rectangulars with thick black borders indicate TEF-1α exons, introns are un-rectangular and unbordered. Labelled arrows indicate the primers used for PCR amplification and sequencing. The TEF-1α gene encodes an essential part of the protein translation machinery. Reviewed from Geiser *et al.* (2004).

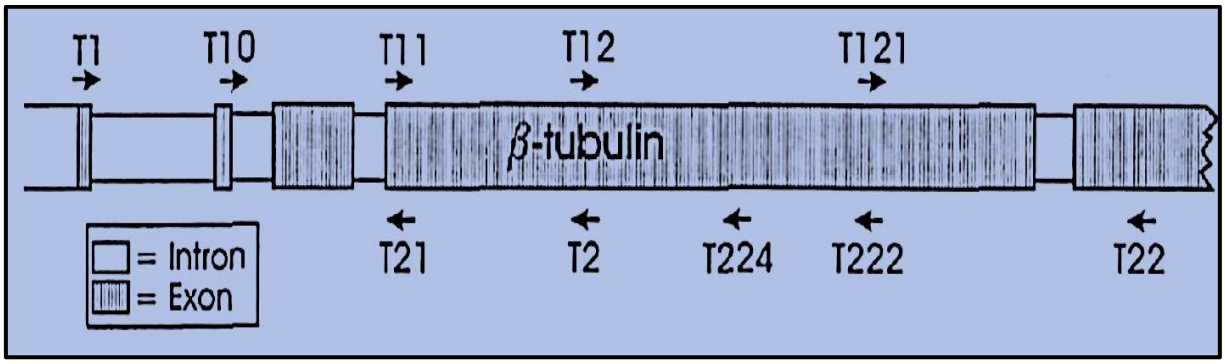


Figure 2.6: Map of the β-tubulin gene region. Stippled boxes indicate β-tubulin exons, introns are unstippled. Labelled arrows indicate the primers used for PCR amplification and sequencing. Reviewed from O'Donnell and Cigelnik (1997).

2.9 Morphological characterisation of *Fusarium oxysporum*

Morphological identification of *Fusarium* is largely based on characteristics such as the shape and size, macroconidia and microconidia, the presence or absence of chlamydosphores as well as colony colour and appearance on specific culture media and growth rates (Leslie and Summerell, 2006; Moretti, 2009; Laurence *et al*., 2016). *Fusarium* had an unclear and unbalanced taxonomic past and was under-estimated by all previous morphological treatments, therefore the identification of *Fusarium* species have been challenging, displaying high level of variation within species

differentiation (Aoki and O'Donnell, 1999; O'Donnell, 2000). The absence of clear morphological characteristics separating species, results in species description that are too wide. In addition, differences and mutation in culture, unites to generate taxonomic classifications that poorly reveal species diversity (Geiser *et al.*, 2004).

Fusarium oxysporum has absence sexual state (Laurence *et al.*, 2014). The *F. oxysporum* morphological characterisation is based on macroconidia that are falcate to straight with usually three septates, with a foot shaped basal cell and a tapered apical cell. The microconidia are oval, elliptical with zero septate and are produced in false heads on short monophialides. The chlamydospores are present with a smooth or rough wall appearance produced singly or in pairs (Leslie and Summerell, 2006; Fourie *et al.*, 2011) as indicated in Figure 2.7. Chlamydospores are formed by the modification of the hyphal and conidial cells through the condensation of their contents (Ohara and Tsuge, 2004). Both macroconidia and microconidia function as secondary inoculum in infecting plants, whereas the chlamydospores are for long term-survival of the organism (Pinaria, 2009).

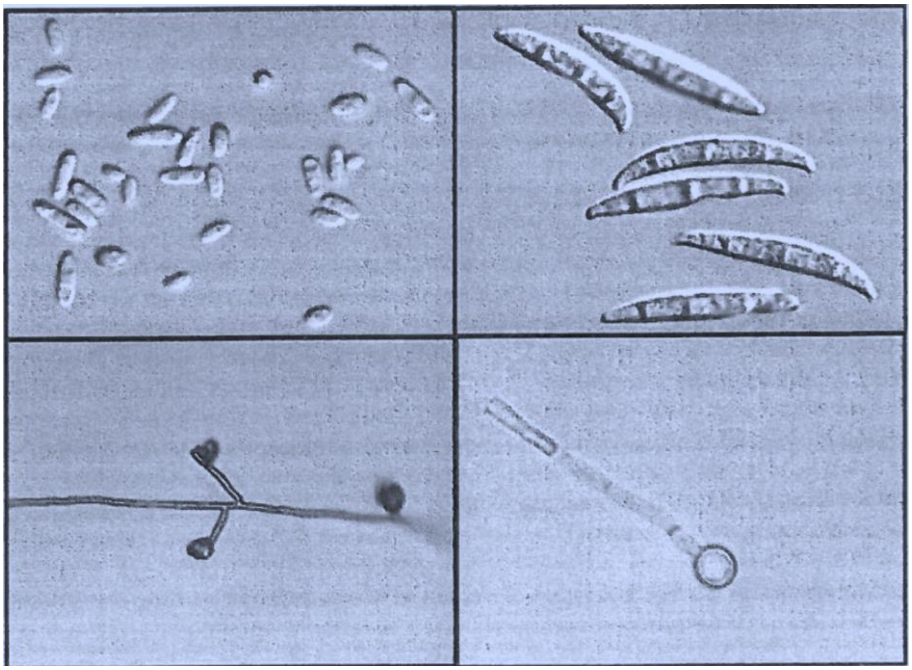


Figure 2.7: Morphological characteristics of *Fusarium oxysporum*. From left to right on top, microconidia and macroconidia. From left to right at the bottom, microconidia produced in false heads on short monophialides and chlamydospore. Reviewed from Fourie *et al.* (2011).

CHAPTER 3
MATERIALS AND METHODS

3.1 Sampling

Stem samples were obtained from naturally infected, symptomatic sweet potato plants from two farms in Eastern Cape Province, one farm in Gauteng Province, eight farms in Limpopo Province, five farms in Mpumalanga Province, two farms in Northen Cape Province and four farms in Western Cape Province during the 2008-2016 production seasons (Figure 3.1). In addition, soil was also collected from sweet potato farms in Gauteng, Limpopo and Mpumalanga provinces of South Africa during the 2015-2016 production seasons. Global Positioning System (GPS) coordinates indicated in Appendix A show the locations of the farms sampled.

Samples were collected during the warmest time of the growing season from November to January because FW is a disease that is favoured by various stress conditions, such as the lack of moisture or high temperatures (30˚C or higher) (Thompson *et al.*, 2011). Sampling from commercial growers consisted of a stratified random sampling method (Snedecor and Cochran, 1980). For resource-poor farmers, a convenience sampling approach (non-probability sampling method) was used (Snedecor and Cochran, 1980).

Standardised transect approach was used to collect the soil samples by collecting the soil in a 15 metres north-south transect crossed by a 15 metres east-west transect (Laurence *et al.*, 2012). Sampling depth was 10-15 cm and soil samples were taken with a core sampler and a small shovel at one metre intermission and then combined together. Each soil sample consisted of two bags per sampling site.

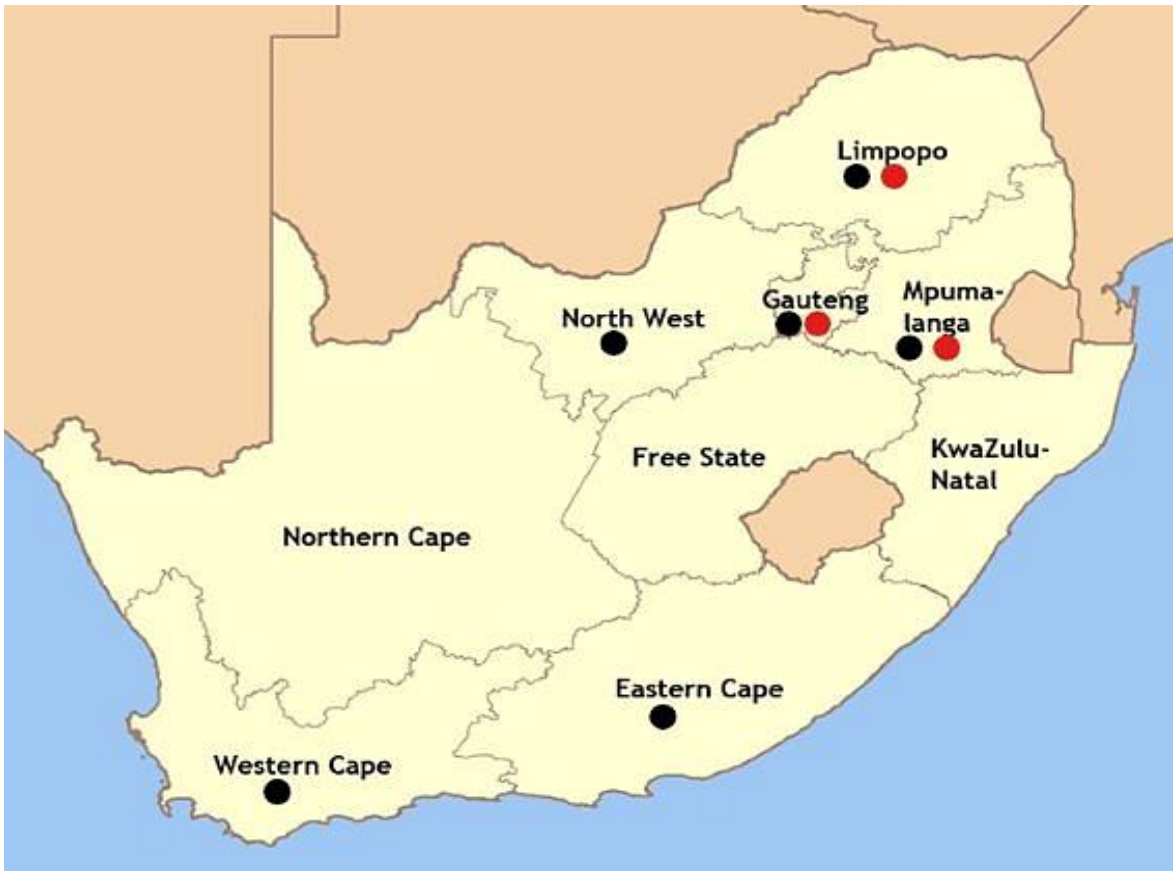


Figure 3.1: Sampling locations of sweet potato plant material (black circles) and soil samples (red circles) from the main sweet potato production areas of South Africa.

3.2 Isolation of fungal isolates from plant material

The lower stem (5 cm long) of selected symptomatic sweet potato plants (Figure 3.2) were surface disinfected for 5 minutes in 0.1% sodium hypochlorite, washed twice in sterile distilled water and air-dried on paper towel in a laminar flow bench. Each stem was split open aseptically, four separate isolations were made along the stem piece length. Pieces were plated onto PDA (Merck, South Africa) containing 0.4% streptomycin sulphate and onto *Fusarium* selective medium (Burgess *et al.*, 1994) and incubated under a 12-hour diurnal light cycle (mixed fluorescent/near Ultraviolet (UV) lighting) at 25°C for five days. Selected colonies were replated onto ¼-strength PDA and incubated at 25°C for five days. A pure culture was obtained from a single conidium to make sure that the culture represented a single genetic entity (Summerell *et al.*, 2003). Single spore cultures were obtained by adding 1 or 2 ml (depending on the culture growth) of sterile water onto the fungal culture and dislodging the spores.

The spore suspension was pipetted onto water agar (WA) and spread with a sterile glass rod. The WA plates were incubated upside down at an angle for 16-24 hours at 25°C. A stereo microscope was used to observe the single spores. A single germinating spore was picked with a sterile sharp needle and transferred onto ¼-strength PDA plates. Per culture, four single spores were transferred onto four separate ¼-strength PDA plates. After 24 hours, each culture plate was observed with a stereo microscope to confirm that the strain was growing from a single spore. The pure cultures were incubated under a 12-hour diurnal light cycle (mixed fluorescent/near UV lighting) at 25°C for seven days and one representative plate was used for preservation and further work.

Figure 3.2: A representative sweet potato plant sample showing browning of vascular tissues in a stem from which isolations were made.

3.3 Isolation of fungal isolates from soil samples

The soil samples were obtained to verify the variety of species. The soil sample were thoroughly mixed within the bag and 5 g of soil was weighed and placed into sterile 15 ml falcon tubes, using three technical repetitions per sample. The weighed soil was sieved through a 450 µm sieve resulting in 6 fragments using empty 90 mm petri dishes i.e. three macro-soil (soil particles larger than 450 µm, including plant debris) and three micro-soil (soil particles smaller than 450 µm excluding plant debris). The soil samples were sprinkled directly onto onto *Fusarium* selective medium (Burgess

et al., 1994). The *Fusarium* selective media culture plates were incubated for four days under light at 25°C. Selected fungal colonies were aseptically transferred onto ¼-strength PDA agar plates and incubated at 25°C for five days. Single spore cultures were prepared from the selected colonies as described previously.

3.4 Preservation methods

A research collection from this study was maintained by preserving fungal isolates as agar plugs under sterile water in 15 ml sterile bottles and as glycerol suspensions stored at ultralow temperatures at Agricultural Research Council-Vegetable and Ornamental Plants (ARC-VOP), Roodeplaat. All the fungal isolates from this study were also deposited in the National Collection of Fungi (NCF), ARC-Plant Health and Protection (PHP), Roodeplaat and are represented by PPRI numbers (Appendix A). For preservation of cultures using the sterile water preservation method (Summerell *et al.*, 2003), 7 ml of water was autoclaved in 15 ml McCartney glass bottles. Agar plugs (10 per bottle) from a pure, actively growing culture were aseptically transferred into the water bottles and stored at 4ºC. For preservation of cultures using the glycerol suspension method (Leslie and Summerell, 2006), 6 ml of 15% sterile glycerol was pipetted into the fungal culture plate and mixed with a pipette to disloged the spores and mycelium. The glycerol suspension was pipetted into the cryovials. Three cryovials tubes were used per isolate. The cryovials were placed into Mr Frosties® container and then stored in an ultralow freezer at -80ºC for 4 hours. After 4 hours, the cryovials were removed and, placed in polypropylene storage boxes and stored in an ultralow freezer at -80ºC (Appendix A).

3.5 Molecular characterisation

3.5.1 DNA extraction

Fungal isolates isolated from the symptomatic sweet potato stems and soil from sweet potato fields, were grown on PDA (Merck, South Africa) at 25°C for 7 days under a 12-hour diurnal light cycle (mixed fluorescent/near UV lighting). The DNA was extracted from the single spored fungal cultures using the DNeasy® Plant Mini Kit (Qiagen, Hilden, Germany) according to manufacturer's recommondations for

fungal samples. The protocol for DNA extraction included the following steps: Mycelium of approximately 100 mg scraped from the fungal culture was placed with a pinch of sterile sand in a sterile 1.5 ml microcentrifuge tube. Buffer AP1 of 400 µl and RNase A of 4 µl were added and the mycelium was disrupted with a sterile microcentrifuge tube grinding stick. The mixture was then vortexed virgorously and incubated for ten minutes at 65°C. The mixture in a microcentrifuge tube was inverted two to three time during this period. 130 µl of Buffer P3 was added to the lysate and the contents were mixed by vortexing and incubated on ice for five minutes. After the incubation, the lysate was centrifuged for five minutes at 20 000 x g (14 000 rpm). The lysate was then pipetted into the QIAshredder Mini spin lilac column, placed in a 2 ml collection tube, and centrifuged for two minutes at 20 000g x g (14 000 rpm). The flow-through portion was transferred into a new 1.5 ml microcentrifuge tube without disturbing the pellet. 675 µl of Buffer AW1 was added to the microcentrifuge tube and the content was mixed by pipetting. Followed by 650 µl of the the mixture of being pipetted into a DNeasy Mini spin column, placed in a 2 ml collection tube, and centrifuged for one minute at 6000 x g (8000 rpm). The flow-through was discarded and the remaining sample was pipetted into a DNeasy Mini spin column placed in a 2 ml collection tube and centrifuged for one minute at 6000 x g (8000 rpm) again. The DNeasy Mini Spin column was placed into a new 2 ml collection tube and a Buffer AW2 of 500 µl was added and centrifuged for one minute at 6000 x g (8000 rpm). The flow-through was discarded, then another Buffer AW2 of 500 µl was added and centrifuged for two minutes at 20 000 x g (14 000 rpm). The DNeasy Mini Spin column was transferred to a new 1.5 ml microcentrifuge tube and then Buffer AE of 50 µl was added and incubated at 25°C for five minutes. After incubation, the DNeasy Mini Spin column and the microcentrifuge tube were centrifuged for one minute at at 6000 x g (8000 rpm). The step was repeated by adding another 50 µl of Buffer AE to complete the elution step. The DNA was eluted in 100 µl of Buffer AE and stored at -40°C. The quality and concentration of genomic DNA was determined by using 1% agarose gel electrophoresis. The 1 kb (Plus) DNA Gene Ruler Ladder (Thermofisher Scientific) was used to determine the size and integrity of the DNA extracted. Loading dye (Sigma Aldrich) (2 µl) was mixed with the PCR amplicons and subjected to gel electrophoresis containing 1X Tris-acetate EDTA (TAE) buffer. Gels were stained with ethidium bromide (0.5 µg/ml) and bands were visualized by UV light. The gel

images were recorded by Cell Biosciences Alpha Innotech AlphaImager HP gel documentation system according to the manufacturer's recommendations.

3.5.2 Polymerase chain reaction

Portions of the TEF-1α, RPB2, β-tubulin and ITS genes were amplified in a 25 µl reaction volumes. Every reaction tube contained 2.5 µl of the 10X PCR buffer, 0.5 µl of 10 mM deoxynucleotide triphosphates (dNTPs) (Thermo Fisher Scientific, South Africa), 0.5 µl each of 10 µM forward oligonucleotide and reverse oligonucleotide (Table 3.1), and 0.2 µl of *Taq* polymerase (2.5 U/µl) (DreamTaq, Thermo Fisher Scientific, South Africa). Two contiguous regions of the RPB2 loci were amplified with the PCR primers 5F and 7CR and primers 7CF and 11AR in separate reactions. The PCR was executed in a thermal cycler under the following cycling steps: initial denaturation at 95°C for four minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing for 30 seconds and elongation at 72°C for 60 seconds and a final extension at 72°C for seven minutes. The specific annealing temperatures for each primer pair are indicated in Table 3.1. All reactions were conducted with an ABI thermocycler, Life Technologies, South Africa. PCR products were electrophoresed in a 1% agarose gel with 1X TAE buffer. Gels were stained with ethidium bromide (0.5 µg/ml) and UV light visualized bands. The gel images were recorded by Cell Biosciences Alpha Innotech AlphaImager HP gel documentation system according to the manufacturer's recommendations. The amplified amplicons were sent to Inqaba Biotec™ for Sanger DNA sequencing.

3.5.3 Sanger DNA sequencing

The PCR amplicons were purified and sequenced at Inqaba Biotec™. Sanger DNA sequences were determined from PCR amplicons using the ABI PRISM ™ Dye Terminator Cycle Sequencing Ready Reaction Kit. The raw DNA sequence data obtained from Inqaba Biotec™ were manually edited via base calling and trimming of ambiguous regions using Molecular Evolutionary Genetics Analysis (MEGA) version 6.0 software (Tamura *et al.*, 2013). The consensus sequences were generated on MEGA version 6.0 software by combining the forward sequence with the reverse sequence to form one clear sequence. The consensus sequences were compared with those sequences on the *Fusarium* MLST database

(http://www.cbs.knaw.nl/*Fusarium*) (O'Donnell *et al.*, 2012) and from *Fusarium*-ID database (http://www.fusarium.cbio.psu.edu) (Geiser *et al.*, 2004). The highest percentage similarity hit was noted.

Table 3.1: Primer sequences that were used in this study

Gene Region	Annealing Temperature (ºC)	Primer pair	Sequence in 5' to3' order	Reference
TEF1-α	52	EF1	ATGGGTAAGGARGACAAGAC	O'Donnell, 1998a
		EF2	GGARGTACCAGTSATCATGTT	
RPB2	55	7CF	ATGGGYAARCAAGCYATGGG	O'Donnell *et al.*, 2010
		11AR	GCRTGGATCTTRTCRTCSACC	
RPB2	50	5F	GAYGAYMGWGATCAYTTYGG	O'Donnell *et al.*, 2010
		7CR	CCCATRGCTTGYTTRCCCAT	
β-tubulin	52	T1	AACATGCGTGAGATTGTAAGT	O'Donnell and Cigelnik, 1997
		T22	TCTGGATGTTGTTGGGAATCC	
ITS	53	ITS1	TCCGTAGGTGAACCTGCGG	White *et al.*, 1990
		ITS4	TCCTCCGCTTATTG TATGC	

3.5.4 Phylogenetic analyses

DNA sequences were aligned using Multiple Alignment Fast Fourier Transform (MAFFT) (Katoh *et al.*, 2002) by inserting gaps. Gaps were treated as missing data in all the phylogenetic analyses. Phylogenetic analyses of FOSC datasets (sweet potato TEF-1α, soil TEF-1α, sweet potato RPB2 (5F and 7CR), sweet potato RPB2 (7CF and 11AR) and sweet potato β-tubulin) was performed based on MP and ML, however, sweet potato ITS phylogenetic analysis was performed based on MP. Maximum Parsimony was performed using Phylogenetic Analysis Using Paup (PAUP) 4.0* software (Swofford, 2002). Heuristic searches were done with random addition of sequences (100 replicates). The tree bisection reconnection (TBR) branch swapping was used to infer MP. The consistency Index (CI) and retention Index (RI) were calculated to demonstrate the amount of homoplasy present in the data set and the tree support. Bootstrap analyses was performed to determine branching point confidence intervals (1000 replicates) for the most parsimonious trees generated for the TEF-1α, RPB2, β-tubulin and ITS data sets. Maximum Liklihood was performed using an online version of PhyML analyses (http://www.atgc-montpellier.fr/phyml/) (Guindon *et al.*, 2010). The best models defined by PhyML for the diseased sweet potato material TEF-1α, soil TEF-1α, RPB2 (5F and 7CR), RPB2 (7CF and 11AR)

and β-tubulin datasets were GTR+G+I, HKY85 +I, GTR +G, TN93 +G and GTR +G, respectively. Phylogenetic trees were rooted using an outgroup for all datasets. The phylogenetic trees were rooted with *Fusaruim* sp. RBG5443 for the TEF-1α and RPB2 datasets. The phylogenetic trees for the β-tubulin and ITS datasets were rooted with *F. graminearum* as monophyletic sister outgroup to the rest of the taxa. The ITS DNA barcode library of the *F. oxysporum* was matched with the reference barcode sequences. MEGA version 6.0 software was used to view the ITS sequences and single nucleotide polymorphisms were noted. A phylogeny ITS tree was constructed via MP analysis using PAUP 4.0* software (Swofford, 2002). A phylogeny TEF-1α, RPB2 and β-tubulin trees were constructed with MEGA version 6.0 software (Tamura *et al.*, 2013) and ITS tree with TreeView (Win32) version 1.6.6 (Page, 2001). The reference sequences were obtained from the highest percentage similarities from *Fusarium* MLST database, *Fusarium*-ID database and from Laurence *et al.* (2014) as indicated in Table 3.2. The species name, isolation host and country of origin were confirmed using the Agricultural Research Service (ARS) culture collection (https://nrrl.ncaur.usda.gov/) website (O'Donnell *et al.*, 2009a). The reference DNA sequences were combined with the *F. oxysporum* isolates obtained from this study to generate the phylogeny of the FOSC in this study.

The *Fusarium* MLST database (http://www.cbs.knaw.nl/*Fusarium*) and the *Fusarium*-ID database (http://isolate.fusariumdb.org) were established to aid the identification of *Fusarium* strains by conducting nBLAST™ queries of the obtained DNA sequences against the verified reference sequences. If the percentage similarity of a single sequence query is at or below 99.4%, a multilocus GCPSR based analysis is recommended to compare more than one gene genealogy (Taylor *et al.*, 2000). If the percentage similarity of a single sequence query is between 99.5% to 99.9%, the context of what sequences are present within the *Fusarium* MLST and *Fusarium*-ID databases should be interpreted thoroughly (http://www.cbs.knaw.nl/*Fusarium*) (O'Donnell *et al.*, 2010; O'Donnell *et al.*, 2012), therefore both of these approaches were used in this study.

Table 3.2: Reference strains within the FOSC and outgroup *Fusarium* sp. and *F. graminearum* included in this study.

Isolate	*Fusarium species*	Isolation host	Country of origin	NCBI GenBank accession			
				TEF-1α	RPB2	β-tubulin	ITS
AUST_744 RBG5443	*Fusarium* sp.	Soi	Australia	KJ397074	KJ397254	N/A	N/A
AUST_590 RBG5697	*F. oxysporum*	Soil	Australia	KJ397064	KJ397244	N/A	N/A
AUST_122 RBG5722	*F. oxysporum*	Soil	Australia	KJ397043	KJ397223	N/A	N/A
AUST_82 RBG5765	*F. oxysporum*	Soil	Australia	KJ397075	KJ397255	N/A	N/A
AUST_103 RBG5768	*F. oxysporum*	Soil	Australia	KJ397040	KJ397220	N/A	N/A
AUST_114 RBG5769	*F. oxysporum*	Soil	Australia	KJ397041	KJ397221	N/A	N/A
AUST_120 RBG5771	*F. oxysporum*	Soil	Australia	KJ397042	KJ397222	N/A	N/A
AUST_142 RBG5774	*F. oxysporum*	Soil	Australia	KJ397044	KJ397224	N/A	N/A
AUST_171 RBG5776	*F. oxysporum*	Soil	Australia	KJ397045	KJ397225	N/A	N/A
AUST_172 RBG5777	*F. oxysporum*	Soil	Australia	KJ397046	KJ397226	N/A	N/A
AUST_181 RBG5778	*F. oxysporum*	Soil	Australia	KJ397047	KJ397227	N/A	N/A
AUST_186 RBG5779	*F. oxysporum*	Soil	Australia	KJ397048	KJ397228	N/A	N/A
AUST_214 RBG5780	*F. oxysporum*	Soil	Australia	KJ397049	KJ397229	N/A	N/A
AUST_217 RBG5781	*F. oxysporum*	Soil	Australia	KJ397050	KJ397230	N/A	N/A
AUST_226 RBG5782	*F. oxysporum*	Soil	Australia	KJ397051	KJ397231	N/A	N/A
AUST_242 RBG5783	*F. oxysporum*	Soil	Australia	KJ397052	KJ397232	N/A	N/A
AUST_293 RBG5784	*F. oxysporum*	Soil	Australia	KJ397053	KJ397233	N/A	N/A
AUST_359 RBG5786	*F. oxysporum*	Soil	Australia	KJ397054	KJ397234	N/A	N/A
AUST_387 RBG5787	*F. oxysporum*	Soil	Australia	KJ397055	KJ397235	N/A	N/A
AUST_449 RBG5789	*F. oxysporum*	Soil	Australia	KJ397056	KJ397236	N/A	N/A
AUST_484 RBG5791	*F. oxysporum*	Soil	Australia	KJ397057	KJ397237	N/A	N/A
AUST_502 RBG5792	*F. oxysporum*	Soil	Australia	KJ397058	KJ397238	N/A	N/A
AUST_508 RBG5793	*F. oxysporum*	Soil	Australia	KJ397059	KJ397239	N/A	N/A
AUST_556 RBG5794	*F. oxysporum*	Soil	Australia	KJ397060	KJ397240	N/A	N/A
AUST_562 RBG5796	*F. oxysporum*	Soil	Australia	KJ397061	KJ397241	N/A	N/A
AUST_582 RBG5801	*F. oxysporum*	Soil	Australia	KJ397062	KJ397242	N/A	N/A
AUST_589 RBG5803	*F. oxysporum*	Soil	Australia	KJ397063	KJ397243	N/A	N/A
AUST_593 RBG5806	*F. oxysporum*	Soil	Australia	KJ397065	KJ397245	N/A	N/A
AUST_595 RBG5807	*F. oxysporum*	Soil	Australia	KJ397066	KJ397246	N/A	N/A
AUST_618 RBG5811	*F. oxysporum*	Soil	Australia	KJ397067	KJ397247	N/A	N/A

Isolate	*Fusarium species*	Isolation host	Country of origin	NCBI GenBank accession			
				TEF-1α	RPB2	β-tubulin	ITS
AUST_638 RBG5813	*F. oxysporum*	Soil	Australia	KJ397068	KJ397248	N/A	N/A
AUST_641 RBG5814	*F. oxysporum*	Soil	Australia	KJ397069	KJ397249	N/A	N/A
AUST_671 RBG5816	*F. oxysporum*	Soil	Australia	KJ397070	KJ397250	N/A	N/A
AUST_676 RBG5817	*F. oxysporum*	Soil	Australia	KJ397071	KJ397251	N/A	N/A
AUST_68 RBG5818	*F. oxysporum*	Soil	Australia	KJ397072	KJ397252	N/A	N/A
AUST_682 RBG5819	*F. oxysporum*	Soil	Australia	KJ397073	KJ397253	N/A	N/A
NRRL 25369	*F.oxysporum*	*Terminalia ivorensis*	Ghana	N/A	N/A	AF008517	N/A
NRRL 25387	*F. oxysporum*	Clinical isolate	New Zealand	N/A	JX171625	N/A	N/A
NRRL 26374	*F. oxysporum*	Clinical isolate	USA	AF008483	N/A	AF008518	N/A
NRRL 34087	*F. oxysporum*	*Gossypium* sp.	USA,	N/A	N/A	N/A	N/A
NRRL 36341	*F. oxysporum*	Unknown	Netherlands	N/A	N/A	N/A	N/A
NRRL 38305	*F. oxysporum*	Guar medicinal plant	Egypt	FJ985376	N/A	N/A	N/A
NRRL 38328	*F. oxysporum*	Nematode cyst on soyabean root	China	FJ985385	N/A	N/A	N/A
NRRL 38477	*F. oxysporum*	*Poaceae*	New Zealand	FJ985397	N/A	N/A	N/A
NRRL 38486	*F. oxysporum*	*Allium cepa*	New Zealand	FJ985400	N/A	N/A	N/A
NRRL 38501	*F. oxysporum*	*Passiflora edulis*	New Zealand	FJ985403	N/A	N/A	N/A
NRRL 38506	*F. oxysporum*	*Pisum sativum*	New Zealand	FJ985404	N/A	N/A	N/A
NRRL 38514	*F. oxysporum*	*Colocasia esculenta*	Cook Island	FJ985406	N/A	N/A	N/A
NRRL 38548	*F. oxysporum*	*Asparagus*	New Zealand	KM092384	N/A	N/A	N/A
NRRL 38592	*F. oxysporum*	*Zea mays*	New Zealand	KM092476	N/A	N/A	N/A
NRRL 38595	*F. oxysporum*	*Zea mays*	New Zealand	FJ985415	N/A	N/A	N/A
NRRL 38596	*F. oxysporum*	*Dianthus caryophyllus*	New Zealand	KM092479	N/A	N/A	N/A
NRRL 38597	*F. oxysporum*	*Cucurbita* sp.	New Zealand	N/A	N/A	N/A	N/A
NRRL 38599	*F. oxysporum*	*Cucurbita maxima*	New Zealand	KM092474	N/A	N/A	N/A
NRRL 39464	*F. oxysporum*	*Dianthus caryophyllus*	Korea	FJ985419	N/A	N/A	N/A
NRRL 40180	*F. oxysporum*	*Lepidozamia peroffskyana*	New Zealand	FJ985420	N/A	N/A	N/A
NRRL 43442	*F. oxysporum*	Corneal scraping	USA	DQ790492	DQ790580	N/A	N/A
NRRL 43499	*F. oxysporum*	Human cornea	USA	DQ790495	DQ790583	N/A	N/A
NRRL 43646	*F. oxysporum*	Unknown	Unknown	N/A	N/A	N/A	EF453129
NRRL 43668	*F. oxysporum*	Unknown	Unknown	N/A	N/A	N/A	EF453151
NRRL 43679	*F. oxysporum*	Unknown	Unknown	N/A	N/A	N/A	EF453158

Isolate	*Fusarium species*	Isolation host	Country of origin	NCBI GenBank accession			
				TEF-1α	RPB2	β-tubulin	ITS
NRRL 45945	*F. oxysporum*	Unknown	Unknown	FJ985430	N/A	N/A	N/A
NRRL 45954	*F. oxysporum*	Unknown	Unknown	FJ985431	N/A	N/A	N/A
AA2I1F1	*F. oxysporum*	Unknown	Unknown	N/A	N/A	N/A	KX421435
A1S3D89	*F. oxysporum*	Unknown	Unknown	N/A	N/A	N/A	KJ774041
By125	*F. oxysporum*	Unknown	Unknown	N/A	N/A	N/A	GQ365156
CA1I1F3	*F. oxysporum*	Unknown	Unknown	N/A	N/A	N/A	KX421434
CJl41109	*F. oxysporum*	Unknown	Unknown	N/A	N/A	N/A	KC767892
DET-20	*F. oxysporum*	Unknown	Unknown	N/A	N/A	N/A	KX385043
DET-25	*F. oxysporum*	Unknown	Unknown	N/A	N/A	N/A	KX385044
ELRF 8	*F. oxysporum*	Unknown	Unknown	N/A	N/A	N/A	KX786247
F1	*F. oxysporum*	Unknown	Unknown	N/A	N/A	N/A	KY810792
FTB2	*F. oxysporum*	Unknown	Unknown	N/A	N/A	N/A	KY810802
F345	*F. oxysporum*	Unknown	Unknown	N/A	N/A	N/A	JX045827
GXF6	*F. oxysporum*	Unknown	Unknown	N/A	N/A	N/A	EU285554
IA6I7F1	*F. oxysporum*	Unknown	Unknown	N/A	N/A	N/A	KX421440
IA7I1F2	*F. oxysporum*	Unknown	Unknown	N/A	N/A	N/A	KX421432
IA8I1F1	*F. oxysporum*	Unknown	Unknown	N/A	N/A	N/A	KX421428
IHEM 957	*F. oxysporum*	Unknown	Unknown	N/A	N/A	N/A	KP132219
IHEM 22401	*F. oxysporum*	Unknown	Unknown	N/A	N/A	N/A	KP132218
ITA 2271	*F. oxysporum*	Unknown	Unknown	N/A	N/A	N/A	KX929698
SHBV2	*F. oxysporum*	Unknown	Unknown	N/A	N/A	N/A	KY090783
SMG1	*F. oxysporum*	Unknown	Unknown	N/A	N/A	N/A	KY090780
2424	*F. oxysporum*	Unknown	Unknown	N/A	N/A	N/A	KT828535
No name	*F. oxysporum*	Unknown	Unknown	N/A	N/A	N/A	AB369259
NRRL 36135	*F. oxysporum f. sp. batatas*	Unknown	Unknown	FJ985332	N/A	N/A	N/A
Foc108	*F. oxysporum f. sp. ciceri*	Unknown	Unknown	N/A	N/A	N/A	JN400681
Foc167	*F. oxysporum f. sp. ciceris*	Unknown	Unknown	N/A	N/A	N/A	JN400697
IHB F 2902	*F. oxysporum f. sp. ciceris*	Unknown	Unknown	N/A	N/A	N/A	KM817208
NRRL 38591	*F. oxysporum f. sp. cucumerinum*	*Cucumis sativus*	New Zealand	FJ985379	N/A	N/A	N/A
ZJ-02	*F. oxysporum f. sp. cucumerinum*	Unknown	Unknown	N/A	N/A	N/A	HM179530
NRRL 26222	*F. oxysporum f. sp. dianthi*	*Dianthus caryophyllus*	Israel	FJ985284	N/A	N/A	N/A

Isolate	*Fusarium species*	Isolation host	Country of origin	NCBI GenBank accession			
				TEF-1α	RPB2	β-tubulin	ITS
NRRL 28365	*F. oxysporum f. sp. dianthi*	*Dianthus caryophyllus*	Netherlands	FJ985303	N/A	N/A	N/A
NRRL 36356	*F. oxysporum f. sp. dianthi*	*Dianthus* sp.	Argentina	FJ985348	N/A	N/A	N/A
NRRL 26574	*F. oxysporum f. sp. erythroxyli*	*Erythroxylum coca*	USA	AF008495	N/A	AF008530	N/A
NRRL 38885	*F. oxysporum f. sp. koae*	*Acacia koa*	USA	FJ985418	N/A	N/A	N/A
FLS52	*F. oxysporum f. sp. lentis*	Unknown	Unknown	N/A	N/A	N/A	KU671041
NRRL 28395	*F. oxysporum f. sp. lilii*	*Lilium* sp.	Italy	EF056788	N/A	N/A	N/A
NRRL 36286	*F. oxysporum f. sp. lini*	*Linum usitatissimum*	Unknown	FJ985344	N/A	N/A	N/A
NRRL 26225	*Fu. oxysporum f. sp. lupini*	*Lupinus* sp.	USA	FJ985285	N/A	N/A	N/A
NRRL 26203	*F. oxysporum f. sp. lycopersici*	*Solanum lycopersicum*	Italy	AF008501	N/A	AF008536	N/A
NRRL 34936	*F. oxysporum f. sp. lycopersici*	Di Pietro	Uknown	N/A	JX171646	N/A	N/A
CBS 42090	*F. oxysporum f. sp. melonis*	*Cucumis melo*	Israel	EF056790	N/A	N/A	N/A
NRRL 22549	*F. oxysporum f. sp. passiflorae*	*Passiflora edulis*	Brazil	N/A	N/A	AF008540	N/A
NRRL 22551	*F. oxysporum f. sp. pini*	*Pinus* sp.	Germany	FJ985272	N/A	N/A	N/A
NRRL 26033	*F. oxysporum f. sp. radicis-lycopersici*	*Solanum lycopersicum*	USA	AF008507	N/A	AF008542	N/A
NRRL 22554	*F. oxysporum f. sp. tracheiphilum*	*Chrysanthemum* sp.	Nigeria	FJ985274	N/A	N/A	N/A
NRRL 22555	*F. oxysporum f. sp. tuberosi*	*Solanum tuberosum*	Iran	AF008511	N/A	AF008546	N/A
NRRL 26448	*F. oxysporum f. sp. vanillae*	*Vanilla* sp.	USA	FJ985300	N/A	N/A	N/A
NRRL 25420	*F. oxysporum f. sp. vasinfectum*	*Gossypium* sp.	USA	AF008512	N/A	AF008547	N/A
NRRL 31084	*F. graminearum*	*Zea mays*	USA	N/A	N/A	HQ141668	N/A
CBS 131778	*F. graminearum*	Unknown	Unknown	N/A	N/A	N/A	JX162395

Adapted from *Fusarium* MLST database; *Fusarium*-ID database and Laurence *et al.* (2014). NRRL and accession numbers were downloaded from NCBI GenBank.

3.5.5 Software and websites used for sequence analyses

3.5.5.1 Software

a) MEGA version 6.0 software was used for sequence editing, generating consensus sequences and for constructing ML phylogenetic trees.
b) Microsoft Office Excel (2010) was used for generating pie and bar graphs.
c) Microsoft Office PowerPoint (2010) was used for editing phylogenetic trees.
d) TreeView (Win32) version 1.6.6 software was used to construct the MP phylogenetic tree.
e) PAUP 4.0* was used for generating MP analyses.

3.5.5.2 Websites

a) ARS culture collection website (https://nrrl.ncaur.usda.gov/) was used to check and confirm the species name, isolation host and country of origin.
b) *Fusarium*-ID website (http://www.*Fusarium*.cbio.psu.edu) and *Fusarium* MLST website (http://www.cbs.knaw.nl/*Fusarium*) were used to compare sequences from this study to the sequences from these reference libraries.
c) NCBI GenBank website (https://www.ncbi.nlm.nih.gov/) was used to retrieve accession numbers and sequences.
d) PhyML analyses of online version (http://www.atgc-montpellier.fr/phyml/) was used to generate ML analyses.

3.6 Morphological characterisation

The single spore cultures were incubated under a 12-hour diurnal light cycle (mixed fluorescent/near UV lighting) at 25°C for 7 days. The media that were used for morphological characterisation was Carnation Leave Agar (CLA), Synthetic Nutrient Agar (SNA) and PDA. Morphological characteristics examined included the shape and size of the macroconidia on CLA (Fisher *et al.*, 1982) the shape and the mode of formation of microconidia on CLA and SNA (Nirenberg, 1976), the production of chlamydospores on CLA, and the colour of the culture on PDA. Descriptions of pigmentation colour was based on the Methuen Handbook of colour (Kornerup and Wanscher, 1978). A total of 30 *F. oxysporum* isolates from diseased sweet potato strains based on phylogenetic analysis clades were selected and measured. Ten *F.*

oxysporum soil isolates per sampling site, from each of the three provinces were randomly selected and measured. Measurements were based on ten macroconidia and ten microconidia per selected fungal isolate. The microscope slides were prepared with lactophenol and the Zeiss Axio Imager A2 compound microscope was used to observe the prepared slides. Photographs were taken with the 40X objective. *Fusarium oxysporum* strains were identified morphologically based on the description in Leslie and Summerell (2006). In addition, other fungal isolates were identified morphologically based on the description in Padwick (1945), Zeller *et al.* (2003) and Laurence *et al.* (2011). The identification of morphologically ambiguous fungal isolates was verified based on DNA sequencing of the TEF-1α (diseased sweet potato and soil) and compared with the *Fusarium* MLST database, using MLST nBLAST™ (O'Donnell *et al.*, 2012) (Appendix A).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Symptoms and isolations

Symptomatic sweet potato plant materials were collected from 20 farms, each showing one or more of the following symptoms, wilting of the plant, stunted growth, a dark to reddish brown discoloration of the vascular tissue in the lower stems, when cut open longitudinally, and yellowing of leaves with dark brown, marginal or interveinal browning as indicated in Figure 3.2, Figure 4.1 and Figure 4.2. These symptoms were similar to FW infected plants worldwide (Clark, 2013; Gerlach and Nirenberg, 1982) and reported locally (Thompson *et al.*, 2011). Soil samples were collected from diseased sweet potato fields in three farms. In total, 89 isolates were obtained from the symptomatic sweet potato plant materials and 189 isolates were obtained from soil.

Figure 4.1: Symptomatic sweet potato plant showing wilting, yellowing of leaves with dark brown and dead leaves.

Figure 4.2: Symptomatic sweet potato field showing the yellowing of leaves.

4.2 DNA extraction and PCR

The extracted DNA was visualised using gel electrophoresis to determine the DNA quality and concentration as indicated in Figure 4.3. The PCR amplification of the TEF-1α, RPB2 (5F and 7CR), RPB2 (7CF and 11AR), β-tubulin and ITS gene regions resulted in PCR amplicon sizes of approximately 700 base pairs (bp), 1500 bp, 1200 bp, 1500 bp and 550 bp, respectively as indicated in Figure 4.4 to Figure 4.8.

The PCR amplification of the TEF-1α gene from the diseased sweet potato and soil isolates was successful with a single band of about 700 bp for most fungal isolates. For the fungal isolates that had a very faint band, e.g. Figure 4.4, in lane 5 (PPRI 9462), the PCR conditions were optimised by decreasing an annealing temperature by 1ºC increment until a single clear band appeared. For the fungal isolates where amplification was unsuccessful, e.g. Figure 4.5 empty lane 12 (PPRI 9469), the initial annealing temperature was decreased by 2ºC increments until a single clear band appeared. For fungal isolates that had multiple bands after PCR amplification, e.g. Figure 4.5, the annealing temperature was increased by 1ºC increments until a single clear band appeared.

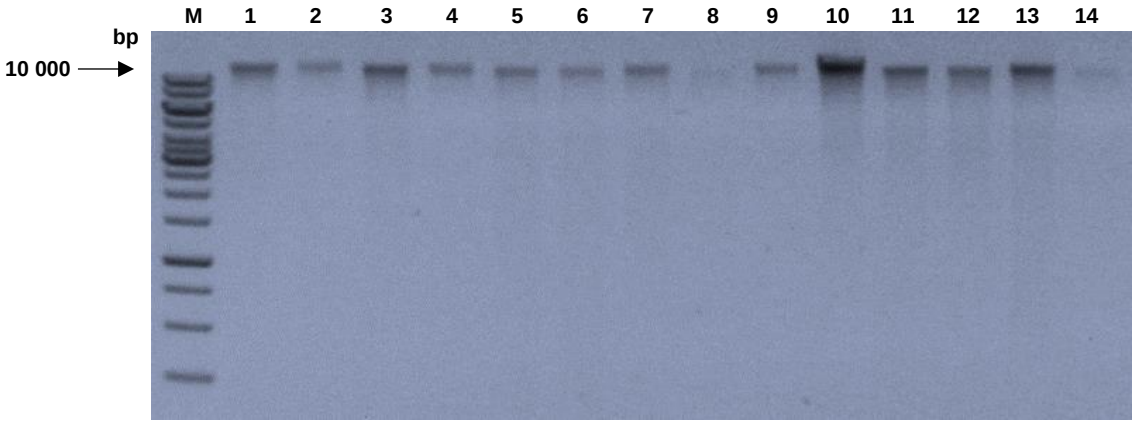


Figure 4.3: DNA extraction from the isolated fungal strains obtained from diseased sweet potato. The genomic DNA bands were visualised on a 1% agarose gel. Lane M = marker (O'GeneRuler 1 kb DNA ladder ready to use, Thermo Scientific). DNA extraction, lane 1 to 14. Lane 1 = PPRI 9458; 2 = PPRI 9459; 3 = PPRI 9460; 4 = PPRI 9461; 5 = PPRI 9462; 6 = PPRI 9463; 7 = PPRI 9464; 8 = PPRI 9465; 9 = PPRI 9466; 10 = PPRI 9467; 11 = PPRI 9468; 12 = PPRI 9469; 13 = PPRI 9470 and 14 = PPRI 9471.

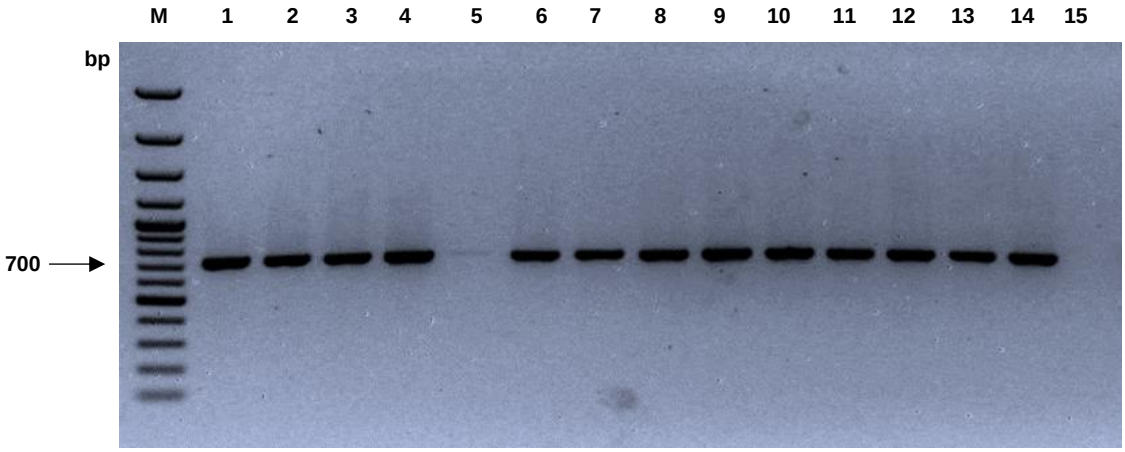


Figure 4.4: PCR amplicons of the TEF-1α from the isolated fungal strains obtained from diseased sweet potato. The PCR amplicons were visualised on a 1% agarose gel. Lane M = marker (O'GeneRuler 100 bp DNA ladder ready to use, Thermo Scientific). PCR product, lane 1 to 14. Lane 1 = PPRI 9458; 2 = PPRI 9459; 3 = PPRI 9460; 4 = PPRI 9461; 5 = PPRI 9462; 6 = PPRI 9463; 7 = PPRI 9464; 8 = PPRI 9465; 9 = PPRI 9466; 10 = PPRI 9467; 11 = PPRI 9468; 12 = PPRI 9469; 13 = PPRI 9470; 14 = PPRI 9471 and 15 = negative control.

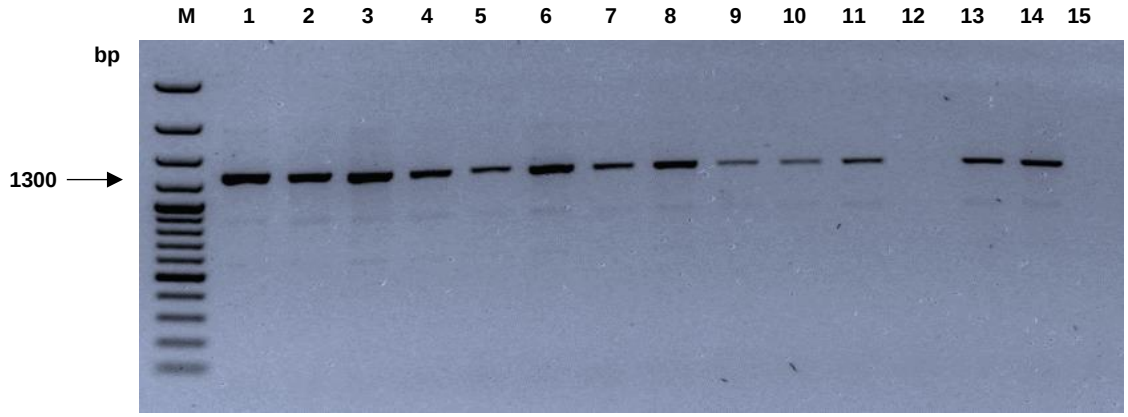


Figure 4.5: PCR amplicons of the RPB2 (5F and 7CR) from the isolated fungal strains obtained from diseased sweet potato. The PCR amplicons were visualised on a 1% agarose gel. Lane M = marker (O'GeneRuler 100 bp DNA ladder ready to use, Thermo Scientific). PCR product, lane 1 to 14. Lane 1 = PPRI 9458; 2 = PPRI 9459; 3 = PPRI 9460; 4 = PPRI 9461; 5 = PPRI 9462; 6 = PPRI 9463; 7 = PPRI 9464; 8 = PPRI 9465; 9 = PPRI 9466; 10 = PPRI 9467; 11 = PPRI 9468; 12 = PPRI 9469; 13 = PPRI 9470; 14 = PPRI 9471 and 15 = negative control.

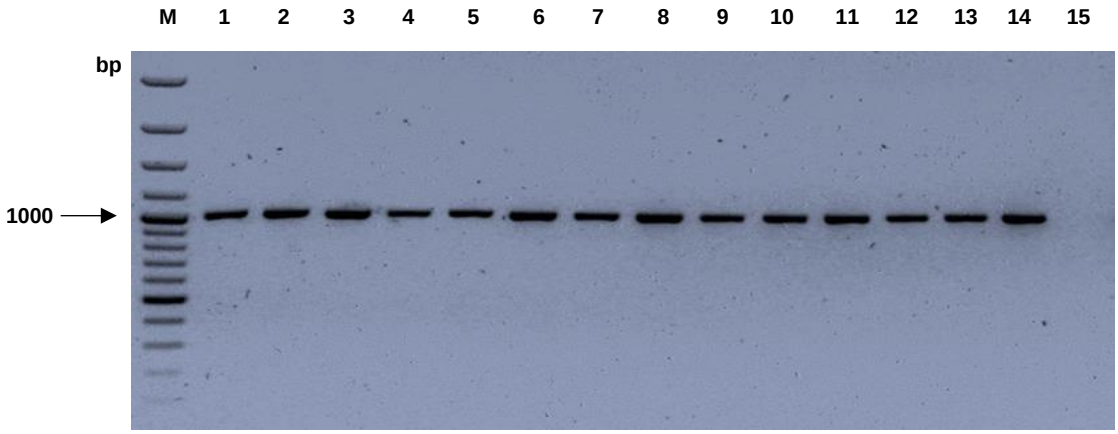


Figure 4.6: PCR amplicons of the RPB2 (7CF and 11AR) from the isolated fungal strains obtained from diseased sweet potato. The PCR amplicons were visualised on a 1% agarose gel. Lane M = marker (O'GeneRuler 100 bp DNA ladder ready to use, Thermo Scientific). PCR product, lane 1 to 14. Lane 1 = PPRI 9458; 2 = PPRI 9459; 3 = PPRI 9460; 4 = PPRI 9461; 5 = PPRI 9462; 6 = PPRI 9463; 7 = PPRI 9464; 8 = PPRI 9465; 9 = PPRI 9466; 10 = PPRI 9467; 11 = PPRI 9468; 12 = PPRI 9469; 13 = PPRI 9470; 14 = PPRI 9471 and 15 = negative control.

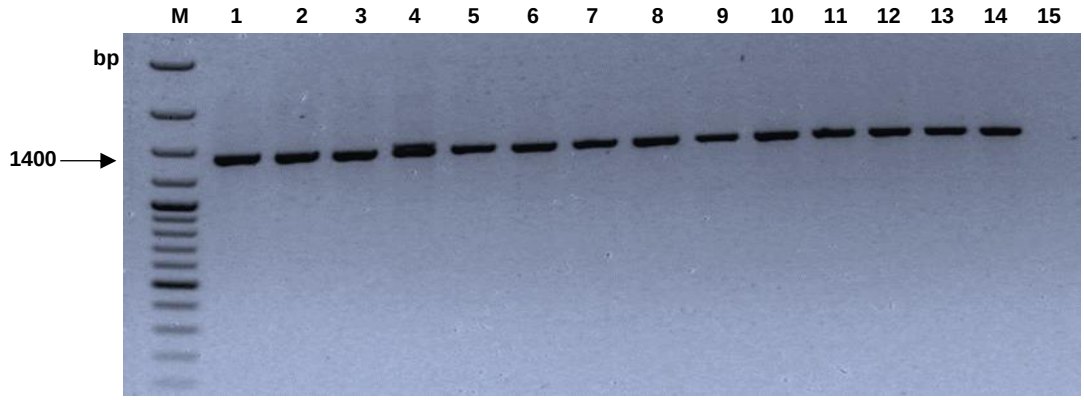


Figure 4.7: PCR amplicons of the β-tubulin from the isolated fungal strains obtained from diseased sweet potato. The PCR amplicons were visualised on a 1% agarose gel. Lane M = marker (O'GeneRuler 100 bp DNA ladder ready to use, Thermo Scientific). PCR product, lane 1 to 14. Lane 1 = PPRI 9458; 2 = PPRI 9459; 3 = PPRI 9460; 4 = PPRI 9461; 5 = PPRI 9462; 6 = PPRI 9463; 7 = PPRI 9464; 8 = PPRI 9465; 9 = PPRI 9466; 10 = PPRI 9467; 11 = PPRI 9468; 12 = PPRI 9469; 13 = PPRI 9470; 14 = PPRI 9471 and 15 = negative control.

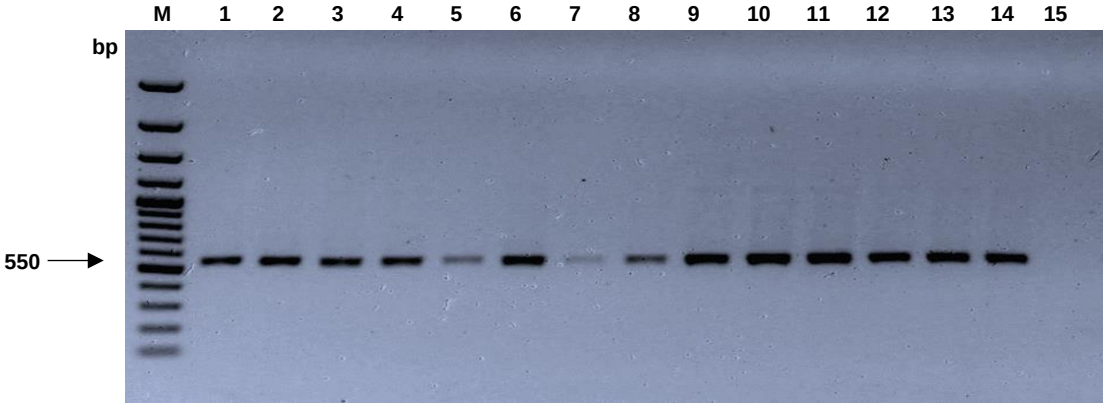


Figure 4.8: PCR amplicons of the ITS from the isolated fungal strains obtained from diseased sweet potato. The PCR amplicons were visualised on a 1% agarose gel. Lane M = marker (O'GeneRuler 100 bp DNA ladder ready to use, Thermo Scientific). PCR product, lane 1 to 14. Lane 1 = PPRI 9458; 2 = PPRI 9459; 3 = PPRI 9460; 4 = PPRI 9461; 5 = PPRI 9462; 6 = PPRI 9463; 7 = PPRI 9464; 8 = PPRI 9465; 9 = PPRI 9466; 10 = PPRI 9467; 11 = PPRI 9468; 12 = PPRI 9469; 13 = PPRI 9470; 14 = PPRI 9471 and 15 = negative control.

4.3 *Fusarium* MLST and *Fusarium*-ID database nBLAST™ results

Fungal isolates obtained from 89 diseased sweet potato plant material and 198 isolates obtained from soil were successfully sequenced and identified by nBLAST™ queries on the *Fusarium* MLST and *Fusarium*-ID databases based on four loci namely, TEF-1α, RPB2, β-tubulin and ITS. *Fusarium* MLST and *Fusarium*-ID database nBLAST™ results are presented in Table 4.1, Table 4.2, Table 4.3, Table 4.4, Table 4.5 and Table 4.6 for sweet potato TEF-1α, soil TEF-1α, sweet potato RPB2 (5F and 7CR), sweet potato RPB2 (7CF and 11AR), sweet potato β-tubulin and sweet potato ITS data, respectively generated with the highest percentage similarities from both databases.

4.3.1 *Fusarium* MLST database nBLAST™ results based on TEF-1α sequences for isolates obtained from sweet potato material

The *Fusarium* MLST database nBLAST™ results based on the TEF-1α sequences of 89 strains obtained from diseased sweet potato material clustered them into four *Fusarium* species complexes represented by seven *Fusarium* species (Table 4.1). The four *Fusarium* species complexes were FFSC, *F. incarnatum-equiseti* species complex (FIESC), FOSC and *F. solani* species complex (FSSC). The species in the complexes were represented by *F. konzum* Zeller, Summerell & J.F. Leslie in the FFSC, *F. lacertarum* Subrahm. and *F. scirpi* Lambotte & Fautrey in the FIESC, *F. cuneirostrum* O'Donnell & T. Aoki and *F. solani* in the FSSC and *F. inflexum* R. Schneid. and *F. oxysporum* in the FOSC (Figure 4.9).

The geographic distribution of the *Fusarium* species recovered, consisted of mostly *F. oxysporum* from all the provinces sampled and included *F. cuneirostrum* and *F. inflexum* recovered from Gauteng Province, *F. konzum* recovered from Gauteng and Limpopo Province and *F. scirpi* recovered from Limpopo Province. Mpumalanga Province was mostly represented by *F. solani* isolates and included *F. lacertarum*, *Fusarium* sp. and unidentified Hypocreales. This is possibly because of the different agricultural practices used in these provinces.

Table 4.1: *Fusarium* MLST and *Fusarium*-ID nBLAST™ results of TEF-1α from diseased sweet potato fungal isolates

	***FUSARIUM* MLST DATABASE**						***FUSARIUM*-ID DATABASE**					
PPRI Number	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**
9458	*F. oxysporum*	FOSC	233	NRRL 40180	FJ985420	99.68	*F. oxysporum*	FOSC	N/A	NRRL 22538	FJ985419	99.70
9459	*F. oxysporum f. sp. erythroxyli*	FOSC	79	NRRL 26574	AF008495	100	*F. oxysporum*	FOSC	232	NRRL 39464	AF008495	100
9460	*F. oxysporum*	FOSC	53	NRRL 43499	DQ790495	100	*F. oxysporum*	FOSC	16	NRRL 38597	N/A	100
9461	*F. oxysporum f. sp. erythroxyli*	FOSC	79	NRRL 26574	AF008495	99.85	*F. oxysporum*	FOSC	232	NRRL 39464	AF008495	99.85
9462	Unidentified Hypocreales	N/A	N/A	N/A	KC461320	100	*F. graminearum*	N/A	N/A	NRRL 5883	N/A	96.06
9463	*F. oxysporum*	FOSC	233	NRRL 40180	FJ985420	99.68	*F. oxysporum*	FOSC	N/A	NRRL 22538	FJ985419	99.70
9464	*F. oxysporum f. sp. vanillae*	FOSC	77	NRRL 26448	FJ985300	100	*F. oxysporum*	FOSC	77	NRRL 36341	N/A	100
9465	*F. oxysporum f. sp. erythroxyli*	FOSC	79	NRRL 26574	AF008495	100	*F. oxysporum*	FOSC	232	NRRL 39464	AF008495	99.85
9466	*F. oxysporum f. sp. vanillae*	FOSC	77	NRRL 26448	FJ985300	100	*F. oxysporum*	FOSC	77	NRRL 36341	N/A	100
9467	*F. oxysporum*	FOSC	233	NRRL 40180	FJ985420	99.68	*F. oxysporum*	FOSC	N/A	NRRL 22538	FJ985419	99.56
9468	*F. oxysporum*	FOSC	53	NRRL 43499	DQ790495	100	*F. oxysporum*	FOSC	16	NRRL 38597	N/A	100
9469	*F. oxysporum*	FOSC	233	NRRL 40180	FJ985420	99.52	*F. oxysporum*	FOSC	N/A	NRRL 22538	FJ985419	99.56
9470	*F. oxysporum*	FOSC	53	NRRL 43499	DQ790495	100	*F. oxysporum*	FOSC	16	NRRL 38597	N/A	100
9471	*F. oxysporum*	FOSC	233	NRRL 40180	FJ985420	99.68	*F. oxysporum*	FOSC	N/A	NRRL 22538	FJ985419	99.70
9472	*F. oxysporum*	FOSC	233	NRRL 40180	FJ985420	99.68	*F. oxysporum*	FOSC	N/A	NRRL 22538	FJ985419	99.56
9473	*F. oxysporum*	FOSC	217	NRRL 38506	FJ985404	100	*F. oxysporum*	FOSC	232	NRRL 39464	AF008495	99.70
10531	*F. oxysporum*	FOSC	213	NRRL 38486	FJ985400	99.66	*F. oxysporum*	FOSC	77	NRRL 36341	N/A	99.54
10532	*F. oxysporum*	FOSC	53	NRRL 43499	DQ790495	100	*F. oxysporum*	FOSC	16	NRRL 38597	N/A	100
10533	*F. oxysporum*	FOSC	53	NRRL 43499	DQ790495	100	*F. oxysporum*	FOSC	16	NRRL 38597	N/A	100
17592	*F. oxysporum f. sp. vanillae*	FOSC	77	NRRL 26448	FJ985300	100	*F. oxysporum*	FOSC	77	NRRL 36341	N/A	100
17593	*F. oxysporum f. sp. vanillae*	FOSC	77	NRRL 26448	FJ985300	100	*F. oxysporum*	FOSC	77	NRRL 36341	N/A	100
17594	*F. oxysporum f. sp. vanillae*	FOSC	77	NRRL 26448	FJ985300	100	*F. oxysporum*	FOSC	77	NRRL 36341	N/A	100
17595	*F. oxysporum f. sp. vanillae*	FOSC	77	NRRL 26448	FJ985300	100	*F. oxysporum*	FOSC	77	NRRL 36341	N/A	100
17596	*F. oxysporum f. sp. vanillae*	FOSC	77	NRRL 26448	FJ985300	100	*F. oxysporum*	FOSC	77	NRRL 36341	N/A	100

	FUSARIUM MLST DATABASE						*FUSARIUM*-ID DATABASE					
PPRI Number	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**
18014	*C. corda*	N/A	N/A	CBS 124754	N/A	100	*Fusarium* sp.	FIESC	13-a	NRRL 43635	GQ505662	94.55
18016	*F. oxysporum f. sp. erythroxyli*	FOSC	79	NRRL 26574	AF008495	100	*F. oxysporum*	FOSC	232	NRRL 39464	AF008495	100
18017	*C. corda*	N/A	N/A	CBS 124754	N/A	100	*Fusarium* sp.	FIESC	13-a	NRRL 43635	GQ505662	94.55
18018	*C. corda*	N/A	N/A	CBS 124754	N/A	99.77	*Fusarium* sp.	FIESC	13-a	NRRL 43635	GQ505662	94.55
18750	*F. oxysporum f. sp. vanillae*	FOSC	77	NRRL 26448	FJ985300	100	*F. oxysporum*	FOSC	77	NRRL 36341	N/A	100
18751	*C. corda*	N/A	N/A	CBS 124754	N/A	98.87	*Fusarium* sp.	FIESC	13-a	NRRL 43635	GQ505662	94.55
18752	*F. oxysporum f. sp. vanillae*	FOSC	77	NRRL 26448	FJ985300	100	*F. oxysporum*	FOSC	77	NRRL 36341	N/A	99.70
18753	*F. oxysporum f. sp. vanillae*	FOSC	77	NRRL 26448	FJ985300	100	*F. oxysporum*	FOSC	77	NRRL 36341	N/A	100
20163	*F. oxysporum f. sp. vasinfectum*	FOSC	28	NRRL 25420	AF008512	100	*F. oxysporum*	FOSC	219	NRRL 38514	FJ985406	100
20164	*F. oxysporum*	FOSC	53	NRRL 43499	DQ790495	100	*F. oxysporum*	FOSC	16	NRRL 38597	N/A	99.85
20165	*F. oxysporum f. sp. radicis-lycopersici*	FOSC	40	NRRL 26033	AF008507	99.52	*F. oxysporum*	FOSC	232	NRRL 39464	AF008495	99.52
20166	*F. oxysporum*	FOSC	188	NRRL 38305	FJ985376	99.53	*F. oxysporum f. sp. vasinfectum*	FOSC	29	NRRL 34087	N/A	99.56
20167	*F. cuneirostrum*	FSSC	N/A	NRRL 31104	DQ452421	90.87	*F. oxysporum*	FOSC	N/A	Zm-20	N/A	98.14
20168	*F. oxysporum*	FOSC	233	NRRL 40180	FJ985420	99.68	*F. oxysporum*	FOSC	N/A	NRRL 22538	FJ985419	99.70
20169	*F. konzum*	FFSC	none	NRRL 53394	N/A	100	*Fusarium* sp.	FOSC	19	NRRL 45881	N/A	100
20170	*F. inflexum*	FOSC	none	NRRL 20433	AF008479	100	*Fusarium* sp.	FOSC	19	NRRL 45881	N/A	100
20171	*F. oxysporum*	FOSC	53	NRRL 43499	DQ790495	100	*F. oxysporum*	FOSC	16	NRRL 38597	N/A	100
20172	*F. oxysporum*	FOSC	244	NRRL 45954	FJ985431	100	*F. oxysporum*	FOSC	232	NRRL 39464	AF008495	99.56
20173	*F. oxysporum f. sp. dianthi*	FOSC	101	NRRL 28365	FJ985303	100	*F. oxysporum f. sp. cucumerinum*	FOSC	191	NRRL 38591	FJ985379	99.85
20174	*F. oxysporum f. sp. dianthi*	FOSC	101	NRRL 28365	FJ985303	96.23	*F. oxysporum f. sp. cucumerinum*	FOSC	191	NRRL 38591	FJ985379	98.05
20175	*F. oxysporum*	FOSC	48	NRRL 43442	DQ790492	99.54	*F. oxysporum*	FOSC	16	NRRL 38597	N/A	99.56
20176	*F. oxysporum f. sp. cucumerinum*	FOSC	191	NRRL 38591	FJ985379	99.84	*F. oxysporum f. sp. cucumerinum*	FOSC	191	NRRL 38591	FJ985379	99.85
20177	*F. oxysporum f. sp. lini*	FOSC	154	NRRL 36286	FJ985344	100	*F. oxysporum*	FOSC	222	NRRL 38592	KM092476	99.70
20178	*F. oxysporum f. sp. batatas*	FOSC	142	NRRL 36135	FJ985332	99.52	*F. oxysporum*	FOSC	16	NRRL 38597	N/A	99.41

	FUSARIUM MLST DATABASE						*FUSARIUM*-ID DATABASE					
PPRI Number	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**
20179	*F. oxysporum*	FOSC	53	NRRL 43499	DQ790495	99.85	*F. oxysporum*	FOSC	16	NRRL 38597	N/A	99.85
23061	*F. scirpi*	FIESC	12-a	NRRL 26921	GQ505600	98.97	*Fusarium* sp.	FIESC	N/A	NRRL 36392	GQ505650	98.96
23062	*F. oxysporum f. sp. tuberosi*	FOSC	21	NRRL 22555	AF008511	100	*F. oxysporum*	FOSC	216	NRRL 38501	FJ985403	100
23063	*F. oxysporum f. sp. dianthi*	FOSC	158	NRRL 36356	FJ985348	100	*F. oxysporum*	FOSC	222	NRRL 38592	KM092476	100
23064	*F. oxysporum f. sp. lilii*	FOSC	107	NRRL 28395	EF056788	99.54	*Fusarium* sp.	FOSC	19	NRRL 45881	EF056788	99.54
23065	*F. oxysporum f. sp. erythroxyli*	FOSC	79	NRRL 26574	AF008495	100	*F. oxysporum*	FOSC	232	NRRL 39464	AF008495	99.85
23066	*F. oxysporum f. sp. vanillae*	FOSC	77	NRRL 26448	FJ985300	100	*F. oxysporum*	FOSC	77	NRRL 36341	N/A	99.85
23067	*F. konzum*	FFSC	none	NRRL 53394	N/A	99.85	*Fusarium* sp.	FOSC	19	NRRL 45881	N/A	99.70
23068	*F. oxysporum f. sp. erythroxyli*	FOSC	79	NRRL 26574	AF008495	100	*F. oxysporum*	FOSC	232	NRRL 39464	AF008495	99.85
23069	*F. oxysporum f. sp. erythroxyli*	FOSC	79	NRRL 26574	AF008495	99.85	*F. oxysporum*	FOSC	232	NRRL 39464	AF008495	99.56
23070	*F. oxysporum f. sp. erythroxyli*	FOSC	79	NRRL 26574	AF008495	100	*F. oxysporum*	FOSC	232	NRRL 39464	AF008495	100
23071	*F. oxysporum f. sp. vanillae*	FOSC	77	NRRL 26448	FJ985300	100	*F. oxysporum*	FOSC	77	NRRL 36341	N/A	99.85
23072	*F. oxysporum f. sp. tracheiphilum*	FOSC	20	NRRL 22554	FJ985274	100	*F. oxysporum f. sp. lupini*	FOSC	47	NRRL 26225	FJ985285	100
23074	*F. oxysporum f. sp. lycopersici*	FOSC	43	NRRL 26203	AF008501	100	*F. oxysporum*	FOSC	43	NRRL 38548	KM092384	100
23076	*F. oxysporum f. sp. erythroxyli*	FOSC	79	NRRL 26574	AF008495	100	*F. oxysporum*	FOSC	232	NRRL 39464	AF008495	100
23077	*F. oxysporum*	FOSC	54	NRRL 26374	AF008483	100	*F. oxysporum*	FOSC	43	NRRL 38548	KM092384	100
23078	*F. oxysporum f. sp. erythroxyli*	FOSC	79	NRRL 26574	AF008495	100	*F. oxysporum*	FOSC	232	NRRL 39464	AF008495	99.70
23473	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	99.11	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	99.11
23474	*F. inflexum*	FOSC	2	NRRL 20433	AF008479	100	*Fusarium* sp.	FOSC	19	NRRL 45881	EF056788	100
23475	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	98.52	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	98.52
23476	*F. solani*	FSSC	N/A	NRRL 28579	DQ246910	100	*F. petroliphilum*	FSSC	1-a	NRRL 28546	DQ246887	100
23477	*F. solani*	FSSC	N/A	NRRL 28579	DQ246910	100	*F. petroliphilum*	FSSC	1-a	NRRL 28546	DQ246887	100
23478	*Fusarium* sp.	FIESC	3-a	NRRL 36323	GQ505648	100	*Fusarium* sp.	FIESC	3-a	NRRL 36323	GQ505648	100
23479	*F. solani*	FSSC	N/A	NRRL 28579	DQ246910	100	*F. petroliphilum*	FSSC	1-a	NRRL 28546	DQ246887	100
23480	*F. solani*	FSSC	N/A	NRRL 28579	DQ246910	100	*F. petroliphilum*	FSSC	1-a	NRRL 28546	DQ246887	100

	FUSARIUM MLST DATABASE						*FUSARIUM*-ID DATABASE					
PPRI Number	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**
23481	*F. solani*	FSSC	N/A	NRRL 28579	DQ246910	100	*F. petroliphilum*	FSSC	1-a	NRRL 28546	DQ246887	100
23482	*F. solani*	FSSC	N/A	NRRL 28579	DQ246910	100	*F. petroliphilum*	FSSC	1-a	NRRL 28546	DQ246887	100
23483	*F. solani*	FSSC	N/A	NRRL 28579	DQ246910	100	*F. petroliphilum*	FSSC	1-a	NRRL 28546	DQ246887	100
23484	*F. solani*	FSSC	N/A	NRRL 28579	DQ246910	100	*F. petroliphilum*	FSSC	1-a	NRRL 28546	DQ246887	100
23485	*F. solani*	FSSC	N/A	NRRL 28579	DQ246910	100	*F. petroliphilum*	FSSC	1-a	NRRL 28546	DQ246887	100
23486	*F. solani*	FSSC	N/A	NRRL 28579	DQ246910	100	*F. petroliphilum*	FSSC	1-a	NRRL 28546	DQ246887	100
23487	*F. solani*	FSSC	N/A	NRRL 28579	DQ246910	100	*F. petroliphilum*	FSSC	1-a	NRRL 28546	DQ246887	100
23488	*F. solani*	FSSC	N/A	NRRL 28579	DQ246910	100	*F. petroliphilum*	FSSC	1-a	NRRL 28546	DQ246887	100
23489	*F. solani*	FSSC	N/A	NRRL 28579	DQ246910	100	*F. petroliphilum*	FSSC	1-a	NRRL 28546	DQ246887	100
23490	*F. solani*	FSSC	N/A	NRRL 28579	DQ246910	100	*F. petroliphilum*	FSSC	1-a	NRRL 28546	DQ246887	100
23491	*F. solani*	FSSC	N/A	NRRL 28579	DQ246910	100	*F. petroliphilum*	FSSC	1-a	NRRL 28546	DQ246887	100
23492	*F. solani*	FSSC	N/A	NRRL 28579	DQ246910	100	*F. petroliphilum*	FSSC	1-a	NRRL 28546	DQ246887	100
23493	*F. solani*	FSSC	N/A	NRRL 28579	DQ246910	100	*F. petroliphilum*	FSSC	1-a	NRRL 28546	DQ246887	100
23494	*F. solani*	FSSC	N/A	NRRL 28579	DQ246910	100	*F. petroliphilum*	FSSC	1-a	NRRL 28546	DQ246887	100
23495	*F. solani*	FSSC	N/A	NRRL 28579	DQ246910	100	*F. petroliphilum*	FSSC	1-a	NRRL 28546	DQ246887	100
23496	*F. solani*	FSSC	N/A	NRRL 28579	DQ246910	100	*F. petroliphilum*	FSSC	1-a	NRRL 28546	DQ246887	100

PPRI=Living fungal collection of the South Africa National Collection of Fungi, Agricultural Research Council-Plant Health and Protection, South Africa, Pretoria

MLST=Multiloci sequence type

nBLAST nBLAST™=Nucleotide Basic Local Alignment Search Tool

NRRL=Agricultural Research Service culture collection, United States Department of Agriculture Illinois, United States of America

CBS=Filamentous fungi and yeast Collection, Westerdijk Fungal Biodiversity Institute, Nertherlands

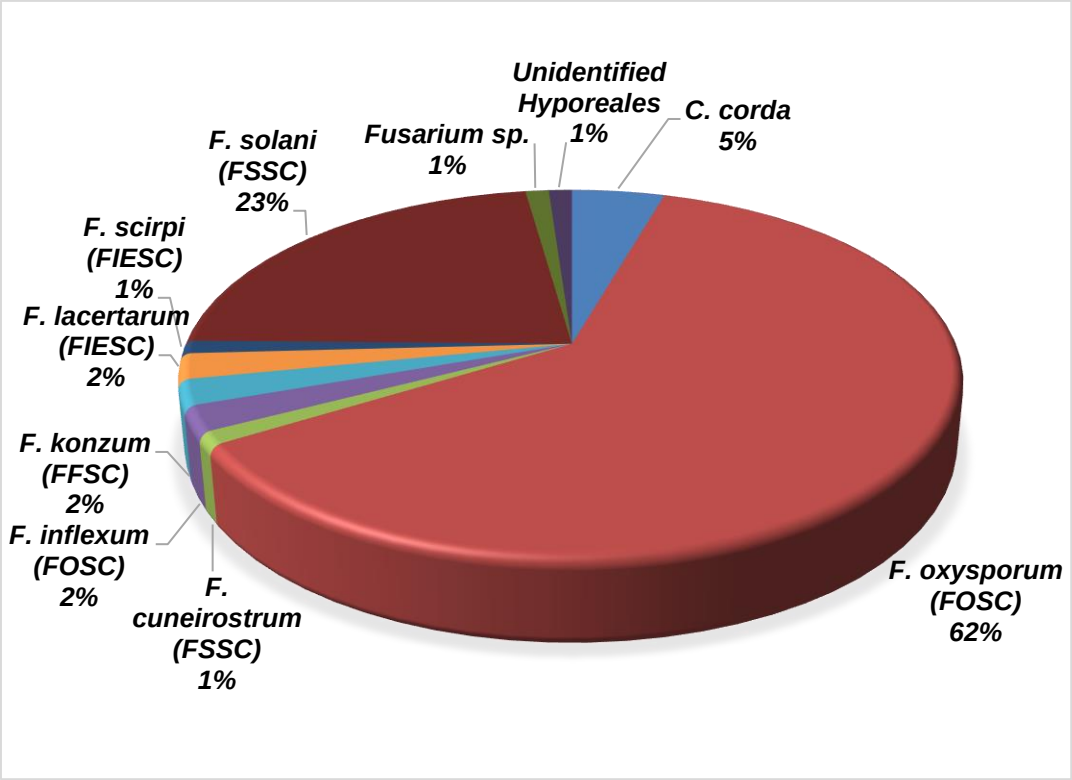


Figure 4.9: *Fusarium* species complexes and other species identified using *Fusarium* MLST database nBLAST™ analyses for isolates recovered from diseased sweet potato material collected from Eastern Cape, Gauteng, Limpopo, Mpumalanga, Northern Cape and Western Cape provinces of South Africa.

The FFSC was represented by two isolates (2%), PPRI 20169 and 23067 that showed significant similarities with *F. konzum* NRRL 53394 from USA, with percentage similarity of 100% and 99.85%, respectively. *Fusarium konzum* was first isolated from native prairie grasses in USA but has not been reported in South Africa. This is the first occurrence of *F. konzum* from South Africa and the first report being associated with sweet potato.

The FIESC was represented by one *F. scirpi* isolate (1%), two *F. lacertarum* isolates (2%) and one *Fusarium* sp. isolate (1%). PPRI 23061 showed a similarity with *F. scirpi* NRRL 26921 MLST type 12-a in the FIESC with a percentage similarity of 98.97%. *Fusarium scirpi* NRRL 26921 was isolated from wheat in Germany. Jacobs *et al.* (2018) recently characterised members of the FIESC from undisturbed soil in South Africa and revealed *F. scirpi* amongst members of the complex. The

association with sweet potato is the first report for South Africa. PPRI 23478 was similar to undescribed *Fusarium* sp. MLST type 3-a in the FIESC with a significant percentage similarity of 100%. PPRI 23473 and 23475 were similar to *F. lacertarum* NRRL 20423 MLST type 4-a in the FSSC with a percentage similarity of 99.11% and 98.52%, respectively. *Fusarium lacertarum* has been reported in India and isolated from lizard skin (O'Donnell *et al.*, 2009b). Furthermore, Favaretto *et al.* (2018) identified *F. lacertarum* as the casual agent of damping-off in *Casuarina equisetifolia* in Brazil. This indicate that the pathogen is associated with plant and animal diseases and now with FW of sweet potato in South Africa. Therefore, this is the first occurrence of *F. lacertarum* associated with FW of sweet potato in South Africa.

The FSSC was represented by one *F. cuneirostrum* isolate (1%) and 20 *F. solani* isolates (22%). The TEF-1α *Fusarium* MLST database nBLAST™ analyses indicated a significant percentage similarity of 99%-100% for most isolates, however, one isolate (PPRI 20167) had a lower percentage similarity of 90.87% and was similar to *F. cuneirostrum* NRRL 31104 in the FSSC. This strain was isolated from bean in Japan and the species has been reported as the causal agent of soybean sudden death syndrome (SDS) in Brazil and the root-rot of dry bean in the USA, Canada and Japan (Aoki *et al.*, 2005; Henriquez *et al.*, 2014). *Fusarium cuneirostrum* has not been reported from South Africa until current. Twenty strains (PPRI 23476, 23477, 23479, 23480, 23481, 23482, 23483, 23484, 23485, 23486, 23487, 23488, 23489, 23490, 23491, 23492, 23493, 23494, 23495 and 23496) showed significant similarity with *F. solani,* NRRL 28579 in the FSSC, with a percentage similarity of 100%. *Fusarium solani* has been found in soil, rotten plant material and as a pathogen of pea, cucurbits, and sweet potato (Zhang *et al.*, 2006). This result suggests that *F. solani* is associated with FW of sweet potato in South Africa. Moreover, the FSSC has been reported to contain over 45 phylogenetically different species scattered amongst three major clades (Zhang *et al.*, 2006). Although outside the scope of the obtained results of the present study, incorporating into the analyses of Zhang *et al*. (2006), will provide a wider phylogenetic view of the complex as the FSSC phylogenetic analysis should be done in the future study.

Furthermore, four fungal isolates (5%) did not belong to any *Fusarium* species complex and were represented by *Clonostachys corda* Corda, and one unidentified Hypocreales (1%) (Table 4.1). Four PPRI strains (PPRI 18014, 18017, 18018 and 18751) showed 98.87-100% similarity to *C. corda* CBS 124754, while PPRI 9462 showed 100% similarity with an unidentified Hypocreales isolate KC461320.

The *Fusarium* MLST database nBLAST™ results for the 89 fungal isolates recognised 57 FOSC isolates that included 55 *F. oxysporum* isolates (62%) and two *F. inflexum* isolates (2%). PPRI 20170 and 23474 had a 100% similarity to *F. inflexum* NRRL 20433. *Fusarium inflexum* was first reported as a causal agent of a vascular wilt of broad bean in Germany (Schneider and Dalchow, 1975). The TEF-1α *Fusarium* MLST database nBLAST™ results revealed a total of 21 sweet potato strains represented by *F. oxysporum* that clustered in the FOSC which were not associated with any *formae speciales,* while 34 sweet potato strains were associated with 13 different *formae speciales* (Figure 4.10).

Twelve sweet potato strains (13%) (PPRI 9464, 9466, 17592, 17593, 17594, 17595, 17596, 18750, 18752, 18753, 23066 and 23071) had 100% similarity to *F. oxysporum f. sp. vanillae* NRRL 26448 MLST type 77 (source: *Vanilla* sp.; origin: USA) (Figure 4.11). This indicated that *F. oxysporum f. sp. vanillae* are associated with FW of sweet potato in South Africa. *Vanilla* is cultivated worldwide (Harris, 1992), valued for its flavour abilities and production of food additives (Ramachandra and Ravishankar, 2000). In South Africa, the cultivation of *Vanilla* is uncommon but the possibility of infection has been identified during this study and indicated that there is a probability of pathogens associated with *Vanilla* in South Africa.

Ten strains (11%) (PPRI 9459, 9461, 9465, 18016, 23065, 23068, 23069, 23070, 23076 and 23078) were similar to *F. oxysporum f. sp. erythroxyli* NRRL 26574 MLST type 79 (source: *Erythroxylum coca*; origin: USA) with similarities ranging from 99.85-100%. Two sweet potato strains (3%), PPRI 20173 and 20174, had percentage similarities of 100% and 96.23%, respectively, with *F. oxysporum f. sp. dianthi* NRRL 28365 MLST type 101 (source: *Dianthus caryophyllus*; origin: Netherlands).

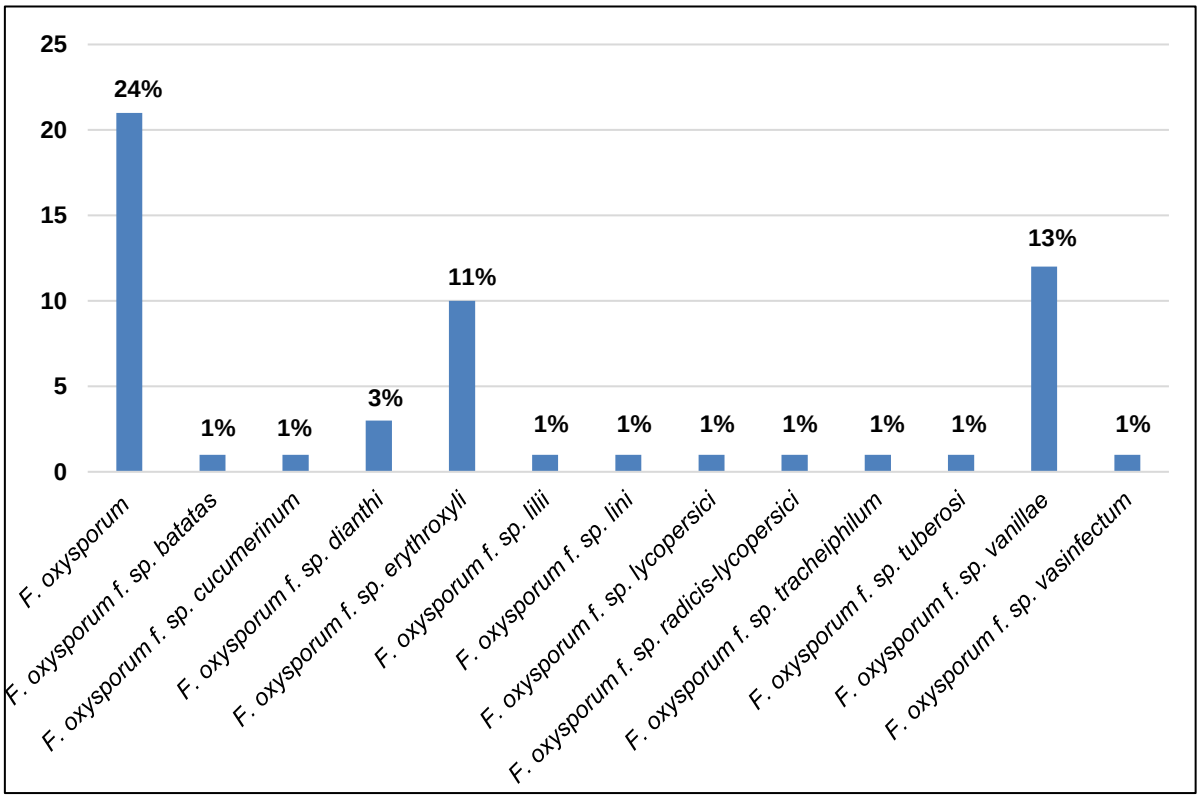


Figure 4.10: FOSC identified using *Fusarium* MLST database nBLAST™ analyses discovered from diseased sweet potato material collected from Eastern Cape, Gauteng, Limpopo, Mpumalanga, Northern Cape and Western Cape provinces of South Africa. The numbers of FOSC are indicated on the Y-axis. The percentages of FOSC are indicated on the X-axis.

Ten *formae speciales* were represented by one strain each. These include PPRI 23063 with 100% similarity to *F. oxysporum f. sp. dianthi* NRRL 36356 MLST type 158 (source: *Dianthus* sp.; origin: Argentina); PPRI 20178 with 99.52% similarity to *F. oxysporum f. sp. batatas* NRRL 36135 MLST type 142 (source: *Ipomoea* sp.; origin; Unknown); PPRI 23064 with 99.54% similarity to *F. oxysporum f. sp. lilii* NRRL 28395 MLST type 107 (source: *Lilium* sp.; origin: Italy); PPRI 20165 with 99.52% similarity to *F. oxysporum f. sp. radicis-lycopersici* NRRL 26033 MLST type 40 (source: *Solanum esculentum*; origin: USA); PPRI 20177 with 100% similarity to *F. oxysporum f. sp. lini* NRRL 36286 MLST type 154 (source: *Linum usitatissimum*; origin: Unknown); PPRI 23074 with 100% similarity to *F. oxysporum f. sp. lycopersici* NRRL 26203 MLST type 43 (source: *Solanum esculentum*; origin: Italy); PPRI 23072 with 100% similarity to *F. oxysporum f. sp. tracheiphilum* NRRL 22554 MLST type 20

(source: *Chrysanthemum* sp.; origin: Nigeria); PPRI 23062 with 100% similarity to *F. oxysporum f. sp. tuberosi* NRRL 22555 MLST type 21 (source: *Solanum tuberosum*; origin: Iran); PPRI 20163 with 100% similarity to *F. oxysporum f. sp. vasinfectum* NRRL 25420 MLST type 28 (source: *Gossypium* sp.; origin: USA) and PPRI 20176 with 99.84% similarity to *F. oxysporum f. sp. cucumerinum* NRRL 38591 MLST type 191 (source: *Cucumis sativus*; origin: New Zealand).

Fusarium oxysporum formae speciales vanillae was the dorminant *formae speciales* discovered from the diseased sweet potato in South Africa, followed by *F. oxysporum f. sp. erythroxyli*. A number of *formae speciales* have been revealed to cross-infect the hosts of other *formae speciales* (O'Donnell *et al.*, 2009a). Isolates designated as a specific *forma specialis* can possibly be classified as another *forma specialis*, for an example *F. oxysporum f. sp. vasinfectum* (Davis *et al.*, 2006).

The FOSC is phylogenetic diverse, hence, MLST offers a useful approach for characterising the genetic diversity within this complex (O'Donnell *et al.*, 2009a). A two loci DNA sequence database, comprising of TEF-1α and IGS sequences indicated that FOSC consists of 256 universal sequence types (STs) amongst 850 isolates, mostly plant pathogens (O'Donnell *et al.*, 2009a). The 256 haplotypes were broken down into seven haplotype groups based on sequence types that were associated with hosts. Halotype group 1 and 2 were associated with a single host, however, the pathogenicity within halotype group 2 was not determined for the 58 STs, except for those isolates revealed as non-pathogenic to a specific host. Halotype groups 3 – 5 were associated with two or more hosts. Haplotype group 6 and 7 contained indoor contaminents or strains who's host/source data is not complete and included ST 54. The ST 54 have been recovered as hospital contaminants and from mycotic infection of humans indicating nosocomiality. Halotype group 7 contained at least one isolate in each ST being recovered from an opportunistic infection of humans or the other animals. Human pathogens within the FOSC are genetically diverse as they are nested within the three major clades that consist of of the phylogenetic breadth of FOSC (O'Donnell *et al.*, 1998b; O'Donnell *et al.*, 2004). ST 48 = FOSC 4-b was recovered from opportunistic infections of humans. Furthermore, Laurence *at al.* (2012) reported an additional 21 STs based on TEF-1α sequences.

The multiloci DNA sequence typing identified 21 MLST types associated with FW of sweet potato in South Africa, based on the *Fusarium* MSLT database (Figure 4.11). Of the 21 MLSTs in the *Fusarium* MLST database, 13 were designated as part of the 68 described *formae speciales* while the remaining eight MLSTs were not designated as a *formae speciales* and were associated with corneal scraping, human cornea, clinical isolate, guar medicinal plant, *Allium cepa*, garden pea, *Lepidozamia peroffskyana* and unknown host based on the origin host description.

O'Donnell *et al.* (2009a) reported that ST 28 was the most common ST represented in the FOSC database and was designated as *F. oxysporum f. sp. vasinfectum* while this study revealed only one ST 28. The sweet potato isolates obtained from South Africa clustered within all of the identified universal MLSTs reported by O'Donnell *et al.* (2009a). MLST 77, with 12 isolates, was the most common MLST represented in the *Fusarium* MLST database, followed by MLST 79 with 10 isolates. Sampling from diseased sweet potato material identified species of approximately 8% of the known universal MLSTs.

There were eleven MLSTs (52%) associated with sweet potato strains obtained from one farm in Gauteng Province. In addition, MLST 53 was the most common MLST found composed of 24% with five isolates. There were ten MLSTs (47.6%) associated with sweet potato strains obtained from eight farms in Limpopo Province. In addition, MLST 77 was the most common MLST found composed of 38% with eight isolates. There were three MLSTs (14%) associated with sweet potato strains obtained from two farms in Mpumalanga Province. There were two MLSTs (9.5%) associated with sweet potato strains obtained from four farms in Western Cape Province. There were two MLSTs (9.5%) associated with sweet potato strains obtained from two farms in Eastern Cape Province. There were nine MLST types associated with the South African sweet potato isolates that clustered into haplotype group 1, namely, MLST 20, 21, 40, 79, 101, 107, 142, 154 and 158 (O'Donnell *et al.*, 2009a). There were five MLST types associated with the South African sweet potato isolates that clustered into haplotype group 2, namely, MLST 188, 213, 217, 233 and 244 (O'Donnell *et al.*, 2009a).

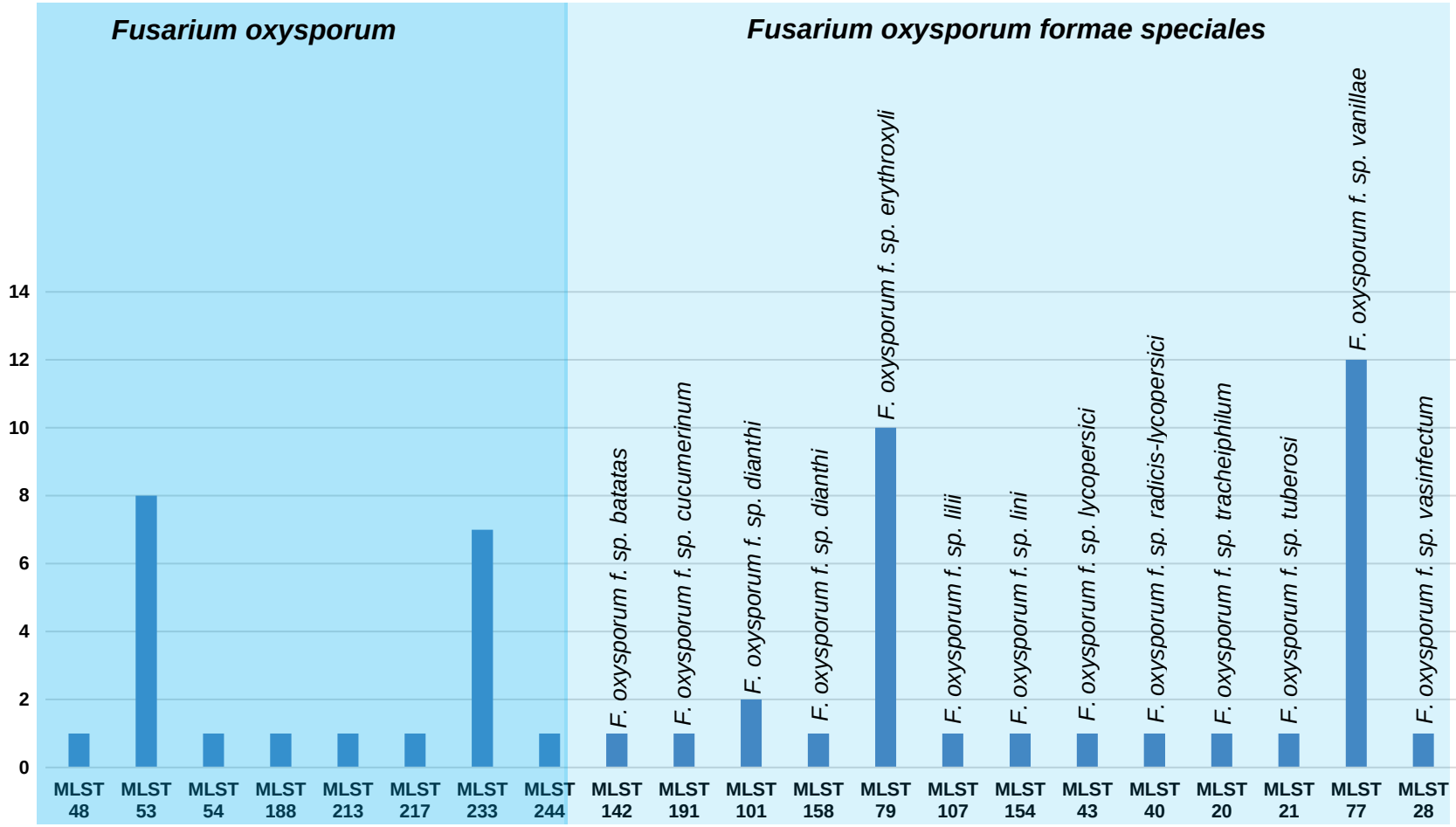


Figure 4.11: Number of MLST types discovered based on the *Fusarium* MLST database. *Fusarium* strains isolated from diseased sweet potato plant material collected from Eastern Cape, Gauteng, Limpopo, Mpumalanga, Northern Cape and Western Cape provinces of South Africa.

Only one MLST type, MLST 43, associated with the South African sweet potato strains, clustered into haplotype group 3 (O'Donnell *et al.*, 2009a). There were three MLST types associated with the South African sweet potato strains that clustered into haplotype group 4, namely, MLST 28, 191 and 77. Lastly, there were three MLST types associated with the South African sweet potato strains that clustered into haplotype group 7, namely, MLST 48, 53 and 54. South African MLST types were present in haplotype group 1, 2, 3, 4 and 7 but were not present in haplotype group 5 and 6. These results indicates that South African MLST types are genetically diverse. The MLST types associated with South Africa are known from Argentinia, Egypt, Iran, Italy, Netherlands, New Zealand, Nigeria, and USA as the countries of origin.

The *Fusarium* MLST database based on TEF-1α sequences was able to reveal nBLAST™ results to genus, species level and *formae speciales* level. The TEF-1α sequences were able to align across the members of the FOSC as it consists of variable introns (Geiser et *al.*, 2004; O'Donnell *et al.*, 2015). The FOSC sequence data results were therefore, further evaluated by conducting a phylogenetic analyses and morphological characterisation.

4.3.2 *Fusarium*-ID database nBLAST™ results based on TEF-1α sequences for isolates obtained from sweet potato material

The *Fusarium*-ID database nBLAST™ results based on the TEF-1α sequences of 89 strains obtained from diseased sweet potato material clustered into four *Fusarium* species complexes and included four *Fusarium* species (Table 4.1). The four *Fusarium* species complexes identified were *F. graminearum* species complex (FGSC), FIESC, FSSC and FOSC. The species in the complexes were represented by *F. graminearum* Schwabe in the FGSC, *F. lacertarum* in the FIESC, *F. petroliphilum* (Q.T. Chen & X.H. Fu) Geiser, O'Donnell, D.P.G. Short & N. Zhang in the FSSC and *F. oxysporum* in the FOSC (Figure 4.12). These results for isolates obtained from diseased sweet potato material indicated a significant percentage similarity of 99.56%-100% for most of the isolates as indicated in Table 4.1.

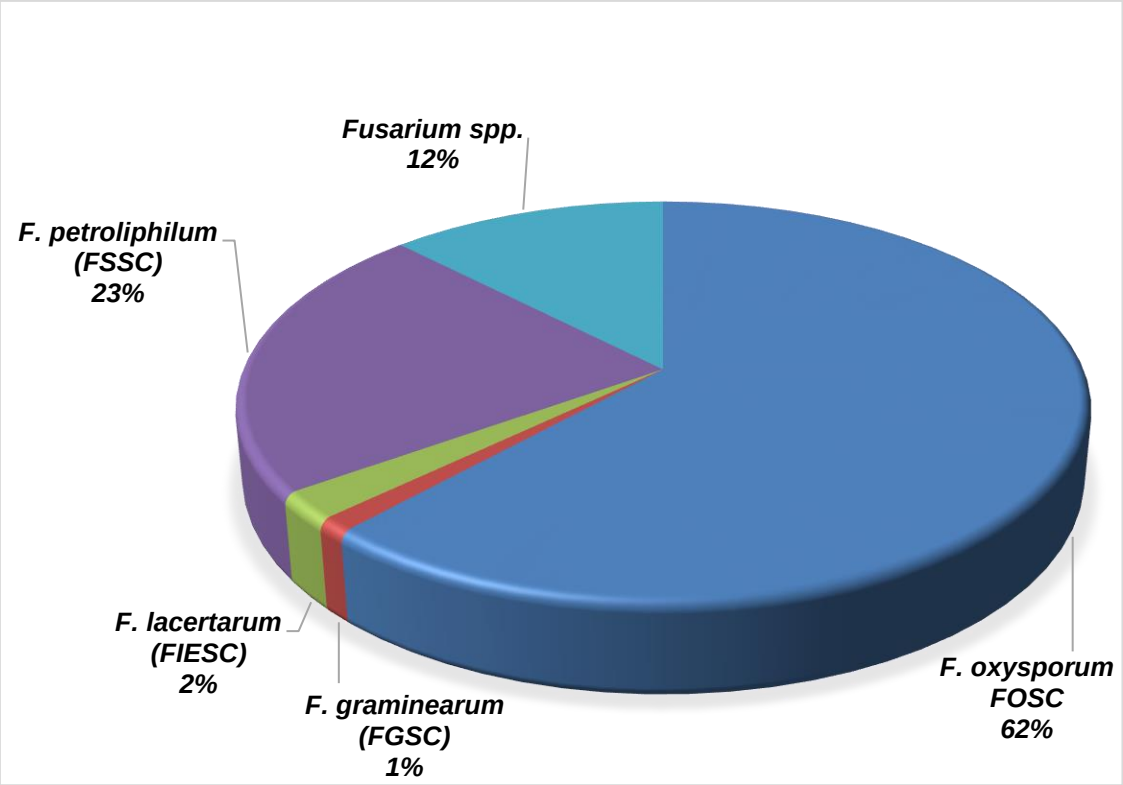


Figure 4.12: *Fusarium* species complexes and other species identified using *Fusarium*-ID database nBLAST™ analyses for isolates recovered from diseased sweet potato material collected from Eastern Cape, Gauteng, Limpopo, Mpumalanga, Northern Cape and Western Cape provinces of South Africa.

The geographic distribution of the *Fusarium* species recovered, consisted of mostly *F. oxysporum* from all the provinces sampled and included *Fusarium* spp. recovered from Gauteng, North West, Limpopo and Mpumalanga Province. Mpumalanga Province was mostly represented by *F. petroliphilum* isolates and included *F. lacertarum* and *F. graminearum*.

The FGSC was represented by PPRI 9462 that was similar to *F. graminearum* NRRL 5883 with a percentage similarity of 96.06%. Two strains (2%) (PPRI 23473 and 23475) had a similarity of 99.11% and 98.52% respectively, to *F. lacertarum* NRRL 20423 MLST type 4-a in the FIESC. Four strains (PPRI 18014, 18017, 18018 and 18751) showed a similarity to *Fusarium* sp. NRRL 43635 MLST type 13-a in the FIESC with 94.55% similarity. One obtained strain (PPRI 23061) was 98.96% similar

to *Fusarium* sp. NRRL 36392 no MLST type in the FIESC, while PPRI 23478 showed a 100% similarity to *Fusarium* sp. NRRL 36323 MLST type 3-a in the FIESC.

The FSSC was represented by 20 (22%) strains and were 100% similar to *F. petroliphilum* NRRL 28546 MLST type 1-a isolates. *Fusarium solani var. petroliphilum* was originally isolated from degraded petroleum, however, *F. solani var. petroliphilum* has also been isolated from oily substrates, in plumbing drain biofilms and outbreaks of contact lens-associated mycotic keratitis (Summerbell and Schroers, 2002; Chang *et al.*, 2006; Khor *et al.*, 2006; Imamura *et al.*, 2008; Ahearn *et al.*, 2009; Short *et al.*, 2013). Short *et al.* (2013) reported *F. petroliphilum* as a plant pathogen that causes fruit rot of cucurbits and later shown to be identical to *F. solani f. sp. cucurbitae* race 2 (O'Donnell, 2000). In addition, *F. solani* has been found in soil, rotten plant material and as a pathogen of cucurbits and sweet potato (Zhang *et al.*, 2006). This study is the first report of *F. petroliphilum* being associated with FW of sweet potato in South Africa.

The FOSC was represented by 55 *F. oxysporum* isolates (62%). Five strains (PPRI 20169, 20170, 23064, 23067 and 23474) were similar to *Fusarium* sp. NRRL 45881 MLST type 19 in the FOSC with percentage similarity ranging from 99.54-100%. A total of eleven obtained strains were represented by *Fusarium* spp. *Fusarium* spp. represents unnamed species based on the *Fusarium*-ID database results therefore, the sequences should be subjected to a GCPSR analysis (Taylor *et al.*, 2000).

The TEF-1α *Fusarium* ID database nBLAST™ results revealed a total of 50 sweet potato strains represented by *F. oxysporum* that clustered in the FOSC which were not associated with any *formae speciales,* while five sweet potato strains were associated with three different *formae speciales* (Figure 4.13). 50 sweet potato strains represented by *F. oxysporum* clustered in the FOSC with a percentage similarity ranging from 98.14-100% (Table 4.1). The TEF-1α *Fusarium*-ID database nBLAST™ results revealed a total of five sweet potato strains represented by *formae speciales* that clustered in the FOSC with a percentage similarity ranging from 98.05-100%. These included three sweet potato strains (PPRI 20173, 20174 and 20176) similar to *F. oxysporum f. sp. cucumerinum* NRRL 38591 MLST 191 with a percentage

similarity of 98.05-99.85%. Two sweet potato strains (PPRI 23072 and 20166) were similar to *F. oxysporum f. sp. lupini* NRRL 26225 MLST 47 (source: *Lupinus* sp.; origin: USA) and *F. oxysporum f. sp. vasinfectum* NRRL 34087 MLST 29 with a percentage similarity of 100% and 99.56%, respectively.

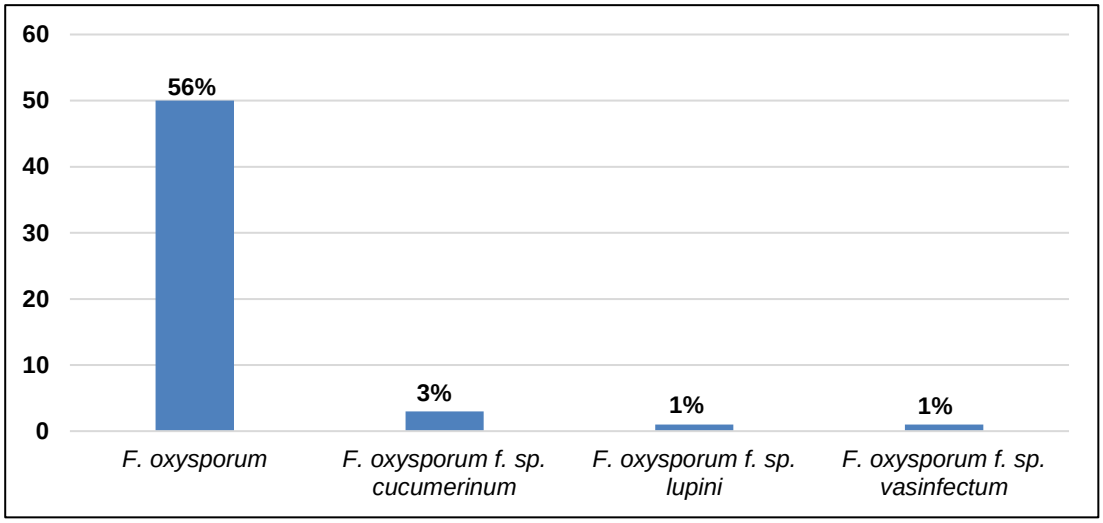


Figure 4.13: FOSC identified using *Fusarium*-ID database nBLAST™ analyses discovered from diseased sweet potato material collected from Eastern Cape, Gauteng, Limpopo, Mpumalanga, Northern Cape and Western Cape provinces of South Africa. The numbers of FOSC are indicated on the Y-axis. The percentages of FOSC are indicated on the X-axis.

Fusarium oxysporum formae speciales lupini and *F. oxysporum f. sp. vasinfectum* were the least represented *formae speciales* recovered. *Fusarium oxysporum formae speciales cucumerinum* was the most recovered *formae speciales* amongst the 55 FOSC isolates. The *Fusarium*-ID database revealed only three *formae speciales*, in contrast to the 13 *formae speciales* revealed by the *Fusarium* MLST database.

Multiloci DNA sequence typing identified ten MLST types associated with FW of sweet potato in South Africa, based on the *Fusarium*-ID database (Figure 4.14.). Of the ten MLSTs, three were designated as part of the 68 described *formae speciales* while the remaining seven MLSTs were not designated as a *formae speciales* within the *Fusarium*-ID database. The sweet potato isolates obtained from South Africa clustered in all of the identified universal STs reported by O'Donnell *et al.* (2009a). Most isolates from the Limpopo Province clustered with MLST 77 and MLST 232, the

most dominant MLSTs. MLST 47 was associated with the South African sweet potato isolates, clustered into haplotype group 1 (O'Donnell *et al.*, 2009a).

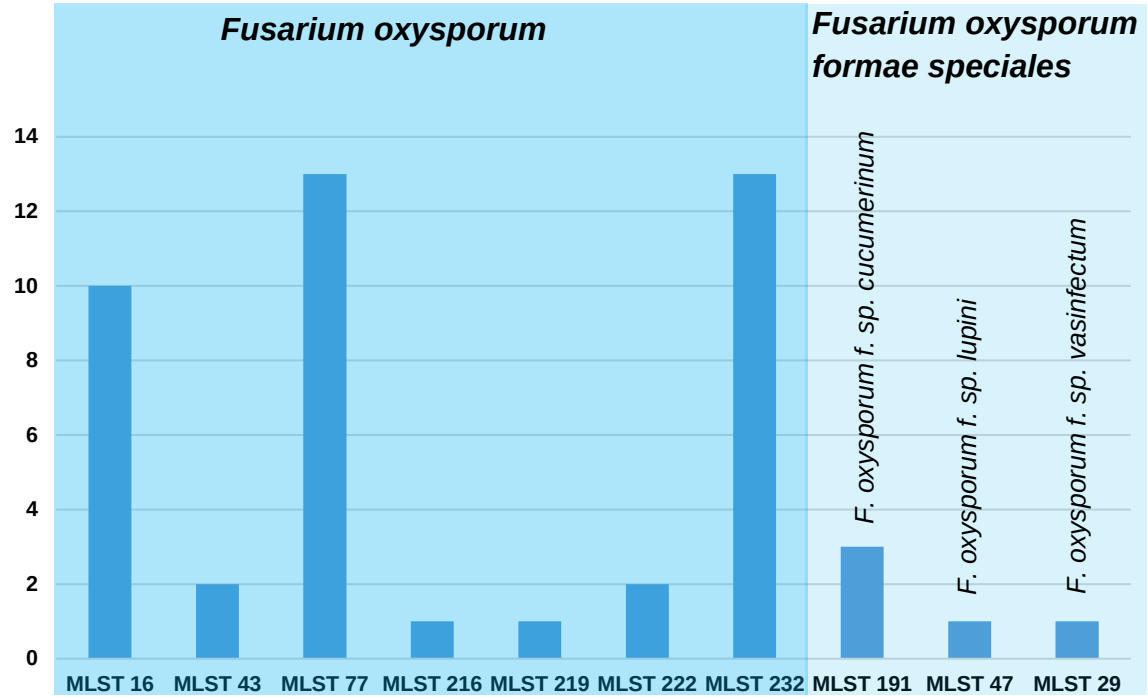


Figure 4.14: Number of MLST types discovered based on the *Fusarium*-ID database. *Fusarium* strains isolated from diseased sweet potato plant material collected from Eastern Cape, Gauteng, Limpopo, Mpumalanga, Northern Cape and Western Cape provinces of South Africa.

There were three MLST types associated with the South African sweet potato isolates that clustered into haplotype group 2, namely, MLST 216, 219 and 232 (O'Donnell *et al.*, 2009a). Only isolates associated with MLST 43, clustered into haplotype group 3 (O'Donnell *et al.*, 2009a). There were four MLST types associated with the South African sweet potato isolates that clustered into haplotype group 4, namely, MLST 16, 29, 77 and 191 (O'Donnell *et al.*, 2009a). Only one MLST type, MLST 222, associated with the South African sweet potato isolates, clustered into haplotype group 5 (O'Donnell *et al.*, 2009a). South African MLST types were present in haplotype group 1, 2, 3, 4 and 5 but were not present in haplotype group 6 and 7 based on the *Fusarium*-ID database. This results indicates that South African MLST types are genetically diverse. *Fusarium*-ID database identified seven *F. oxysporum* MLSTs associated with *Dianthus caryophyllus*, *Asparagus*, *Passiflora edulis*,

Colocasia esculenta, *Zea mays*, and unknown hosts plants based on the origin host description.

The *Fusarium* MLST database supported the results obtained from the *Fusarium*-ID database as both databases resulted in 62% of the FOSC isolate identifications. Discrepancies included the different *Fusarium* spp. represented by two strains, PPRI 20167 and 23064. PPRI 20167 was identified as *F. cuneirostrum* based on the the *Fusarium* MLST database whereas the *Fusarium*-ID database identified it as part of the *F. oxysporum* in the FOSC. The second strain PPRI 23064 was identified as *F. oxysporum f. sp. lilii* in the FOSC based on the *Fusarium* MLST database whereas *Fusarium*-ID database identified the same strain as *Fusarium* sp. in the FOSC. Differences were also observed in the identifications for PPRI 9462 as it was identified as an unidentified Hypocreales the *Fusarium*-MLST database whereas the *Fusarium*-ID database identified it as *F. graminearum*. *Fusarium* MLST database revealed four *C. corda* isolates whereas the identifications based on the *Fusarium*-ID database revealed these four same isolates as *Fusarium* species within FIESC. In some cases, the *Fusarium* MLST database determined the identification of isolates to species level whereas *Fusarium*-ID determined the identification of isolates to genus level.

4.3.3 *Fusarium*-MLST database nBLAST™ results based on TEF-1α sequences for isolates obtained from soil

The *Fusarium* MLST database nBLAST™ results based on the TEF-1α sequences of 189 strains obtained from soil clustered into five *Fusarium* species complexes that comprised of seven *Fusarium* species and one species that did not belong to any species complex. The TEF-1α *Fusarium* MLST database nBLAST™ analyses indicated a significant percentage similarities ranging from 99%-100% for most isolates (Table 4.2). The five *Fusarium* species complexes were FFSC, FIESC, *F. sambucinum* species complex (FSASC), FSSC and FOSC. The species in the complexes were represented by *F. nygamai* L.W. Burgess & Trimboli in the FFSC, *F. lacertarum* in the FIESC, *F. brachygibbosum* Padwick in the FSASC, *F. falciforme* (Carrión) Summerb. & Schroers and *F. solani* in the FSSC and *F. inflexum* and *F. oxysporum* in the FOSC (Figure 4.15). *Fusarium burgessii* M.H. Laurence, Summerell & E.C.Y. Liew did not belong to any *Fusarium* species complex.

Table 4.2: *Fusarium* MLST and *Fusarium*-ID nBLAST™ results of TEF-1α from soil fungal isolates

	***FUSARIUM* MLST DATABASE**						***FUSARIUM*-ID DATABASE**					
PPRI Number	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**
21929	*F. oxysporum f. sp. melonis*	FOSC	167	CBS 420.90	EF056790	99.69	*F. oxysporum f. sp. koae*	FOSC	231	NRRL 38885	FJ985418	99.70
21930	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	99.85	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	99.85
21931	*F. oxysporum*	FOSC	210	NRRL 38477	FJ985397	100	*F. oxysporum f. sp. cucumerinum*	FOSC	191	NRRL 38591	FJ985379	100
21932	*F. oxysporum f. sp. cucumerinum*	FOSC	191	NRRL 38591	FJ985379	100	*F. oxysporum f. sp. cucumerinum*	FOSC	191	NRRL 38591	FJ985379	100
21933	*F. oxysporum*	FOSC	210	NRRL 38477	FJ985397	100	*F. oxysporum f. sp. cucumerinum*	FOSC	191	NRRL 38591	FJ985379	100
21934	*F. oxysporum f. sp. lini*	FOSC	154	NRRL 36286	FJ985344	100	*F. oxysporum*	FOSC	222	NRRL 38592	KM092476	99.85
21935	*F. oxysporum f. sp. cucumerinum*	FOSC	191	NRRL 38591	FJ985379	100	*F. oxysporum f. sp. cucumerinum*	FOSC	191	NRRL 38591	FJ985379	100
21936	*F. oxysporum f. sp. cucumerinum*	FOSC	191	NRRL 38591	FJ985379	100	*F. oxysporum f. sp. cucumerinum*	FOSC	191	NRRL 38591	FJ985379	100
21937	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	99.22	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	99.22
21938	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	100	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	100
21939	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	97.92	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	97.92
21940	*F. falciforme*	FSSC	3+4-ss	NRRL 32729	DQ247049	99.55	*F. falciforme*	FSSC	3+4-rr	NRRL 32727	DQ247047	99.40
21941	*F. oxysporum f. sp. cucumerinum*	FOSC	191	NRRL 38591	FJ985379	100	*F. oxysporum f. sp. cucumerinum*	FOSC	191	NRRL 38591	FJ985379	100
21942	*F. falciforme*	FSSC	3+4-ss	NRRL 32729	DQ247049	99.53	*F. falciforme*	FSSC	3+4-rr	NRRL 32727	DQ247047	99.37
21943	*F. falciforme*	FSSC	3+4-r	NRRL 28565	DQ246906	99.85	*F. falciforme*	FSSC	3+4-r	NRRL 28565	DQ246906	99.85
21944	*F. nygamai*	FFSC	N/A	CBS 140.95	HM347121	100	*F. nygamai*	FFSC	N/A	NRRL 26421	HM347121	100
21945	*F. oxysporum*	FOSC	210	NRRL 38477	FJ985397	100	*F. oxysporum f. sp. cucumerinum*	FOSC	191	NRRL 38591	FJ985397	100
21946	*F. oxysporum f. sp. erythroxyli*	FOSC	79	NRRL 26574	AF008495	100	*F. oxysporum*	FOSC	232	NRRL 39464	FJ985419	100
21947	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	97.92	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	97.91
21948	*F. inflexum*	FOSC	N/A	CBS 716.74	AF008479	99.85	*Fusarium* sp.	FOSC	19	NRRL 45881	N/A	99.85
21949	*F. falciforme*	FSSC	3+4-ss	NRRL 32729	DQ247049	99.55	*F. falciforme*	FSSC	3+4-rr	NRRL 32727	DQ247047	99.40

	***FUSARIUM* MLST DATABASE**						***FUSARIUM*-ID DATABASE**					
PPRI Number	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**
21950	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	99.25	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	99.25
21951	*F. oxysporum*	FOSC	210	NRRL 38477	FJ985397	100	*F. oxysporum f. sp. cucumerinum*	FOSC	191	NRRL 38591	FJ985379	100
21952	*F. falciforme*	FSSC	3+4-k	NRRL 28548	DQ246889	100	*F. falciforme*	FSSC	3+4-y	NRRL 32331	DQ246959	100
21953	*F. inflexum*	FOSC	N/A	CBS 716.74	AF008479	99.85	*Fusarium* sp.	FOSC	19	NRRL 45881	N/A	99.85
21954	*F. oxysporum*	FOSC	210	NRRL 38477	FJ985397	100	*F. oxysporum f. sp. cucumerinum*	FOSC	191	NRRL 38591	FJ985379	100
21955	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	97.64	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	99.64
24308	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	97.92	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	97.92
21956	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	97.93	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	97.92
21957	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	97.69	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	97.69
21958	*F. oxysporum f. sp. lini*	FOSC	154	NRRL 36286	FJ985344	100	*F. oxysporum*	FOSC	222	NRRL 38592	KM092476	99.85
21959	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	99.26	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	99.25
21960	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	99.85	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	99.85
21961	*Fusarium* sp.	FIESC	3-b	NRRL 28029	GQ505602	100	*Fusarium* sp.	FIESC	3-b	NRRL 28029	GQ505602	100
21962	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	97.92	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	97.91
21963	*F. inflexum*	FOSC	N/A	CBS 716.74	AF008479	100	*Fusarium* sp.	FOSC	19	NRRL 45881	N/A	100
21964	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	97.92	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	97.92
21965	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	97.92	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	97.91
21966	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	97.92	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	97.92
21968	*F. oxysporum f. sp. vasinfectum*	FOSC	28	NRRL 25420	AF008512	100	*F. oxysporum*	FOSC	219	NRRL 38514	FJ985406	100
21969	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	100	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	100
21970	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	99.70	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	99.70
21971	*F. oxysporum*	FOSC	210	NRRL 38477	FJ985397	100	*F. oxysporum f. sp. cucumerinum*	FOSC	191	NRRL 38591	FJ985379	100
21972	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	97.92	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	97.92
21973	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	100	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	100
21974	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	99.70	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	99.70

	FUSARIUM MLST DATABASE						*FUSARIUM*-ID DATABASE					
PPRI Number	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**
21975	*F. oxysporum f. sp. erythroxyli*	FOSC	79	NRRL 26574	AF008495	100	*F. oxysporum*	FOSC	232	NRRL 39464	FJ985419	100
21976	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	100	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	100
21977	*F. inflexum*	FOSC	N/A	CBS 716.74	AF008479	99.69	*Fusarium* sp.	FOSC	19	NRRL 45881	N/A	99.56
21992	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	97.91	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	97.91
22319	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	97.92	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	97.92
22320	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	97.92	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	97.92
22321	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	99.70	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	99.70
22322	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	99.24	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	99.24
22323	*F. oxysporum*	FOSC	210	NRRL 38477	FJ985397	100	*F. oxysporum f. sp. cucumerinum*	FOSC	191	NRRL 38591	FJ985379	100
22324	*F. oxysporum*	FOSC	210	NRRL 38477	FJ985397	100	*F. oxysporum f. sp. cucumerinum*	FOSC	191	NRRL 38591	FJ985379	100
22325	*F. falciforme*	FSSC	3+4-k	NRRL 28548	DQ246889	100	*F. falciforme*	FSSC	3+4-y	NRRL 32331	DQ246959	100
22326	*F. oxysporum f. sp. melonis*	FOSC	167	CBS 420.90	EF056790	99.54	*F. oxysporum f. sp. koae*	FOSC	231	NRRL 38885	FJ985418	99.55
22327	*F. oxysporum f. sp. lini*	FOSC	154	NRRL 36286	FJ985344	100	*F. oxysporum*	FOSC	222	NRRL 38592	KM092476	99.85
22328	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	97.90	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	97.89
22329	*F. oxysporum f. sp. cucumerinum*	FOSC	191	NRRL 38591	FJ985379	100	*F. oxysporum f. sp. cucumerinum*	FOSC	191	NRRL 38591	FJ985379	100
22330	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	100	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	100
22331	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	99.26	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	99.25
23578	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	99.56	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	99.55
23579	*F. falciforme*	FSSC	3+4-k	NRRL 28548	DQ246889	100	*F. falciforme*	FSSC	3+4-y	NRRL 32331	DQ246959	100
23580	*F. inflexum*	FOSC	N/A	CBS 716.74	AF008479	100	*Fusarium* sp.	FOSC	19	NRRL 45881	N/A	100
23581	*F. falciforme*	FSSC	3+4-v	NRRL 32308	DQ246936	100	*F. falciforme*	FSSC	3+4-v	NRRL 32308	DQ246936	100
23582	*F. oxysporum f. sp. tuberosi*	FOSC	21	NRRL 22555	AF008511	100	*F. oxysporum*	FOSC	216	NRRL 38501	FJ985403	100
23583	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	99.70	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	99.70
23584	*F. oxysporum f. sp. vanillae*	FOSC	77	NRRL 26448	FJ985300	100	*F. oxysporum*	FOSC	77	NRRL 36341	N/A	100

	FUSARIUM MLST DATABASE						*FUSARIUM*-ID DATABASE					
PPRI Number	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**
23585	*F. solani*	FSSC	N/A	CBS 318.73	DQ247642	100	*F. falciforme*	FSSC	3+4-ii	NRRL 32542	KR673929	100
23586	*F. solani*	FSSC	N/A	CBS 318.73	DQ247642	100	*F. falciforme*	FSSC	3+4-ii	NRRL 32542	KR673929	100
23587	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	99.56	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	99.55
23588	*F. falciforme*	FSSC	3+4-k	NRRL 28548	DQ246889	100	*F. falciforme*	FSSC	3+4-y	NRRL 32331	DQ246959	100
23589	*F. oxysporum f. sp. erythroxyli*	FOSC	79	NRRL 26574	AF008495	100	*F. oxysporum*	FOSC	232	NRRL 39464	FJ985419	100
23590	*F. oxysporum f. sp. erythroxyli*	FOSC	79	NRRL 26574	AF008495	100	*F. oxysporum*	FOSC	232	NRRL 39464	FJ985419	100
23591	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	99.56	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	99.55
23592	*F. oxysporum*	FOSC	243	NRRL 45945	FJ985430	100	*F. oxysporum*	FOSC	67	NRRL 38599	KM092474	100
23593	*F. oxysporum f. sp. erythroxyli*	FOSC	79	NRRL 26574	AF008495	100	*F. oxysporum*	FOSC	232	NRRL 39464	FJ985419	100
23594	*F. inflexum*	FOSC	N/A	CBS 716.74	AF008479	100	*Fusarium* sp.	FOSC	19	NRRL 45881	N/A	100
23595	*F. oxysporum f. sp. erythroxyli*	FOSC	79	NRRL 26574	AF008495	100	*F. oxysporum*	FOSC	232	NRRL 39464	FJ985419	100
23596	*F. oxysporum f. sp. erythroxyli*	FOSC	79	NRRL 26574	AF008495	100	*F. oxysporum*	FOSC	232	NRRL 39464	FJ985419	100
23597	*F. inflexum*	FOSC	N/A	CBS 716.74	AF008479	100	*Fusarium* sp.	FOSC	19	NRRL 45881	N/A	100
23614	*F. oxysporum f. sp. erythroxyli*	FOSC	79	NRRL 26574	AF008495	100	*F. oxysporum*	FOSC	232	NRRL 39464	FJ985419	100
23615	*F. oxysporum f. sp. tuberosi*	FOSC	21	CBS 797.70	AF008511	100	*F. oxysporum*	FOSC	216	NRRL 38501	FJ985403	100
23616	*F. oxysporum f. sp. erythroxyli*	FOSC	79	NRRL 26574	FJ985379	100	*F. oxysporum*	FOSC	232	NRRL 39464	FJ985419	100
23617	*F. inflexum*	FOSC	N/A	CBS 716.74	AF008479	100	*Fusarium* sp.	FOSC	19	NRRL 45881	N/A	100
23618	*Fusarium* sp.	FFSC	none	NRRL 26756	N/A	99.85	*Fusarium* sp.	N/A	N/A	NRRL 26061	AF160303	99.84
23619	*F. oxysporum f. sp. erythroxyli*	FOSC	79	NRRL 26574	AF008495	100	*F. oxysporum*	FOSC	232	NRRL 39464	FJ985419	100
23620	*F. oxysporum f. sp. erythroxyli*	FOSC	79	NRRL 26574	AF008495	100	*F. oxysporum*	FOSC	232	NRRL 39464	FJ985419	100
23621	*F. oxysporum f. sp. cucumerinum*	FOSC	191	NRRL 38591	FJ985379	100	*F. oxysporum f. sp. cucumerinum*	FOSC	191	NRRL 38591	FJ985379	100
23622	*F. oxysporum*	FOSC	228	NRRL 38595	FJ985415	100	*F. oxysporum*	FOSC	228	NRRL 38595	FJ985415	100
23623	*F. inflexum*	FOSC	N/A	CBS 716.74	AF008479	100	*Fusarium* sp.	FOSC	19	NRRL 45881	N/A	100

	FUSARIUM MLST DATABASE						*FUSARIUM*-ID DATABASE					
PPRI Number	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**
23624	*F. oxysporum f. sp. erythroxyli*	FOSC	79	NRRL 26574	AF008495	100	*F. oxysporum*	FOSC	232	NRRL 39464	FJ985419	100
23625	*F. oxysporum*	FOSC	228	NRRL 38595	FJ985415	100	*F. oxysporum*	FOSC	228	NRRL 38595	FJ985415	100
23626	*F. oxysporum f. sp. erythroxyli*	FOSC	79	NRRL 26574	AF008495	100	*F. oxysporum*	FOSC	232	NRRL 39464	FJ985419	100
23627	*Fusarium* sp.	FFSC	none	NRRL 26756	N/A	99.85	*Fusarium* sp.	N/A	N/A	NRRL 26061	AF160303	99.84
23628	*F. inflexum*	FOSC	N/A	CBS 716.74	AF008479	99.69	*Fusarium* sp.	FOSC	19	NRRL 45881	N/A	99.70
23629	*F. oxysporum f. sp. dianthi*	FOSC	158	NRRL 36356	FJ985348	100	*F. oxysporum*	FOSC	222	NRRL 38592	KM092476	100
23630	*F. oxysporum f. sp. erythroxyli*	FOSC	79	NRRL 26574	AF008495	100	*F. oxysporum*	FOSC	232	NRRL 39464	FJ985419	100
23631	*F. oxysporum f. sp. erythroxyli*	FOSC	79	NRRL 26574	AF008495	100	*F. oxysporum*	FOSC	232	NRRL 39464	FJ985419	100
23872	*Fusarium* sp.	FIESC	22-a	NRRL 34002	GQ505626	98.39	*Fusarium* sp.	FIESC	22-a	NRRL 34002	GQ505626	98.39
23873	*F. oxysporum f. sp. tuberosi*	FOSC	21	CBS 797.70	AF008511	100	*F. oxysporum*	FOSC	216	NRRL 38501	FJ985403	100
23874	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	99.56	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	99.70
23875	*F. oxysporum f. sp. erythroxyli*	FOSC	79	NRRL 26574	AF008495	100	*F. oxysporum*	FOSC	232	NRRL 39464	FJ985419	100
23804	*F. oxysporum*	FOSC	243	NRRL 45945	FJ985430	100	*F. oxysporum*	FOSC	67	NRRL 38599	KM092474	100
23805	*F. oxysporum f. sp. tuberosi*	FOSC	21	CBS 797.70	AF008511	100	*F. oxysporum*	FOSC	216	NRRL 38501	FJ985403	100
23806	*F. oxysporum f. sp. erythroxyli*	FOSC	79	NRRL 26574	AF008495	100	*F. oxysporum*	FOSC	232	NRRL 39464	FJ985419	100
23807	*F. oxysporum f. sp. radicis-lycopersici*	FOSC	40	NRRL 26033	AF008507	100	*F. oxysporum*	FOSC	232	NRRL 39464	FJ985419	100
23808	*F. falciforme*	FSSC	3+4-ss	NRRL 32729	DQ247049	99.55	*F. falciforme*	FSSC	3+4-rr	NRRL 32727	DQ247047	99.40
23809	*Fusarium sp.*	FIESC	5-f	NRRL 45997	GQ505672	99.56	*Fusarium sp.*	FIESC	5-f	NRRL 45997	GQ505672	99.55
23876	*F. nygamai*	FFSC	N/A	CBS 140.95	HM347121	100	*F. nygamai*	FFSC	N/A	NRRL 26421	HM347121	100
23877	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	99.70	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	99.70
23878	*F. oxysporum f. sp. erythroxyli*	FOSC	79	NRRL 26574	AF008495	100	*F. oxysporum*	FOSC	232	NRRL 39464	FJ985419	100
23879	*Fusarium* sp.	FFSC	N/A	NRRL 26756	N/A	99.85	*Fusarium* sp.	N/A	N/A	NRRL 26061	AF160303	99.84
23880	*F. nygamai*	FFSC	N/A	CBS 140.95	HM347121	100	*F. nygamai*	FFSC	N/A	NRRL 26421	HM347121	100

	FUSARIUM MLST DATABASE						*FUSARIUM*-ID DATABASE					
PPRI Number	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**
23881	*F. falciforme*	FSSC	3+4-k	NRRL 28548	DQ246889	100	*F. falciforme*	FSSC	3+4-y	NRRL 32331	DQ246959	100
23810	*F. oxysporum f. sp. erythroxyli*	FOSC	79	NRRL 26574	AF008495	100	*F. oxysporum*	FOSC	232	NRRL 39464	FJ985419	100
23811	*F. oxysporum f. sp. erythroxyli*	FOSC	79	NRRL 26574	AF008495	99.85	*F. oxysporum*	FOSC	232	NRRL 39464	FJ985419	99.85
23812	*F. oxysporum f. sp. erythroxyli*	FOSC	79	NRRL 26574	AF008495	100	*F. oxysporum*	FOSC	232	NRRL 39464	FJ985419	99.85
23813	*F.oxysporum f. sp. erythroxyli*	FOSC	79	NRRL 26574	AF008495	99.85	*F. oxysporum*	FOSC	232	NRRL 39464	FJ985419	99.85
23814	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	99.56	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	99.55
23815	*F. inflexum*	FOSC	N/A	CBS 716.74	AF008479	100	*Fusarium* sp.	FOSC	19	NRRL 45881	N/A	100
23816	*F. inflexum*	FOSC	N/A	CBS 716.74	AF008479	100	*Fusarium* sp.	FOSC	19	NRRL 45881	N/A	100
23817	*F. inflexum*	FOSC	N/A	CBS 716.74	AF008479	100	*Fusarium* sp.	FOSC	19	NRRL 45881	N/A	100
23818	*Fusarium* sp.	FFSC	none	NRRL 26756	N/A	99.85	*Fusarium* sp.	N/A	N/A	NRRL 26061	AF160303	99.84
23819	*F. oxysporum*	FOSC	48	NRRL 43442	DQ790492	100	*F. oxysporum*	FOSC	16	NRRL 38597	N/A	100
23820	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	99.56	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	99.55
23821	*F. inflexum*	FOSC	2	NRRL 20433	AF008479	100	*Fusarium* sp.	FOSC	19	NRRL 45881	N/A	100
23822	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	99.55	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	99.55
23823	*F. oxysporum f. sp. dianthi*	FOSC	46	NRRL 26222	FJ985284	97.63	*F. oxysporum f. sp. dianthi*	FOSC	46	NRRL 38596	KM092479	97.82
23972	*F. brachygibbosum*	FSASC	none	NRRL 34033	GQ505418	94.39	*F. brachygibbosum*	FSASC	N/A	NRRL 34033	GQ505418	94.38
23973	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	99.55	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	99.54
23974	*F. inflexum*	FOSC	N/A	CBS 716.74	AF008479	100	*Fusarium* sp.	FOSC	19	NRRL 45881	N/A	100
23975	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	99.55	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	99.55
23976	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	99.40	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	99.39
23977	*F. oxysporum*	FOSC	210	NRRL 38477	FJ985397	100	*F. oxysporum f. sp. cucumerinum*	FOSC	191	NRRL 38591	FJ985379	100
23978	*F. falciforme*	FSSC	3+4-r	NRRL 28565	DQ246906	99.85	*F. falciforme*	FSSC	3+4-r	NRRL 28565	DQ246906	99.85
23979	*F. nygamai*	FFSC	N/A	CBS 140.95	HM347121	99.85	*F. nygamai*	FFSC	N/A	NRRL 26421	HM347121	99.84
23980	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	98.49	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	98.48

	FUSARIUM MLST DATABASE						*FUSARIUM*-ID DATABASE					
PPRI Number	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**
23981	*F. inflexum*	FOSC	N/A	CBS 716.74	AF008479	100	*Fusarium* sp.	FOSC	19	NRRL 45881	N/A	100
23982	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	99.55	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	99.55
23983	*F. oxysporum*	FOSC	53	NRRL 43499	DQ790495	100	*F. oxysporum*	FOSC	16	NRRL 38597	N/A	100
23984	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	99.40	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	99.39
23985	*F. oxysporum*	FOSC	53	NRRL 43499	DQ790495	100	*F. oxysporum*	FOSC	16	NRRL 38597	N/A	100
23986	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	99.55	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	99.55
23987	*F. falciforme*	FSSC	3+4-ee	NRRL 32505	DQ247002	99.84	*F. falciforme*	FSSC	3+4-ee	NRRL 32505	DQ247002	99.84
23988	*F. falciforme*	FSSC	3+4-k	NRRL 28548	DQ246889	100	*F. falciforme*	FSSC	3+4-y	NRRL 32331	DQ246959	100
23989	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	99.55	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	99.55
23990	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	99.40	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	99.39
23991	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	98.53	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	98.52
23992	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	98.53	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	98.52
23993	*F. inflexum*	FOSC	N/A	CBS 716.74	AF008479	100	*Fusarium* sp.	FOSC	19	NRRL 45881	N/A	100
24199	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	98.95	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	98.95
24200	*Fusarium* sp.	FCSC	2-a	NRRL 43630	GQ505426	100	*Fusarium* sp.	FCSC	2-a	NRRL 43630	GQ505426	100
24201	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	99.55	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	99.54
24202	*F. oxysporum f. sp. erythroxyli*	FOSC	79	NRRL 26574	AF008495	100	*F. oxysporum*	FOSC	232	NRRL 39464	FJ985419	100
24203	*F. falciforme*	FSSC	3+4-k	NRRL 28548	DQ246889	99.85	*F. falciforme*	FSSC	3+4-g	NRRL 22938	DQ246855	99.85
24204	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	99.55	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	99.55
24205	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	99.55	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	99.55
24206	*F. oxysporum f. sp. erythroxyli*	FOSC	79	NRRL 26574	AF008495	100	*F. oxysporum*	FOSC	232	NRRL 39464	FJ985419	100
24207	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	98.49	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	98.48
24208	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	98.65	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	98.64
24209	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	99.41	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	99.40
24210	*F. falciforme*	FSSC	3+4-mm	NRRL 32714	DQ247034	99.70	*F. falciforme*	FSSC	3+4-uu	NRRL 32743	DQ247062	99.70

	FUSARIUM MLST DATABASE						*FUSARIUM*-ID DATABASE					
PPRI Number	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**
24211	*F. brachygibbosum*	FSASC	none	NRRL 34033	GQ505418	98.36	*F. brachygibbosum*	FSASC	N/A	NRRL 34033	GQ505418	99.10
24212	*F. oxysporum*	FOSC	197	NRRL 38328	FJ985385	100	*F. oxysporum*	FOSC	197	NRRL 38328	FJ985385	100
24213	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	99.55	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	99.54
24214	*F. oxysporum*	FOSC	197	NRRL 38328	FJ985385	100	*F. oxysporum*	FOSC	197	NRRL 38328	FJ985385	100
24215	*Fusarium* sp.	FIESC	6-a	NRRL 43638	GQ505665	98.02	*Fusarium* sp.	FIESC	6-b	NRRL 45998	GQ505673	98.02
24216	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	99.55	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	99.54
24217	*F lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	98.63	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	98.63
24218	*F. falciforme*	FSSC	3+4-r	NRRL 28565	DQ246906	99.84	*F. falciforme*	FSSC	3+4-r	NRRL 28565	DQ246906	99.84
24219	*F. inflexum*	FOSC	2	NRRL 20433	AF008479	100	*Fusarium* sp.	FOSC	19	NRRL 45881	N/A	100
24220	*F. inflexum*	FOSC	N/A	CBS 716.74	AF008479	100	*Fusarium* sp.	FOSC	19	NRRL 45881	N/A	100
24221	*F. falciforme*	FSSC	3+4-r	NRRL 28565	DQ246906	99.85	*F. falciforme*	FSSC	3+4-r	NRRL 28565	DQ246906	99.85
24222	*F. oxysporum f. sp. pini*	FOSC	18	NRRL 22551	FJ985272	100	*F. oxysporum*	FOSC	18	NRRL 22551	FJ985272	100
24223	*F. inflexum*	FOSC	N/A	CBS 716.74	AF008479	100	*Fusarium* sp.	FOSC	19	NRRL 45881	N/A	100
24224	*F. falciforme*	FSSC	3+4-ff	NRRL 32506	DQ247003	99.85	*F. falciforme*	FSSC	3+4-ff	NRRL 32506	DQ247003	99.85
24225	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	98.51	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	98.50
24307	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	98.51	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	98.66
24226	*F. falciforme*	FSSC	3+4-mm	NRRL 32714	DQ247034	99.70	*F. falciforme*	FSSC	3+4-uu	NRRL 32743	DQ247062	99.70
24227	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	98.66	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	98.66
24228	*F. falciforme*	FSSC	3+4-g	NRRL 22938	DQ246855	99.70	*F. falciforme*	FSSC	3+4-g	NRRL 22938	DQ246855	99.70
24229	*F. inflexum*	FOSC	N/A	CBS 716.74	AF008479	100	*Fusarium* sp.	FOSC	19	NRRL 45881	N/A	100
24230	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	99.56	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	99.55
24231	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	99.55	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	99.55
24232	*Fusarium* sp.	FIESC	22-a	NRRL 34002	GQ505626	98.09	*Fusarium* sp.	FIESC	22-a	NRRL 34002	GQ505626	98.08
24233	*F. burgessii*	FBSC	N/A	CBS 125537	N/A	96.92	*F. hostae*	N/A	N/A	NRRL 29889	AY329034	90.22
24234	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	98.52	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	98.51

	FUSARIUM MLST DATABASE						*FUSARIUM*-ID DATABASE					
PPRI Number	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**
24235	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	99.70	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	99.70
24236	*F. oxysporum f. sp. tracheiphilum*	FOSC	20	CBS 130.81	FJ985274	100	*F. oxysporum f. sp. lupini*	FOSC	47	NRRL 26225	FJ985285	100
24237	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	98.51	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	98.51
24238	*F. oxysporum*	FOSC	53	NRRL 43499	DQ790495	100	*F. oxysporum*	FOSC	16	NRRL 38597	N/A	100
24239	*Fusarium* sp.	FIESC	6-f	NRRL 37640	FJ240355	97.64	*Fusarium* sp.	FIESC	6-f	NRRL 37640	FJ240355	97.64
24240	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	98.67	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	98.66
24241	*F. falciforme*	FSSC	3+4-g	NRRL 22938	DQ246855	99.70	*F. falciforme*	FSSC	3+4-g	NRRL 22938	DQ246855	99.70
24242	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	98.67	*F. lacertarum*	FIESC	4-a	NRRL 20423	GQ505593	98.50

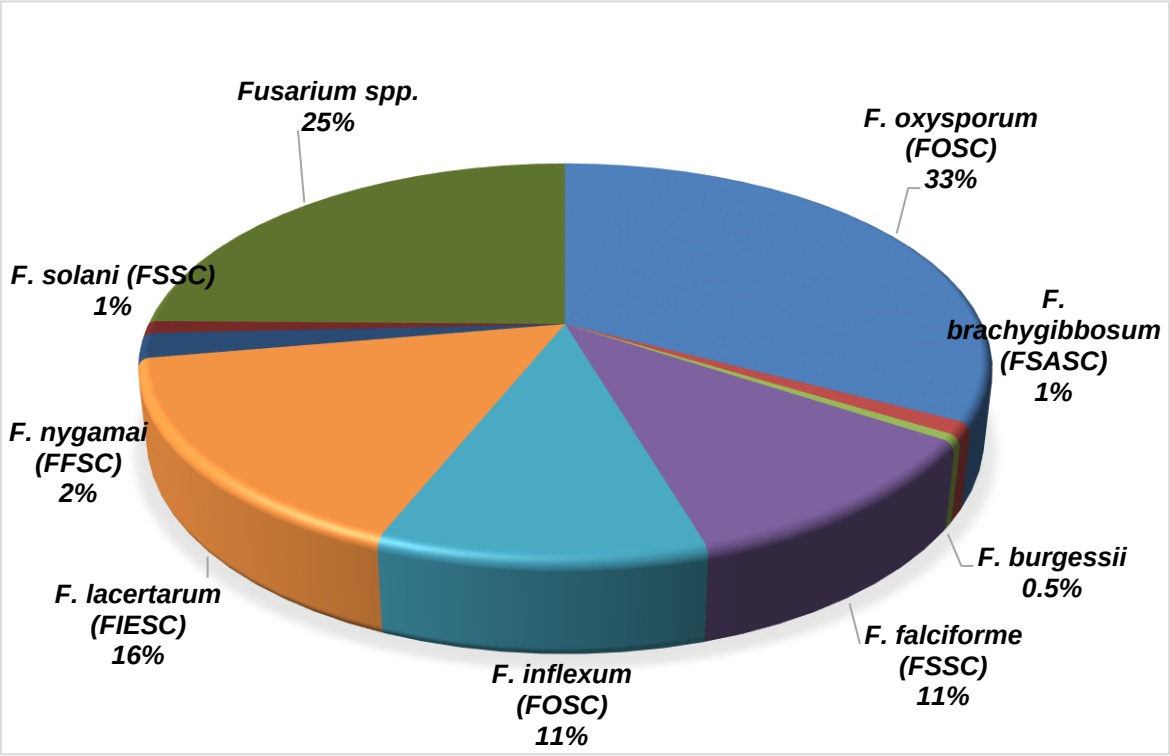


Figure 4.15: *Fusarium* species complexes and other species identified using *Fusarium* MLST database nBLAST™ analyses for isolates recovered from soil collected from Gauteng, Limpopo, Mpumalanga provinces of South Africa.

The FFSC was represented by four *F. nygamai* (2%) isolates (PPRI 21944, 23876, 23880 and 23979) that were similar to *F. nygamai* CBS 140.95 with a percentage similarity ranging from 99.85-100%. *Fusarium nygamai* is an agriculturally important soilborne pathogen (Klaasen and Nelson, 1996), and is associated with millet (Marasas *et al.*, 1988; Onyike *et al.*, 1991) and sorghum (Onyike and Nelson, 1992).

The FSASC was represented by two *F. brachygibbosum* (1%) isolates (PPRI 23972 and 24211) that were similar to *F. brachygibbosum* NRRL 34033 with a percentage similarity of 94.39% and 98.36%, respectively. *Fusarium brachygibbosum* was recently reported to cause a basal rot of onion in Mexico (Tirado-Ramirez *et al.*, 2019). PPRI 24233 was similar to *F. burgessii* CBS 125537 with a low percentage similarity of 96.92% and was the least recovered from soil. *Fusarium burgessii* is associated with soil in non-cultivated environments in Australia. Furthermore, the RPB2 phylogenetic analysis indicated that *F. burgessii* does not form a sister group

relationship with the FOSC but forms a distinctive monophyletic lineage (Laurence *et al.*, 2011).

The FSSC was represented by 22 *F. falciforme* (11%) isolates (PPRI 21940, 21942, 21943, 21949, 21952, 22325, 23579, 23581, 23588, 23808, 23881, 23978, 23987, 23988, 24203, 24210, 24218, 24221, 24224, 24226, 24228 and 24241) with a percentage similarity ranging from 99.53-100% and two *F. solani* (1%) (PPRI 23585 and 23586) with a percentage similarity of 100%. The FIESC was represented by 32 soil strains (PPRI 21937, 21950, 21959, 22322, 22331, 23578, 23814, 23975, 23976, 23980, 23982, 23984, 23986, 23989, 23990, 24199, 24204, 24207, 24208, 24209, 24213, 24217, 24225, 24307, 24227, 24230, 24231, 24234, 24235, 24237, 24240 and 24242) that were similar to *F. lacertarum* NRRL 20423 MLST type 4-a with a percentage similarity ranging from 98.49-100%. This indicate that the pathogen might be associated with FW and can be found in soil.

The FOSC was represented by 65 *F. oxysporum* isolates (33%) and 21 *F. inflexum* isolates (11%). Nineteen soil strains (PPRI 21948, 21953, 21963, 21977, 23580, 23594, 23597, 23617, 23623, 23628, 23815, 23816, 23817, 23974, 23981, 23993, 24220, 24223 and 24229) were similar to *F. inflexum* CBS 716.74 in the FOSC with a percentage similarities ranging from 99.69-100%, while two soil strains (PPRI 23821 and 24219) were similar to *F. inflexum* NRRL 20433 MLST type 2 with a percentage similarity of 100%. *Fusarium inflexum* NRRL 20433 (CBS 716.74) was isolated from bean in Germany and known to cause vascular wilt.

The TEF-1α *Fusarium* MLST database nBLAST™ results revealed a total of 19 soil strains represented by *F. oxysporum* that clustered in the FOSC which were not associated with any *formae speciales,* while 46 soil strains were associated with 11 different *formae speciales* (Figure 4.16).

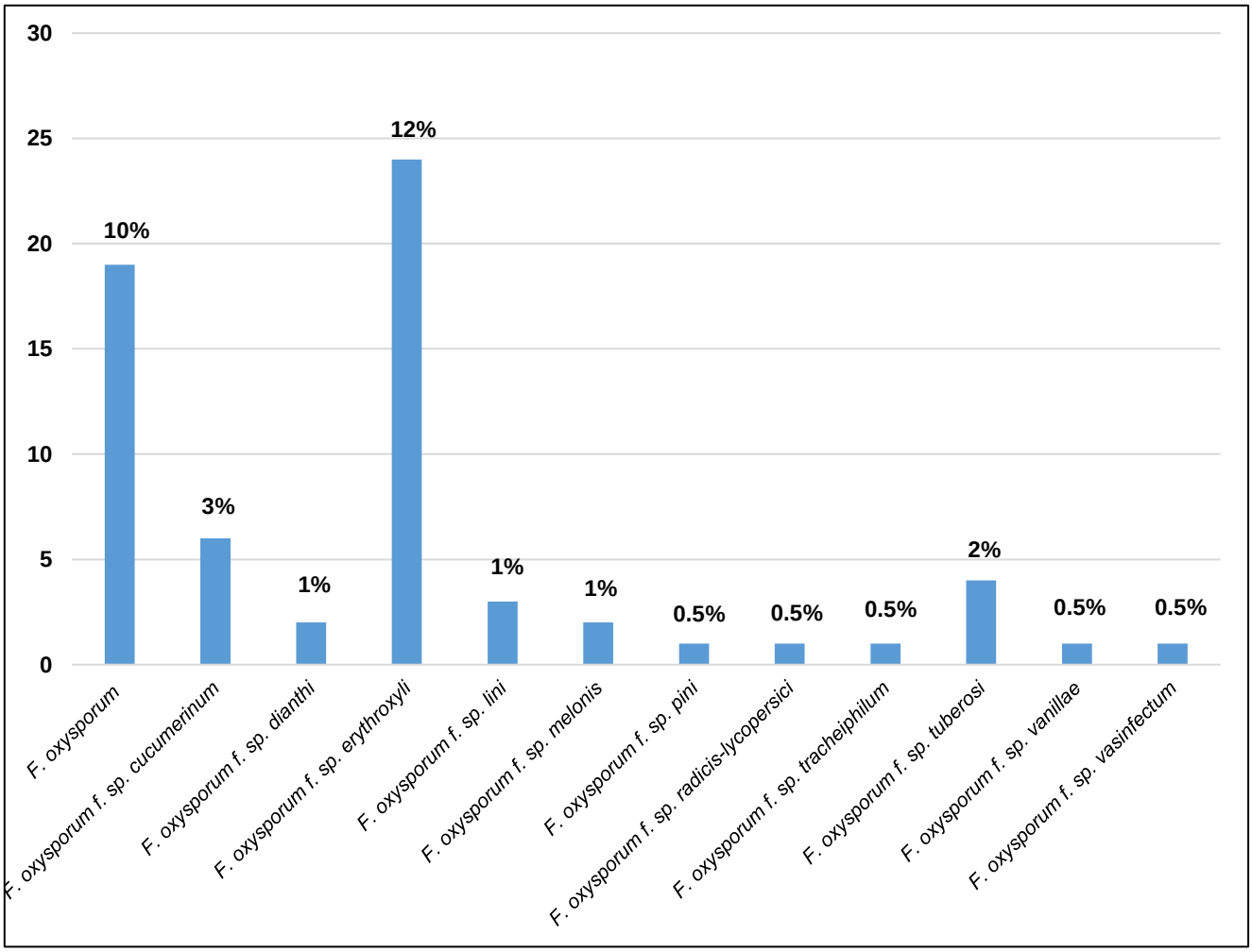


Figure 4.16: FOSC identified using *Fusarium* MLST database nBLAST™ analyses from soil collected from Gauteng, Limpopo and Mpumalanga provinces of South Africa. The numbers of FOSC are indicated in bold black font on the Y-axis. The percentages of FOSC are indicated on the X-axis.

The TEF-1α *Fusarium*-MLST database nBLAST™ results revealed a total of 19 soil strains represented by *F. oxysporum* that clustered in the FOSC with a significant percentage similarity of 100% (Table 4.2). Nine soil strains (PPRI 21931, 21933, 21945, 21951, 21954, 21971, 22323, 22324 and 23977) were similar to *F. oxysporum* NRRL 38477 MLST type 210 (source: *Poaceae*; origin: New Zealand), three strains (PPRI 23983, 23985 and 24238) were similar to *F. oxysporum* NRRL 43499 MLST type 53 (source: Cornea; origin: USA), two strains (PPRI 23592 and 23804) were similar to *F. oxysporum* NRRL 45945 MLST type 243 (source: *Cotula* sp.; origin: New Zealand), two strains (PPRI 23622 and 23625) were similar to *F. oxysporum* NRRL

38595 MLST type 228 (source: *Zea mays*; origin: New Zealand), two strains (PPRI 24212 and 24214) were similar to *F. oxysporum* NRRL 38328 MLST type 197 (source: Glycine max; origin: China) and one strain (PPRI 23819) was similar to *F. oxysporum* NRRL 43442 MLST type 48 (source: Cornea; origin: USA). These results did not follow any pattern as the the data does not group according to the MLSTs, geographical distribution or hosts.

The TEF-1α *Fusarium* MLST database nBLAST™ results revealed a total of 46 soil strains represented by *formae speciales* that clustered in the FOSC with a percentage similarity range of 99.54-100% except PPRI 23823 similar to *F. oxysporum f. sp. dianthi* NRRL 26222 MLST 46 with a percentage similarity of 97.63%. Twenty-four obtained soil strains (PPRI 21946, 21975, 23589, 23590, 23593, 23595, 23596, 23614, 23616, 23619, 23620, 23624, 23626, 23630, 23631, 23875, 23806, 23810, 23811, 23812, 23813, 23878, 24202 and 24206) had a significant similarity to *F. oxysporum f. sp. erythroxyli* NRRL 26574 MLST type 79 with a percentage similarity of 99.85-100%. Our results indicate that *F. oxysporum f. sp. erythroxyli* can be recovered from soil and this suggest that infested soil was a source of inoculum. The pathogen is reported to cause vascular wilt of the narcotic plant *Erythroxylum coca* (Gracia-Garza *et al.*, 1999). Six PPRI isolates (PPRI 21932, 21935, 21936, 21941, 22329 and 23621) were similar to *F. oxysporum f. sp. cucumerinum* NRRL 38591 MLST type 191 with a percentage similarity of 100%. The results indicate that *F. oxysporum f. sp. cucumerinum* can be recovered from soil and is associated with FW of sweet potato. *Fusarium oxysporum forma specialis cucumerinum* is the soil borne fungus responsible for FW of cucumber (Owen, 1956). The pathogen has been identified in all cucumber growing regions around the world (Martyn, 1996).

Four soil strains (PPRI 23582, 23615, 23873 and 23805) were similar to *F. oxysporum f. sp. tuberosi* NRRL 22555 MLST type 21 with a percentage similarity of 100%. Three strains (PPRI 21934, 21958 and 22327) were similar to *F. oxysporum f. sp. lini* NRRL 36286 MLST type 154 with a percentage similarity of 100%. PPRI 23629 was similar to *F. oxysporum f. sp. dianthi* NRRL 36356 MLST type 158 with percentage similarity of 100%. Two soil strains (PPRI 21929 and 22326) were represented by *F. oxysporum f. sp. melonis* W.C. Snyder & H.N. Hansen and were

similar to *F. oxysporum f. sp. melonis* CBS 420.90 MLST type 167 with a percentage similarity of 99.69% and 99.54%, respectively. Furthermore, the solo soil strains had a percentage similarity of 100% that included PPRI 24222 that was similar to *F. oxysporum f. sp. pini* NRRL 22551MLST type 18 (source: *Pinus* sp.; origin: Germany); PPRI 23807 similar to *F. oxysporum f. sp. radicis-lycopersici* NRRL 26033 MLST type 40; PPRI 24236 similar to *F. oxysporum f. sp. tracheiphilum* CBS 130.81 MLST type 20; PPRI 23584 similar to *F. oxysporum f. sp. vanillae* NRRL 26448 MLST type 77 and PPRI 21968 similar to *F. oxysporum f. sp. vasinfectum* NRRL 25420 MLST type 28 as shown in Table 4.2. The *F. oxysporum f. sp. erythroxyli* were the dorminant *formae speciales* discovered from the soil in Gauteng, Limpopo and Mpumalanga Provinces of South Africa followed by *F. oxysporum f. sp. cucumerinum*. There are genetic differences amongst the FOSC strains, therefore, these strains were further investigated by using multiloci phylogenies.

Multiloci DNA sequence typing identified 18 MLST types associated with FW on soil, based on the *Fusarium* MLST database (Figure 4.17). Of the 18 MLSTs, 12 were were designated as part of the 68 described *formae speciales* while the remaining six MLSTs were not designated as *formae speciales* in the *Fusarium* MLST database. The soil isolates obtained from Gauteng, Limpopo and Mpumalanga provinces of South Africa clustered within all of the identified universal MLSTs reported by O'Donnell *et al.* (2009a). MLST 79, with 24 isolates, was the most common MLST represented in the *Fusarium* MLST database. Sampling from soil identified species of approximately 7% of the known universal STs.

There were eight MLST types associated with soil strains that clustered into haplotype group 1, namely, MLST 18, 20, 21, 40, 79, 154, 158 and 167 (O'Donnell *et al.*, 2009a). Only one MLST type, MLST 197 associated with soil isolates clustered into haplotype group 2 (O'Donnell *et al.*, 2009a). There were four MLST types associated with soil isolates that clustered into haplotype group 4, namely, MLST 28, 46, 77 and 191 (O'Donnell *et al.*, 2009a). There were three MLST types associated with soil isolates that clustered into haplotype group 5, namely, MLST 210, 228 and 243 (O'Donnell *et al.*, 2009a).

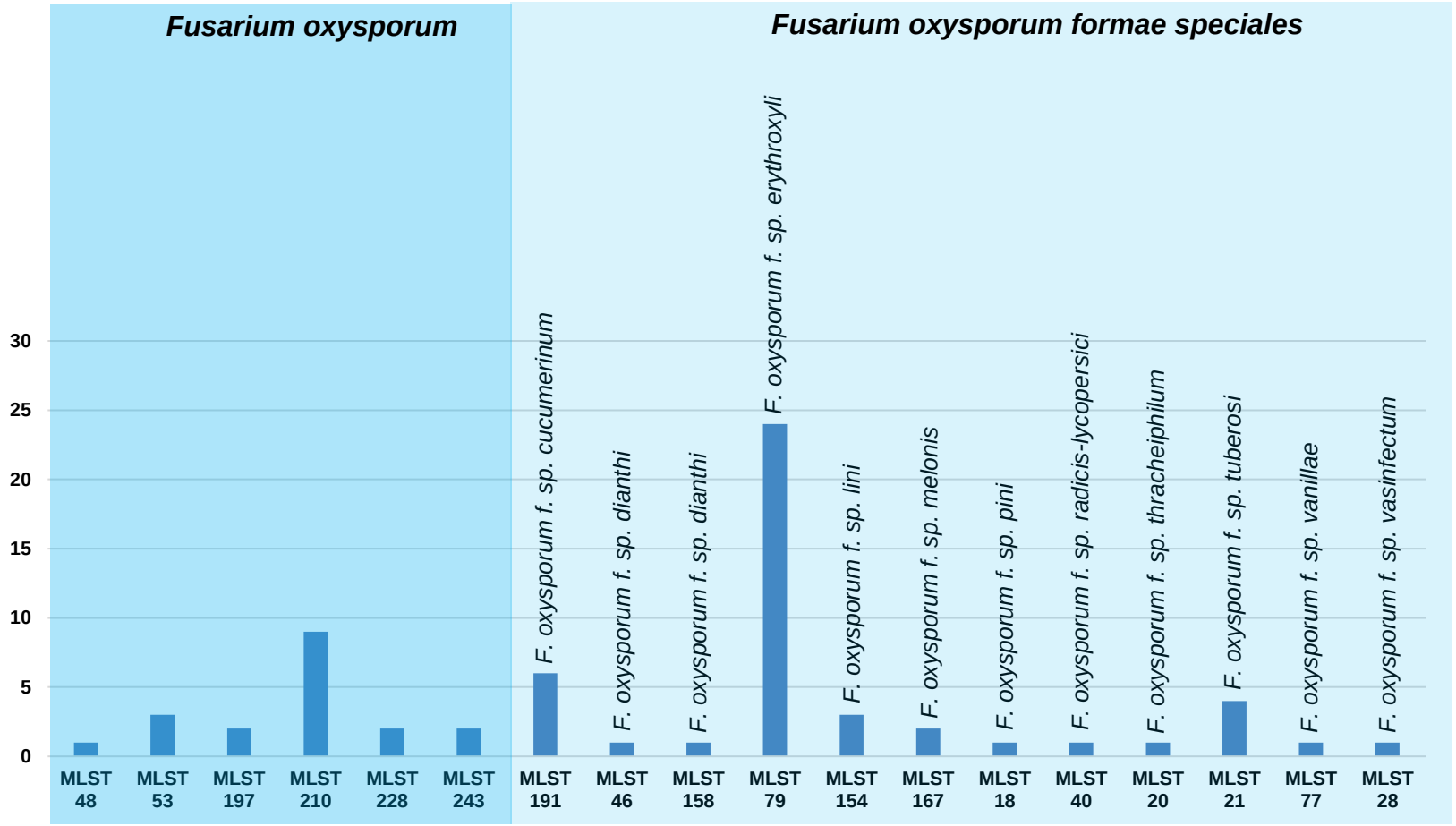


Figure 4.17: Number of MLST types discovered from soil isolates based on the *Fusarium* MLST database and samples collected from Gauteng, Limpopo and Mpumalanga provinces of South Africa.

Lastly, there were two MLST types associated with soil isolates that clustered into haplotype group 7, namely, MLST 48 and 53 (O'Donnell *et al.*, 2009a). MLST 79, with 24 isolates, was the most commom MLST represented in the *Fusarium* MLST database and was designated as *F. oxysporum f. sp. erythroxyli*. South African MLST types were present in haplotype group 1, 2, 4, 5 and 7 but were not present in haplotype group 3 and 6 based on the *Fusarium* MLST database. This results indicates that South African MLST types are genetic diverse.

4.3.4 *Fusarium*-ID database nBLAST™ results based on TEF-1α sequences for isolates obtained from soil

The *Fusarium*-ID database nBLAST™ results based on the TEF-1α sequences of 189 strains obtained from soil clustered into six *Fusarium* species complexes that comprised six *Fusarium* species. The six *Fusarium* species complexes were FFSC, FIESC, *F. redolens* species complex (FRSC), FSASC, FSSC and FOSC. The species in the complexes were represented by *F. nygamai* in the FFSC, *F. lacertarum* in the FIESC, *F. hostae* Geiser & Juba, in the FRSC, *F. brachygibbosum* in the FSASC, *F. falciforme* in the FSSC and *F. oxysporum* in the FOSC (Figure 4.18). The TEF-1α *Fusarium*-ID database nBLAST™ analyses indicated a significant percentage similarity of 99.50%-100% for most of the isolates except one isolate (0.5%) (PPRI 24233) with the lowest percentage similarity of 90.22% represented by *F. hostae* NRRL 29889 in the FRSC.

The FIESC was represented by 32 (16%) strains that were similar to *F. lacertarum* NRRL 20423 MLST type 4-a with a percentage similarity ranging from 98.48-99.85%. The FSSC was represented by 24 *F. falciforme* strains (12%) with a percentage similarity ranging from 99.37-100%, of which six strains (PPRI 21952, 22325, 23579, 23588, 23881 and 23988) were similar to NRRL 32331 MLST type 3+4-y; four strains (PPRI 21940, 21942, 21949 and 23808) were similar to NRRL 32727 MLST type 3+4-rr; four strains (PPRI 21943, 23978, 24218 and 24221) were similar to NRRL 28565 MLST type 3+4-r; three strains (PPRI 24203, 24228 and 24241) were similar to NRRL 22938 MLST type 3+4-g; two strains (PPRI 23585 and 23586) were similar to NRRL 32542 MLST type 3+4-ii; two strains (PPRI 24210 and 24226) were similar to NRRL 32743 MLST type 3+4-uu; PPRI 23987 was similar to

NRRL 32505 MLST type 3+4-ee; PPRI 24224 was similar to NRRL 32506 MLST type 3+4-ff; PPRI 23581 was similar to NRRL 32308 MLST 3+4-v.

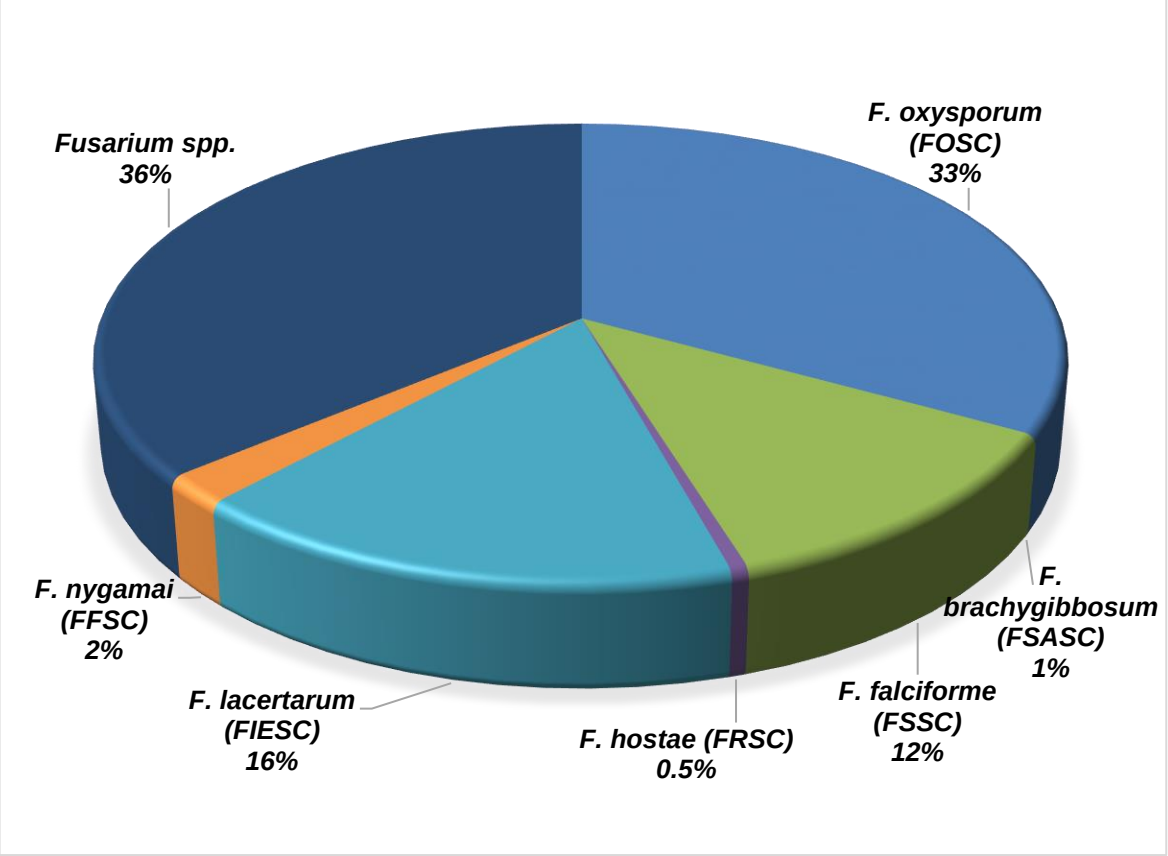


Figure 4.18: *Fusarium* species complexes and other species identified using *Fusarium*-ID database nBLAST™ analyses for isolates recovered from soil collected from Gauteng, Limpopo and Mpumalanga provinces of South Africa.

The FFSC was represented by four strains (2%) (PPRI 21944, 23876, 23880 and 23979) that were similar to *F. nygamai* CBS 140.95 with a percentage similarity of 99.84-100%. The FSASC was represented by two strains (1%) (PPRI 23972 and 24211) that were similar to *F. brachygibbosum* NRRL 34033 with a percentage similarity of 94.38% and 99.10%, respectively. A total of 71 soil strains (36%) were represented by *Fusarium* spp. with a percentage similarity ranging from 97.69-100%. The unnamed species sequences should be subjected to a GCPSR analysis (Taylor *et al.*, 2000).

The FOSC was represented by 65 *F. oxysporum* isolates (33%) obtained from soil, of which 45 strains were not represented by any *formae speciales* according to the TEF-1α *Fusarium*-ID database nBLAST™ results (Table 4.1), including nine MLSTs associated with *Dianthus caryophyllus*, *Cucurbita* sp., *Passiflora edulis*, *Zea mays*, *Cucurbita maxima*, Glycine max, and *Pinus* sp. hosts plants based on the origin host description.

The TEF-1α *Fusarium* ID database nBLAST™ results revealed a total of 45 soil strains represented by *F. oxysporum* that clustered in the FOSC which were not associated with any *formae speciales,* while 19 soil strains were associated with four different *formae speciales* (Figure 4.19). The 19 soil strains represented by *formae speciales* clustered in the FOSC with a percentage similarity range of 99.55-100%, except PPRI 23823 (0.5%) with a low percentage similarity of 97.82% to *F. oxysporum f. sp. dianthi* NRRL 38596 MLST type 46 (source: *Dianthus caryophyllus*; origin: New Zealand) (Table 4.1). Fifteen soil strains (7%) (PPRI 21931, 21932, 21933, 21935, 21936, 21941, 21945, 21951, 21954, 21971, 22323, 22324, 22329, 23621 and 23977) were similar to *F. oxysporum f. sp. cucumerinum* NRRL 38591 MLST type 191 with a percentage similarity of 100%. Two soil strains (1%) (PPRI 21929 and 22326) were similar to *F. oxysporum f. sp. koae* NRRL 38885 MLST type 231 (source: *Acacia koa*; origin: USA) with a percentage similarity of 99.70% and 99.55%, respectively. Lastly, PPRI 24236 was similar to *F. oxysporum f. sp. lupini* NRRL 26225 MLST type 47 with a percentage similarity of 100%. There is a genetic difference amongst the FOSC isolates therefore, these isolates were further investigated by using multiloci phylogenies. *Fusarium oxysporum formae speciales dianthi* and *F. oxysporum f. sp. lupini* were the least represented *formae speciales* recovered. *Fusarium oxysporum formae speciales cucumerinum* was the most recovered *formae speciales* amongst the 65 FOSC isolates. The *Fusarium*-ID database revealed only four *formae speciales*, in contrast to the 11 *formae speciales* revealed by the *Fusarium* MLST database.

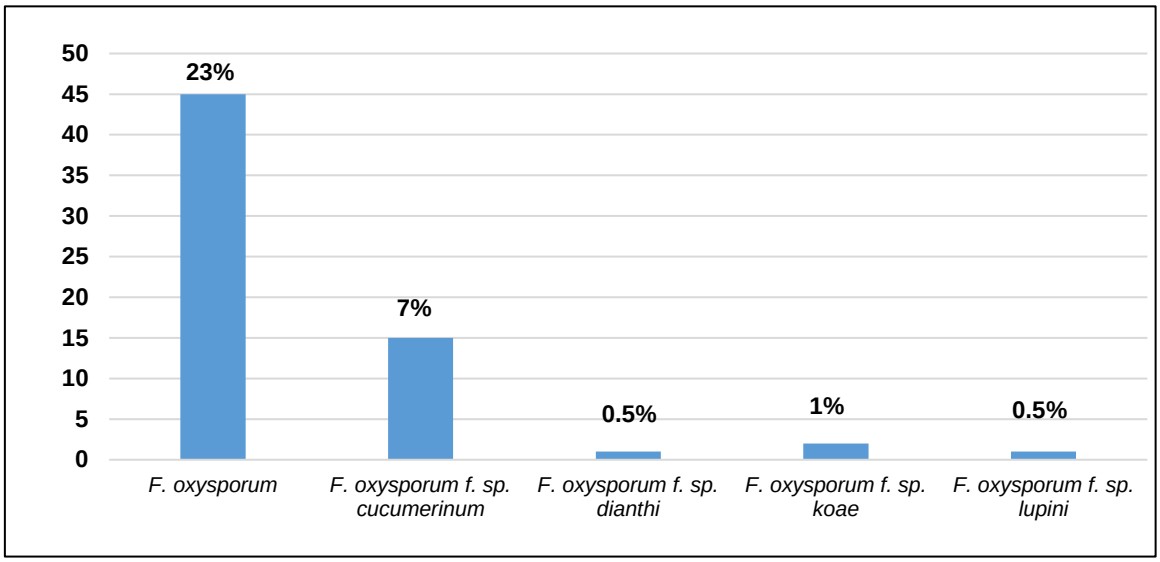


Figure 4.19: FOSC identified using *Fusarium*-ID database nBLAST™ analyses discovered from soil collected from Gauteng, Limpopo and Mpumalanga provinces of South Africa. The numbers of FOSC are indicated on the Y-axis. The percentages of FOSC are indicated on the X-axis.

Multiloci DNA sequence typing identified 14 MLST types associated with FW on soil, based on the *Fusarium*-ID database (Figure 4.20). Of the 14 MLSTs, four were designated as part of the 68 described *formae speciales* while the remaining ten MLSTs were not designated as *formae speciales* in the *Fusarium* MLST database. The soil isolates clustered within all of the identified universal STs reported by O'Donnell *et al.* (2009a). Sampling from soil identified species of approximately 5% of the known universal STs.

There were two MLST types associated with the South African soil isolates that clustered into haplotype group 1, namely, MLST 18 and 47 (O'Donnell *et al.*, 2009a). There were five MLST types associated with the South African soil isolates that clustered into haplotype group 2, namely, MLST 197, 216, 219, 231 and 232 (O'Donnell *et al.*, 2009a). There were five MLST types associated with the South African soil isolates that clustered into haplotype group 4, namely, MLST 16, 46, 67, 77 and 191 (O'Donnell *et al.*, 2009a). Lastly, there were two MLST types associated with the South African soil isolates that clustered into haplotype

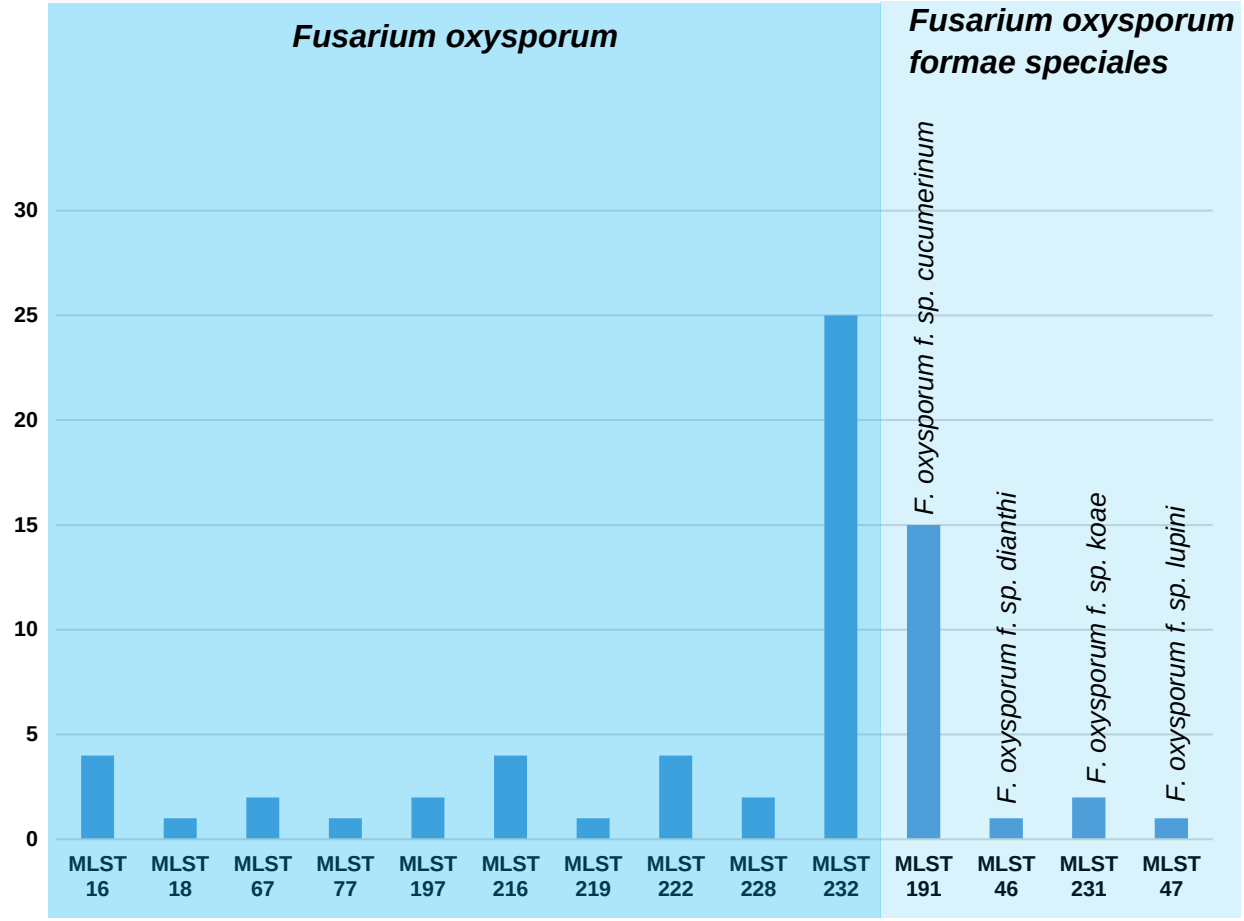


Figure 4.20: Number of MLST types discovered from soil isolates based on the *Fusarium*-ID database and samples collected from Gauteng, Limpopo and Mpumalanga provinces of South Africa.

group 5, namely, MLST 222 and 228 (O'Donnell *et al.*, 2009a). MLST 232 with 25 isolates, was the most common ST represented in the *Fusarium*-ID database and was not designated as *formae speciales*. South African MLST types were present in haplotype group 1, 2, 4 and 5 but were not present in haplotype group 3, 6 and 7 based on the *Fusarium*-ID database. The South African MLST types from isolates obtained from soil were distributed in all the groups except group 3 and 6. This results indicates that South African MLST types are genetic diverse.

Multiloci DNA sequence typing identified six MLST types in the FIESC associated with FW on soil, based on the *Fusarium*-ID database (Figure 4.16). These were MLST 3-b, 4-a, 5-f, 6-b, 6-f and 22-a. Of the 46 strains within the FIESC MLST types, 32

strains were designated as *F. lacertarum,* while the remaining MLSTs were designated as *Fusarium* sp.

Multiloci DNA sequence typing identified nine MLST types within FSSC associated with FW on soil, based on the *Fusarium*-ID database (Figure 4.16). These were MLST 3+4-ee, 3+4-ff, 3+4-g, 3+4-ii, 3+4-r, 3+4-rr, 3+4-uu, 3+4-v and 3+4-y. All of the 24 soil isolates within FSSC were designated as *F. falciforme*.

The nucleotide BLAST results from *Fusarium* MLST database and *Fusarium*-ID database were similar for most soil isolates. Both databases placed the query sequences within the same *Fusarium* species complexes. Both databases had significant percentage similarities for most of the isolates. Both databases resolved the identification of *Fusarium* isolates to species level as they both had the same species with similar percentage similarities namely, *F. brachygibbosum*, *F. nygamai*, *F. falciforme* and *F. lacertarum* as indicated in Table 4.2. In some instances, *Fusarium* MLST database resolved identification of species complex isolates to species level whereas the *Fusarium*-ID database resolved the identification of species complex isolates to genus level. The two databases gave contradicting results for *formae speciales* as the databases where revealing different *formae speciales* for the same strain. The two databases gave one major contradicting nBLAST™ result for PPRI 24233, whereas the *Fusarium* MLST database revealed *F. burgessii* in contrast to the *Fusarium*-ID database that revealed *F. hostae*.

4.3.5 *Fusarium* MLST database nBLAST™ results based on RPB2 (5F and 7CR) sequences for isolates obtained from sweet potato

The *Fusarium* MLST database nBLAST™ results based on the RPB2 (5F and 7CR) sequences of 89 strains obtained from diseased sweet potato material clustered into three *Fusarium* species complexes, namely FIESC, FSSC and FOSC. The species in the complexes were represented by *F. lacertarum* in the FIESC, *F. petroliphilum* in the FSSC and *F. inflexum* and *F. oxysporum* in the FOSC. The RPB2 (5F and 7CR) *Fusarium* MLST database nBLAST™ analyses indicated a percentage similarity of 99-100% for most of the isolates except two strains (PPRI

23064 and 23065) that were similar to *F. inflexum* NRRL 20433 with no MLST type and *F. oxysporum f. sp. lycopersici* NRRL 34936 in the FOSC with the low percentage similarity of 98.93% and 98.90%, respectively (Table 4.3).

PPRI 23473 was 99.78% similar to *F. lacertarum* NRRL 36123 MLST type 4-b in the FIESC. The FIESC was also represented by two strains similar to unnamed *Fusarium* spp. PPRI 23475 and PPRI 23478 had a percentage similarity of 99.44% and 99.89% respectively and were similar to *Fusarium* sp. NRRL 36401 MLST type 2-a and *Fusarium* sp. NRRL 36323 MLST type 3-a. The FSSC was represented by 20 (22%) strains (PPRI 23476, 23477, 23479, 23480, 23481, 23482, 23483, 23484, 23485, 23486, 23487, 23488, 23489, 23490, 23491, 23492, 23493, 23494, 23495 and 23496) that were similar to *F. petroliphilum* NRRL 34095 MLST type 1-b with a percentage similarity ranging from 99.73-100%. The FOSC was represented by ten strains (PPRI 9462, 20163, 20165, 20173, 20176, 20177, 23063, 23064, 23072 and 23474) were similar to *F. inflexum* NRRL 20433 with a percentage similarity ranging from 98.93-99.78%.

The RPB2 (5F and 7CR) *Fusarium* MLST database nBLAST™ results revealed a total of two sweet potato strains (PPRI 20174 and 23067) represented by *F. oxysporum* that clustered in the FOSC and were similar to *F. oxysporum* NRRL 25387 MLST type 27 associated with clinical isolate with a percentage similarity of 99.54% and 99.77%, respectively. The FOSC was also represented by 49 *F. oxysporum f. sp. lycopersici* that clustered in the FOSC that were similar to NRRL 34936 MLST 63 with a percentage similarity ranging from 98.90-99.78% (Table 4.3), including MLST 63 associated with a clinical isolate based on the origin host description.

Table 4.3: *Fusarium* MLST and *Fusarium*-ID nBLAST™ results of RPB2 (5F and 7CR) from diseased sweet potato fungal isolates

	***FUSARIUM* MLST DATABASE**						***FUSARIUM*-ID DATABASE**					
PPRI Number	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**
9458	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.78	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.77
9459	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.56	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.55
9460	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.78	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.77
9461	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.56	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.55
9462	*F. inflexum*	FOSC	none	NRRL 20433	JX171583	99.78	*F. inflexum*	FOSC	N/A	NRRL 20433	JX171583	99.77
9463	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.78	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.77
9464	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.78	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.77
9465	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.34	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.33
9466	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.78	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.77
9467	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.78	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.77
9468	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.78	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.77
9469	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.78	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.77
9470	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.78	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.77
9471	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.78	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.77
9472	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.78	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.66
9473	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.78	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.44
10531	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.78	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.77
10532	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.78	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.77
10533	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.78	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.77
17592	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.78	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.77
17593	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.78	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.77
17594	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.78	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.77
17595	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.78	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.77
17596	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.78	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.77

	FUSARIUM MLST DATABASE						*FUSARIUM*-ID DATABASE					
PPRI Number	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**
18016	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.56	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.55
18750	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.78	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.77
18752	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.78	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.77
18753	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.78	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.77
20163	*F. inflexum*	FOSC	none	NRRL 20433	JX171583	99.67	*F. inflexum*	FOSC	N/A	NRRL 20433	JX171583	99.66
20164	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.78	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.77
20165	*F. inflexum*	FOSC	none	NRRL 20433	JX171583	99.78	*F. inflexum*	FOSC	N/A	NRRL 20433	JX171583	99.77
20166	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.78	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.77
20167	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.56	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.55
20168	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.78	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.77
20169	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.78	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.77
20170	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.56	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.55
20171	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.78	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.77
20172	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.56	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.55
20173	*F. inflexum*	FOSC	none	NRRL 20433	JX171583	99.67	*F. inflexum*	FOSC	N/A	NRRL 20433	JX171583	99.66
20174	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	99.54	*F. inflexum*	FOSC	N/A	NRRL 20433	JX171583	99.33
20175	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.56	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.55
20176	*F. inflexum*	FOSC	none	NRRL 20433	JX171583	99.56	*F. inflexum*	FOSC	N/A	NRRL 20433	JX171583	99.55
20177	*F. inflexum*	FOSC	none	NRRL 20433	JX171583	99.45	*F. inflexum*	FOSC	N/A	NRRL 20433	JX171583	99.44
20178	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.67	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.66
20179	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.76	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.55
23062	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.56	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.55
23063	*F. inflexum*	FOSC	none	NRRL 20433	JX171583	99.64	*F. inflexum*	FOSC	N/A	NRRL 20433	JX171583	99.63
23064	*F. inflexum*	FOSC	none	NRRL 20433	JX171583	98.93	*F. inflexum*	FOSC	N/A	NRRL 20433	JX171583	98.92
23065	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	98.90	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	98.70
23066	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.56	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.55

	FUSARIUM MLST DATABASE						*FUSARIUM*-ID DATABASE					
PPRI Number	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**
23067	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	99.77	*F. inflexum*	FOSC	N/A	NRRL 20433	JX171583	99.66
23068	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.56	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.55
23069	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.56	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.44
23070	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.56	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.55
23071	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.78	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.77
23072	*F. inflexum*	FOSC	none	NRRL 20433	JX171583	99.78	*F. inflexum*	FOSC	N/A	NRRL 20433	JX171583	99.77
23074	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.77	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.55
23076	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.56	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.55
23077	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.78	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.77
23078	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.56	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.55
23473	*F. lacertarum*	FESC	4-b	NRRL 36123	GQ505821	99.78	*F. lacertarum*	FIESC	4-a	NRRL 20423	JX171581	99.77
23474	*F. inflexum*	FOSC	none	NRRL 20433	JX171583	99.78	*F. inflexum*	FOSC	N/A	NRRL 20433	JX171583	99.77
23475	*Fusarium* sp.	FIESC	2-a	NRRL 36401	GQ505829	99.44	*F. lacertarum*	FIESC	4-a	NRRL 20423	JX171581	99.20
23476	*F. petroliphilum*	FSSC	1-b	NRRL 34095	FJ240404	100	*F. petroliphilum*	FSSC	1-c	NRRL 43812	EF470093	100
23477	*F. petroliphilum*	FSSC	1-b	NRRL 34095	FJ240404	100	*F. petroliphilum*	FSSC	1-c	NRRL 43812	EF470093	99.86
23478	*Fusarium* sp.	FIESC	3-a	NRRL 36323	GQ505826	99.89	*F. lacertarum*	FIESC	4-a	NRRL 20423	JX171581	99.31
23479	*F. petroliphilum*	FSSC	1-b	NRRL 34095	FJ240404	100	*F. petroliphilum*	FSSC	1-c	NRRL 43812	EF470093	100
23480	*F. petroliphilum*	FSSC	1-b	NRRL 34095	FJ240404	99.73	*F. petroliphilum*	FSSC	1-c	NRRL 43812	EF470093	99.72
23481	*F. petroliphilum*	FSSC	1-b	NRRL 34095	FJ240404	100	*F. petroliphilum*	FSSC	1-c	NRRL 43812	EF470093	100
23482	*F. petroliphilum*	FSSC	1-b	NRRL 34095	FJ240404	100	*F. petroliphilum*	FSSC	1-c	NRRL 43812	EF470093	100
23483	*F. petroliphilum*	FSSC	1-b	NRRL 34095	FJ240404	100	*F. petroliphilum*	FSSC	1-c	NRRL 43812	EF470093	99.22
23484	*F. petroliphilum*	FSSC	1-b	NRRL 34095	FJ240404	100	*F. petroliphilum*	FSSC	1-c	NRRL 43812	EF470093	100
23485	*F. petroliphilum*	FSSC	1-b	NRRL 34095	FJ240404	100	*F. petroliphilum*	FSSC	1-c	NRRL 43812	EF470093	99.22
23486	*F. petroliphilum*	FSSC	1-b	NRRL 34095	FJ240404	100	*F. petroliphilum*	FSSC	1-c	NRRL 43812	EF470093	100
23487	*F. petroliphilum*	FSSC	1-b	NRRL 34095	FJ240404	100	*F. petroliphilum*	FSSC	1-c	NRRL 43812	EF470093	100
23488	*F. petroliphilum*	FSSC	1-b	NRRL 34095	FJ240404	100	*F. petroliphilum*	FSSC	1-c	NRRL 43812	EF470093	100

	FUSARIUM MLST DATABASE						*FUSARIUM*-ID DATABASE					
PPRI Number	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**
23489	*F. petroliphilum*	FSSC	1-b	NRRL 34095	FJ240404	100	*F. petroliphilum*	FSSC	1-c	NRRL 43812	EF470093	100
23490	*F. petroliphilum*	FSSC	1-b	NRRL 34095	FJ240404	100	*F. petroliphilum*	FSSC	1-c	NRRL 43812	EF470093	100
23491	*F. petroliphilum*	FSSC	1-b	NRRL 34095	FJ240404	100	*F. petroliphilum*	FSSC	1-c	NRRL 43812	EF470093	100
23492	*F. petroliphilum*	FSSC	1-b	NRRL 34095	FJ240404	100	*F. petroliphilum*	FSSC	1-c	NRRL 43812	EF470093	100
23493	*F. petroliphilum*	FSSC	1-b	NRRL 34095	FJ240404	100	*F. petroliphilum*	FSSC	1-c	NRRL 43812	EF470093	100
23494	*F. petroliphilum*	FSSC	1-b	NRRL 34095	FJ240404	100	*F. petroliphilum*	FSSC	1-c	NRRL 43812	EF470093	100
23495	*F. petroliphilum*	FSSC	1-b	NRRL 34095	FJ240404	100	*F. petroliphilum*	FSSC	1-c	NRRL 43812	EF470093	100
23496	*F. petroliphilum*	FSSC	1-b	NRRL 34095	FJ240404	100	*F. petroliphilum*	FSSC	1-c	NRRL 43812	EF470093	100

4.3.6 *Fusarium*-ID database nBLAST™ results bases on RPB2 (5F and 7CR) sequences for isolates obtained from sweet potato material

The *Fusarium*-ID database nBLAST™ results based on the RPB2 (5F and 7CR) sequences of 89 strains obtained from diseased sweet potato material clustered into three *Fusarium* species complexes, namely FIESC, FSSC and FOSC. The species in the complexes were represented by *F. lacertarum* in the FIESC, *F. petroliphilum* in the FSSC and *F. inflexum* and *F. oxysporum* in the FOSC (Table 4.3). The RPB2 (5F and 7CR) *Fusarium*-ID database nBLAST™ analyses indicated a significant percentage similarity of 99.20-100% for most of the isolates. The FIESC was represented by three strains (PPR 23473, 23475 and 23478) were similar to *F. lacertarum* NRRL 20423 MLST type 4-a with a percentage similarity of 99.20-99.77%. The FSSC was represented by 20 (22%) strains (PPRI 23476, 23477, 23479, 23480, 23481, 23482, 23483, 23484, 23485, 23486, 23487, 23488, 23489, 23490, 23491, 23492, 23493, 23494, 23495 and 23496) that were similar to *F. petroliphilum* NRRL 43812 MLST type 1-c in the FSSC with a percentage similarity of 99.22-100%. The FOSC was represented by 12 (13%) strains (PPRI 9462, 20163, 20165, 20173, 20174, 20176, 20177, 23063, 23064, 23067, 23072 and 23474) that were similar to *F. inflexum* NRRL 20433 with a percentage similarity ranging from 98.92-99.77%.

The *Fusarium*-ID database nBLAST™ results based on the RPB2 (5F and 7CR) sequences of 89 fungal strains clustered into 49 *F. oxysporum* in the FOSC. Fourty-nine sweet potato strains were similar to *F. oxysporum f. sp. lycopersici* NRRL 34936 MLST 63 in the FOSC with a percentage similarity ranging from 98.70-99.77%. The *Fusarium* MLST and *Fusarium*-ID database nBLAST™ results based on the RPB2 (5F and 7CR) sequences did not reveal the expected results compared to TEF-1α sequences. Both *Fusarium*-ID and *Fusarium* MLST databases revealed only one *forma specialis*, namely, *F. oxysporum f. sp. lycopersici*.

4.3.7 *Fusarium* MLST database nBLAST™ results based on RPB2 (7CF and 11AR) sequences for isolates obtained from sweet potato

The *Fusarium* MLST database nBLAST™ results based on the RPB2 (7CF and 11AR) sequences of 89 strains obtained from diseased sweet potato material

clustered into three *Fusarium* species complexes, namely FIESC, FSSC and FOSC. The species in the complexes were represented by *F. lacertarum* and *F. scirpi* in the FIESC, *F. keratoplasticum* and *F. petroliphilum* in the FSSC and *F. inflexum* and *F. oxysporum* in the FOSC. The RPB2 (7CF and 11AR) *Fusarium* MLST database nBLAST™ analyses indicated a percentage similarity of 99-100% for most of the isolates (Table 4.4). PPRI 23473 was 99.64% similar to *F. lacertarum* NRRL 20423 MLST type 4-a and PPRI 23061 was 99.43% similar to *F. scirpi* CBS 731.87 MLST type 12-a in the FIESC. PPRI 23475 and 23478 were similar to *Fusarium* sp. NRRL 36401 MLST type 2-a and *Fusarium* sp. NRRL 28029 MLST type 3-b in the FIESC with a similarity of 99.42 and 100%, respectively. Twenty strains (PPRI 23476, 23477, 23479, 23480, 23481, 23482, 23483, 23484, 23485, 23486, 23487, 23488, 23489, 23490, 23491, 23492, 23493, 23494, 23495 and 23496) were similar to *F. petroliphilum* NRRL 22142 MLST type 1-b in the FSSC with a percentage similarity ranging from 99.89-100%. Four strains (PPRI 18014, 18017, 18018 and 18751) were similar to *F. solani* 001AFUS, 001DFUS, CBS 490.63 and 001DFUS, respectively, in the FSSC with a low percentage similarity of 83.35-83.49%.

The RPB2 (7CF and 11AR) *Fusarium* MLST database nBLAST™ results revealed a total of 37 sweet potato strains represented by *F. oxysporum* NRRL 25387 MLST type 27 that clustered in the FOSC with a percentage similarity ranging from 99.34-100%. Twenty-three sweet potato strains (PPRI 9461, 9465, 17592, 17593, 17594, 17595, 17596, 10816, 18750, 18752, 20164, 20168, 20170, 20171, 20172, 20175, 20178, 23062, 23065, 23068, 23069, 23070 and 23074) were similar to *F. oxysporum f. sp. lycopersici* NRRL 34936 MLST type 63 in the FOSC with a percentage similarity ranging from 99.26-100%. The *Fusarium* MLST and *Fusarium*-ID database nBLAST™ results based on the RPB2 (7CF and 11AR) sequences did not reveal the expected results compared to TEF-1α sequences.

Table 4.4: *Fusarium* MLST and *Fusarium*-ID nBLAST™ results of RPB2 (7CF and 11AR) from diseased sweet potato fungal isolates

	***FUSARIUM* MLST DATABASE**						***FUSARIUM*-ID DATABASE**					
PPRI Number	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**
9458	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	99.52	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.76
9459	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	99.52	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.76
9460	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	99.52	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.76
9461	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	100	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.53
9462	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	99.88	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	99.84
9463	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	99.34	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.65
9464	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	99.64	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.88
9465	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.75	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.76
9466	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	99.64	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.88
9467	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	99.52	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.76
9468	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	99.52	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.76
9469	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	99.52	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.76
9470	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	99.52	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.76
9471	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	99.52	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	99.76
9472	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	99.52	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	99.76
9473	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	99.52	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	99.76
10531	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	99.52	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.88
10532	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	99.52	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.76
10533	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	99.52	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.76
17592	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.88	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.88
17593	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.88	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.88
17594	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.88	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.88
17595	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.88	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.88
17596	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.88	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.88

	FUSARIUM MLST DATABASE						*FUSARIUM*-ID DATABASE					
PPRI Number	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**
18014	*F. solani*	FSSC	N/A	001AFUS	JN985499	83.46	*F. keratoplasticum*	FSSC	2-r	NRRL 32862	EU329631	83.90
18016	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	100	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	100
18017	*F. solani*	FSSC	N/A	001DFUS	JN985497	83.35	*F. keratoplasticum*	FSSC	2-r	NRRL 32862	EU329631	83.90`
18018	*F. solani*	FSSC	N/A	CBS 490.63	EU329524	83.49	*F. keratoplasticum*	FSSC	2-r	NRRL 32862	EU329631	83.86
18750	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.26	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.30
18751	*F. solani*	FSSC	N/A	001DFUS	JN985497	83.39	*F. keratoplasticum*	FSSC	2-r	NRRL 32862	EU329631	83.90
18752	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.75	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.76
18753	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	99.64	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.88
20163	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	100	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	100
20164	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.74	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.76
20165	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	100	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	100
20166	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	99.51	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.53
20167	*F. inflexum*	FOSC	N/A	NRRL 20433	JX171583	99.34	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	99.34
20168	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.75	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.76
20169	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	100	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	100
20170	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	100	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	100
20171	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.75	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.76
20172	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.75	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.76
20173	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	100	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	99.88
20174	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	99.64	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.65
20175	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.75	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.76
20176	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	100	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	100
20177	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	100	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	100
20178	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.67	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.66
20179	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	99.64	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.65
23061	*F. scirpi*	FIESC	12-a	CBS 731.87	GQ505778	99.43	*F. equiseti*	FIESC	14-b	NRRL 20697	JX171595	99.83

	***FUSARIUM* MLST DATABASE**						***FUSARIUM*-ID DATABASE**					
PPRI Number	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**
23062	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.87	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.87
23063	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	99.87	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	99.88
23064	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	99.76	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.76
23065	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.88	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.88
23066	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	99.64	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.88
23067	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	99.64	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.65
23068	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.75	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.76
23069	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.75	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.76
23070	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.75	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.76
23071	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	99.64	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.88
23072	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	100	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	100
23074	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.88	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.76
23076	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	99.52	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.76
23077	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	99.52	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.76
23078	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	99.52	*F. oxysporum f. sp. lycopersici*	FOSC	63	NRRL 34936	JX171646	99.76
23473	*F. lacertarum*	FIESC	4-a	NRRL 20423	JX171581	99.64	*F. lacertarum*	FIESC	4-a	NRRL 20423	JX171581	99.53
23474	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	100	*F. oxysporum*	FOSC	27	NRRL 25387	JX171625	100
23475	*Fusarium* sp.	FIESC	2-a	NRRL 36401	GQ505829	99.42	*F. lacertarum*	FIESC	4-a	NRRL 20423	JX171581	99.40
23476	*F. petroliphilum*	FSSC	1-b	NRRL 22142	FJ240379	100	*F. petroliphilum*	FSSC	1-c	NRRL 43812	EF470093	100
23477	*F. petroliphilum*	FSSC	1-b	NRRL 22142	FJ240379	100	*F. petroliphilum*	FSSC	1-c	NRRL 43812	EF470093	100
23478	*Fusarium* sp.	FIESC	3-b	NRRL 28029	GQ505780	100	*F. lacertarum*	FIESC	4-a	NRRL 20423	JX171581	99.40
23479	*F. petroliphilum*	FSSC	1-b	NRRL 22142	FJ240379	100	*F. petroliphilum*	FSSC	1-c	NRRL 43812	EF470093	100
23480	*F. petroliphilum*	FSSC	1-b	NRRL 22142	FJ240379	100	*F. petroliphilum*	FSSC	1-c	NRRL 43812	EF470093	100
23481	*F. petroliphilum*	FSSC	1-b	NRRL 22142	FJ240379	100	*F. petroliphilum*	FSSC	1-c	NRRL 43812	EF470093	99.88
23482	*F. petroliphilum*	FSSC	1-b	NRRL 22142	FJ240379	100	*F. petroliphilum*	FSSC	1-c	NRRL 43812	EF470093	100
23483	*F. petroliphilum*	FSSC	1-b	NRRL 22142	FJ240379	100	*F. petroliphilum*	FSSC	1-c	NRRL 43812	EF470093	100

	***FUSARIUM* MLST DATABASE**						***FUSARIUM*-ID DATABASE**					
PPRI Number	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**
23484	*F. petroliphilum*	FSSC	1-b	NRRL 22142	FJ240379	100	*F. petroliphilum*	FSSC	1-c	NRRL 43812	EF470093	100
23485	*F. petroliphilum*	FSSC	1-b	NRRL 22142	FJ240379	100	*F. petroliphilum*	FSSC	1-c	NRRL 43812	EF470093	100
23486	*F. petroliphilum*	FSSC	1-b	NRRL 22142	FJ240379	100	*F. petroliphilum*	FSSC	1-c	NRRL 43812	EF470093	100
23487	*F. petroliphilum*	FSSC	1-b	NRRL 22142	FJ240379	100	*F. petroliphilum*	FSSC	1-c	NRRL 43812	EF470093	100
23488	*F. petroliphilum*	FSSC	1-b	NRRL 22142	FJ240379	100	*F. petroliphilum*	FSSC	1-c	NRRL 43812	EF470093	100
23489	*F. petroliphilum*	FSSC	1-b	NRRL 22142	FJ240379	100	*F. petroliphilum*	FSSC	1-c	NRRL 43812	EF470093	100
23490	*F. petroliphilum*	FSSC	1-b	NRRL 22142	FJ240379	100	*F. petroliphilum*	FSSC	1-c	NRRL 43812	EF470093	100
23491	*F. petroliphilum*	FSSC	1-b	NRRL 22142	FJ240379	99.89	*F. petroliphilum*	FSSC	1-c	NRRL 43812	EF470093	99.88
23492	*F. petroliphilum*	FSSC	1-b	NRRL 22142	FJ240379	99.89	*F. petroliphilum*	FSSC	1-c	NRRL 43812	EF470093	99.88
23493	*F. petroliphilum*	FSSC	1-b	NRRL 22142	FJ240379	99.89	*F. petroliphilum*	FSSC	1-c	NRRL 43812	EF470093	99.88
23494	*F. petroliphilum*	FSSC	1-b	NRRL 22142	FJ240379	99.89	*F. petroliphilum*	FSSC	1-c	NRRL 43812	EF470093	99.88
23495	*F. petroliphilum*	FSSC	1-b	NRRL 22142	FJ240379	99.89	*F. petroliphilum*	FSSC	1-c	NRRL 43812	EF470093	99.88
23496	*F. petroliphilum*	FSSC	1-b	NRRL 22142	FJ240379	99.89	*F. petroliphilum*	FSSC	1-c	NRRL 43812	EF470093	99.88

4.3.8 *Fusarium*-ID database nBLAST™ results based on RPB2 (7CF and 11AR) sequences for isolates obtained from sweet potato

The *Fusarium*-ID database nBLAST™ results based on the RPB2 (5F and 7CR) sequences of 89 strains obtained from diseased sweet potato material clustered into three *Fusarium* species complexes, namely FIESC, FSSC and FOSC. The species in the complexes were represented by *F. equiseti* and *F. lacertarum* in the FIESC, *F. keratoplasticum* Geiser, O'Donnell, D.P.G. Short & Ning Zhang and *F. petroliphilum* in the FSSC and *F. inflexum* and *F. oxsysporum* in the FOSC. PPRI 23061 was similar to *F. equiseti* NRRL 20697 MLST type 14-b in the FIESC with a percentage similarity of 99.83%. Three strains (PPRI 23473, 23475 and 23478) were similar to *F. lacertarum* NRRL 20423 MLST type 4-a in the FIESC with a similarity of 99.40-99.53%. Four sweet potato strains (PPRI 18014, 18017, 18018 and 18751) were similar to *F. keratoplasticum* NRRL 32862 MLST type 2-r in the FSSC with a low percentage similarity of 83.86-83.90%. *Fusarium keratoplasticum* was isolated in drains biofilms and occurrences of contact lens-associated mycotic keratitis (Short *et al.*, 2013).

The FOSC was represented by 14 strains (PPRI 9462, 9471, 9472, 9473, 20163, 20165, 20167, 20169, 20173, 20176, 20177, 23063, 23072 and 23474) that were similar to *F. oxysporum* NRRL 25387 MLST type 27 with a percentage similarity of 99.34-100%. Fourty-seven strains (PPRI 9458, 9459, 9460, 9461, 9463, 9464, 9465, 9466, 9467, 9468, 9469, 9470, 10531, 10532, 10533, 17592, 17593, 17594, 17595, 17596, 10816, 18750, 18752, 18753, 20164, 20166, 20168, 20170, 20171, 20172, 20174, 20175, 20178, 20179, 23062, 23064, 23065, 23066, 23067, 23068, 23069, 23070, 23071, 23074, 23076, 23077 and 23078) were similar to *F. oxysporum f. sp. lycopersici* MLST 63 with a percentage similarity ranging from 99.30-100%. Both *Fusarium*-ID and *Fusarium* MLST databases revealed only one *forma specialis*, namely, *F. oxysporum f. sp. lycopersici* (Table 4.4).

4.3.9 *Fusarium*-MLST database nBLAST™ results based on β-tubulin sequences for isolates obtained from sweet potato

The *Fusarium*-MLST database nBLAST™ results based on the β-tubulin sequences of 89 strains obtained from diseased sweet potato material clustered into two

Fusarium species complexes, namely *F. dimerum species* complex (FDSC) and FOSC. The species in the complexes were represented by *F. biseptatum* Sawada, *F. cf. lunatum*, *F. delphinoides* Schroers, Summerb., O'Donnell & Lampr., *F. domesticum* (Fr.) H.P. Bachm., *F. dimerum var. violaceum* Wollenw., *F. lunatum* (Ellis & Everh.) Arx in the FDSC and *F. oxysporum* in the FOSC. *Calonectria amazoniensis*, *Calonectria tereticornis, Chaetosphaeria pymaea*, *Seimatosporium anomalum*, *Zopfiella ebriosa* did not belong to any of the *Fusarium* species complex. Two obtained strains (PPRI 9461 and 9473) were similar to *F. oxysporum f. sp. passiflorae* NRRL MLST 16 and *F. oxysporum f. sp. tuberosi* NRRL 22555 MLST 21, respectively, with a percentage similarity of 100%.

The *Fusarium*-MLST database nBLAST™ results based on the β-tubulin sequences did not reveal the relevant results and had a low percentage similarity ranging from 83.25-90.58%. *Fusarium dimerum* species complex was represented by 63 sweet potato strains that were similar to *F. cf. delphinoides*, *F. cf. lunatum*, *F. lunatum*, *F. dimerum var. violaceum*, *F. delphinoides*, *F. domesticum* and *F. biseptatum* with a low percentage similarity ranging from 83.25-90.58%. Thirteen PPRI sweet potato strains (PPRI 23476, 23477, 23479, 23481, 23482, 23484, 23485, 23486, 23487, 23488, 23491, 23492 and 23494) displayed a similarity with *C. pymaea* with the low percentage similarity of 90.53%. PPRI 23478 was 88.42% similar to *C. amazoniensis* and PPRI 23480 was 72.45% similar to *S. anomalum*. Five sweet potato strains (PPRI 23483, 23489, 23490, 23493 and 23495) were similar to *Z. ebriosa* with a percentage similarity of 90.74% (Table 4.5). The results indicate that new tools that allow a more distinguished grouping of species based on β-tubulin in *Fusarium* are needed and more sequences based on β-tubulin should be deposited into the database.

4.3.10 *Fusarium*-ID database nBLAST™ results based on β-tubulin sequences for isolates obtained from sweet potato

The *Fusarium*-ID database nBLAST™ results based on the β-tubulin sequences of 89 strains obtained from diseased sweet potato material clustered into two *Fusarium* species complexes, namely FDSC and FOSC. The species in the complexes were represented by *F. cf. lunatum*, *F. lunatum* and *F. domesticum* in

the FDSC and *F. oxysporum* in the FOSC. *Fusarium dimerum* species complex was represented by 86 sweet potato strains with a low percentage similarity of 83.04-93.57%. Only three sweet potato strains (PPRI 9461, 9466 and 9473) were similar to *F. oxysporum* NRRL 25369 with the percentage similarity of 99.44%, 98.89% and 99.26%, respectively. The rest of the β-tubulin sequences from *Fusarium*-ID database nBLAST™ did not reveal the relevant results and had a lower percentage similarity of 83.04-93.57% for *F. domesticum*, *F. cf. lunatum*, and *F. lunatum* within FDSC as indicated in Table 4.5.

The β-tubulin data set from Laurence *et al.* (2014) had only one PIC and therefore, was excluded from the GCPSR analysis (Laurence *et al.*, 2014), however, β-tubulin is an excellent informative locus in other *Fusarium* species complexes (O'Donnell *et al.*, 1998a; O'Donnell, 2000). In addition, β-tubulin gene region has the ability to resolve closely related species (Lima *et al.*, 2009; Walsh *et al.*, 2010). Geiser *et al.* (2004) reported that species that are poorly characterised are listed in the *Fusarium*-ID database as '*Fusarium* sp. *cf*' since there is unsurity of their correct identification until morphological and multilocus phylogenetic analyses studies are done (Geiser *et al.*, 2004). Therefore, the nBLAST™ results based on the *Fusarium* MLST and *Fusarium*-ID databases suggest that the query β-tubulin sequences corresponded to a species that were poorly defined (Geiser *et al.*, 2004). The *Fusarium*-ID database revealed no *formae speciales*, in contrast to the two *formae speciales* revealed by the *Fusarium* MLST database.

Table 4.5: *Fusarium* MLST and *Fusarium*-ID nBLAST™ results of β-tubulin from diseased sweet potato fungal isolates

	***FUSARIUM* MLST DATABASE**						***FUSARIUM*-ID DATABASE**					
PPRI Number	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**
9458	*F. cf. lunatum*	FDSC	none	NRRL 36185/34031	EU926356	85.03	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	91.56
9459	*F. delphinoides*	FDSC	N/A	NRRL 36172	EU926371	85.19	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	91.44
9460	*F. cf. lunatum*	FDSC	N/A	NRRL 36185/34031	EU926356	85.13	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	91.44
9461	*F. oxysporum f. sp. passiflorae*	FOSC	16	NRRL 22549	AF008540	100	*F. oxysporum*	FOSC	N/A	NRRL 25369	AF008517	99.44
9462	*F. cf. delphinoides*	FDSC	none	NRRL 36191	EU926379	85.58	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	92.55
9463	*F. cf. lunatum*	FDSC	none	NRRL 36185/34031	EU926356	85.04	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	91.44
9464	*F. delphinoides*	FDSC	N/A	NRRL 53291	EU926363	84.80	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	91.22
9465	*F. delphinoides*	FDSC	N/A	NRRL 53291	EU926363	84.71	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	91.44
9466	*F. cf. lunatum*	FDSC	none	NRRL 36185/34031	EU926356	84.69	*F. oxysporum*	FOSC	N/A	NRRL 25369	AF008517	98.89
9467	*F. cf. lunatum*	FDSC	none	NRRL 36185/34031	EU926356	85.65	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	91.56
9468	*F. delphinoides*	FDSC	N/A	NRRL 53291	EU926363	85.18	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	91.56
9469	*F. cf. lunatum*	FDSC	none	NRRL 36185/34031	EU926356	85.05	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	91.33
9470	*F. cf. lunatum*	FDSC	none	NRRL 36185/34031	EU926356	85.13	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	91.44
9471	*F. cf. lunatum*	FDSC	none	NRRL 36185/34031	EU926356	85.52	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	91.57
9472	*F. delphinoides*	FDSC	N/A	NRRL 53290	EU926362	85.16	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	91.56
9473	*F. oxysporum f. sp. tuberosi*	FOSC	21	NRRL 22555	AF008546	100	*F. oxysporum*	FOSC	N/A	NRRL 25369	AF008517	99.26
10531	*F. cf. lunatum*	FDSC	none	NRRL 36185/34031	EU926356	85.58	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	91.44
10532	*F. cf. lunatum*	FDSC	none	NRRL 36185/34031	EU926356	85.39	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	91.22
10533	*C. tereticornis*	N/A	N/A	CBS 111301	KX784664	90.52	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	91.33
17592	*F. delphinoides*	FDSC	N/A	NRRL 53291	EU926363	85.23	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	91.56
17593	*F. delphinoides*	FDSC	N/A	NRRL 53291	EU926363	85.19	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	91.56
17594	*F. delphinoides*	FDSC	N/A	NRRL 53291	EU926363	85.19	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	91.56
17595	*F. delphinoides*	FDSC	N/A	NRRL 53291	EU926363	85.19	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	91.56
17596	*F. delphinoides*	FDSC	N/A	NRRL 53291	EU926363	85.22	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	91.56

	***FUSARIUM* MLST DATABASE**						***FUSARIUM*-ID DATABASE**					
PPRI Number	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**
18014	*C. tereticornis*	N/A	N/A	CBS 111301	KX784664	89.76	*F. lunatum*	FDSC	N/A	NRRL 20690/36168/37067	KM232057	93.33
18016	*F. delphinoides*	FDSC	N/A	NRRL 22260	EU926374	83.25	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	91.23
18017	*F. lunatum/F. dimerum var. violaceum*	FDSC	none	NRRL 20690/36168/37067	EU926357	89.64	*F. lunatum*	FDSC	N/A	NRRL 20690/36168/37067	KM232057	93.23
18018	*F. lunatum/F. dimerum var. violaceum*	FDSC	none	NRRL 20690/36168/37067	EU926357	89.64	*F. lunatum*	FDSC	N/A	NRRL 20690/36168/37067	KM232057	93.23
18750	*F. delphinoides*	FDSC	N/A	NRRL 53291	EU926363	85.25	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	91.56
18751	*C. tereticornis*	N/A	N/A	CBS 111301	KX784664	89.76	*F. lunatum*	FDSC	N/A	NRRL 20690/36168/37067	KM232057	93.33
18752	*F. delphinoides*	FDSC	N/A	NRRL 53291	EU926363	85.19	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	91.56
18753	*F. delphinoides*	FDSC	N/A	NRRL 53291	EU926363	85.22	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	91.56
20163	*F. cf. lunatum*	FDSC	none	NRRL 34031	EU926356	85.87	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	92.32
20164	*F. cf. lunatum*	FDSC	none	NRRL 36185/34031	EU926356	85.00	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	91.56
20165	*F. cf. lunatum*	FDSC	none	NRRL 36185/34031	EU926356	85.98	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	92.55
20166	*F. delphinoides*	FDSC	N/A	NRRL 53291	EU926363	85.23	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	91.56
20167	*F. domesticum*	FDSC	none	NRRL 29976	EU926353	87.71	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	91.56
20168	*F. domesticum*	FDSC	none	NRRL 37583	EU926354	89.46	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	91.56
20169	*F. cf. lunatum*	FDSC	none	NRRL 36185/34031	EU926356	85.99	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	91.56
20170	*F. cf. lunatum*	FDSC	none	NRRL 36185/34031	EU926356	85.99	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	92.55
20171	*F. domesticum*	FDSC	none	NRRL 37583	EU926354	89.47	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	91.56
20172	*F. lunatum/F. dimerum var. violaceum*	FDSC	none	NRRL 20690/36168/37067	EU926357	89.01	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	91.56
20173	*F. domesticum*	FDSC	none	NRRL 29976	EU926353	90.58	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	92.55
20174	*F. domesticum*	FDSC	none	NRRL 29976	EU926353	90.45	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	92.55
20175	*F. delphinoides*	FDSC	N/A	NRRL 53291	EU926363	85.25	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	91.56
20176	*F. cf. lunatum*	FDSC	none	NRRL 36185/34031	EU926356	85.97	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	92.44
20177	*F. cf. lunatum*	FDSC	none	NRRL 36185/34031	EU926356	86.03	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	92.55

	FUSARIUM MLST DATABASE						*FUSARIUM*-ID DATABASE					
PPRI Number	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**
20178	*F. delphinoides*	FDSC	N/A	NRRL 53291	EU926363	85.20	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	91.56
20179	*F. delphinoides*	FDSC	N/A	NRRL 53291	EU926363	85.25	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	91.56
23061	*F. delphinoides*	FDSC	N/A	NRRL 36191	EU926379	87.94	*F. cf. lunatum*	FDSC	N/A	NRRL 36185/34031	EU926356	93.32
23062	*F. delphinoides*	FDSC	N/A	NRRL 53291	EU926363	85.14	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	91.56
23063	*F. cf. lunatum*	FDSC	none	NRRL 36185/34031	EU926356	85.96	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	92.55
23064	*F. cf. lunatum*	FDSC	none	NRRL 36185/34031	EU926356	85.96	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	92.55
23065	*F. delphinoides*	FDSC	N/A	NRRL 53291	EU926363	85.23	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	91.56
23066	*F. delphinoides*	FDSC	N/A	NRRL 53291	EU926363	85.42	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	91.56
23067	*F. cf. lunatum*	FDSC	none	NRRL 36185/34031	EU926356	85.96	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	92.55
23068	*F. delphinoides*	FDSC	N/A	NRRL 36191	EU926379	85.31	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	91.56
23069	*F. delphinoides*	FDSC	N/A	NRRL 36191	EU926379	85.24	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	91.56
23070	*F. delphinoides*	FDSC	N/A	NRRL 53291	EU926363	85.24	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	91.56
23071	*F. delphinoides*	FDSC	N/A	NRRL 53291	EU926363	85.24	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	91.56
23072	*F. cf. lunatum*	FDSC	none	NRRL 36185/34031	EU926356	86.02	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	92.43
23074	*F. cf. lunatum*	FDSC	none	NRRL 36185/34031	EU926356	85.20	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	91.56
23076	*F. delphinoides*	FDSC	N/A	NRRL 53291	EU926363	85.24	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	91.56
23077	*F. delphinoides*	FDSC	N/A	NRRL 53291	EU926363	85.23	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	91.56
23078	*F. cf. lunatum*	N/A	none	NRRL 36185/34031	EU926356	85.31	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	91.56
23473	*F. biseptatum*	FDSC	none	NRRL 36158	EU926384	87.63	*F. cf. lunatum*	FDSC	N/A	NRRL 36185/34031	EU926356	93.57
23474	*F. cf. lunatum*	FDSC	none	NRRL 36185/34031	EU926356	85.20	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	92.55
23475	*F. biseptatum*	FDSC	none	NRRL 36164	EU926386	87.44	*F. cf. lunatum*	FDSC	N/A	NRRL 36185/34031	EU926356	93.32
23476	*C. pymaea*	N/A	N/A	CBS 114451	AF466040	90.53	*F. lunatum*	FDSC	N/A	NRRL 20690/36168/37067	KM232057	93.39
23477	*C. pymaea*	N/A	N/A	CBS 114451	AF466040	90.53	*F. lunatum*	FDSC	N/A	NRRL 20690/36168/37067	KM232057	93.39
23478	*C. amazoniensis*	N/A	N/A	CBS 115438	KX784613	88.42	*F. cf. lunatum*	FDSC	N/A	NRRL 36185/34031	EU926356	93.32
23479	*C. pymaea*	N/A	N/A	CBS 114451	AF466040	90.53	*F. lunatum*	FDSC	N/A	NRRL 20690/36168/37067	KM232057	93.39

	***FUSARIUM* MLST DATABASE**						***FUSARIUM*-ID DATABASE**					
PPRI Number	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**
23480	*S. anomalum*	N/A	N/A	CBS 437.87		72.45	*F. domesticum*	FDSC	N/A	NRRL 37582	EU926355	83.04
23481	*C. pymaea*	N/A	N/A	CBS 114451	AF466040	90.53	*F. lunatum*	FDSC	N/A	NRRL 20690/36168/37067	KM232057	93.39
23482	*C. pymaea*	N/A	N/A	CBS 114451	AF466040	90.53	*F. lunatum*	FDSC	N/A	NRRL 20690/36168/37067	KM232057	93.39
23483	*Z. ebriosa*	N/A	N/A	CBS 111.75	AY780146	90.74	*F. lunatum*	FDSC	N/A	NRRL 20690/36168/37067	KM232057	93.39
23484	*C. pymaea*	N/A	N/A	CBS 114451	AF466040	90.53	*F. lunatum*	FDSC	N/A	NRRL 20690/36168/37067	KM232057	93.39
23485	*C. pymaea*	N/A	N/A	CBS 114451	AF466040	90.53	*F. lunatum*	FDSC	N/A	NRRL 20690/36168/37067	KM232057	93.39
23486	*C. pymaea*	N/A	N/A	CBS 114451	AF466040	90.53	*F. lunatum*	FDSC	N/A	NRRL 20690/36168/37067	KM232057	93.39
23487	*C. pymaea*	N/A	N/A	CBS 114451	AF466040	90.53	*F. lunatum*	FDSC	N/A	NRRL 20690/36168/37067	KM232057	93.39
23488	*C. pymaea*	N/A	N/A	CBS 114451	AF466040	90.53	*F. lunatum*	FDSC	N/A	NRRL 20690/36168/37067	KM232057	93.39
23489	*Z. ebriosa*	N/A	N/A	CBS 111.75	AY780146	90.74	*F. lunatum*	FDSC	N/A	NRRL 20690/36168/37067	KM232057	93.39
23490	*Z. ebriosa*	N/A	N/A	CBS 111.75	AY780146	90.74	*F. lunatum*	FDSC	N/A	NRRL 20690/36168/37067	KM232057	93.39
23491	*C. pymaea*	N/A	N/A	CBS 114451	AF466040	90.53	*F. lunatum*	FDSC	N/A	NRRL 20690/36168/37067	KM232057	93.39
23492	*C. pymaea*	N/A	N/A	CBS 114451	AF466040	90.53	*F. lunatum*	FDSC	N/A	NRRL 20690/36168/37067	KM232057	93.39
23493	*Z. ebriosa*	N/A	N/A	CBS 111.75	AY780146	90.74	*F. lunatum*	FDSC	N/A	NRRL 20690/36168/37067	KM232057	93.39
23494	*C. pymaea*	N/A	N/A	CBS 114451	AF466040	90.53	*F. lunatum*	FDSC	N/A	NRRL 20690/36168/37067	KM232057	93.39
23495	*Z. ebriosa*	N/A	N/A	CBS 111.75	AY780146	90.74	*F. lunatum*	FDSC	N/A	NRRL 20690/36168/37067	KM232057	93.39
23496	*F. delphinoides*	FDSC	N/A	NRRL 53289	EU926361	87.91	*F. lunatum*	FDSC	N/A	NRRL 20690/36168/37067	KM232057	93.26

Table 4.6: *Fusarium* MLST and *Fusarium*-ID nBLAST™ results of ITS from diseased sweet potato fungal isolates

	***FUSARIUM* MLST DATABASE**						***FUSARIUM*-ID DATABASE**					
PPRI Number	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**
9458	*F. oxysporum f. sp. ciceris*	FOSC	N/A	Foc167	JN400697	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.58
9459	*Fusarium* sp.	N/A	N/A	D2I10	HM131981	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.43
9460	*F. oxysporum*	FOSC	N/A	IA6I7F1	KX421440	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.39
9461	*F. oxysporum f. sp. ciceris*	FOSC	N/A	Foc108	JN400681	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.24
9462	*F. oxysporum*	FOSC	N/A	SMG1	KY090780	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.24
9463	*Fusarium* sp.	N/A	N/A	D2I10	HM131981	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.43
9464	*Fusarium* sp.	N/A	N/A	D1I22	HM132001	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.46
9465	*Fusarium* sp.	N/A	N/A	D2I7	HM131987	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.61
9466	*Fusarium* sp.	N/A	N/A	D1I22	HM132001	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.46
9467	*Fusarium* sp.	N/A	N/A	D2I10	HM131981	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.43
9468	*Fusarium* sp.	N/A	N/A	D2I10	HM131981	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.43
9469	*Fusarium* sp.	N/A	N/A	D2I10	HM131981	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.43
9470	*F. oxysporum f. sp. lentis*	FOSC	N/A	FLS52	KU671041	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.38
9471	*Fusarium* sp.	N/A	N/A	D2I10	HM131981	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.43
9472	*Fusarium* sp.	N/A	N/A	D1I22	HM132001	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.46
9473	*F. oxysporum*	FOSC	N/A	CJl41109	KC767892	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.53
10531	*F. oxysporum*	FOSC	237	NRRL 43668	EF453151	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.24
10532	*F. oxysporum*	FOSC	N/A	IA8I1F1	KX421428	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.27
10533	*F. oxysporum*	FOSC	N/A	AA2I1F1	KX421435	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.24
17592	*F. oxysporum*	FOSC	N/A	By125	GQ365156	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.42
17593	*F. oxysporum*	FOSC	N/A	FTB2	KY810802	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.53
17594	*F. oxysporum*	FOSC	N/A	DET-20	KX385043	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.56
17595	*F. oxysporum*	FOSC	N/A	CA1I1F3	KX421434	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.53
17596	*Fusarium* sp.	N/A	N/A	DZF18	EU543261	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.53

	***FUSARIUM* MLST DATABASE**						***FUSARIUM*-ID DATABASE**					
PPRI Number	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**
18014	*Clonostachys* sp.	N/A	N/A	PAPOCHF 04	HQ731632	100	*Fusarium* sp.	FCSC	1-i	NRRL 45992	GQ505431	95.67
18016	*F. oxysporum*	FOSC	N/A	DET-20	KX385043	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.56
18017	*Clonostachys* sp.	N/A	N/A	PAPOCHF 04	HQ731632	100	*Fusarium* sp.	FCSC	1-i	NRRL 45992	GQ505431	95.67
18018	*Clonostachys* sp.	N/A	N/A	PAPOCHF 04	HQ731632	100	*Fusarium* sp.	FCSC	1-i	NRRL 45992	GQ505431	95.67
18750	*F. oxysporum*	FOSC	N/A	DET-20	KX385043	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.56
18751	*C. rosea*	N/A	N/A	CR0814M	KP670432	100	*Fusarium* sp.	FCSC	1-i	NRRL 45992	GQ505431	95.67
18752	*F. oxysporum*	FOSC	N/A	FTB2	KY810802	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.53
18753	*F. oxysporum*	FOSC	N/A	ISOLATE 2424	KT828535	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.61
20163	*F. oxysporum*	FOSC	N/A	IA8I1F1	KX421428	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.28
20164	*F. oxysporum*	FOSC	N/A	F1	KY810792	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.3
20165	*F. oxysporum*	FOSC	N/A	IHEM 9571	KP132219	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.11
20166	*F. oxysporum f. sp. ciceris*	FOSC	N/A	IHB F 2902	KM817208	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.17
20167	*F. oxysporum*	FOSC	N/A	IHEM 22401	KP132218	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.58
20168	*F. oxysporum*	FOSC	N/A	BY125	GQ365156	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.42
20169	*F. oxysporum*	FOSC	N/A	2271	KX929698	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.28
20170	*F. oxysporum*	FOSC	N/A	ELRF 8	KX786247	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.33
20171	*F. oxysporum f. sp. cucumerinum*	FOSC	N/A	ZJ-04	HM179530	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.29
20172	*F. oxysporum*	FOSC	N/A	DET-25	KX385044	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.54
20173	*F. oxysporum*	FOSC	N/A	IHEM 9571	KP132219	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	94.99
20174	*F. oxysporum f. sp. ciceris*	FOSC	N/A	IHB F 2902	KM817208	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.26
20175	*F. oxysporum*	FOSC	1	NRRL 43646	EF453129	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.05
20176	*F. oxysporum*	FOSC	N/A	GXF-6	EU285554	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.52
20177	*F. oxysporum*	FOSC	N/A	CJL41109	KC767892	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.53
20178	*F. oxysporum*	FOSC	N/A	ELRF 8	KX786247	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.34
20179	*Fusarium* sp.	N/A	N/A	T22	KT351621	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.39
23061	*F. equiseti*	FIESC	N/A	ISOLATE 32	KY318493	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.33

	***FUSARIUM* MLST DATABASE**						***FUSARIUM*-ID DATABASE**					
PPRI Number	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**
23062	*F. oxysporum*	FOSC	N/A	ELRF 8	KX786247	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.33
23063	*F. oxysporum*	FOSC	N/A	IA7I1F2	KX421432	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.40
23064	*F. oxysporum*	FOSC	N/A	F345	JX045827	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.25
23065	*F. oxysporum*	FOSC	N/A	F345	JX045827	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.26
23066	*F. oxysporum*	FOSC	N/A	A1S3-D89	KJ774041	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.37
23067	*F. oxysporum*	FOSC	N/A	SHBV2	KY090783	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.28
23068	*F. oxysporum*	FOSC	N/A	IHEM 22401	KP132218	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.48
23069	*F. oxysporum*	FOSC	N/A	IHEM 22401	KP132218	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.19
23070	*F. oxysporum*	FOSC	N/A	BY125	GQ365156	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.42
23071	*F. oxysporum*	FOSC	N/A	N/A	AB369259	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.37
23072	*Fusarium* sp.	N/A	N/A	184GP/F	GQ352492	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.41
23074	*F. oxysporum*	FOSC	N/A	BY125	GQ365156	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.42
23076	*F. oxysporum*	FOSC	237	NRRL 43679	EF453158	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.53
23077	*F. oxysporum*	FOSC	237	NRRL 43668	EF453151	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.53
23078	*F. oxysporum*	FOSC	N/A	BY125	GQ365156	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.42
23473	*Neurospora* sp.	N/A	N/A	FSP14	KX058050	100	*Fusarium* sp.	FIESC	5-b	NRRL 45995	GQ505670	100
23474	*F. oxysporum*	FOSC	N/A	AA2I1F1	KX421435	100	*Fusarium* sp.	FIESC	16-c	NRRL 43730	GQ505669	95.24
23475	*Neurospora* sp.	N/A	N/A	FSP14	KX058050	100	*Fusarium* sp.	FIESC	5-b	NRRL 45995	GQ505670	100
23476	*F. petroliphilum*	FSSC	N/A	HCPF 11106	KC254043	100	*F. solani*	FSSC	N/A	Ppf28	GU586832	95.93
23477	*F. solani*	FSSC	N/A	A2-5	KT876631	100	*F. solani*	FSSC	N/A	Ppf28	GU586832	95.98
23478	*F. equiseti*	FIESC	N/A	SI1008	KU041631	100	*Fusarium* sp.	FIESC	5-f	NRRL 45997	GQ505672	100
23479	*F. solani*	FSSC	N/A	A2-5	KT876631	100	*F. solani*	FSSC	N/A	Ppf28	GU586832	96.03
23480	*F. petroliphilum*	FSSC	N/A	HCPF 11106	KC254043	100	*F. solani*	FSSC	N/A	Ppf28	GU586832	95.89
23481	*F. petroliphilum*	FSSC	N/A	HCPF 11106	KC254043	100	*F. solani*	FSSC	N/A	Ppf28	GU586832	95.93
23482	*F. petroliphilum*	FSSC	N/A	HCPF 11106	KC254043	100	*F. solani*	FSSC	N/A	Ppf28	GU586832	96.52
23483	*F. petroliphilum*	FSSC	N/A	HCPF 11106	KC254043	100	*F. solani*	FSSC	N/A	Ppf28	GU586832	95.98

	***FUSARIUM* MLST DATABASE**						***FUSARIUM*-ID DATABASE**					
PPRI Number	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**	**Species**	**Species Complex**	**MLST Type**	**Strain**	**Accession Number**	**Percentage Similarity (%)**
23484	*Fusarium* sp.	N/A	N/A	NRRL 43724	EF453187	100	*F. solani*	FSSC	N/A	Ppf28	GU586832	95.97
23485	*F. solani*	FSSC	N/A	B9-3	KT876634	100	*F. solani*	FSSC	N/A	Ppf28	GU586832	95.98
23486	*F. petroliphilum*	FSSC	N/A	HCPF 11106	KC254043	100	*F. solani*	FSSC	N/A	Ppf28	GU586832	95.98
23487	*F. solani*	FSSC	N/A	A2-5	KT876631	100	*F. solani*	FSSC	N/A	Ppf28	GU586832	96.03
23488	*F. petroliphilum*	FSSC	N/A	HCPF 11106	KC254043	100	*F. solani*	FSSC	N/A	Ppf28	GU586832	95.89
23489	*F. solani*	FSSC	N/A	BR01	JX282605	100	*F. solani*	FSSC	N/A	Ppf28	GU586832	95.99
23490	*F. petroliphilum*	FSSC	N/A	HCPF 11106	KC254043	100	*F. solani*	FSSC	N/A	Ppf28	GU586832	95.84
23491	*F. petroliphilum*	FSSC	N/A	HCPF 11106	KC254043	100	*F. solani*	FSSC	N/A	Ppf28	GU586832	96.03
23492	*F. petroliphilum*	FSSC	N/A	HCPF 11106	KC254043	100	*F. solani*	FSSC	N/A	Ppf28	GU586832	95.98
23493	*F. petroliphilum*	FSSC	N/A	HCPF 11106	KC254043	100	*F. solani*	FSSC	N/A	Ppf28	GU586832	95.98
23494	*F. petroliphilum*	FSSC	N/A	HCPF 11106	KC254043	100	*F. solani*	FSSC	N/A	Ppf28	GU586832	95.93
23495	*F. petroliphilum*	FSSC	N/A	HCPF 11106	KC254043	100	*F. solani*	FSSC	N/A	Ppf28	GU586832	95.97
23496	*F. petroliphilum*	FSSC	N/A	HCPF 11106	KC254043	100	*F. solani*	FSSC	N/A	Ppf28	GU586832	95.98

4.3.11 *Fusarium*-MLST database nBLAST™ results based on ITS sequences for isolates obtained from sweet potato

The *Fusarium*-MLST database nBLAST™ results based on the ITS sequences of 89 strains obtained from diseased sweet potato material clustered into three *Fusarium* species complexes, namely FIESC, FSSC and FOSC. The species in the complexes were represented by *F. equiseti* in the FIESC, *F. petroliphilum* and *F. solani* in the FSSC and *F. oxysporum* in the FOSC. All the strains had a percentage similarity of 100% as indicated in Table 4.6. PPRI 23061 and 23478 were similar to *F. equiseti* in the FIESC. Fourteen sweet potato strains (PPRI 23476, 23480, 23481, 23482, 23483, 23486, 23488, 23490, 23491, 23492, 23493, 23494, 23495 and 23496) were similar to *F. petroliphilum* in the FSSC. Five sweet potato strains (PPRI 43477, 23479, 23485, 23487 and 23489) were similar to *F. solani* in the FSSC. Fourteen sweet potato strains (PPRI 9459, 9463, 9464, 9465, 9466, 9467, 9468, 9469, 9471, 9472, 17596, 20179, 23072 and 23484 were similar to *Fusarium* spp. Three sweet potato strains (PPRI 18014, 18017 and 18018) were similar to *Clonostachys* sp. and PPRI 18751 was similar to *C. rosea*. PPRI 23473 and 23475 were similar to *Neurospora* sp.

The *Fusarium* MLST database nBLAST™ results based on the ITS sequences revealed a total of 42 sweet potato strains represented by *F. oxysporum* that clustered in the FOSC. Five strains (PPRI 9458, 9461, 9470, 20166 and 20174) were similar to *F. oxysporum f. sp. ciceris* Matuo & K. Satô 1962. PPRI 9470 and 20171 were similar to *F. oxysporum f. sp. lentis* W.L. Gordon 1965 and *F. oxysporum f. sp. cucumerinum,* respectively.

4.3.12 *Fusarium*-ID database nBLAST™ results based on ITS sequences for isolates obtained from sweet potato

The *Fusarium*-ID database nBLAST™ results based on the ITS sequences of 89 strains obtained from diseased sweet potato material clustered into three *Fusarium* species complexes, namely FCSC, FIESC and FSSC. The species in the complexes were represented by *Fusarium* sp. in the FCSC, *Fusarium* sp. in the FIESC and *F. solani* in the FSSC as indicated in Table 4.6**.** Four *Fusarium* spp. isolates (PPRI

18014, 18017, 18018 and 18751) were similar to *Fusarium* sp. NRRL 45992 MLST type 1-i in the FCSC with the percentage similarity of 95.67%.

The FIESC was represented by 64 sweet potato strains that were similar to *Fusarium* spp. percentage similarity ranging from 94.99-100%. Twenty sweet potato strains (PPRI 23476, 23477, 23479, 23480, 23481, 23482, 23483, 23484, 23485, 23486, 23487, 23488, 23489, 23490, 23491, 23492, 23493, 23494, 23495 and 23496) were similar to *F. solani* in the FSSC with a percentage similarity ranging from 95.93%-96.52% (Table 4.6). ITS *Fusarium*-ID database nBLAST™ results did not reveal any *F. oxysporum* outcome. The *Fusarium* MLST database nBLAST™ results from both databases showed that DNA sequences of ITS region lack phylogenetic signal to determine FOSC isolates. The *Fusarium*-ID database revealed no *formae speciales*, in contrast to the two *formae speciales* revealed by the *Fusarium* MLST database.

4.4 Phylogenetic analyses

Maximum Likelihood and Maximum Parsimony analyses of the separate TEF-1α, RPB2 and β-tubulin gene regions (Figure 4.21-4.25) were done to determine the phylogenetic placement of the South African *F. oxysporum* isolates from diseased sweet potato, including the genetic related *formae speciales* among the selected reference strains. Maximum Parsimony analysis of the separate ITS region was done to determine the phylogenetic placement of the South African *F. oxysporum* isolates from diseased sweet potato, including the genetic related *formae speciales* among the selected reference strains. The reference sequences were obtained from the highest percentage similarities from the *Fusarium* MLST database, *Fusarium*-ID database and Laurence *et al.* (2014). Phylogenetic MP trees statistics are summarised in Table 4.7. *Fusarium oxysporum formae speciales* on different hosts discovered from this study collected from diseased sweet potato material and soil are summarised in Table 4.8.

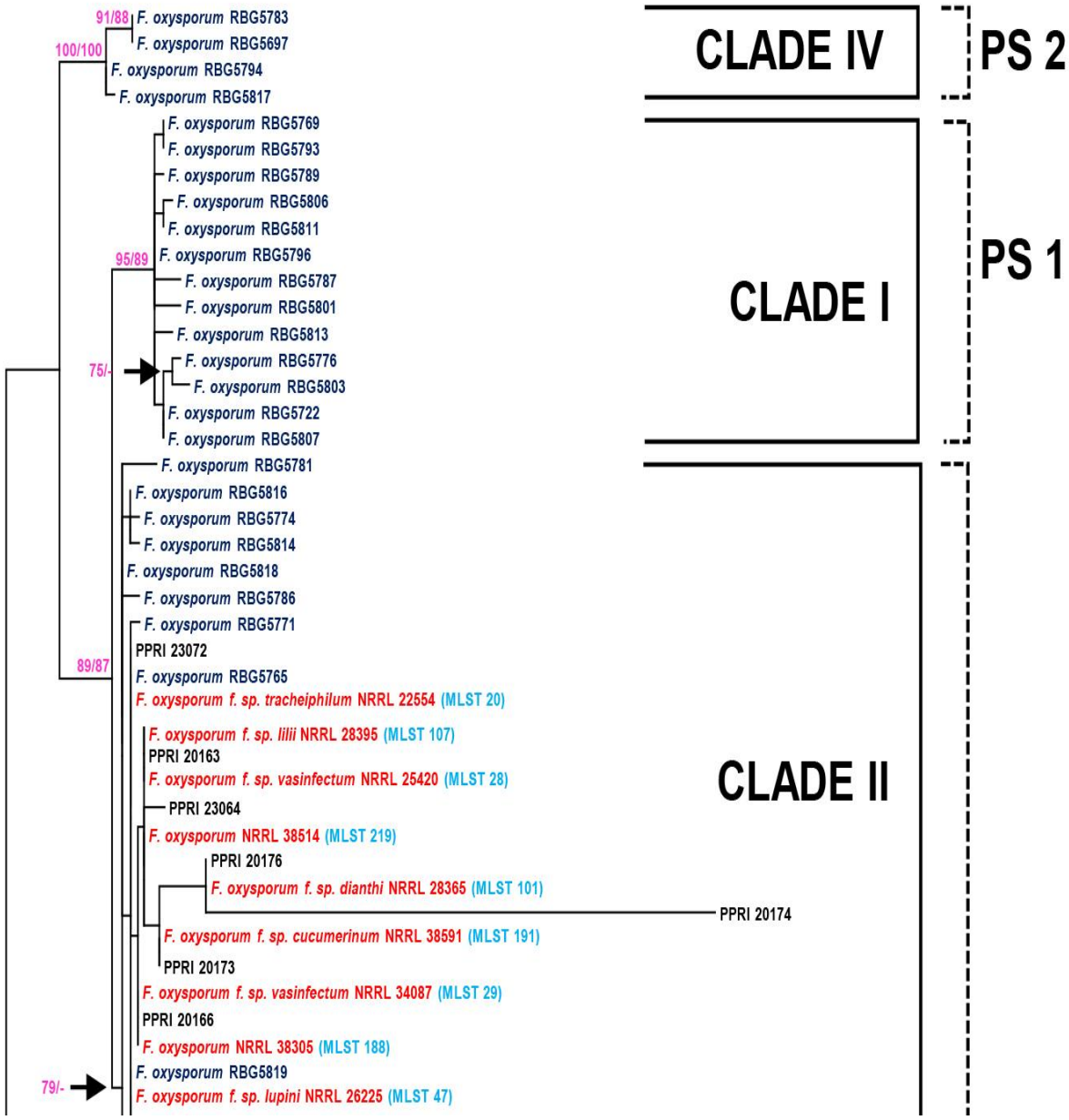


Figure 4.21: Phylogenetic tree based on the ML and MP analyses of the TEF-1α region of *F. oxysporum* and related *formae speciales* associated with FW of sweet potato in South Africa. Bootstrap support of higher than 70% are indicated with values in bold pink above the nodes for ML and MP analyses (ML/MP). The tree is rooted with *Fusarium* sp. RBG5443 (Laurence *et al.*, 2014). The PPRI isolates from South Africa are indicated in bold black. The RBG reference strains obtained from Laurence *et al.* (2014) are in bold blue. The NRRL and CBS strains are in bold red. Clade designation is according to O'Donnell *et al.* (1998b, 2004) and Laurence *et al.* (2014). The Phylogenetic Species boundaries is according to Laurence *et al.* (2014) is indicated as PS 1 and PS 2.

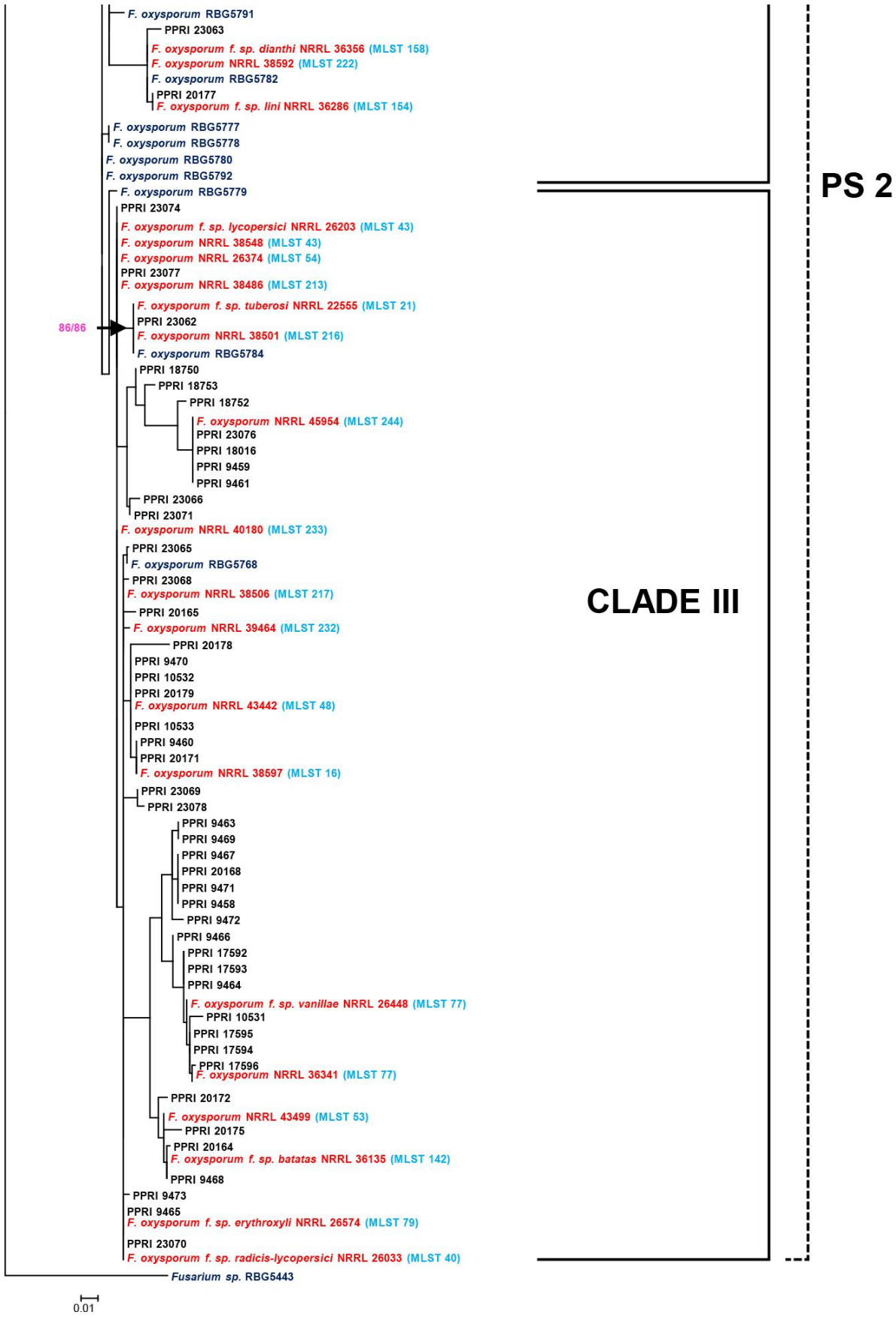


Figure 4.21: (Continued).

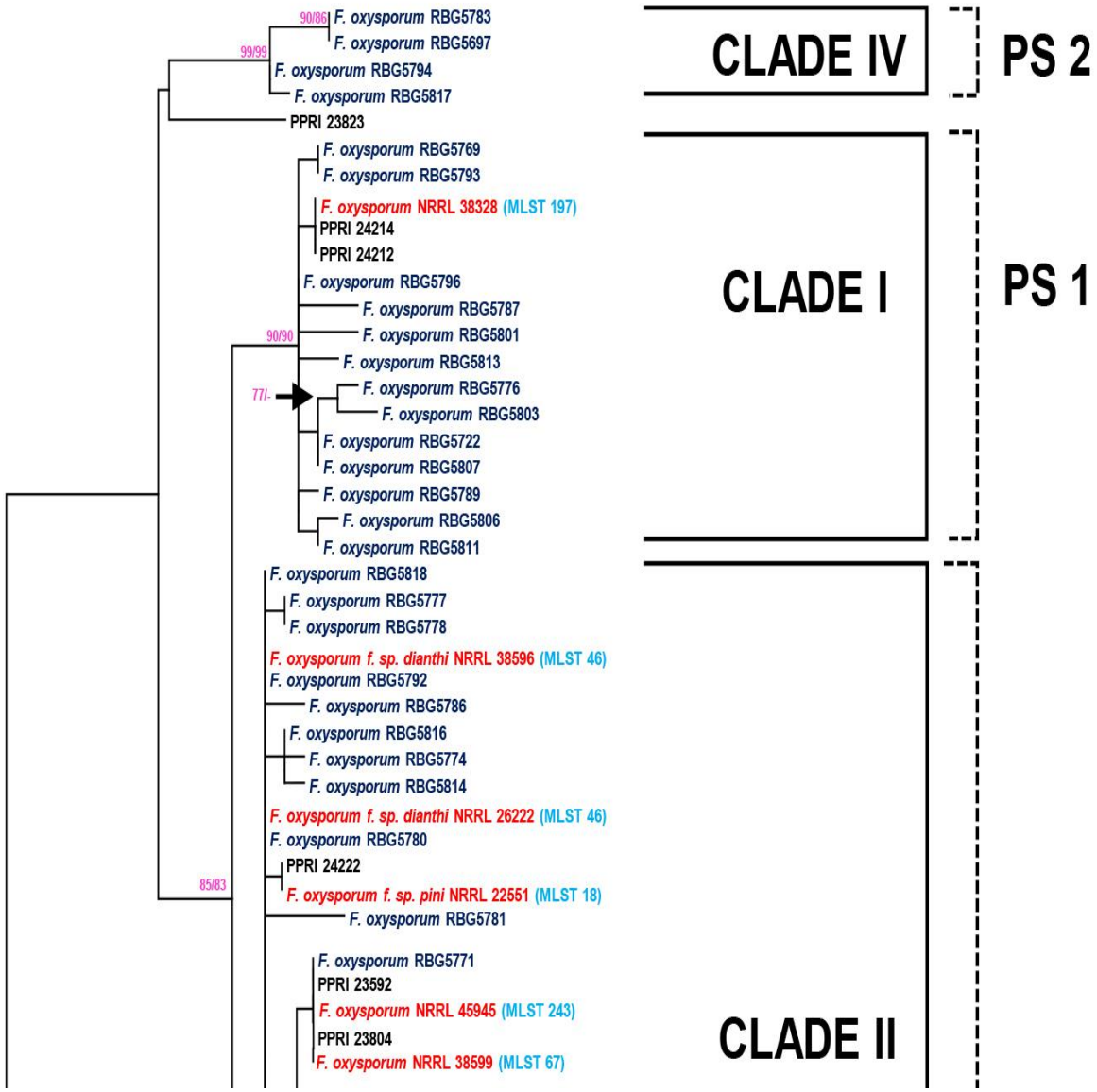


Figure 4.22: Phylogenetic tree based on the ML and MP analyses of the TEF-1α region of *F. oxysporum* and related *formae speciales* associated with soil. Bootstrap support of higher than 70% are indicated with values in bold pink above the nodes for ML and MP analyses (ML/MP). The tree is rooted with *Fusarium* sp. RBG5443 (Laurence *et al.*, 2014). The PPRI isolates from South Africa are indicated in bold black. The RBG reference strains obtained from Laurence *et al.* (2014) are in bold blue. The NRRL and CBS stains are in bold red. Clade designation is according to O'Donnell *et al.* (1998b), O'Donnell *et al.* (2004) and Laurence *et al.* (2014). The Phylogenetic Species boundaries is according to Laurence *et al.* (2014) is indicated as PS 1 and PS 2.

Figure 4.22: (Continued).

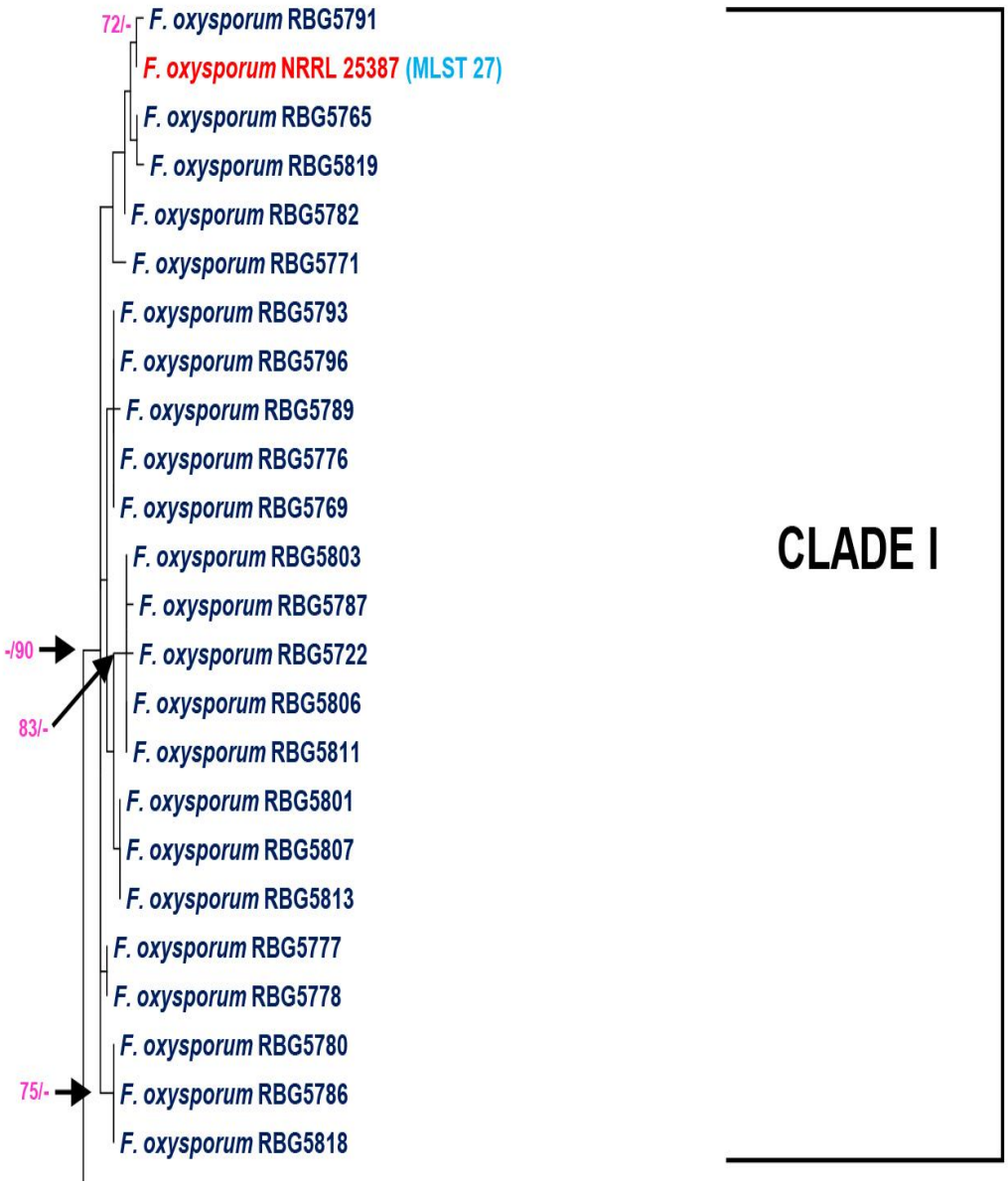


Figure 4.23: Phylogenetic tree based on the ML and MP analyses of the RPB2 (5F and 7CR) region of *F. oxysporum* and related *formae speciales* associated with FW of sweet potato in South Africa. Bootstrap support of higher than 70% are indicated with values in bold pink above the nodes for ML and MP analyses (ML/MP). The tree is rooted with *Fusarium* sp. RBG5443 (Laurence *et al.*, 2014). The PPRI isolates from South Africa are indicated in bold black. The RBG reference strains obtained from Laurence *et al.* (2014) are in bold blue. The NRRL and CBS strains are in bold red.

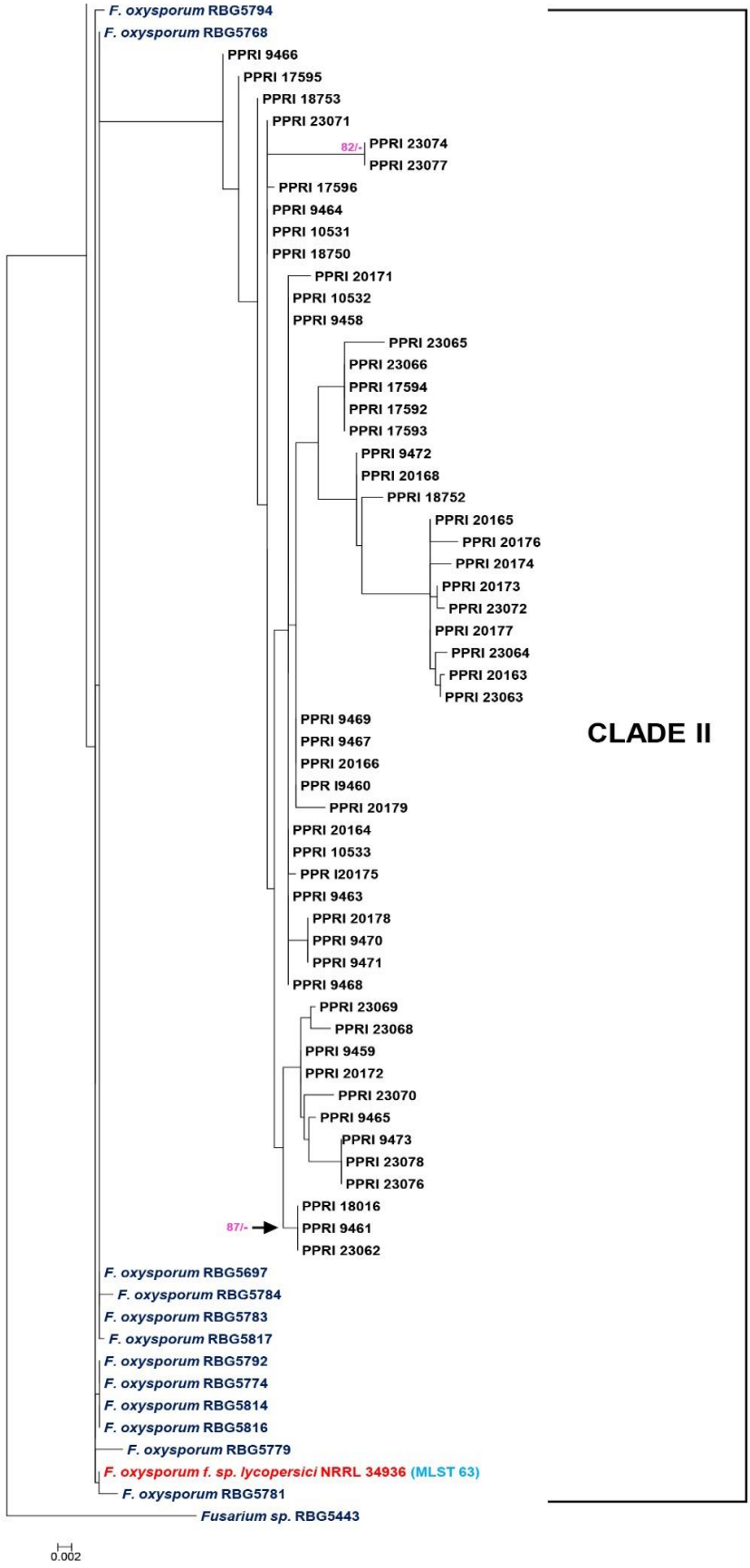


Figure 4.23: (Continued).

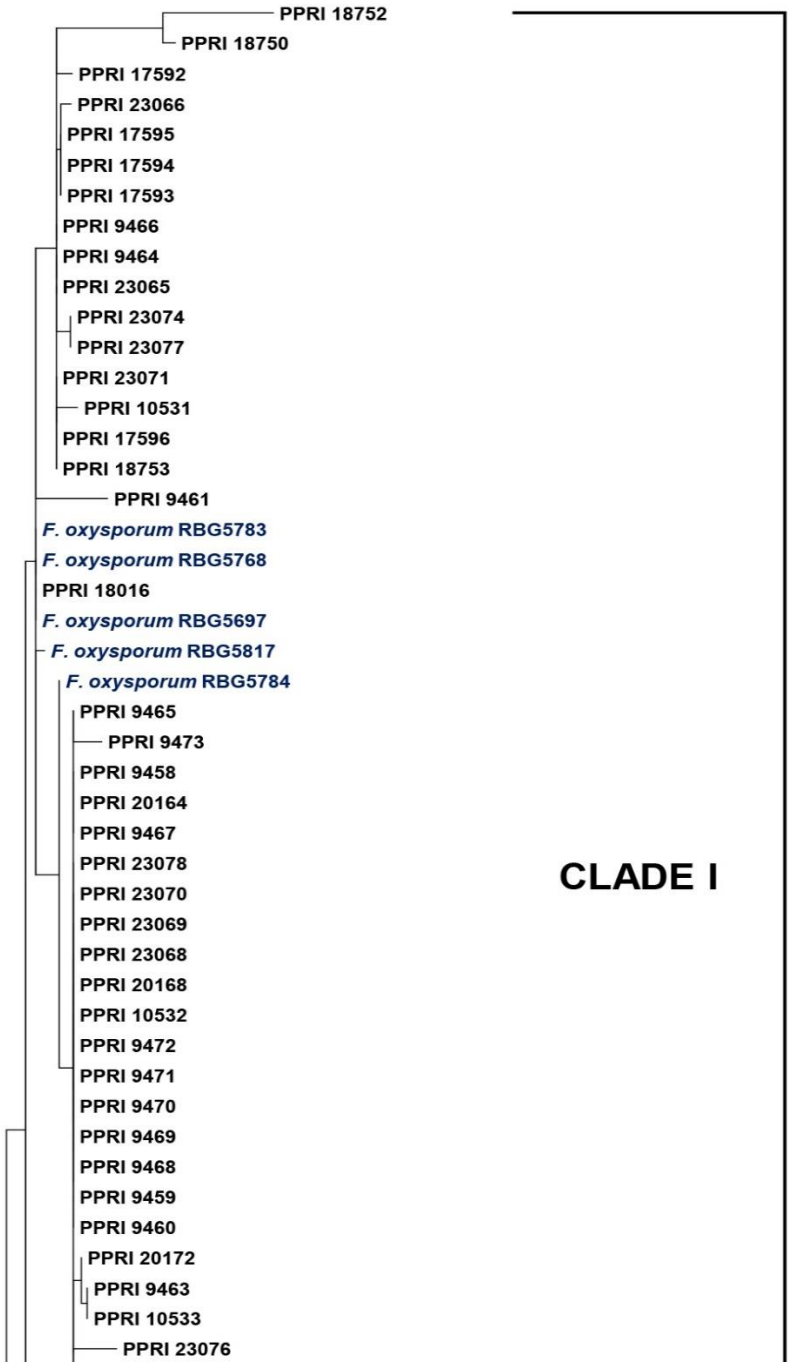


Figure 4.24: Phylogenetic tree based on the ML and MP analyses of the RPB2 (7CF and 11AR) region of *F. oxysporum* and related *formae speciales* associated with FW of sweet potato in South Africa. Bootstrap support of higher than 70% are indicated with values in bold pink above the nodes for ML and MP analyses (ML/MP). The tree is rooted with *Fusarium* sp. RBG5443 (Laurence *et al.*, 2014). The PPRI isolates from South Africa are indicated in bold black. The RBG reference strains obtained from Laurence *et al.* (2014) are in bold blue. The NRRL and CBS strains are in bold red.

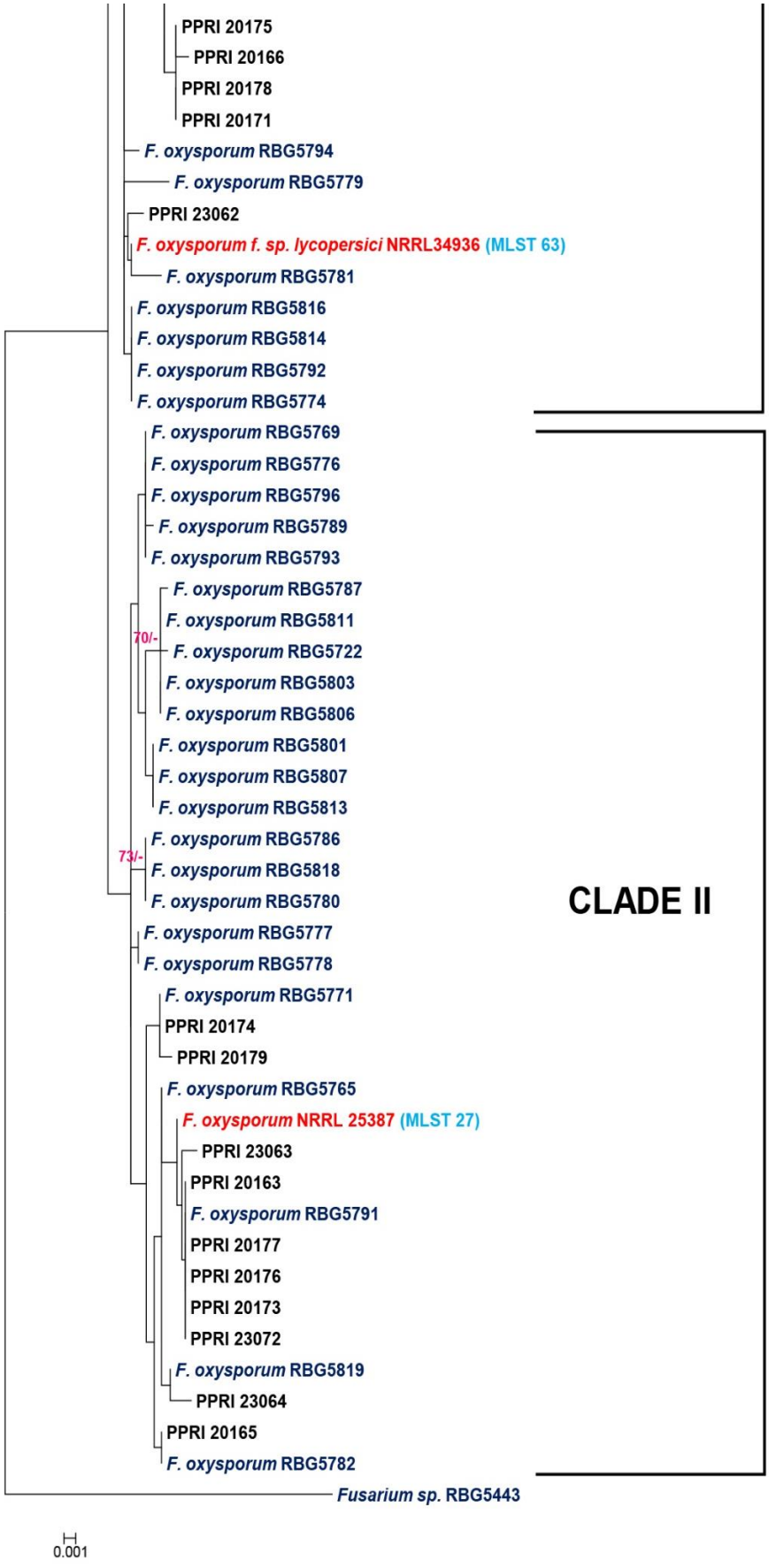


Figure 4.24: (Continued).

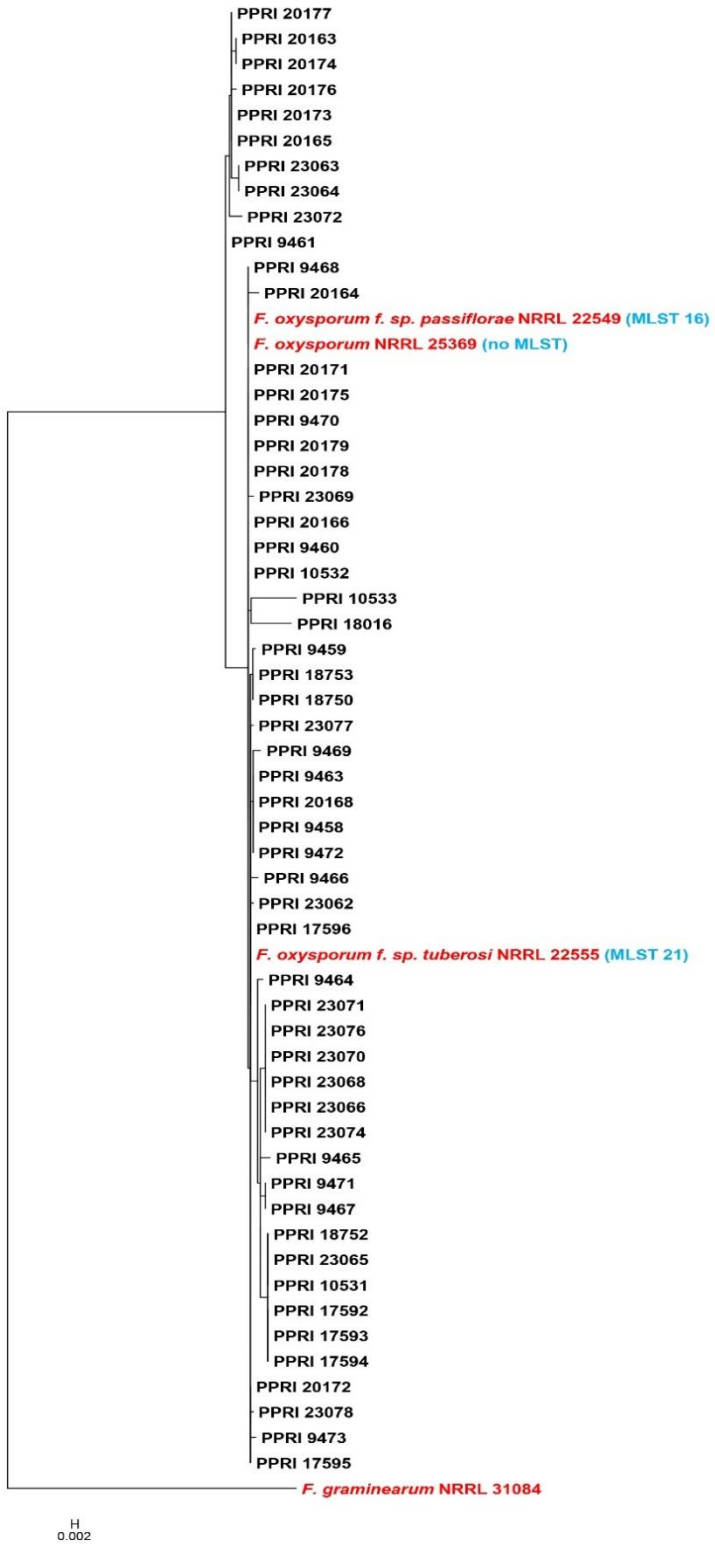


Figure 4.25: Phylogenetic tree based on the ML and MP analyses of the β-tubulin region of *F. oxysporum* and related *formae speciales* associated with FW of sweet potato in South Africa. The tree is rooted with *F. graminearum* NRRL 31084. The PPRI isolates from South Africa are indicated in bold black. The NRRL and CBS reference strains are in bold red.

Table 4.7: Summary sequence and Maximum Parsimony tree statistics

Data set	taxa	characters	PUC[a]	PIC[b]	MPTs	Tree length	CI	RI
TEF1-α: Sweet potato	112	738	697	41	1	56	0.8571	0.9758
TEF1-α: Soil	120	710	670	40	7	54	0.8704	0.9809
RPB2: 5F and 7CR	94	2159	2151	8	1	9	1.0000	1.0000
RPB2: 7CF and 11AR	94	1979	1966	13	6	15	0.8667	0.9854
β-tubulin	64	1394	1389	5	1	7	0.8571	0.9000
ITS	72	563	562	1	1	1	1.0000	1.0000

PUC=Parsimony un-informative characters

PIC=Parsimony informative characters

MPTs=Most-parsimonious trees

CI=Consistency index

RI=Retention index

Table 4.8: *Fusarium oxysporum formae speciales* on different hosts discovered from this study collected from diseased sweet potato material and soil

Formae speciales	**Host**	**References**
F. oxysporum f. sp. batatas	Sweet potato	Wollenweber, (1914) amended by Snyder and Hasen (1940)
F. oxysporum f. sp. cucumerinum	Cucurbits	Owen, (1956)
F. oxysporum f. sp. dianthi	Carnation	Prillieux and Delacroix, (1899) amended by Wollenweber and Reinking, (1935)
F. oxysporum f. sp. erythroxyli	coca	Bazán de Segura, (1959)
F. oxysporum f. sp. koae	Acacia koa	Gardner, (1980)
F. oxysporum f. sp. lilii	Lilly	Imle, (1942)
F. oxysporum f. sp. lini	Flax	Bolley, (1901) amended by Snyder and Hasen (1940)
F. oxysporum f. sp. lupini	Lupin	Snyder and Hasen, (1940)
F. oxysporum f. sp. lycopersici	Tomato	(Saccardo) amended by Snyder and Hasen (1940)
F. oxysporum f. sp. melonis	Melon	Leach and Currence, (1938) amended by Snyder and Hasen (1940)
F. oxysporum f. sp. pini	Pinus	(Hartig) amended by Snyder and Hasen (1940)
F. oxysporum f. sp. radicis-lycopersici	Tomato	Jarvis and Shoemaker, (1978)
F. oxysporum f. sp. tracheiphilum	Cowpea	Smith (1899) amended by Snyder and Hansen (1940)
F. oxysporum f. sp. tuberosi	Potato	(Wollenweber) amended by Snyder and Hasen (1940)
F. oxysporum f. sp. vanillae	Vanilla	Tucker, (1927) amended by Gordon 1965
F. oxysporum f. sp. vasinfectum	Cotton	Atkinson, (1892) amended by Snyder and Hasen (1940)

The phylogenetic analysis of the diseased sweet potato TEF-1α gene dataset resolved the FOSC dataset into four distinct clades as previously described by O'Donnell *et al.* (2004) and Laurence *et al.* (2014), indicating some partial level of genetic variation among the FOSC isolates in South Africa. The dataset consisted of 118 isolates that included 55 PPRI FOSC strains from the current study, 36 reference strains from Laurence *et al.* (2014) and 30 reference strains from the

Fusarium MLST and *Fusarium*-ID databases (Figure 4.21). The clades comprised of various *F. oxysporum* and *F. oxysporum formae speciales* as well as a range of MLST types. Both ML and MP analysis provided limited bootstrap support, with only seven clades with bootstrap support above 70%. Clade I represented only RBG isolates from Laurence *et al.* (2014) representing PS 1 with a significant bootstrap support of 95% for ML analysis and 85% for the MP analysis. A sub-clade within clade one was supported by a bootstrap support of 75% for ML analyses but not by the MP analyses.

Clade II, housed eight South African isolates from the current study, 14 RBG isolates from Laurence *et al.* (2014) that belong to PS 2, and eleven isolates from the *Fusarium* MLST and *Fusarium*-ID databases. Clade II comprised of seven diverse *F. oxysporum formae speciales* that included *F. oxysporum f. sp. cucumerinum* NNRL 38591, *F. oxysporum f. sp. dianthi* NRRL 36356, *F. oxysporum f. sp. dianthi* NRRL 28365, *F. oxysporum f. sp. lilii* NRRL 28395, *F. oxysporum f. sp. lini* NRRL 36286, *F. oxysporum f. sp. lupini* NRRL 26225, *F. oxysporum f. sp. tracheiphilum* NRRL 22554 and *F. oxysporum f. sp. vasinfectum* NRRL 25420. Therefore, Clade II comprised of FOSC isolates associated with the plant family Fabaceae, Caryophyllaceae Asteraceae, Cucurbitaceae, Linaceae and Malvaceae. The result suggests that FOSC is of diverse plant family distribution.

Clade III comprised of 46 South African isolates, three RBG isolates that belonged to PS 2, and sixteen reference strains. One South African strain, PPRI 23062, in Clade III clustered with *F. oxysporum f. sp. tuberosi* NRRL 22555 MLST type 21, *F. oxysporum* NRRL 38501 MLST type 216 and *F. oxysporum* RBG 5784 with a significant bootstrap support of 86% for both ML and MP analyses. The strain PPRI 23062 was identified via *Fusarium* MLST nBLAST™ results as *F. oxysporum f. sp. tuberosi* NRRL 22555 and via *Fusarium*-ID nBLAST™ results as *F. oxysporum* NRRL 38501. The results indicate that *F. oxysporum f. sp. tuberosi* NRRL 22555 is closely associated with isolate PPRI 23062 therefore, presenting a possible close association of *F. oxysporum f. sp. tuberosi* with FW of sweet potato in South Africa.

Most of the South African strains were clustered in Clade III, the most diverse phylogenetically clade (O'Donnell *et al.*, 2004). Clade III consisted of two sub-clades that can be distinguished, indicating large genetic variability. Therefore, there will be an impact on any plant resistance breeding programme, as the diverse polulation of genetic varation within *F. oxysporum* should be taken into consideration. Clade III comprised of six diverse South African isolates representing *F. oxysporum f. sp. batatas* NRRL 36135 (origin: Unknown; host: Unknown; family: Unknown), *F. oxysporum f. sp. erythroxyli* NRRL 26574 (origin: USA; host: *Erythroxylum coca*; family: Erythroxylaceae), *F. oxysporum f. sp. lycopersici* NRRL 26203 (origin: Italy; host: *Solanum lycopersicum*; family: Solanaceae), *F. oxysporum f. sp. radicis-lycopersici* NRRL 26033 (origin: USA; host: *Solanum lycopersicum*; family: Solanaceae), *F. oxysporum f. sp. tuberosi* NRRL 22555 (origin: Iran; host: *Solanum tuberosum*; family: Solanaceae) *and F. oxysporum f. sp. vanillae* NRRL 26448 (origin: USA; host: *Vanilla* sp.; family: Orchidaceae). Clade III grouped together the three *formae speciales* from Solanaceae family and individual *forma specialis* from the Erythroxylaceae and Orchidaceae families.

Clade IV comprised of only four RBG isolates that belong to PS 2 with a significant bootstrap support of 100% for both the ML and MP analyses. South African strains and various *formae speciales* of the FOSC were distributed in Clade II and Clade III. The TEF-1α results followed the similar pattern as Laurence *et al.* (2014). Some differences were found in the sequences of the South African isolates as Clade III consisted of four sub-clades and only one clade was supported by bootstrap value of 86% for both ML and MP analysis. This results indicate the genetic variation within FOSC associated with FW of sweet potato in South Africa. The results are aligned with the report of O'Donnell *et al.* (2004) who discovered 4 clades of the FOSC.

Only nine PPRI isolates were clustered in Clade II and the rest of the 47 PPRI isolates were clustered in Clade III. The results indicate that the South African isolates are genetically diverse and generated phylogeny similar to the previously reported *formae speciales* namely, *F. oxysporum f. sp. batatas* NRRL 36135 (O'Donnell *et al.*, 2009a), *F. oxysporum f. sp. erythroxyli* NRRL 26574 (O'Donnell *et*

al., 1998b), *F. oxysporum f. sp. lycopersici* NRRL 26203 (O'Donnell *et al.*, 1998b), *F. oxysporum f. sp. radicis-lycopersici* NRRL 26033 (O'Donnell *et al.*, 2004), *F. oxysporum f. sp. tuberosi* NRRL 22555 (O'Donnell *et al.*, 1998b), *F. oxysporum f. sp. vanillae* NRRL 26448 (O'Donnell *et al.*, 2009a) and *F. oxysporum f. sp. vasinfectum* NRRL 25420 (Pinaria *et al.*, 2015). O'Donnell *et al.* (2009a) consist of all the MLST types and *F. oxysporum formae speciales* reference strains from TEF-1α sequences obtained from diseased sweet potato material from this study. The relationships were not fully supported by a bootstrap value of above 70%, however supported by a significant percentage similarity of 95.5-100% from *Fusarium* MLST and *Fusarium*-ID databases using the nucleotide BLAST results. Furthermore, the host plant families were randomly distributed in Clade II and III and did not group according to host plant family. The results suggest that there was some partial degree of genetic variation among the FOSC isolates in South Africa as the South African isolates were distributed between two clades.

The hosts linked with *formae speciales* reported in this study were all commom in that they belong in Angiosperm plant group flowering plants (Table 4.8). Phylogenetically, the hosts plant families cluster in the similar manner as *formae speciales*. Chase *et al.* (2016) reported Angiosperm plant groups that comprised of different plant familes. The plant families phylogeny grouped Fabaceae, Cucurbitaceae and Malvaceae in Superrosids clade, which is a clade that consist of rosids and saxifragales. Furthermore, Asteraceae, Caryophyllaceae and Solanaceae grouped in Superasterids clade, which is a clade that consist of Asterids, Berberidopsidales and Santalales. In this study Fabaceae (*F. oxysporum f. sp. lupini*), Cucurbitaceae (*F. oxysporum f. sp. cucumerinum*) and Malvaceae (*F. oxysporum f. sp. vasinfectum*) all grouped in clade II. Caryophyllaceae (*F. oxysporum f. sp. dianthi*) also grouped in Clade II. Asteraceae (*F. oxysporum f. sp. tracheiphilum*) and Solanaceae (*F. oxysporum f. sp. lycopersici, F. oxysporum f. sp. radicis-lycopersici* and *F. oxysporum f. sp. tuberosi)* grouped in clade III.

In addition, a study by Dau (2016) indicated that FOSC isolates that were pathogenic were present therefore, isolates showed a continuous variation in virulence from most virulent, intermediate virulent to least virulent. Some of the

isolates were from this study obtained from diseased sweet potato plant material collected from Eastern Cape, Gauteng, Limpopo, Mpumalanga, Northern Cape and Western Cape provinces of South Africa, five isolates (PPRI 9458, 9463, 9467, 9471 and 18750) were most virulent, 15 isolates (PPRI 9459, 9460, 9461, 9464, 9465, 9466, 9469, 9470, 9472, 9473, 10532, 10533, 18016, 18752 and 18753) were intermediate virulent and three isolates (PPRI 9462, 9468 and 10531) were least virulent (Dau, 2016). All of this strains clustered in Clade III therefore, the strains were not grouping according to pathogenic or non-pathogenic organisation.

The phylogenetic analysis of the soil TEF-1α gene dataset resolved the FOSC dataset into four distinct clades as previously described by O'Donnell *et al.* (2004) and Laurence *et al.* (2014) indicating some partial level of genetic variation among the FOSC isolates in South Africa. The dataset consisted of 128 isolates that included 65 PPRI FOSC isolates from the current study, 36 isolates from Laurence *et al.* (2014) and 29 isolates from the *Fusarium* MLST and *Fusarium*-ID databases (Figure 4.22). The reference strains are listed in Table 3.3. The clades comprised of various *F. oxysporum* and *F. oxysporum formae speciales* as well as a range of MLST types. The clades comprised of various *F. oxysporum* and *formae speciales* of the FOSC that included a range of MLSTs. Maximum Parsimony analysis of the soil TEF-1α gene dataset generated a CI and RI values indicated in Table 4.7. Both ML and MP analyses provided a partial bootstrap support. Maximum Likelihood analyses had higher bootstrap values than MP analyses.

Clade I included two South African strains (PPRI 24214 and 24212), thirteen RBG isolates that belong to PS 1 and one isolate *F. oxysporum* NRRL 38328 isolated from soyabean in China from both databases with a significant bootstrap support of 90% for both ML and MP analyses. Clade I comprised of a sub-clade with a bootstrap support of 77% for ML analysis and less than 70% bootstrap support for the MP analysis for the RBG5776 and RBG5803 isolates.

Clade II, contained 26 PPRI isolates, fifteen RBG isolates that belong to PS 2, and 17 isolates from *Fusarium* MLST and *Fusarium*-ID databases. A sub-clade within Clade II comprised of 15 PPRI isolates that grouped with *F. oxysporum f. sp.*

cucumerinum NRRL 38591 and *F. oxysporum* NRRL 38477 with a significant bootstrap support of 86% for the ML analysis and 85% for the MP analysis. In addition, PPRI 21929 and 22326 clustered together with a bootstrap support of 86% for the ML and 88% for the MP analysis, respectively. In terms of *formae speciales* linked to nBLAST™ analyses, Clade II comprised of nine *formae speciales* namely *F. oxysporum f. sp. cucumerinum* NNRL 38591 (origin: New Zealand; host: *Cucumis sativus*; family: Cucurbitaceae), *F. oxysporum f. sp. dianthi* NRRL 26222 (origin: Israel; host: *Dianthus caryophyllus*; family: Caryophyllaceae)*, F. oxysporum f. sp. dianthi* NRRL 36356 (origin: Unknown; host: Unknown; family: Unknown). *F. oxysporum f. sp. koae* NRRL 38885 (origin: USA; host: *Acacia koa*; family: Fabaceae), *F. oxysporum f. sp. lini* NRRL 36286 (origin: Unknown; host: *Linum usitatissimum*; family: Linaceae), *F. oxysporum f. sp. lupini* NRRL 26225 (origin: USA; host: *Lupinus* sp.; family: Fabaceae), *F. oxysporum f. sp. melonis* CBS 420.90 (origin: Israel; host: *Cucumis melo*; family: Cucurbitaceae), *F. oxysporum f. sp. pini* NRRL 22551 (origin: Germany; host: *Pinus* sp.; family: Pinaceae), *F. oxysporum f. sp. tracheiphilum* NRRL 22554 (origin: Nigeria; host: *Chrysanthemum* sp.; family: Asteraceae) and *F. oxysporum f. sp. vasinfectum* NRRL 25420 (origin: USA; host: Uknown; family: Unknown). Therefore, Clade II comprised of the plant family Cucurbitaceae from two *formae speciales* and Fabaceae from two *formae speciales*. The rest of the *formae speciales* individually comprised of Asteraceae, Caryophyllaceae, Linaceae and Pinaceae plant family. Two *formae speciales* from Clade II originated in USA, two from Israel and individuals from Germany, New Zealand, Nigeria and USA. In addition, *F. oxysporum f. sp. dianthi* had two different MLST type indicating a genetic diversity.

Clade III comprised of four sub-clades and one big clade that indicates a large genetic variability amongst the strains. Clade III also included three RBG strains and 11 strains from *Fusarium* MLST and *Fusarium*-ID databases. Clade III consisted of four *formae speciales* namely *F. oxysporum f. sp. erythroxyli* NRRL 26574 (origin: USA; host: *Erythroxylum coca*; family: Erythroxylaceae), *F. oxysporum f. sp. radicis-lycopersici* NRRL 26033 (origin: USA; host: *Solanum lycopersicum*; family: Solanaceae), *F. oxysporum f. sp. tuberosi* NRRL 22555 (origin: Iran; host: *Solanum tuberosum*; family: Solanaceae) *and F. oxysporum f. sp.*

vanillae NRRL 26448 (origin: USA; host: *Vanilla* sp.; family: Orchidaceae). Therefore, the variety of *formae speciales* were accommodated in Clade II than Clade III. Twenty-six PPRI strains grouped in Clade II and 36 PPRI strains were accomodated in Clade III.

Four South African strains (PPRI 23582, 23873, 23805 and 23615) in Clade III grouped with *F. oxysporum f. sp. tuberosi* NRRL 22555, *F. oxysporum* NRRL 38501 and *F. oxysporum* RBG5784 with a significant bootstrap support of 85% for the ML and 88% for the MP analyses. The results suggest that *F. oxysporum f. sp. tuberosi* NRRL 22555 is genetically more closely associated with these four isolates. One South African strain in Clade III, PPRI 23584, clustered with *F. oxysporum f. sp. vanillae* NRRL 26448 with a bootstrap support of 86% for the ML analyses and 87% for the MP analyses. The results suggest that *F. oxysporum f. sp. vanillae* NRRL 26448 is genetically more closely associated with PPRI 23584. Most of the South African strains were clustered in Clade III, the most phylogenetically diverse clade (O'Donnell *et al.*, 2004).

Clade IV comprised of only four RBG isolates that belong to PS 2 with a significant bootstrap support of 99% for both ML analyses and MP analyses. The South African strains were distributed with the Laurence *et al.* (2014) strains within the PS 1 and PS 2 in Clade I, Clade II and Clade III. The soil TEF-1α phylogenetic analysis results formed a similar pattern as Laurence et *al.* (2014). There are over 100 *F. oxysorum formae speciales* in PS 2 and there is only three in PS 1 namely *F. oxysporum f. sp. canariensis*, *F. oxysporum f. sp. cubense* and *F. oxysporum f. sp. perniciosum*. Australian *F. oxysporum f. sp. vasinfectum* (Laurence *et al.*, 2014) belongs to PS 1, as well as two of the South African strains from this study. Therefore, the results suggest that South African isolates are genetically diverse and are related to more than one *forma specialis*.

Twenty-six PPRI isolates were clustered in Clade II and the rest of the 36 PPRI isolates were clustered in Clade III. The results indicate that the South African isolates are genetically diverse and generated phylogeny similar to the previously reported *formae speciales* namely, *F. oxysporum f. sp. erythroxyli* NRRL 26574

(O'Donnell *et al.*, 1998b), *F. oxysporum f. sp. radicis-lycopersici* NRRL 26033 (O'Donnell *et al.*, 2004), *F. oxysporum f. sp. tuberosi* NRRL 22555 (O'Donnell *et al.*, 1998b), *F. oxysporum f. sp. vanillae* NRRL 26448 (O'Donnell *et al.*, 2009a) and *F. oxysporum f. sp. vasinfectum* NRRL 25420 (Pinaria *et al.*, 2015). O'Donnell *et al.* (2009a) consist of all the MLST types and *F. oxysporum formae speciales* reference strains from TEF-1α sequences obtained from soil from this study (Table 4.8). Most *F. oxysporum formae speciales* are pathogenic to a single crop, however, some attack more than one crop for example, *F. oxysporum f. sp. cucumerinum* was reported that it affects both cucumber and melon (Cafri *et al.*, 2005).

The relationships were not fully not supported by a bootstrap value of above 70% however supported by a significant percentage similarity of 95.5-100% from *Fusarium* MLST and *Fusarium*-ID databases using the nBLAST™ results. Furthermore, the host plant families were un-evenly distributed in Clade II and III and did not group according to host plant family. The results suggest that there was some partial degree of genetic variation among the FOSC soil isolates in South Africa as the South African isolates were distributed between three clades. The phylogenetic tree of TEF-1α with strains recovered from diseased sweet potato formed a similar pattern with the phylogenetic tree of TEF-1α with isolates recovered from the soil. Both of these trees had a significant bootstrap support for the *F. oxysporum f. sp. tuberosi* and GBG5784 with one PPRI isolate from diseased sweet potato and four PPRI isolates from soil. *Fusarium oxysporum formae speciales vanillae* from soil isolates also formed a significant bootstrap support and clustered with PPRI 23584 Therefore, these results suggest that *F. oxysporum f. sp. tuberosi* and *F. oxysporum f. sp. vanillae* are genetically more closely associated with some of the South African isolates and more closely associated with FW of sweet potato in South Africa. Some differences were found in the sequences of the South African isolates, most likely indicating genetic variation. Only one South African strain, PPRI 23823, was phylogenetically unresolved as it did not belong to any of the four clades and did not have any bootstrap support. The PPRI 23823 was identified as *F. oxysporum f. sp. dianthi* via nBLAST™ results of *Fusarium* MLST and *Fusarium*-ID databases however, supported by a percentange similarity of 97.63% and 97.82%, respectively. The results suggest that the query sequence might be from an

undescribed and phylogenetically distinct species that is not present in the databases (Geiser *et al.*, 2004). In addition, PPRI 23823 appeared to be basal to Clade IV. Therefore, this isolate requires further investigation. The basal split between Clade I and IV suggests that the lineage may be descended from one of the earliest divergences within FOSC and might be an ancentral area (O'Donnell *et al.*, 1998b). The phylogeny generation between Clade II and III suggests an early divergence and supported by a significant bootstrap support (O'Donnell *et al.*, 1998b).

Comparing the findings of the present study, it can be concluded that ML and MP analyses was able to partially reveal some degree of genetic diversity among the South African FOSC strains. The phylogenetic analyses for TEF-1α from diseased sweet potato and soil formed a similar pattern. However, none of the South African isolates from diseased sweet potato grouped with Clade I whereas two South African isolates from soil did group with Clade I.

Based on the 55 FOSC isolates from diseased sweet potato material, the RPB2 (5F and 7CR) phylogenetic analysis formed two distinct clades. The dataset consisted of 93 isolates that included 55 PPRI FOSC strains from the current study, 36 reference strains from Laurence *et al.* (2014) and two reference strains from the *Fusarium* MLST and *Fusarium*-ID databases (Figure 4.23). Maximum Parsimony analysis of the diseased sweet potato RPB2 (5F and 7CR) gene dataset generated a CI and RI values indicated in Table 4.7.

Clade I consisted of all the Australian strains that belong to PS 1 and ten of the Australian isolates that belong to PS 2. It also included a strain from *Fusarium* MLST database *F. oxysprom* NRRL 25387 MLST 27, a clinical strain from New Zealand that grouped with RBG5791 with a bootstrap support of 72% for the ML analyses and less than 70% for the MP analyses. Clade I had a bootstrap support of less than 70% for the ML analyses and had 90% for the MP analyses. All the South African strains were clustered together in the middle of the reference strains within Clade II and formed a sub-clade as indicated in Figure 4.23. The RPB2 (5F and 7CR) only demonstrated partial genetic variation and did not completely resolve the

FOSC phylogeny, however RPB2 is a phylogenetically informative orthologous gene that can resolve near species-level and can be used across the phylogenetic breadth of *Fusarium* (Geiser *et al.*, 2004). RNA polymerase II second largest subunit together with RPB1 has resolved 20 monophyletic species complexes and nine monotypic lineages (O'Donnell *et al.*, 2013). Both RPB2 and RPB1 has provided the initial robust genus-wide framework for evaluating if the traditional morphology based sectional classification accurately reflects evolutionary relationships within *Fusarium* and most of the clades identified did cut across *Fusarium* sectional boundaries (Gerlach and Nirenberg, 1982; O'Donnell *et al.*, 2013). RNA polymerase II second largest subunit is a gene region that is used to resolve the entire *Fusarium* genus (O'Donnell *et al.*, 2007) however, cannot resolve the *F. oxysporum* within the FOSC. RNA polymerase II second largest subunit gene is sufficiently conserved so that the genus-wide alignments reflect positional homology however with enough phylogenetic signal to generate solid supported phylogenies (Laurence *et al.*, 2011). The FOSC South African strains demonstrated genetic variation and possible that some strains retain aberration of alleles in a population which are appropriate for the certain area. The origin of the species is significant as it retains a better genetic diversity compared to the recently emerged species.

Based on the 55 FOSC isolates from diseased sweet potato material, the RPB2 (7CF and 11AR) phylogenetic analysis formed two distinct clades. The dataset consisted of 93 isolates that included 55 PPRI FOSC strains from the current study, 36 reference strains from Laurence *et al.* (2014) and two reference strains from the *Fusarium* MLST and *Fusarium*-ID databases (Figure 4.24). Maximum Parsimony analysis of the diseased sweet potato RPB2 (7CF and 11AR) gene dataset generated a CI and RI values indicated in Table 4.7. Clade I had no bootstrap support and consisted of five Australian isolates and 44 PPRI isolates. Most of the South African isolates were distributed in Clade I however did not have a bootstrap support.

Clade II comprised of 30 RBG, 11 PPRI and two strains from databases. Clade II comprised of sub-clades including two sub-clades with a bootstrap support of 70% and 73% for ML analyses and less that 70% for the MP analyses as indicated in

Figure 4.24. Most of the RBG strains were clustered together. RNA polymerase II second largest subunit (7CF and 11AR) had a partially genetic variation and did not completely resolve the FOSC phylogeny, however RPB2 is a phylogenetically informative orthologous gene that can resolve near species-level and can be used across the phylogenetic breadth of *Fusarium* (O'Donnell *et al.*, 2013).

The β-tubulin gene region only had one clade with a low bootstrap support of below 70%. The dataset consisted of 58 isolates that included 55 PPRI FOSC strains from the current study and three reference strains from the *Fusarium* MLST and *Fusarium*-ID databases (Figure 4.25). Maximum Parsimony analysis of the diseased sweet potato β-tubulin gene dataset generated a CI and RI values indicated in Table 4.7. Most of the South African isolates grouped together as indicated in Figure 4.25. The reference strains included *F. oxysporum f. sp. passiflorae* NRRL 22549 MLST type 16 (origin: Brazil; host: *Passiflora edulis*; family: Passifloraceae), *F. oxysporum f. sp. tuberosi* NRRL 22555 MLST type 21 and *F. oxysporum* NRRL 25369 with no MLST type. Beta-tubulin did not have enough reference sequences since most isolates had the similar nBLAST™ results and only revealed three reference sequences. Beta-tubulin did not deliver the accurate reflection of determining evolutionary relationships within FOSC associated with FW of sweet potato in South Africa. Laurence *at al.* (2014) found that β-tubulin data set had only one parsimony informative character in the phylogenetic analyses, therefore β-tubulin data was excluded in the GCPSR analyses. However, previous studies have showed that β-tubulin gene can be highly informative in other *Fusarium* species complexes (O'Donnell *et al.*, 1998a; O'Donnell, 2000). The β-tubulin gene has also showed the ability to resolve closely related species (Lima *et al.*, 2009; Walsh *et al.*, 2010). Furthermore, β-tubulin gene was the first protein-encoding gene that was used for molecular phylogenetics in *Fusarium* genus (O'Donnell and Cigelnik, 1997; O'Donnell *et al.*, 1998a). The TEF-1α dataset provided much better resolution of the relationships amongst the FOSC isolates while RPB2 provided little resolution and β-tubulin dataset provided no resolution.

The phylogenetic analysis of *F. oxysporum formae species* associated with FW improved the knowledge of FOSC in South Africa. This work emphasizes the

importance of identification other *formae speciales* that are present in South Africa and also understanding their genetic groupings. Preliminary identification of South African strains showed that there could be other *formae speciales* associated with FW of sweet potato besides *F. oxysporum f. sp. batatas* (Thompson *et al.*, 2011). These findings raised questions as to whether the occurrence of the disease is throughout South Africa, and which *F. oxysporum formae speciales* are associated with FW of sweet potato besides *F. oxysporum f. sp. batatas.* This study was built on and expanded on the previous research done by Narayanin (2008) and Thompson *et al.* (2011). The results indicated that there are more than one *F oxysporum formae speciales* associated FW on sweet potato in South Africa. The results also suggested that *F. oxysporum f. sp. tuberosi* and *F. oxysporum f. sp. vanillae* are more phylogenetically related to South African isolates therefore, closely associated with FW of sweet potato in South Africa.

Phylogenetic species two has over 100 *formae speciales* compared to only three in PS 1 namely *F. oxysporum f. sp. canariensis*, *F. oxysporum f. sp. cubense* and *F. oxysporum f. sp. perniciousum*. The Australian *F. oxysporum f. sp. vasinfectum* also belong to PS 1 whereas international *F. oxysporum f. sp. vasinfectum* belong to PS 2 (Laurence *et al.*, 2014). South Africa had only two strains from this study namely PPRI 24212 and 24214 recovered from soil that belong to PS 1. Therefore, further investigation is needed to determine if PPRI 24214 and 24212 are pathogenic to sweet potato. Mojela (2017), reported three South African strains (PPRI 22778, 20540 and 20715) in the PS 1 and these strains were from undisturbed soil in the Willem Pretorius Nature Reserve. Only one South African soil strain, PPRI 23823, did not group with any of the phylogenetic species and did not have a bootstrap support. This proves that FOSC is phylogenetically diverse and the *formae speciales* are not always correlated with phylogenetic analyses (Baayen *et al.*, 2000). In addition, the acknowledgement of two PS recommended that lineages within the FOSC have recently diverged (Laurence *et al.*, 2014).

The FOSC dispersal pattern was inconsistent as the phylogenetic tree of TEF-1α indicated that the isolates from the same region were distributed between the clades. Host specificity did not play role in FOSC strains as the *F. oxysporum*

formae speciales were from different hosts in different plant families. Some *F. oxysporum formae speciales* are polyphyletic (O'Donnell *et al.*, 1998b; Baayen *et al.*, 2000) therefore, a taxonomic value of the *F. oxysporum formae speciales* naming system is in question (O'Donnell *et al.*, 2009a). In addition, the FOSC database had the poor resolution to distinguish the *formae speciales* (O'Donnell *et al.*, 2009a). The challenge of the FOSC is that the phylogenetic history seems to be characterised by many host obstacles based on geographic proximity rather than taxonomic relatedness (O'Donnell *et al.*, 1998b; Baayen *et al.*, 2000) and by the horizontal gene transfer adding to host specificity (van der Does *et al.*, 2008). Molecular studies suggested that horizontal gene transfer is capable of shaping the evolutionary history of *F. oxysporum* (Fourie *et al.*, 2011). Taylor *et al.* (2000) indicated that recombination can contribute to the evolution of FOSC. Lastly, the origins and nature of genetic variation in FOSC is significant for future study (Fourie *et al.*, 2011).

4.5 Morphological characterisation

The morphological characterisation of selected *F. oxysporum* strains obtained from diseased sweet potato and soil in this study was done to confirm the molecular results and to provide an outline of some of the morphological characteristics of *Fusarium* species. The morphological characterisation was based on the *Fusarium* MLST database based on the observable morphological characteristics formed on selected fungal cultures grown on CLA, SNA and PDA media. Fungal cultural characteristics used for the morphological identifications are indicated in Figure 4.26 (A-K) and Figure 4.27 (A-N). Fungal macroconidia and microconidia are indicated in Figure 4.28 (A-K) and Figure 4.29 (A-N). Morphological characteristics observed included the following *Fusarium* spp.: *F. brachygibbosum*, *F. burgessii*, *F. cuneirostrum*, *F. falciforme*, *F. fujikuroi*, *F. inflexum*, *F. konzum*, *F. lacertarum*, *F. nygamai*, *F. oxysporum*, *F. scirpi* and *F. solani*. False heads on short monophialide of *F. oxysporum* and chlamydospores are indicated in Figure 4.30 (A-B).

Fusarium brachygibbosum had macroconidia that were rare, falcate to moderately curved with 3 to 5 septate The apical cells were slightly curved. The basal cells were

foot shaped. On SNA, the size of the macroconidia ranged from 32.1-46.0 x 3.8-4.7 µm, with 27.0-40.8 x 3.5-4.5 µm on average, (33.36)38.6-43.84 x (4.03)4.32-4.61 µm. Microconidia were elliptical, ovoid, and fusiform. They were zero to one septate. On SNA, the size of the microconidia ranged from 11.5-14.9 x 2.5-3.5 µm, with 10.7-13.9 x 2.1-3.2 µm on average, (11.92)12.92-13.92 x (2.53)2.93-3.33 µm. Chlamydospores were present. The colony colour ranged from initially white to pale orange (5A3) with abundant cottony aerial mycelium on PDA (Kornerup and Wanscher, 1978) as indicated in Figure 4.26 A. Macroconidia and microconidia of *F. brachygibbosum* are indicated in Figure 4.28 A. Morphological characteristics of the isolates were similar to the features of *F. brachygibbosum* described by Padwick (1945).

Fusarium burgessii had macroconidia that were slender, straight to slightly curved and in shape. They were usually 3 septate. The apical cells were tapered slightly hooked. The basal cells were foot shaped and pointed. On SNA, the size of the macroconidia ranged from 27.7-37.1 x 3.3-4.65 µm, with 24.4-33.7 x 2.9-4.2 µm on average, (27.16)30.5-33.84 x (3.72)4.1-4.48 µm. Microconidia were elliptical and fusiform. They were zero to one septate. On SNA, the size of the microconidia ranged from 8.6-11.9 x 2.7-3.3 µm, with 7.6-10.8 x 2.5-3.1 µm on average, (9.3)10.3-11.3 x (2.83)3.0-3.17 µm. Chlamydospores were present in all the *F. burgessii* isolates. The colony colour was white to (1A1) orange white (5A2) on PDA (Kornerup and Wanscher, 1978) as indicated in Figure 4.26 B. Macroconidia and microconidia of *F. burgessii* are indicated in Figure 4.28 B. The morphological characteristics had similar features as Laurence *et al*. (2011). *Fusarium burgessii* has similar morphological characters as *F. oxysporum* as it has ovoid, elliptical and reniform microconidia formed in false heads on short monophialides (Laurence *et al*., 2011). However, the formation of polyphialides separates *F. burgessii* from all members of the FOSC. The presence of polyphialides, long monophialides and the production of a yellow pigment on PDA matches with *F. hostae*. *Fusarium hostae* is morphologically similar to *F. nygamai* but does not produce microconidia in chains as *F. nygamai* (Leslie and Summerell, 2006).

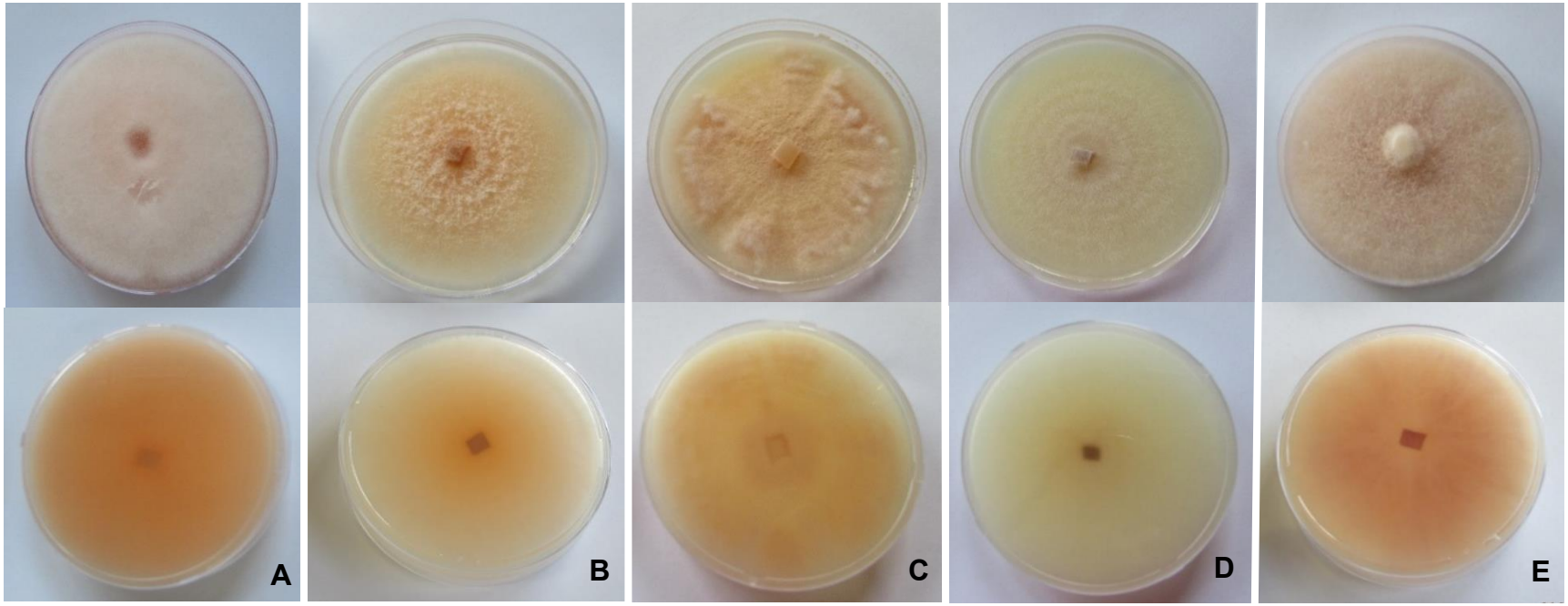


Figure 4.26 (A-K): Colony pigmentation of *Fusarium* species from this study on PDA at 25 ºC after 7 days of top of the colony (top plates) and reverse of the colony (bottom plates). Descriptions of pigmentation colour was based on the Methuen Handbook of colour (Kornerup and Wanscher, 1978). (A) *F. brachygibbosum*, pale orange (5A3), (B) *F. burgessii*, orange white (5A2), (C) *F. cuneirostrum*, orange white (6A2), (D) *F. falciforme*, pinkish white (7A2), (E) *F. inflexum*, pinkish white (9A2), (F) *F. konzum*, light orange (6A5), (G) *F. lacertarum*, light orange (6A5), (H) *F. nygamai*, pale orange (6A3), (I) *F. oxysporum*, pink=rose (13A3), (J) *F. scirpi*, light orange (6A5), (K) *F. solani*, white (A1).

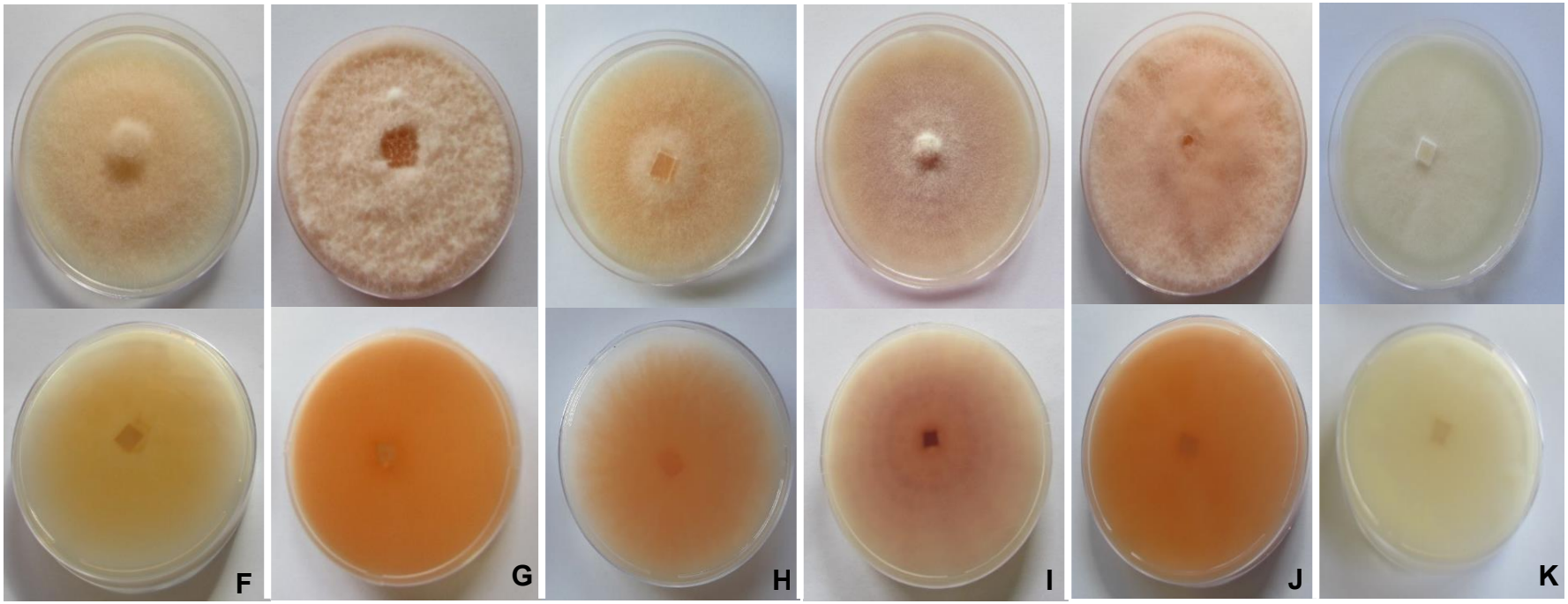


Figure 4.26: (Continued).

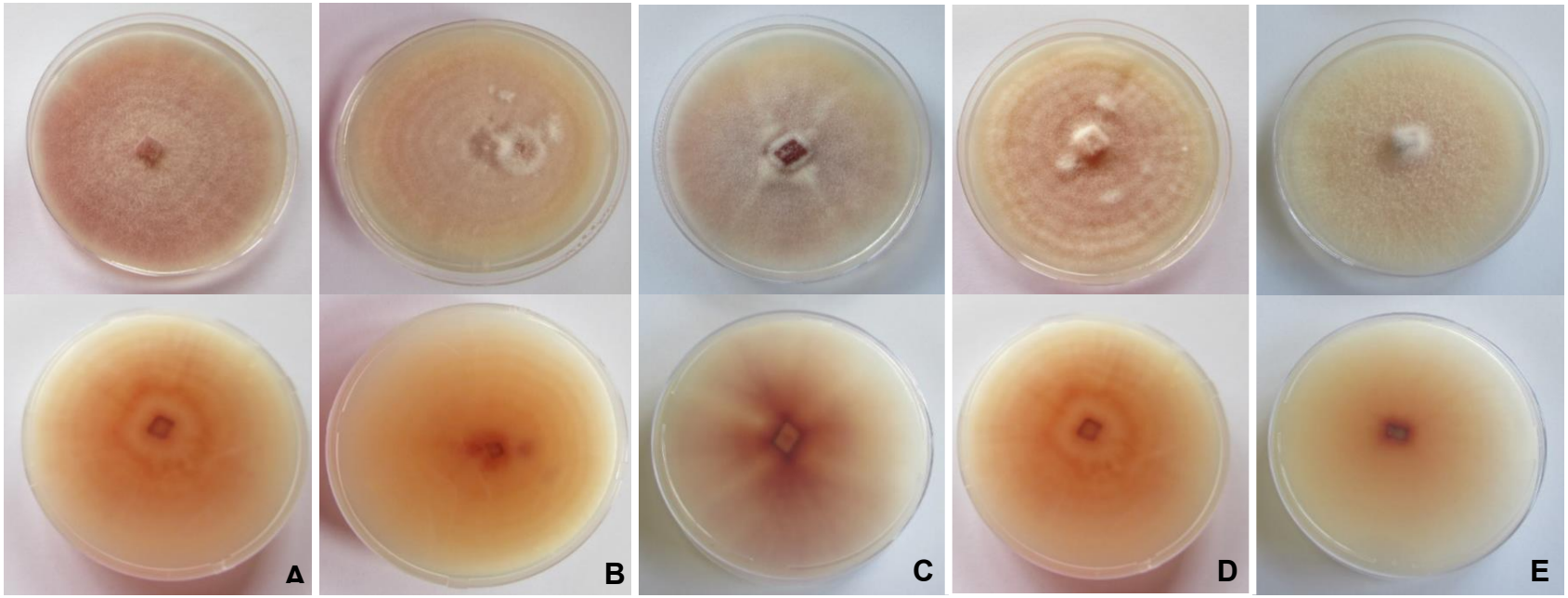


Figure 4.27 (A-N): Colony pigmentation of *F. oxysporum formae speciales* from this study on PDA at 25 ºC after 7 days of top of the colony (top plates) and reverse of the colony (bottom plates). Descriptions of pigmentation colour was based on the Methuen Handbook of colour (Kornerup and Wanscher, 1978). (A) *F. oxysporum f. sp. batatas*, pink=rose-pale red (124A), (B) *F. oxysporum f. sp. cucumerinum*, purplish pink (14A4), (C) *F. oxysporum f. sp. dianthi*, purplish red (12A6), (D) *F. oxysporum f. sp. erythroxyli*, pinkish white (13A2), (E) *F. oxysporum f. sp. lilii*, purplish white (14A2), (F) *F. oxysporum f. sp. lini*, pink=rose-pale red (12A4), (G) *F. oxysporum f. sp. lycopersici*, pinkish white (12A2), (H) *F. oxysporum f. sp. melonis*, purplish white (14A2), (I) *F. oxysporum f. sp. pini*, orange white (6A2), (J) *F. oxysporum f. sp. radicis-lycopersici*, pinkish white (7A2), (K) *F. oxysporum f. sp. tracheiphilum*, orange white (6A2), (L) *F. oxysporum f. sp. tuberosi*, orange white (5A2), (M) *F. oxysporum f. sp. vanillae*, orange white (6A2) and *F. oxysporum f. sp. vasinfectum*, pinkish white (8A2).

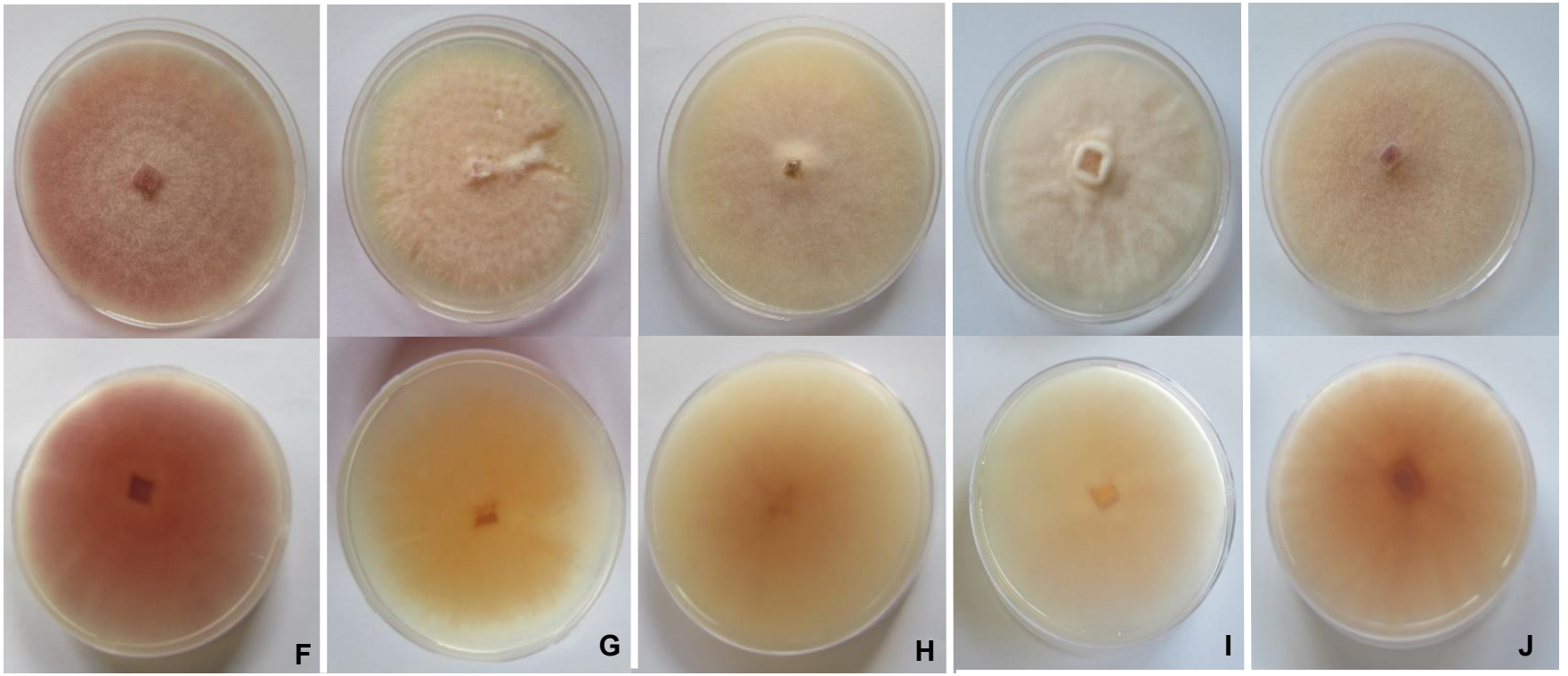


Figure 4.27: (Continued).

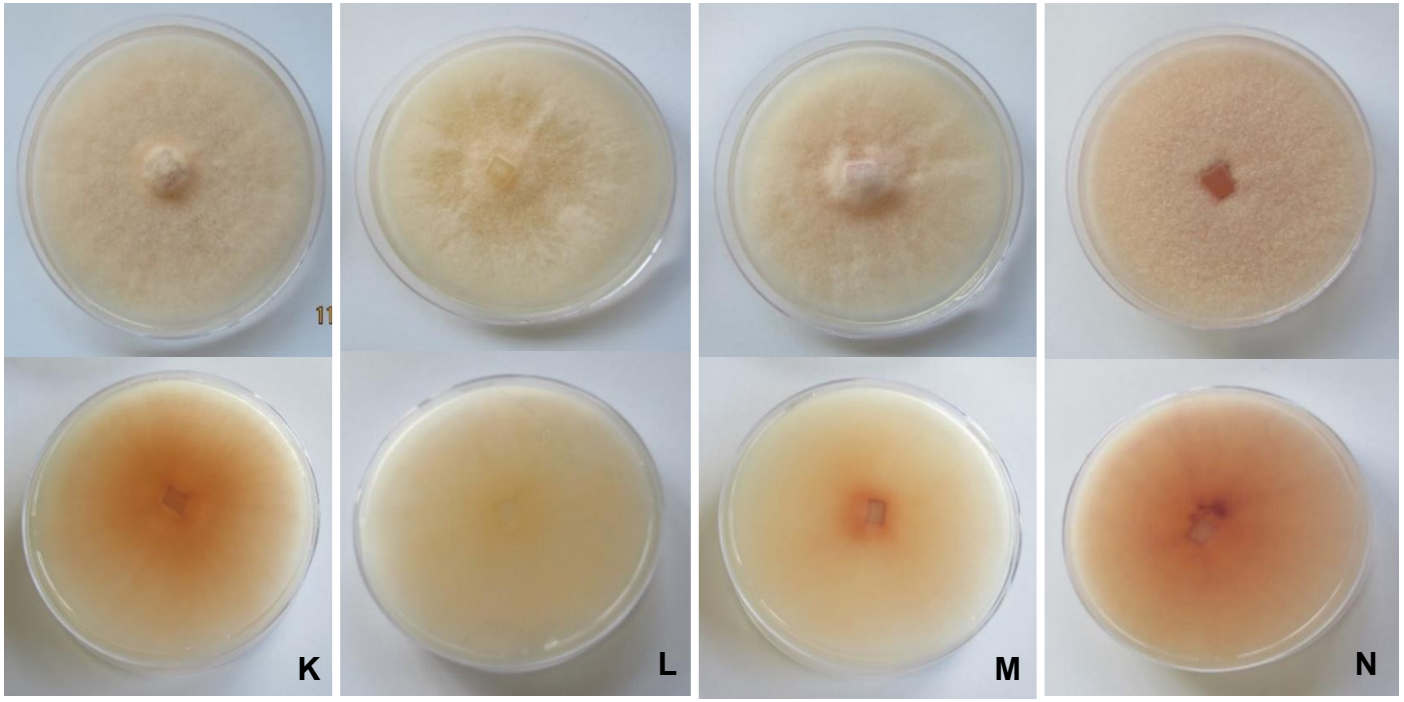


Figure 4.27: (Continued).

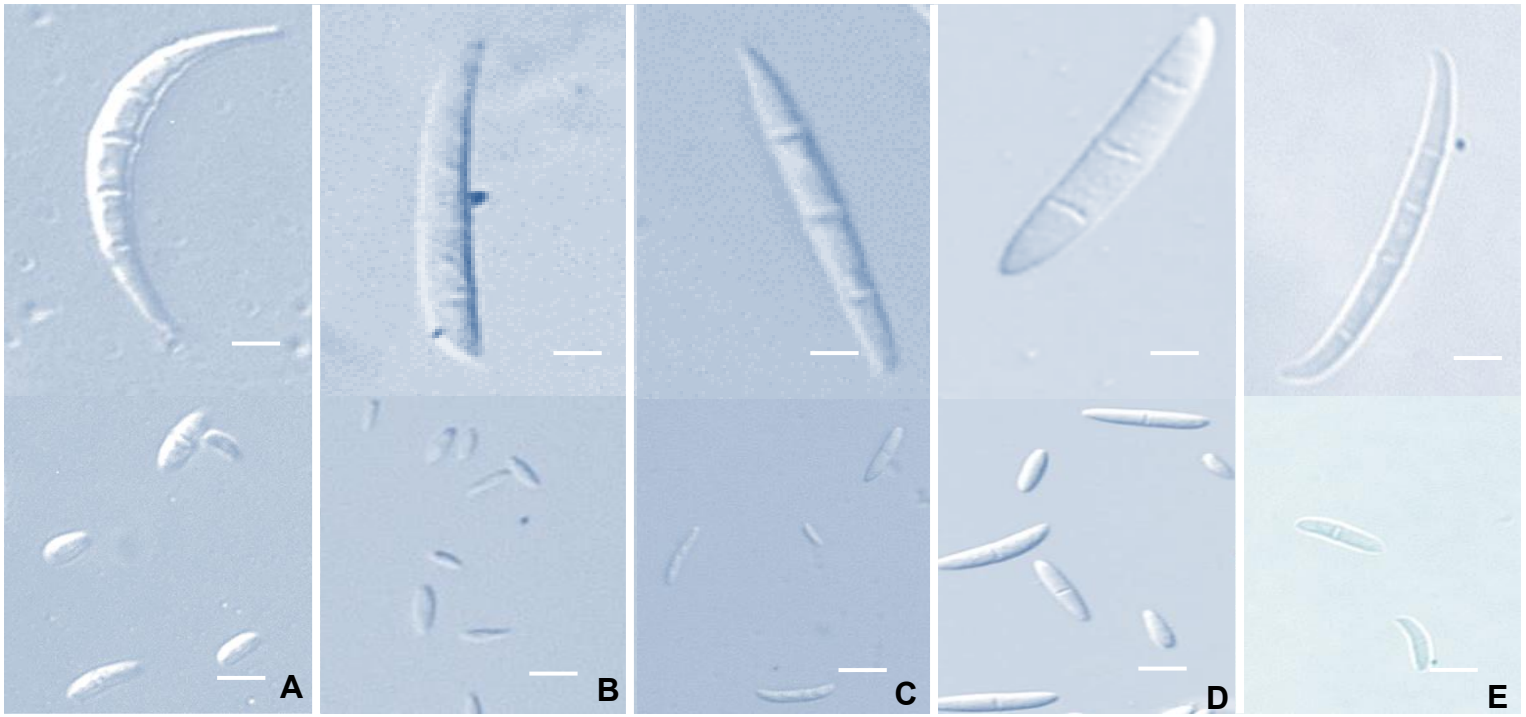


Figure 4.28 (A-K): Morphological characters of *Fusarium* species from this study. Macroconidia and microconidia on CLA. Bars = 20 µm. (A) *F. brachygibbosum*, pale orange (5A3), (B) *F. burgessii*, orange white (5A2), (C) *F. cuneirostrum*, orange white (6A2), (D) *F. falciforme*, pinkish white (7A2), E) *F. inflexum*, pinkish white (9A2), (F) *F. konzum*, light orange (6A5), (G) *F. lacertarum*, light orange (6A5), (H) *F. nygamai*, pale orange (6A3), (I) *F. oxysporum*, pink=rose (13A3), (J) *F. scirpi*, light orange (6A5), (K) *F. solani*, white (A1).

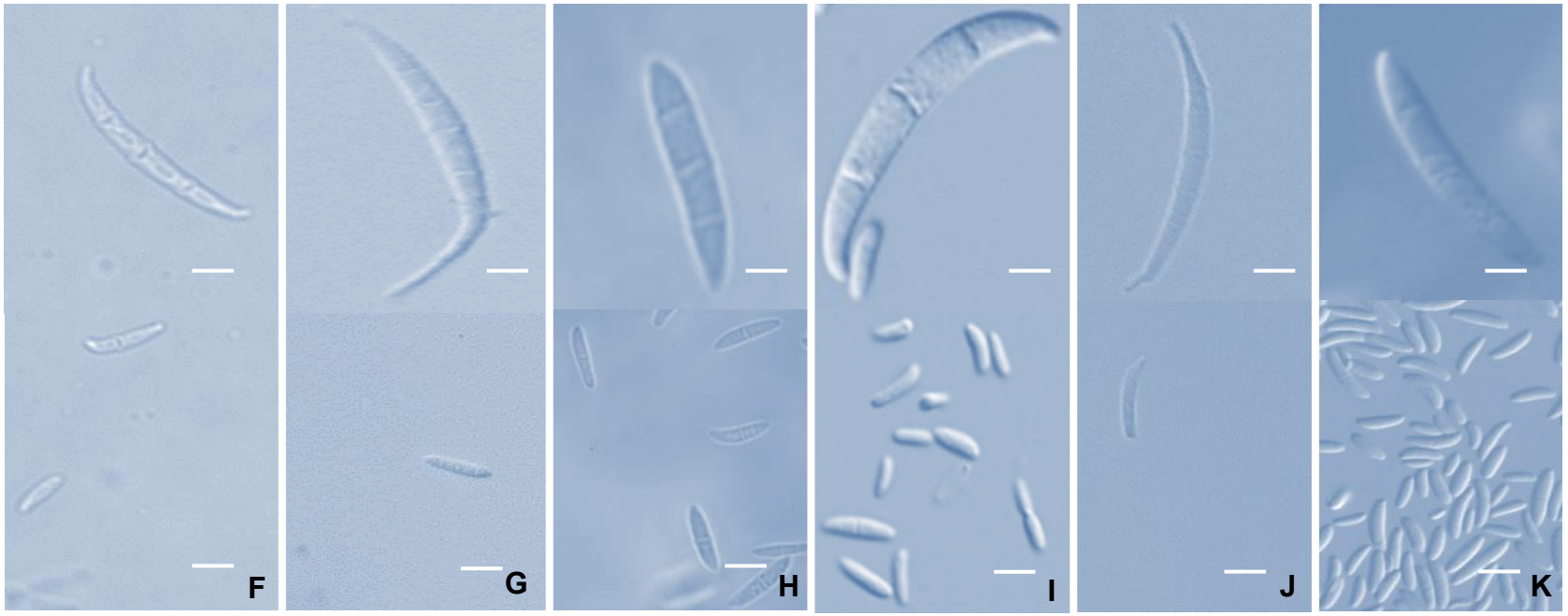


Figure 4.28: (Continued).

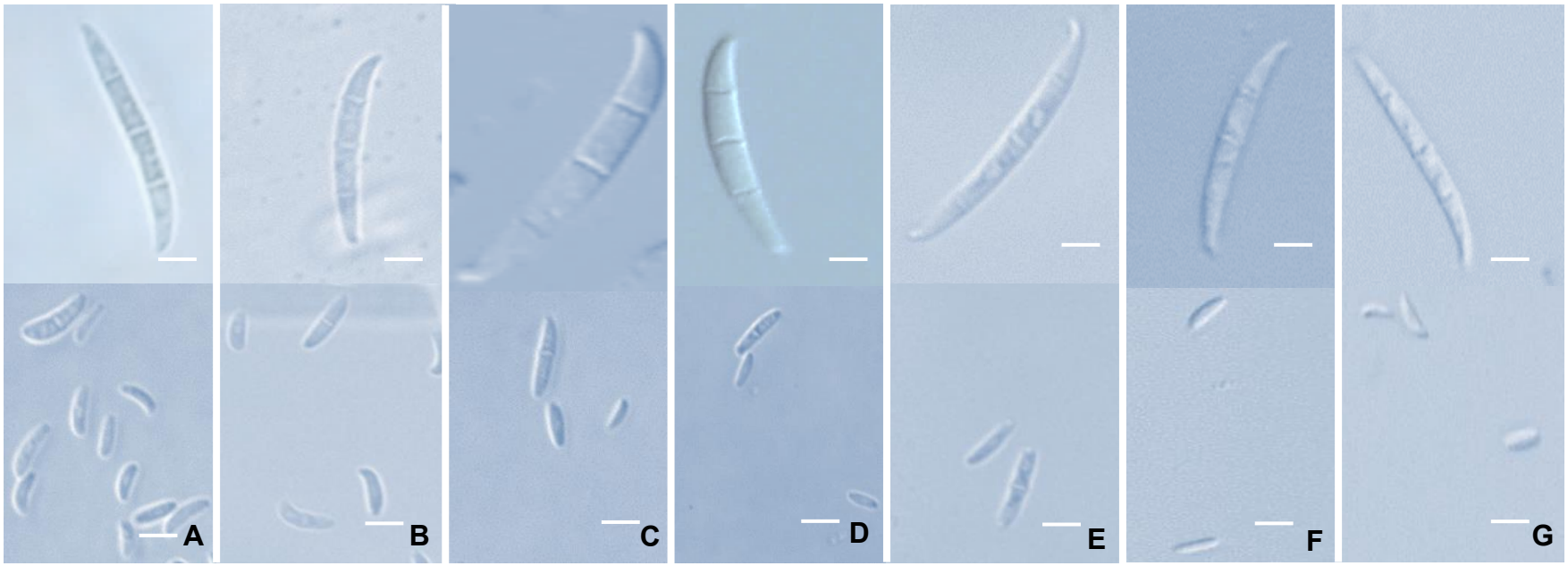


Figure 4.29 (A-N): Morphological characters of *F. oxysporum formae speciales* from this study. Macroconidia and microconidia on CLA. Bars = 20 µm. (A) *F. oxysporum f. sp. batatas*, pink=rose-pale red (124A), (B) *F. oxysporum f. sp. cucumerinum*, purplish pink (14A4), (C) *F. oxysporum f. sp. dianthi*, purplish red (12A6), (D) *F. oxysporum f. sp. erythroxyli*, pinkish white (13A2), (E) *F. oxysporum f. sp. lilii*, purplish white (14A2), (F) *F. oxysporum f. sp. lini*, pink=rose-pale red (12A4), (G) *F. oxysporum f. sp. lycoersici*, pinkish white (12A2), (H) *F. oxysporum f. sp. melonis*, purplish white (14A2), (I) *F. oxysporum f. sp. pini*, orange white (6A2), (J) *F. oxysporum f. sp. radicis-lycopersici*, pinkish white (7A2), (K) *F. oxysporum f. sp. tracheiphilum*, orange white (6A2), (L) *F. oxysporum f. sp. tuberosi*, orange white (5A2), (M) *F. oxysporum f. sp. vanillae*, orange white (6A2) and *F. oxysporum f. sp. vasinfectum*, pinkish white (8A2).

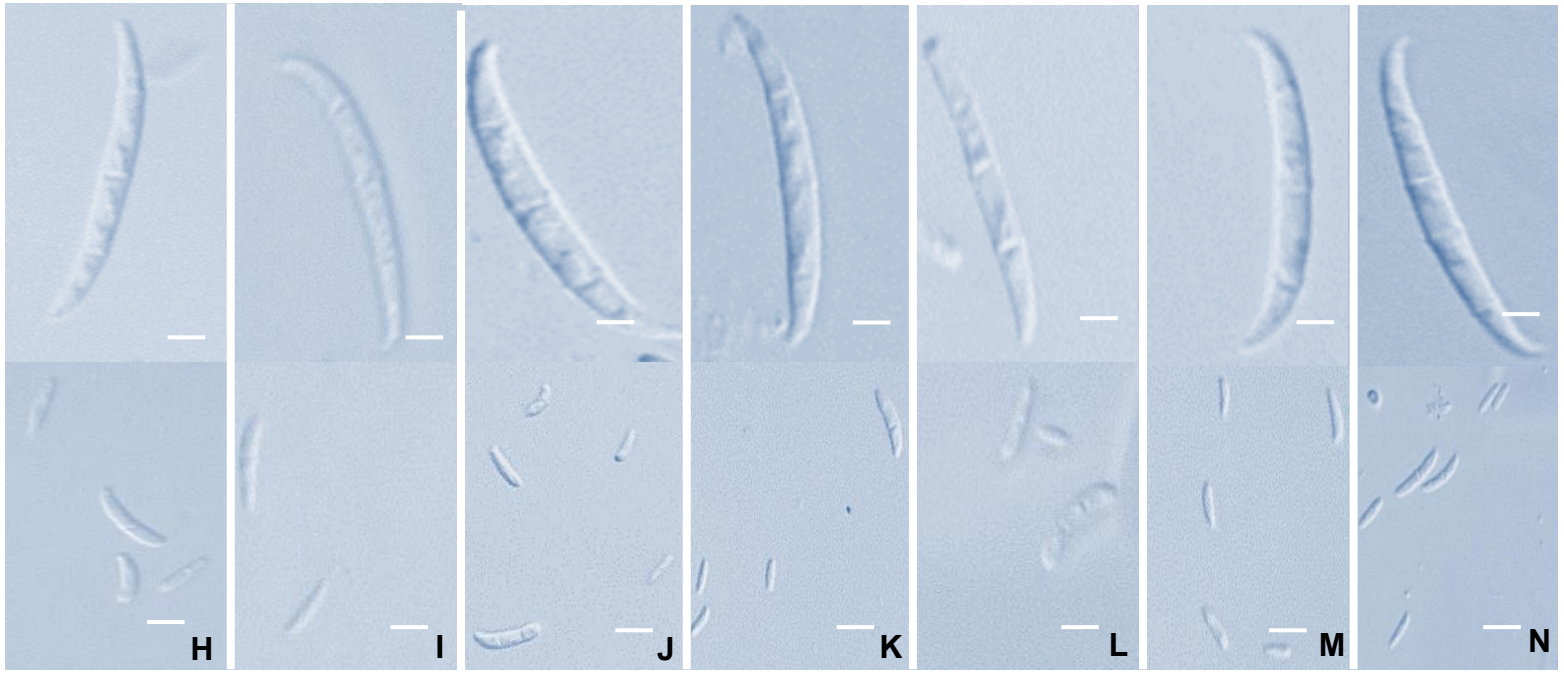


Figure 4.29: (Continued).

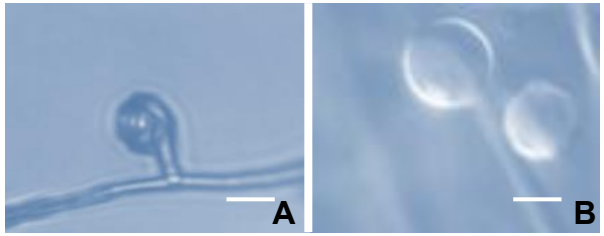


Figure 4.30 (A-B): (A) False heads on short monophialide of *F. oxysporum*. (b) Chlamydospores of *F. oxysporum*. Bar 20 µm.

Fusarium cuneirostrum had macroconidia that were usually falcate, cylindrical, gradually curved and wide. They were usually 3 septate. The apical cells were tapered to pointed. The basal cells were slightly bulged and pointed. On SNA, the size of the macroconidia ranged from 38.9-56.4 x 3.5-4.7 µm, with 33.1-50.4 x 3.1-4.3 µm on average, (37.6)43.4-49.2 x (3.61)4.0-4.39 µm. Microconidia were ellipsoid and short. They were zero septate or one septate. On SNA, the size of the microconidia ranged from 7.0-13.4 x 2.3-3.0 µm, with 4.7-11.0 x 2.0-2.7 µm on average, (8.1)10.5-12.9 x (2.37)2.6-2.83 µm. Chlamydospores were present. The colony colour had white (1A1) to orange white (6A2) on PDA (Kornerup and Wanscher, 1978). Colony margin was undulate and did not cover the entire plate. The reverse pigmentation was brownish-orange (5C3-6) to yellowish-brown (5D-E5-6) as indicated in Figure 4.26 C. Macroconidia and microconidia of *F. cuneirostrum* are indicated in Figure 4.28 C.

Fusarium falciforme had macroconidia that were wide, short, dorsiventral and falcate. Macroconidia were mostly 3 septate and can be 3 to 4 septate. They were oval and elliptical in shape. The apical cells were blunt and rounded. The basal cells were foot shaped, straight and had rounded ends. On SNA, the size of the macroconidia ranged from 36.5-49.6 x 5.1-6.1 µm, with 32.5-45.5 x 4.9-5.8 µm on average, (35.9)39.9-43.9 x (5.26)5.5-5.74 µm. Microconidia were cylindrical to oval in shape. They were zero septate or one septate. On SNA, the size of the microconidia ranged from 16.2-18.8 x 3.0-5.7 µm, with 15.5-18.0 x 2.3-5.0 µm on average, (16.48)17.2-17.92 x (3.62)4.3-4.98 µm. Chlamydospores were present in all the *F. falciforme* and were globose, smooth and rough walled, formed singly and in pairs. The colony colour was pinkish white (7A2) with concentric rings on PDA (Kornerup and Wanscher, 1978). The mycelium was raised, fluffy, cottony and covered the entire plate as indicated in Figure 4.26 D. Macroconidia and microconidia of *F. falciforme* are indicated in Figure 4.28 D.

Fusarium inflexum had macroconidia that were relatively slender, slight wide and falcate. On SNA, the size of the macroconidia ranged from 31.5-39.7 x 3.9-5.5 µm, with 28.6-36.8 x 3.5-5.0 µm on average, (33.0)35.9-38.8 x (4.23)4.7-5.17 µm. They were mostly three septate. The apical cells were tapered and slightly curved. The

basal cells were foot shaped. The microconidia were zero septate, elliptical and slightly hooked. On SNA, the size of the microconidia ranged from 8.3-11.8 x 2.5-3.7 µm, with 7.3-10.8 x 2.1-3.2 µm on average, (9)10.0-11 x (2.59)3.0-3.14 µm. Chlamydospores were present in all the *F. inflexum* isolates. The colony colour of the *F. inflexum* isolates were pinkish white (9A2) on PDA. The mycelium was raised, fluffy, cottony and covered the entire plate as indicated in Figure 4.26 E. Macroconidia and microconidia of *F. inflexum* is indicated in Figure 4.28 E.

Fusarium konzum had macroconidia that were falcate, slender, slightly curved and rare. They were 3 septate. The apical cells were slightly curved. The basal cells were foot shaped. On SNA, the size of the macroconidia ranged from 26.3-32.7 x 3.1-3.8 µm, with 24.1-30.4 x 2.8-3.5 µm on average, (27.74)30.0-32.26 x (3.06)3.3-3.54 µm. Microconidia were elliptical and ovoid. They were zero to one septate. On SNA, the size of the microconidia from 8.8-12.7 x 2.4-3.2 µm, with 7.3-11.2 x 2.1-2.9 µm on average, (9.01)10.5-11.99 x (2.54)2.8-3.06 µm. Chlamydospores were absent. The colony colour was light orange (6A5) on PDA (Kornerup and Wanscher, 1978) as indicated in Figure 4.26 F. Macroconidia and microconidia of *F. konzum* are indicated in Figure 4.28 F. The mycelium was raised, fluffy and did not cover the entire plate. Morphological characteristics of the isolates were similar to the features of *F. konzum* described by Zeller *et al.* (2003) and Leslie and Summerell (2006). *Fusarium konzum* is morphologically similar to *F. anthophilum* because of its pyriform microconidia, however, the longated mono-phialides and more enlarged polyphialides found in *F. konzum* differentiate them (Zeller *et al*., 2003).

Fusarium lacertarum had macroconidia that were long and slender and had a dorsiventral curvature. They were 5 to 7 septate but mostly 5 septate. The apical cells were tapered, filamentous and whip-like. The basal cells were foot shaped. On SNA, the size of the macroconidia ranged from 35.2-44.6 x 3.8-4.6 µm, with 31.7-41.1 x 3.5-4.3 µm on average, (35.19)38.7-42.21 x (3.98)4.3-4.62 µm. Microconidia were are oblongate, fusiform and elliptical. They were three septate. On SNA, the size of the microconidia ranged from 11.8-13.2 x 2.9-3.4 µm, with 11.4-12.8 x 2.7-3.2 µm on average, (12.07)12.5-12.93 x (2.96)3.1-3.24 µm. Leslie and Summerell (2006) stated that microconidia are absent but some other isolates of *F. equiseti*

can produce microconidia (Leslie and Summerell, 2006). The colony colour was white (1A1) to light orange (6A5) (Kornerup and Wanscher, 1978). The mycelium was abundant, raised, woolly, fluffy and covered the entire plate as indicated in Figure 4.26 G. Macroconidia and microconidia of *F. lacertarum* are indicated in Figure 4.28 G. Some isolates form a very long macroconidia with filamentous or whip-like apical cell and might resemble the macroconidida formed by *F. longipes* (Leslie and Summerell, 2006).

Fusarium nygamai had macroconidia that were falcate to almost straight in shape and slender. Macroconidia were 3 to 5 septate but usually 3 septate. The apical cells were tapered. The basal cells were foot shaped and notched. On SNA, the size of the macroconidia ranged from 23.6-28.5 x 2.9-3.3 µm, with 22.1-27.0 x 2.7-3.2 µm on average, (23.54)25.0-26.46) x (2.87)3.0-3.13 µm. Microconidia were elliptical and usually zero to septate. On SNA, the size of the microconidia ranged from 7.6-10.5 x 2.4-3.3 µm, with 6.7-9.6 x 2.2-3.0 µm on average, (8.39)9.3-10.21 x (2.55)2.8-3.05 µm. Chlamydospores were present. The colony colour was white (1A1) to pale orange (6A3) on PDA (Kornerup and Wanscher, 1978) as indicated in Figure 4.26 H. Macroconidia and microconidia of *F. nygamai* is indicated in Figure 4.28 H. Leslie and Summerell (2006) states that microconidia are formed in false heads on monophialides. Polyphialides and short chains can be observed in older cultures or at the edges of the colony. The micro-conidia of *F. nygamai* matches those of *F. verticillioides*. *Fusarium nygamai* cannot be incorporated in section *Liseola* as it forms chlamydospores and cannot be incorporated in section *Elegans* as it forms chains of micro-conidia. Furthermore, Burgess and Trimboli (1986) defined the production of microconidia in short chains as a consistent and reliable criterion for identification of *F. nygamai*.

Fusarium oxysporum had macroconidia that were falcate to almost straight and moderately slender. They were thin walled with 3 to 5 septate but mostly were usually 3-septate. The apical cells were tapered and slightly hooked. The basal cells were foot shaped and pointed. On SNA, the size of the macroconidia ranged from 20.5-60.3 x 2.9-5.1 µm, with 27.8-50.3 x 2.9-5.1 µm on average, (32.09)38.22-44.35 x (3.03)3.72-4.41 µm. The microconidia were kidney-shaped, elliptical, fusiform,

curved and straight. They were usually zero to 1-septate. Conidiogenous cells were monophialides. Microconidia were formed in false heads on short monophialides (Figure 30 A). Chlamydospores were present in all the FOSC isolates (Figure 30 B). They were globose shaped and formed singly or in pairs or were smooth to roughed walls. Chlamydospores were observed after seven days of incubation under white light, but according to Leslie and Summerell (2006), chlamydospores can be observed after two to four weeks. On SNA, the size of the microconidia ranged from 7.5 - 16.0 x 2.5-4.5, with 12.7-6.4 x 3.5-1.9 µm on average, (8.18)10.20-12.22 x (2.13)2.64-3.15 µm.

The colony colour of the *F. oxysporum* isolates on PDA included pink (13A3), pale red (12A4), purplish pink (14A4), purplish red (12A6), pinkish white (13A2), purplish white (14A2), brownish violet (11D7), orange white (6A2) on PDA (Kornerup and Wanscher, 1978) (Figure 4.27 A-K). The mycelium was raised and fluffy and covered the entire plate except *F. oxysporum f. sp. melonis*. The mycelium was cottony (filamentous) for some of the cultures namely *F. oxysporum f. sp. lycopersici*, *F. oxysporum f. sp. radicis-lycopersici* and *F. oxysporum f. sp. lupini* and *F. oxysporum f. sp. tracheiphilum*. Most of the FOSC isolates had concentric rings namely the representatives of *F. oxysporum*, *F. oxysporum f. sp. batatas*, *F. oxysporum f. sp. cucumerinum*, *F. oxysporum f. sp. erythroxyli*, *F. oxysporum f. sp. lini*, and *F. oxysporum f. sp. lycopersici*. Colony pigmentation of *F. oxysporum formae speciales* is indicated in Figure 4.27 (A-N). Macroconidia and microconidia of *F. oxysporum formae speciales* is indicated in Figure 4.29 (A-N).

Fusarium scirpi had macroconidia that were long, slender and dorsi-ventral curvature. The apical cells were long and tapered. The basal cells were foot shaped. Macroconidia were usually 6 - 7 septate. On SNA, the size of the macroconidia ranged from 39.1-42.1 x 3.0-4.3 µm, with 38.1-41.0 x 2.7-3.9 µm on average, (39.58)40.6-41.62 x (3.53)3.9-4.27 µm. Microconidia were elliptical. They were usually zero to 3 septate. On SNA, the size of the microconidia ranged from 9.8-16.1 x 2.9-3.8 µm, with 7.5-13.9 x 2.6-3.5 µm on average, (9.45)11.7-13.95 x (3.1)3.4-3.7 µm. Chlamydospores were present is all the *F. scirpi* isolates. The colony colour was white (1A1) to light orange (6A5) (Kornerup and Wanscher, 1978)

as indicated in Figure 4.26 J. Macroconidia and microconidia of *F. scirpi* are indicated in Figure 4.28 J. The mycelium was abundant, raised, woolly, fluffy and covered the entire plate. Chlamydospores were observed under microscope after seven days of incubation under light but according to Leslie and Summerell (2006), chlamydospores can be observed after two to four weeks. *Fusarium scirpi* might be confused with *F. equiseti* as the macroconidia are of similar size and shape and the PDA cultures are also similar. Microconidia conidiogenous cells are monophialides and polyphialides. *Fusarium scirpi* have a lot of microconidia and have a diagnostic of short and cross-shaped polyphialides (Leslie and Summerell, 2006).

Fusarium solani had macroconidia that were moderately wide, elliptical to straight, reniform and sturdy. They were 1 to 5 septate and can be 3-7 septate. On SNA, the size of the macroconidia ranged from 38.4-54.0 x 4.5-5.5 µm, with 32.7-48.2 x 4.17-5.2 µm on average, (38.36)44.1-49.84 x (4.67)5.0-5.33 µm. The apical cells were blunt and rounded. The basal cells were discrete foot shaped, straight, almost cylindrical and rounded ends. Microconidia were cylindrical to oval in shape and also fusiform. They were zero to one septate. On SNA, the size of the microconidia ranged from 14.6-16.7 x 4.0-4.9 µm, with 13.9-16.0 x 3.7-4.6 µm on average, (14.72)15.4-16.08 x (4.08)4.4-4.72 µm. Chlamydospores were globose and present in all the *F. solani* isolates. They had smooth appearance and roughed walled. The colony colour was white (A1) to cream white cream white (1A1) colour on PDA (Kornerup and Wanscher, 1978). The mycelium was raised, fluffy, cottony and covered the entire plate as indicated in Figure 4.26 K. Macroconidia and microconidia of *F. solani* are indicated in Figure 4.28 K. Microconidia are formed in false heads on long monophialides when compared with *F. oxysporum* (Leslie and Summerell, 2006).

Morphological characterisation of the *Fusarium* isolates under current study were in agreement with the previously studied *Fusarium* species. When morphologically characterising strains of *Fusarium*, spore type and morphology are usually observed as the significant features (Summerell *et al.*, 2003). Observed morphological characteristics indicated *Fusarium* species identification (Summerell and Leslie, 2011).

4.6 DNA barcoding analysis

DNA barcoding is an approach used to identify organisms based on a short, uniform fragment of genomic DNA. This study used the DNA barcoding approach through ITS gene region to develop the possible discrimination of *F. oxysporum* within the FOSC by determining the presence or absence of distinct single nucleotide polymorphisms. ITS gene region is extensively used in fungal taxonomy and molecular phylogenetic analyses as it is easy to amplify because of the high copy number of rRNA genes, and has a high degree of genetic variation between closely related species. The ITS gene region can be used to resolve other fungal species (Das and Deb, 2015) as it is an effective DNA barcode in some lichenized lineages (Kelly *et al.*, 2011). Schoch *et al.* (2012) reported ITS region as a universal DNA barcode marker for Fungi. DNA barcode criteria listed by Letourneau *et al.* (2010) states that the barcode should be between 500-800 bp, easily amplifiable, must have a low intraspecific variation and a higher interspecific variation than interspecific variation.

The aligned ITS sequences had only one site base difference at about 375 site when viewed with MEGA version 6.0 software as indicated in Appendices B-D and resolved the South African and reference sequences into two clades due to base difference of Thymine (T) nucleotice and Cytocine (C) nucleotide. The MP phylogenetic analysis of the diseased sweet potato ITS gene dataset resolved the FOSC dataset into two distinct clades. Clade I comprised of nineteen South African isolates that grouped together with twelve reference strains of *F. oxysporum* and three *formae speciales* namely, two *F. oxysporum f. sp. ciceris*, *F. oxysporum f. sp. cucumerinum* and *F. oxysporum f. sp. lentis* with a significant bootstrap value of 100% (Figure 4.31). Clade I was based on the common T-base pair (Appendices B-D). Clade II comprised of thirty-six South African isolates that grouped together with thirteen reference strains of *F. oxysporum* and only one *F. oxysporum f. sp. ciceris* with no bootstrap support. Clade II was based on the common C-base pair that if found at about 375 base site (Appendices B-D). Therefore, ITS MP analysis partially supported DNA barcoding by grouping into two clades based on C-base pair or T-base pair. The reference strains were associated with mostly *F. oxysporum* and only three *formae speciales*, unknown hosts and origin.

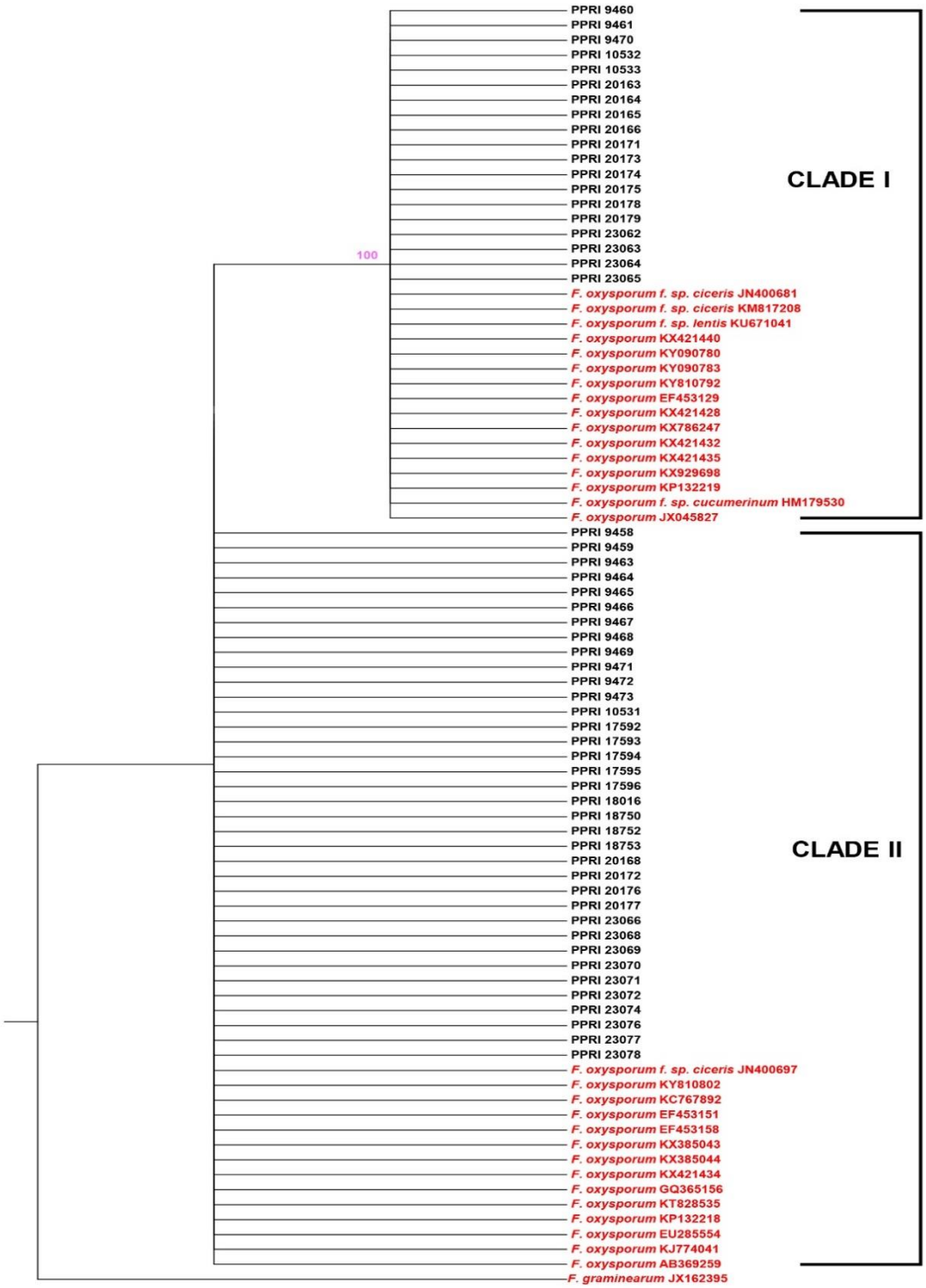


Figure 4.31: Phylogenetic tree based on MP analyses of *F. oxysporum* associated with FW of sweet potato in South Africa based on the ITS gene region. The tree is rooted to KU254606 *F. graminearum*. The PPRI isolates in bold black are from South Africa and were recovered from diseased sweet potato plant stems. The NRRL isolates in bold red are reference strains obtained from *Fusarium* MLST database.

Most of the South African strains were clustered in Clade II. DNA barcoding based on ITS gene region cannot be used as an identification or classification tool in FOSC as there was limited correlation between two clades of a single base pair difference and there was no correlation regarding the *formae speciales*, hosts and geographic regions. This is in contrast to several other fungal species that can be successfully be resolved by the ITS region (Kelly *et al.*, 2011; Das and Deb, 2015). This could be as a results of the ITS sequences that are identical in many *Fusarium* complexes and they do not tend to evolve at a rate correlated with speciation (Al-Hatmi *et al.*, 2016). A study by Al-Hatmi *et al.* (2016) concluded that TEF-1α, TOP1 and PGK gene regions can be used as a barcoding markers for accurate identification of *Fusarium* spp.

In summary, this study recovered fungal isolates from diseased sweet potato and soil, which were characterised based on morphological and molecular data. Isolates were identified with *Fusarium* MLST and *Fusarium*-ID databases. Both databases revealed similar results, although some did not correspond. The species complexes that were disclosed via database's nBLAST™ results included FDSC, FFSC, FGSC, FIESC, FOSC, FRSC, FSASC and FSSC. The *Fusarium* species in this study were presented by *F. brachygibbosum*, *F. burgessii*, *F. cuneirostrum*, *F. falciforme*, *F. graminearum*, *F. inflexum*, *F. konzum*, *F. lacertarum*, *F. nygamai*, *F. oxysporum*, *F. scirpi* and *F. solani* associated with different hosts. *Fusarium cuneirostrum* and *F. konzum* have not been reported in South Africa, therefore, this is the first report of these species associated with FW of sweet potato in South Africa. This study revealed different *F. oxysporum formae speciales*, including *F. oxysporum f. sp. batatas*, *F. oxysporum f. sp. cucumerinum*, *F. oxysporum f. sp. dianthi*, *F. oxysporum f. sp. erythroxyli*, *F. oxysporum f. sp. lilii*, *F. oxysporum f. sp. lini*, *F. oxysporum f. sp. lycopersici*, *F. oxysporum f. sp. melonis*, *F. oxysporum f. sp. pini, F. oxysporum f. sp. radicis-lycopersici*, *F. oxysporum f. sp. tracheiphilum*, *F. oxysporum f. sp. tuberosi*, *F. oxysporum f. sp. vanillae* and *F. oxysporum f. sp. vasinfectum* associated with sweet potato, however very few *F. oxysporum formae speciales* have been reported in South Africa.

Our study found 21 MLSTs and 10 MLSTs from diseased sweet potato based on the *Fusarium* MLST and *Fusarium*-ID databases respectively, based on the TEF-1α gene region. These MLSTs are associated with various hosts. Furthermore, this study found 18 MLSTs and 14 MLSTs from soil based on the *Fusarium* MLST and *Fusarium*-ID databases respectively, based on the TEF-1α gene region. These MLSTs are associated with various hosts. Only two MLSTs was discovered from diseased sweet potato based on the *Fusarium* MLST database and *Fusarium*-ID database of the RPB2 and ITS gene region. *Fusarium* MLST database revealed more *Fusarium* species complexes and *F. oxysporum formae speciales* than *Fusarium*-ID database therefore *Fusarium* MLST database was useful in providing the information relating to the South African fungal isolates.

Maximum Likelihood and Maximum Parsimony analyses of the separate TEF-1α, RPB2, β-tubulin and ITS phylogenetic trees showed that TEF-1α provided the best phylogenetic grouping with bootstrap support compared to the other gene regions, followed by RPB2 trees. The β-tubulin and ITS phylogenetic trees did not cluster into different genetic groups. The ITS sequence data generated supported the DNA barcoding approach.

Morphological observation provided the confirmation of the FOSC and *Fusarium* species identification. The morphological characteristics observed were in agreement with the literature.

CHAPTER 5
CONCLUSION

This study has shown that apart from *F. oxysporum f. sp. batatas*, two other *formae speciales* namely, *F. oxysporum f. sp. tuberosi* and *F. oxysporum f. sp. vanillae*, are associated with FW of sweet potato in South Africa. The characterisation of the FOSC isolates using phylogenetic analyses indicated that the TEF-1α gene region was the best gene region amongst all the other gene regions to resolve the FOSC dataset. This was followed by the RPB2 gene region which was also able to partially group South African isolates but the clustering was not well supported. The β-tubulin gene region was unable to distinguish between the different South African isolates. Therefore, the approach of molecular characterisation of FOSC using the TEF-1α gene served as a significant tool for identification of FOSC from South Africa. The ITS sequence data used as the barcoding gene in fungi was able to distinguish two clades amongst the FOSC isolates and supported the DNA barcoding approach.

The morphological characterisation was useful in confirming the South African FOSC isolates, however more useful in indicating the other *Fusarium* species and can provide additional information for describing and distinguishing known and new species. This study contributes information about the composition and diversity FOSC in diseased sweet potato and soil in the sweet potato production areas of South Africa. This study has recovered eight *Fusarium* species complexes, several important *Fusarium* species which are reported plant pathogens on other crops and *Fusarium* species that have not been reported in South Africa.

This work contributes to a new body of knowledge in the management of pest and diseases by improved our current understanding of FOSC. The identification of new *formae speciales* associated with FW of sweet potato in South Africa can have an impact on South African agriculture as it should be considered in determining risk evaluation approaches, control measures for farmers and assist breeders in making informed choices on which *F. oxysporum formae speciales* associated with FW of sweet potato to use when screening during resistence breeding to FW.

Future work in this area should include the characterisation of the FIESC, FSSC and other *Fusarium* species obtained in this study based on comprehensive phylogenetic analyses and detailed morphological characterisation. The future work should also include, pathogenicity glasshouse trials on sweet potato and other hosts such as potato, tomato and indigenous vegetables and testing for the pathogenicity-related genes.

CHAPTER 6

REFERENCES

Adejuwon, J.O., Olawole, A.O. and Adeoye, N.O., 2019. Changing Pattern in Sweet Potato Cultivation in Semi-Arid Environment, Nigeria. *Journal of Applied Sciences and Environmental Management*, 23 (7), pp.1233-1238.

Ahearn, D.G., Zhang, S., Ward, M.A., Simmons, R.B. and Stulting, R.D., 2009. Hyphal penetration of worn hydrogel contact lenses by *Fusarium*. *The Journal of Cornea and External Disease*, 28 (8), pp.914-917.

Alabouvette, C., Schippers, B., Lemanceau, P. and Bakker, P.A.H.M., 1998. *Biological control of Fusarium wilts. Plant-Microbe Interactions and Biological Control.* (Edition): Boland, G.J. and Kuykendall, L.D. Marcel Dekker, New York, pp.15-36.

Al-Hatmi, A.M., Van Den Ende, A.G., Stielow, J.B., Van Diepeningen, A.D., Seifert, K.A., McCormick, W., Assabgui, R., Grafenhan, T., De Hoog, G.S. and Levesque, C.A., 2016. Evaluation of two novel barcodes for species recognition of opportunistic pathogens in *Fusarium*. *Fungal Biology*, 120 (2), pp.231-245.

Amini, J. and Sidovich, D., 2010. The effects of fungicides on *Fusarium oxysporum f. sp. lycopersici* associated with *Fusarium* wilt of tomato. *Journal of Plant Protection Research*, 50 (2), pp.172-178.

Aoki, T. and O'Donnell, K., 1999. Morphological and molecular characterisation of *Fusarium pseudograminearum* sp. nov., formerly recognized as the Group 1 population of *F. graminearum*. *Mycologia*, 91 (4), pp.597-609.

Aoki, T., O'Donnell, K. and Scandiani, M.M., 2005. Sudden death syndrome of soybean in South America is caused by four species of *Fusarium*: *Fusarium brasiliense* sp. nov., *F. cuneirostrum* sp. nov., *F. tucumaniae*, and *F. virguliforme*. *Mycoscience*, 46 (3), pp.162-183.

Armstrong, G.M. and Armstrong, J.K., 1981. *Formae speciales and races of Fusarium oxysporum causing wilt diseases.* (Edition): Nelson, P.E., Toussoun, T.A. and Cook, R.J. *Fusarium: Diseases, Biology and Taxonomy.* University Park, United States of America: The Pennsylvania State University Press. pp.392-399.

Atkinson, G.F., 1892. *Some diseases of cotton* (No. 41). Agricultural Experiment Station of the Agricultural and Mechanical College. Alabama. The Brown Printing. pp.5-9

Austin, D.F., 1983. Variability in sweet potatoes in America. *Proceedings of the American Society for Horticultural Science*, *Tropical Region*, 27, pp.15-27.

Austin, D.F., 1988. *The taxonomy, evolution and genetic diversity of sweet potatoes and related wild species. Exploration, Maintenance, and Utilization of Sweetpotato Genetic Resources*. International Potato Centre (Report of the first Sweet Potato Planning Conference 1987). Lima. Peru. pp.27-60.

Baayen, R.P., O'Donnell, K., Bonants, P.J., Cigelnik, E., Kroon, L.P., Roebroeck, E.J. and Waalwijk, C., 2000. Gene genealogies and AFLP analyses in the *Fusarium oxysporum* complex identify monophyletic and nonmonophyletic *formae speciales* causing wilt and rot disease. *Phytopathology*, 90 (8), pp.891-900.

Baayen, R.P., O'Donnell, K., Breeuwsma, S., Geiser, D.M. and Waalwijk, C., 2001. Molecular relationships of fungi within the *Fusarium redolens-F. hostae* clade. *Phytopathology*, 91 (11), pp.1037-1044.

Bazán de Segura, C., 1959. *Principales Enfermedades de las Plantas en el Perú. Fitopatóloga de las Estaciones Experimentales Agrícloas de las Asociaciones de Agricultores de Cañete e Ica y del Comité de Defensa Técnica del Algodonero de la Sociedad Nacional Agraria*.

Benhamou, N. and Garand, C., 2001. Cytological analysis of defense-related mechanisms induced in pea root tissues in response to colonization by nonpathogenic *Fusarium oxysporum* Fo47. *Phytopathology*, 91 (8), pp.730-740.

Bertoldo, C., Gilardi, G., Spadaro, D., Gullino, M.L. and Garibaldi, A., 2015. Genetic diversity and virulence of Italian strains of *Fusarium oxysporum* isolated from *Eustoma grandiflorum*. *European Journal of Plant Pathology*, 141 (1), pp.83-97.

Bogale, M., Wingfield, B.D., Wingfield, M.J. and Steenkamp, E.T., 2005. Simple sequence repeat markers for species in the *Fusarium oxysporum* complex. *Molecular Ecology Notes*, 5 (3), pp.622-624.

Bolley, H.L., 1901. A Preliminary Note on the Cause of "flax-sick" Soil: *Fusarium lini* sp. nov. Proceedings of the Annual Meeting of the Society for the Promotion of Agricultural Science, pp.42-46.

Booth, C., 1971. *The genus Fusarium.* Commonwealth Mycological Institute, Kew, UK. pp.237.

Brayford, D., 1992. IMI descriptions of fungi and bacteria, set 112, nos 1111–1120. *Mycopathologia*, 118 (1), pp.37-64.

Burgess, L.W. and Trimboli, D., 1986. Characterisation and distribution of *Fusarium nygamai* sp. nov. *Mycologia*, 78 (2), pp.223-229.

Burgess, L., Summerell, B., Bullock, S., Gott, K. and Backhouse, D., 1994. Laboratory Manual for *Fusarium* Research, Third Edition, *Fusarium* Research Laboratory, *Department of Crop Science, University of Sydney and Royal Botanic Gardens*, Sydney.

Bushula, V.S., 2008. *Native Fusarium species from indigenous fynbos soils of the Western Cape*. Doctoral Dissertation, Stellenbosch University. South Africa.

Cafri, D., Katan, J. and Katan, T., 2005. Cross-pathogenicity between *formae speciales* of *Fusarium oxysporum*, the pathogens of cucumber and melon. *Journal of Phytopathology*, 153 (10), pp.615-622.

Cai, G., Gale, L.R., Schneider, R.W., Kistler, H.C., Davis, R.M., Elias, K.S. and Miyao, E.M., 2003. Origin of race 3 of *Fusarium oxysporum f. sp. lycopersici* at a single site in California. *Phytopathology*, 93 (8), pp.1014-1022.

Chang, D.C., Grant, G.B., O'Donnell, K., Wannemuehler, K.A., Noble-Wang, J., Rao, C.Y., Jacobson, L.M., Crowell, C.S., Sneed, R.S., Lewis, F.M. and Schaffzin, J.K., 2006. Multistate outbreak of *Fusarium keratitis* associated with use of a contact lens solution. *Jama*, 296 (8), pp.953-963.

Chase, M.W., Christenhusz, M.J.M., Fay, M.F., Byng, J.W., Judd, W.S., Soltis, D.E., Mabberley, D.J., Sennikov, A.N., Soltis, P.S. and Stevens, P.F., 2016. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Botanical Journal of the Linnean Society*, 181 (1), pp.1-20.

Clark, C.A., 2013. *Compendium of Sweet Potato Diseases.* (Second Edition): Clark, C.A., Ferrin, D.M., Smith, T.P. and Holmes, G.J. APS Press. The American Phytopathological Society, St. Paul, Minnesota.

Clark, C.A. and Moyer, J.W., 1988. *Compendium of sweet potato diseases*. The American Phytopathological Society, St. Paul, Minnesota, pp.74.

Correll, J.C., 1991. The relationship between *formae speciales*, races, and vegetative compatibility groups in *Fusarium oxysporum*. *Phytopathology*, 81 (9), pp.1061-1064.

Das, S. and Deb, B., 2015. DNA barcoding of fungi using Ribosomal ITS Marker for genetic diversity analysis: A Review. *International Journal of Pure and Applied Bioscience*, 3, pp.160-167.

Dau, N., 2016. *Characterisation, pathogenicity and cultivar screening of Fusarium wilt of sweet potato in South Africa.* Master of Technology Dissertation. Tshwane University of Technology. South Africa.

Davis, R.M., Colyer, P.D., Rothrock, C.S., Kochman, J.K., 2006. *Fusarium* wilt of cotton: Population diversity and implications for management. *Plant Disease*, 90 (6), pp.692–703.

Department of Agriculture, Fisheries and Forestry, 2019. Abtract of Agricultural Statistics.Available:https://www.daff.gov.za/Daffweb3/Portals/0/Statistics%20and%20Economic%20Analysis/Statistical%20Information/Abstract%202019.pdf.Date Accesed: October 2019.

Di Primo, P., Cartia, G. and Katan, T., 2001. Vegetative compatibility and heterokaryon stability in *Fusarium oxysporum f. sp. radicis-lycopersici* from Italy. *Plant Pathology*, 50 (3), pp.371-382.

Favaretto, R.F., Muniz, M.F.B., Poletto, T., Krahn, J.R.T., Fantinel, V.S., Rabuske, J.E. and Sarzi, J.S., 2018. Morphological, physiological and molecular characterisation of *Fusarium lacertarum* causal agent of damping-off in Casuarina equisetifolia. *Brazilian Journal of Agricultural Sciences*, 13 (2), pp.1-6.

Fisher, N.L., Burgess, L.W., Toussoun, T.A. and Nelson, P.E., 1982. Carnation leaves as a substrate and for preserving cultures of *Fusarium* species. *Phytopathology*, 72 (1), pp.151-153.

Fourie, G., Steenkamp, E.T., Gordon, T.R. and Viljoen, A., 2009. Evolutionary relationships among the vegetative compatibility groups of *Fusarium oxysporum f. sp. cubense*. *Applied and Environmental Microbiology*, pp.4770-4781.

Fourie, G., Steenkamp, E.T., Ploetz, R.C., Gordon, T.R. and Viljoen, A. 2011. Current status of the taxonomic position of *Fusarium oxysporum formae specialis cubense*

within the *Fusarium oxysporum* complex. *Infection, Genetics and Evolution*, 11 (3), pp.533-542.

Fravel, D., Olivain, C. and Alabouvette, C., 2003. *Fusarium oxysporum* and its biocontrol. *New Phytologist*, 157 (3), pp.493-502.

Fries, E.M., 1821. EM 1821-1832. *Systema ycologicum*, 3.

Gams, W., Klamer, M. and O'Donnell, K., 1999. *Fusarium miscanthi* sp. nov. from Miscanthus litter. *Mycologia*, 91 (2), pp.263-268.

Gardner, D.E., 1980. *Acacia koa* seedling wilt caused by *Fusarium oxysporum f. sp. koae*, f. sp. nov. *Phytopathology*, 70 (7), pp.594-597.

Geiser, D.M., del Mar Jiménez-Gasco, M., Kang, S., Makalowska, I., Veeraraghavan, N., Ward, T.J., Zhang, N., Kuldau, G.A. and O'donnell, K., 2004. *FUSARIUM*-ID v. 1.0: a DNA sequence database for identifying *Fusarium*. *European Journal of Plant Pathology*, 110 (5-6), pp.473-479.

Geiser, P., Lacazette, A. and Vermilye, J., 2012. Beyond ‘dots in a box’: An empirical view of reservoir permeability with tomographic fracture imaging. *First Break*, 30 (7), pp.63-69.

Geiser, D.M., Aoki, T., Bacon, C.W., Baker, S.E., Bhattacharyya, M.K., Brandt, M.E., Brown, D.W., Burgess, L.W., Chulze, S., Coleman, J.J. and Correll, J.C., 2013. One fungus, one name: defining the genus *Fusarium* in a scientifically robust way that preserves longstanding use. *Phytopathology*, 103 (5), pp.400-408.

Gerlach, W. and Nirenberg, H., 1982. *The genus Fusarium-a pictorial atlas. Mitteilungen aus der Biologischen Bundesanstalt fur Land-und Forstwirtschaft Berlin-Dahlem*, Berlin-Dahlem, German (209), pp.406.

Gichuki, S.T., Berenyi, M., Zhang, D., Hermann, M., Schmidt, J., Glössl, J. and Burg, K., 2003. Genetic diversity in sweetpotato [*Ipomoea batatas* (L.) Lam.] in relationship to geographic sources as assessed with RAPD markers. *Genetic Resources and Crop Evolution*, 50 (4), pp.429-437.

Gordon, T.R. and Martyn, R.D., 1997. The evolutionary biology of *Fusarium oxysporum*. *Annual Review of Phytopathology*, 35 (1), pp.111-128.

Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W. and Gascuel, O., 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology*, 59 (3), pp.307-321.

Gracia-Garza, J.A., Fravel, D.R., Nelson, A.J., Elias, K.S., Bailey, B.A., Gardini, E.A. and Darlington, L.C., 1999. Potential for dispersal of *Fusarium oxysporum f. sp. erythroxyli* by infested seed. *Plant Disease*, 83 (5), pp.451-455.

Grafenhan, T., Schroers, H.J., Nirenberg, H.I. and Seifert, K.A., 2011. An overview of the taxonomy, phylogeny, and typification of nectriaceous fungi in *Cosmospora*, *Acremonium*, *Fusarium*, *Stilbella*, and *Volutella*. *Studies in Mycology*, 68, pp.79-113.

Hafizi, R., Salleh, B. and Latiffah, Z., 2013. Morphological and molecular characterisation of *Fusarium solani* and *F. oxysporum* associated with crown disease of oil palm. *Brazilian Journal of Microbiology*, 44 (3), pp.959-968.

Halsted, B.D., 1890. Some fungus diseases of the sweet potato. *Some fungus diseases of the sweet potato*, (76), pp.25-27.

Harris, P. M., 1992. *Mineral nutrition.* (Edition): Harris, P. M., *The Potato Crop*: The Scientific Basis for Improvement, second edition. Chapman & Hall, *London, Springer, Dordrecht,* pp.163-213.

Harter, L.L. and Field. E.C., 1913. *Fusarium batatatis Woll. Ms. Not Nectria impoeae Hals. the cause of sweetpotato stem rot. Phytopathology*.

Harter, L.L. and Field, E.C., 1914. The stem-rot of the sweet potato (*Ipomoea batatas*). *Phytopathology*, 4 (4), pp.279-304.

Harter, L.L. and Weimer, J.L., 1929. *A monographic study of sweet-potato diseases and their control*, Technical Bulletin, (99), pp.1-118.

Hebert, P.D., Cywinska, A., Ball, S.L. and De Waard, J.R., 2003. Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London. Biological Sciences Series*, 270 (1512), pp.313-321.

Hedge, V., Misra, R.S. and Jeeva, M.L., 2012. *Sweet potato diseases: Diagnosis and management. Fruit Vegetable Cereal Science and Biotechnology Global Science Books*, 6, pp.65-78.

Henriquez, M.A., McLaren, D.L., Conner, R.L., Balasubramanian, P.M., Chang, K.F., Hwang, S.F. and Strelkov, S.E., 2014. First report of *Fusarium cuneirostrum* causing root rot disease in dry bean (*Phaseolus vulgaris*) in Canada. *Plant Disease*, 98 (2), pp.278-278.

Ilgen, P., Maier, F. and Schäfer, W., 2008. Trichothecenes and lipases are host-induced and secreted virulence factors of *Fusarium graminearum*. *Cereal Research Communications*, 36 (6), pp.421-428.

Imamura, Y., Chandra, J., Mukherjee, P.K., Lattif, A.A., Szczotka-Flynn, L.B., Pearlman, E., Lass, J.H., O'Donnell, K. and Ghannoum, M.A., 2008. *Fusarium* and *Candida albicans* biofilms on soft contact lenses: model development, influence of lens type, and susceptibility to lens care solutions. *Antimicrobial Agents and Chemotherapy*, 52 (1), pp.171-182.

Imle, E.P., 1942. *Bulb rot diseases of lilies.* American Horticultural Society.

Jackson, D.M., Bohac, J.R., Thies, J.A. and Harrison, H.F., 2010. 'Charleston Scarlet' Sweetpotato. *American Society for Horticultural Science*, 45 (2), pp.306-309.

Jackson, D.M., Harrison, H.F., Thies, J.A., Bohac, J.R. and Mueller, J.D., 2011. 'Liberty'dry-fleshed sweetpotato. *American Society for Horticultural Science*, 46 (1), pp.125-129.

Jacobs, A., Van Wyk, P.S., Marasas, W.F., Wingfield, B.D., Wingfield, M.J. and Coutinho, T.A., 2010. *Fusarium ananatum* sp. nov. in the *Gibberella fujikuroi* species complex from pineapples with fruit rot in South Africa. *Fungal Biology*, 114 (7), pp.515-527.

Jacobs, A., Govender, R. and van Heerden, S.W., 2013. *Fusarium oxysporum f. sp. lycopersici* race 3 causing tomato wilt in South Africa. *Australasian Plant Disease Notes*, 8 (1), pp.145-147.

Jacobs, A., Mojela, L., Summerell, B. and Venter, E., 2018. Characterisation of members of the *Fusarium incarnatum*-equiseti species complex from undisturbed soils in South Africa. *Antonie van Leeuwenhoek*, 111 (11), pp.1999-2008.

Jaganathan, D., Immanuel, S., More, S.J. and Sivakumar, P.S., 2019. Assessment of Livelihood Capitals of Sweet Potato and Paddy Growers in Karnataka. *Indian Research Journal of Extension Education*, 19 (4), pp.43-48.

James, T.Y., Kauff, F., Schoch, C.L., Matheny, P.B., Hofstetter, V., Cox, C.J., Celio, G., Gueidan, C., Fraker, E., Miadlikowska, J. and Lumbsch, H.T., 2006. Reconstructing the early evolution of Fungi using a six-gene phylogeny. *Nature*, 443 (7113), pp.818.

Jarvis W.R. and Shoemaker R.A., 1978. Taxonomic status of *Fusarium oxysporum* causing foot and root rot of tomato. *Phytopathology*, 68, pp.1679–1680.

Jimenez-Gasco, M.M., Milgroom, M.G. and Jimenez-Diaz, R.M., 2002. Gene genealogies support *Fusarium oxysporum f. sp. ciceris* as a monophyletic group. *Plant Pathology*, 51 (1), pp.72-77.

Kagimbo, F., Shimelis, H. and Sibiya, J., 2018. Sweet Potato Weevil Damage, Production Constraints, and Variety Preferences in Western Tanzania: Farmers' Perception. *Journal of Crop Improvement*, 32 (1), pp.107-123.

Katoh, K., Misawa, K., Kuma, K.I. and Miyata, T., 2002. MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research*, 30 (14), pp.3059-3066.

Kawabe, M., Kobayashi, Y., Okada, G., Yamaguchi, I., Teraoka, T. and Arie, T., 2005. Three evolutionary lineages of tomato wilt pathogen, *Fusarium oxysporum f. sp. lycopersici*, based on sequences of IGS, MAT1, and pg1, are each composed of isolates of a single mating type and a single or closely related vegetative compatibility group. *Journal of General Plant Pathology*, 71 (4), pp.263-272.

Kelly, L.J., Hollingsworth, P.M., Coppins, B.J., Ellis, C.J., Harrold, P., Tosh, J. and Yahr, R., 2011. DNA barcoding of lichenized fungi demonstrates high identification success in a floristic context. *New Phytologist*, 191 (1), pp.288-300.

Khor, W.B., Aung, T., Saw, S.M., Wong, T.Y., Tambyah, P.A., Tan, A.L., Beuerman, R., Lim, L., Chan, W.K., Heng, W.J. and Lim, J., 2006. An outbreak of *Fusarium keratitis* associated with contact lens wear in Singapore. *Jama*, 295 (24), pp.2867-2873.

Kim, Y., Hutmacher, R.B. and Davis, R.M., 2005. Characterisation of California isolates of *Fusarium oxysporum f. sp. vasinfectum*. *Plant Disease*, 89 (4), pp.366-372.

Klaasen, J.A. and Nelson, P.E., 1996. Identification of a mating population, *Gibberella nygamai* sp. nov. within the *Fusarium nygamai* anamorph. *Mycologia*, 88 (6), pp.965-969.

Kornerup, A. and Wanscher, J.H., 1978. Methuen handbook of colour. 3rd Edition London: Eyre Methuen.

Koyyappurath, S., Atuahiva, T., Le Guen, R., Batina, H., Le Squin, S., Gautheron, N., Edel Hermann, V., Peribe, J., Jahiel, M., Steinberg, C. and Liew, E.C.Y., 2016. *Fusarium oxysporum f. sp. radicis-vanillae* is the causal agent of root and stem rot of vanilla. *Plant Pathology*, 65 (4), pp.612-625.

Kreuze, J., 2002. *Molecular studies on the sweet potato virus disease and it two causal agents.* Doctoral Thesis, Swedish University of Agricultural Sciences. Agrari.

Laurence, M.H., Summerell, B.A., Burgess, L.W. and Liew, E.C.Y., 2011. *Fusarium burgessii* sp. nov. representing a novel lineage in the genus *Fusarium*. *Fungal Diversity*, 49 (1), pp.101-112.

Laurence, M.H., Burgess, L.W., Summerell, B.A. and Liew, E.C., 2012. High levels of diversity in *Fusarium oxysporum* from non-cultivated ecosystems in Australia. *Fungal Biology*, 116 (2), pp.289-297.

Laurence, M.H., Summerell, B.A., Burgess, L.W. and Liew, E.C., 2014. Genealogical concordance phylogenetic species recognition in the *Fusarium oxysporum* species complex. *Fungal Biology*, 118 (4), pp.374-384.

Laurence, M.H., Walsh, J.L., Shuttleworth, L.A., Robinson, D.M., Johansen, R.M., Petrovic, T., Vu, T.T.H., Burgess, L.W., B. A. Summerell, B.A. and Liew, E.C.Y., 2015. Six novel species of *Fusarium* from natural ecosystems in Australia. *Fungal Diversity*, 77 (1), pp.349-366.

Laurence, M.H., Walsh, J.L., Shuttleworth, L.A., Robinson, D.M., Johansen, R.M., Petrovic, T., Vu, T.T.H., Burgess, L.W., Summerell, B.A. and Liew, E.C.Y., 2016. Six novel species of *Fusarium* from natural ecosystems in Australia. *Fungal Diversity*, 77 (1), pp.349-366.

Laurie, S.M., 2010. *Agronomic performance, consumer acceptability and nutrient content of new sweet potato varieties in South Africa*. Doctoral Dissertation, University of the Free State, South Africa.

Laurie, S.M., Van Jaarsveld, P.J., Faber, M., Philpott, M.F. and Labuschagne, M.T., 2012. Trans-β-carotene, selected mineral content and potential nutritional contribution of 12 sweetpotato varieties. *Journal of Food Composition and Analysis*, 27 (2), pp.151-159.

Laurie, S.M., Booyse, M., Labuschagne, M.T. and Greyling, M.M., 2015a. Multienvironment performance of new orange-fleshed sweetpotato cultivars in South Africa. *Crop Science*, 55 (4), pp.1585-1595.

Laurie, S.M., Faber, M., Adebola, P. and Belete, A., 2015b. Biofortification of sweet potato for food and nutrition security in South Africa. *Food Research International*, 76, pp.962-970.

Laurie, S.M., Calitz, F., Mtileni, M., Mphela, W. and Tjale, S., 2017. Performance of informal market sweet potato cultivars in on-farm trials in South Africa. *Open Agriculture*, 2 (1), pp.431-441.

Laurie, S.M., Faber, M. and Claasen, N., 2018. Incorporating orange-fleshed sweet potato into the food system as a strategy for improved nutrition: The context of South Africa. *Food Research International*, 104, pp.77-85.

Leach, J.G. and Currence, T.M., 1938. *Fusarium wilt of muskmelon in Minnesota.* University of Minnesota Agricultural Experimental Station, Minnesotta, Technical Bulletin, 129, pp.1-32.

Lee, H.U., Chung, M.N., Lee, J.S., Ahn, S.H., Kim, J.M., Han, S.K., Yang, J.W., Song, Y.S., Shin, H.M., Moon, J.Y. and Choi, K.H., 2018. A Sweetpotato Variety Resistant to *Fusarium* Wilt, 'Yeseumi'. *Korean Society of Breeding Science*, 50 (2), pp.131-138.

Leslie, J.F., Zeller K.A. and Summerell, B.A., 2001. Icebergs and species in populations of *Fusarium*. *Physiol. Molecular Plant Pathology*, 59, pp.107–17

Leslie, J.F. and Summerell, B.A., 2006. *Fusarium* laboratory manual. Blackwell Publishing, Ames, Iowa.

Letourneau, A., Seena, S., Marvanová, L. and Bärlocher, F., 2010. Potential use of barcoding to identify aquatic hyphomycetes. *Fungal Diversity*, 40 (1), pp.51-64.

Lievens, B. and Thomma, B.P., 2005. Recent developments in pathogen detection arrays: implications for fungal plant pathogens and use in practice. *Phytopathology*, 95 (12), pp.1374-1380.

Lievens, B., Rep, M. and Thomma, B.P., 2008. Recent developments in the molecular discrimination of *formae speciales* of *Fusarium oxysporum*. *Pest Management Science:* formerly *Pesticide Science*, 64 (8), pp.781-788.

Lima, C.S., Pfenning, L.H., Costa, S.S., Campos, M.A. and Leslie, J.F., 2009. A new *Fusarium* lineage within the *Gibberella fujikuroi* species complex is the main causal agent of mango malformation disease in Brazil. *Plant Pathology*, 58 (1), pp.33-42.

Link, H.F., 1809. *Observationes in ordines plantarum naturales.* Gesellschaft Naturforschenden Freunde Berlin, Germany, 3, pp.1-42.

Liu, Y.J., Whelen, S. and Hall, B.D., 1999. Phylogenetic relationships among ascomycetes: evidence from an RNA polymerse II subunit. *Molecular Biology and Evolution*, 16 (12), pp.1799-1808.

Lombard, L., Van der Merwe, N.A., Groenewald, J.Z. and Crous, P.W., 2015. Generic concepts in Nectriaceae. *Studies in Mycology*, 80, pp.189-245.

Lombard, L., Sandoval-Denis, M., Lamprecht, S.C. and Crous, P.W., 2019. Epitypification of *Fusarium oxysporum*–clearing the taxonomic chaos. *Persoonia: Molecular Phylogeny and Evolution of Fungi*, *43*, pp.1.

Lutzoni, F., Kauff, F., Cox, C.J., McLaughlin, D., Celio, G., Dentinger, B., Padamsee, M., Hibbett, D., James, T.Y., Baloch, E. and Grube, M., 2004. Assembling the fungal tree of life: progress, classification, and evolution of subcellular traits. *American Journal of Botany*, 91 (10), pp.1446-1480.

Ma, L.J., Geiser, D.M., Proctor, R.H., Rooney, A.P., O'Donnell, K., Trail, F., Gardiner, D.M., Manners, J.M. and Kazan, K., 2013. *Fusarium* pathogenomics. *Annual Review of Microbiology*, 67, pp.399-416.

Maitlo, S., Syed, R., Rustamani, M., Khuhro, R. and Lodhi, A., 2014. Comparative efficacy of different fungicides against *Fusarium* wilt of chickpea (*Cicer arietinum* L.). *Pakistan Journal of Botany*, 46 (6), pp.2305-2312.

Mandeel, Q. and Baker, R., 1991. Mechanisms involved in biological control of *Fusarium* wilt of cucumber with strains of nonpathogenic *Fusarium oxysporum*. *Phytopathology*, 81 (4), pp.462-469.

Marasas, W.F.O., Rabie, C.J., Lubben, A., Nelson, P.E., Toussoun, T.A. and Van Wyk, P.S., 1988. *Fusarium nygamai* from millet in southern Africa. *Mycologia*, 80 (2), pp.263-266.

Martin, K.J. and Rygiewicz, P.T., 2005. Fungal-specific PCR primers developed for analysis of the ITS region of environmental DNA extracts. *BioMedCentral Microbiology*, 5 (1), pp.28.

Martyn, R.D. 1996. *Fusarium wilt of cucumber.* (Edition): Zitter, T.A., Hopkins, D.L. & C. E. Thomas, C.E., *Compendium of cucurbit diseases.* St. Paul*: The American Phytopathological Society Press*. pp.15-16

Martyn, R.D., 1998. *Fusarium* wilt of melon. *Compendium of cucurbit diseases*, pp.14-15.

Maurya, S., Dubey, S., Kumari, R. and Verma, R., 2019. Management tactics for fusarium wilt of tomato caused by *Fusarium oxysporum f. sp. lycopersici* (Sacc.): A Review. *Management*, 4 (5).

McNeill, J., Barrie, F.R., Burdet, H.M., Demoulin, V., Hawksworth, D.L., Marhold, K., Nicolson, D.H., Prado, J., Silva, P.C., Skog, J.E., Wiersema, J.H. and Turland, N.J., 2006. International Code of Botanical Nomenclature (Vienna Code), [Regnum vegetabile no. 146.] Ruggell: A.R.G. Gantner Verlag Scientific Books.

McNeill, J., Barrie, F.R., Buck, W.R., Demoulin, V., Greuter, W., Hawksworth, D.L., Herendeen, P.S., Knapp, S., Marhold, K., Prado, J. and Prud'homme Van Reine, W.F., 2012. International Code of Nomenclature for algae, fungi and plants. *Regnum Vegetabile*, 154.

Menzies, J.G., Koch, C. and Seywerd, F., 1990. Additions to the host range of *Fusarium oxysporum f. sp. radicis-lycopersici*. *Plant Disease*, 74 (8), pp.569-572.

Mes, J.J., Joosten, M.H., de Wit, P.J., Honée, G. and Cornelissen, B.J., 1999. Expression of the *Cladosporium fulvum* avirulence genes Avr4 and Avr9 in *Fusarium oxysporum* does not affect virulence on tomato with the matching resistance genes. *Molecular aspects of the interaction between tomato and Fusarium oxysporum f. sp. lycopersici*, pp.95.

Min, X.J. and Hickey, D.A., 2007. Assessing the effect of varying sequence length on DNA barcoding of fungi. *Molecular Ecology Notes*, 7 (3), pp.365-373.

Minemba, D., Gleeson, D.B., Veneklaas, E. and Ryan, M.H., 2019. Variation in morphological and physiological root traits and organic acid exudation of three sweet potato (*Ipomoea batatas*) cultivars under seven phosphorus levels. *Scientia Horticulturae*, 256, pp.108572.

Minuto, A., Migheli, Q. and Garibaldi, A., 1995. Evaluation of antagonistic strains of *Fusarium* spp. in the biological and integrated control of *Fusarium* wilt of cyclamen. *Crop Protection*, 14 (3), pp.221-226.

Mojela, M.L., 2017. *The diversity of the genus Fusarium in soil from the Willem Pretorius Nature Reserve, South Africa*. Doctoral Dissertation, University of Johannesburg. South Africa.

Moretti, A.N., 2009. Taxonomy of *Fusarium* genus: A continuous fight between lumpers and splitters. *Journal for Natural Sciences*, 117, pp.7-13.

Mulabisana, M.J., Cloete, M., Laurie, S.M., Mphela, W., Maserumule, M.M., Nhlapo, T.F., Cochrane, N.M., Oelofse, D. and Rey, M.E.C., 2019. Yield evaluation of multiple and co-infections of begomoviruses and potyviruses on sweet potato varieties under field conditions and confirmation of multiple infection by NGS. *Crop Protection*, 119, pp.102-112.

Narayanin, C.D., 2008. *Fusarium wilt (Fusarium oxysporum f. sp. batatas) and Alternaria blight (Alternaria bataticola) on sweet potato (Ipomoea batatas) in South Africa.* Master of Technology Dissertation. Tshwane University of Technology. South Africa.

Nelson, P.E., Toussoun, T.A. and Marasas, W.F.O., 1983. *Fusarium Species: An Illustrated Manual for Identification.* Pennsylvania State University, University Park.

Nhlapo, T.F., Rees, D.J.G., Odeny, D.A., Mulabisana, J.M. and Rey, M.E.C., 2018. Viral metagenomics reveals sweet potato virus diversity in the Eastern and Western Cape provinces of South Africa. *South African Journal of Botany*, 117, pp.256-267.

Nimmakayala, P., Vajja, G. and Reddy, U.K., 2011. *Ipomoea*. Wild crop relatives: Genomic and Breeding Resources, Industrial crops, Berlin. Springer, pp.123-132.

Nirenberg, H.I., 1976. Untersuchungen über die morphologische und biologische. Differenzierung in der *Fusarium*-Sektion *Liseola*. *Mitteilungen aus der Biologischen Bundesanstalt fur Landund Forstwirtschaft, Berlin-Dahlem.* 169, pp.1-117.

Nirenberg, H.I. and Aoki, T., 1997. *Fusaium nisikadoi*, a new species from Japan. *Mycoscience*, 38 (3), pp.329-333.

Nirenberg, H.I. and O'Donnell, K., 1998. New *Fusarium* species and combinations within the *Fusarium fujikuroi* species complex. *Mycologia,* 90 (3), pp.434–458.

Norvell, L.L., 2011. Fungal nomenclature. 1. Melbourne approves a new Code. *Mycotaxon*, 116 (1), pp.481-490.

O'Donnell, K., 2000. Molecular phylogeny of the *Nectria haematococca-Fusarium solani* species complex. *Mycologia*, 92 (5), pp.919-938.

O'Donnell, K. and Cigelnik, E., 1997. Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. *Molecular Phylogenetics and Evolution*, 7 (1), pp.103-116.

O'Donnell, K., Cigelnik, E. and Nirenberg, H.I., 1998a. Molecular systematics and phylogeography of the *Gibberella fujikuroi* species complex. *Mycologia*, 90 (3), pp.465-493.

O'Donnell, K., Kistler, H.C., Cigelnik, E. and Ploetz, R.C., 1998b. Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. *Proceedings of the National Academy of Sciences*, 95 (5), pp.2044-2049.

O'Donnell, K., Nirenberg, H.I., Aoki, T. and Cigelnik, E., 2000. A multigene phylogeny of the *Gibberella fujikuroi* species complex: Detection of additional phylogenetically distinct species. *Mycoscience*, 41 (1), pp.61-78.

O'Donnell, K., Sutton, D.A., Rinaldi, M.G., Magnon, K.C., Cox, P.A., Revankar, S.G., Sanche, S., Geiser, D.M., Juba, J.H., Van Burik, J.A.H. and Padhye, A., 2004. Genetic diversity of human pathogenic members of the *Fusarium oxysporum* complex inferred from multilocus DNA sequence data and amplified fragment length polymorphism analyses: evidence for the recent dispersion of a geographically widespread clonal lineage and nosocomial origin. *Journal of Clinical Microbiology*, 42 (11), pp.5109-5120.

O'Donnell, K., Sarver, B.A., Brandt, M., Chang, D.C., Noble-Wang, J., Park, B.J., Sutton, D.A., Benjamin, L., Lindsley, M., Padhye, A. and Geiser, D.M., 2007. Phylogenetic diversity and microsphere array-based genotyping of human pathogenic fusaria, including isolates from the multistate contact lens-associated US keratitis outbreaks of 2005 and 2006. *Journal of Clinical Microbiology*, 45 (7), pp.2235-2248.

O'Donnell, K., Gueidan, C., Sink, S., Johnston, P.R., Crous, P.W., Glenn, A., Riley, R., Zitomer, N.C., Colyer, P., Waalwijk, C. and Van Der Lee, T., 2009a. A two-locus DNA sequence database for typing plant and human pathogens within the *Fusarium oxysporum* species complex. *Fungal Genetics and Biology*, 46 (12), pp.936-948.

O'Donnell, K., Sutton, D.A., Rinaldi, M.G., Gueidan, C., Crous, P.W. and Geiser, D.M., 2009b. Novel multilocus sequence typing scheme reveals high genetic diversity of human pathogenic members of the *Fusarium incarnatum-equiseti* and *F. chlamydosporum* species complexes within the United States. *Journal of Clinical Microbiology*, 47 (12), pp.3851-3861.

O'Donnell, K., Sutton, D.A., Rinaldi, M.G., Sarver, B.A., Balajee, S.A., Schroers, H.J., Summerbell, R.C., Robert, V.A., Crous, P.W., Zhang, N. and Aoki, T., 2010. Internet-

accessible DNA sequence database for identifying fusaria from human and animal infections. *Journal of Clinical Microbiology*, 48 (10), pp.3708-3718.

O'Donnell, K., Humber, R.A., Geiser, D.M., Kang, S., Park, B., Robert, V.A., Crous, P.W., Johnston, P.R., Aoki, T., Rooney, A.P. and Rehner, S.A., 2012. Phylogenetic diversity of insecticolous fusaria inferred from multilocus DNA sequence data and their molecular identification via *FUSARIUM*-ID and *Fusarium* MLST. *Mycologia*, 104 (2), pp.427-445.

O'Donnell, K., Rooney, A.P., Proctor, R.H., Brown, D.W., McCormick, S.P., Ward, T.J., Frandsen, R.J., Lysøe, E., Rehner, S.A., Aoki, T. and Robert, V.A., 2013. Phylogenetic analyses of RPB1 and RPB2 support a middle Cretaceous origin for a clade comprising all agriculturally and medically important fusaria. *Fungal Genetics and Biology*, *52*, pp.20-31.

O'Donnell, K., Ward, T.J., Robert, V.A., Crous, P.W., Geiser, D.M. and Kang, S., 2015. DNA sequence-based identification of *Fusarium*: Current status and future directions. *Phytoparasitica*, 43 (5), pp.583-595.

Ogawa, K., Watanabe, K. and Komada, H., 1996, Formulation of nonpathogenic *Fusarium oxysporum*, a biocontrol agent for commercial use. In *Proceedings of the 1st International Workshop on Fusarium Biocontrol,* pp. 28-31.

Ohara, T. and Tsuge, T., 2004. FoSTUA, encoding a basic helix-loop-helix protein, differentially regulates development of three kinds of asexual spores, macroconidia, microconidia, and chlamydospores, in the fungal plant pathogen *Fusarium oxysporum*. *Eukaryotic Cell*, 3 (6), pp.1412-1422.

Onyike, N.B.N., Nelson, P.E. and Marasas, W.F.O., 1991. *Fusarium* species associated with millet grain from Nigeria, Lesotho, and Zimbabwe. *Mycologia*, 83 (6), pp.708-712.

Onyike, N.B.N. and Nelson, P.E., 1992. *Fusarium* species associated with sorthum grain from Nigeria, Lesotho, and Zimbabwe. *Mycologia*, 84, pp.452-459.

Orjeda, G., Freyre, R. and Iwanaga, M., 1990. Production of 2n pollen in diploid *Ipomoea trifida*, a putative wild ancestor of sweet potato. *Journal of Heredity*, 81 (6), pp.462-467.

Owen, J.H., 1956. Cucumber wilt, caused by *Fusarium oxysporum f. cucumerinum*. *Phytopathology*, 46, pp.153-157.

Padwick, G.W., 1945. Notes on Indian fungi III. *Mycological Papers.* 12, pp.1-15.

Page, R.D., 2001. TreeView. Glasgow University, Glasgow, United Kingdom.

Pinaria, A.G., 2009*. Fusarium-vanilla.* Doctoral Dissertation University of Sydney, Sydney.

Pinaria, A.G., Laurence, M.H., Burgess, L.W. and Liew, E.C.Y., 2015. Phylogeny and origin of *Fusarium oxysporum f. sp. vanillae* in Indonesia. *Plant Pathology*, 64 (6), pp.1358-1365.

Prillieux, É. and Delacroix, G., 1899. *La maladie des Œeillets à Antibes*. Gauthier-Villars et Fils.

Purseglove, J.W., 1968. *Convolvulaceae*. In: Tropical crops; *Dicotyledons*. Longman, London, pp.78-88.

Ramachandra Rao, S. and Ravishankar, G.A., 2000. Vanilla flavour: production by conventional and biotechnological routes. *Journal of the Science of Food and Agriculture*, 80 (3), pp.289-304.

Riaz, T., Shehzad, W., Viari, A., Pompanon, F., Taberlet, P. and Coissac, E., 2011. ecoPrimers: inference of new DNA barcode markers from whole genome sequence analysis. *Nucleic Acids Research*, 39 (21), pp.145-145.

Robert, P.L., Donald, L.H. and Frank, N.M., 1996. Suppression of *Fusarium* wilt of watermelon by nonpathogenic *Fusarium oxysporum* and other microorganism recovered from a disease suppressive soil. *Phytopathology*, 86, pp.812-819.

Rossman, A.Y., Seifert, K.A., Samuels, G.J., Minnis, A.M., Schroers, H.J., Lombard, L., Crous, P.W., Põldmaa, K., Cannon, P.F., Summerbell, R.C. and Geiser, D.M., 2013. Genera in Bionectriaceae, Hypocreaceae, and Nectriaceae (Hypocreales) proposed for acceptance or rejection. *International Mycological Association Fungus*, 4 (1), pp.41-51.

Rowe, R.C., 1980. Comparative pathogenicity and host ranges of *Fusarium oxysporum* isolates causing crown and root rot of greenhouse and field-grown tomatoes in North America and Japan. *Phytopathology*, 70 (12), pp.1143-1148.

Saikia, R. and Kadoo, N., 2010. Molecular detection and identification of *Fusarium oxysporum*. In *Molecular Identification of Fungi* Springer, Berlin, Heidelberg. pp.131-157.

Sandoval-Denis, M., Swart, W.J. and Crous, P.W., 2018. New *Fusarium* species from the Kruger National Park, South Africa. *MycoKeys*, (34), pp.63.

Schardl, C.L., Leuchtmann, A., Tsai, H.F., Collett, M.A., Watt, D.M. and Scott, D.B., 1994. Origin of a fungal symbiont of perennial ryegrass by interspecific hybridization of a mutualist with the ryegrass choke pathogen, *Epichloe typhina*. *Genetics*, 136 (4), pp.1307-1317.

Schneider, R. and Dalchow, J., 1975. *Fusarium inflexum spec*, nov., als Erreger einer Welkekrankheit an Vicia faba L. in Deutschland. *Journal of Phytopathology*, 82 (1), pp.70-82.

Schoch, C.L., Seifert, K.A., Huhndorf, S., Robert, V., Spouge, J.L., Levesque, C.A., Chen, W., Bolchacova, E., Voigt, K., Crous, P.W. and Miller, A.N., 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences*, 109 (16), pp.6241-6246.

Short, D.P., O'Donnell, K., Thrane, U., Nielsen, K.F., Zhang, N., Juba, J.H. and Geiser, D.M., 2013. Phylogenetic relationships among members of the *Fusarium solani* species complex in human infections and the descriptions of *F. keratoplasticum sp.* nov. and *F. petroliphilum stat.* nov. *Fungal Genetics and Biology*, 53, pp.59-70.

Silva, J.C.D. and Bettiol, W., 2005. Potential of non-pathogenic *Fusarium oxysporum* isolates for control of *Fusarium* wilt of tomato. *Fitopatologia Brasileira*, 30 (4), pp.409-412.

Skovgaard, K., Nirenberg, H.I., O'Donnell, K. and Rosendahl, S., 2001. Evolution of *Fusarium oxysporum f. sp. vasinfectum* races inferred from multigene genealogies. *Phytopathology*, 91 (12), pp.1231-1237.

Skovgaard, K., Bodker, L. and Rosendahl, S., 2002. Population structure and pathogenicity of members of the *Fusarium oxysporum* complex isolated from soil and root necrosis of pea (*Pisum sativum* L.). *FEMS Microbiology Ecology*, 42 (3), pp.367-374.

Smith, E.F., 1899. Wilt disease of cotton, watermelon and cowpea (Neocosmospora nov. gen.). *USA Department of Agriculture Division of Vegetable Physiology Pathology Bulletin*, 17, pp.1-54.

Snedecor, G.W. and Cochran. W.G., 1980. Statistical Methods, seventh edition. University Press, Iowa.

Snyder, W.C. and Hansen, H.N., 1940. The species concept in *Fusarium*. *American Journal of Botany*, 27 (2), pp.64-67.

Srinivasan, K., Gilardi, G., Spadaro, D., Gullino, M.L. and Garibaldi, A., 2010. Molecular characterisation through IGS sequencing of *formae speciales* of *Fusarium oxysporum* pathogenic on lamb's lettuce. *Phytopathologia Mediterranea*, 49 (3), pp.309-320.

Ssali, R.T., Sseruwu, G., Yada, B., Ssemakula, G., Wasonga, C., Grüneberg, W.J., Eyzaguirre, R., Low, J.W. and Mwanga, R.O.M., 2019. Efficiency of the Polycross and Controlled Hybridization Methods in Sweetpotato Breeding in Uganda. *Journal of Agricultural Science*, 11, (17), pp.123-134.

Stewart, J.E., Kim, M.S., James, R.L., Dumroese, R.K. and Klopfenstein, N.B., 2006. Molecular characterisation of *Fusarium oxysporum* and *Fusarium commune* isolates from a conifer nursery. *Phytopathology*, 96 (10), pp.1124-1133.

Summerbell, R.C. and Schroers, H.J., 2002. Analysis of phylogenetic relationship of *Cylindrocarpon lichenicola* and *Acremonium falciforme* to the *Fusarium solani* species complex and a review of similarities in the spectrum of opportunistic infections caused by these fungi. *Journal of Clinical Microbiology*, 40 (8), pp.2866-2875.

Summerell, B.A., 2019. Resolving *Fusarium*: Current Status of the Genus. *Annual Review of Phytopathology*, 57, pp.323-339.

Summerell, B.A., Salleh, B. and Leslie, J.F., 2003. A utilitarian approach to *Fusarium* identification. *Plant Disease*, 87 (2), pp.117-128.

Summerell, B.A. and Leslie, J.F., 2011. Fifty years of *Fusarium*: How could nine species have ever been enough? *Fungal Diversity*, 50 (1), pp.135-144.

Swofford, D.L., 2002. Phylogenetic Analysis Using Parsimony (and other methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.

Taha, E.M., Rabie, W., Mousa, A.S., Yasser, M.M. and Fahmy, Z.M., 2016. Phylogenetic diversity among Egyptian isolates of *Fusarium* species from sugar beet. *International Journal of Agricultural Technology*, 12 (2), pp.365-385.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S., 2013. MEGA 6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, 30 (12), pp.2725-2729.

Taylor, J.W., Jacobson, D.J., Kroken, S., Kasuga, T., Geiser, D.M., Hibbett, D.S. and Fisher, M.C., 2000. Phylogenetic species recognition and species concepts in fungi. *Fungal Genetics and Biology*, 31 (1), pp.21-32.

Thompson, A.H. 2004. *Fungal diseases*. (Edition): J.G. Niederwieser. *Guide to sweet potato production in South Africa* Pretoria: ARC-Roodeplaat, pp. 81-84.

Thompson, A.H., Narayanin, C.D., Smith, M.F. and Slabbert, M.M., 2011. A disease survey of *Fusarium* wilt and *Alternaria* blight on sweet potato in South Africa. *Crop Protection*, 30 (11), pp.1409-1413.

Tirado-Ramirez, M.A., Lopez-Orona, C.A., Diaz-Valdes, T., Velarde-Felix, S., Martinez-Campos, A.R., Romero-Gomez, S.J. and Retes-Manjarrez, J.E., 2019. First Report of Basal Rot of Onion Caused by *Fusarium brachygibbosum* in Sinaloa, Mexico. *Plant Disease*, 103 (3), pp.582-582.

Tombe, M. and Liew, E.C., 2010. *Fungal diseases of Vanilla.* Vanilla, Medicinal and Aromatic Plants-Industrial Profiles. CRC Press. Taylor and Francis Group. Boca Raton, London, New York, pp.125-140.

Tsai, H.F., Liu, J.S., Staben, C., Christensen, M.J., Latch, G.C., Siegel, M.R. and Schardl, C.L., 1994. Evolutionary diversification of fungal endophytes of tall fescue

grass by hybridization with Epichloë species. *Proceedings of the National Academy of Sciences*, 91 (7), pp.2542-2546.

Tucker, C.M., 1927. Vanilla root rot. *Journal of Agricultural Research*, 35 (12), pp.1121-1136.

Turland, N.J., Wiersema, J.H., Barrie, F.R., Greuter, W., Hawksworth, D.L., Herendeen, P.S., Knapp, S., Kusber, W.H., Li, D.Z., Marhold, K. and May, T.W., 2018. *International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017*. Koeltz Botanical Books.

Veasey, E.A., Borges, A., Rosa, M.S., Queiroz-Silva, J.R., Bressan, E.D.A. and Peroni, N., 2008. Genetic diversity in Brazilian sweet potato (*Ipomoea batatas* (L.) Lam., Solanales, Convolvulaceae) landraces assessed with microsatellite markers. *Genetics and Molecular Biology*, 31 (3), pp.725-733.

Valentini, A., Miquel, C., Nawaz, M.A., Bellemain, E.V.A., Coissac, E., Pompanon, F., Gielly, L., Cruaud, C., Nascetti, G., Wincker, P. and Swenson, J.E., 2009. New perspectives in diet analysis based on DNA barcoding and parallel pyrosequencing: the trnL approach. *Molecular Ecology Resources*, 9 (1), pp.51-60.

Van Der Does, H.C., Lievens, B., Claes, L., Houterman, P.M., Cornelissen, B.J. and Rep, M., 2008. The presence of a virulence locus discriminates *Fusarium oxysporum* isolates causing tomato wilt from other isolates. *Environmental Microbiology*, 10 (6), pp.1475-1485.

Vimala, B.A., Sreekanth, H. Binu and G. Wolfgang. G., 2011. Variability in 42 orange-fleshed sweet potato hybrids for the tuber yield and carotene and dry mass content. *Geneconserve*, 40, pp.190-200.

Walsh, J.L., Laurence, M.H., Liew, E.C., Sangalang, A.E., Burgess, L.W., Summerell, B.A. and Petrovic, T., 2010. *Fusarium*: Two endophytic novel species from tropical grasses of northern Australia. *Fungal Diversity*, 44 (1), pp.149-159.

Wechter, W.P., Kousik, C., McMillan, M. and Levi, A., 2012. Identification of resistance to *Fusarium oxysporum f. sp. niveum* race 2 in *Citrullus lanatus* var. citroides plant introductions. *American Society for Horticultural Science*, 47 (3), pp.334-338.

White, T.J., Bruns, T., Lee, S.J.W.T. and Taylor, J.L., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: A guide to methods and applications*, 18 (1), pp.315-322.

Wollenweber, H.W. and Reinking, O.A., 1935. Die *Fusarien*, Ihre Beschreibung, Schadwirkung und Bekampfung. Berlin: Paul Parey.

Wollenweber, H.W., 1914. Identification of species of *Fusarium* occurring on sweetpotato, *Ipomoea batatas*. *Journal of Agricultural Research*, 2 (4), pp.251-285.

Wright, P.J., Falloon, R.E. and Hedderley, D., 2017. A long-term vegetable crop rotation study to determine effects on soil microbial communities and soilborne diseases of potato and onion. *New Zealand Journal of Crop and Horticultural Science*, 45 (1), pp.29-54.

Yadav, M.K., Singh, V.K. and Arora, D.K., 2017. Development of *Fusarium oxysporum* species complex (FOC) specific primers using Topoisomerase-II gene. *Journal of Microbiology and Biomedical Research*, 3 (1), pp.01-09.

Zeller, K.A., Summerell, B.A., Bullock, S. and Leslie, J.F., 2003. *Gibberella konza* (*Fusarium konzum*) sp. nov. from prairie grasses, a new species in the *Gibberella fujikuroi* species complex. *Mycologia*, 95 (5), pp.943-954.

Zhang, N., O'Donnell, K., Sutton, D.A., Nalim, F.A., Summerbell, R.C., Padhye, A.A. and Geiser, D.M., 2006. Members of the *Fusarium solani* species complex that cause infections in both humans and plants are common in the environment. *Journal of Clinical Microbiology*, 44 (6), pp.2186-2190.

CHAPTER 7

APPENDICES

Appendix A: *Fusarium* isolates obtained from diseased sweet potato plants and soil collected from Eastern Cape, Gauteng, Limpopo, Mpumalanga, Northern Cape and Western Cape provinces of South Africa.

PPRI no.	Province	Region	Substrate	Collection Year	GPS co-ordinates
9472	Eastern Cape	Kirkwood	sweet potato	2008	S 33°23'56.67"; E 25°26'35.38"
9473	Eastern Cape	Malan	sweet potato	2008	S 34°01'08.86"; E 24°55'08.63"
9458	Gauteng	Roodeplaat	sweet potato	2006	S 25°35'53.18"; E 28°21'31.19"
10532	Gauteng	Roodeplaat	sweet potato	2008	S 25°35'53.18"; E 28°21'31.19"
10533	Gauteng	Roodeplaat	sweet potato	2008	S 25°35'53.18"; E 28°21'31.19"
20163	Gauteng	Roodeplaat	sweet potato	2015	S 25°35'53.18"; E 28°21'31.19"
20164	Gauteng	Roodeplaat	sweet potato	2015	S 25°35'53.18"; E 28°21'31.19"
20165	Gauteng	Roodeplaat	sweet potato	2015	S 25°35'53.18"; E 28°21'31.19"
20166	Gauteng	Roodeplaat	sweet potato	2015	S 25°35'53.18"; E 28°21'31.19"
20167	Gauteng	Roodeplaat	sweet potato	2015	S 25°35'53.18"; E 28°21'31.19"
20168	Gauteng	Roodeplaat	sweet potato	2015	S 25°35'53.18"; E 28°21'31.19"
20169	Gauteng	Roodeplaat	sweet potato	2015	S 25°35'53.18"; E 28°21'31.19"
20170	Gauteng	Roodeplaat	sweet potato	2015	S 25°35'53.18"; E 28°21'31.19"
20171	Gauteng	Roodeplaat	sweet potato	2015	S 25°35'53.18"; E 28°21'31.19"
20172	Gauteng	Roodeplaat	sweet potato	2015	S 25°35'53.18"; E 28°21'31.19"
20173	Gauteng	Roodeplaat	sweet potato	2015	S 25°35'53.18"; E 28°21'31.19"
20174	Gauteng	Roodeplaat	sweet potato	2015	S 25°35'53.18"; E 28°21'31.19"
20175	Gauteng	Roodeplaat	sweet potato	2015	S 25°35'53.18"; E 28°21'31.19"
20176	Gauteng	Roodeplaat	sweet potato	2015	S 25°35'53.18"; E 28°21'31.19"
20177	Gauteng	Roodeplaat	sweet potato	2015	S 25°35'53.18"; E 28°21'31.19"
20178	Gauteng	Roodeplaat	sweet potato	2015	S 25°35'53.18"; E 28°21'31.19"
20179	Gauteng	Roodeplaat	sweet potato	2015	S 25°35'53.18"; E 28°21'31.19"
21929	Gauteng	Roodeplaat	soil	2016	S 25°35'53.18"; E 28°21'31.19"
21930	Gauteng	Roodeplaat	soil	2016	S 25°35'53.18"; E 28°21'31.19"
21931	Gauteng	Roodeplaat	soil	2016	S 25°35'53.18"; E 28°21'31.19"
21932	Gauteng	Roodeplaat	soil	2016	S 25°35'53.18"; E 28°21'31.19"
21933	Gauteng	Roodeplaat	soil	2016	S 25°35'53.18"; E 28°21'31.19"
21934	Gauteng	Roodeplaat	soil	2016	S 25°35'53.18"; E 28°21'31.19"
21935	Gauteng	Roodeplaat	soil	2016	S 25°35'53.18"; E 28°21'31.19"
21936	Gauteng	Roodeplaat	soil	2016	S 25°35'53.18"; E 28°21'31.19"
21937	Gauteng	Roodeplaat	soil	2016	S 25°35'53.18"; E 28°21'31.19"
21938	Gauteng	Roodeplaat	soil	2016	S 25°35'53.18"; E 28°21'31.19"
21939	Gauteng	Roodeplaat	soil	2016	S 25°35'53.18"; E 28°21'31.19"
21940	Gauteng	Roodeplaat	soil	2016	S 25°35'53.18"; E 28°21'31.19"
21941	Gauteng	Roodeplaat	soil	2016	S 25°35'53.18"; E 28°21'31.19"
21942	Gauteng	Roodeplaat	soil	2016	S 25°35'53.18"; E 28°21'31.19"

PPRI no.	Province	Region	Substrate	Collection Year	GPS co-ordinates
21943	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
21944	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
21945	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
21946	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
21947	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
21948	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
21949	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
21950	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
21951	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
21952	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
21953	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
21954	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
21955	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
24308	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
21956	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
21957	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
21958	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
21959	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
21960	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
21961	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
21962	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
21963	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
21964	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
21965	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
21966	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
21968	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
21969	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
21970	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
21971	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
21972	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
21973	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
21974	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
21975	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
21976	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
21977	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
21992	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
22319	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
22320	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
22321	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
22322	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
22323	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
22324	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
22325	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”

PPRI no.	Province	Region	Substrate	Collection Year	GPS co-ordinates
22326	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
22327	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
22328	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
22329	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
22330	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
22331	Gauteng	Roodeplaat	soil	2016	S 25°35’53.18”; E 28°21’31.19”
9461	Limpopo	Mara	sweet potato	2007	S 23°05.15.11”; E 29°23’55.84”
9463	Limpopo	Naboomspruit	sweet potato	2007	S 24°30’59.30”; E 28°43’02.63”
9465	Limpopo	Bylsteel	sweet potato	2007	S 23°31’35.40”; E 29°30’50.83”
9465	Limpopo	Mara	sweet potato	2007	S 23°05’15.11”; E 29°23’55.84”
9466	Limpopo	Levubu	sweet potato	2007	S 23°05’00.00”; E 30°17’00.00”
10531	Limpopo	Tom Bourke	sweet potato	2008	S 23°37’04.37”; E 30°11’32.59”
17592	Limpopo	Tom Bourke	sweet potato	2014	S 23°37’04.37”; E 30°11’32.59”
17593	Limpopo	Tom Bourke	sweet potato	2014	S 23°37’04.37”; E 30°11’32.59”
17594	Limpopo	Tom Bourke	sweet potato	2014	S 23°37’04.37”; E 30°11’32.59”
17595	Limpopo	Tom Bourke	sweet potato	2014	S 23°37’04.37”; E 30°11’32.59”
17596	Limpopo	Tom Bourke	sweet potato	2014	S 23°37’04.37”; E 30°11’32.59”
23061	Limpopo	Soekmekaar	sweet potato	2016	S 23º27’.69.30”; E 29º57’.89.0”
23062	Limpopo	Soekmekaar	sweet potato	2016	S 23º27’.69.30”; E 29º57’.89.0”
23063	Limpopo	Soekmekaar	sweet potato	2016	S 23º27’.69.30”; E 29º57’.89.0”
23064	Limpopo	Soekmekaar	sweet potato	2016	S 23º27’.69.30”; E 29º57’.89.0”
23065	Limpopo	Soekmekaar	sweet potato	2016	S 23º27’.69.30”; E 29º57’.89.0”
23066	Limpopo	Groblers Bridge	sweet potato	2016	S 22º58’26.52”; E 27º59’37.26”
23067	Limpopo	Groblers Bridge	sweet potato	2016	S 22º58’26.52”; E 27º59’37.26”
23068	Limpopo	Groblers Bridge	sweet potato	2016	S 22º58’26.52”; E 27º59’37.26”
23069	Limpopo	Groblers Bridge	sweet potato	2016	S 22º58’26.52”; E 27º59’37.26”
23070	Limpopo	Groblers Bridge	sweet potato	2016	S 22º58’26.52”; E 27º59’37.26”
23071	Limpopo	Groblers Bridge	sweet potato	2016	S 22º58’26.52”; E 27º59’37.26”
23072	Limpopo	Baltimore	sweet potato	2016	S 23º13’49.33”; E 28º24’05.90”
23074	Limpopo	Baltimore	sweet potato	2016	S 23º13’49.33”; E 28º24’05.90”
23076	Limpopo	Baltimore	sweet potato	2016	S 23º13’49.33”; E 28º24’05.90”
23077	Limpopo	Baltimore	sweet potato	2016	S 23º13’49.33”; E 28º24’05.90”
23078	Limpopo	Baltimore	sweet potato	2016	S 23º13’49.33”; E 28º24’05.90”
23578	Limpopo	Groblers Bridge	soil	2016	S 22º58’26.52”; E 27º59’37.26”
23579	Limpopo	Groblers Bridge	soil	2016	S 22º58’26.52”; E 27º59’37.26”
23580	Limpopo	Groblers Bridge	soil	2016	S 22º58’26.52”; E 27º59’37.26”
23581	Limpopo	Groblers Bridge	soil	2016	S 22º58’26.52”; E 27º59’37.26”
23582	Limpopo	Groblers Bridge	soil	2016	S 22º58’26.52”; E 27º59’37.26”
23583	Limpopo	Groblers Bridge	soil	2016	S 22º58’26.52”; E 27º59’37.26”
23584	Limpopo	Groblers Bridge	soil	2016	S 22º58’26.52”; E 27º59’37.26”
23585	Limpopo	Groblers Bridge	soil	2016	S 22º58’26.52”; E 27º59’37.26”
23586	Limpopo	Groblers Bridge	soil	2016	S 22º58’26.52”; E 27º59’37.26”
23587	Limpopo	Groblers Bridge	soil	2016	S 22º58’26.52”; E 27º59’37.26”

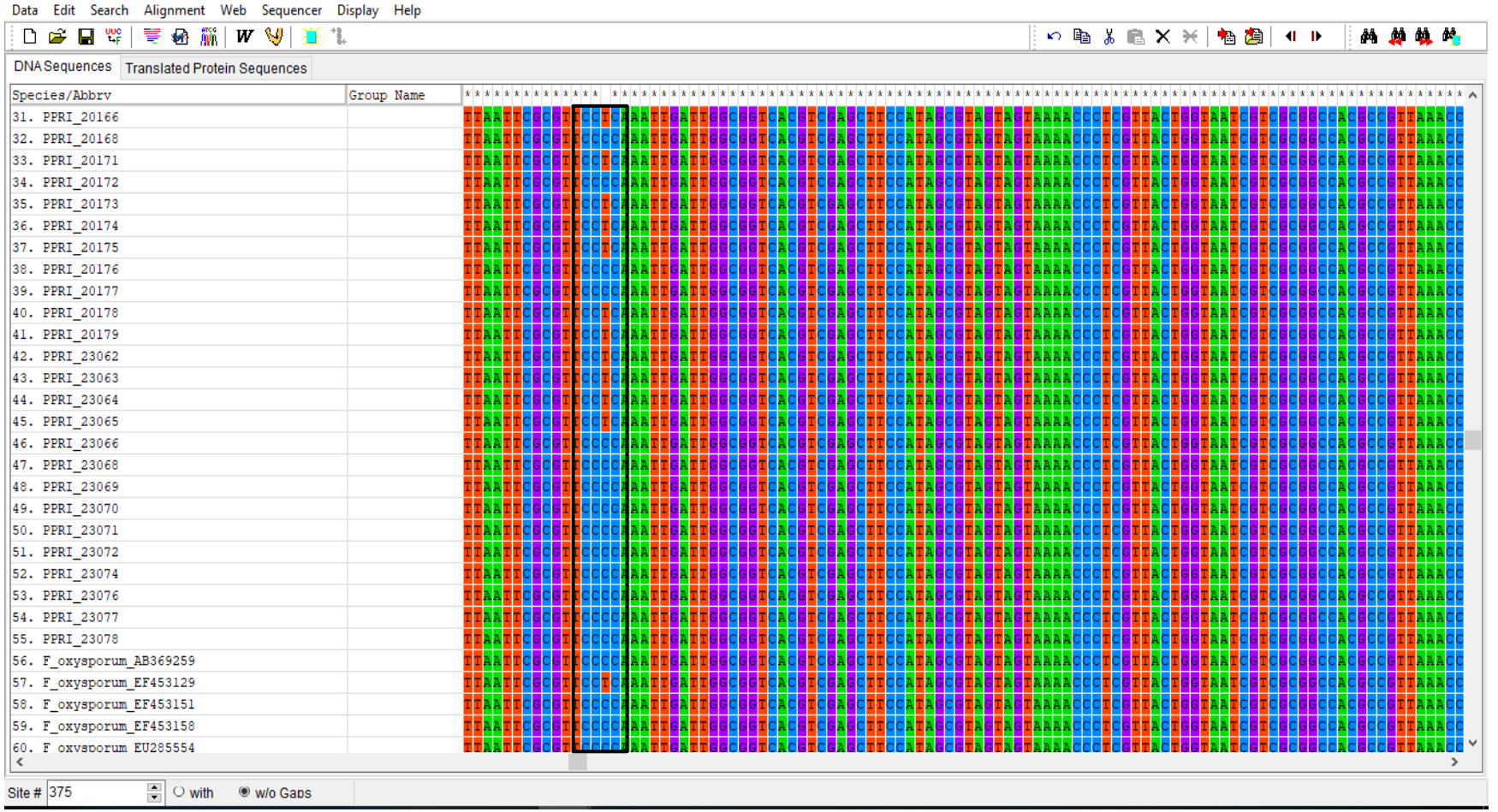
PPRI no.	Province	Region	Substrate	Collection Year	GPS co-ordinates
23588	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23589	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23590	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23591	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23592	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23593	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23594	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23595	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23596	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23597	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23614	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23615	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23616	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23617	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23618	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23619	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23620	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23621	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23622	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23623	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23624	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23625	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23626	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23627	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23628	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23629	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23630	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23631	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23872	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23873	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23874	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23875	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23804	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23805	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23806	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23807	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23808	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23876	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23809	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23877	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23878	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23879	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23880	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"

PPRI no.	Province	Region	Substrate	Collection Year	GPS co-ordinates
23881	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23810	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23811	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23812	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23813	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23814	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23815	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23816	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23817	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23818	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23819	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23820	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23821	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23822	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
23823	Limpopo	Groblers Bridge	soil	2016	S 22º58'26.52"; E 27º59'37.26"
9459	Mpumalanga	Hoedspruit	sweet potato	2006	S 24º21'27.00"; E 30º56'04.00"
9460	Mpumalanga	Marble Hall	sweet potato	2006	S 24º57'33.12"; E 29º16'42.87"
9462	Mpumalanga	White River	sweet potato	2007	S 25º20'27.28"; E 31º00'15.64"
9467	Mpumalanga	Marble Hall	sweet potato	2007	S 24º57'33.12"; E 29º16'42.87"
23473	Mpumalanga	Mkhuhlu	sweet potato	2016	S 24º58'57.25"; E 31º14'56.77"
23474	Mpumalanga	Mkhuhlu	sweet potato	2016	S 24º58'57.25"; E 31º14'56.77"
23475	Mpumalanga	Mkhuhlu	sweet potato	2016	S 24º58'57.25"; E 31º14'56.77"
23476	Mpumalanga	Mkhuhlu	sweet potato	2016	S 24º58'57.25"; E 31º14'56.77"
23477	Mpumalanga	Mkhuhlu	sweet potato	2016	S 24º58'57.25"; E 31º14'56.77"
23478	Mpumalanga	Mkhuhlu	sweet potato	2016	S 24º58'57.25"; E 31º14'56.77"
23479	Mpumalanga	Mkhuhlu	sweet potato	2016	S 24º58'57.25"; E 31º14'56.77"
23480	Mpumalanga	Mangweni	sweet potato	2016	S 25º43'17.36"; E 31º48'55.43"
23481	Mpumalanga	Mangweni	sweet potato	2016	S 25º43'17.36"; E 31º48'55.43"
23482	Mpumalanga	Mangweni	sweet potato	2016	S 25º43'17.36"; E 31º48'55.43"
23483	Mpumalanga	Mangweni	sweet potato	2016	S 25º43'17.36"; E 31º48'55.43"
23484	Mpumalanga	Mangweni	sweet potato	2016	S 25º43'17.36"; E 31º48'55.43"
23485	Mpumalanga	Mangweni	sweet potato	2016	S 25º43'17.36"; E 31º48'55.43"
23486	Mpumalanga	Mangweni	sweet potato	2016	S 25º43'17.36"; E 31º48'55.43"
23487	Mpumalanga	Mangweni	sweet potato	2016	S 25º43'17.36"; E 31º48'55.43"
23488	Mpumalanga	Mangweni	sweet potato	2016	S 25º43'17.36"; E 31º48'55.43"
23489	Mpumalanga	Mangweni	sweet potato	2016	S 25º43'17.36"; E 31º48'55.43"
23490	Mpumalanga	Mangweni	sweet potato	2016	S 25º43'17.36"; E 31º48'55.43"
23491	Mpumalanga	Mangweni	sweet potato	2016	S 25º43'17.36"; E 31º48'55.43"
23492	Mpumalanga	Mangweni	sweet potato	2016	S 25º43'17.36"; E 31º48'55.43"
23493	Mpumalanga	Mangweni	sweet potato	2016	S 25º43'17.36"; E 31º48'55.43"
23494	Mpumalanga	Mangweni	sweet potato	2016	S 25º43'17.36"; E 31º48'55.43"
23495	Mpumalanga	Mangweni	sweet potato	2016	S 25º43'17.36"; E 31º48'55.43"
23496	Mpumalanga	Mangweni	sweet potato	2016	S 25º43'17.36"; E 31º48'55.43"

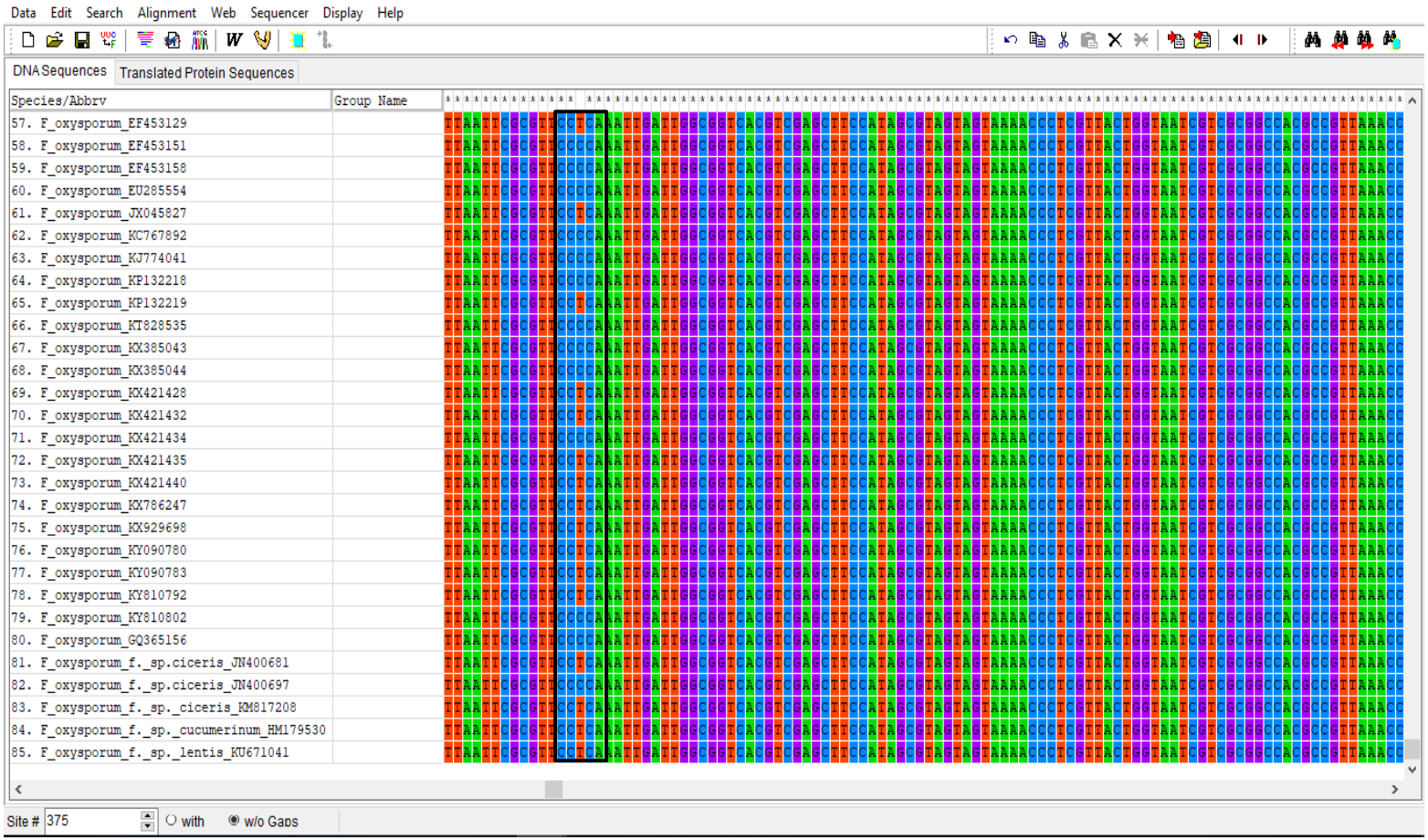
PPRI no.	Province	Region	Substrate	Collection Year	GPS co-ordinates
23972	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
23973	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
23974	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
23975	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
23976	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
23977	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
23978	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
23979	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
23980	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
23981	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
23982	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
23983	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
23984	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
23985	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
23986	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
23987	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
23988	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
23989	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
23990	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
23991	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
23992	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
23993	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
24199	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
24200	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
24201	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
24202	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
24203	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
24204	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
24205	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
24206	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
24207	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
24208	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
24209	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
24210	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
24211	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
24212	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
24213	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
24214	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
24215	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
24216	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
24217	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
24218	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
24219	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”

PPRI no.	Province	Region	Substrate	Collection Year	GPS co-ordinates
24220	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
24221	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
24222	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
24223	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
24224	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
24225	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
24307	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
24226	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
24227	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
24228	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
24229	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
24230	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
24231	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
24232	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
24233	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
24234	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
24235	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
24236	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
24237	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
24238	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
24239	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
24240	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
24241	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
24242	Mpumalanga	Mangweni	soil	2016	S 25º43’17.36”; E 31º48’55.43”
18014	Northern Cape	Sonop	sweet potato	2014	S 28º42’28.44”; E 20º58’04.42”
18016	Northern Cape	Wolwekop	sweet potato	2014	S 30º20’09.83”; E 24º35’01.34”
18017	Northern Cape	Sonop	sweet potato	2014	S 28º42’28.44”; E 20º58’04.42”
18018	Northern Cape	Sonop	sweet potato	2014	S 28º42’28.44”; E 20º58’04.42”
18750	Northern Cape	Sonop	sweet potato	2014	S 28º42’28.44”; E 20º58’04.42”
18751	Northern Cape	Sonop	sweet potato	2014	S 28º42’28.44”; E 20º58’04.42”
18752	Northern Cape	Sonop	sweet potato	2014	S 28º42’28.44”; E 20º58’04.42”
18753	Northern Cape	Wolwekop	sweet potato	2014	S 30º20’09.83”; E 24º35’01.34”
9468	Western Cape	Hartbeeskraal	sweet potato	2008	S 33º46’46.80”; E 19º00’06.94”
9469	Western Cape	Wellington	sweet potato	2008	S 33º38’46.12”; E 19º01’10.48”
9470	Western Cape	Lutouw	sweet potato	2008	S 31º33’15.22”; E 18º20’10.13”
9471	Western Cape	Saron	sweet potato	2008	S 23º11’24.07”; E 19º00’29.37”

Appendix B: Aligned PPRI sequences using MEGA version 6.0 software for the first 30 sequences showing the middle bases of the sequences including the base differences in a black rectangle.



Appendix C: Aligned PPRI sequences and reference sequences using MEGA version 6.0 software for the sequences showing the middle bases of the sequences including the base differences in a black rectangle.



Appendix D: Aligned reference sequences using MEGA version 6.0 software for the sequences showing the middle bases of the sequences including the base differences in a black rectangle.