CHARACTERIZATION OF WILT PATHOGENS AFFECTING EGGPLANTS IN ARUMERU DISTRICT, TANZANIA AND IDENTIFICATION OF SOURCES OF RESISTANCE

PHOEBE KIRIGO MWANIKI

EGERTON UNIVERSITY LIBRARY
19 NOV 2014
P.O. BOX 536
EGERTON
AFRICANA

A thesis submitted to the Graduate School in partial fulfilment for the requirements of the Master of Science agronomy degree in Crop Protection of Egerton University

EULIB
036 866
EGERTON UNIVERSITY LIBRARY
2013/95020

EGERTON UNIVERSITY

DECEMBER 2011

DECLARATION AND RECOMMENDATION

I declare that this thesis is my original work and has not been presented to any University for any degree or any other award.

Signature .....Phoebe..... Date ...15/03/2012...

Phoebe Kirigo Mwaniki

KM12/2197/08

RECOMMENDATION

This thesis has been submitted with our approval as university supervisors

Signature ..... Date 15/3/2012

Dr. Isabel N. Wagara

Egerton University, Njoro

Signature ..... Date 19/03/2012

Dr. Joseph N. Wolukau

Egerton University, Njoro

Signature Mabang Date 15/3/2012

Dr. Mathew Abang

AVRDC- The World Vegetable Center,

Regional Center for Africa, Arusha, Tanzania

2013/95020

EGERTON UNIVERSITY LIBRARY

COPYRIGHT

Phoebe Kirigo Mwaniki © 2012

All rights reserved, no part of this work may be produced, stored in any retrieval system or transmitted in any form or any means including electronic, recording, photocopying or otherwise without the prior written permission of the author or Egerton University on behalf of the author.

ACKNOWLEDGEMENT

I give all the glory to God for being with me, giving me new opportunities and giving me strength to see each new day. He has been faithful and his promises are true and Amen.

I am extremely grateful to my supervisors Dr Isabel N. Wagara, Dr Joseph N. Wolukau of Egerton University and Dr Mathew M. Abang of AVRDC-The World Vegetable Center, Arusha, Tanzania under whose guidance I undertook this research and wrote this thesis. They offered me consistent mentorship throughout my studies and supported me all through my research. I am indebted to AVRDC - Regional Center for Africa (AVRDC-RCA), Tanzania which funded this research. I would like to acknowledge the technical assistance accorded to me by Juma William in my work at the laboratory and Ijumaa Omari in the field and nurseries.

I also wish to register my profound appreciation to all my family members, my darling daughter and son, Celine and Einstein, my parents Charles and Loise, and all my brothers without whose contribution through academic, material and moral support this work would not have been completed.

ABSTRACT

In Arumeru district of Tanzania, as in many other parts of Africa, eggplant (*Solanum* spp.) production is severely affected by wilting of unknown etiology. Wilting observed in farmers' fields may be verticillium wilt, fusarium wilt, or bacterial wilt but the symptoms are remarkably similar, which leads to confusion as to the causal agent of the disease. Knowledge of pathogen identity is a necessary precondition for the development of host resistance in eggplant cultivars and for sustainable disease management. The objective of this study was to determine the predominant pathogen(s) associated with wilting of African eggplant in Arumeru district, Tanzania and identify new sources of wilt resistance within the African eggplant germplasm held at Asian Vegetable Research & Development Centre (AVRDC), Regional Center for Africa. Thirty two pathogen isolates obtained from wilting *Solanum macrocarpon* L., *S. aethiopicum* L., *S. dasyPhyllum, S. anguivi* and *S. melongena* in Arumeru district were characterized based on their morphological, cultural and genetic characteristics. Both Fusaria (*Fusarium oxysporum* f.sp. *melongenae* [FOM], *F. solani* [FS] and *Fusarium incarnatum-F. equiseti* species complex [FIESC]) and non-Fusaria (mainly *Phoma sorghina*) were identified and their identity confirmed by sequence analysis of the rDNA ITS region and the α-elongation factor (TEF). There was 100% ITS and TEF sequence similarity with reference isolates but FOM isolates differed from each other by just two substitutions. Of the 27 *Fusarium* spp. isolates, 53% were *Fusarium oxysporum*, 22% were *Fusarium solani* and 25% belonged to FIESC. Pathogenicity tests were carried out using six Fusaria (two FOM, two FIESC, and two FS). The pathogenicity tests indicated that Fs 27 in the FIESC was pathogenic to *S. macrocapon* (MM 1131) and *S. anguivi* (N 19). *Fusarium solani* caused wilting in *S. anguivi*. *Fusarium oxysporum* was pathogenic to an accession in *Solanum macrocarpon* and *S. anguivi*. *Solanum aethiopicum* gr. Gilo, (accession Sivonkwe) showed significant resistance to all the isolates. This is the first confirmed report of FOM and *F. solani* causing wilting of eggplant in Tanzania and the first report of FIESC causing wilting in African eggplant. A virulent FOM isolate (Fs40) was selected and used in screening 93 eggplant accessions (comprising five *Solanum* spp.) under screen house conditions. Thirteen accessions including MM 1161, MM 1616, UG AE– 21, MM 1119, SOS1 MM 1164, MM 12126, MM 1119, MM 10079, MM 1692, MM 1062, MM 1044, MM 11044 and MM 10260 from different species (*S. macrocarpon, S. dasyphyllum* and *S. aethiopicum*) were found to be

resistant. Eggplant accessions that showed high levels of resistance could potentially serve as valuable sources of Fusarium wilt resistance in eggplant breeding programs in Tanzania and beyond.

TABLE OF CONTENTS

DECLARATION AND RECOMMENDATION ii
COPYRIGHT iii
ABSTRACT v
TABLE OF CONTENTS vii
LIST OF TABLES ix
LIST OF FIGURES x
LIST OF ABBREVIATIONS AND ACRONYMS xii
CHAPTER ONE 1
INTRODUCTION 1
1.1 Economic Importance of Eggplant 1
1.2 Importance of Wilting in Eggplants 2
1.3 Statement of the problem 3
1.4 Objectives 4
1.5 Hypotheses 4
1.4 Justification 5
CHAPTER TWO 6
LITERATURE REVIEW 6
2.1 African Eggplant 6
2.1.1 Taxonomy of African Eggplant 6
2.1.2 Production of Eggplant (*Solanum* spp.) 7
2.2 Fusarium Wilt in Eggplants 11
2.2.1 Host Range of *Fusarium oxysporum* 11
2.2.2 Description of *Fusarium oxysporum* f.sp *melongenae* in Culture 12
2.2.3 Economic importance of *Fusarium* species 12
2.2.4 Dissemination of *Fusarium oxysporum* 13
2.2.5 Characters Used in Identification of *Fusarium* Species Using Synoptic Keys 13
2.3 Taxonomy of *Verticillium* Species 15
2.3.1 Description of *Verticillium* Species 15
2.3.2 Host Range and Dissemination of *Verticillium* Species 15
2.3.3 Disease cycle of *Verticillium dahliae* 16
2.3.4 Characteristics of *Verticillium* Species in Culture 17
2.4 Host Resistance of Eggplants to Wilt Pathogens 18

MATERIALS AND METHODS **20**
3.1 Experimental Site Description 20
3.2 Pathogen Isolation 20
3.3 Determination of the Predominant Pathogen 21
3.4. Morphological and Cultural Characterization of Collected Isolates 21
3.5 Analysis of Data on Morphological and Cultural Characteristics 24
3.6 Molecular characterization 24
3.7. DNA Isolation 25
3.7.2 Molecular identification 27
3.8 Pathogenicity Tests 27
CHAPTER FOUR **30**
RESULTS **30**
4.1 Predominant wilt pathogen 30
4.2 Characteristics of isolates collected 31
4.3 Molecular Characterization of representative isolates 35
4.4 Pathogenicity Tests 37
4.5 Reaction of Eggplant Accessions to *Fusarium oxysporum* f.sp *melongenae* 40
CHAPTER FIVE **48**
DISCUSSION **48**
5.1 Predominant Pathogen causing wilt in Eggplant 48
5.2 Significance of the Morphological Characterization of the isolates 48
5.3 Molecular characterization of the representative isolates 49
5.4 Pathogenicity of *Fusarium* species 51
5.5 Screening eggplant accessions for reaction to *Fusarium oxysporum* 52
CHAPTER SIX **54**
CONCLUSIONS AND RECOMMENDATIONS **54**
REFERENCES **56**
APPENDICES **66**

LIST OF TABLES

Table 1: Nutrients composition per 100 g of edible portion of eggplants (*Solanum* spp.) compared with headed cabbage (*Brassica oleraceae*) 2

Table 2: Categories of morphological characteristics used to compare isolates collected 22

Table 3: Source and number of isolates collected (July to November 2009) 30

Table 4: Cultural and morphological characteristics of *Fusarium* spp..... 33

Table 5: Molecular characterization of representative Fusarium isolates 37

Table 6: Disease severity scores of eggplant accessions after artificial inoculation with isolates of *Fusarium* spp 39

Table 7: Characaterization of the Fusaria and non Fusaria isolates..... 39

Table 8: Differences between the two trials expressed as disease index for trials 1 and 2 41

Table 9: Reaction of eggplant accessions to *Fusarium oxysporum* f.sp *melongenae*..... 42

LIST OF FIGURES

Figure 1. 1: A farmers field in Arumeru district with wilting eggplant (*Solanum melongena*.....3

Figure 2. 1: *Solanum aethiopicum* cultivars: 10

Figure 2. 2: *Solanum macrocapon* and *Solanum anguivi* cultivars 10

Figure 2. 3: Expression of wilting in eggplants 10

Figure 2. 4: Asexual spores of *Fusarium oxysporum* 13

Figure 3. 1: Map of the translation elongation factor region that was amplified, sequenced, and used in the identification of *Fusarium* species in this study. 24

Figure 3. 2: Flowchart describing the process of using TEF sequences used to identify *Fusarium* species in this study..... 25

Figure 4. 1: Plant samples from which isolates were collected 30

Figure 4. 2: Pigmentation of aerial mycelium of some of Fusarium cultures collected 31

Figure 4. 3: Shapes of *Fusarium* spp. macroconidia collected..... 31

Figure 4. 4: Shapes of apical cell of *Fusarium* spp 31

Figure 4. 5:Shapes of basal cell of *Fusarium* spp..... 31

Figure 4. 6: Other features of the *Fusarium* sporulation apparatus..... 32

Figure 4. 7: Macroconidia of representative *Fusarium* spp. isolates 34

Figure 4. 8: Pictures of *Phoma sorghina* 34

Figure 4. 9: Dendrogram based on pooled data of morphological and cultural characteristics of *Fusarium* spp. and *Phoma sorghina* isolates as the out group using the UPGMA algorithm 35

Figure 4. 10: Polymerase chain reaction identification of *Fusarium* spp. isolates..... 36

Figure 4. 11: Stages of pathogenicity test,..... 38

Figure 4. 12: Wilting in seedlings inoculated with the different isolates..... 38

Figure 4. 13: Wilting in eggplant accessions..... 41

Figure 4. 14: Mean monthly temperatures during the duration the two trials were carried out.....42

Figure 4. 15: Disease development on eggplant seedlings accessions inoculated with *F. oxysporum* f.sp *melongenae* 47

LIST OF ABBREVIATIONS AND ACRONYMS

AFLP	Amplified Fragment Length Polymorphism
AVRDC-RCA	Asian Vegetable Research and Development Center -The World Vegetable Center, Regional Centre for Africa
BLAST	Basic Local Alignment Search Tool
CAPS	Cleaved Amplified Polymorphic Sequences
CLA	Carnation Leaf Agar
FAO	Food and Agricultural Organization of the United Nations
FAOSTAT	Statistical database of FAO
FIESC	*Fusarium incarnatum* - *F. equiseti* species complex
FOM	*Fusarium oxysporum* f.sp *melongenae*
FSB	Fruit Shoot Borer
IPGRI	International Plant Genetic Research Institute
ITS	Internal Transcribed Spacer
NCBI	National Center for Biotechnology Information
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PROTA 2	Plant Resource of Tropical Africa 2
RAPD	Random Amplified Polymorphic DNA
rDNA	ribosomal Deoxy-ribonucleic Acid
SNA	Synthetic Nutrient Agar
SRAP	Sequence – Related Amplified Polymorphism
SRAP-RGA	Sequence Related Amplified Polymorphism- Resistance Gene Analog

TEF	Translation Elongation Factor
USDA	United States Department of Agriculture
VCG	Vegetative Compatibility Groups
WHO	World Health Organization
ZEA	Zearalenone

CHAPTER ONE

INTRODUCTION

1.1 Economic Importance of Eggplant

Eggplant (*Solanum* spp.) is a multi-species, diploid (2n=24) and seed propagated crop that is cultivated widely in sub-saharan Africa. African eggplant (*Solanum aethiopicum* L. and *Solanum macrocarpon)* are the most popular native traditional vegetables in West and Central Africa. *Solanum aethiopicum* is especially important as a source of vitamins, fibre and minerals. Eggplant is the number one vegetable crop in Senegal and number two in Ghana (Shippers, 2002) in terms of area and volume of production. According to Grubben and Denton (2004), annual fruit production of African eggplant was 8 000 tonnes in Senegal, 60 000 tonnes in Cote d'Ivore and 4,500 tonnes in Burkina Faso. Gboma eggplant (*Solanum macrocarpon*) fruits are exported from Senegal and Uganda to European markets such as Paris and Brussels. The African eggplant is widely cultivated as a major source of food rich in vitamins, fibers and minerals (Table 1); moreover, some fruits of bitter cultivars are used as medicine in some African countries (Chadha and Mndiga, 2006). Production of African eggplants has also created job opportunities for the growers, casual laborers and retailers. African eggplants are indigenous to Africa and deserve to be researched and popularized on the continent.

Research done indicated that among the indigenous vegetables grown in Tanzania, African eggplant (*Solanum aethiopicum* L.*)* was se ond in fruit and leaf yield (9.5 tonnes/ha) to Ethiopian mustard which had a leaf yield of 11.6 tonnes/ha ((Weinberger and Msuya, 2004) African eggplant also has the highest sales in Tanzanian markets with 82% sales followed by Ethiopian mustard with 63% sales. The net returns per hectare (Tshs.1, 331,176) have been shown to be the highest among the indigenous vegetables, followed by Ethiopian mustard (T shs. 1,290,704). In Arumeru district, farmers in Manyire village growing Tengeru white (*Solanum aethiopicum*) earn an average of Tshs 1,800,000ha/season which lasts six months (Weinberger and Msuya, 2004).

Table 1: Nutrients composition per 100 g of edible portion of eggplants (*Solanum* spp.) compared with headed cabbage (*Brassica oleraceae*)

Minerals	Scarlet eggplant (*Solanum aethiopicum*)		Gboma eggplant (*Solanum macrocarpon*)		Brinjal eggplant (*Solanum melongena*)	Headed Cabbage (*Brassica oleraceae*)
	Fruits	Leaves	Fruits	Leaves	Fruits	Raw leaves
Calories	24.0 kcal	-	40 kcal	42 kcal	15 kcal	50 kcal
Moisture content	90.6%,	82.1%	89.0 %	85.6 %	92.9 %	84.5 %
Carbohydrates	7.2 g	10.3 g	8.0 g	6.4 g	2.2 g	10.0 g
Protein	1.5 g	4.8 g	1.4 g	4.6 g	0.9 g	3.3 g
Fat	0.1 g	4.8 g	1.0 g	1.0 g	0.4 g	0.7 g
Fibre	2.0 g	2.4 g	1.5 g	1.6 g	2.3 g	2.0 g
Calcium	28.0 mg	523 mg	13 mg	391 mg	10 mg	135 mg
Phosphorus	47.0 mg	94 mg	-	49 mg	16 mg	56 mg
Iron	1.5 mg	6.0 mg	-	-	0.3 mg	1.7 mg
Vitamin B Complex-Thiamine	0.047 mg	0.23 mg	-	-	0.02 mg	0.11
Riboflavin	0.06 mg	0.44 mg	-	-	0.01 mg	0.13 mg
β-carotene	0.35 µg	6.40 µg	-	-	70 µg	
Vitamin c	8.0 mg	67.0 mg	-	-	4 mg	120 mg
Niacin	0.8 mg	1.8 mg	-	-	0.1 mg	1.0 mg

Table compiled from these sources: Grubben and Denton, 2004; USDA, 2003

1.2 Importance of Wilting in Eggplants

Eggplant production is constrained by various diseases and insect pests. These include pests such as fruit and shoot borers, Epilancha beetle, two spotted spider mite and root knot nematodes. The diseases are phomopsis blight, southern blight, late blight, leaf spots, and wilts such as bacterial wilt, Verticillium wilt, Fusarium wilts (Figure 1) and damping off. Wilt-associated pathogens cause severe losses in eggplant in terms of quality and quantity. Verticillium wilt losses are rated 50% - 70% in severely infested soils (Liu, 2001). Bacterial wilt is most severe in tropical and subtropical countries. In the Philippines, particularly in Central Luzon, losses in eggplant production due to bacterial wilt were estimated at 30-80% in 1949 and 10% in 1969 (Rao *et al.*,

1976). Losses of 75% to 81% have also been reported in India (IPM CRSP, 2007). Losses in eggplant production in Africa due to wilt diseases have not been statistically evaluated. Previous research has shown that Bacterial wilt, Fusarium wilt and Vertcillium wilt pathogens are the predominant causal agents of wilting in eggplants (Fugro *et al.,* 1994). The search for sources of resistance to Fusarium wilt pathogens has been done using the wild relatives of *S. melongena* and two genes carrying wilt resistance have recently been tagged (Mutlu *et al.,* 2008; Toppino *et al.,* 2008). *Solanum anguivi* and *Solanum aethiopicum* have been utilized in breeding programmes for development of disease resistant eggplant varieties (Mochizuki and Yamakawa, 1979; Toppino *et al.,* 2008). Several eggplant accessions have also been utilized in the production of disease resistant rootstocks for grafting high yielding varieties (Monma *et al.,* 1996; Yoshida *et al.,* 2004).

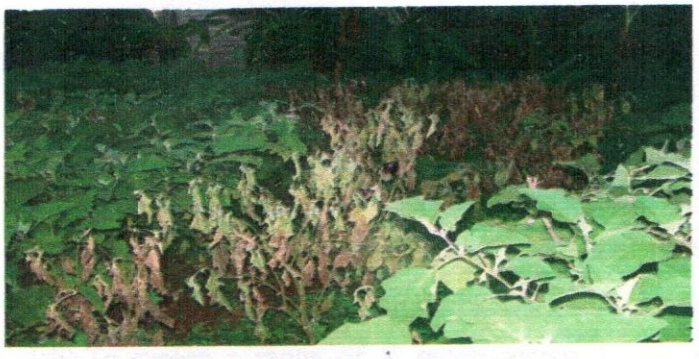


Figure 1. 1: A farmers field in Arumeru district with wilting eggplant (*Solanum melongena*)

1.3 Statement of the problem

Eggplant production in Arumeru district and other parts of Africa has been affected enormously by wilting diseases which have not been identified and there have been major losses in the farmers' fields. Symptoms observed in farmers' fields have caused confusion in wilting diseases' diagnosis due to similarities between Verticillium wilt, Fusarium wilt or yellows and bacterial wilt. Lack of information on pathogen identity and on the characteristic symptoms associated with each fungal pathogen has hindered proper management of wilting eggplants. Morphological, cultural and pathological characterization of the fungal pathogens associated with wilting in eggplants is necessary for the efficient management of wilts and the development of host resistance in eggplant cultivars. Lack of high yielding wilt-resistant eggplant cultivars

with farmer- preferred quality attributes is a major drawback to eggplant production in these areas. Most of the popular cultivars such as 'black beauty' are susceptible to wilt; therefore lack of wilt resistant eggplant varieties has contributed to major losses in eggplant yield and quality. Other control methods are ineffective and disease reistance is an attarctice option. There is need to search for genes that confer resistance to wilt pathogens.

1.4 Objectives

Broad Objective

To characterize the primary wilt causing soilborne fungal pathogen of African eggplant *S. macrocarpon* L., *S. aethiopicum* L., *S. dasyphyllum* L., *S. anguivi* L., and *S. melongena*, and screen eggplant accessions for resistance to the predominant wilt pathogen.

Specific Objectives

(i) To identify the predominant fungal pathogen causing wilt of African eggplants in Arumeru district, Tanzania.

(ii) To characterize isolates of the predominant pathogen causing wilt of African eggplant based on cultural, morphological, molecular and pathological characteristics.

(iii) To screen a range of *S. macrocarpon* L., *S. aethiopicum* L., *S. dasyphyllum* L., *S. anguivi* L. and *S. melongena* accessions for resistance to the predominant wilt pathogen.

1.5 Hypotheses

Null hypothesis:

(i) There is no predominant fungal pathogen causing wilting of African eggplants in Arumeru District, Tanzania.

(ii) Isolates of the fungal pathogens causing wilting in eggplant do not have distinguishing cultural, morphological, molecular and pathological characteristics.

(iii) There are no *S. macrocarpon*, *S. aethiopicum*, *S. dasyphyllum*, *S. anguivi* and *S. melongena* accessions in the locally available germplasm collection with resistance to wilt.

1.4 Justification

Allard (1960) stated that "knowledge of two closely related phenomena, variations in the pathogenic capabilities of parasitic organisms and differences within host species in resistance to infectious disease, are basic to the intelligent planning of breeding programmes designed to develop disease resistant varieties." Variability of the fungal pathogens causing wilt in *S. macrocarpon* L., *S. aethiopicum* L., *S. dasyphyllum* L., *S. anguivi* L. and *S. melongena* L. increases the difficulty of breeding for resistance and deployment of improved cultivars. Moreover, the development of resistant varieties, together with chemical and cultural control measures has so far been undertaken without adequate knowledge of the range of pathogen species in the population that can match particular cultivars. For studies on genetic variability to be of any practical relevance, however, they must be related to phenotypic (morphology and virulence) variation within pathogen populations (Rozlinah and Sariah, 2006). Morphological and pathogenicity characterization of the fungal pathogens associated with wilting in eggplants is important for efficient management of wilts and the development of host resistance in eggplant cultivars. The development of resistant rootstocks 'Daitaro' and 'Daizaborou' is a proof of the importance of resistance genes within the wild eggplant large diversity (Monma *et al.*, 1996; Yoshida *et al.*, 2004). The tagging of the resistance genes to *Fusarium oxysporum* f.sp *melongenae* designated as *Rof sal* (Toppino *et al.*, 2008) and F.O.M (Mutlu *et al.*, 2008) is another major step towards developing resistant eggplant varieties. The products of the screening envisaged in this study would provide the building blocks for the development of resistant varieties against the predominant wilt causing pathogen in Arumeru district. These resistant accessions may be utilized to introgress wilt resistance into the cultivated varieties and may be used as rootstocks (Yoshida *et al.*, 2004). This could form the basis of wilt disease management strategies for *S. melongena* L., *S macrocarpon* L., *S. aethiopicum* L., *S. dasyphyllum* L. and *S. anguivi* L. eggplant species in Tanzania and beyond.

CHAPTER TWO

LITERATURE REVIEW

2.1 African Eggplant

There are several domesticated eggplant species in Africa in the genus *Solanum.* The species include *Solanum melongena* L., *Solanum aethiopicum* L. and *Solanum macrocarpon* L. (Grubbenand Denton 2004). Its wild relatives are are *Solanum anguivi, Solanum dasyphyllum, Solanum torvum* L. and others. African eggplants *S. aethiopicum* L. and *S. macrocarpon* L. are the most popular native, traditional vegetables in West and Central Africa, but the productivity of these crops in Africa is still relatively low although the yields per area have not been widely statistically evaluated (Agnieszka *et al.,* 2007). African eggplant is a native crop in East Africa that is being promoted for domestication by AVRDC- The World Vegetable Center, FARM AFRICA, FARM CONCERN and other partners.

The common names for eggplant (*Solanum melongena* L.*)* are Asian eggplant, Aubergine, Brinjal eggplant, Easter eggplant, eggplant and Tropical Egg. The eggplant is native to India and Pakistan and was first domesticated over 4000 years ago (Huntrods, 2008). The fruits were introduced to China around 500 B.C. *Solanum melongena* L. may have been indirectly derived from the wild *Solanum incanum* domesticated in India and Southeast of China. The common names for African eggplant *S. aethiopicum* are scarlet eggplants, garden eggs, jakatu or Nakati (Shippers, 2002). The common names of *Solanum macrocarpon* L. are African eggplant, terong asam, terong iban, aubergine gboma, anthora, antrua, Scarlet eggplant, Terong rapoh, Terong engkol, Terong kelapa, Aubergine d'Afrique, Sennari nasu, Berenjena, Melanzana petonciano, Afrikaanse aubergine and Antroewa (Tropilab INC). *Solanum aethiopicum* L. and *S. macrocarpon* L. were domesticated in Africa from their wild relatives *S. anguivi* L. and *S. dasyphyllum* L. respectively (Lester *et al.,* 1988; Agnieszka *et al.,* 2007).

2.1.1 Taxonomy of African Eggplant

Solanum aethiopicum and *Solanum anguivi* belong to section Oliganthes, *Solanum macrocarpon* and *Solanum melongena* belong to the section Melongena and all the four species are in the

subgenus Leptostemonum. *Solanum aethiopicum* is an erect biennial herb, 90-120cm in height (Tindall, 1983) and comprises of four groups (Figure 2.1): *Solanum aethiopicum* group *gilo*, *Solanum zuccagnianum* also known as shum group, *Solanum aethiopicum* group kumba and *Solanum aethiopicum* group *aculeatum* (*S. integrifolium*) (Lester, 1986). *Solanum macrocarpon* is widely grown in Asia and tropical America, too.

Solanum macrocarpon is a perennial branched herb up to 150cm in height (Tindall, 1983) and is less morphologically diverse than *Solanum aethiopicum.* It has three groups: *S. macrocarpon* (semi wild group), *S. macrocarpon* (*'Mukono'* cv. Group) and *S. macrocarpon 'Nabingo'* cv. Group (Bukenya and Carasco, 1994) (Figure 2.2a). The wild ancestor of *S. macrocarpon* is *S. dasyphyllum* Schum. and Thonn., which originated probably in East Africa. *Solanum anguivi* is found in the non arid areas of Africa especially in Madagascar and is considered to be the ancestor of *S. aethiopicum*. *Solanum anguivi* has small (7-15mm) bitter berries normally occurring in clusters of up to 20 fruits. *Solanum anguivi* is important in breeding programmes due to its high number of fruits and resistance to *Ralstonia solanacearum*. Its fruits are normally used for medicinal purposes to control high blood pressure (Shippers, 2002, Figure 2.2b). Singh *et al.* (2006) conducted genetic analysis of *S. melongena* and *S. incanum* using Random Amplified Polymorphic DNA (RAPD) markers and found a close genetic relationship between the two species. Molecular characterization using Amplified Fragment Length Polymorphism (AFLP) markers indicated that *S. macrocarpon* is more related to *S. aethiopicum* (section *Oliganthes*) than to *S. melongena* (Singh *et al.*, 2006). These results were similar to those of a study conducted by Mace *et al.* (1999) based on the AFLP analysis of genetic relationship among the cultivated eggplants and their wild relatives.

2.1.2 Production of Eggplant (*Solanum* spp.)

Eggplant is a warm-season crop that requires 65 to 85 days to reach market maturity from transplanting. Following pollination, 25 to 40 days are required for fruit to reach maturity for harvesting. Optimal temperature for eggplant production is 26°C during the days and 20°C at nights. Plant growth slows and pollination problems occur at temperatures below 17°C or above 35°C (Aguiar *et al.*, 1998). Eggplant can tolerate drought and excessive rainfall, but struggles to grow when temperatures exceed 30°C. When temperatures and humidity are high, growth of

eggplant tends to become more vegetative (Kemble *et al.,* 1998).Well-drained sandy loam, loam, or clay loam soils with a pH of 6.0 to 6.5 with a good supply of organic matter are best for growing eggplants (Kemble *et al.,* 1998).

Eggplant production suffers yield losses from pests, diseases and unfavourable environmental conditions. It is affected by many diseases which are most commonly associated with solanaceous crops, for example; phomopsis blight caused by *Phomopsis vexans* which attacks the stems of eggplant, causing plants to wilt. Blossom end rot caused by low concentration of calcium in the fruit (Miller *et al.,* 1996) affects ripe fruit resulting in round, leathery, sunken spots appearing on fruit ends with the affected fruit eventually dropping from the plant. Southern blight caused by *Sclerotium rolfsii* manifests itself as softening of the crown and root tissues. Mold may also be seen on the stems and surrounding soil. Phytophthora blight caused by *Phytophthora parasitica* quickly destroys eggplants. Infected plants get dark streaks prior to collapsing and dying. There are four different types of leaf spots in eggplant caused by *Alternaria melongenae, Alternaria solani, Cercospora solani-melongenae,* and *Cercospora solani.* The *Alternaria* leaf spots produce characteristic spots with concentric rings. The spots are mostly irregular, 4-8 mm in diameter and usually enlarge and cover a large area of the leaf blade. The leaves may drop under severe infection. The *Cercospora* leaf spots are characteristically chlorotic lesions, angular to irregular in shape, and later turn grayish brown with profuse sporulation at the center of the spot. Severely infected leaves drop off prematurely resulting in the reduction of yield (Chen and Li, 1995).

Eggplant is also susceptible to a number of wilt diseases: bacterial wilt which is caused by *Ralstonia solanacearum* is very destructive especially in hot, wet season that causes plants to wilt and die suddenly. When newly infected stems are cut crosswise and placed in water bacterial ooze appears (Figure 2.3a). Verticillium wilt caused by *Verticillium dahliae* is a soil-borne fungal disease causing characteristic V-shaped necrotic lesions on the leaves (Figure 2.3b). Plants may become stunted, turn yellow, and wilt. Damping off fungi (*Pythium, Phytophthora* and *Rhizoctonia*) attack germinating seeds, creating lesions on the stems and the seedlings collapse. Fusarium wilt caused by *Fusarium oxysporum* f.sp *melongenae* induces symptoms that begin as slight vein clearing on outer leaflets (Figure 2.3c) and drooping of leaf petioles. Later

the lower leaves wilt, turn yellow and die and the entire plant may be killed, often before the plant reaches maturity. In many cases a single shoot wilts before the rest of the plant shows symptoms or one side of the plant is affected first (Miller *et al.,* 1996). Because *Fusarium oxysporum* and *Verticillium dahliae* fungi are widespread and persist several years in soil, a long crop rotation (4 to 6 years) is necessary to reduce populations of these fungi. The rotations should not include any solanaceous crop, for example; potato, tomato, pepper or eggplant (Chen *et al.,* 2002). Removal and destruction of infested crop debris, reduction of irrigation frequency and amount of water, proper drainage of fields, disinfection of pruning tools, maintenance of high level of plant vigor with appropriate fertilization, adequate irrigation, soil fumigation and soil solarization are important means to control the pathogens.



(a)



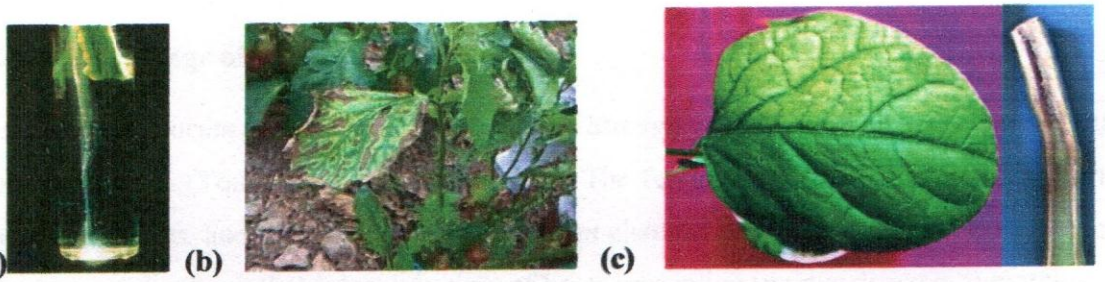
(b) (c) (d)

Figure 2. 1: *Solanum aethiopicum* cultivars: a: *Solanum aethiopicum* group gilo, b: *Solanum aethiopicum* group kumba, c: *Solanum aethiopicum* group shum, d: *Solanum aethiopicum* group aculeatum (*S. integrifollium*). Source: Porcher, 2009.



(a) (b)

Figure 2. 2: *Solanum macrocapon* and *Solanum anguivi* cultivars
(a) *Solanum macrocarpon,* (b) *Solanum anguivi* : Source: Porcher, 2009



(a) (b) (c)

Figure 2. 3: Expression of wilting in eggplants (a) Bacterial ooze streaming in a glass tube, (b) V- Shaped lesions on leaves of plant infected by verticillium wilt, (c) Symptoms of Fusarium wilt on eggplant leaf and shoot.

Source: (b) Camele *et al.,* (2005) (c) Miller *et al.,* (1996).

2.2 Fusarium Wilt in Eggplants

Fusarium wilt is caused by *Fusarium oxysporum,* a genus of the class hyphomycetes, subdivision Deuteromycotina in the section Elegans (Lester *et al.,* 1988). *Fusarium oxysporum* is an imperfect fungus (one with no known sexual stage) affiliated to the hypocreales (ascomycetes). Most of the anamorphs are species of Gibberella and the teleomorphs belong to the genus Nectria (Seifert, 1996). *Fusarium oxysporum* is an abundant and active saprophyte in soil and organic matter, with most of its specific forms being plant pathogens (Smith *et al.,* 1988). Fusarium wilt in eggplants is caused by *Fusarium oxysporum* f.sp *melongenae* and spreads by means of asexual spores (microconidia, macroconidia and clamydospores). Clamydospores can survive in the soil for many years until the conditions become favourable and mycelium can survive and overwinter in the debris from infected plants. The fungus is quite cosmopolite and once it becomes established in an area, it is difficult to eliminate it. It is a warm-weather pathogen, prevalent on acid, sandy soils and requires soil and air temperatures of 28°C. Fusarium enters the plant through the root and then spreads throughout the plant through the vascular system. The pathogen colonizes water conducting vessels of the plant and blocks the xylem (Mui-yun, 2003). Affected plants show symptoms such as leaf wilting, yellowing and eventually plant death.

2.2.1 Host Range of *Fusarium oxysporum*

Fusarium oxysporum is a fungus that causes *Fusarium* wilt disease in more than a hundred species of plants (Toussoun and Nelson, 1978). The fungus attacks crops including vegetables, ornamental plants, and trees. *Fusarium oxysporum* also has several specialized forms - known as *formae specialis* (f.sp.) that infect a variety of hosts presenting various diseases symptoms. These include: *Fusarium oxysporum* f.sp. *asparagi* (fusarium yellows on asparagus); f.sp. *callistephi* (wilt on China aster); f.sp. *cubense* (Panama wilt disease on banana); f.sp. *dianthi* (wilt on carnation); f.sp. *koae* (wilt on koa); f.sp. *lycopersici* (wilt on tomato); f.sp. *melonis* (Fusarium wilt on muskmelon); f.sp. *niveum* (fusarium wilt on watermelon); f.sp. *pisi* (wilt on edible-podded pea); f.sp. *tracheiphilum* (wilt on Glycine max); and f.sp. *zingiberi* (Fusarium yellows on ginger) (Toussoun and Nelson, 1978; Raabe *et al.,* 1981). Fusarium wilt of tomato is the most economically significant disease caused by *F. oxysporum*, but it shows a large number of *formae*

speciale that are specific pathogens of other plants including: asparagus, bananas, carnations, cotton, curcubits, ginger, snowpeas and soybean. *Fusarium oxysporum* was first described from a *Solanum melongena* plant suffering from vascular wilt (Cline, 2006). *Fusarium oxysporum* f.sp *melongenae* attacks eggplants.

2.2.2 Description of *Fusarium oxysporum* f.sp *melongenae* in Culture

Fusarium oxysporum f.sp *melongenae* is characterized by the production of three to five septate, sickle shaped macroconidia, foot shaped basal cell, ellipsoid macroconidia borne on false heads on short monophilalides, and clamydospores in culture (Altinok, 2005). In solid media culture, such as potato dextrose agar (PDA), the different special forms of *F. oxysporum* may vary in colony morphology; however, *formae specialis* cannot be distinguished based on morphology.

Fusarium oxysporum produces three types of asexual spores: microconidia, macroconidia, and clamydospores (Agrios, 1988) (Figure 2.4). Microconidia are one or two celled, and it is also the type of spore most frequently produced within the vascular bundles of infected plants. Macroconidia are three to five celled, gradually pointed and curved toward the ends. These spores are commonly found on the surface of plants killed by this pathogen as well as in sporodochialike groups. Chlamydospores are round, thick-walled spores, produced either terminally or intercalary on older mycelium or in macroconidia. These spores are either one or two celled (Agrios, 1988).

2.2.3 Economic importance of *Fusarium* species

Fusarium species are important disease pathogens of almost all cultivated crops. Mycotoxigenic *Fusarium* species cause significant quantitative and qualitative losses to global agriculture and also cause serious animal and human diseases by producing harmful mycotoxins (Saharan *et al.*, 2005). Once Fusarium is introduced into the soil it becomes difficult to eradicate and failure to control the build-up of the disease results in fields becoming less suitable for crop production.

2.2.4 Dissemination of *Fusarium oxysporum*

Fusarium oxysporum is primarily spread over short distances by irrigation water, rain drop splash and contaminated farm equipment. Its dispersal to other fields is fast via irrigation systems. The fungus can also be spread over long distances either in infected transplants or in soil. Although the fungus can sometimes infect the fruit and contaminate its seed, the spread of the fungus by way of the seed is very rare (Agrios, 1988). Fusarium spores my also be spread by wind (Lester *et al.*, 1988).

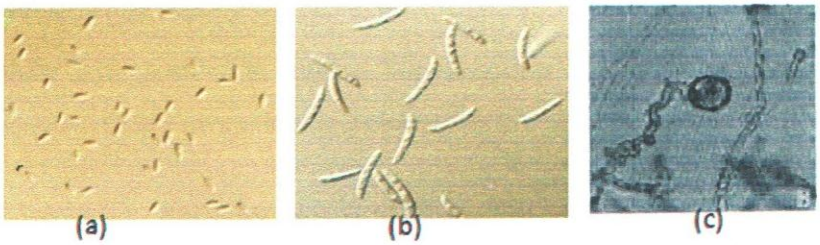


Figure 2. 4: Asexual spores of *Fusarium oxysporum*: (a) Microconidia, (b) Macroconidia (c) "Terminal" Clamydospore
Source: Toussoun and Nelson, 1978

2.2.5 Characters Used in Identification of *Fusarium* Species Using Synoptic Keys

Identifying cultures of Fusarium species is typically based on manuals which provide a series of identification keys: synoptic keys (Nelson *et al.*, 1983; Seifert, 1996), tabular keys (Lester *et al.*, 1988) among others. The most commonly used keys are the synoptic keys since they are compatible with computer applications. Below are the parameters considered in the study of the colony morphology in the synoptic keys:

- *Colony characters on PDA*:- These characters on PDA include growth rate, formation of aerial mycelium i.e. growth of hyphae above the agar surface, colony reverse i.e. the colour of the colony backside which is normally highly variable within species (Seifert, 1996).
- *Macroconidia from sporodochia*: - These include the colour of the sporondochial conidial masses, the macroconidial shape which is a critical character for identification of *Fusarium* spp., widest part of macroconidial where some macroconidia are wedge shaped while others are broadest below the centre of the spore, macroconidial septation, shape of

the base of the cell which can either be blunt papillate, extended foot or foot shaped, length of apical cell which describes the relative length of the apical cell compared with the cell next to it (the penultimate cell) and shape of apical cell which can be hooked, nipple like, blunt or conical (Seifert, 1996).

- *Microconidia from aerial mycelium*: - These include the relative abundance of microconidia in aerial mycelium; microconidial arrangement, which may be in chains or beads, microconidia shape and length of conidiophores in the aerial mycelium (Seifert, 1996).
- *Clamydospores* are storage cells, usually thick walled and used in identification based on their arrangement in agar. Clamydospores can exist in chains, clumps, in pairs and singles. Other miscellaneous characters are also used (Seifert, 1996).

2.2.6 Molecular characterization of *Fusarium* species

The translation elongation factor 1-α (TEF) gene is commonly used for DNA sequence-based identification in *Fusarium* spp. because there is frequently insufficient variation in the internal transcribed spacer (ITS) region. The TEF gene, which encodes an essential part of the protein translation machinery, has high phylogenetic utility because it is (i) highly informative at the species level in *Fusarium*; (ii) non-orthologous copies of the gene have not been detected in the genus; and (iii) universal primers have been designed that work across the phylogenetic breadth of the genus (Geiser *et al.*, 2004). Primers were first developed in the fungi to investigate lineages within the *F. oxysporum* complex (O'Donnell *et al.*, 1998). The ef1 and ef2 primers were designed based on sites shared in exons between *Trichoderma reesei* and *Histoplasma capsulatum*, which can be applied to a wide variety of filamentous ascomycetes. These primers amplify the ~700 bp region of TEF, flanking three introns that total over half of the amplicon's length, in all known fusaria. This gene appears to be consistently single-copy in *Fusarium*, and it shows a high level of sequence polymorphism among closely related species, even in comparison to the intron-rich portions of protein-coding genes such as calmodulin and beta-tubulin. For these reasons, TEF has become the marker of choice as a single-locus identification tool in *Fusarium* and several other fungi.

2.3 Taxonomy of *Verticillium* Species

Verticillium is a heterogeneous anamorphic genus and information on its classification is still limited though it has been placed in two orders in the subdivision deuteromycotina. The phytopathogenic and saprophytic *Verticillium* spp. i.e. *V. albo-atrum*, *V. dahliae*, *V. longisporum*, *V. nigrescens*, *V. nubilum*, *V. tricorpus* and *V. theobromae* have been placed in the order phyllachorales whereas the species that parasitize insects, fungi, and nematodes are placed in the order Hypocreales, for example; *Leucanicillium,, V. fungicola, Pochonia, Haptocillium* and *Simplicillium* (Pantou *et al.,* 2005). The two most notorious species attacking plants are *V. dahliae* and *V. albo-atrum*, which cause billions of dollars in annual crop losses worldwide. Yield losses in potato crops may reach 50%, but are more commonly in the range of 10–15% whereas in lettuce, losses can easily reach 100% (Klostermann *et al.,* 2009).

2.3.1 Description of *Verticillium* Species

Verticillium dahliae on natural hosts has few completely hyaline conidiophores with small conidia, abundant black microsclerotia; a higher temperature range for growth on media and pathogenicity; a very extensive host range and a lower pathogenicity than *V. albo-atrum* on many hosts (Smith, 1965). They both produce one-celled, colourless conidia that are short-lived. *Verticillium albo-atrum* is a cool weather organism that grows best when soil temperatures are between 18°C and 23°C while *V. dahliae* is more active between 23°C and 28°C (Miller *et al.,* 1996). There are many variations among strains of the same species in virulence and other characteristics. Eggplants infected by *V. dahliae* strains exhibit lesions which have a characteristic V-shaped pattern which is widest at the leaf margin. Brown, necrotic tissue within lesions is surrounded by a large, irregular area of yellowing due to a systemic leaf toxin produced by the fungi. When the stems of infected plants are cut lengthwise, the vascular tissue exhibits a brown discoloration caused by *Verticillium dahliae* (Miller *et al.,* 1996).

2.3.2 Host Range and Dissemination of *Verticillium* Species

Verticillium wilt is a serious disease that affects over 300 host plants in numerous plant families. The host range includes trees, shrubs, ground covers and vines, vegetables, field crops, fruits,

herbaceous ornamentals, and many weeds (Smith, 1965). Propagules of *Verticillium* spp. are disseminated by flowing water, for example; irrigation furrows and by strong winds where, contaminated seed, vegetative cuttings, transplants, tubers, scions, buds, nursery plants, root balls, or the bare roots of infected plants also serve as means of dispersal. In addition, mycelia and microsclerotia may be transported by normal tillage operations, hand tools, or farm machinery.

2.3.3 Disease cycle of *Verticillium dahliae*

Verticillium dahliae is a monocyclic pathogen and can be divided into two phases: inside the vascular tissue as an endophyte during the earlier stages of the disease and a semi-necrotrophic phase at the later stages of disease, where microsclerotia are formed due to increased senescence of the plant. The germination of microsclerotia in the soil is stimulated by root exudates (Mol and Vanriessen, 1995). *Verticillium dahliae* and *V. albo-atrum* penetrate the root systems directly and through wounds.

The lifecycle begins with dormant microsclerotia in the soil or embedded within the plant debris. The microsclerotia germinates in the presence of a host and produces one or several hyphae that extend towards the host roots. Colonization of the root surface has been observed at or near root tips or following root hairs to the root surface, with hyphae growing between epidermal cells (Eynck *et al.*, 2007; Klosterman *et al.*, 2009). The hypha spreads through the xylems and moves upward through the plant thereby blocking the water conducting tissues. As water moves up the xylem, it transports with it conidia which become lodged in the vessels, germinate and affect other parts of the plant. The fungus produces toxins that cause tyloses or gums to form in the xylem which limits water flow resulting in wilting (the characteristic symptom of the disease), and often death of the host. Because *Verticillium dahliae* affects the water-conducting vessels, symptoms may appear on one side of the plant or on one side of a leaf but not the other. *Verticillium dahliae* does not cause root rot (Olsen and Deborah, 1998). As wilting progresses the host dies and as the growing season ends, the fungi survive as mycelia overwintering in dead plant parts.

The pathogen may also live saprophytically in the soil. Survival can occur in roots of non host species in which systemic infection does not occur. *Verticillium* is known to naturally colonize soils where susceptible hosts have never been grown. The microsclerotia can survive for up to 15 years in the soil without contact with a host plant. Once conditions are conducive and in the presence of a host, the fungus infects the root system, progressing upward and the cycle begins (University of Illinois, 1997).

2.3.4 Characteristics of *Verticillium* Species in Culture

Colonies of *Verticillium* grow moderately rapidly. At 25°C on potato dextrose agar, the colonies are velvety to woolly. The front color is white initially and becomes yellowish, red, pinkish-brown, or green. Colony reverse is white or brown (rust color) (Larone, 1995). Colonies of *V. dahliae* can be identified by the presence of subglobose to elongate, black microsclerotia (18-41 x 9-17 μm), which are produced on single, hyaline hyphae (Termorshuizen, 1998). The optimal incubation temperature of *V. dahliae* is about 23°C and cultures are degraded in prolonged culturing which cause irreversible changes such as reduction or complete loss of the ability to produce microsclerotia, conidia become completely hyaline and there is reduction of pathogenicity (Harris, 1993).

Use of fresh isolates is necessary during identification since plant pathogenic *Verticillium* species may lose some of their distinctive characters in culture. Camele *et al.* (2005) attempted identification of *Verticillium dahliae* causing Verticillium wilt in *Solanum aethiopicum.* The study was based on morphological and molecular characterization using ITS5/ITS4 based on the fungal nuclear ribosomal DNA (rDNA) repeat sequences. Sequence analysis of the ITS regions revealed that the isolate of *V. dahliae* infecting African eggplant (GenBank accession no. AJ970308) is identical to a Greek strain of *V. dahliae* (GenBank accession no. AF104926). There is much evidence for variation in pathogenicity in *V. dahliae* which may be quantitative or qualitative (Barbara *et al.,* 1998). Isolates are often found to be more pathogenic to the host from which they were isolated than to other hosts. Vegetative compatibility has been widely used to examine populations of *V. dahliae.* Four main vegetative compatibility groups (VCG) of *V. dahliae* have been identified based on nitrate nonutilising (*nit*) mutants (Barbara *et al.,* 1998). It was also possible to distinguish defoliating types from non-defoliating types by several

morphological and physiological traits: the shape of microsclerotia, optimum temperature for growth, growth rate and fluorescence on sanguinarine-amended media (Barbara *et al.,* 1998).

Morphological characterization of 67 *V. dahliae* isolates from pepper based on VCG indicated that 67% belonged to VCG 2, 22% to VCG 4, and 11% to a new group which was designated VCG 6. Pathogenicity tests were done on one month-old bell pepper (cv. Cal Wonder) and tomato (cv. EP 7) seedlings. Seedlings of cv. 'Cal Wonder' were susceptible only to the isolates of *V. dahliae* from pepper, whereas seedlings of cv. 'EP 7' were susceptible to both pepper and tomato isolates. Isolates of pepper belonging to VCG 2, VCG 4, and VCG 6 were found to be highly pathogenic to bell pepper and chili pepper. Molecular characterization of the isolates was also done by use of RAPD analysis which showed minor variations among the isolates; however there were unique polymorphic banding patterns found on isolates in VCG 6 (Bhat *et al.,* 2003).

2.4 Host Resistance of Eggplants to Wilt Pathogens

There is the potential of introgressing genes for disease resistance from wild relatives, although crosses of *S. melongena* with the wild species are limited by loss of heterosis in subsequent crosses as observed by Oyelana and Ogborogho (2008). Wild relatives may be used as rootstocks, although this is also limited by graft incompatibility, thin stems, and slow growth (Sakata, 1991). Nonetheless, successful crosses have been made in several studies. Fertile interspecific crosses of *S. macrocarpon* with *S. aethiopicum* L. and *S. melongena* have been accomplished and *S. macrocarpon* has been found to be a good genitor for inducing disease and pest resistance in eggplants (*S. melongena*) (Grubben and Denton, 2004). F_1 and F_2 hybrids between the various groups of *S. macrocarpon* (including wild *S. dasyphyllum*) show heterosis and F_2 superior hybrids have been isolated as candidates for future breeding programmes.

Two eggplant accessions of *S. aethiopicum* gr. Gilo and *S. aethiopicum* gr. Aculeatum *(S. integrifolium*) are known to carry a gene for resistance designated as *Rfo-sa1,* to the fungal wilt disease caused by *Fusarium oxysporum* f. sp. *melongenae* (Toppino *et al.,* 2008). The resistant trait was introgressed into the cultivated eggplant *S. melongena* L. by backcrossing it to the two resistant accessions. The resistance genes from *S. aethiopicum* and *S. integrifolium* were found to be alleles of the same locus. In this study of Toppino *et al.*(2008), sequence-related amplified

polymorphism (SRAP), sequence-related amplified polymorphism-resistance gene analog (SRAP-RGA), random amplified polymorphic DNA (RAPD) and cleaved amplified polymorphic sequences (CAPS) markers were identified to represent useful tools for indirect selection of *Fusarium* resistance in eggplant since they confirmed the monogenic inheritance of the resistance. Mutlu *et al.* (2008) used these markers to tag the Fusarium wilt resistant gene designated as FOM. A study to screen eggplants *Solanum* spp. on inoculation against four isolates of *Fusarium oxysporum* f. sp. *melongenae* (FOM) found an accession from *S. aethiopicum* L group Gilo that was resistant to all four fungal isolates (Stravato and Cappelli, 2000). The sources of resistance found in this wild *Solanum* spp are useful in breeding aubergine cultivars resistant to Fusarium wilt.

An accession of *S. aethiopicum* group Aculeatum is also known to be resistant to Fusarium wilt and therefore its rootstock is used in propagation of eggplants in Japan. The Fusarium wilt resistant accession LS2436 and bacterial wilt resistant accession LS1934 of *S. melongena* L were identified by Yoshiteru *et al.* (1996). The two accessions are of limited commercial value on account of slow growth and low yields; therefore they could only serve as sources of resistance in breeding programs. A cross between LS1934 from Malaysia which is resistant to bacterial and Fusarium wilt with WGCR 112-8 from India which is bacterial wilt resistant produced F1 hybrids that showed high levels of resistance to both bacterial and Fusarium wilts. Its rootstock 'Daitaro' has successfully been used to graft scions of high yielding cultivars. It is highly resistant to both bacterial and Fusarium wilt and highly adaptable to mechanical grafting (Monma *et al.,* 1996). Another rootstock used for its resistance to bacterial wilt and Fusarium wilt is 'Diazaburou'. Diazaburou has been found to be more superior to 'Daitaro' since it has a higher level of resistance to bacterial wilt (Yoshida *et al.,* 2004). Three wild species (*S. abutiloides*, *S. scabrum*, and *S. toxicarium*) have been found to have a high level of resistance to bacterial wilt and *Verticillium* wilt (Mochizuki and Yamakawa 1979; Sakata 1991). An efficient plant regeneration system via protoplast culture has been developed for *S. integrifolium* and its wild relatives that are resistant to wilt diseases (Iwamoto and Ezura, 2006). This shows there is a high potential of disease resistance from the large diversity (Appendix 10) of eggplants that could be introgressed in to the cultivated varieties and therefore the need to invest time and resources in their study.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental Site Description

The study was conducted at the Asian Vegetable and Research Development Centre- Regional Centre for Africa, (AVRDC-RCA) in Arusha, Tanzania. AVRDC-RCA is located at Madiira Farm, Arumeru district, which is 15 km from Arusha town. The area is situated at an altitude of 1,290 m asl, latitude 4.8°S and longitude 37°E. The average annual rainfall is 700-1000 mm per annum and temperatures range from 14.1°C to 30.5°C. The soil type is clay loam with a pH between 6.0 and 6.5 (Oluoch and Chadha, 2006). The study was carried out between June 2009 to June 2010.

3.2 Pathogen Isolation

Wilting eggplant samples were collected from AVRDC-RCA experimental fields, RCA seed production fields, RCA Demonstration fields, Manyani village, Manyire village, Rwadarige village and Shangalai village in Arumeru district, Tanzania (Appendix 11) between July and November 2009. Isolates were obtained from discoloured vascular tissue of eggplant stems. Tissues from discoloured stem lesions of the plant collar were cut, washed with tap water to remove soil, surface sterilized in a petri dish containing 10% sodium hypochlorite solution (Clorox ®) for 5 minutes, placed in 75% alcohol for 30 seconds, and rinsed three times with sterile distilled water. The excised segments were then placed on sterilized air-dried filter paper to dry. The pathogens were isolated using the methods described by Lester *et al.* (1988). The media and incubation conditions used favoured the growth and sporulation of a wide range of fungal and bacterial pathogens (with the exception of oomycetes like *Phytophthora* spp. that require very low temperature to grow and sporulate). Potato Dextrose Agar (PDA) was prepared and autoclaved for 15 minutes at 121°C, allowed to cool to 45°C and 300ppm of streptomycin sulphate was added to inhibit bacterial growth. The media was dispensed into 9 cm plastic petri dishes and allowed to solidify. The surface sterilized tissues were transferred onto petri dishes containing PDA and placed in a lit culture room without humidity control for 5 days at 25°C.

Identification through cultural and microscopic features was done to differentiate expected eggplant pathogens from the other pathogenic and non pathogenic species. Sub-culturing was done to obtain pure cultures on 25% PDA for 7-10 days at 25°C (Larone, 1995). For consistent sporulation and pigmentation, culture plates were kept 40 cm below cool white fluorescent tubes (1000 lux) and illuminated for 12 hour periods of 25°C day/20°C night for 10 days. After incubation, single spores were obtained by preparing five serial dilutions of conidia suspensions. Three drops of the last dilution were pipetted onto the surface of a petri dish containing water agar (15 g of Bacto agar per liter of double distilled water (ddH_2O), and the plates were shaken horizontally on a laboratory bench to disperse the drops containing conidia. The water agar dishes were incubated for 18 to 20 hours at 25°C (Singleton *et al.*, 1992) in the dark to allow for conidia germination. Single germinating conidia were picked and transferred to fresh PDA media and incubated at 25°C to produce single spore cultures. The resulting single spore cultures were preserved at 4°C for future use.

3.3 Determination of the Predominant Pathogen

Cultural and microscopic identification of the isolates collected was carried out in the laboratory. The number of single spore cultures collected of *Fusarium* spp. and *Phoma sorghina* were counted and the cultures with the highest frequency determined. The isolates were characterized based on cultural and morphological characters and further confirmation was done by sequence analysis of the rDNA ITS region and the α-Translation Elongation Factor (TEF).

3.4. Morphological and Cultural Characterization of Collected Isolates

Cultures were incubated at 25°C for 10 days at an alternating 12 hour periods of 25°C day/20°C night. Microconidia and macroconidia were observed in 10 day old cultures grown on SNA (Singleton *et al.*, 1992). Microscopic slide preparations were examined on spores mounted on distilled water at x40 magnification. Observations were done for characteristics (Table 2) of the macroconidial shape on sporodochia, macroconidial septation, shape of basal cell, shape of apical cell, presence of microconidia, microconidial shape, conidiophores in the aerial mycelium and presence or absence of clamydospores according to Seifert (1996). Measurements were taken from a total of 30 ma- and microconidia per isolate.

EGERTON UNIVERSITY LIBRARY

Colony characteristics were determined for all single spore isolates. The spores were grown on PDA media and the plates were incubated at 25°C for 10 days (Nelson *et al.,* 1983; Singleton *et al.,* 1992; Ghoneem *et al.,* 2009). After incubation, cultures were examined for growth rate, colony reverse colour, texture, colour and shape of aerial mycelium and the development of pigments in the agar medium (Seifert, 1996).

Table 2: Categories of morphological characteristics used to compare isolates collected

Characteristic	Details	Character state
Pigmentation of aerial mycelium	Blank	Factor
	Violet	"
	White	"
	Orange	"
	Cream	"
	Black	"
	Pink	"
	Green	"
Pigmentation on colony reverse	Blank	Factor
	Cream	"
	Brown	"
	Orange	"
	Violet	"
	White	"
	Pink	"
	Black	"
Colony diameter after 10 days		Ordered Factor
	Blank	1
	< 3 cm	2
	≥ 3 cm to 7 cm	3
	> 7 cm	4
Aerial mycelium on PDA		Ordered Factor
	Blank	1
	Abundant	2
	Sparse	3
Prescence of clamydospores		Ordered Factor
	Absent	1

Table 2 contd.	Present	2
Shape of microconidia		
According to Seifert, (1996)		Factor
	A	"
	B	"
	C	"
	D	"
	E	"
	F	"
	G	"
	H (absent)	
Shape of macroconidia		Ordered Factor
According to Seifert, (1996)	A	"
	B	"
	C	"
	D	"
	E	"
	F	"
	G	"
	H (absent)	"
Macroconidia septation		Ordered Factor
	3 to 7	1
	> 7	2
	None	3
Shape of apical cell		Factor
	Blunt	1
	Hooked	2
	Conical	3
	Nipple like	4
	None	5
Shape of basal cell		Factor
	Notched/ foot shaped	1
	Blunt	2
	Papillate	3
	Extended foot	4
	None	5
Macroconidia length		Numerical
Macroconidia width		Numerical

3.5 Analysis of Data on Morphological and Cultural Characteristics

The qualitative data on mophological characteristics were pooled in a square matrix, with equal weight. A dissimilarity matrix was generated in the CLUSTER ANALYSIS module of the R console version 2.11.1, which used the percent disagreement (simple mismatch coefficient [SM]) calculated as SM = m/n where m = the number of mismatches between paired isolates and n = the total number of characters. The matrix was analyzed using the UPGMA method to generate a phenogram. Percent disagreement was used as distance measure because the data for the dimensions included in the analysis were categorical in nature.

3.6 Molecular characterization

Selected isolates of nine *Fusarium* spp and five *Phoma sorghina* isolates that represented a range of morphological types were characterized by analysis of the ITS region and α – translation elongation factor (Figure 3.1 and 3.2). The representative isolates were sent to the Agricultural Institute of Slovenia, Ljubljana, Slovenia for molecular characterization. The same approach was applied to identify the non-fusaria using the GenBank database. It involved obtaining a pure culture of an isolate, extracting genomic DNA, amplification of the TEF gene region, and sequencing. BLAST was then used to identify the closest matches between the unknown sequence and those contained in the FUSARIUM-ID sequence database.

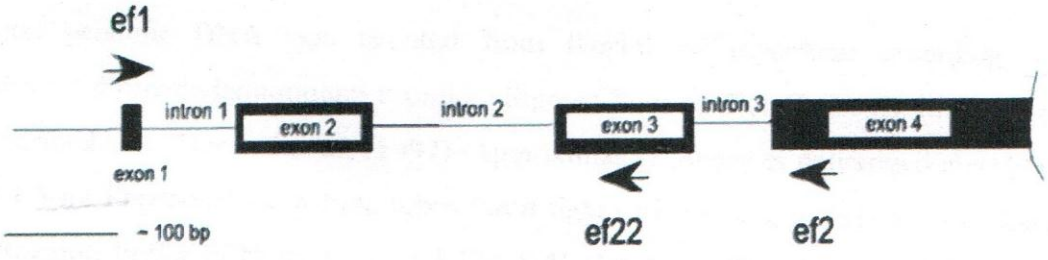


Figure 3. 1: Map of the translation elongation factor region that was amplified, sequenced, and used in the identification of *Fusarium* species in this study.

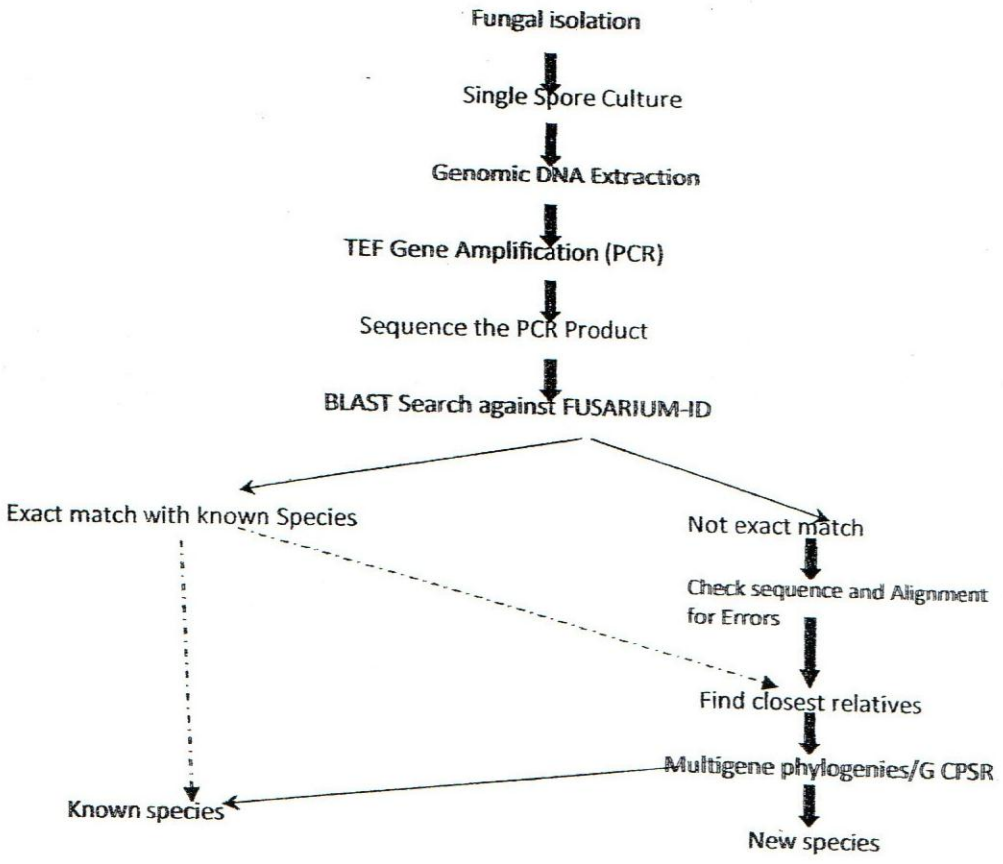


Figure 3. 2: Flowchart describing the process of using TEF sequences used to identify *Fusarium* species in this study. (Geiser *et al.*, 2004).

3.7. DNA Isolation

Total genomic DNA was isolated from lyophilized mycelium according to the CTAB (hexacetyltrimethylammonium bromide; Sigma Chemical Co., St. Louis, Mo.) miniprep protocol described by O'Donnell *et al.* (1997). Approximately 50 mg of pulverized mycelium was placed in 1.5-ml Eppendorf microfuge tubes (with tight fitting caps), resuspended in 700 ml of CTAB extraction buffer (100 mM Tris-Cl [Ph 8.4], 1.4 M NaCl, 25 mM EDTA, 2% CTAB) and vortexed for 10 s. After extraction, an equal volume of chloroform was added to each tube and vortexed for 5 s, followed by centrifugation at 12,000 *g* for 10 min. A 500-ml portion of the upper phase was removed to a new 1.5-ml tube, and DNA was precipitated by the addition of an

equal volume of -20°C isopropanol. After the DNA was pelleted at 12,000 *g* in the microcentrifuge for 1 min, the supernatant was discarded and the pellet was gently washed with 70% ethanol and resuspended in 200 ml of TE buffer (10 mM Tris-Cl [pH 8.0]–1 mM EDTA [pH 8.0]). For PCR amplifications, 8 ml of the genomic DNA stock was diluted in 1 ml of deionized water and stored at -20°C. For PCR , 25 ml of the diluted genomic DNA was added to an equal volume of a 23 PCR master mix.

3.7.1 PCR and DNA sequencing

The primer pairs ef1 (forward primer; 5' -ATGGGT AAGGA (A/ G)GAC AAGA C-3') and ef2 (reverse primer; 5' -GGA(G/A)G TACCA GT(G/C)ATCA TGTT-3') were used for PCR amplifications (O'Donnell *et al.* 1998) of a partial fragment of the translation elongation factor 1-alpha gene intron and exons (*tef1*). A PCR System 9700 (PE Applied Biosystems, Foster City, California) with ramp speeds of the PCR System 9600 was used for PCR amplification with the following program: an initial denaturation step at 94°C for 2 min, 35 cycles of denaturation at 94°C for 35 s, annealing at 52°C for 50 s, extension at 72°C for 2 min, and a final extension at 72°C for 6 min. Each 50 µL PCR reaction contained 1 µL genomic DNA extract, 25 pmol of each PCR primer, 200 µmol of each dNTP (Amersham Biosciences, Freiburg, Germany), 1 U of Taq polymerase (Super Taq, HT Biotechnology, UK) and 1 x standard PCR buffer supplied with the Taq polymerase. All amplicons were viewed over a UV transilluminator after electrophoresis through a 1% agarose gel and ethidium bromide staining. An ~ 700 bp product was amplified from all isolates. The PCR fragments or amplicons were purified with the GFX® purification kit (Amersham Pharmacia Biotech Inc., Roosendaal, Netherlands), sequenced with the BigDye terminator cycle sequencing kit (PE Applied Biosystems) and analyzed on an automated ABI Prism 3700 (Applied Biosystems) capillary DNA sequencer. The sequencing primers ef1 and ef2 were used for sequencing of the *tef1* region. Using the latest capillary technology, a DNA sequence generated from the ef2 primer alone usually produces high enough quality data to produce an unambiguous identification. However, sequencing from both directions using the ef1 and ef2 primers, as was the case in this study, provided an internal check for sequence quality.

3.7.2 Molecular identification

The BLAST search tools of the FUSARIUM-ID server at http://fusarium.cbio.psu.edu and the GenBank database that is accessible via the Entrez website at the US National Center for Biotechnology Information (NCBI): http://www.ncbi.nlm.nih.gov/Entrez/ were used to query unknown sequences against the database. FUSARIUM-ID was relied upon for identification of *Fusarium* species because it contains vouchered and well-characterized sequences that correspond to publicly available cultures that can be used for confirmation. When the FUSARIUM-ID search results indicated clearly that an isolate was non-fusaria, a BLAST search was conducted using GenBank to verify the identity of the isolate.

3.8 Pathogenicity Tests

Pathogenicity tests were carried out in a screen house covered at the top with a UV-resistant polyethylene. Plug trays were filled with sterilized media (forest soil mixed with sand at the ratio of 3:1). Seeds of two African eggplants (MM 1131 and N19) observed to be susceptible and one observed to be resistant (SIVONKWE) were planted in these trays and maintained in a screen house. For each isolate tested, fifteen seedlings of each of the three eggplants were used. They were watered to field capacity and fertilized weekly. Selected *Fusarium* spp. isolates (n=6) that represented a range of the predominant species were tested for pathogenicity on the eggplant seedlings. Conidia were harvested from 14 day old cultures grown on PDA at 25°C by adding sterile water to the plates and scraping the surface of the culture with a sterile glass slide. The resulting conidial suspension was filtered through two layers of cheesecloth to remove mycelia fragments. Spore concentrations were then determined using a hemacytometer and adjusted in sterile water to a concentration of 1×10^6 conidia per ml.

Inoculation was done on seedlings at the six leaf stage (Altnolk, 2005). The seedlings were lifted gently from the trays and the soil washed off. The root tip of each seedling was cut to two thirds its length and immersed into 5mls of the conidial suspension for three minutes (Yoshiteru *et al.*, 1996; Romberg and Davis, 2007). The seedlings were planted in 15cm diameter plastic pots containing sterile soil and placed in a screen house. The experiment was laid in a Randomized complete block design with five replicates. The experiment was repeated once. The plants were

monitored daily for wilt development and the type of symptoms and extent of disease severity recorded. To confirm the pathogenicity of the isolates, infected root tissues were randomly cultured on PDA and colonies of *Fusarium* spp. regenerated were identified based on the cultural and morphological characteristics.

Foliar symptoms of each plant were scored at the 4^{th}, 8^{th} and 12^{th} week after inoculation on a scale of 1 to 5 as follows: 1 = no symptoms; 2 = slight wilt and yellowing of the lowest leaves; 3 = half of the leaves wilted or showing yellowing; 4 = almost all the leaves wilted or showing yellowing; and 5 = all the leaves wilted, showing yellowing or plant died. The response for each accession and cultivar was determined against a mean severity index (MSI) calculated according to the following formula (Matsubara *et al.*, 2004):

$$MSI = \frac{\Sigma(\text{Number of plants x Degree of symptoms})}{\text{Total number of plants x 5}}$$

3.9 Screening for Disease Resistance

Evaluation of eggplants for wilt resistance was done according to Yoshiteru *et al.*, (1996) with modifications. 93 eggplant accessions of *S. macrocarpon, S. aethiopicum, S. anguivi, S. dasyphyllum* and *S. melongena* (Appendix 10) were inoculated at 5-week-old stage (2 to 3 true leaves stage) using root dip inoculation technique. Isolate Fs 40 collected from *S. melongena* was used in inoculating the accessions. Six seedlings of each accession were planted in 15-cm-diameter plastic pots (1 plant per pot) filled with sterilized soil. The soil was drenched with 5ml of inoculum ($1x10^6$spores ml^{-1}) and the seedlings grown in a screen house. The experiment was laid out in a randomized complete block design with three replicates. The experiment was repeated once. The response of each accession and cultivar was determined against a percentage disease index (PDI) calculated according to the following formula (Matsubara *et al.*, 2004):

$$PDI = \frac{\Sigma(\text{Number of plants x Degree of symptoms})}{\text{Total number of plants x 5}} \times 100$$

The scoring for PDI was as follows: Less than 20% = symptomless; 20% - 40% = slight wilting and yellowing of the lowest leaves; 40% - 60% = half of the leaves wilted or showing yellowing; 60 – 80% = almost all the leaves wilted or showing yellowing and 80% – 100% all the leaves wilted, showing yellowing or plant died. Accessions with a mean disease index ≤ 2 were considered resistant and a disease index ≥ 2 were considered susceptible. The response of plants was also determined against a mean disease index (MSI).

3.10 Data Analysis

Statistical data was analyzed for significant differences using analysis of variance (ANOVA) and comparison of means among accessions was done using Tukeys HSD. Co STAT statistical package (COHORT Software, Minneapolis, USA), was used to analyze the data. The level of probability was set at $P \leq 0.05$. The area under disease progress curve (AUDPC) was assessed for the second trial in screening for resistance experiment. Using the same data of the second trial, a disease progress curve was plotted based on the following formula:

$$\sum_{i=1}^{n-1} [(ti + 1 - ti)\ (yi + yi + 1)/2]$$

Where "t" is time in days of each reading, "y" is the average disease index and "n" is the number of readings (Campbell and Madden, 1990).

CHAPTER FOUR

RESULTS

4.1 Predominant wilt pathogen

A total of 57 isolates were collected from the seven sample sites that were not less than 10 km among each of them. Among the isolates collected from seven farms, 37 were *Fusarium* spp. and 19 were *Phoma sorghina* (Table 3). Isolates were collected from plants exhibiting chorosis, necrosis and wilting (Figure 4.1).

Table 3: Source and number of isolates collected (July to November 2009)

NO.	SITE	*Fusarium* spp.	*Phoma sorghina*
1.	RCA experimental fields	9	4
2.	RCA seed production fields	6	4
3.	RCA Demonstration fields	4	3
4.	Manyani	5	3
5.	Manyire	4	0
6.	Rwadarige	3	3
7.	Shangalai	6	2
TOTAL		37	19

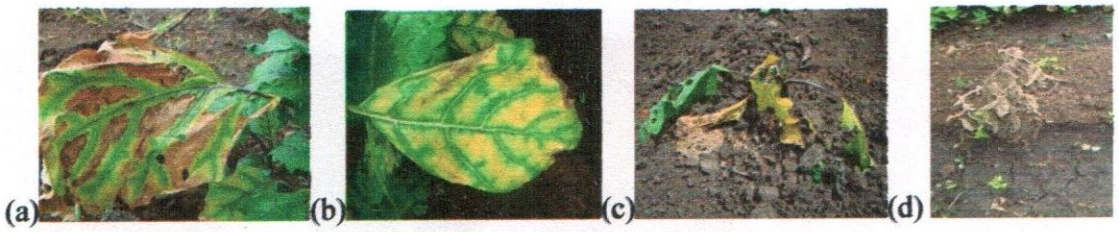


Figure 4. 1: Plant samples from which isolates were collected (a & b) Symptoms of wilting on leaves of diseased plants (c) Partially wilted plant, (d) Completely wilted plant.

4.2 Characteristics of isolates collected

Morphological and cultural characterization was done on the colony and sporulation apparatus. Figures 4.2, 4.3, 4.4, 4.5, 4.6 and 4.7 show some of the cultural and morphological characteristics of the Fusarium isolates collected that were used in the characterization.

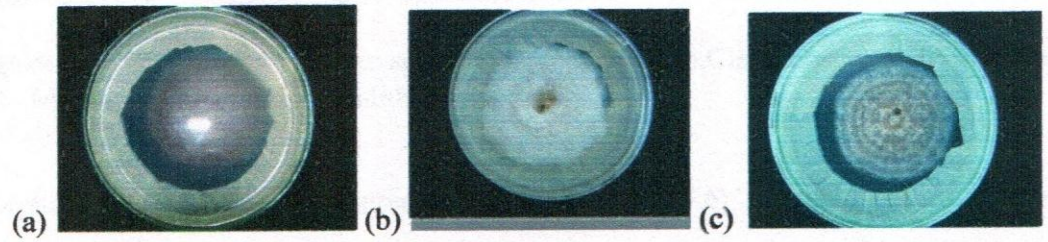


Figure 4. 2: Pigmentation of aerial mycelium of some of Fusarium cultures collected: (a) Purple, (b) Cream, (c) Orange.

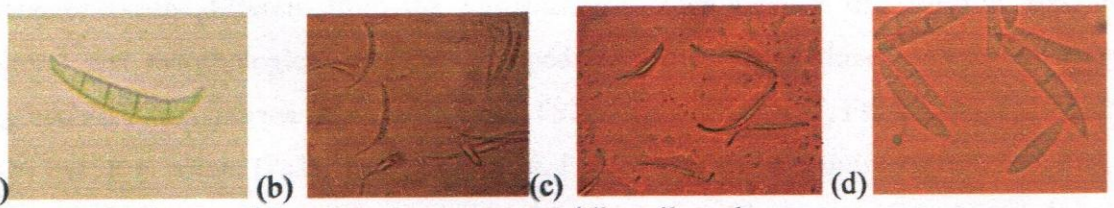


Figure 4. 3: Shapes of *Fusarium* spp. macroconidia collected

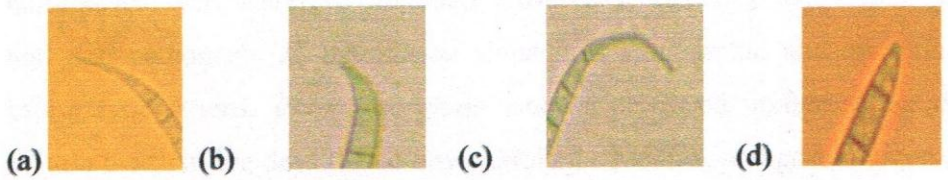


Figure 4. 4: Shapes of apical cell of *Fusarium* spp: (a) Conical, (b & c) Hooked (d) Blunt

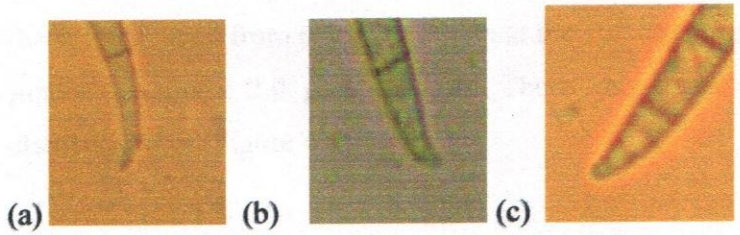


Figure 4.5: Shapes of basal cell of *Fusarium* spp: (a) Extended foot, (b) Notched foot, (C) Blunt foot

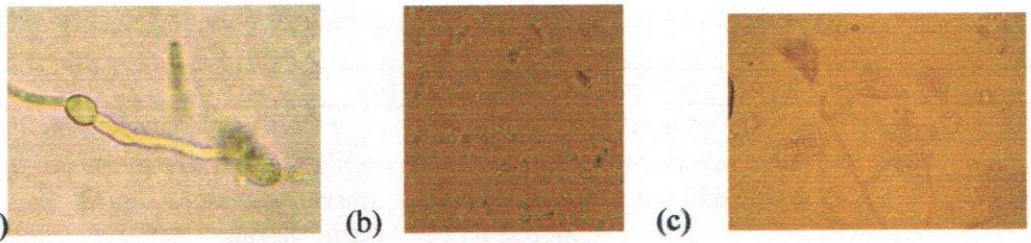


Figure 4. 6: Other features of the sporulation apparatus (a) Clamydospores (b) Microconidia (c) Microconidia on monophialides

Morphological characters (Figures 4.3, 4.4, 4.5, and 4.6) appeared inconsistent among the isolates. Marked differences between the *Fusarium* spp. were observed in colour, macroconidia shape, apical and basal cell shape. The out group was *Phoma sorghina* (Figure 4.8) which was characteristically different from the *Fusarium* spp. Identification of *Fusarium* spp. through cultural and morphological analysis, grouped the isolates into three representatives species aggregates; *F. oxysporum* (Fs9, Fs18, Fs20. FS24, Fs12, Fs30, Fs7, Fs26, Fs40, Fs10 Fs29 Fs1, Fs26 Fs15), *F. solani* (Fs35, Fs22, Fs2, Fs16, Fs17, Fs34) and *F. equisseti* (Fs4, Fs5, Fs3, Fs14, Fs27, Fs23 Fs 8) (Table 4a, b and c). Ten of the thirty seven isolates collected were degenerated cultures and were therefore discarded. However it was not possible to distinguish the pathogenic and non pathogenic *F. oxysporum* since it is anamorphic and comprises of pathogenic and saprophytic strains. *Phoma sorghina* isolates produced immersed aerial mycelium on PDA. Within the first five days the colony exhibited a reddish, orange and green diurnal ring of colours and by the 10^{th} day the colony colours turned olivaceous green to smoke grey. The colony reverse had a coral appearance. Abundant black pycnidia was scattered over the culture. Colony diameters ranged from 60mm to 70mm at the 10^{th} day. Conidia length and width ranged from 5.6 µm ~ 6.1 µm x 2.0 µm ~2.9 µm. There was formation of both intercalary and terminal clamydospores (Figure 4.8).

Table 4: Cultural and morphological characteristics of *Fusarium* spp.

(a) *Fusarium solani* (see also Figure 4.7 a)

Cultural characteristics on PDA	Morphological characteristics on SNA	Isolates identified
Pigmentation; Aerial mycelium varied from white to cream. Colony reverse; varied from white to brown. Growth rate: Colony diameter >7cm on the 10th day on PDA: Aerial mycelium was sparse.	Macroconidia; Apical and basal cell were blunt with a sausage like shape. 2 to 4 septate. Dimensions ranged from 28.5 µm ~ 32.5 µm x 4.1 µm ~ 5.1 µm Microconidia: 1 to 2 celled; shapes: oval to ellipsoidal.	Fs35 Fs22 Fs16 Fs17 Fs34 Fs2

(b) *Fusarium oxysporum* (see also Figure 4.7 b)

Cultural characteristics on PDA	Morphological characteristics on SNA	Isolates identified
Pigmentation; Aerial mycelium varied from white, pink, cream and orange.	Macroconidia; The basal cells varied from Blunt to foot-notched and the apical cell were blunt to conical.	Fs10 Fs12 Fs30
	Three to five septate	Fs24
Colony reverse; orange and violet	Macroconidia shape; Narrow and slightly curved.	Fs20
	Macroconidia dimensions; ranged from 36. 3µm ~ 47.3 µm x 4.3 µm ~4.7 µm.	Fs28 Fs26
Growth rate; The aerial mycelium was fast growing and abundant. Colony diameter was greater than 7 cm within the 10th day	Abundant prescence of macroconidia, microconidia and clamydospores within the 10th day.	Fs15 Fs40 Fs7
	Microconidia; The microconidia were formed abundantly. Single celled to kidney shaped and formed on false heads.	Fs18 Fs9 Fs 29, Fs 1
	Conidiophores; The monophialides were short compared to those of *F. solani*	

(c): *Fusarium equiseti* (See Figure 4.7 c)

Cultural characteristics on PDA	Morphological characteristics on SNA	Isolates identified
Pigmentation; Aerial mycelium was constantly orange Colony reverse; brown.	Macroconidia; Apical cell was characteristically long and whip like. Basal cell was an extended foot.	Fs4 Fs3 Fs5
	Macroconidia 4 to 5 septate	Fs14
Growth rate; Rapid growth	Macroconidia dimensions; ranged from 39.2 µm ~ 56.7 µm x 4.1 µm ~ 5.9 µm	Fs27 Fs 23
Colony diameter was greater than 7 cm within the 10th day. Aerial mycelium was abundant	Microconidia; Absent in some isolates and in others there was the prescence of ellipsoidal microconidia.	Fs 8

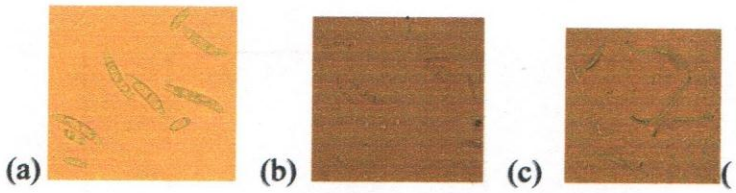


Figure 4. 7: Macroconidia of representative *Fusarium* spp. isolates (a) *Fusarium solani* (b) *Fusarium oxysporum* (c) *Fusarium equiseti*



Figure 4. 8: Pictures of *Phoma sorghina* (a) *Phoma sorghina* culture colony reverse on 14th day in ¼ PDA. (b). *Phoma sorghina* culture aerial mycelium on 5th day in ¼ PDA , (c,) Conidia, (d) Chain of unicellular intercalary clamydospores, (e) Chain of unicellular terminal clamydospores, (f) Pycnidia

A dendrogram generated from cluster analysis of the pooled data grouped the fungal isolates in clusters according to the assigned species and groups (Figure 4.9). Groups 1, 2, 3, 4 were assigned as *Phoma sorghina*, *Fusarium equiseti, Fusarium oxysporum* and *Fusarium solani* respectively. *Phoma sorghina* isolates (Group 1) appeared to be genetically distant from all the Fusaria (distance of 0.60) while the Fusaria appeared to be closely related to each other. There was a distance of 0.47 between *F. equiseti* (Group 2) and *F. oxysporum* (Group 3) and 0.08 between *F. oxysporum* and *F. solani* (Group 4).

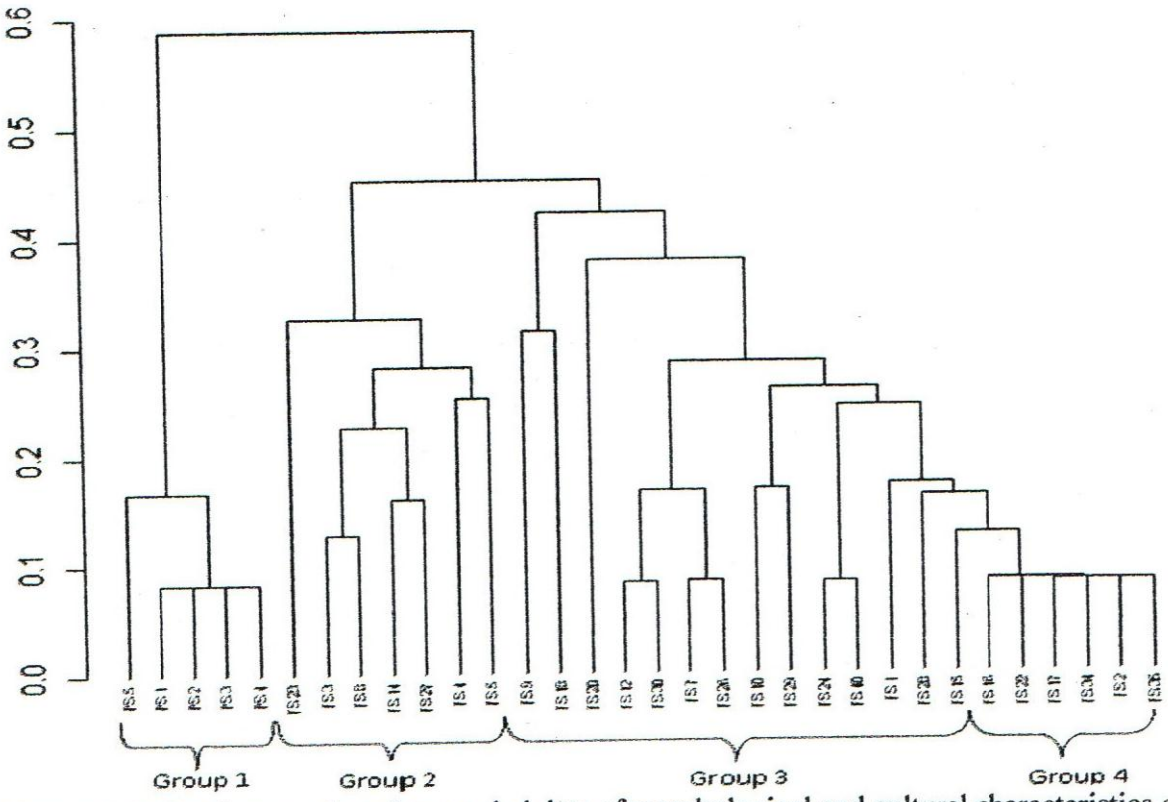


Figure 4. 9: Dendrogram based on pooled data of morphological and cultural characteristics of *Fusarium* spp. and *Phoma sorghina* isolates as the out group using the UPGMA algorithm

4.3 Molecular Characterization of representative isolates

The ITS and TEF sequences (Figure 4.10; Appendix 10) from each of the 14 representative isolates were copied and pasted into a web browser connected to the FUSARIUM-ID and GenBank BLAST servers and used as a query for comparison to the database. Nucleotide BLAST retrieved the closest matches to the query sequence, and presented the matches as a series of DNA alignments with corresponding percentage match information. In some cases, an exact sequence match was observed between a query sequence and a known isolate in the FUSARIUM-ID and/or GenBank database, which is suggestive of an unambiguous species identification. In some cases, TEF nucleotide sequences differed by one or a few nucleotides. In both instances, species ID suggested by the closest DNA sequence match was confirmed by reference to the morphology and pathogenicity of the isolate. Of the 14 isolates (Table 5), one

was identified as belonging to *F. solani* species, two to *F. oxysporum* species (FOM), six to the *F. incarnatum-equiseti* species complex (FIESC) and five to *Phoma sorghina.*

Representative isolates morphologically considered to be *F. equiseti* were identified as belonging to the *Fusarium incarnatum-F. equiseti* species complex. FS 23 was close to NRRL 20423, 52753 (JF740828) differing by 3 substitutions, when it was compared with NRRL 52753 the identities were 645/652 with a 99% similarity. When compared with NRRL 20423: The identities were 633/641 with a 99% similarity. Fs 3 was close to NRRL 20423, 52753 (JF740828) differing by 3 substitutions. When compared with NRRL 52753: the identities were 645/652 with a 99% similarity, compared with NRRL 20423: it had 633/641 identities with a 99% similarity. Fs 14 was close to NRRL 20423, 52753 (JF740828) differing by three substitutions, when compared with NRRL 52753, identities were 645/652 with a 99% similarity. When compared with NRRL 20423, the identities were 633/641 with a 99% similairity. Fs 34 was close to NRRL 20423, 52753 (JF740828) differing by three substitutions, when compared with NRRL 52753, identities were 679/682 with 99% similaity. Fs 5 was an unpublished EF haplotype, Fs 40 was similar to NRRL 46605 and some others f. sp. *pisi* and *melonis*. FS 24 had a 100% simillarity to NRRL 44900 (GU170541). When Fs 35 was compared with NRRL 43536 there were 701/703 identities with 99% similarity.

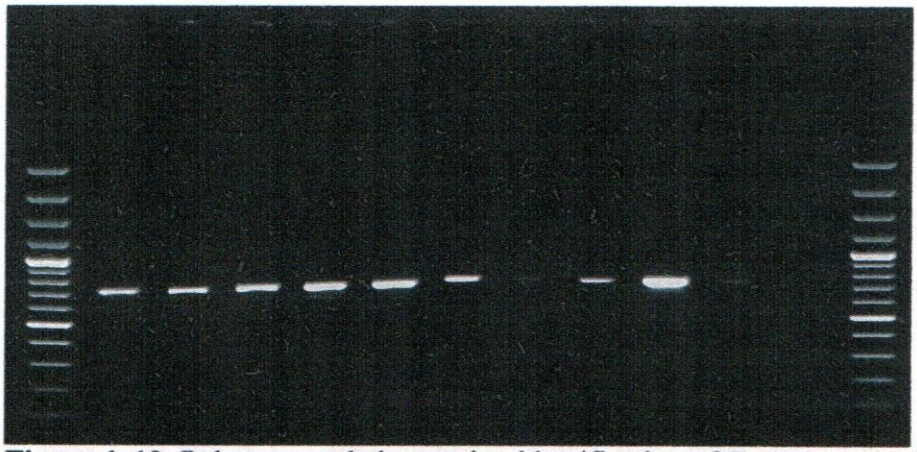


Figure 4. 10: Polymerase chain reaction identification of *Fusarium* spp. isolates

Table 5: Molecular characterization of representative *Fusarium* isolates

FS 27	Morphologically *F. equiseti*-like
FS 23	*F. lacertarum* (morphologically *F. equiseti* like)
FS 3	*F. lacertarum* (morphologically *F. equiseti* like)
FS 14	*F. lacertarum* (morphologically *F. equiseti* like)
FS 34	*F. lacertarum* (morphologically *F. equiseti* like)
FS 5	FIESC, inbetween "Incarnatum group" and "Equiseti group"
FS 40	*Fusarium oxysporum* species group
FS 24	*Fusarium oxysporum* species group
FS 35	*Fusarium solani* species group
Ps 1	*Phoma sorghina*
Ps 2	*Phoma sorghina*
Ps 3	*Phoma sorghina*
Ps 5	*Phoma sorghina*

Molecular characterization of isolates done at the Agricultural Institute of Slovenia, Ljubljana. The isolates were submitted to the NCBI genebank (Appendix 11).

The non Fusaria isolates were identified as *Phoma sorghina.* The blast results showed 100% identity with Ps 1, Ps 2, Ps 3, Ps 5 (Appendix 11)

4.4 Pathogenicity Tests

Pathogenicity tests were conducted with representative isolates of *Fusarium* spp. (FS 24, FS 40, FS22, FS 27, FS 35, FS 3) on accessions MM 1131 (*S. macrocarpon*), N 19 (*S. anguivi*) and Sivonkwe (*Solanum aethiopicum)* (Figure 4.11). The mean severity index was used to evaluate resistance. There were no significant differences between the two trials. The tests confirmed pathogenicity of FS 27, FS 40, FS 24 FS 35 and FS 22 to specific accessions, FS 3 being non-pathogenic to all the accessions and the control causing no wilting (Figure 4.12, Table 6). There was rapid symptom development on accession MM1131 inoculated with isolate Fs 27 compared to the other isolates. Typical symptoms of eggplant Fusarium wilt were observed when seedlings of MM 1131 and N 19 (susceptible lines) were inoculated with isolates Fs 24, Fs 22, Fs 35, Fs 27, and Fs 40. Symptom development of the inoculated seedlings included drooping of leaves starting from the apical part of the plant and progressing downward, yellowing of the leaves which began from one side of the leaf and final wilting of the whole plant.

The disease development on the three accessions revealed variability in virulence amongst the isolates. Of the six isolates; Fs 27, Fs 40 and Fs 24 were identified to be highly virulent to their hosts, Fs 22 and Fs 35 were moderately virulent and Fs 3 appeared to be non-pathogenic. There was no symptom development in the control plants (Table 6). There was no variability in the virulence of the isolates characterized to be within the same species. Fs 40 and Fs 24, which were identified as *Fusarium oxysporum*, were pathogenic to the same hosts MM 1131 (*S. macrocarpon*) and *S. anguivi* (N 19). The two isolates were collected from the same host *S. melongena* (Table 7). The pathogenicity tests (Table 6) indicated that isolate Fs 27 in the FIESC was pathogenic to *S. macrocapon* (MM 1131) and *S. anguivi* (N 19). *Fusarium solani* caused wilting in *Solanum anguivi* (N19). Re isolation and culturing of the pathogen from the artificially infected stem tissues on PDA yielded colonies of the inoculated pathogens *F. oxysporum*, *F. solani* and *FIESC* and therefore comfirmed Koch's postulates.



Figure 4. 11: Stages of pathogenicity test (a) Seedlings at the 2 to 3 true leaf stage ready for inoculation, (b) Early symptoms on the leaf (C) All the leaves yellowing,

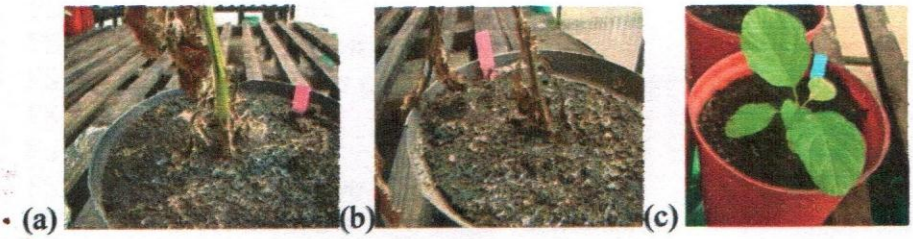


Figure 4. 12: Wilting in seedlings inoculated with the different isolates
(a) Plant inoculated with isolate FS 24, (b) Plant inoculated with FS 27, (c) Control plant.

Table 6: Disease severity scores of eggplant accessions after artificial inoculation with isolates of *Fusarium* spp.

	Mean Disease severity (1 – 5) Isolates tested for pathogenicity						
Accession code	Fs 24 *F.oxy*	Fs 35 *F.sol*	Fs 22 *F.sol*	Fs 27 *F. equi*	Fs 3 *F. lac*	Fs 40 *F.oxy*	Control dH_2O
MM 1131	3 ab	1.2 cd	1.2 cd	3 ab	1.0 d	3 ab	1.2 cd
N 19	2.8 abc	2.6 abcd	2.8 abc	4 a	1.6 bcd	2.8 abc	1.4 bcd
SIVONKWE	1.2 cd	1.8 bcd	1.4 bcd	2.4 abcd	1.4 bcd	1.4 bcd	1.0 d

*Means followed by the same letter are not significantly different following Tukeys test, ($P \leq 0.05$)
Foliar symptom scale: higher numbers indicate severity of disease
DH_2O: Distilled sterilized water

Table 7: Characaterization of the Fusaria and non Fusaria isolates

Isolate code	Host	Collection Site	Morphological Identification	Pathogenicity Test
FS 1	*S. aethiopicum* gr. Gilo	RCA	*F. oxysporum*	Nt
FS 2	*S. melongena*	Shangarai	*F. solani*	Nt
FS 3	*S. macrocarpon*	RCA	FIESC *	+
FS 4	*S. aethiopicum* gr. Kumba	Rwandarige	FIESC	Nt
FS 5	*S. aethiopicum* gr. Kumba	Manyire	FIESC*	Nt
FS 7	*S. melongena*	Manyani	*F. oxysporum*	Nt
FS 8	*S. macrocarpon*	Manyire	FIESC	Nt
FS 9	*S. aethiopicum* gr.Gilo	Rwandarige	*F. oxysporum*	Nt
FS 10	*S. melongena*	RCA	*F. oxysporum*	Nt
FS 12	*S. melongena*	Shangalai	*F. oxysporum*	Nt
FS 14	*S. macrocarpon*	RCA	FIESC *	Nt
FS 15	*S. aethiopicum*	Rwandarige	*F. oxysporum*	Nt
FS 16	*S. anguivi*	RCA	*F. solani*	Nt
FS 17	*S. anguivi*	RCA	*F. solani*	Nt
FS 18	*S. aethiopicum* gr.Gilo	Manyire	*F. oxysporum*	Nt
FS 20	*S. melongena*	Shangarai	*F. oxysporum*	Nt
FS 22	*S. anguivi*	RCA	*F. solani*	+
FS 23	*S. macrocarpon*	RCA	FIESC *	Nt
FS 24	*S. melongena*	Shangarai	*F. oxysporum*	+
FS 26	*S. melongena*	Manyani	*F. oxysporum* *	Nt
FS 27	*S. aethiopicum* gr.Kumba	RCA	FIESC *	+

Table 7 contd.

FS 28	*S. aethiopicum* gr Gilo	Nabala	*F. oxysporum*	Nt
FS 29	*S. melongena*	RCA	*F. oxysporum*	Nt
FS 30	*S. melongena*	RCA	*F. oxysporum*	Nt
FS 31	*S. melongena*	Manyani	*F. oxyporum*	Nt
FS 33	*S. anguivi*	RCA	*F. solani*	Nt
FS 34	*S. aethiopicum* gr Gilo	RCA	FIESC *	Nt
FS 35	*S. anguivi*	RCA	*F. solani**	+
FS 40	*S. melongena*	Shangalai	*F. oxysporum* *	+
PS 1	*S. aethiopicum* gr.Gilo	Manyani	*P. sorghina* *	Nt
PS 2	*S. melongena*	Shangalai	*P. sorghina* *	Nt
PS 3	*S. aethiopicum* gr.Kumba	RCA	*P. sorghina* *	Nt
PS 4	*S. aethiopicum* gr.Kumba	RCA	*P. sorghina* *	Nt
PS 5	*S. melongena*	Shangalai	*P. sorghina* *	Nt

Identification followed by an asterick were isolates confirmed by analysis of the ITS region and the α – elongation factor

\+ Isolate tested for pathogenicity

Nt Isolate not tested for pathogenicity

Reference (FIESC): O'Donnell *et al.* (2009)

4.5 Reaction of Eggplant Accessions to *Fusarium oxysporum* f.sp *melongenae*

Ninety three accessions were screened for reaction to *Fusarium oxysporum* f.sp *melongenae*. Among the 93 accessions, 47 were *Solanum aethiopicum*, four were *Solanum anguivi*, two were *Solanum dasyphyllum*, 12 were *S. macrocarpon*, 12 were *S. melongena* and 16 accessions whose species is unknown (Appendix 13). Inoculation was done at the 2 to 3 leaf stage using the root dip technique. Symptoms were observed weekly and some species exhibited early wilting whereas others had late wilting (Figure 4.13). Characteristic necrosis of the leaves from the edges towards the midrib was consistently observed within *S. aethiopicum* and *S. macrocarpon* accessions.

The inoculation method adopted gave good disease incidences in all trials, and provided a useful screening system for resistance to Fusarium wilt. Symptoms started after 7 days of inoculation. There were significant differences (Appendix 2, 3, 4) in disease severity between the two trials and this was attributed to the different prevailing weather conditions at the time the two trials were carried out (Table 8, Figure 4.14). Temperatures were higher and the condition were drier during the duration the 1st trial was carried out compared to the 2nd trial which was characterized

by heavy rains and temperatures as low as 13°C during the nights. The maximum and minimum temperatures in the 2nd trial ranged from 19°C to 29°C while those in the 1st trial ranged from 21°C to 34°C. Since the environment in the 1st trial presented the optimum conditions for Fusarium prevalence, it led to a higher disease severity than in the 2nd trial (Table 9).

The two trials showed that strain Fs 40 was virulent to the accessions (Table 9). There were significant differences ($P \leq 0.05$) in disease severity of accessions within the same species in their reaction to *Fusarium oxysporum* f.sp *melongenae* inoculation except for *S. dasyphyllum* (Appendix 5, 6, 7, 8 and 9; Table 10). *Solanum macrocarpon* species contained accessions that were highly susceptible such as MM 855 and MM 283. Among the accessions evaluated within the *S. melongena*, none was found to be resistant. Accessions MM 1044, MM 11044 and MM 10260 within the *S. macrocarpon* accessions were found to be resistant to *F. oxysporum* f.sp *melongenae* (Table 9). Accessions MM 1161, MM 1616, UG AE – 21, MM 1119 and SOS1 in the *S. aethiopicum* species were observed to be resistant (Table 10).



Figure 4. 13: Wilting in eggplant accessions (a) Symptoms on the lower leaves (b) Wilting at five weeks after inoculation (c) Wilting at 12 weeks after inoculation

Table 8: Differences between the two trials expressed as disease index for trials 1 and 2

Trial	Start of the trial	Average temperature °C	Disease index (Appendix 2)
1	Oct – Dec 09	26 °C	a*
2	Dec- Feb 10	24°C	b*

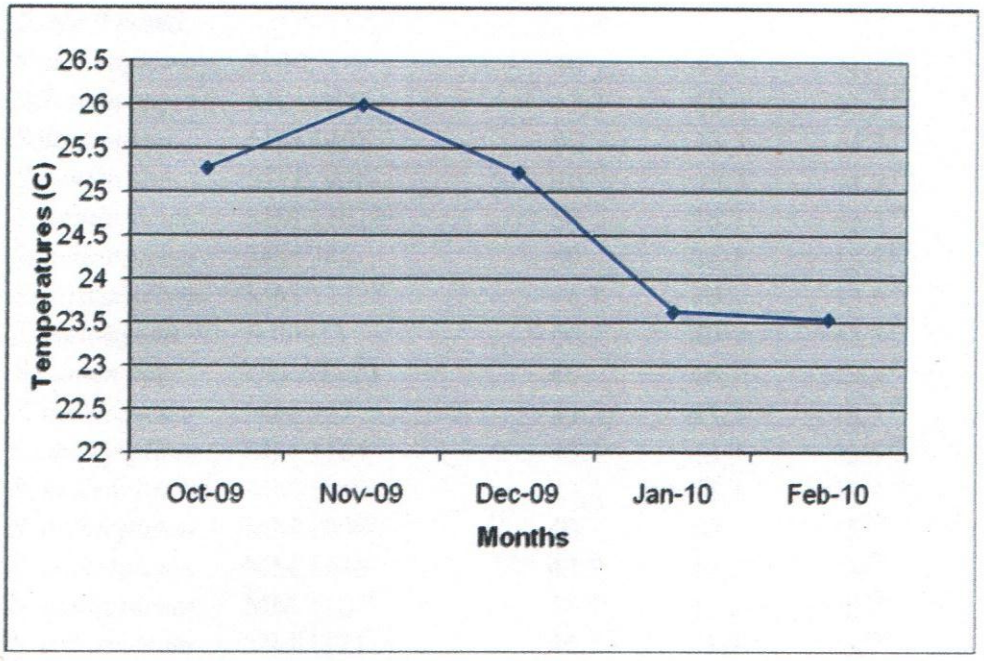


Figure 4. 14: Mean monthly temperatures during the duration the two trials were carried out

Table 9: Reaction of eggplant accessions to *Fusarium oxysporum* f.sp *melongenae*

		Disease incidence (0 – 100%)			
Species	Accession code	Trial 1	Trial 2	Average	Tukeys test
S. macrocarpon	MM 10260	36.7	20	28.3^{R}	e
S. aethiopicum	MM 1119	33.3	23.3	28.3^{R}	e
Solanum spp.	UG AE 6	33.3	26.7	30^{R}	e
S. aethiopicum	SOS1	30	33.3	31.7^{R}	e
S. aethiopicum	MM 1616	43.3	20	31.7^{R}	e
Solanum spp.	ML AE 6	40	26.7	33.3^{R}	e
Solanum spp.	ML AE 4	30	40	35^{R}	de
S. aethiopicum	MM 1161	36.7	36.7	36.7^{R}	de
S. macrocarpon	MM 11044	36.7	36.7	36.7^{R}	de
S. aethiopicum	MM 10079	40	33.3	36.7^{R}	de
S. aethiopicum	MM 1207	40	33.3	36.7^{R}	de
S. aethiopicum	UG AE-21	30	43.3	36.7^{R}	de
Solanum spp.	MM 1692	40	36.7	38.3^{R}	de
S. aethiopicum	TZ SMN AE 3-10	50	30	40^{R}	de
S. aethiopicum	MM 11008	36.7	43.3	40^{R}	de
S. macrocarpon	MM 1044	46.7	33.3	40^{R}	de
Solanum spp.	LANDRACE	50	30	40^{R}	de
Solanum spp.	MM 01139	36.7	46.7	41.7^{PR}	de
S. aethiopicum	MM 1160	43.3	40	41.7^{PR}	de

Table 9 contd.

S. aethiopicum	N 20	40	43.3	41.7 [PR]	de
Solanum spp.	ML AE 9	43.3	40	41.7 [PR]	de
Solanum spp.	MM 1498	50	33.3	41.7 [PR]	de
Solanum spp.	SITE 101	40	43.3	41.7 [PR]	de
S. aethiopicum	MM 11010	50	36.7	43.3 [PR]	de
S. aethiopicum	MM 348	50	36.7	43.3 [PR]	de
S. dasyphyllum	MM 12126	46.7	40	43.3 [PR]	de
S. melongena	S 00813	46.7	40	43.3 [PR]	de
Solanum spp.	UG AE-23	40	46.7	43.3 [PR]	de
S. aethiopicum	MM 347	43.3	43.3	43.3 [PR]	de
S. dasyphyllum	MM 1164	43.3	43.3	43.3 [PR]	de
S. macrocarpon	MM 1062	43.3	43.3	43.3 [PR]	de
S. aethiopicum	MM 10086	40	50	45 [PR]	de
S. aethiopicum	MM 1106	43.3	46.7	45 [PR]	de
S. aethiopicum	MM 1107	46.7	43.3	45 [PR]	de
S. aethiopicum	MM 1121	46.7	43.3	45 [PR]	de
S. aethiopicum	MM 1162	50	40	45 [PR]	de
S. aethiopicum	MM 1642	43.3	46.7	45 [PR]	de
S. aethiopicum	MM 803	46.7	43.3	45 [PR]	de
S. aethiopicum	SMALL OVAL TYPE	50	40	45 [PR]	cde
S. aethiopicum	TZ SMN-AE 2-8	56.7	33.3	45 [PR]	cde
S. aethiopicum	TZ SMN AE 52-3	50	40	45 [PR]	cde
S. aethiopicum	MM 10252	46.7	43.3	45 [PR]	cde
S. macrocarpon	MM 1048	50	40	45 [PR]	cde
S. macrocarpon	MM 12209	40	50	45 [PR]	cde
S. macrocarpon	MM 132	36.7	53.3	45 [PR]	cde
Solanum spp.	MM 1007	40	50	45 [PR]	cde
S. aethiopicum	MM 10213	46.7	46.7	46.7 [PR]	cde
Solanum spp.	ML AE 5 29-7	46.7	46.7	46.7 [PR]	cde
S. aethiopicum	MM 1102	50	43.3	46.7 [PR]	cde
S. aethiopicum	MM 870	50	43.3	46.7 [PR]	cde
S. anguivi	MM 159	50	43.3	46.7 [PR]	cde
S. melongena	S 00736	50	43.3	46.7 [PR]	cde
Solanum spp.	UG AE- 5	43.3	50	46.7 [PR]	cde
Solanum spp.	UG AE-14	43.3	50	46.7 [PR]	cde
S. aethiopicum	MM 574	50	46.7	48.3 [PR]	cde
S. melongena	OOO17	43.3	53.3	48.3 [PR]	cde
S. melongena	S 00718	43.3	53.3	48.3 [PR]	cde
S. melongena	TZ 00567	30	66.7	48.3 [PR]	cde
Solanum spp.	UG AE – 24	43.3	53.3	48.3 [PR]	cde

Table 9 contd

S. aethiopicum	MANYIRE GREEN	53.3	46.7	50 PR	bcde
S. aethiopicum	MM 10245	60	40	50 PR	bcde
S. aethiopicum	UG AE-10	60	40	50 PR	bcde
S. aethiopicum	MM 196	46.7	56.7	51.7 S	bcde
S. melongena	TS 00131	56.7	46.7	51.7 S	bcde
Solanum spp.	MM 138	56.7	46.7	51.7 S	bcde
S. aethiopicum	MM 1158	60	46.7	53.3 S	bcde
S. melongena	OO 204	46.7	60	53.3 S	bcde
S. aethiopicum	MM 868	53.3	53.3	53.3 S	bcde
S. aethiopicum	MM 1188	56.7	53.3	55 S	bcde
S. aethiopicum	MM 1483	60	50	55 S	bcde
S. aethiopicum	MM 1615	50	60	55 S	bcde
S. melongena	OO 677	50	60	55 S	bcde
S. aethiopicum	MM 1371	56.7	56.7	56.7 S	abcde
S. anguivi	MM 905	56.7	56.7	56.7 S	abcde
S. macrocarpon	MM 150	56.7	56.7	56.7 S	abcde
S. aethiopicum	MM 134	63.3	50	56.7 S	abcde
S. aethiopicum	MM 1480	66.7	46.7	56.7 S	abcde
S. aethiopicum	TZ SMN AE 75-7	63.3	50	56.7 S	abcde
S. melongena	OO 567	60	53.3	56.7 S	abcde
S. melongena	S 00735	60	53.3	56.7 S	abcde
S. aethiopicum	MM 1474	56.7	60	58.3 S	abcde
S. aethiopicum	MM 267	46.7	70	58.3 S	abcde
S. macrocarpon	CR 001	56.7	60	58.3 S	abcde
S. aethiopicum	MM 10181	63.3	56.7	60 S	abcde
S. aethiopicum	DB 3	63.3	56.7	60 S	abcde
S. anguivi	MM 1103	60	60	60 S	abcde
S. melongena	BLACK BEAUTY	60	60	60 S	abcde
S. anguivi	N 19	66.7	63.3	65 S	abcd
S. macrocarpon	MM 1131	63.3	66.7	65 S	abcd
S. aethiopicum	MM 1308	66.7	70	68.3 S	ab
S. macrocarpon	MM 283	70	76.7	73.3 S	ab
S. melongena	OO 256	80	73.3	76.7 S	a
S. macrocarpon	MM 855	80	83.3	58.7 S	a

Each value is a mean of six plants.

*R: resistant (< 40%); PR: partially resistant (40%-50%); S: susceptible (>50%)

*Means on the same column followed by the same letter are not significantly different following Tukeys test, ($P \leq 0.05$)

*12 % of the accessions were considered as resistant while 71% were partially resistant and 10% susceptible.

Table 10: Disease reaction of eggplant accessions to inoculation with *Fusarium oxysporum* f.sp *melongenae* within the individual species

Isolate code	Mean Disease Severity Index of 12 seedlings	Significance ranges in mean separation
S. macrocarpon		
MM 855	4.17	a
MM 283	3.42	ab
MM 1131	3	bc
MM150	2.83	bcd
CR 001	2.83	bcd
MM 12209	2.5	bcd
MM 1048	2.33	bcd
MM 132	2.25	cd
MM 1062	2	cd
MM 1044	1.83	d
MM 11044	1.83	d
MM 10260	1.75	d
S. aethiopicum		
MM 1308	3.42	a
MM 1480	3.08	ab
MM 10181	3.08	ab
MM 1474	2.83	abc
MM 1615	2.83	abc
MM 1371	2.67	abc
MM 267	2.67	abc
MM 1188	2.67	abc
MM 868	2.67	abc
MM 1483	2.67	abc
MM 134	2.67	abc
MM 1158	2.67	abc
DB 3	2.58	abc
UG AE – 10	2.58	abc
MANYIRE GREEN	2.58	abc
MM 347	2.58	abc
MM 10245	2.5	abc
MM 1107	2.5	abc
MM 1642	2.42	abc
MM 10252	2.42	abc
MM 574	2.42	abc
MM 870	2.33	abc
MM 196	2.33	abc
MM 10213	2.33	abc
TZZ SMN-AE	2.25	abc
MM 348	2.25	abc
MM 1102	2.25	abc

Table 10 contd.

TZ SMN AE	2.25	abc
MM 1121	2.17	abc
MM 10086	2.17	abc
MM 11010	2.17	abc
TZ SMN-AE	2.17	abc
MM 1162	2.17	abc
SMALL OVAL	2.17	abc
MM 803	2.17	abc
N 20	2.17	abc
MM 1160	2.08	abc
MM 1106	2.08	abc
MM 11008	2.08	abc
MM 1207	2.08	abc
MM 10079	2	abc
MM 1161	1.92	abc
MM 1616	1.83	bc
UG AE – 21	1.67	bc
SOS1	1.67	bc
MM 1119	1.5	c
S. melongena		
OO 256	3.75	ab
BLACK BEAUTY	3.08	ab
OO 567	3.0	ab
S 00735	2.83	ab
OO 204	2.67	ab
00017	2.58	ab
OO 677	2.58	ab
TS 00131	2.5	b
S 00736	2.42	b
S 00718	2.33	b
S 00813	2.25	b
TZ 00567	2.25	b
S. anguivi		
N 19	3.25	a
MM 1103	3.08	a
MM 905	2.67	ab
MM 159	2.42	ab
S. dasyphyllum		
MM 1164	2.33	a
MM 12126	2.08	a

Foliar symptom scale: higher numbers indicate severity of disease

Means followed by the same letter are not significantly different following Tukeys test, ($P \leq 0.05$).

There was rapid symptom development on the susceptible accessions. By the 35th day there was a 50% - 70 % disease incidence and within the 84th day all the plants had exhibited total wilting. Among the resistant accessions by the 84th day, there was less than 40% disease incidence and the disease progression was insignificant. Disease progression was gradual and did not proceed to total wilting of the plants in the accessions evaluated to be partially resistant.

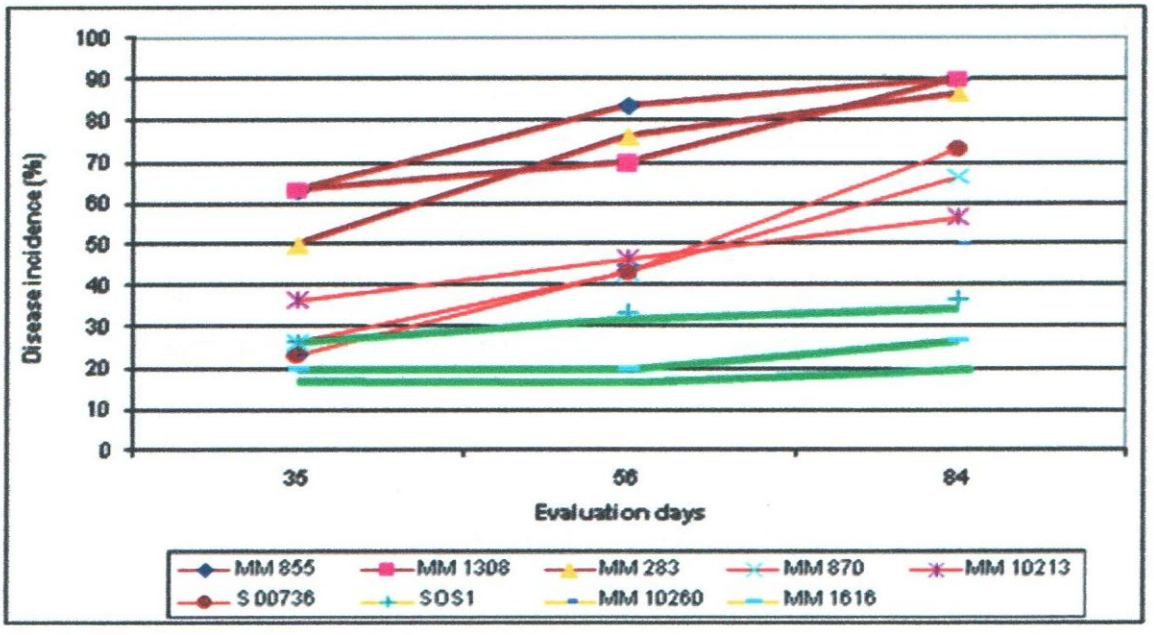


Figure 4. 15: Disease development on eggplant seedlings accessions inoculated with *F. oxysporum* f.sp *melongenae* isolate FS 40

Figure 4.15 above shows the variability in progression of disease incidence in some of the resistant (MM 10260, MM SOS1, MM 1616), partially resistant (MM 870, MM 10213, S 00736), and susceptible (MM 1308, MM 855, MM 283) accessions.

CHAPTER FIVE

DISCUSSION

5.1 Predominant Pathogen causing wilt in Eggplant

This work encompassed studies on the diversity of soil borne pathogen isolates recovered from eggplant farms affected by wilting diseases in Arumeru district in Tanzania. An important feature of this study was the determination of the most predominant pathogen causing wilting of eggplants based on samples collected from several farms. Based on the preliminary identification of the colony appearance and form of the sporulation apparatus, *Fusarium* spp. and *Phoma sorghina* isolates were differentiated from the other soil borne pathogens and saprophytes. *Fusarium* spp. was found to be the most common pathogen associated with wilt in *Solanum melongena, Solanum aethiopicum, Solanum anguivi* and *Solanum macrocarpon* accessions in Arumeru district.

Despite the fact that Verticillium is a major cause of wilting in eggplant (Camele *et al.*, 2005; Fradin and Thoma, 2006), there were no Verticillium isolates collected from the eggplant farms. This could be attributed to the clay loam soil in these farms which have a pH of 6.0 to 6.5 (Oluoch and Chadha, 2006) and that eggplant production in these fields is done in furrow irrigated fields. Jones and Overman (1984) reported that Verticillium wilt is suppressed at low pH and thrives well at a high pH of approximately 7.5. Though it may thrive at a pH of 5.5 as observed by Dutta (2007), it's disease severity was found to be much less in wet soils compared with plants grown in medium moisture level and dry soil. Since eggplant production in these farms is done in furrow irrigated fields, Verticillium may have been suppressed by the furrow irrigated soils. As observed by Farkya *et al.* (1996) and Pandav (2002), pH of 5.5 to 7.0 led to an increase in incidence of Fusarium wilt and therefore the soils provided a friendly environment for Fusarium wilt rather than Verticillium wilt.

5.2 Significance of the Morphological Characterization of the isolates

The precise definition of the pathogen culture conditions was important for the characterization of colony morphology and sporulation habit since *Fusarium* spp. varyconsiderably in their

morphology depending on the changes in the environment (Lester *et al.*, 1988; Mui-yun, 2003; Gyoung, 2005). Culturing on PDA was important for colony morphology, pigmentation and diameter (Lester *et al.*, 1988) and the conidia morphology was studied on SNA which gave relatively uniform results than those on PDA. In general, all the isolates of *Fusarium* spp. presented few similar characteristics such as the formation of macroconidia and formation of aerial mycelium. There was great variability in the colony morphology, shape and dimensions of the sporulation apparatus among the isolates. This variability was also found within isolates grouped into the same species. Isolates of *F. equiseti* produced long macroconidia with a filamentous apical cell and were 4-7 septate. *Fusarium oxysporum* had characteristic foot shaped basal cell, 3-4 septate and microconidia were formed on short monophialides which is characteristic of the species (Lester *et al,* 1988; Seifert, 1996). The macroconidia of *F. solani* had characteristically blunt ends and the microconidia were formed on long monophialides.

UPGMA clustering analysis revealed a high degree of interspecific diversity within the *Fusarium* spp. at the interspecific level and a high intraspecific variability in the three species groups. This variability is also reported in Malaysian Fusarium isolates collected from tomato (Rozlianah and Sariah, 2006). Despite this variability, *F. oxysporum* was found to be more morphologically close to *F. solani* than to *F. equisseti* as confirmed by Balali and Iranpoor (2005). The most distinguishing character was the short monophialides in *F. oxysporum* compared to phialides of *F. solani*. Morphological characterization of the isolates is an important preliminary identification of pathogens prior to further characterization using molecular tools.

5.3 Molecular characterization of the representative isolates

UPGMA clustering based on morphology was able to characterize all the 32 isolates and grouped them together into the respective species. However, due to the great variability of *Fusarium* spp. in culture, morphological characterization has been proved to be inadequate for unambiguous identification at the species level. The inaccuracies of identifying *Fusarium* spp. solely by morphological and cultural criteria have been largely overcome by the use of molecular methods for differentiating *Fusarium* species. The alpha-elongation factor gene has been used extensively for species delineation within Fusarium and genera of other ascomycetes (O'Donnell *et al.*,

1998). Analysis of these variable genomic regions provides sufficient information to infer phylogenetic relationships among fusaria and non-fusaria isolates (O'Donnell *et al.*, 1998 and references therein). For that reason, alpha-elongation factor sequence analysis was used to verify the identity of the pathogen(s) responsible for wilting of African eggplant.

Based on pathogenicity (of fusaria isolates) and sequence analysis of the ITS and TEF regions, one isolate was identified as belonging to *F. solani*, two to *F. oxysporum* f.sp. *melongenae* (FOM), six to the *F. incarnatum-equiseti* species complex (FIESC), and five to *Phoma sorghina*. This is the first confirmed report of FOM and *F. solani* causing wilting of eggplant in Tanzania. Representative isolates morphologically characterized as *F. equiseti* were identified as belonging to the *Fusarium incarnatum-F. equiseti* species complex (O'Donnell, *et al.*, 2009). This is also the first report of FIESC causing wilting in African eggplant. Confirmation of the identity of the *Fusarium* spp. causing wilt in African eggplant has important implications for the development and implementation of appropriate disease management strategies. Knowledge on the variability of the fungal pathogens causing wilt in the African eggplants that are rapidly being domesticated reduces the difficulty of breeding for resistance and deployment of improved cultivars. This is because it facilitates a targeted search for resistance genes that can be deployed against a specific *Fusarium* species on a given African eggplant species. Chemical control also is more effective when it is based on knowledge of the most prevalent soil borne pathogen causing wilt in eggplants. Verification and confirmation of the identity of African eggplant wilt pathogens provides the necessary basis for future studies on the population structure of *Fusarium* species attacking eggplant in Tanzania. It also satisfies an important pre-condition for the development and deployment of eggplant cultivars with improved resistance to Fusarium wilt.

Considering the importance of host plant resistance as the most economical and environmentally friendly strategy for wilt disease management, plant breeders, pathologists, and genetic resource specialists should focus on the search for sources of Fusarium resistance in African eggplant. Findings of this study may also be relevant to plant quarantine and phytosanitary measures in Tanzania. However, the relevance would dependent on whether African eggplant Fusaria are seed borne, and this needs to be investigated. The findings of this study do not definitively exclude the possibility that Verticillium wilt may be present and damaging to eggplants in

Tanzania. Further research is warranted to survey a wider geographic area of the country and analyze a larger population of wilt-associated pathogen isolates in order to confirm that Fusaria are the sole causal agents of wilt disease of African eggplant in Tanzania.

5.4 Pathogenicity of *Fusarium* species

Pathogenicity tests are the only means of determining the pathological effect of disease causing pathogens, for example; fungal strains present in diseased plants or in soil samples. Root dip inoculation technique was effective for screening for wilt resistance under controlled conditions. This study identified the various *Fusarium* spp. associated with wilting of *S. melongena*, *S. aethiopicum* and *S. macrocarpon* species. There were significant differences in the reaction of the eggplant cultivars to the isolates. The pathogenicity tests indicated that Fs 27, which was morphologically identified as *Fusarium equisseti* and found to be within the FIESC, was aggressive to both *S. macrocapon* (MM 1131) and *S. anguivi* (N19). *Fusarium equiseti* has been reported to cause rots of cucurbits fruits in contact with the soil (Lester *et al.*, 1988). *Fusarium equiseti* and *F. solani* have also been reported to cause heavy wilting and severe seedling root rot respectively in sunflower (Sharfun-nahar and Mushtaq, 2007). Pathogenicity of *F. equiseti* has also been reported on capsicum, date palm and soybean cultivars (Abbas *et al.*, 1991; Hamman *et al.*, 1995; Mushtaq and Hashmi, 1997). *Fusarium equiseti* pathogenicity has been linked to a pathogenicity factor known as equisetin and trichothecenes (Hestbjerg *et al.*, 2002; Wheeler *et al.*, 2008). Among the isolates used for the pathogenicity test, *F. equiseti* caused the highest wilting in *S. macrocarpon* and *S. anguivi* followed by *F. oxysporum* f.sp. *melongenae*. Fs 22 and Fs 35, which were identified as *F. solani,* were moderately pathogenic to N 19 (*S. anguivi*).

Solanum macrocarpon has been reported to be attacked by several pests and diseases including *Fusarium* spp. (Shippers, 2002; Grubben and Denton, 2004). However, the pathogen causing Fusarium wilt in *S. macrocarpon* has not been described to the species level (Shippers, 2002). This study reveals that *F. solani* and *F. equiseti* are capable of causing wilt in *S. macrocarpon* and *S. anguivi*. Previous work has reported *F. solani* to be pathogenic to eggplant (*S. melongena*) resulting to crown rot, vascular discolouration and wilt (Hundoo and Dwivedi, 1992; Romberg and Davis, 2007; Chakraborty, *et al.*, 2008). Isolates Fs 40 and Fs 24 which were identified as

Fusarium oxysporum were pathogenic to the same host MM 1131 (*S. macrocarpon*). The two isolates were collected from the same host *S. melongena*. *Solanum macrocarpon* is a species known to be within the same section of Melongena together with *S. melongena* (aubergine) (Tindall, 1983). This study confirms previous work showing accessions of *S. aethiopicum* gr. Gilo to carry resistance to *Fusarium oxysporum* f.sp *melongenae* though other accessions have also been found to be susceptible (Stravato and Capelli, 2000; Toppino *et al.*, 2008).

Phoma sorghina, a saprobic soil organism in the group Epicoccum is a secondary invader of weak or diseased plants. In this study it was abundantly found in the fields. It is associated with leaf and stem spots, glume blotch, root rot and dying off in rice (Amaral *et al.*, 2005).

5.5 Screening eggplant accessions for reaction to *Fusarium oxysporum*

Thirteen out of the 93 eggplant accessions screened under screen house conditions were found to be resistant to *Fusarium oxysporum* f.sp. *melongenae,* and they belonged to different species; *S. aethiopicum, S. dasyphyllum* and *S. macrocarpon*. *Solanum aethiopicum* is a species known to possess accessions with resistance to wilt caused by *Fusarium* spp (Mutlu *et al.*, 2008). However, some of the common African aethiopicum species under cultivation and *S. melongena* cultivars in Arumeru district have been affected by wilting (Growers observation). The numerous *Solanum* species which are more or less related to aubergine constitute a potential valuable reserve of genetic variability for resistance that would be exploited for the improvement of aubergine and other cultivated African eggplants. The survival and activity of *Fusarium* spp. is greatly dependant on many factors, with the most important being soil moisture and soil and air temperatures (Mui-Yun, 2003). *Fusarium oxysporum* is a warm weather pathogen and wilting is more prevalent when the temperatures are high and in moisture stressed soils. Infected plants may remain symptomless in wet seasons (Lester *et al.*, 1988). The significant differences in wilting within the two trials were as a result of the prevailing environmental conditions during the time the two trials were carried out. Between October and December 2009 (first trial), the mean monthly temperatures were above 25°C while within the second trial temperatures below 25°C were recorded. This probably explains the higher disease severity of the accessions in the

first trial compared to the second trial. This confirms reports that *Fusarium* spp. requires soil and air temperatures of 25 -28°C (Mui-yun, 2003) to effectively infect their hosts.

Solanum dasyphyllum accessions (MM 1164, MM 12126) were observed to be resistant to the isolate *Fusarium oxysporum* f.sp *melongenae* used in the inoculation. *Solanum dasyphyllum* is a wild eggplant species which has not been cultivated but these accessions (MM 1164 and MM12126) would be valuable if used as rootstocks. *Solanum aethiopicum* has also been found to have accessions carrying resistance to *Fusarium oxysporum* f. sp *melongenae* (Sakata *et al.*, 1996; Toppino *et al.*,, 2008). Among the *S. aethiopicum*, accession MM 1119, MM1616, MM 10079, MM 1692, MM 1062 and MM 1161 were observed to be resistant to *F. oxysporum* f.sp *melongenae* and their further evaluation for resistance to Verticillium and bacterial wilts would be important for eggplant improvement. These accessions would be very useful in crossing with the cultivated *S. aethiopicum* cultivars. Among the *S. aethiopicum* groups, Shum and Kumba were found to carry a higher resistance than Group Gilo and Aculeatum. Gilo accessions exhibited the whole range of reactions i.e resistance, partial resistance and susceptibility. Similar results were reported by Stravato and Cappelli (2000). This may also be explained by the existence of genetic variability within the *S. aethiopicum* groups Gilo and Shum as reported by Sekara *et al.* (2007).

Accessions of *S. macrocarpon* (Accession MM 1044, MM 10260) were also observed to carry resistance to *Fusarium oxysporum* f.sp *melongenae*. *Solanum macrocarpon* is reported to be resistant to most diseases such as damping off caused by *Thielaviopsis basicola* (Shippers, 2002). Certain cultivars of *S. macrocarpon* have been reported to be resistant to Fusarium wilt (Grubben and Denton, 2004). Interspecific hybridization between *S. macrocarpon* and *S. melongena* is known to produce fertile hybrids and therefore can be used in transfer of the resistant traits to cultivated eggplants. *Solanum macrocarpon* and *S. dasyphyllum* can be crossed easily and therefore produce fully fertile hybrids (Shippers, 2002). In this case the *S. dasyphyllum* accessions (MM 1164, MM 12126) can be used in crosses with cultivated *S. macrocarpon* cultivars. Accession SOS1 (*S. aethiopicum* gr. Gilo X *S. aethiopicum* gr. Aculeatum) reported to be resistant by Toppino *et al.* (2008) was also resistant in the present study.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

This study showed that among other soil borne pathogens causing wilt in eggplant in farmers fields in Arumeru district, *Fusarium* spp. is a common and prevalent causal agent of wilting in eggplants in these farms. Correct identification of the causal agent of wilting will make management of wilting in eggplant production farms of Arumeru district more effective. The different eggplant species evaluated in this study were found to be affected by different *Fusarium* spp. *Fusarium oxysporum* was pathogenic to *S. macrocarpon*, *F. solani* was pathogenic to *S. anguivi* and an isolate in the FIESC was pathogenic to *S. macrocapon* (MM 1131) and *S. anguivi.* Accession SIVONKWE in the *S. aethiopicum* gr. Gilo species was resistant to the three isolates tested and can be used in breeding for resistance for the *S. aethiopicum* cultivars. Accessions MM 1161, MM 1616, UG AE– 21, MM 1119, SOS1 MM 1164, MM 12126 MM 1119, MM1616, MM 10079, MM 1692, MM 1062 and MM 1161, MM 1044, MM 11044 and MM 10260 were resistant to *Fusarium oxysporum* f.sp *melongenae* and are valuable in the development of resistant varieties and for use as rootstocks.

The following recommendations can be made:

1. Further evaluation of the resistant eggplant accessions should be done to determine their resistance to other eggplant diseases such as Verticillium wilt, Phomopsis blight, southern blight, leaf spots etc.

2. Eggplant being a major vegetable produced in Tanzania, this study needs to be carried out in other parts of the country where there is large scale production of the crop. The economic importance of wilt diseases on eggplant production in other African countries need to be assessed for proper policy decision making to curb losses and improve yields.

3. It would be important to carry out further research on the host specificity of the *Fusarium* spp. on the various eggplants *(Solanum* spp.) and determine strains/races of *Fusarium oxysporum* f.sp *melongenae.*

4. Compatibility of the resistant accessions identified in this study with the cultivated eggplant varieties in breeding or for use as rootstocks needs to be assessed to enable utilization of the identified sources of resistance.

5. *Phoma sorghina* being a common foliar pathogen of the eggplants and since this study demonstrates its abundance in the eggplant fields, its pathogenicity and economic importance to the crop should be assessed.

6. Previous work has shown *Fusarium oxysporum* f.sp *melongenae* to be the causal agent of Fusarium wilt in eggplant in other parts of the world and sources of resistance have been identified in some countries. It would be necessary to undertake comparative analysis of FOM populations from Tanzania and those from other parts of the world, in view of introducing those sources of resistance to Tanzania.

7. Further studies to confirm the pathogenicity of *Fusarium equiseti* and its range of virulence on the different species of eggplants would provide useful insights for improved management of Fusarium wilt in the cultivated and wild eggplants.

REFERENCES

Abbas, I.H., Mouhi, M.N., Al-Roubaie, J.T., Hama, N.N. and El-Bahadli, A.H. (1991). *Phomopsis Phoenicola* and *Fusarium equiseti*, new pathogens on date palm in Iraq, *Mycological research* **4** :509

Agnieszka, S., Cebula S. and Kunicki E. (2007). Cultivated eggplants: origin, breeding objectives and genetic resources, A review, *Folia Horticurturae* **19**;: 97-114.

Agrios, G.N., (1988). Plant Pathology, 3rd. edition. Academic Press, Inc.: New York. 803 pp.

Aguiar, J.A., Molinar, and R.,Valencia J. (1998). Eggplant production in California. Vegetable research and information Centre. University of California, Division of agriculture and resources. Publication 7235.

Allard, R.W (1960). Principles of Plant breeding. John wiley and sons, Inc, New York, 485pp.

Altınok, H.H. (2005) First report of Fusarium wilt of eggplant caused by *Fusarium oxysporum* f. sp. *melongenae* in Turkey. *Plant pathology* **54**; 577.

Amaral, A. L., Dal Soglio, F. K., De Carli, M. L. and Barbosa Neto, J. F. (2005). Pathogenic fungi causing symptoms similar to Phaeosphaeria leaf spot of maize in Brazil. *Plant Diseases.* **89**: 44-49.

Balali, G.R. and Iranpoor, M. (2005). Application of pectic zymogram in the identification and genetic variation of *Fusarium* species. *Journal of Agricultural Technology* **1**: 127-143.

Barbara, D.J., Paplomatas, E.J. and Jiménez-Díaz R.M. (1998) Variability in *V. dahliae*. In: Hiemstra J.A., Harris D.C. (eds.). *A compendium of Verticillium wilts in tree species*, 43-45. Ponsen and Looijen, Wageningen, Netherlands.

Bhat, R.G., Davis, Smith R.F., Koike S.T., Wu B.W. and Subbarao K.V. (2003) Characterization of *Verticillium dahliae* isolates and wilt epidemics of Pepper. *Plant Disease*, **87**:789-797.

Bukenya, Z.R. and Carasco J.F. (1994) Biosystematic study of *Solanum macrocarpon- S. dasyphyllum* complex in Uganda and relations with *Solanum linnaeanum*. *East African Agricultural Forestry Journal* **59**: 187-204.

Camele, I., Marcone C., Caponero, A., Ambrico, A., Nigro, C. and Rana, G. L. (2005). First report of *Verticillium dahliae* causing Verticillium wilt of *Solanum aethiopicum* in Italy. *Plant Pathology* **55**: 581.

Campbell and Madden (1990*) Introduction to plant disease epidemiology*. John Wiley and sons, New York: 531 pp

Chadha, M. L. and Mndiga, H.H. (2006). African Eggplant - From underutilized to a commercially profitable venture. *Acta Horticulturae* (ISHS) **752**:521-524.

Chakraborty M. R., Chatterjee, N.C., and Quimio T.H. (2008). Integrated management of fusarial wilt of eggplant (*Solanum melongena*) with soil solarization. *Micología Aplicada Internacional* **21**: 25-36

Chen, N.C. and Li, H.M. (1995) Evaluation of eggplant germplasm and commercial varieties. In: EUCARPIA: IXth meeting on genetics and breeding on Capsicum and eggplant. *AVRDCAannual Staff Publication*: EUCARPIA. 30-33.

Cline, E., (2006) *Fusarium oxysporum f.sp. melongenae*. Systematic Mycology and Microbiology Laboratory, ARS, USDA. Retrieved June 19, 2011, from /sbmlweb/OnlineResources/Fungi Online.cfm .

Dutta, B.K. (2007) Effect of the chemical and physical condition of the soil on Verticillium wilt of antirrhinum. *Plant and Soil* **63**: 217-225

Eynck, C., Koopmann B., Grunewaldt-Stocker G., Karlovsky P. and Von Tiedemann A. (2007) Differential interactions of *Verticillium longisporum* and *V. dahliae* with *Brassica napus* detected with molecular and histological techniques. *European Journal of Plant pathology* **188**: 259-274.

Farkya, S., Pandey A. K. and Rajak R.S. (1996) Mycoherbicides potential of *Fusarium* spp. against *Parthenium*: Factors affecting *in vitro* growth and sporulation. *Bioved 7: 1–9.*

Fradin, F.E. and Thoma B. (2006) PhHysiology and molecular aspects of Verticillium wilt diseases caused by *V. dahliae* and *V. albo-artrum. Molecular plant pathology* **7**: 71-86

Fugro, P.A., Rajput, J.C., Patil, V.H. and Jadhav D. (1994) New bacterial wilt resistant F1 hybrids of eggplant- *Solanum melongena* L. *Capsicum and Eggplant Newsletter*, **13**: 107-109.

Geiser M, Kang, M.M., Makalowska, S. 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****: 473–479*

Ghoneem, M. K., Saber, I.A. and Elwakil, M.A. (2009) Alkaline Seed-Bed: An Innovative Technique for Manifesting *Verticillium dahliae* on Fennel Seeds. *Plant Pathology Journal* **8**: 22-26.

Grubben, G.J.H. and Denton, O.A, (2004) Plant Resources of Tropical Africa Vegetables 2. PROTA Foundation, Wagenigen, Netherlands/Backhuys Publishers, Leiden, Netherlands /CTA, Wagenigen, Netherlands.668 pp.

Gyoung, H. K., Jae-Seoun, H., Woobong, C., and Young, J. K. (2005) Fusarium Wilt of Winter DaPhne (Daphne odora Thunb.) Caused by *Fusarium oxysporum. The Plant Pathology Journal* **21**: 102-105

Hamman, B., Eicker, A. and Van de Venter H. A. (1995). First Report of *Fusarium equiseti* on South African Soybean Cultivars. *Plant Diseases* **79**:754

Harris, D.C, (1993). *Manipulation of V. dahliae.* In: Hiemstra J.A., Harris D.C. (eds.).*A compendium of Verticillium wilts in tree species*, 49-50. Ponsen and Looijen, Wageningen, The Netherlands.

Hestbjerg, H., Nielsen, K. F., Thrane, U. and Elmholt S. (2002). Production of trichothecenes and other secondary metabolites by *Fusarium culmorum* and *Fusarium equiseti* on common laboratory media and soil organic matter agar: an ecological interpretation. *Journal of Agricultural Food Chemistry*. **50**: 7593-7599.

Hundoo, S. and Dwivedi, R.S, (1992). Chemical control of *Fusarium solani*, the incitant of root-rot of eggplant (*Solanum melongena* L.). *Journal of Phytopathology*, **5** :89-92.

Huntrods, D. (2008) Eggplant profile. Vegetables and melons situation and outlook year book. ERS, USDA. Agricultural Marketing resource Centre. IOWA State University.

IPM CRSP, (2007) Bacterial wilt of eggplant. http://mail.openacademy.ph/ Accessed 3rd march 2009.

Iwamoto, Y. and Ezura, H. (2006) Efficient plant regeneration from protoplasts of eggplant rootstock cultivar and its wild relatives. *Plant Biotechnology,* **23**: 525–529.

Jones, J. P. and Overman, A. J., (1984) Management of Fusarium wilt, Fusarium crown rot, verticillium wilt (race 2), southern blight, and root-knot of tomato on fine sandy soils. *Proceedings of the Florida State horticultural Society*. **98**: 229-231.

Kemble, J. M., Sikora, E. J., Simmone, E. H., Zehnder, G. W. and Pattersson M.G. (1998) Guide to eggplant commercial production. Aces publications, 8. http://www.aces.edu/ Accessed 22nd may 2009.

Klosterman, J.S., Atallah, Z. K., Vallad, G.E. and Subbarao, K.V. (2009) Diversity, pathogenecity and management of *Verticillium* species. *Annual Reviews of Phytopathology* **43**: 39-62.

Larone, D. H. (1995) *Medically Important Fungi - A Guide to Identification*, ASM press: Washington DC, 3rd edition. 214pp.

Lester R.N., 1986. Taxonomy of scarlet eggplants, *Solanum aethiopicum* L. *Acta Horticulture (ISHS)* **182**: 125-132.

Lester, W.B., Craig, L., Brett, A. and Summerell. (1988) *Laboratory manual for Fusarium research: incorporating a key and descriptions of common species found in Australasia.* University of Sydney Press, 2nd edition. 156pp.

Liu, F. Z. (2001) *Elite Eggplant varieties and Practical Cultivation Technology* .China Labour and Welfare Press, Beijing, China.

Mace, E.S., Lester, R.N. and Gebhardt, C.G. (1999) AFLP analysis of genetic mapping of domestication traits in eggplant. *Genetics* **161**: 1712-1726.

Matsubara, T., Hirano, I.,Sassa, D. and Koshikawa, K. (2004) Increased tolerance to Fusarium wilt in mycorrhizal strawberry plants raised by capillary watering methods. *Environmental Control in Biology.* **42**:185-191

Miller, S.A., Rowe C. R. and Riedel M.R. (1996) Fusarium and Verticillium wilt of tomato, potato, pepper and eggplant. The Ohio University Extension. Fact sheet.

Migheli, Q., Balmas, V., Harak, H., Sanna, S., Scherm, B., Aoki T and O'Donell K., (2010) Molecular phylogenetic diversity of Dermatological and other human pathogenic Fusarial isolates from hospitals in Nothern and Central Italy. *Journal of Clinical Microbiology* **48**: 1076-1084

Mochizuki, H. and Yamakawa, K. (1979) Resistance of selected eggplant cultivars and related wild *Solanum* species to bacterial wilt (*Pseudomonas solanacearum*). *Bulletin: Vegetable & Ornamental Crops Resistance* **6**: 1–10.

Mol, L. and Vanriessen, H.W. (1995) Effect of plant-roots in the germination of microsclerotia of *Verticillium dahliae*. *European Journal of Plant Pathology* **101**: 673- 678.

Monma, S., Akazawa, S., Simogaka, K., Sakata, Y. and Matsunaga, H. (1996) 'Daitaro', a bacterial wilt- and Fusarium wilt- resistant hybrid eggplant for rootstock. *Acta Horticulture (ISHS)* **659**: 514-23.

Mui-Yun, W. (2003) *Fusarium oxysporum* f.sp *lycopersici* (Sacc.) Snyder and Hans. 728. Soilborne plant pathogen class project. N C State University.

Mushtaq, M. and Hashmi M. H. (1997. Fungi associated with wilt diseases of capsicum in Sindh. *Pakistan Journal of Botany* **29**: 217-222

Mutlu, N., Boyaci, F.H., Gocmen, M., Abak, K. (2008) Development of SRAP, SRAP-RGA, RAPD and SCAR markers linked with a Fusarium wilt resistance gene in eggplant. *Theoretical and Applied Genetics* **117**: 1303-12.

Nelson, P.E., Toussoun, T.A. and Marasas, W.F.O. (1983). *Fusarium* species*: An illustrated Manual for identification.* Pennysylvania State University Press, University Park.193pp.

O'Donnell, K., Cigelnik, E., Weber, N.S. and Trappe J.M. (1997) PHylogenetic relationships among ascomycetous truffles and the true and false morels from 18S and 28S ribosomal DNA sequence analysis. *Mycologia **89**:* 48–65

O'Donnell, K., Kistler, H.C., Cigelnik, E. and Ploetz, R.C. (1998) 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 of the United States of America **95**:* 2044–2049.

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

Olsen, M.W., and Deborah J.Y. (1998). Plant Disease Management: Horticultural Crops Verticillium Wilt. Co operate extension. Publication No. AZ1034.

Oluoch, M.O. and Chadha M.L. (2006). Evaluation of African Eggplant for yield and quality characteristics AVRDC-The World Vegetable Center, AVRDC Annual Report.

Oyelana, O.A. and Ugborogho R.E. (2008). Phenotypic variation of F_1 and F_2 populations from three species of Solanum L. (Solanaceae). *African Journal of Biotechnology* **7**: 2359-2367.

Pandav, F.J. (2002) (*Vigna unguiculata* L. Walp) under South Gujarat condition. *M.Sc. (Agric.) thesis*, G.A.U., S.K. Nagar, India, 262 pp.

Pantou, P., Strunnikova, O.K., Shakhnazarova, V.Y., Vishnevskaya, N.A., Papalouka, V.G. and Typas, M.A. (2005). Molecular and immunochemical Phylogeny of Verticillium species. *Mycological Research* **109**: 889-902.

Porcher, M. H., (2009) Know your eggplants: http://www.plantnames.unimelb.edu.au/new/sorting/catalogue. Accessed June 2009.

Raabe, R.D., Conners, I.L, and Martinez. A.P. (1981). *Checklist of plant diseases in Hawaii: including records of microorganisms, principally fungi, found in the state.* Hawaii Institute of Tropical Agriculture and Human Resources (CTAHR), Information Text Series **022**. 313pp.

Rao, M.V.B., Sohi, W.S. and Vijay. O.P. (1976). Reaction of some varieties of brinjal (*Solanum melongena_* L.) to *Pseudomonas solanacearum. Vegetable Science* **3**: 61-64.

Romberg, M.K. and Davis, R.M. (2007). Host range and Phylogeny of *Fusarium solani* f. sp. *eumartii* from potato and tomato in California. *The American Phytopathological Society. Plant Disease* **91**: 585-592.

Rozalinah, F.S. and Sariah M. (2006). Characterization of Malaysian isolates of Fusarium from Tomato and Pathogenecity Testing. *ResearchJournal of Microbiology* **3**: 266-272.

Sakata, Y., (1991). Wild relatives of *Solanum melongena* as gene resources. *Bio Hortculture* **5**: 38–41.

Saharan, M.S., Naef, A., Kumar, J. and Tiwari R. (2005). Characterization of variability among isolates of *Fusarium graminearum* associated with head scab of wheat using DNA markers . *Current Science* **230**: 92.

Seifert, K., (1996). FUSKEY Fusarium Interactive key. Agriculture and Agri- Food Canada *Product Development Unit, Now taxonomic Information Systems*. 65pp.

Sękara A., Cebula, S. and Kunicki, E. (2007). Cultivated eggplants – origin, breeding objectives and genetic resources, a review. *Foliar Horticulture* **9** :97-114

Sharfun-nahar and Mushtaq, M. (2007). Pathogenic effects and transmission studies of seed-borne *Fusarium* species in sunflower. *Pakistan Journal of Botany*. **39**: 645-649.

Shippers, R.R. (2002). African indigenous vegetables, An overview of the cultivated species 2002- Revised version on CD-ROM. Natural Resources International Limited, Aylesford,UK.

Singh, A.K., Singh, M., Singh, A.K., Singh, R., Kumar, S. and Kalloo, G. (2006) Genetic diversity within the genus *Solanum* (*Solanaceae*) as revealed by RAPD markers. *Current Science* **90**: 711-716.

Singleton, L.L., Mihail J.D. and Rush C.M. (1992). Methods for research on soilborne Phytopathogenic fungi. *The American Phytopathological Society*. 265pp.

Smith, H.C. (1965). *The morphology* of *Verticillium albo-artrum*, *V. dahliae*, and *V. tricopus*. *New Zealand Journal of Agricultural Research* **8**: 450-478.

Smith, I.M., J. Dunez, D.H., Phillips, R.A. Lelliott, S.A. and Archer, A. 1988. *European handbook of plant diseases.* Blackwell Scientific Publications: Oxford. 583pp.

Stravato, V.M. and Cappelli C., (2000). Behaviour of *Solanum* spp. on inoculation with different isolates of *Fusarium oxysporum* f. sp. *melongenae. Bulletin OEPPEPPO* **30**, 247-249.

Termorshuizen, A.J, (1998) Disease diagnosis and identification of the pathogen. *A compendium of Verticillium wilts in tree species.* In: Hiemstra J.A., Harris D.C. (eds.) 41-42. Ponsen and Looijen, Wageningen, The Netherlands.

Tindall, H.D. (1983). *Vegetables in the tropics.* The Macmillan Press Ltd. 533pp.

Toppino, L., Giampiero, V. and Rotino, G. L., (2008) Inheritance of Fusarium wilt resistance introgressed from *Solanum aethiopicum Gilo* and *Aculeatum* groups into cultivated eggplant (*S. melongena*) and development of associated PCR-based markers. *Molecular Breeding Journal* **22**: 237-250.

Toussoun, T. A. and Nelson, P. E. (1978). A Pictorial Guide to the Identification of Fusarium species according to the taxonomic system of Snyder and Hansen. The Pennsylvania State Universtiy Press: University Park, Pennsylvania. 51 pp.

University Of Illinois Extension, Department of Crop Sciences, (1997). Report On Plant Disease- Verticillium Wilt. Publication No. 1010.

USDA, (2003). USDA national nutrient database for standard reference, release 16. { Internet } U.S Department of Agriculture, Beltsville Md, United States. http://www.nal.usda.gov/fnic/food comp. Accessed August 2009.

Weiberger, K. and Msuya, J., (2004). Indigenous vegetables in Tanzania, significance and prospects. *Technical bulletin no. 31. AVRDC Publication*, 04-600.70pp.

Wheeler, M. H., Stipanovic R. D., Puckhaber L. S. (2008). Phytotoxicity of equisetin and epi-equisetin isolated from *Fusarium equiseti* and *F. pallidoroseum. Mycological Research* **103**: 967-973.

Yoshida, T., Monma, S., Matsunaga, H., Sakata, Y. and Saito, T. (2004). Development of a new rootstock eggplant cultivar 'Daizaburou' with high resistance to bacterial wilt and Fusarium wilt. *Yasai Chagyo Kenkyujo Kenkyu Hokoku* **3:** 199-211.

Yoshiteru, S., Monma S., Tomoaki, N. and Komochi S. (1996). Evaluation of resistance to bacterial wilt and Verticillium wilt in Eggplants (*Solanum melongena* L.) collected in Malaysia. *Japan Society of Horticultural Science* **65**: 81-88.

APPENDICES

Appendix 1: Anova of disease severity of eggplant accessions after artificial inoculation with isolates of *Fusarium spp.*

Source	df	SS	MS	F	P
Blocks	4	1.2	0.3	0.5310	.7133 ns
Treatments	20	70.5143	3.5257	6.2402	.0000 ***
Error	80	45.2	0.565		
Total	104	116.9143			

Appendix 2: Anova of disease severity of eggplant accessions in the two trials

Source	df	SS	MS	F	P
Blocks (Trials)	1	688.8889	688.8889	5.1491	.0237 *
Accessions	92	53943.0108	586.3371	4.3826	.0000 ***
Error	464	62077.7777	133.7883		
Total	557	116709.6774			

There were significant differences ($P \leq 0.05$) in the two trials.

Appendix 3: Anova of disease severity of the eggplant accessions tested in 1st trial

Source	df	SS	MS	F	P
Blocks	2	112.5448	56.272401	0.4116	.6632 ns
Accession	92	30043.7276	326.5623	2.38878	.0000 ***
Error	184	25154.1219	136.7072		
Total	278	55310.3943			

Appendix 4: Anova of disease severity of the eggplant accessions tested in 2nd trial

Source	df	SS	MS	F	P
Blocks	2	149.1039	74.5520	0.6316	.5329 ns
Accession	92	38843.7276	422.2144	3.5772	.0000 ***
Error	184	21717.5627	118.0302		
Total	278	60710.3943			

Appendix 5: Anova of mean disease severity of the *Solanum aethiopicum* accessions

Source	df	SS	MS	F	P
Blocks	2	0.1773	0.0887	0.2080	.8124 ns
Accession	46	39.1454	0.8510	1.9963	.0005 ***
Error	233	99.3227	0.4263		
Total	281	138.6453901			

Appendix 6: Anova of mean disease severity of the *Solanum melongena* accessions

Source	df	SS	MS	F	P
Blocks	2	0.8125	0.4063	1.0405	.3598 ns
Accession	11	12.2604	1.1146	2.8546	.0047 **
Error	58	22.6458	0.3904		
Total	71	35.71875			

Appendix 7: Anova of mean disease severity of the *Solanum anguivi* accessions

Source	df	SS	MS	F	P
Blocks	2	0.3958	0.1979	1.3053	.2955 ns
Accession	3	2.6146	0.8715	5.7481	.0061 **
Error	18	2.7292	0.1516		
Total	23	5.7396			

Appendix 8: Anova of mean disease severity of the *Solanum. dasyphyllum* accessions.

Source	df	SS	MS	F	P
Blocks	2	1.2917	0.6458	4.1333	.0585 ns
Accession	1	0.1875	0.1875	1.2	.3052 ns
Error	8	1.25	0.1563		
Total	11	2.7292			

Appendix 9: Anova of mean disease severity of the *Solanum macrocarpon* accessions.

Source	**df**	**SS**	**MS**	**F**	**P**
Blocks	2	1.3333	0.6667	2.1333	.1276 ns
Accession	11	35.0104	3.1828	10.1848	.0000 ***
Error	58	18.125	0.3125		
Total	71	54.46875			

Appendix 10: Sequences of the TEF region of the *Fusarium* spp isolates.

FS24

CAAGACTCACCTTAACGTCGTCGTCATCGGCCACGTCGACTCTGGCAAGTCGACCACTGTGAGTACTCTCCTCGAC
AATGAGCATATCTGCCATCGTCAATCCCGACCAAGACCTGGCGGGGTATTTCTCAAAGTCAACA
TACTGACATCGTTTCACAGACCGGTCACTTGATCTACCAGTGCGGTGGTATCGACAAGCGAACCATCGAG
AAGTTCGAGAAGGTTAGTCACTTTCCCTTCAATCGCGCGTCCTTTGCCCATCGATTTCCCCTACGACTCG
AAACGTGCCCGCTACCCCGCTCGAGACCAAAAATTTTGCAATATGACCGTAATTTTTTTGGTGGGGCACT
TACCCCGCCACTTGAGCGAAGGGAGCGTTTGCCCTCTTACCATTCTCACAACCTCAATGAGTGCGTCGTC
ACGTGTCAAGCAGTCACTAACCATTCAACAATAGGAAGCCGCTGAGCTCGGTAAGGGTTCCTTCAAGTAC
GCCTGGGTTCTTGACAAGCTCAAGGCCGAGCGTGAGCGTGGTATCACCATCGATATTGCTCTCTGGAAGT
TCGAGACTCCTCGCTACTATGTCACCGTCATTGGTATGTTGTCGCTCATGCTTCATTCTACTTCTCTTCG
TACTAACATATCACTCAGAC

FS27

CAAGACTCACCTTAACGTCGTCGTCATCGGCCACGTCGACTCTGGCAAGTCGACCACTGTGAGTACTACC
CTCAATGACCTGCTTATCAGCAGTCATCAACCCCGCCATACGTGGTGGGGTTAATTAACTTACACATTTG
CTGACAAGATTGCATAGACCGGTCACTTGATCTACCAGTGCGGTGGTATCGACAAGCGAACCATCGAGAA
GTTCGAGAAGGTTGGTTTCCATTTTCCTCGATCGCACGCCCTCTGCCCATCGATCCATCACCCGAATTAG
TCTCGACGACTGAACATGCGCCTGTTACCCCGCTCGAGTACAAAATTTTGCGGTTCAATCGTAATTTTTT
GGTGGGGCTTATACCCCGCTACTCGAGTGACAGGCGCTTGCCCTCTTCCCACAAAATCACTTGCGCATCA
CGTGTCAATCAGTCACTAACCACTCGACAATAGGAAGCCGCCGAGCTCGGTAAGGGTTCCTTCAAGTACG

CCTGGGTTCTTGACAAGCTCAAGGCCGAGCGTGAGCGTGGTATCACCATCGATATCGCTCTCTGGAAGTT
CGAGACTCCTCGCTACTATGTCACCGTCATTGGTATGTTGTCACCACTTGCACTCATTACCTTCTCATGC
TAACATGTATTCCAGACGCTCCCGGTCACCGTGATTTCATCAAGAACATGATCA

FS34

GAGGACAAGACTCACCTTAACGTCGTCGTCATCGGCCACGTCGACTCTGGCAAGTCGACCACTGTGAGTA
CTATCCTCAATGACCTGCTTATCAGCAGTCATCAACCCCGCCATACGTGGTGGGGTAAATTCAACTTATA
CATTTGCTGACAAGATTGTATAGACCGGTCACTTGATCTACCAGTGCGGTGGTATCGACAAGCGAACCAT
CGAGAAGTTCGAGAAGGTTGGTTTCCATTTTCCTCGATCGCACGCCCTCTGCCCATCGATCCATCACCCG
AATCAGTCTCGACGACTGAACATGCGCCTGTTACCCCGCTCGAGTACAAAATTTTGCGGTTCAATCGTAA
TTTTTTGGTGGGGCTCATACCCCGCTACTCGAGTGACAGGCGCTTGCCCTCTTCCCACAAAATCACTTGC
GCATCACGTGTCAATCAGTCACTAACCACCTGACAATAGGAAGCCGCCGAGCTCGGTAAGGGTTCCTTCA
AGTACGCCTGGGTTCTTGACAAGCTCAAGGCCGAGCGTGAGCGTGGTATCACCATCGATATCGCTCTCTG
GAAGTTCGAGACTCCTCGCTACTATGTCACCGTCATTGGTATGTTGTCACCACTTCCACTCATTACCTTC
TCATGCTAACATGTATTCCAGACGCTCCCGGTCACCGTGATTTCATCAAGAACATGAT

FS23

CACGTCGACTCTGGCAAGTCGACCACTGTGAGTACTATCCTCAATGACCTGCTTATCAGCAGTCATTAAC
CCGGCCATACGTGGTGGGGTAAATTCAACTTATACATTTGCTGACAAGATTGTATAGACCGGTCACTTGA
TCTACCAGTGCGGTGGTATCGACAAGCGAACCATCGAGAAGTTCGAGAAGGTTGGTTTCCATTTTCCTCG
ATCGCACGCCCTCTGCCCATCGATCCATCACCCGAATCAGTCTCGACGACTGAACATGCGCCTGTTACCC
CGCTCGAGTACAAAATTTTGCGGTTCAATCGTAATTTTTTGGTGGGGCCCATACCCCGCTACTCGAGTGA
CAGGCGCTTGCCCTCTTCCCACAAAATCACTTGCGCATCACGTGTCAATCAGTCACTAACCACCTGACAA
TAGGAAGCCGCCGAGCTCGGTAAGGGTTCCTTCAAGTACGCCTGGGTTCTTGACAAGCTCAAGGCCGAGC
GTGAGCGTGGTATCACCATCGATATCGCTCTCTGGAAGTTCGAGACTCCTCGCTACTATGTCACCGTCAT
TGGTATGTTGTCACCACTTCCACTCATTACCTTCTCATGCTAACATGTATTCCAGACGCTCCCGGTCACC
GTGATTTCATCAAGAACATGAT

FS3

GAGGACAAGACTCACCTTAACGTCGTCGTCATCGGCCACGTCGACTCTGGCAAGTCGACCACTGTGAGTA
CTATCCTCAATGACCTGCTTATCAGCAGTCATTAACCCGGCCATACGTGGTGGGGTAAATTCAACTTATA
CATTTGCTGACAAGATTGTATAGACCGGTCACTTGATCTACCAGTGCGGTGGTATCGACAAGCGAACCAT
CGAGAAGTTCGAGAAGGTTGGTTTCCATTTTCCTCGATCGCACGCCCTCTGCCCATCGATCCATCACCCG
AATCAGTCTCGACGACTGAACATGCGCCTGTTACCCCGCTCGAGTACAAAATTTTGCGGTTCAATCGTAA
TTTTTTGGTGGGGCCCATACCCCGCTACTCGAGTGACAGGCGCTTGCCCTCTTCCCACAAAATCACTTGC
GCATCACGTGTCAATCAGTCACTAACCACCTGACAATAGGAAGCCGCCGAGCTCGGTAAGGGTTCCTTCA
AGTACGCCTGGGTTCTTGACAAGCTCAAGGCCGAGCGTGAGCGTGGTATCACCATCGATATCGCTCTCTG
GAAGTTCGAGACTCCTCGCTACTATGTCACCGTCATTGGTATGTTGTCACCACTTCCACTCATTACCTTC
TCATGCTAACATGTATTCCAGACGCTCCC

FS14

GAGGACAAGACTCACCTTAACGTCGTCGTCATCGGCCACGTCGACTCTGGCAAGTCGACCACTGTGAGTA
CTATCCTCAATGACCTGCTTATCAGCAGTCATTAACCCGGCCATACGTGGTGGGGTAAATTCAACTTATA
CATTTGCTGACAAGATTGTATAGACCGGTCACTTGATCTACCAGTGCGGTGGTATCGACAAGCGAACCAT
CGAGAAGTTCGAGAAGGTTGGTTTCCATTTTCCTCGATCGCACGCCCTCTGCCCATCGATCCATCACCCG
AATCAGTCTCGACGACTGAACATGCGCCTGTTACCCCGCTCGAGTACAAAATTTTGCGGTTCAATCGTAA
TTTTTTGGTGGGGCCCATACCCCGCTACTCGAGTGACAGGCGCTTGCCCTCTTCCCACAAAATCACTTGC
GCATCACGTGTCAATCAGTCACTAACCACCTGACAATAGGAAGCCGCCGAGCTCGGTAAGGGTTCCTTCA
AGTACGCCTGGGTTCTTGACAAGCTCAAGGCCGAGCGTGAGCGTGGTATCACCATCGATATCGCTCTCTG
GAAGTTCGAGACTCCTCGCTACTATGTCACCGTCATTGGTATGTTGTCACCACTTCCACTCATTACCTTC
TCATGCTAACATGTATTCCAGACGCTCCC

FS40

CAAGACTCACCTTAACGTCGTCGTCATCGGCCACGTCGACTCTGGCAAGTCGACCACTGTGAGTACTCTC
CTCGACAATGAGCATATCTGCCATCGTCAATCCCGACCAAGACCTGGCGGGGTATTTCTCAAAGTCAACA
TACTGACATCGTTTCACAGACCGGTCACTTGATCTACCAGTGCGGTGGTATCGACAAGCGAACCATCGAG
AAGTTCGAGAAGGTTAGTCACTTTCCCTTCAATCGCGCGTCCTTTGCCCATCGATTTCCCCTACGACTCG
AAACGTGCCCGCTACCCCGCTCGAGACCAAAAATTTTGCAATATGACCGTAATTTTTTTGGTGGGGCACT
TACCCCGCCACTTGAGCGAAGGGAGCGTTTGCCCTCTTACCATTCTCACAACCTCAATGAGTGCGTCGTC
ACGTGTGAAGCAGTCACTAACCATTCAATAATAGGAAGCCGCTGAGCTCGGTAAGGGTTCCTTCAAGTAC
GCCTGGGTTCTTGACAAGCTCAAGGCCGAGCGTGAGCGTGGTATCACCATCGATATTGCTCTCTGGAAGT
TCGAGACTCCTCGCTACTATGTCACCGTCATTGGTATGTTGTCGCTCATGCTTCATTCTACTTCTCTTCG
TACTAACATATCACTCAGACGCTCCCGGTCACCGTGATTTCATCAAGAACATGATC

FS35

CAAGACTCACCTCAACGTCGTCGTCATCGGCCACGTCGACTCTGGCAAGTCGACCACCGTAAGTCAAACC
CTCATCGCGATCTGCTTATCTCGGGTCGTGGAACCCCGCCTGGCATCTCGGGCGGGGTATTCATCAGTCA
CTTCATGCTGACAATCATCTACAGACCGGTCACTTGATCTACCAGTGCGGTGGTATCGACAAGCGAACCA
TCGAGAAGTTCGAGAAGGTTGGTGACATCTCCCCCGATCGCGCCTTGCTATTCCACAACGAATTCCCTCC
CTCGCGATACGCTCTGCGCCCGCTTCTCCCGAGTTCCAAAATTTTTGCGGTCCGACCGTAATTTTTTTTT
TGGTGGGGCATTTACCCCGCCACTCGGGCGACGTTGGACAAAGCCCTGATCCCTGCACACAAAAACACCA
AACCCTCTTGGCGCGCATCATCACGTGGTTCACAACAGACGCTAACCGGTCCAACAATAGGAAGCCGCTG
AGCTCGGTAAGGGTTCCTTCAAGTACGCCTGGGTCCTTGACAAGCTCAAGGCCGAGCGTGAGCGTGGTAT
CACCATCGACATTGCCCTCTGGAAGTTCGAGACTCCCCGCTACTATGTCACCGTCATTGGTATGTTGCTG
TCACCTCTGTCACACATGTCTCACCACTAACAATCAACAGACGCCCCCGGCCACCGTGACTTCATCAAGA
ACATGAT

FS5

CAAGACTCACCTTAACGTCGTCGTCATCGGCCACGTCGACTCTGGCAAGTCGACCACTGTGAGTACTACC
CTCGATGACCTGCTTATCAGCAGTCATCAACCCCCCATACGTGGCGGGGTAATTTCAACTTGAATATTGG
CTGACAAAATTGCATAGACCGGTCACTTGATCTACCAGTGCGGTGGTATCGACAAGCGAACCATCGAGAA
GTTCGAGAAGGTTGGTTTCCATTTTCCTCGATCGCACGCCCTCTGCCCACCGATCCATCACTCGAATCCG
TCTCACGACGACTGAATATGCGCCTGTTACCCCGCTCGAGTACAAAATTTTGCGGTTCAATCGTAATTTT
TTTGGTGGGGCTTATACCCCGCTACTCGAGTGACAGGCGTTTGCCCTTTCCCACAAAATCATCTCTTGCG
CATCACGTGTCAAACAGTCACTAACCACCCGACAATAGGAAGCCGCCGAGCTCGGTAAGGGTTCCTTCAA
GTACGCTTGGGTTCTTGACAAGCTCAAGGCCGAGCGTGAGCGTGGTATCACCATCGATATCGCCCTCTGG
AAGTTCGAGACTCCTCGCTACTATGTCACCGTCATTGGTATGTTGTCATCGCTTACACTCATTACCTTCT
CATGCTAACATGTGCTTCAGACGCTCCCGGTCACCGTGATTTCATCAAGAACATGATCACTG

Appendix 11: ITS sequences of *Phoma sorghina* isolates

Ps1

ATCATTACCTAGAGTTGTAGGCTTTGCCTGCTATCTCTTACCCATGTCTTTTGAGTACCTTACGTTTCCTCGGTGGGT
TCGCCCACCGATTGGACAAATTTAAACCCTTTGCAGTTGAAATCAGCGTCTGAAAAAACTTAATAGTTACAACTTT
CAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAGTGTGAATTGCAGAATT
CAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCTTGGTATTCCATGGGGCATGCCTGTTCGAGCGTCATTTG
TACCTTCAAGCTCTGCTTGGTGTTGGGTGTTTGTCTCCTGTAGACTCGCCTTAAAACAATTGGCAGCCGGCGTATTG
ATTTCGGAGCGCAGTACATCTCGCGCTTTGCACTCATAACGACGACATCCAAAAGTACATTTTTACACTCTTGACC

Ps 2

ATCATTACCTAGAGTTGTAGGCTTTGCCTGCTATCTCTTACCCATGTCTTTTGAGTACCTTACGTTTCCTCG
GTGGGTTCGCCCACCGATTGGACAAATTTAAACCCTTTGCAGTTGAAATCAGCGTCTGAAAAAACTTAATAGTTAC
AACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAGTGTGAATTGCA
GAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCTTGGTATTCCATGGGGCATGCCTGTTCGAGCGT
CATTTGTACCTTCAAGCTCTGCTTGGTGTTGGGTGTTTGTCTCCTGTAGACTCGCCTTAAAACAATTGGCAGCCGGC
GTATTGATTTCGGAGCGCAGTACATCTCGCGCTTTGCACTCATAACGACGACATCCAAAAGTACATTTTTACACTCT
TGACC

Ps5

ATCATTACCTAGAGTTGTAGGCTTTGCCTGCTATCTCTTACCCATGTCTTTTGAGTACCTTACGTTTCCTCG
GTGGGTTCGCCCACCGATTGGACAAATTTAAACCCTTTGCAGTTGAAATCAGCGTCTGAAAAAACTTAATAGTTAC
AACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAGTGTGAATTGCA
GAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCTTGGTATTCCATGGGGCATGCCTGTTCGAGCGT
CATTTGTACCTTCAAGCTCTGCTTGGTGTTGGGTGTTTGTCTCCTGTAGACTCGCCTTAAAACAATTGGCAGCCGGC

GTATTGATTTCGGAGCGCAGTACATCTCGCGCTTTGCACTCATAACGACGACATCCAAAAGTACATTTTTACACTCT
TGACC

Ps3

ATCATTACCTAGAGTTGTAGGCTTTGCCTGCTATCTCTTACCCATGTCTTTTGAGTACCTTACGTTTCCTCG
GTGGGTTCGCCCACCGATTGGACAAATTTAAACCCTTTGCAGTTGAAATCAGCGTCTGAAAAAACTTAATAGTTAC
AACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAGTGTGAATTGCA
GAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCTTGGTATTCCATGGGGCATGCCTGTTCGAGCGT
CATTTGTACCTTCAAGCTCTGCTTGGTGTTGGGTGTTTGTCTCCTGTAGACTCGCCTTAAAACAATTGGCAGCCGGC
GTATTGATTTCGGAGCGCAGTACATCTCGCGCTTTGCACTCATAACGACGACATCCAAAAGTACATTTTTACACTCT
TGACC

Appendix 12: Genebank codes for isolates submitted to NCBI

Sequence	Isolate code	Genebank code
ef.sqn	FS24	JQ244840
ef.sqn	FS34	JQ244842
ef.sqn	FS23	JQ244843
ef.sqn	FS3	JQ244844
ef.sqn	FS14	JQ244845
ef.sqn	FS40	JQ244846
ef.sqn	FS35	JQ244847
ef.sqn	FS5	JQ244848
ITS.sqn	Ps1	JQ244849
ITS.sqn	Ps2	JQ244850
ITS.sqn	Ps3	JQ244851
ITS.sqn	Ps5	JQ244852
ITS.sqn	FS5	JQ244854
ITS.sqn	FS3	JQ244855
ITS.sqn	FS27	JQ244856

Appendix 13: List of eggplant accessions screened for resistance to wilt

No.	Species	Accession/ cultivar code	Origin
1.	*S. aethiopicum*	SOS 1	Origin unknown*
2.	*S. aethiopicum*	UG-AE-4	Uganda
3.	*S. aethiopicum*	UG-AE-10	Uganda
4.	*S. aethiopicum*	UG-AE-21	Uganda
5.	*S. aethiopicum*	TZSMN 2-8	Tanzania
6.	*S. aethiopicum*	TZSMN 3-10	Tanzania
7.	*S. aethiopicum*	TZSMN 75-7	Tanzania
8.	*S. aethiopicum*	ML-AE-5	Malawi
9.	*S. aethiopicum*	ML-AE-12	Malawi
10.	*S. aethiopicum*	DB3	Ghana
11.	*S. aethiopicum*	Manyire Green	Tanzania
12.	*Solanum spp.*	Landrace	Tanzania
13.	*S. aethiopicum*	OAA (089)	Cameroon
14.	*S. aethiopicum*	Small oval	Tanzania
15.	*S. aethiopicum*	N20	Origin unknown*
16.	*S. aethiopicum*	Heart shaped	Tanzania
17.	*S. aethiopicum*	Sivonkwe	Origin unknown*
18.	*S. aethiopicum gr. shum*	MM347	Congo
19.	*S. aethiopicum*	MM01150	Origin unknown*
20.	*S. aethiopicum gr. Gilo*	MM1371	Tanzania
21.	*S. aethiopicum*	MM1106	Origin unknown*
22.	*S. aethiopicum gr. Aculeatum*	MM134	France
23.	*S. aethiopicum gr. Aculeatum*	MM1474	INDE
24.	*S. aethiopicum gr. Aculeatum*	MM1102	Burkina Faso
25.	*S. aethiopicum gr. Aculeatum*	MM1483	Inconnue
26.	*S. aethiopicum gr. Gilo*	MM10181	Ghana
27.	*S. aethiopicum gr. Gilo*	MM803	Gabon
28.	*S. aethiopicum gr. Gilo*	MM870	Madagascar
29.	*S. aethiopicum gr. Gilo*	MM1641	Africa(Ouest)
30.	*S. aethiopicum gr. Gilo*	MM10086	Togo
31.	*S. aethiopicum gr. Gilo*	MM10245	Zambia
32.	*S. aethiopicum gr. Gilo*	MM1480	Inconnue
33.	*S. aethiopicum gr. Gilo*	MM1188	Inconnue
34.	*S. aethiopicum gr. Gilo*	MM11010	Cote D' Voire
35.	*S. aethiopicum gr. Gilo*	MM196 TER	Burkina Faso
36.	*S. aethiopicum gr. Gilo*	MM868	Tchad (Bousso)
37.	*S. aethiopicum gr. Gilo*	MM458	Japon
38.	*S. aethiopicum gr. Gilo*	MM10079	Togo
39.	*S. aethiopicum gr. Gilo*	MM10213	Ghana

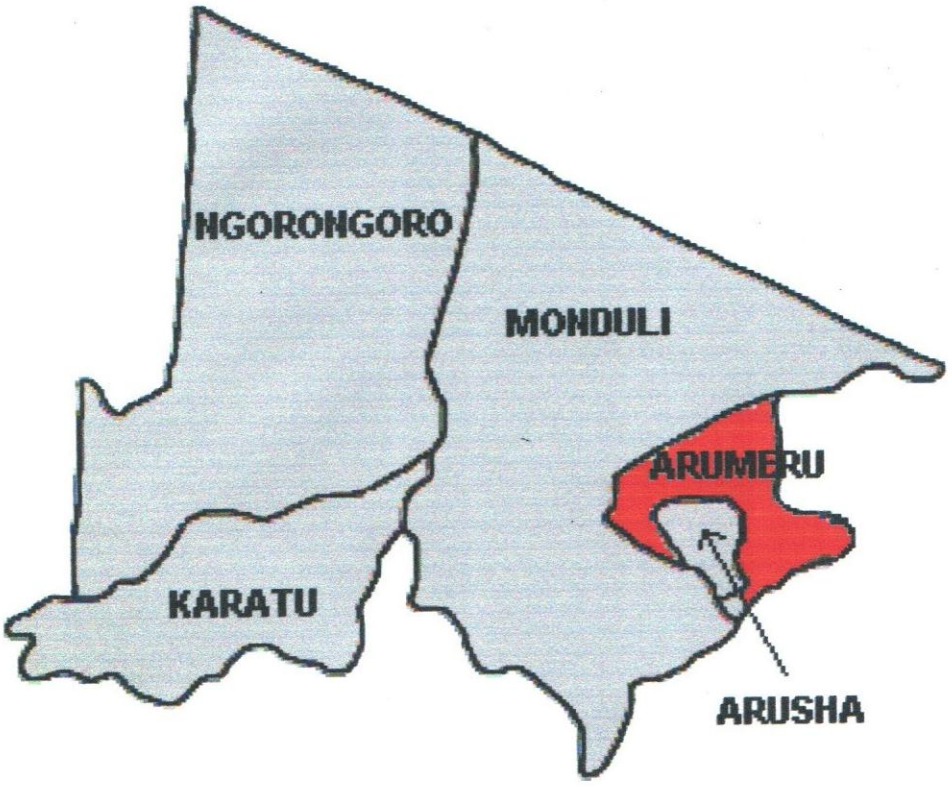
40.	*S. aethiopicum gr. Gilo*	MM1162	Uganda
41.	*S. aethiopicum gr. Kumba*	MM574	Senegal
42.	*S. aethiopicum gr. Kumba*	MM1642	Africa(Ouest)
43.	*S. aethiopicum gr. Kumba*	MM1107	Burkina Faso
44.	*S. aethiopicum gr. Kumba*	MM1207	Mali
45.	*S. aethiopicum gr. Kumba*	MM267	Mauritania
46.	*S. aethiopicum gr. Shum*	MM1121	Zambia
47.	*S. aethiopicum gr. Shum*	MM1119	Togo
48.	*S. aethiopicum gr. Shum*	MM1161	Bernin
49.	*S. aethiopicum*	UG-AE-1	Uganda
50.	*S. aethiopicum*	UG-AE-3	Uganda
51.	*S. aethiopicum*	UG-AE-14	Uganda
52.	*S. aethiopicum*	UG-AE-15	Uganda
53.	*S. aethiopicum*	UG-AE-23	Uganda
54.	*S. aethiopicum*	UG-AE-24	Uganda
55.	*S. melongena*	Black beauty	Tanzania
56.	*S. dasyphyllum*	MM1164	Togo
57.	*S.dasyphyllum*	MM12126	Uganda
58.	*S. macrocarpon*	MM10256	Ghana
59.	*S. macrocarpon*	MM714	Zimbabwe
60.	*S. macrocarpon*	MM10260	Ghana
61.	*S. macrocarpon*	MM11044	Cote D' Voire
62.	*S. macrocarpon*	MM132	France
63.	*S. macrocarpon*	MM1131	Togo
64.	*S. macrocarpon*	MM 150	Cote D' Voire
65.	*S. macrocarpon*	MM283	Origin unknown*
66.	*S. macrocarpon*	MM12209	Zaire
67.	*S. macrocarpon*	MM252	Ghana
68.	*S. macrocarpon*	MM855	Togo
69.	*S. macrocarpon*	UG-AE-6	Uganda
70.	*S. macrocarpon*	UVPP	Tanzania
71.	*S. macrocarpon*	CR001	Cameroon
72.	*S. macrocarpon*	MM01139	Origin unknown*
73.	*S. macrocarpon*	MM01064	Origin unknown*
74.	*S.melongena*	S. 00677	Origin unknown*
75.	*S.melongena*	S. 00718	Origin unknown*
76.	*S.melongena*	S. 00735	Origin unknown*
77.	*S.melongena*	S. 00811	Origin unknown*
78.	*S.melongena*	S. 00017	Origin unknown*
79.	*S.melongena*	S. 0052	Origin unknown*
80.	*S.melongena*	S. 00204	Origin unknown*
81.	*S.melongena*	S. 00256	Origin unknown*
82.	*S.melongena*	S. 00736	Origin unknown*
83.	*S.melongena*	S. 00567	Origin unknown*
84.	*S.melongena*	TS00567	Origin unknown*

85.	*S.melongena*	TS00131	Origin unknown*
86.	*Solanum anguivi*	N19	Origin unknown*
87.	*Solanum anguivi*	MM1103	Burkina Faso
88.	*Solanum anguivi*	MM905	Origin unknown*
89.	*Solanum anguivi*	MM159	Origin unknown*
90.	*Solanum spp*	TZSMN 15-2	Tanzania
91.	*Solanum spp*	ML-AE-4	Malawi
92.	*Solanum spp*	ML-AE-6	Malawi
93.	*Solanum spp*	ML-AE-9	Malawi

*Germplasm without place of origin data.

EGERTON UNIVERSITY LIBRARY

Appendix 14 : Arumeru District Map



Source: Arumeru District homepage for the Tanzania National census

UNIVERSITY LIBRARY