CHARACTERIZATION OF *FUSARIUM UDUM* BUTLER ISOLATES AND WILT RESISTANCE IN PIGEONPEA IN KENYA

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B.Sc., M.Sc. (Nairobi)

A Thesis Submitted in Fulfilment of the Requirements for the

Degree of Doctor of Philosophy

Department of Crop Protection

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2001

Declaration

This thesis is my original work and has not been presented for a degree in any other university

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Acknowledgements

I wish to express my sincere appreciation to Prof. A.W. Mwang'ombe, Prof. J.P. Baudoin, Prof. P.M. Kimani and Dr. G. Mergeai for their guidance, invaluable suggestions and encouragement during the period of this study. I am indebted with much gratitude to Prof. Mwang'ombe's efforts in getting me interested in a Ph.D. programme and Prof. Baudoin, as the coordinator of the project "Genetic improvement of pigeonpea and management of intercropping systems in semi arid areas of East Africa", for an award of a Scholarship. I greatly appreciate the assistance of Dr. J.M. Jacquemin and Dr. A. Maquet in the AFLP analysis of the fungal isolates, Prof. P. Lepoivre for using the facilities in his laboratory for fungal DNA extraction, and Mr. C. Wells for training me on transmission electron microscopy (TEM).

I am grateful to all members of staff in the Unit of Phytotechnie des Regions Intertropicales and Unit of Phytopathologie (Faculte Universitaire des Sciences Agronomiques de Gembloux, Belgium), Centre de Recherches Agronomiques (Gembloux, Belgium), Unit of Electron Microscopy (ILRI, Nairobi, Kenya), and Department of Crop Protection and Department of Crop Science (University of Nairobi, Kenya) for their help and encouragement during the period of my study.

I wish to express my gratitude to the INCO-DC of European Commission (contract number ERBIC18CT960130) and AGCD (Government of Belgium) for financial support that was crucial to the accomplishment of my Ph.D. programme.

My thanks go to Dr. S.N. Silim and the staff at ICRISAT (Nairobi, Kenya) for their collaboration and for providing pigeonpea seeds used in this study; and the International Mycological Institute (United Kingdom) for providing two strains of *Fusarium udum*.

I am grateful to Moi University (Eldoret, Kenya) who offered me study leave in order to undertake my Ph.D. programme at the University of Nairobi.

Special thanks to my son Peter Kibillioch, and daughters Daisy Jepkoech, Phyllis Jepchumba and Millicent Jebet for their patience, understanding and learning to do without me during most times of my student life.

Dedication

Dedicated to my wife:

Gladys Kabon Kiptoo

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List of abbreviations

AEZ: Agro-ecological zone.

AFLP: Amplified fragment length polymorphism.

ANOVA: Analysis of variance.

ASAL: Arid and semi-arid lands.

ATP: Adenosine 5'-trisphosphate.

CDAZ: Czapek Dox plus AZ liquid.

CMI: Commonwealth Mycological Institute.

CTAB: 3-D-cetyltrimethylammonium bromide

DMP: Dimethylpolysiloxane.

DNA: Deoxyribonucleic acid.

dNTP: Deoxynucleoside triphosphate.

EDTA: Ethylenediaminetetraacetic acid.

GLM: General linear model.

HaGiS: Habgood-Gilmour Spreadsheet.

ICRISAT: International Crops Research Institute for the Semi-Arid Tropics.

ILRI: International Livestock Research Institute.

IMI: International Mycological Institute.

KARI: Kenya Agricultural Research Institute

LM: Light microscope/micrograph.

LSD: Least significant difference test.

MM: Minimal medium.

MMC: Minimal medium with chlorate.

NDFRC: National Dryland Farming Research Centre

NTSYS: Numerical taxonomy and multivariate analysis system.

PCNB: Peptone chloronitrobenzene.

PCR: Polymerase chain reaction

PDA: Potato dextrose agar.

PIPES: Piperazine-N, N′-bis (2-ethane-sulfonic acid).

RFLP: Restriction fragment length polymorphism.

RNA: Ribonucleic acid.

RNase: Ribonuclease.

SAT: Semi-arid tropics.

SIMQUAL: Similarity for qualitative data.

SNA: Spezieller Nahrastofarmer agar.

SNK: Student-Newman-Keuls test.

TAE: Tris-acetate-EDTA.

TBE: Tris-borate-EDTA.

TE: Tris-EDTA.

TEM: Transmission electron microscope/micropgraph.

TEMED: N,N,N′,N′-tetramethylethylenediamine.

UPGMA: Unweighted paired group method with arithmetic averages.

UV: Ultra violet light.

VCG: Vegetative compatibility group(s)/grouping.

Abstract

The present study was undertaken to determine the variability among pathogenic isolates of *Fusarium udum* causing *Fusarium* wilt of pigeonpea in Kenya using various techniques, and to find out the nature of mechanical resistance in pigeonpea plants.

A survey of *Fusarium* wilt of pigeonpea was carried out on 86 farms located in 13 pigeonpea growing districts in Eastern, Coast, Central and Nairobi provinces during the flowering, pod setting and dry pod stages between July and September 1997. The disease was recorded in 12 districts, 8 AEZs and from 55 farms (64% prevalence). There were significant differences ($P \leq 0.05$) in *Fusarium* wilt incidences among the farms, districts, AEZs, altitude and cropping system, but no significant differences among pigeonpea types. The wilt incidence ranged from 0 to 96.1% with a mean of 8.4%.

Inoculation techniques were assessed, and root-dip inoculation technique was found to be very effective and reliable in inoculating pigeonpea plants with *F. udum* isolates during pathogenicity and virulence studies under glasshouse conditions. The wilt incidence on pigeonpea plants was significantly different ($P \leq 0.05$) between the two inoculation techniques, with means of 48.1% and 14.4% for root-dip technique and colonized whole rice grain technique, respectively.

Cultural characteristics of *F. udum* isolates grown on PDA medium were used to classify 79 single-spore isolates into: 3 groups by aerial mycelial growth, 2 groups by mycelial texture, 3 groups by radial mycelial growth (colony diameter), 5 groups by mycelium colour, 3 groups by substrate colour, and 4 groups by sporulation. Macroconidial length was used to classify 79 isolates of *F. udum* grown on PDA medium into 3 groups.

All the 79 isolates of *F. udum* were found to be pathogenic to susceptible pigeonpea variety KAT 60/8. Pathogenic variability was evident among *F. udum* isolates resulting in significant differences ($P \leq 0.05$) in wilt incidence with a mean of 75% wilt. The isolates were classified into 2 virulence groups. Seven pigeonpea varieties obtained from and recommended by ICRISAT were used as differentials in order to determine the possible existence of races in *F. udum* in Kenya. Twenty-one isolates were used for inoculation in a glasshouse experiment.

Fusarium wilt incidence was significantly different ($P \leq 0.05$) among the isolates and varieties, with a mean of 24.9% wilt at six weeks after root-dip inoculation. Pathogenic variability was observed among the isolates. Eleven physiologic races of *F. udum* were identified with race 0 being dominant and independent, while the remaining 10 races were closely related.

The *Nit* mutants of 79 isolates of *F. udum* were generated by selecting chlorate-resistant sectors on minimal medium amended with 1.5% potassium chlorate (MMC). The *nit* mutants were grouped into *nit*1 (79% mutants), *nit*3 (18% mutants) and NitM (3% mutants). A total of 8 NitM tester mutants from Kenya and India were paired with *nit*1 and/or *nit*3 mutants of different isolates for vegetative compatibility reactions on minimal medium (MM). The 75 isolates from Kenya, and two isolates each from Malawi and India were grouped into a single vegetative compatibility group, VCG 1.

AFLP analysis of the DNA of 56 isolates of *F. udum* using 7 primer combinations with *Eco*R I + 2 and *Mse* I + 3 selective nucleotides at the 3'-end of the primers revealed 326 amplified bands with 121 being polymorphic (37% polymorphism). Based on AFLP analysis three groups were identified with group I having 40 isolates, group II had 9 isolates and group III had 7 isolates.

The presence of *F. udum* in wilt susceptible pigeonpea plants was characterized by mycelia and conidia in the xylem vessels, plugging in some vessels, disintegration of xylem parenchyma cells in the infected areas, and the formation of cavities due to heavy colonization in the vascular bundle, the pith and the cortex. Resistance to *F. udum* in the roots and stems of wilt resistant pigeonpea plants was associated with low fungal colonization, and higher occlusion due to tyloses and gels in the xylem vessels than in susceptible plants. There were significant differences ($P \leq 0.05$) in the number of xylem vessels occluded by tyloses in resistant and susceptible plants with a maximum of 22.5% and 8.0% occlusion, respectively

1.0 INTRODUCTION

1.1 Pigeonpea production in Kenya

Pigeonpea (*Cajanus cajan* (L.) Millsp.) is one of the most widely grown and eaten grain legume in the semi-arid tropics (SAT) of the world. Kenya is the world's second largest producer after India (Omanga and Matata, 1987; Nene and Sheila, 1990). Pigeonpea in Kenya is second to common bean (*Phaseolus vulgaris* L.) in yield and third to common bean and cowpea (*Vigna unguiculata* (L.) Wallp.) in acreage (Anon., 1990). In 1990, total acreage and yield of pigeonpea in Kenya were 87,530 ha and 90,430 tones, respectively (Anon., 1990) (Appendix 8.1). During the same year, acreage and yield of common bean were 589,800 ha and 466,057 tones, respectively, while that of cowpea were 119,420 ha and 64,640 tones, respectively. Over 80% of the crop is cultivated in Eastern province mainly in Machakos, Kitui, Mwingi, Makueni, Embu, Tharaka-Nithi, Nyambene, Mbeere and Meru districts. Other districts that grow pigeonpeas are Muranga, Thika, Taita-Taveta, Kilifi, Tana River, Kirinyaga, Kwale, Marsabit, Kiambu, Baringo, West Pokot and Kajiado. Majority of these districts are in the marginal rainfall zones or arid and semi-arid lands (ASAL). Pigeonpea is mainly grown by small-scale farmers in mixtures with maize (*Zea mays* L.), sorghum (*Sorghum bicolor* (Linn.) Moench), cotton (*Gossypium hirsutum* L.), and other legumes such as common bean (Onim, 1981). The crop is mainly grown for home consumption with surpluses being sold off.

1.2 Pigeonpea adaptation and uses

Pigeonpea is widely adapted to lowland tropical conditions. It is notably tolerant to heat, drought and poor soil, with a deep root system that permits good growth in semi-arid regions (Allen, 1983). It is often the only crop that gives some grain yield during years with low rainfall when other crops such as maize and common bean will have wilted and probably dried up.

Although pigeonpea ranks sixth in the world in area and production in comparison to other grain legumes such as beans, peas, and chickpeas, it is used in more diverse ways than others (Nene and Sheila, 1990). Pigeonpea is primarily grown for dry seeds. These are usually either boiled mixed with maize, cassava, sweet potatoes and bananas or fried and eaten as sauce. The green immature seeds can also be harvested and eaten as vegetables or taken to canning industry that has a market potential for export. The crop is often grown as boundary plants, hedges or windbreaks, while the woody stems are used as fuelwood and for roofing and making baskets.

The crop is utilised in other ways elsewhere in the world (Whyte *et al.*, 1953; Saville and Wright, 1955; Khan and Rachie, 1972). In India the dry seeds are split and made into 'dhal'. The dried husks and rejected seed can be compounded with other feed and fed to cattle, poultry and pigs (Whiteman and Norton, 1981; Onim, 1982). The crop can be used as fodder, hay or silage; and as browse plants. It is also planted as green manure, cover crop, and for control of soil erosion. It is also grown as a rotation crop for improvement of soil fertility and as a fallow crop (Webster and Wilson, 1966). In Thailand and eastern India, pigeonpea crops grown for 2 to3 years, serve as an important host for scale insects that produces lac (Nene and Sheila, 1990). Pigeonpea leaves are also used to feed silkworms. Morton (1976) lists many folk medicinal uses for pigeonpea. Dry roots, leaves, flowers, and seeds are used in different countries to treat a wide range of ailments of the skin, liver, lungs, and kidney.

The role of pigeonpea and other pulse in human and animal nutrition is largely for the supply of protein. Protein content of dry seeds of pigeonpea varies from 19.8% to 23.6% (Deosthale and Rao, 1981).

1.3 Pigeonpea production constraints in Kenya

Although the average of pigeonpea yields of 1,000 kg/ha in Kenya are higher than the world average of 684 kg/ha, they are below half of what has been realised under research conditions which is in the range of 2700 - 2939 kg/ha (Onim, 1981; Kimani,

1991). This has been attributed to lack of suitable varieties, diseases, insect pests, moisture stress/drought and poor agronomic practices.

Unfortunately, crop scientists did not recognise diseases as a major problem in countries of Eastern Africa (Acland, 1971; Rachie and Roberts, 1974). Surveys carried out by Kannaiyan *et al.* (1984) showed *Fusarium* wilt (*Fusarium udum* Butler), cercospora leaf spot (*Mycevellosiella cajani* (Henn) Rangel ex. Trotter (Syn. *Cercospora cajani* = *Vellosiella cajani* Rangel) and powdery mildew (*Oidiopsis taurica* (Lev.) Salmon) to be the most common diseases in the region.

1.4 *Fusarium* wilt disease

Fusarium wilt of pigeonpea has been reported from 15 countries; Bangladesh, Ghana, Grenada, India, Indonesia, Kenya, Malawi, Mauritius, Nepal, Nevis, Tanzania, Trinidad, Uganda, and Venezuela (Nene *et al.*, 1989), but it is apparently more important in India and Eastern Africa. *Fusarium* wilt is the most important disease of pigeonpea in Kenya (Onim, 1981) and India (Sen Gupta, 1974). In Eastern Africa, wilt incidence has been found to be quite high in Kenya (15.9%), Malawi (36.3%) and Tanzania (20.4%) (Kannaiyan *et al.*, 1984). In some fields, wilt incidence could be as high as 90%. The annual losses caused by this disease in these countries were estimated at over US$ 5 million. Losses due to wilt vary from a negligible amount to 100% depending on the stage at which the crop is attacked (Kannaiyan and Nene, 1981). *Fusarium* wilt has been identified as one of the main causes of low yields in farmer's fields in Kenya (Onim, 1981). A survey carried out by Onim (1981) in Kenya showed an incidence of between 5% and 60% with a mean of 12%. It was also noted that the disease incidence seemed to increase during wetter years. Songa *et al.* (1991) carried a *Fusarium* wilt survey in Machakos and Kitui districts in Kenya and noted a wilt incidence of 6 to 55%. *Fusarium* wilt was found to be the most widely distributed disease of pigeonpea in all the 13 districts studied in Malawi with a mean wilt incidence of 5.4% (Changaya-Banda *et al.*, 1996).

Control of wilt could increase productivity of pigeonpea. Acland (1971) considered crop rotation as the only method to avoid *Fusarium* wilt. Crop rotation of the legume with cereals such as sorghum or tobacco and also intercropping with cereals such as sorghum are known to reduce wilt incidence in India (Reddy, 1991). However, the multiplication and survival ability of the wilt pathogen is known to vary with soils. Control of the pathogen by soil organic amendments, and by application of trace elements such as boron, manganese and zinc to the soil have been reported (McRae and Shaw, 1933; Sarojini, 1951).

The breeding for resistant varieties is the best method of plant disease control. Breeding for *Fusarium* wilt resistant varieties has been conducted since the turn of the last century. In most cases it was mainly through routine methods of selection in wilt-infested fields and 'wilt-sick' soils in glasshouses (Butler, 1908; Mukherjee *et al.*, 1971; Nene and Kannaiyan, 1982; Okiror, 1986; Reddy, 1991). In all cases resistant and/or tolerant cultivars/lines were reportedly identified. In Kenya, the pigeonpea improvement programs at the University of Nairobi and the National Dryland Research Centre, Katumani (KARI) have undertaken the screening and improvement of pigeonpea varieties against *Fusarium* wilt. Songa *et al.*(1995) reported 26 pigeonpea lines that were resistant to *Fusarium* wilt. Kimani *et al.*(1994) identified ten pigeonpea lines that were resistant or tolerant to *Fusarium* wilt, and these lines were early maturing, short in height, high yielding and with large, white/beige seeds. Evaluation of extensive collections of pigeonpea germplasm at ICRISAT centre in India has confirmed that adequate wilt resistance is available within *C. cajan* (Nene and Kannaiyan, 1982).

It is important to note that screening plant germplasm against plant pathogens should be an ongoing process as the pathogens are biological entities that tend to vary or alter their mode of action/attack. Thus, germplasm known to be resistant to the disease in one locality could succumb to the same pathogen in another locality. Some pigeonpea varieties resistant to wilt at one location have been found to be susceptible at other locations (Nene *et al*, 1979; Songa *et al.*, 1995).

There is evidence that resistance to *F. udum* for which most information is available, has a physiologic basis. Murthy and Bagyaraj (1980) demonstrated that flavanols, as well as alkaloids, occur at higher concentrations in wilt-resistant than in wilt susceptible pigeonpea cultivars infected with *F. udum*. Preston (1977) showed that the phytoalexin, cajanone, has the ability to completely inhibit the growth of *F. udum*. Marley and Hillocks (1993) indicated that resistance to *Fusarium* wilt in pigeonpea appears to depend primarily on the rapid synthesis of cajanol and other phytoalexins. They reported that vascular occlusion, the main physical response to infection, may be unimportant, or may serve to complement the antifungal effect of cajanol. Information is not available implicating vascular occlusion (mechanical barriers) due to the production of tyloses or gels (or gums) in xylem vessels of resistant plants of pigeonpea infected with *F. udum*, and yet vascular occlusion have been implicated in resistance of other plants to wilt diseases induced by *Fusarium* (Beckman *et al.*, 1962; Mehrotra, 1980) or *Verticillium* sp (Blackhurst and Wood, 1963; Mace, 1978). There is no data on systematic investigation on *F. udum* colonization of pigeonpea lines with varying resistance or susceptibility.

1.5 Variability in *Fusarium udum*

Management of soilborne diseases is a continuous challenge to growers. The structural, physical, and biological complexity of the soil environment in which pathogens interact with plant roots inherently limits the options available for disease control. Some of the most proven control measures include resistance and, in some situations, fungicide application. Despite great efforts to effectively combine these options with other control strategies, soilborne diseases such as *Fusarium* wilt of pigeonpea continue to constrain crop production. Pathogen populations must constantly adapt to the changes in their environment to survive. In agricultural ecosystems, environmental changes may include resistant varieties, applications of fungicides and fertilisers, irrigation, and crop rotation. It is clear that agricultural systems impose strong directional selection on pathogen populations (McDonald, 1997). Control strategies for

Fusarium wilt of pigeonpea must therefore target populations of *F. udum* instead of individual isolates if they are to be effective.

Continuous efforts towards pathogenic variability and identification of distinct strains (groups) or races present in *F. udum* are therefore needed to develop a proper strategy for pigeonpea resistance breeding and also utilization of resistant lines. *F. udum* shows a great deal of variation in cultural and morphology characteristics. The characteristics that have been used to study cultural variation in *F. udum* include colony diameter (radial mycelial growth), aerial mycelial growth, mycelial dry weight, mycelium colour, substrate colour and sporulation. Isolates of *F. udum* have been classified into 3 to 25 groups (pathotypes) on the basis of cultural characteristics (Subramanian, 1963; Shit and Sen Gupta, 1978; Nene *et al.*, 1979; Gupta *et al.*, 1988, Gaur and Sharma, 1989; Changaya-Banda *et al.*, 1996). Other reports have also indicated high variation of this pathogen in culture (Butler, 1910; Sarojini, 1951, Subramanian, 1955; Baldev and Amin, 1974; Jeswani *et al.*, 1977; Reddy and Chaudhary, 1985; Okiror, 1986). The conidial lengths have been reported to vary considerably in *F. udum*, ranging from 3.9 to 15.0 µm and 8.5 to 50.0 µm for microconidia and macroconidia, respectively (Booth, 1978; Mehrotra, 1980; Rai and Upadhyay, 1982; Gaur and Sharma, 1989). The high variation in cultural and morphology characteristics of this pathogen could be due to environmental conditions, the age of the isolates, subculturing, method of storage and culturing conditions. These phenotypic characteristics are therefore likely to be subject to selection and hence appear unreliable for the characterization of this pathogen into distinct pathogenic groups.

F. udum is pathogenic only to pigeonpea (Booth, 1971; Subramanian, 1971; Gerlach and Nirenberg, 1982). Wide variations in virulence (pathogenicity) to different genotypes of pigeonpea among *F. udum* isolates have been reported, and therefore the possibility of the existence of physiologic races and/ or distinct strains (Sarojini, 1951, Subramanian, 1963; Mukherjee *et al.*, 1971; Baldev and Amin, 1974; Shit and Sen Gupta, 1978; Okiror, 1986; ICRISAT, 1987; Gaur and Sharma, 1989; Reddy and Raju, 1993, Changaya-Banda *et al.*, 1996). However, gene (s) that governs race-specific

resistance to *Fusarium* wilt in pigeonpea have not been identified (Allen, 1983), and hence standard pigeonpea differentials for race typing has not been developed. Virulence levels of the pathogen in pigeonpea plants could also be affected by the environmental conditions and the inoculation techniques.

To overcome the problems associated with the standard methods such as cultural and morphological characterization, and virulence/race typing, attempts have been made to use the natural variation present in the DNA as a means for grouping fungal pathogens into species, forma specialis, races or pathotypes. The genetic markers that have been used to characterize phytopathogenic fungal species include vegetative compatibility groups (VCG), isozymes, restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs) and amplified fragment length polymorphisms (AFLPs). Among these markers, only isozyme techniques have been used to identify genetic variation within *F. udum* (Shit and Sen Gupta, 1980; Okiror, 1986).

Most studies on the variability of *F. udum* have used cultural and morphology characteristics, and virulence as a basis to distinguish between different isolates or strains (Sarojini, 1951; Subramanian, 1963; Mukherjee *et al.*, 1971; Baldev and Amin, 1974; Shit and Sen Gupta, 1978; Nene *et al.*, 1979; Gupta *et al.*, 1988; Gaur and Sharma, 1989; Reddy and Raju, 1993). Majority of these studies have been undertaken in India. Studies reported from Kenya on variability of this pathogen included 12 isolates obtained from three districts, and cultural characteristics, virulence on different pigeonpea varieties and isozymes (protein profiles) were used as markers (Okiror, 1986). The use of genetic markers such as AFLP, RFLP, RAPD or VCG to characterize *F. udum* isolates could greatly help in the understanding of the variability within this fungus and could help pigeonpea improvement programs aimed at breeding for wilt resistance.

1.6 Objectives

In view of the economic importance of wilt as a constraint to increased pigeonpea yields and the fact that little has been reported on the pathogenic variability of *F. udum*

and mechanisms of resistance to the fungus in pigeonpea, this research study was undertaken with the following objectives:

1. To undertake a field survey to determine the current status on occurrence, prevalence and incidence of *Fusarium* wilt on pigeonpea in Kenya.
2. To isolate *F. udum* from infected pigeonpea plants collected from various agro-ecological zones and verify indeed that it is the causal agent.
3. To validate two inoculation techniques as possible methods for screening pigeonpea for resistance to *Fusarium* wilt.
4. To characterize *F. udum* isolates into distinct pathogenic groups and/or physiologic races by using: (i) Cultural characteristics, (ii) Conidial measurements, (iii) Virulence on wilt susceptible pigeonpea variety, (iv) Physiological race typing (v) Vegetative compatibility groups (VCG), and (vi) Amplified fragment length polymorphism (AFLP)
5. To study tissue colonization by *F. udum* and the nature of mechanical resistance (vascular occlusion) in pigeonpea plants.

2.0 LITERATURE REVIEW

2.1 Pigeonpea

2.1.1 Origin and world distribution of pigeonpea

Pigeonpea (*Cajanus cajan* (L.) Millsp.) originated in India, and this is based on the presence of several wild relatives, the large diversity of the crop gene pool, and the wide usage in daily cuisine (Van der Maesen, 1990). A secondary centre of diversity of pigeonpea is found in eastern Africa (Van der Maesen, 1990).

Pigeonpea is grown in 111 countries in the world although data on acreage and production is available from 26 countries (Van der Maesen, 1983, 1986; Nyabyenda, 1987; Nene *et al.*, 1989). It is widely grown in the Indian sub-continent and accounts for almost 90% of the world's crop. Other regions where pigeonpea is grown are South East Asia, Africa, and the Americas. There is substantial area under pigeonpea in Kenya, Uganda, and Malawi in eastern Africa, and in the Dominican Republic and Puerto Rico in Central America. In most other countries, pigeonpea is grown in small areas and as a backyard crop. In Kenya, it is the most important pulse crop after beans, and is widely grown in Eastern and Coast Provinces, and the drier parts of Central and Rift Valley Provinces (Anon., 1990).

2.1.2 Importance of pigeonpea

Pigeonpea being a legume, fixes atmospheric nitrogen. In one experiment where maize followed pigeonpea, the residual nitrogen was estimated to be approximately 40 kg/ha (Kumar Rao *et al.*, 1981). Its deep root system break the plough pans and improve soil structure and allows for optimum moisture and nutrient utilization. Extensive ground cover by pigeonpea prevents soil erosion by wind and water, encourages water filtration, minimize sedimentation, and smoothers weeds (Nene and Sheila, 1990).

Pigeonpea landraces and traditional cultivars enables the production of multiple harvests because of their perrenial nature, and are almost always grown as intercrops or in mixed cropping systems with shorter-duration crops. The new short-duration cultivars

are grown as sole crops. Intercropping sorghum and pigeonpea leads to significant reduction in incidence of *Fusarium* wilt of pigeonpea (Natarajan *et al.*, 1985). In rainfed situations, pigeonpea provides more stability of production over environments and seasons than cereals with which it is intercropped (Singh and Subba Reddy, 1988).

2.1.3 Diseases of pigeonpea

Pigeonpea is subject to attack by more than 100 pathogens (Nene *et al.*, 1989). These include fungi, bacteria, viruses, nematodes, and mycoplasma-like organisms. Fortunately, only a few of them cause considerable economic losses (Kannaiyan *et al.*, 1984), and the distribution of the most important diseases is geographically restricted. Diseases of economic importance in eastern Africa are *Fusarium* wilt (*F. udum* Butler) and *Cercospora* leaf spot (*Mycevellosiella cajani* (Henn) Rangel ex. Trotter. The major diseases of pigeonpea in Kenya (in descending order of importance) are *Fusarium* wilt, *Cercospora* leaf spot and powdery mildew (*Oidiopsis taurica* (Lev.) Salmon) (Songa and King, 1994).

2.2 History and geographical distribution of *Fusarium* wilt of pigeonpea

According to available reports, pigeonpea wilt caused by *F. udum* could have occurred before the beginning of the last century (Butler, 1906). This is supported by the fact that by 1902 efforts were already being directed at breeding resistant cultivars through selection in wilt-infested fields. Butler is credited as being the first scientist to isolate, identify and name the fungus causing pigeonpea wilt (Butler, 1906). According to him, pigeonpea wilt was caused by a member of genus *Fusarium* and had no other known host. This fungus was subsequently named *F. udum* Butler. A detailed account of the disease and the pathogen was given later (Butler, 1918).

Research work on *Fusarium* wilt of pigeonpea covers more than 90 years (1906-2000) and has focused on various topics especially the methods of disease control such as breeding for resistance, cultural methods, biological control and chemical control.

Other areas of study on *Fusarium* wilt included physiologic aspects of the pathogen, infection, pathological histology, physiologic races and the discovery of the perfect state of the pathogen as *Gibberella indica* (Rai and Upadhyay, 1982). However, it is important to note that despite much information on the disease, there is no successful method of its control/management and it remains a serious problem in pigeonpea growing areas. *Fusarium* wilt widely occurs in Asia and Africa (CMI, 1985). The disease is apparently more important in India and eastern Africa.

2.3 Economic importance

Fusarium wilt is considered to be the most important disease of pigeonpea in India (Butler, 1906; Mundkur, 1967). National mean values for wilt incidence in India vary between 1.1 and 22.61% (Nene, 1977). However, extremely high incidences have been reported. In Madhaya Pradesh (India), incidences of about 50% have been recorded (Sharma *et al.*, 1977). Mukherjee *et al.*, (1971) reported *Fusarium* wilt incidences of between 93 and 100% in West Bengal (India). Kannaiyan *et al.* (1984) reported wilt incidence in Kenya, Malawi and Tanzania of 15.9% (0-90%), 36.3% (0-90%) and 20.4% (0-60%), respectively, with an annual loss estimated at US$ 5 million. The same workers reported an annual loss in the crop production due to the combined effect of wilt and sterility mosaic in India to be equivalent to US$ 113 million. Like in India, *Fusarium* wilt is the most important disease of pigeonpea in Kenya (Onim, 1981).

2.4 Symptomatology

The main symptom of the disease is the wilting of seedlings and adult plants as if from water shortage even when plenty of it may be present in the soil, and is exacerbated by periods of drought (Booth, 1971; Mehrotra, 1980; Allen, 1983). The wilting is characterized by gradual, sometimes sudden yellowing, withering and drying of leaves followed by the drying of the entire plant or some of its branches. On peeling off the bark, black streaks are found to extend in the vascular tissue from the dead branches downward to one or more infected lateral roots. Later, other roots become infected

Infection of the taproot usually causes withering of the entire plant, which remains standing with the dried leaves still attached (Butler, 1906; Nene, 1980).

Discolored portions of the stem and roots contain the pathogen, concentrated within the vascular vessels. Systemic infection may lead to the development of spore masses externally on the bark. Aerial infection apparently plays little part in disease development (Mohanty, 1946). The fungus can be retrieved from the roots of a susceptible cultivar within 15-30 days after sowing in 'wilt-sick' soil whereas symptoms develop two weeks later (Nene, 1980).

2.5 The causal organism

2.5.1 Nomenclature

The pathogen responsible for the wilt of pigeonpea is called *F. udum* Butler (Butler, 1910; Booth, 1978). Its synonyms are *Fusarium butleri, Fusarium uncinatum, Fusarium lateratium* var. *uncinatum, Fusarium oxysporum* f.sp. *udum, Fusarium lateratium* f.sp. *cajani, F. udum* var. *cajani*. It is now accepted that *F. udum* is the imperfect state of the pathogen. The perfect state (perithecial state) have been described as *Gibberella indica* Rai and Upadhyay (Rai and Upadhyay, 1982; CMI, 1985).

2.5.2 Cultural characteristics

The fungus can easily be cultured on various synthetic media, for example, in Nash and Snyder's peptone chloronitrobenzene (PCNB) agar, potato dextrose agar (PDA), potato sucrose agar (PSA), and various other media (Booth, 1971; Shit and Sen Gupta, 1978; Dhingra and Sinclair, 1985). The cultures are white to violet purple, aerial mycelium felted or almost absent and usually with profuse development of pionnote sporodochia. The colonies could have smooth or irregular margins (Booth, 1971; Gerlach and Nirenberg, 1982).

2.5.3 Morphological characteristics

The pathogen produces both macroconidia and microconidia. The macroconidia are linear, curved or fusoid, pointed at both ends and knotched at the base, thin walled, septate with 3-4 septa, measuring 15-50 x 3-5 µm (Butler, 1906; Subramanian, 1971; Booth, 1978; Gerlach and Nirenberg, 1982). The microconidia are small, elliptical or curved, thin walled, unicellular or with 1 or 2 septa and measure 5-15 x 2-4 µm. The chlamydospores are formed in culture as well as in host tissue. They are spherical to oval, thick and smooth walled, single or in chains of 2-3 terminal or intercalary. Sporodochia and pionnotes, which are salmon to rose buff coloured, are frequently formed.

2.5.4 Survival, spread and epidemiology

F. udum is not internally seedborne although it may be carried-over as a contaminant on pigeonpea seed (Mohanty, 1946; Nene, 1980). Studies at ICRISAT Centre (India) have, however, indicated that the pathogen is also internally seedborne in seeds of tolerant cultivars but not in susceptible and resistant ones (ICRISAT, 1987). The fungus occurs as a saprophyte in the soil where it survives for at least three years, and its persistence is governed by soil type and nutrient status (Sarojini, 1951; Nene, 1980), and presumably also by its propensity to form chlamydospores (Padwick, 1940). McRae and Shaw (1933) reported that the fungus could survive for up to 20 years in the soil. Clamydospores are produced in the vascular tissue of old infected plants (Butler, 1906). Clamydospores are also formed in the hyphae and reproductive structures of other soil-inhabiting fungi (Upadhyay *et al.*, 1983).

F. udum spreads through the soil at the rate of about 3 metres during the growing season but this is dependant in part on soil composition and organic matter content (McRae and Shaw, 1933; Shukla, 1975). Soil antagonists influence its growth, especially the bacterium *Bacillus subtilis* that produces the antibiotic bulbiformin (Vasudeva and Roy, 1950). The presence of this antibiotic results in a reduced incidence of wilt

The wilt is more common in areas where the crop is grown year after year. The fungus can withstand widely varying adverse conditions, including a pH range of 4.6-9.0 and soil temperatures as high as 35°C. Mundkur (1935) reported that wilt of pigeonpea develops at a wide range of temperature. He reported that 17 to 29°C temperatures were conducive to disease development. Shukla (1975), using pot experiments observed more wilt inoculum in sand (94%) than in heavy black soil (18%). Singh and Bhargava (1981) observed the highest fungal population at 30% soil water-holding capacity and at the soil temperatures between 20 and 30°C.

The fungus can be retrieved from the roots of a susceptible cultivar within 15-30 days after sowing in 'wilt-sick' soil, and symptoms develop two weeks later (Nene, 1980). However, wilting is more common during the crop's reproductive phase (Mundkur, 1935). Sheldrake *et al.* (1978) demonstrated that disease development is influenced by the physiologic state of the plant. The plant was less liable to wilt if pod development was prevented by flower removal, whereas wilting was increased by defoliation during the reproductive phase. The time of wilting strongly influences the extent of crop loss. The yield loss depends on the stage at which the plants wilt; it can approach 100% when wilt occurs at the pre-pod stage, about 67% when wilt occurs at maturity, and 30% when it occurs at the pre-harvest stages (Kannaiyan and Nene, 1981).

2.6 Disease control

2.6.1 Cultural control

Crop rotation and mixed cropping are traditional practices to reduce *Fusarium* wilt of pigeonpea. Mundkur (1967) suggested that if long rotations are practised the disease could be effectively reduced in intensity. The crop should not as a rule be sown in the same soil for at least 3 years in succession. The type of crop used in crop rotation greatly influences the effectiveness of rotation. Bose (1938) reported that rotation of pigeonpea with tobacco after every 3 to 4 years significantly suppresses the disease. It was observed that pigeonpea intercropped with sorghum had only 24% wilt against 85% in sole crop treatment, which was consistent across 14 susceptible pigeonpea genotypes

(Natarajan *et al.*, 1985). It has been recorded that a one-year break between pigeonpea crop by fallowing or by growing sorghum or tobacco reduces the wilt incidence to 22%, 20%, and 44% respectively (ICRISAT, 1987). A break with cotton or maize was not very effective. Mixed cropping of pigeonpea with *Crotalaria madicaginea* also suppresses the disease to a good extent (Upadhyay and Rai, 1981).

Management of the disease by soil amendments has also been reported. A considerable reduction in the incidence of wilt disease was observed in fields receiving green manure and addition of compost or the leaves of *Crotalaria madicaginea* (Upadhyay and Rai, 1981).

Sowing susceptible cultivars in the post rainy season resulted in much lower *Fusarium* wilt incidence than when the same cultivars were sown earlier in the year (Kannaiyan and Nene, 1985).

2.6.2 Chemical control

Seed treatment with a mixture of benomyl and thiram at equivalent rates completely eradicates the fungus (ICRISAT, 1987). Addition of boron, manganese or zinc to the soil provides protection to seedlings during emergence (Sarojini, 1951). Sinha (1975) observed a significant control of the disease by Bavistin applied as a soil drench at 2,000 ppm ten days before inoculation of pigeonpea with *F. udum*. Upadhyay and Rai (1981) observed a considerable reduction in *Fusarium* wilt incidence by Phygon XL, Dithane-78, and Zincop when applied as soil treatments. However, the effect of the fungicides applied at the time of sowing does not persist for the whole cropping season and thus a single treatment does not provide remedy for the disease control.

Antibiotics such as griseofulvin (Chakrabarti and Nandi, 1969) and bulbiformin (Vasudeva *et al.*, 1962) have been reported to be effective in controlling the disease. However, these antibiotics do not provide an economic approach to disease management. Therefore, it may be concluded that fungicides and antibiotics are not econmical for *Fusarium* wilt control, and other methods of disease management should be explored.

2.6.3 Biological control

Biological control remains an attractive possibility for management of soil-borne plant pathogens. Some encouraging results have been reported for wilt causing fusaria. Soil antagonists influence *F. udum* growth, especially the bacterium *Bacillus subtilis* that produces the antibiotic bulbiformin, which lowers the incidence of wilt (Vasudeva and Roy, 1950; Singh and Singh, 1981). A suitable medium for the growth of *B. subtilis* favouring production of antibiotics against *F. udum* has been described (Vasudeva *et al*., 1962). Inoculation of *B. subtilis* into autoclaved soil amended with molasses and sweet clover roots and groundnut-cake reduced pigeonpea wilt by 88%. Unfortunately, work has not been pursued to use *B. subtilis* for biological control of this disease.

Another antagonist reported to have potential biocontrol of *F. udum* is *Micromonospora globosa*, which kills and destroys *F. udum* even in its resting stage (Upadhyay and Rai, 1978). Certain soil fungi are also known to suppress the development of wilt through induction of resistance (Vasudeva and Govindaswamy, 1953; ICRISAT, 1987).

Solarization-induced control of *Fusarium* wilt has been observed at ICRISAT (1986, 1987). This method reduced *Fusarium* wilt in a susceptible pigeonpea genotype (LRG 30) and enhanced growth and yield in a wilt-resistant genotype ICPV 1 (ICP 8863) (ICRISAT, 1985). Hot weather cultivation with deep ploughing in the summer is also recommended.

2.6.4 Resistant varieties

Selection of cultivars with *Fusarium* wilt resistance began in 1905 at Poona, India (Butler, 1908). Subsequent screening has been conducted at many centres in India, and in other countries including Kenya. Some 33 lines have proved to be consistently resistant in 'wilt-sick' plots at ICRISAT Centre, India (Nene, 1980; Nene and Kannaiyan, 1982). Pigeonpea lines found to be resistant to *Fusarium* wilt at Katumani, Kenya, are ICP 8864, ICP 9145, ICP 9155, ICP 10957 and ICP 10960 (Reddy *et al*., 1990). The Kenyan pigeonpea lines showing promise for wilt resistance are NPP 675/2,

NPP 675/6, NPP 668/4, NPP 690/1, NPP 691/2, NPP 693/3, NPP 696/2, NPP 699/1, NPP 699/2 (ICRISAT, 1987). Many other lines have been reported to be resistant to wilt at various locations in Kenya (Okiror, 1986; Kimani *et al.*, 1994; Songa *et al.*, 1995).

Inconsistencies in reaction are common, and it is evident that resistance is environmentally unstable (Sheldrake *et al*, 1978; Nene, 1980). There is possible existence of pathogenic strains or races because the fungus is a highly variable species (Padwick, 1940; Sarojini, 1951; Subramanian, 1955). In addition pathogenicity may also be cultivar-specific (Shit and Sen Gupta, 1978).

Several cultivars that were earlier claimed to possess resistance against the wilt failed to give uniform performance at ICRISAT Centre (Nene *et al.*, 1979). Some lines have shown broad-based resistance, for example, ICP 4769, ICP 7182, ICP 8863, ICP 9168, ICP 10958 and ICP 11299. Frequently, due to the segregation, some resistant varieties lose their resistance during later generations (Nene *et al.*, 1979). Mundkur (1946) reported that cultivars D-16-12-2, PT-12, and D-33-4-22 lost resistance when grown in the field. Cultivars that were earlier reported to be resistant or tolerant and later became susceptible to the wilt include C-11, C-28, C-36, F-18, NP(WR)-15, NP-41, and T-17 (Singh and Mishra, 1976). A similar observation has been made for many lines at ICRISAT Centre during co-ordinated varietal trials (ICRISAT, 1986, 1987).

Screening pigeonpea for resistance to date has shown that reaction to wilt can vary considerably between regions for example India and Africa, between sites within regions for example Kenya and Malawi in Africa, and even between sites within the same country for example Kiboko and Katumani in Kenya. Songa *et al.* (1995) reported pigeonpea line ICP 9145 to be wilt-resistant at Katumani (Kenya), ICRISAT Asia Center (India), and Malawi but highly susceptible (71% wilt) at Kiboko (Kenya). They also found that line ICP 2376, susceptible in India, had an average wilt incidence of 47.5% at Katumani (Kenya) compared with 100% usually reported at ICRISAT Asia Centre (India).

Another problem associated with this method of control is that many of the resistant lines do not possess resistance against many other diseases of pigeonpea, some

of which, like *Phytopthora* blight and sterility mosaic are prevalent and serious (Upadhyay and Rai, 1992). For example, out of 52 entries of pigeonpea evaluated for resistance against wilt, sterility mosaic and *Phytopthora* blight during multilocational trials, only ICP 8860 and ICP 8861 were observed to be highly resistant to these diseases (incidence less than 20%) at all the sites (ICRISAT, 1986). However, many other lines were promising for more than one pathogen. Nevertheless the emphasis in the control of *Fusarium* wilt lies in the continued development of resistant varieties.

2.7 Variability in *F. udum*

2.7.1 Variation in cultural and morphology characteristics

F. udum shows a great deal of variation in cultural characteristics such as aerial mycelial growth, mycelial texture, mycelium colour, substrate colour, colony diameter (radial mycelial growth), mycelial dry weight and sporulation. Based on cultural characteristics, Subramanian (1963) and Gupta *et al.* (1988) reported 7 groups of *F. udum* from 100 and 71 isolates from India, respectively. Based on cultural characteristics, Nene *et al.* (1979) classified some isolates *F. udum* into 12 distinct groups. Changaya-Banda *et al.* (1996) grouped 75 isolates of *F. udum* from Malawi into 25 pathotypes on the basis of cultural characteristics. Variation of this pathogen in cultural characteristics has also been reported by other workers (Butler, 1910; Baldev and Amin, 1974; Jeswani *et al.*, 1977; Shit and Sen Gupta, 1978; Reddy and Chaudhary, 1985; Okiror, 1986; Gaur and Sharma, 1989). Isolates capable of profuse mycelial growth are often less pathogenic than those producing scanty mycelium, but no correlation has yet been shown between conidial production and pathogenicity (Shit and Sen Gupta, 1978). Morphological variation in *F. udum* has been observed with respect to its ability to produce chlamydospores (Padwick, 1940), and on conidial measurements. Microconidia and macroconidia measurements of *F. udum* have been reported to be quite variable with 3.9-15.0 x 1.5-4.0 µm and 8.5-50.0 x 1.5-5.0 µm, respectively (Booth, 1978, Mehrotra, 1980; Rai and Upadhyay, 1982; Gaur and Sharma, 1989).

2.7.2 Genetic variation

Many factors contribute to genetic change (evolution) within populations. These factors include mutation, mating systems, gene flow or migration, population size, and selection. The genetic diversity (variation) in phytopathogenic fungi such as *F. udum* could be determined within and among its populations using methods/markers that include race typing/virulence, VCG, isozymes and DNA based markers.

2.7.2.1 Physiologic races

Variability in virulence of *F. udum* isolates from pigeonpea has been reported, suggesting possible genetic variation within this fungus. Gupta *et al.* (1988) reported 7 strains of *F. udum* determined by virulence on different varieties of pigeonpea in Madhya Pradesh (India). Reddy and Raju (1993) reported two distinct strains of *F. udum* after virulence tests of 13 isolates collected from various parts of India. Okiror (1986) observed variation in pathogenicity of 12 isolates of *F. udum* obtained from 3 districts in Kenya. Physiologic races in *F. udum* have been suggested to exist but not determined conclusively (Mukhurjee *et al.*, 1971; Baldev and Amin, 1974; Shit and Sen Gupta, 1978; ICRISAT, 1987).

2.7.2.2 Vegetative compatibility groups (VCG)

Vegetative compatibility groups (VCG) has been used frequently as a means of identifying isolates of a fungus that are closely related (Leslie, 1990, 1993). This trend has been accelerated by the development of faster methods of identifying compatible isolates using nitrate non-utilizing (*nit*) mutants (Cove, 1976; Puhalla, 1985; Correll *et al.*, 1987). To be compatible isolates must show a common allele at each of the loci. If pairs of *nit* mutants from different isolates fuse to produce a heterokaryon on minimal medium, complementation result in wild-type growth. Such isolates are determined to be in the same VCG. The more closely related two isolates were genetically the more likely they are to be vegetatively compatible. Therefore, VCG determination may reflect genetic similarities, although not the degree of genetic differences, among isolates of the

species (Kistler, 1997). If asexual reproduction is the rule, strains related by clonal descent should be within the same VCG, because clonally derived strains would be isogenic, or nearly so, and therefore, vegetative compatible (unless they happen to be vegetatively self-incompatible) (Leslie, 1993). Additionally, isolates within separate clonal lineages and in different VCGs might then be isolated genetically due to vegetative incompatibility. Clonal reproduction and, more importantly, the lack of meiotic recombination would greatly limit or preclude reassortment of genes for heterokaryon incompatibility.

This methodology has been applied in the characterization of soilborne pathogenic fungi such as *F. oxysporum* formae speciales and *Verticilium* species. Fungal species reported to have a single VCG include *F. oxysporum* f.sp. *canariensis* (Plyler *et al.*, 2000), and *F. oxysporum* f.sp. *tracheiphilum* (Puhalla, 1985); and those with two or more VCGs include *F. oxysporum* f.sp. *apii* (Corell *et al.*, 1987), *F. oxysporum* f.sp. *radicis-lycopersici* (Katan and Katan, 1999) and *Verticillium dahliae* (Elena and Paplomatas, 1998). Since *F. udum* reproduce asexually it would be possible to determine its genetic variation by VCG technique.

2.7.2.3 Molecular markers

Molecular markers (techniques) have been used to detect genetic variation among *Fusarium* species and forma specialis, and other pathogenic fungi. These markers include isozymes and deoxyribose nucleic acid (DNA) markers such as randomly amplified polymorphic DNAs (RAPDs), restriction fragments length polymorphisms (RFLPs) and amplified fragments length polymorphisms (AFLPs).

Isozymes (isoenzymes) are structurally different molecular forms of an enzyme system with, qualitatively, the same catalytic function. Isozymes function well as nuclear-specific markers in the quantification of heterozygosity, genetic diversity, genetic differentiation and other measures to quantify intra- and inter-populational genetic variation (Muller-Starck, 1998). Although isozyme markers are relatively cheap and easy to use, they tend to reveal low levels of polymorphisms in pathogenic fungi

(Burdon and Roelfs, 1985a, b; Tooley *et al.*, 1985; Newton, 1987). Okiror (1986) found low variation in protein profiles of 12 isolates of *F. udum* from Kenya. Shit and Sen Gupta (1980) found some enzymatic variations in 7 isolates of *F. udum* from India.

DNA markers are methods based solely on the detection of naturally occurring DNA polymorphisms. These polymorphisms are a result of point mutations or rearrangements such as insertion or deletion in the DNA and can be detected by scoring band presence versus absence in banding patterns that are generated by either restriction enzyme digestion or DNA amplification procedures, or both. The underlying idea is that variations in banding patterns are a direct reflection of the genetic relationship between the fungal strains/species examined and therefore these banding patterns can be considered as 'genomic fingerprints' allowing numerical analysis for the evaluation of levels of genetic diversity and phylogenetic relationships within and between species, and to identify particular races and pathotypes (Majer *et al.*, 1996).

RAPD markers (Williams *et al.*, 1990) have become popular because this polymerase chain reaction (PCR) technology is relatively easy to implement. RAPDs can be assayed using very small amounts of fungal biomass (20 ng), making them an ideal tool for obligate biotrops such as rusts and mildews. Because a large number of amplicons can be screened in a relatively short time, RAPDs are especially useful in differentiating clonal lineages for fungi that reproduce asexually (McDonald, 1997). RAPD data are also easy to interpret. Unfortunately, RAPDs have many drawbacks that must be weighed against their relative ease of use. There are several technical limitations associated with RAPDs that make them difficult to reproduce between laboratories and sometimes within laboratories (Devos and Gale, 1992; Penner *et al.*, 1993; Muralidharan and Wakeland, 1993; Ellesworth *et al.*, 1993; Micheli *et al.*, 1994; McDonald, 1997). RAPDs are dominant, so they cannot differentiate homozygotes and heterozygotes without a progeny test (Breyne *et al.*, 1997; McDonald, 1997). This is a disadvantage especially for measures of genetic diversity affected by the number of alleles at a locus. Despite their drawbacks, RAPDs have been used to determine genetic diversity among many fungal species such as *F. oxysporum* f.sp. *vasinfectum* (Assigbetse *et al.*, 1994),

Colletotrichum lindemuthianum (Balardin *et al*., 1997), and *Phaeoisariopsis griseola* (Busogoro *et al*., 1999).

RFLPs in nuclear and mitochondria (mt) genomes have been used in many studies of pathogenic fungi. Because RFLPs are based on DNA-DNA hybridisation, they are technically more difficult than RAPDs but offer the advantage of being more reproducible (McDonald, 1997). Like RAPDs, RFLPs are easy to interpret. The properties of codominance and multiple alleles make RFLP markers advantageous compared to RAPDs for most studies in population genetics (McDonald, 1997). The disadvantages of RFLPs include the relatively large amounts (5 to 10 g) of DNA required from each isolate, more technical expertise, and expensive nylon membranes for Southern blotting. RFLP markers may be highly informative if appropriate DNA probes are available (Hulbert and Michelmore, 1988; Garber and Yoder, 1983), and the results can be reproduced in other laboratories using the same probes and restriction enzymes. RFLPs have been used to study the genetic diversity of pathogenic fungi such as *F. oxysporum* f.sp. *pisi* (Coddington and Gould, 1992), and *F. oxysporum* f.sp. *phaseoli* (Woo *et al*., 1996).

AFLP technique is a recently developed molecular marker that makes use of the reliability of RFLP technique combined with the power of polymerase chain reaction (PCR) technique (Vos *et al*., 1995). AFLP is a DNA fingerprinting technique that detects genomic restriction fragments and resembles in that respect to RFLP, with the major difference that PCR amplification instead of Southern hybridization is used for detecting fragments. It is a very effective tool to reveal restriction fragment polymophisms. These polymorphisms (AFLP markers) can be used to construct high-density genetic maps of genomes or genome fragments. In most organisms AFLP will prove to be the most effective way to construct genetic DNA marker maps compared to other existing marker technologies. AFLPs have been reported as dominant (McDonald, 1997) or mixed (dominant and codominant) (Breyne *et al*., 1997). AFLPs usually have two alleles per locus. AFLP technique has been used to determine genetic variability between and within pathogenic fungal species such as *Cladosporium fulvum* and *Pyrenopeziza*

brassicae (Majer *et al.*, 1996); *Colletotrichum* species (O'Neill *et al.*, 1997); *Colletotrichum lindemuthianum* (Gonzalez *et al.*, 1998), and *Pyrenopeziza brassicae* (Majer *et al.*, 1998).

The advantages of AFLP fingerprinting technique over the other molecular markers (RFLP or RAPD) are that variability is assessed at a large number of independent loci; AFLP markers are 'neutral' (that is they are not subject to natural selection); variation is revealed in any part of the genome; the data are obtained very fast; and are extremely reproducible (Vos *et al.*, 1995; Janssen *et al.*, 1996; Majer *et al.*, 1996). The main disadvantage of AFLP markers is that alleles are not easily recognized (Majer *et al.*, 1996). Allelic fragments will be scored as independent, although in reality they are not. This could lead to an overestimation of variation. Variation detected may also deviate from the true level of variation between individuals due to loss of restriction sites, insertions or deletions. This is due to the fact that 'fragment' changes rather than 'site' changes are scored. The loss of an *Eco*R I site, for example, could result in the disappearance of two fragments (assuming all *Eco*R I-*Mse* I fragments are detected).

In AFLP technique, genomic DNA is isolated and digested simultaneously with two restriction endonuclease, a six-base cutter such as *Eco*R I and a four-base cutter such as *Mse* I. A typical fungal genome of about 40,000 kb is cut into over 150,000 fragments, the majority being less than 500 bp long (Majer *et al.*, 1996). This step generates the required substrate for ligation and subsequent amplification. Due to primer design and amplification strategy, *Eco*R I-*Mse* I fragments are preferably amplified rather than *Eco*R I-*Eco*R I or *Mse* I-*Mse* I fragments (Life Technologies, 1995). The genomic DNA fragments are ligated to *Eco*R I and *Mse* I adapters to generate template DNA for amplification. A two-step amplification strategy, preamplification and selective amplification, results in consistently cleaner and more reproducible fingerprints. Products from the selective amplification are separated on a 5% or 6% denaturing polyacrylamide (sequencing) gel. The resultant banding pattern ("fingerprint") obtained after autoradiography can be analysed for polymorphism

either manually or using analytical software (Vos *et al.*, 1995). The number of fragments amplified per sample per primer pair averages 50 with a range of 10 to 100, but it depends on the selective nucleotides in the primers used and the complexity of the genome (Vos *et al.*, 1995; Majer *et al.*, 1996). The composition of C and G of the selective nucleotides also affect the number of amplified fragments. In general, the more Cs and Gs used as selective nucleotides in the amplification primers, the fewer DNA fragments amplified. Also, the smaller the genome being analysed, the fewer fragments amplified and the simpler the fingerprint.

2.8 Host-pathogen interaction

2.8.1 Mode of infection

F. udum is a soil-borne pathogen that normally infects the host through the roots, but it can also enter the stem when it is injured with infected objects. Booth (1978) indicated that infection of the host by *F. udum* follows similar patterns to infections by *F. oxysporum*. The infection is systemic, occurring through fine lateral roots by the germ hyphae, produced commonly either from conidia or chlamydospores (Singh, 1975). The fungus becomes systemic invading taproot, lateral roots, collar, main stem, branches, leaflets, petioles, rachis, pedicel, and pod hull (Mehrotra, 1980; Nene *et al.*, 1979). Nene *et al.* (1979) established that infection occurs fairly early in the seedlings although symptoms are not visible till later. Nene *et al.* (1979) isolated the fungus in 15 day-old seedlings infected with *F. udum*. Although Mohanty (1946) reported that aerial infection play little part in *Fusarium* wilt development, objects with the pathogen inoculum that injure healthy pigeonpea plants, for example ratooning knives, could aid in systemic infection and wilt occurrence (Okiror, 1986).

Toxins and enzymes have also been implicated in other *Fusarium* infections and it is probable that these may also be involved in the disease syndrome (Manners, 1993; Isaac, 1992). Fusaric acid has been suggested as the operative toxin in *Fusarium* wilt of tomatoes (Gaumann, 1957). In tomato plants suffering from *Fusarium* wilt, it has been reported that water transport is decreased (Page, 1959; Scheffer and Walker, 1953). The

evidence suggests that fusaric acid plays some part in wilting in this disease, but not the principal cause. Even when the pathogen is restricted to the roots, symptoms like vein clearing and epinasty are manifested on the leaves. This appears to be due to toxic metabolites translocated to distant parts of the plant from the locus of infection (Scheffer, 1983).

2.8.2 Tissue colonization

F. udum is mainly confined to the vascular tissues and is both intercellular and intracellular (Mehrotra, 1980; Upadhyaya and Rai, 1992). It is suspected that once infection has occurred in the roots, the fungus then produces and releases microconidia into the sap stream and this is believed to be the means of rapid colonization of the above ground parts. The septate hyphae run across the cells, growing rapidly along the inside of the walls of the larger vessels. The vessels are partially plugged with these hyphae. The profuse growth of the mycelium within the lumen of the xylem vessels and the consequent plugging by matted coils of hyphae interfere with the free flow of water to the green parts of the plants so that a dropping and wilting of the leaves result. Kaiser and Sen Gupta (1975) observed hyphae of *F. udum* in the epidermis, the inner cortex and vascular bundle of pigeonpea stem. Plants with external symptoms of *Fusarium* wilt normally have masses of mycelia, conidia and chlamydospores on their surfaces particularly on the stem, collar region and the roots (Mohanty, 1946; Upadhyaya and Rai, 1992).

2.8.3 Nature of resistance to *Fusarium* wilt

Evaluation of extensive collections of pigeonpea germplasm at ICRISAT Centre (India) has confirmed that adequate wilt resistance is available within *Cajanus cajan* (Nene and Kannaiyan, 1982). There is evidence that resistance to wilt, on which most information is available, has a physiologic basis. Subramanian (1963) showed that a vascular difusate from a resistant (cultivar NP-15) but not from a susceptible (cultivar NP-24) plant was partially inhibitory to conidial germination in *F. udum*, especially

when the difusate was obtained from an infected plant. Murthy and Bagyaraj (1980) have demonstrated that flavanols, as well as alkaloids, occur at higher concentrations at all stages of growth of a wilt-resistant cultivar (C-11-6) than in the susceptible cultivar (TS-136-1). Ingham (1976) has identified various antifungal isoflavanoids, including cajanol, from infected stems. Preston (1977) showed that the phytoalexin, cajanone, which is the major phenolic compound in the roots of pigeonpea, had ability to completely inhibit the growth of *F. udum*.

Vascular occlusion (mechanical barriers) due to the formation of tyloses, gels or gums in the xylem vessels have not been implicated in resistance in pigeonpea to *F. udum* pathogen. However, Marley and Hillocks (1993) indicated that resistance to *Fusarium* wilt caused by *F. udum* in pigeonpea appears to depend primarily on the rapid synthesis of cajanol and other phytoalexins, and vascular occlusion, the main physical response to infection, may be unimportant, or may serve to complement the antifungal effect of cajanol. Tyloses are the overgrowth of the protoplast of adjacent living parenchymatous cells, which protrude into xylem vessels through half bordered pits, forming ballon-like structures which commonly have well-developed cell walls, sometimes with reticulate lignified thickening, dense cell contents and often masses of starch grains (Talboys, 1972; Rioux *et al.*, 1998). The formation of tyloses involves considerable synthesis of cell wall and cytoplasmic material. They may fill the vessels completely and present the appearance of a parenchymatous tissue. Evidence for the importance of vessel tyloses as a defence mechanism is available for vascular wilt of tomatoes. Blackhurst and Wood (1963) reported that the percentage of vessels occluded by tyloses, 19 days after root inoculation with *Verticillium albo-atrum* was higher in wilt resistant variety, Loran Blood (36%) than in the susceptible variety, Ailsa Craig (23%). In sweet potato wilt caused by *Fusarium oxysporum* f.sp. *batatas*, tyloses in some varieties are formed abundantly and quickly before the pathogen, and thus bring about resistance as this prevents the further spread of the pathogen (Mehrotra, 1980).

Occluding gums are formed by living cells adjacent to the vessels and are apparently extruded through the pits into the lumena of the vessels (Talboys, 1972). Gels

arise from perforation plates, end-walls and pit membranes by a process of distension of primary wall and middle lamella constituents (Beckman and Zaroogian, 1967; Van der Molen *et al.*, 1977; Rioux *et al.*, 1998). Swelling of these pre-formed constituents can account for complete occlusion of vessel lumina. The formation of gels and gums as a host response to vascular infection occurs in a wide variety of hosts including banana (Beckman and Zaroogian, 1967) and cotton (Mace, 1978). Rioux *et al.* (1998) have proposed that the term 'gel' instead of 'gum' be used in future studies to describe the occluding material secreted by ray and paratracheal parenchyma cells. This is because of the confusion that still exists in the literature over the use of these two terms.

The effects of vascular occlusions depend upon their rates of formation and the numbers of vessels that are affected. If they form in advance of the pathogen at a rate sufficient to prevent the transport of a new 'generation' of conidia in the transpiration stream they will contribute to resistance (Beckman *et al.*, 1962). They will in any case reduce the capacity of the vascular system for water transport. If few vessels are affected this will not cause damage to the plant, but if many are affected the plant may collapse as a result of water shortage but nevertheless, if new xylem is produced at a sufficiently high rate, severe damage may be prevented (Talboys, 1958; Schoeneweiss, 1959). According to the circumstances, therefore, occlusion may contribute to pathogenesis or to resistance. Occlusion responses are non-specific which can be induced in many plants by many fungi (Beckman, 1964) and correspond with the wound-response of the host.

3.0 MATERIALS AND METHODS

3.1 Field survey of *Fusarium* wilt of pigeonpea in Kenya

The present study aimed at establishing the current status on the occurrence, prevalence and incidence of *Fusarium* wilt in the pigeonpea-growing districts of Eastern, Coast, Central and Nairobi provinces in Kenya. Diseased pigeonpea plant samples with *Fusarium* wilt symptoms were collected for verification of Koch's postulates, and the pathogenic isolates of *F. udum* were retained for further characterization into distinct groups and/or races.

3.1.1 Occurrence and prevalence of *Fusarium* wilt

The *Fusarium* wilt survey was carried out in the major pigeonpea growing districts in Eastern, Coast, Central and Nairobi Provinces using the transect approach, starting from June to September 1997, during flowering, pod setting and dry pod stages of pigeonpea. A total of 86 farms with pigeonpea were visited during the survey in 13 districts spread in nine agro-ecological zones (AEZs) (Figure 1). The surveyed farms in a particular district or AEZ were selected at random and they were on average five kilometres away from each other along accessible routes. The altitude of each farm was verified using an altimeter. The climatic data for each farm was based on the nearest meteorological station in any given district or AEZ.

3.1.2 *Fusarium* wilt incidence and collection of samples

Wilt incidence (%) on each farm surveyed was recorded as the average per cent wilt due to *F. udum* in a 30-metre row of pigeonpea plants in four replications (4 rows). The data gathered in each farm included, pigeonpea variety (local or improved), stage of plant growth and cropping system (intercrop or monocrop). One to three samples per farm, each of 5 to 10 cm stem length, were cut from plants showing vascular symptoms of *Fusarium* wilt. The samples were placed in paper bags and transported in a keep cool box to the laboratory at University of Nairobi for isolation.

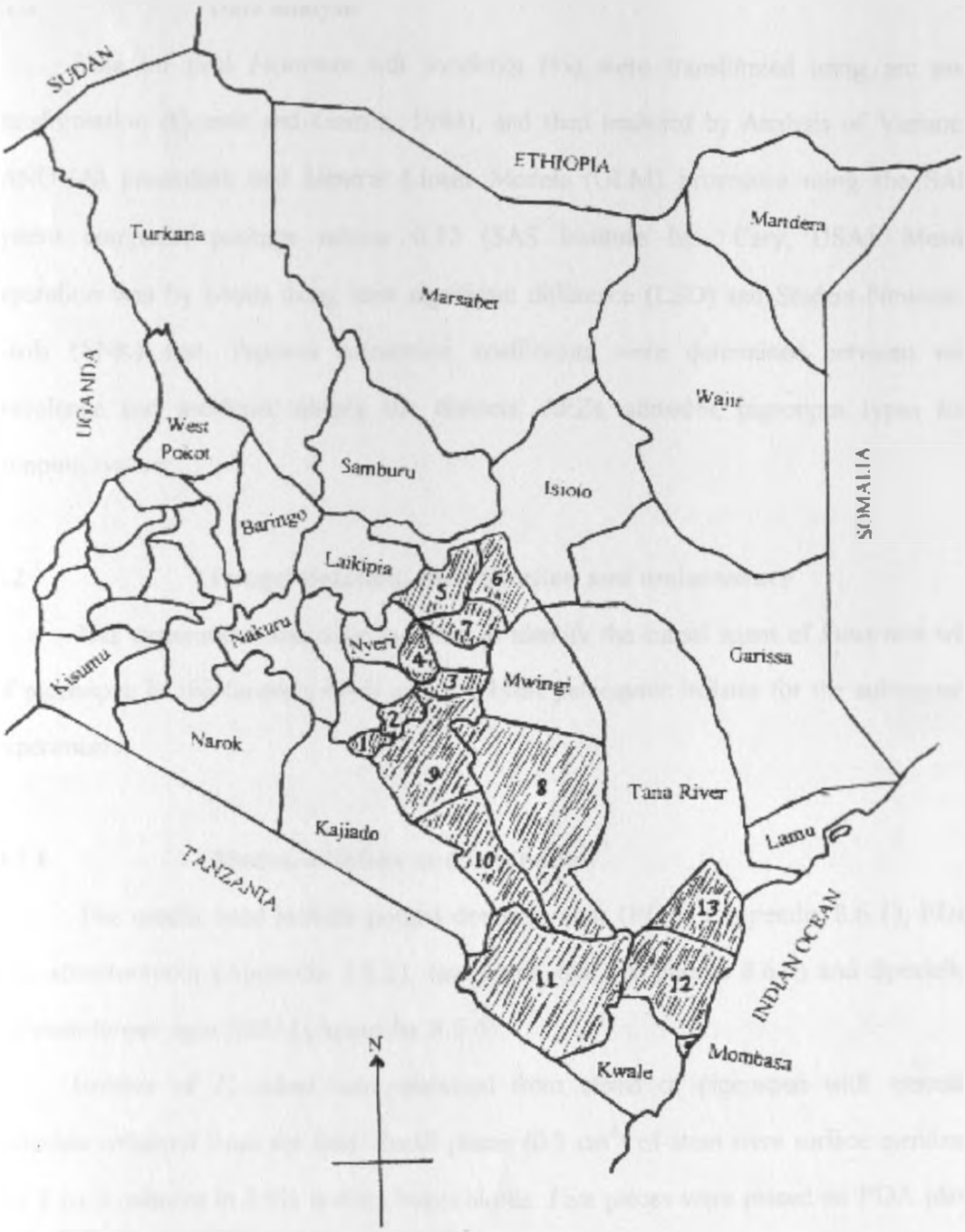


Figure 1. Map of Kenya showing the districts (shaded) where survey of *Fusarium* wilt of pigeonpea was carried out and field isolates of *F. udum* collected, June to September 1997.

3.1.3 Data analysis

Data on field *Fusarium* wilt incidence (%) were transformed using arc sine transformation (Gomez and Gomez, 1984), and then analysed by Analysis of Variance (ANOVA) procedure and General Linear Models (GLM) procedure using the SAS system computer package release 6.12 (SAS Institute Inc., Cary, USA). Means separation was by t-tests using least significant difference (LSD) and Student-Newman-Keuls (SNK) test. Pearson correlation coefficients were determined between wilt prevalence and incidence among the districts, AEZs, altitudes, pigeonpea types and cropping system.

3.2 Fungal isolation, identification and maintenance

This experiment was done in order to identify the causal agent of *Fusarium* wilt of pigeonpea in the farmer's fields and to obtain pathogenic isolates for the subsequent experiments.

3.2.1 Media, isolation and incubation

The media used include potato dextrose agar (PDA) (Appendix 8.6.1), PDA plus streptomycin (Appendix 8.6.2), tap water agar (Appendix 8.6.3) and Spezieller Nahrastofarmer agar (SNA) (Appendix 8.6.4).

Isolates of *F. udum* were obtained from stems of pigeonpea with vascular infection collected from the field. Small pieces (0.5 cm^2) of stem were surface sterilized for 2 to 3 minutes in 2.5% sodium hypochlorite. Five pieces were placed on PDA plate containing 133 mg/l streptomycin. The petri plates were incubated at 25°C in a 12-hour light/dark cycle for 36 to 48 hours. Colonies showing growth and morphology typical of *F. udum* according to the description of the fungus by Booth (1978) and Gerlach and Nirenberg (1982) were transferred to fresh PDA, where they were maintained until sporulation was achieved. A suspension of spores was prepared from these cultures and dispersed on tap water agar. After 24 to 36 hours, individual germinating spores were transferred to fresh PDA. Two isolates namely IMI No. 275452 from Malawi and IMI

No. 205514 from India designated MAL01 and IND01, respectively in this study, were obtained from International Mycological Institute, United Kingdom.

Seventy-five single-spore isolates of *F. udum* were isolated from the 55-pigeonpea farms that had plants showing wilt symptoms. Two separate single-spore isolates were obtained from each of the strains from Malawi and India, to make a total of 79 isolates.

3.2.2 Maintenance

Colonies produced from single spores of the field isolates of *F. udum* were maintained as a pure isolates and stored in sterile fine soil at room temperature, and the duplicate on low nutrient medium SNA (Nirenberg, 1976) at –20°C. These isolates were used to assess the inoculation techniques, pathogenicity tests, cultural and morphological characteristics, VCG, AFLP and tissue colonization.

3.2.3 Identification

The single-spore isolates of *F. udum* were subcultured onto PDA and SNA for cultural and morphological studies, for the purpose of identification. The cultures were incubated at 25°C in a 12 hour light/dark cycle for up to 4 weeks. Each single-spore isolate was inoculated onto 5 seedlings of wilt susceptible pigeonpea variety KAT 60/8 using root-dip inoculation technique as described in section 3.3.2, and the fungus re-isolated onto PDA in order to fulfil the Koch's postulates.

3.3 Validation of *Fusarium* wilt inoculation techniques in the glasshouse

Fast and accurate glasshouse inoculation techniques are necessary for screening large populations of germplasm emanating from breeding programs. Various inoculation techniques for vascular plant diseases have been developed. In this study, the effectiveness of the root-dip inoculation technique widely used for screening pigeonpea germplasm against *F. udum* (Nene *et al.*, 1981; Reddy and Raju, 1993) was compared to

colonized whole rice grain which proved to be highly successful in screening common bean against *Macrophomina phaseolina* (Songa, 1995) under the glasshouse conditions.

3.3.1 Experimental site, pigeonpea varieties and *F. udum* isolates

The experiment was conducted in a glasshouse whose prevailing temperatures were 22 to 30°C with about 12 hours photoperiod (natural light) at the Field Station, Kabete Campus, University of Nairobi at 1820 m.a.s.l.

Two wilt susceptible (KAT 60/8 and NPP 670) and two wilt resistant (ICP 8863 and ICP 9174) pigeonpea varieties were used in this experiment. Varieties ICP 8863, ICP 9174 and KAT 60/8 were obtained from ICRISAT Experimental Station (Kiboko, Kenya) while NPP 670 was obtained from the Department of Crop Science, University of Nairobi. Two *F. udum* isolates used in the study were MR01 and TT01. These isolates were selected at random to represent the highly pathogenic isolates.

3.3.2 Root-dip inoculation technique

3.3.2.1 Inoculum preparation

The inoculum was prepared from each isolate by flooding 7 day-old PDA cultures with 100 ml of sterile distilled water. The conidial suspension was filtered through cheese cloth, and the inoculum concentration determined using a haemocytometer and then adjusted to 1.0×10^6 conidia/ml.

3.3.2.2 Seed pregermination

The pigeonpea seeds were surface sterilized for one minute in 1% sodium hypochlorite and washed in two series of sterile distilled water before planting in 12 cm diameter polythene bags filled with sterilized riverbed sand. The bags were kept in the glasshouse and watered at regular intervals with sterile distilled water.

3.3.2.3 Inoculation and transplanting of seedlings

Seven-day-old seedlings were uprooted carefully from the sand medium and the roots washed in running sterile distilled water. The roots were trimmed to about 4 cm from the collar region, and then dipped into 1.0 x 10^6 conidial suspension for 30 minutes before transplanting them into 20 cm diameter polythene bags containing sterilized mixture of red soil (Vertisol) and riverbed sand (3:1 v/v) (Dhingra and Sinclair, 1985; ICRISAT, 1990). For each *F. udum* isolate five seedlings were inoculated and this was replicated four times. Control seedlings were dipped in sterile distilled water. The pots were placed in the glasshouse and watered at regular intervals with sterile distilled water.

3.3.3 Colonized whole rice grain inoculation technique

3.3.3.1 Inoculum preparation

Whole rice grains were autoclaved at 121°C for 20 minutes in water in a ratio of 1:1 (w/v) in a 250 ml beaker and cooled to room temperature. Agar blocks of 1 cm^3 were cut from 7 day-old single-spore PDA (Appendix 8.6.1) cultures of *F. udum* isolates MR01 and TT01 and transferred into beakers containing the autoclaved rice seeds and incubated at 28°C in a 12-hour light/dark cycle for 15 days.

3.3.3.2 Inoculation of seeds and planting

Three rice seeds colonized by *F. udum* were placed with one seed of pigeonpea during planting before covering with soil/sand mixture. Eight pigeonpea seeds were planted per 20 cm polythene bag containing sterilized mixture of red soil and riverbed sand (3:1 v/v) and replicated four times. Seeds of control plants were planted with un-colonized sterilized whole rice grains. The polythene bags were placed in the glasshouse and watered at regular intervals with sterile distilled water. Seven-day-old seedlings were thinned to 5 per polythene bag.

3.3.4 Experimental design and data collection

The experiment was laid out in a four-factor completely randomized design in which the level of inoculation techniques was split in replicates, pigeonpea variety and *F. udum* isolates. Observations on the symptom development were carried out daily until the initial symptoms were noted. The records on per cent wilt incidence on seedlings due to *F. udum* were taken thereafter on a weekly basis from the third week after inoculation and the final records taken eight weeks later. The experiment was repeated once.

3.3.5 Data analysis

Data were analysed by the four-factor ANOVA procedure and means separated by LSD using the SAS system computer package release 6.12 (SAS Institute Inc., Cary, USA).

3.4 Characterization of *F. udum* isolates by cultural characteristics

This study aimed at grouping of 75 isolates of *F. udum* from various districts, AEZs and altitudes in Kenya and 2 isolates each from Malawi and India using cultural characteristics that included aerial mycelial growth, mycelial texture, pigmentation, colony diameter (radial mycelial growth) and sporulation.

3.4.1 Inoculum preparation, plate inoculation and experimental design

The isolates were subcultured onto PDA medium (Appendix 8.6.1) and incubated for 7 days at 25°C in a 12-hour light/dark cycle. A disc of 6 mm (diameter) was cut from these cultures using a cork borer and then transferred onto the centre of a fresh PDA plate. One disc per isolate was transferred to one PDA plate and replicated three times. The experimental design was a completely randomized design. The experiment was repeated once.

3.4.2 Incubation and data collection

The inoculated PDA plates were incubated at 25°C in a 12-hour light/dark cycle for 8 days. The growth of aerial mycelia, mycelial texture and pigmentation was determined by visual observation. The radial mycelial growth was measured in terms of the colony diameter in millimetres. Vernier calliper was used to measure the colony diameter by taking an average of four measurements.

To estimate sporulation, conidial suspension was prepared from the cultures using a modification of the method described by Calpouzos and Stallknecht (1965). Two discs of 6 mm diameter each were cut from the opposite sides of the centre of the colony, but 1.5 cm from the middle point, and crushed with the tip of a sterile glass rod before suspending them in a universal bottle with 10 ml of sterile distilled water. The universal bottles were put on a rotary shaker (Orbital Shaker SO1, STUART Scientific, UK) at 250 rpm for 20 minutes, the spore suspension then filtered through two layers of sterile cheesecloth and the conidial concentration determined using haemocytometer. A mean of 4 counts was obtained per colony.

3.4.3 Data analysis

The pigment observed on the mycelia and substrate was determined with the help of a mycological colour chart (Rayner, 1970). The data on radial mycelial growth and sporulation were analysed by ANOVA and GLM procedures using the SAS system computer package release 6.12 (SAS Institute Inc., Cary, USA). Means separation was by LSD and SNK. Radial mycelial growth and sporulation of *F. udum* isolates were classified into groups on the basis of inter-quartile ranges obtained from one-sample T-tests, and stem and leaf plot by SYSTAT version 8.0 program (SPSS Inc., Chicago, IL, USA).

3.5 Characterization of *F. udum* isolates by conidial measurements

The study aimed at grouping 79 isolates of *F. udum* using the conidial length

3.5.1 Inoculum preparation, plate inoculation, incubation and experimental design

The isolates were sub-cultured onto PDA medium (Appendix 8.6.1) and incubated for 7 days at 25°C in a 12-hour light/dark cycle. Blocks of 0.5 cm^3 of PDA cultures were then subcultured onto fresh plates having PDA medium. Three blocks of the cultures of each isolate were transferred to separate plates of PDA. The plates were incubated at 25°C in a 12-hour light/dark cycle for upto 4 weeks. The experiment was repeated once.

3.5.2 Data collection and analysis

A block of 1 cm^3 of agar from ten days old PDA culture was cut and mounted directly on a slide with a drop of cotton blue in lactophenol stain and covered with a cover slip and viewed under a light microscope. Morphology of macroconidia and microconidia were described and measurements made in micrometres (µm). Fifty conidia per isolate were measured to determine the length, width and the number of septa, using a light microscope with a micrometer. Determination of the width was done by measuring the widest section of the conidia. The mean and range of conidial length and width, and septation were determined. The presence of chlamydospores was dertermined by a transfer of small mycelia from 2 to 4 weeks old PDA cultures onto a slide, stained with cotton blue in lactophenol and viewed under a light microscope (Gerlach and Nirenberg, 1982).

3.6 Characterization of *F. udum* isolates by virulence on a wilt susceptible pigeonpea variety KAT 60/8

This experiment aimed at determining the ability of each of the 79 isolates of *F. udum* to induce wilt in susceptible pigeonpea variety KAT 60/8, and their levels of virulence/aggressiveness.

3.6.1 Pigeonpea variety and *F. udum* isolates

The experiment was conducted in a glasshouse as given under section 3.3.1. The *Fusarium* wilt susceptible pigeonpea variety used was KAT 60/8, kindly supplied by ICRISAT Experimental Station (Kiboko, Kenya). Seventy-five isolates of *F. udum* from various districts, AEZs and altitudes in Kenya and 2 isolates each from Malawi and India were used for this study.

3.6.2 Inoculum preparation and seed pregermination

The inoculum was prepared as described in section 3.3.2.1. The pigeonpea seeds were pregerminated as described in section 3.3.2.2.

3.6.3 Inoculation, transplanting of seedlings and experimental design

Inoculation and transplanting of seedlings were done as described in section 3.3.2.3. The experimental design was completely randomised design and was replicated three times. The experiment was repeated once.

3.6.4 *Fusarium* wilt assessment

Observations on the symptom development were carried out daily until the initial symptoms were noted. The records on per cent wilt incidence on seedlings due to *F. udum* were taken six weeks after root-dip inoculation. The disease scale used was a modification of the one used by Reddy and Raju (1993, 1997), where 0-10% = resistant plants, 11-20% = moderately resistant plats, 21-30% = moderately susceptible plants, 31-60% = susceptible plants, and 61-100% = highly susceptible plants.

3.6.5 Data analysis

The wilt incidence data was analysed by ANOVA and GLM procedures using the SAS system computer package release 6.12 (SAS Institute Inc., Cary, USA). Means separation was by LSD and SNK. Pearson correlation coefficient was determined

between the virulence (% wilt), and colony diameter (radial mycelial growth), sporulation and macroconidial length.

3.7 Characterization of *F. udum* isolates by physiological race typing

Twenty-one isolates of *F. udum* were further characterized using selected host differentials to determine any differences in virulence towards various pigeonpea genotypes, and to find out the possible existence of physiologic races.

3.7.1 Pigeonpea differentials and *F. udum* isolates

The experiment was conducted in a glasshouse as given under section 3.3.1. The seven pigeonpea genotypes used in this experiment were kindly supplied by ICRISAT Experimental Station (Kiboko, Kenya). These were: *Fusarium* wilt resistant varieties ICP 8863, ICP 9174, ICP 8858, ICPL 87105, C-11 (ICPL 138) and ICEAP 00040, and the wilt susceptible variety KAT 60/8.

Nineteen isolates of *F. udum* from Kenya that were used in this study were selected randomly on the basis of their cultural characteristics, virulence on wilt susceptible variety and geographic origin (districts and/or AEZs), and one isolate each from Malawi and India. The isolates were NY02, TK02, MK10, NB01, MR04, NB03, MAL01a, KT05, TN05, MR02, TT02, ML01, MS10, TT08, MK02, MS04, TN01, NY07, KR03, IND01b and MB05.

3.7.2 Inoculum preparation and seed pregermination

The inoculum was prepared as described in section 3.3.2.1. The pigeonpea seeds were pregerminated as described in section 3.3.2.2.

3.7.3 Inoculation, transplanting of seedlings and experimental design

Inoculation and transplanting of seedlings were done as described in section 3.3.2.3. The experimental design was completely randomised design and was replicated three times. The experiment was repeated once.

3.7.4 *Fusarium* wilt assessment

Observations on the symptom development were carried out daily until the initial symptoms were noted. The records on per cent wilt incidence on seedlings due to *F. udum* were taken thereafter on a weekly basis from the third week after inoculation and the final records taken six weeks later. The disease scale used was a modification of the one used by Reddy and Raju (1993, 1997) as described under section 3.6.4.

3.7.5 Data analysis

The wilt incidence data was analysed by ANOVA and GLM procedures using the SAS system computer package release 6.12 (SAS Institute Inc., Cary, USA). Means separation was by LSD and SNK. In order to determine the races of the isolates, the race typing computer package Habgood-Gilmour Spreadsheet (HaGiS) was used (Habgood, 1970; Gilmour, 1973; Herrmann *et al.*, 1999).

3.8 Characterization of *F. udum* isolates by vegetative compatibility groups (VCG)

Vegetative compatibility grouping (VCG) has been used frequently as a means of identifying isolates of a fungus that are closely related (Leslie, 1990, 1993). This method has not been used to characterize *F. udum* isolates but appears as a promising genetic technique that could assist in the understanding of the genetic variability within this fungus. The present study aimed to establish the vegetative compatibility groups (VCGs) of *F. udum* isolates.

3.8.1 Generation of *nit* mutants from *F. udum* isolates

Vegetative compatibility was determined by Puhalla's modification of Cove's method of complementation tests between *nit* mutants (Cove, 1976; Puhalla, 1985; Correll *et al.*, 1987; Clark *et al.*, 1995;) (Appendix 8.3).

3.8.1.1 *F. udum* isolates and media

Seventy-five *F. udum* isolates from various districts, AEZs and altitudes in Kenya and 2 isolates each from Malawi and India were used for this study. The media used include PDA medium (Appendix 8.6.1), basal medium (Appendix 8.6.6), minimal agar medium with chlorate (MMC) (Appendix 8.6.7) and minimal agar medium (MM) (Appendix 8.6.8).

3.8.1.2 Inoculum preparation, plate inoculation and incubation

The isolates were subcultured onto PDA medium and incubated for 5 days at 25°C in a 12-hour light/dark cycle. Nitrate non-utilising mutants of each isolate were generated by a mycelial transfer from the edge of the colony of a single block of 5 mm^2 of PDA cultures onto the centre of 9 cm (diameter) petri plate having minimal agar medium with chlorate (MMC). Ten MMC plates with single mycelial blocks were used per isolate. The plates were incubated at 25°C in a 12-hour dark/ light cycle and examined periodically for up to 3 weeks for the appearance of fast-growing sectors (chlorate-resistant) from the initially restricted colony.

3.8.1.3 Subculturing and incubation

Transfers were made from the leading margin of any fast-growing sectors on MMC medium onto minimal medium (MM), each sector on a separate plate and examined after 3 to 7 days of incubation at 25°C in a 12-hour light/dark cycle. Colonies that were having a thin expansive growth with no aerial mycelium on MM were considered *nit* mutants (Correll *et al.*, 1987).

3.8.1.4 Plate assessment and data analysis

The number of chlorate-resistant sectors on MMC medium was recorded per colony and per isolate. The average sectoring frequency per colony and per isolate was determined. The number of chlorate-resistant sectors that were *nit* mutants, as determined by their growth on MM, was recorded for each isolate.

3.8.2 Phenotypic classification of *nit* mutants

The *nit* mutants were classified into *nit*1, *nit*3 and NitM phenotypes on the basis of their growth on media containing one of the five different nitrogen sources (Correll *et al.*, 1987).

3.8.2.1 *Nit* mutants and media

The chlorate-resistant sectors obtained from the 79 isolates of *F. udum* that were *nit* mutants were 505. These were subjected to phenotypic classification. The media used include nitrate medium (Appendix 8.6.8), nitrite medium (Appendix 8.6.9), hypoxanthine medium (Appendix 8.6.10), ammonium medium (Appendix 8.6.11) and uric acid medium (Appendix 8.6.12).

3.8.2.2 Plate inoculation and incubation

A mycelial transfer of 5-mm^2 block from MM of each *nit* mutant was placed onto 9 cm petri plates containing each of the five media. Four *nit* mutants were placed 3 cm apart onto a single plate. The plates were incubated at 25°C in a 12-hour light/dark cycle for four to seven days. A mycelial block of each *nit* mutant was also transferred onto the PDA medium and incubated as described above in order to determine if they would have a wild-type growth as the parent isolate.

3.8.2.3 Plate assessments and data analysis

The colony morphology of each *nit* mutant was scored relative to the wild-type parent isolate. The *nit* mutants were scored as having either a wild-type growth or thin

growth with no aerial mycelium on the respective media containing various nitrogen sources. The data was recorded on the number and percentage of each class of *nit* mutant obtained per isolate.

3.8.3 Complementation tests between *nit* mutants

Vegetative compatibility or heterokaryon formation was determined between selected NitM tester mutants and *nit* mutants of all isolates.

3.8.3.1 *Nit* mutants

Eight NitM mutants that were used as testers include MS05/1, IND01a/1, MB01/3, KR03/1, TT04/1, MR06/1, MS07/4 and TK03/1. The NitM mutants were selected based on the total number generated and picking only one per isolate. The *nit* mutants that were paired with the testers were *nit*1 and/or *nit*3 or NitM from each of the 79 isolates of *F. udum*.

3.8.3.2 Media, plate inoculation and incubation

To test for vegetative complementation reaction or heterokaryon formation between *nit* mutants of different isolates of *F. udum*, a 5-mm^2 block of mycelium was transferred from MM to a fresh 9 cm petri plate having MM with a NitM tester mutant in the centre with a daisy configuration and four *nit*1 and/or *nit*3 or NitM mutants on the outer circle at 3 cm apart. The plates were incubated in a 12-hour dark/ light cycle at 25°C for up to 20 days. All the *nit* mutants recovered from the same parent were paired with at least one *nit*1, one *nit*3 and one NitM mutant from that parent, and some *nit* mutants of the same phenotype were also paired.

3.8.3.3 Plate assessment and data analysis

Four types of scoring were made: these include (1) wild-type growth (++) if a continuous line of robust aerial mycelia developed within 4 to 7 days where the thin expansive growth of the *nit* mutants converge; (2) moderate or weak wild-type growth

(+) if a continuous or broken line of aerial mycelia developed within 8 to 20 days; (3) no wild-type growth (-) after 20 days of incubation; and (4) uncertain (+-). If a line of robust growth, continuous or broken line, appeared when complementary *nit* mutants (NitM and *nit*1 or *nit*3) from different isolates were paired on MM, then the isolates were of the same VCG. If a thin growth appeared at the intersection of colonies, then the isolates were of different VCG. Complementation within and between *nit* mutants of isolates of *F. udum* was recorded. Percentage vegetative compatibility or heterokaryosis (positive reaction) was calculated for each isolate. NitM testers were used to classify *F. udum* isolates into VCG. Each complementation reaction between *nit* mutants was replicated three times and repeated twice.

3.9 Characterization of *F. udum* isolates by amplified fragment length polymorphism (AFLP)

AFLP is a molecular technique that has been used to detect genetic variation between and within species of fungi (Majer *et al.*, 1996), and to group isolates of plant pathogenic fungi into distinct haplotypes/groups such as *Colletotrichum lindemuthianum* (Gonzalez *et al.*, 1998) and *Pyrenopeziza brassicae* (Majer *et al.*, 1998). AFLP technique has not been used to detect genetic variation within *F. udum* isolates, and therefore the present experiment aimed at achieving this using isolates from Kenya. The experiments were conducted at the Faculte Universiraire des Sciences Agronomiques de Gembloux (Gembloux, Belgium).

3.9.1 Production of fungal mycelia

3.9.1.1 *F. udum* isolates and media

Fifty-six isolates of *F. udum* were used in this experiment. The selection of the isolates for AFLP analysis was based on their geographical origin (AEZ), cultural characteristics and virulence on wilt susceptible variety KAT 60/8. Fifty-five isolates were from Kenya and one isolate from Malawi. The media used include PDA medium (Appendix 8.6.1) and Czapek Dox plus AZ liquid medium (CDAZ) (Appendix 8.6.5).

3.9.1.2 Plate inoculation and incubation

A small plug of preserved SNA cultures (at –20°C) of *F. udum* isolates were transferred onto PDA medium and incubated at 25°C in a 12-hour light/dark cycle. After 7 days of growth on PDA medium, a 5-mm^2 block was excised from the margins of the colonies and transferred into 500 ml Erlenmeyer flasks containing 200 ml of Czapek Dox plus AZ liquid medium (CDAZ) (Coddington and Gould, 1992). The inoculated flasks were incubated at 23°C to 25°C in the dark on a rotary shaker (Gerhard, Bonn, Germany) at 120 rpm for 7 days.

3.9.1.3 Harvesting of fungal mycelia

The contents of the flasks were filtered through two layers of sterile cheesecloth to collect the mycelia. The mycelia were placed into 50-ml screw cap polypropyline tubes and stored at –70°C.

3.9.2 Extraction of fungal genomic DNA

The method of DNA extraction used in this experiment was a modification of the procedures of Doyle and Doyle (1990) and Rogers and Bendich (1985). Ten millilitres of CTAB extraction buffer (Appendix 8.7.2.1), was pre-heated in a sterile 50 ml capacity screw cap polypropylene tube in a water bath at 65°C. Three grams of the mycelial sample was then frozen in liquid nitrogen and ground to a fine powder with a mortar and a pestle. Mycelial powder, 2.5 g, was transferred to the pre-heated extraction buffer and the tube placed in a water bath at 65°C for 30 to 60 minutes with occasional gentle swirling. An equal volume (10 ml) of chloroform:isoamyl alcohol (24:1) (Appendix 8.7.2.2) was added at room temperature and mixed gently but thoroughly for 10 minutes. The mixture was transferred into sterile 30 ml glass centrifuge tubes (Beckman) and centrifuged at 2,987 x g using Sorvel RC-5B Refrigerated Super speed centrifuge (Sorval Inc., Newtown, CT, USA) for 20 minutes at room temperature. The upper aqueous phase was transferred using a wide-bore pipette tip into a fresh 50 ml screw cap polypropylene tube and a second chloroform:isoamyl alcohol extraction was performed

After the second mixture was centrifuged, the upper aqueous phase was transferred into a fresh sterile 30 ml glass centrifuge tube and 2/3 volume of cold isopropanol added and mixed by 4 to 5 quick, gentle inversions and kept at room temperature for 1 to 2 hours, and then centrifuged at 1,075 x g for 10 minutes at room temperature. The supernatant was gently poured off and 15 ml of washing buffer (Appendix 8.7.2.4) added to the pellet and the tube put on a vertical shaker at 20 rpm for 30 to 60 minutes, and then centrifuged at 746 x g for 10 minutes at room temperature. The supernatant was carefully poured off and the pellet vacuum dried in a Speedvac evaporater (Savant) for 10 minutes. The DNA pellet was dissolved in 400 µl TE buffer (Appendix 8.7.2.5).

The DNA solution was transferred into a sterile 1.5 ml Eppendorf tube using a wide-bore pipette tip and 10 µl of 20 mg/ml RNase A (Appendix 8.7.2.6) added and incubated at 37°C for 30 minutes. To eliminate RNase A and other contaminants, 400 µl phenol:chloroform:isoamyl alcohol (25:24:1) (Appendix 8.7.2.3) was added and the solution inverted gently for 5 minutes, and centrifuged at 13,624 x g for 10 minutes at 4°C. The upper aqueous phase was transferred to a new Eppendorf tube, measuring the volume recovered and an equal volume of chloroform:isoamyl alcohol (24:1) added. The solution was inverted gently for 5 minutes and then centrifuged at 13,624 x g for 10 minutes at 4°C. The upper aqueous phase was transferred to a new Eppendorf tube and 1/10 volume of 3 M sodium acetate (pH 8.0) and 3 volumes of cold 100% ethanol added and kept for 1 hour at –70°C. The mixture was then centrifuged at 15,989 x g for 15 minutes at 4°C, the supernatant discarded and the pellet washed twice with 700 µl of cold 70% ethanol. The pellet was vacuum dried in a Speedvac evaporator for 10 minutes and then dissolved in 100 µl TE buffer. The DNA solution was stored at –20°C.

3.9.3 Estimation of the amount of DNA extracted

Agarose gel (1%) (Appendix 8.7.2.10) was prepared by dissolving 0.5 g of agarose in 50 ml TAE 1x. The mixture was heated in a microwave until completely dissolved and cooled to about 65°C before adding 5 µl ethidium bromide (Appendix 8.7.2.7). The agarose was poured onto horizontal trays and allowed to set for at least 30 minutes. After

removing the comb and the tape, the gel was placed into the electrophoresis tank, and TAE 1x buffer poured until the gel was completely submerged. The samples were prepared by mixing 3 µl sample DNA with 1 µl of loading dye (Appendix 8.7.2.11) and loaded to the wells of the gel. One well of the gel was loaded with 6 µl undigested λ *Hind* III DNA (20 ng/µl) (Roche Diagnostics, Gmbh, Mannheim, Germany). The gel was run at 100 mA for 45 to 60 minutes. After the run the gel was illuminated with UV light and photographed. The intensity of the DNA bands of the samples were compared with the intensity of the λ DNA. The following equation was used to approximate the DNA in the samples: sample DNA = (q/x) ng/µl; where q is the concentration of DNA estimated by comparing the two band intensities, that is, the sample DNA and the λ DNA, and x is the volume of DNA analysed. The DNA concentration in the samples was adjusted to 250 ng in ≤18 µl TE buffer.

3.9.4 AFLP analysis

AFLP assays were performed with AFLP Analysis System II GibcoBRL (Life Technologies Inc., Merelbeke, Belgium) following the manufacturer's instructions and as described by Lin and Kuo (1995) and Vos *et al.* (1995) (Appendix 8.4). The procedures in the AFLP Instruction Manual were adopted (Life Technologies, 1995). Some reagents and solutions for AFLP analysis were provided in the kit (Appendix 8.7.3).

3.9.4.1 Restriction digestion of genomic DNA

The components of the restriction digestion were prepared by adding, to a 1.5-ml microcentrifuge tube, 5 µl 5X reaction buffer, ≤18 µl sample DNA (250 ng in ≤18 µl TE buffer), 2 µl *Eco*R I/*Mse* I and filled with distilled water to 25 µl. The contents were mixed gently and incubated for 2 hours at 37°C. The mixture was then incubated at 70°C for 15 minutes to inactivate the restriction endonucleases.

3.9.4.2 Ligation of adapters

The adapter ligation solution (24 µl) and T4 DNA ligase (1 µl) were added to the digested DNA. The components were mixed gently at room temperature and incubated at 20°C ± 2°C for 2 hours. A 1:10 dilution of the ligated mixture was performed by a transfer of 10 µl of the reaction mixture to a 1.5-ml microcentrifuge tube and adding 90 µl TE buffer. The components were mixed gently.

3.9.4.3 Preamplification reactions

The following were added into a 0.5-ml thin-walled microcentrifuge tube: 5 µl diluted template DNA, 40 µl pre-amp primer mix, 5 µl 10X PCR buffer plus Mg and 1 µl Taq DNA polymerase (1 unit/µl). The contents were mixed gently and the tube overlaid with 2 to 3 drops of silicone oil. The preamplification reaction was performed in a GeneAmp PCR system 9600 (Perkin-Elmer, Cetus, CT, USA) with 20 cycles at temperature profile of 94°C for 30 seconds, 56°C for 60 seconds, and 72°C for 60 seconds, and a soak temperature at 4°C. A 1:50 dilution was performed by a transfer of 3 µl to a 1.5-ml microcentrifuge tube containing 147 µl TE buffer.

3.9.4.4 Primer labelling and selective AFLP amplification

The *Eco*RI + 2 primers that were used in the selective AFLP amplification were radioactively labelled with [γ-^{32}P]ATP by using T4 kinase (Appendix 8.7.2.15). The primers for selective amplification were provided in the kit. The following components were added to a 1.5-ml microcentrifuge tube: 18 µl *Eco*R I primer, 10 µl 5X kinase buffer, 20 µl [γ-^{32}P]ATP (3,000 Ci/mmol) and 2 µl T4 kinase. These were mixed gently and incubated at 37°C for 1 hour. Heating at 70°C for 10 minutes inactivated the enzyme in the mixture.

Selective amplification was performed with 5 µl of the diluted preamplified template DNA, 5 µl of a mixture of ^{32}P-labeled *Eco*R I + 2 primer (5 µl) and unlabeled *Mse* I + 3 primer (contains dNTPs) (45 µl), and 10 µl *Taq* DNA polymerase mixture [79 µl distilled water, 20 µl 10X PCR buffer plus Mg and 1µl *Taq* DNA polymerase (5

units/µl)]. The components were mixed gently and the tubes overlaid with 2 to 3 drops of silicone oil. The PCR amplification temperature profile was one cycle at 94°C for 30 seconds, 56°C for 30 seconds, and 72°C for 60 seconds and then the annealing temperature was lowered during each cycle by 0.7°C for 12 cycles to give a touch down phase of 13 cycles. The remainder of the amplification was 23 cycles at 94°C for 30 seconds, 56°C for 30 seconds, and 72°C for 60 seconds. The primer combinations used were: *Eco*R I + TA/*Mse* I + CAT, *Eco*R I + AA/*Mse* I + CAG, *Eco*R I + AG/*Mse* I + CAA, *Eco*R I + AG/*Mse* I + CAG, *Eco*R I + AC/*Mse* I + CAG, *Eco*R I + TG/*Mse* I + CAC, and *Eco*R I + AG/*Mse* I + CAC. These primer combinations were selected based on the results of the preliminary experiments using 20 primer combinations. Primer combinations revealing high number of bands after polyacrylamide gel electrophoresis were selected

3.9.4.5 Electrophoresis of PCR products

The amplified fragments were analysed on 6% polyacrylamide (sequencing) gel (Appendix 8.7.2.12). To carry out polyacrylamide gel electrophoresis, two glass plates (one treated with 10 ml repel silane (Appendix 8.7.2.17) and the other with 6.26 ml bind silane) (Appendix 8.7.2.18) were assembled and separated by two 0.4 mm thick spacers at each side, and held together by clamps. Polyacrylamide gel (6%) was prepared by adding 9 ml 40% acrylamide (Appendix 8.7.2.13) and 6 ml TBE 10X (Appendix 8.7.2.14) to 25.2 g urea, filled with sterile distilled water to 40 ml and stirred until the urea was completely dissolved, and then filled to 60 ml using sterile distilled water. For the gel to polymerise, 250 µl 10% ammonium persulfate and 50 µl TEMED were added, and immediately poured in between the two glass plates. A 0.4 mm thick comb was inserted between the glass plates and the gel left for 45 to 60 minutes at room temperature to polymerize. The gel was pre-electrophoresed in TBE 1X buffer for 45 minutes at constant power (80 W) using Model SQ sequencing gel unit (Life Technologies Inc., Merelbeke, Belgium). Prior to gel loading an equal volume (20 µl) of formamide dye (Appendix 8.7.2.19) was added to each of the reaction mixtures, heated

for 3 minutes at 95°C and then rapidly cooled on ice to prevent nucleic acid secondary structures from reannealing. The samples of each reaction mixture, 3 µl each, were then loaded on the wells of the gel. A 100 bp molecular weight marker *λ Hind* III DNA labelled with [γ-^{32}P]ATP (Appendix 8.7.2.16) was loaded (3 µl) in the first and the last wells of the gel. Gels were run for about 150 minutes until the xylene cyanol (slower dye) was two-thirds down the length of the gel.

The gel was removed from the sequencing unit and fixed in 10% acetic acid (Appendix 8.7.2.20) for 20 minutes, with regular shaking at 5 minutes interval. Placing it in an oven at 80°C for 30 to 60 minutes dried the gel.

3.9.4.6 Data collection by autoradiography

Autoradiographs were obtained by exposing Kodak Biomax MR film (Eastman Kodak Co., USA) to the dried gel in an exposure cassette for 15 to 17 hours at room temperature. The films were developed by placing them in 20% Kodak liquid X-ray developer for 5 minutes (no agitation), then on running water for 30 seconds, followed by 30% Kodak rapid fixer for 5-10 minutes (moderate agitation), and last on running water for 5 to 10 minutes (moderate agitation). The films were dried at room temperature. AFLP reactions were repeated twice.

3.9.4.7 Data (fingerprint) analysis

Bands observed by AFLP analysis were assigned a number in relation to their migration within the gel. Bands with the highest molecular weight were assigned number 1 and so on in ascending order until the band of the lowest molecular weight was assigned. It was assumed that bands of the same molecular weight in different individuals were identical. For each individual, the presence or absence of each band was determined and designated 1, present or 0, absent in order to obtain binary banding data. Similarity matrices from binary banding data of each of the seven primer combinations were derived with the Similarity for Qualitative Data Program (SIMQUAL) in the Numerical Taxonomy and Multivariate Analysis System for

personal computer (NTSYS-pc) version 1.80 (Exeter Software, Stauket, NY, USA). The dendrograms (clusters) were then generated by Unweighted Paired Group Method with Arithmetic Averages (UPGMA) based on DICE similarity coefficient: $S_D = 2nAB/(nA + nB)$, where S_D is the similarity between two *F. udum* isolates, nAB is the number of shared bands (fragments) between isolate A and B, nA is the total number of bands in isolate A, and nB is the total number of bands in isolate B. The dendrograms were generated for single primer combinations and for combined primer combinations in order to determine the similarity among *F. udum* isolates.

Binary banding data from seven primer combinations were combined in order to determine the genetic relationships (distances) among the *F. udum* isolates. A pairwise distance matrix was generated with RFLPfrag program (X. Vekemans, ULB, Belgium) using Nei and Li distance index (Nei and Li, 1979): $Dx = 1 - (2Nxy/(Nx + Ny))$, where Nxy is the number of bands shared between a pair of isolates, and Nx and Ny are the number of bands in isolate x and y, respectively. The genetic distance between the isolates in the combined matrix was subjected to cluster analysis by UPGMA method using NEIGHBOR program in the PHYLIP (Phylogeny Inference Package) version 3.5c computer software (Felsenstein, University of Washington, Washington DC, USA). A consensus tree was generated using the Majority-rule and Strict Consensus tree program. To determine the statistical significance of the dendrogram branches, the data were bootstrapped with 1,000 replications.

3.10 Colonization of pigeonpea by *F. udum*

The extent to which *F. udum* pathogen colonizes susceptible and resistant plants of pigeonpea is still not clearly understood. A study was undertaken to establish the extent of fungal colonization in resistant and susceptible pigeonpea plants inoculated with *F. udum*, and to determine if tyloses and gels play a role in *Fusarium* wilt resistance in pigeonpea.

3.10.1 Histology of inoculated and non-inoculated pigeonpea plants

3.10.1.1 Experimental sites, pigeonpea varieties and *F. udum* isolates

Planting and inoculation of pigeonpea plants was done in a glasshouse whose prevailing temperatures were 23°C to 30°C with about 12 hours photoperiod (natural light) at the Field Station, Kabete Campus, University of Nairobi. The pigeonpea varieties used were wilt resistant ICP 8863 and C-11, and wilt susceptible KAT 60/8. The *F. udum* isolates used were MS04 and TN05. They were selected on the basis of their geographical origin, cultural characteristics and virulence.

3.10.1.2 Inoculum preparation and seed pregermination

The inoculum was prepared as described in section 3.3.2.1. The inoculum concentration was adjusted to 2 x 10^6 conidia/ml for this experiment (Marley and Hillocks, 1993). The pigeonpea seeds were pregerminated as described in section 3.3.2.2.

3.10.1.3 Inoculation, transplanting of seedlings and experimental design

Inoculation and transplanting of seedlings were done as described in section 3.3.2.3. The experimental design was completely randomised design and was replicated three times. The experiment was repeated once.

3.10.1.4 Sampling for microscopy

Freshly harvested infected and control pigeonpea seedlings were taken for the preparation and observation under LM and TEM at intervals of 1, 3, 7, 14 and 21 days.

3.10.1.5 Light microscopy (LM)

The histology of inoculated and non-inoculated pigeonpea plants using *F. udum* isolates was studied using light and transmission electron microscopy at ILRI, Kenya

The taproots and stems were cut into small pieces (3-5 mm in length) at 2 cm above the inoculation point and 2 cm above the collar region (6 cm above the inoculation point), respectively using a sharp sterile scalpel in a petri dish having the primary fixative. The specimens were transferred immediately into small glass vials containing the primary fixative (2% sucrose in 5% glutaraldehyde buffered in 0.1 M PIPES pH 8.0) (Appendix 8.8.1), and stored on ice (4°C) for 4 hours (Roberts and Wilson, 1993). The specimens were washed in 2% sucrose in 0.1 M PIPES, pH 8.0 (Appendix 8.8.2) for 30 minutes, and this process was done three times. The specimens were then fixed in the secondary fixative (2% osmium tetraoxide (OsO_4) buffered in 0.2 M PIPES, pH 6.8) (Appendix 8.8.3) at 4°C (ice) for 2 hours and then washed in distilled water for 30 minutes, and this process was done three times. The specimens were then fixed and block stained in 5% uranyl acetate overnight followed by a wash in distilled water for 30 minutes, and this process was done three times.

The specimens were gradually dehydrated using different concentrations of acetone viz: 50% acetone for 25 minutes; 70% acetone for 15 minutes; 95% acetone for 15 minutes; 100% acetone for 15 minutes, and this process was done three times; and 100% propylene oxide (optional) for 10 minutes, and this process was done two times. The specimens were treated in equal volumes of propylene oxide and resin (2 to 4 drops of DMP-30 added) and the glass vials placed on a rotary shaker at 2 rpm for 2 hours. The resin (100%) (2 to 4 drops of DMP-30 added) was poured into the specimens and placed on a rotary shaker at 2 rpm for 1 day with 3 to 4 changes of the resin. The specimens were embedded in 100% resin (Appendix 8.8.4) (2 to 4 drops of DMP-30 added) and kept in an oven at 60°C overnight for full polymerization to occur. Half of the specimens were placed horizontally and another half vertically in the wells of the comb.

Transverse and longitudinal sections of 5 µm thick were cut with a rotary Reichert Ultracut E microtome equiped with glass knives made on a LKB Knifemaker 7800 (LKB Ltd, Bromma, Sweden).

The specimens were placed on a slide, dried on a hot plate and then stained using a solution of 1% toluene blue O in 1% borax at pH 8.5. After about 1 minute, the stain was washed thoroughly in distilled water and dried again briefly. The specimens were mounted on deepex and viewed with a Nikon Microphot FX light microscope (Nikon Ltd, Tokyo, Japan) with a Hitachi colour camera DK-5050 (Hitachi Denshi Ltd, Tokyo, Japan).

3.10.1.6 Transmission electron microscopy (TEM)

The root and stem specimens for TEM were fixed, dehydared and embedded as for LM (section 3.10.1.5). Transverse and longitudinal sections of 0.5 μm were cut from the specimens with a rotary Reichert Ultracut E microtome (Reichert-Jung, Vienna, Austria) equiped with a Micro Star 3 mm diamond knife (TAAB Ltd, Aldermaston, UK). The specimens (gold in colour) were placed on copper grids and stained for 20 minutes in urenyl acetate in 50% ethanol. The specimens were washed in distilled water and then stained in 0.2% lead citrate for about 20 seconds inside a petri plate having sodium hydroxide pellets. The specimens were then washed in distilled water and viewed under a JEOL 1010 TEM (JEOL, Tokyo, Japan).

3.10.1.7 Data collection and analysis

The images observed under TEM were captured with SIS pro 2.1 (Soft Imaging Systems GmbH, Muter, Germany) image analysis system, and those observed under LM captured on a Macintosh Powermarc computer using the Openlab System version 2.1 (Improvision Ltd, Coventry, UK). Observations were made on the extent and intensity of fungal colonization in different tissues of the tap root and the stem; the presence of fungal hyphae or conidia, tyloses, gels in the vascular system; and the percentage of xylem vessels having tyloses at each site of sectioning. The term 'gel' was used as a synonym of 'gum' (Rioux *et al.*, 1998). Both transverse and longitudinal sections of the roots and stems were examined. Observations were recorded from at least 4 sections from each site and the results expressed as a mean of 4 pigeonpea

plants per isolate. Photomicrographs were made from LM and TEM. Data on percentage of xylem vessels with tyloses was analysed by ANOVA procedure using the SAS System computer software (SAS Institute Inc., Cary, USA).

3.10.2 Determination of the presence of *F. udum* at different heights of pigeonpea plants

3.10.2.1 Pigeonpea varieties and *F. udum* isolates

The experiment was conducted in a glasshouse as described in section 3.10.1.1. The three pigeonpea varieties used were wilt resistant ICP 8863 and C-11, and wilt susceptible KAT 60/8. Five *F. udum* isolates selected were MS04, TN05, MAL01a, MK10 and KT05.

3.10.2.2 Inoculum preparation and seed pregermination

The inoculum was prepared as described in section 3.3.2.1. The pigeonpea seeds were pregerminated as described in section 3.3.2.2.

3.10.2.3 Inoculation, transplanting of seedlings and experimental design

Inoculation and transplanting of seedlings were done as described in section 3.3.2.3. The experimental design was completely randomised design and was replicated three times. The experiment was repeated once.

3.10.2.4 Incubation and wilt assessment

The pots were placed in the glasshouse and watered at regular intervals with sterile distilled water for 8 weeks. The wilt incidence of inoculated and control plants were determined on the eigth week by using a modification of *Fusarium* wilt scale of Reddy and Raju (1993, 1997) as described in section 3.6.4.

3.10.2.5 Sampling of inoculated plants

Four plants (with or without wilt symptoms) per variety per isolate were uprooted from the pots and taken to the laboratory for further examination. One piece (0.5 cm) of stem was cut at the following heights (upwards) from each plant: 0 cm (collar region), 5 cm, 10 cm, 15 cm, 20 cm and 25 cm.

3.10.2.6 Plate inoculation and incubation

The six pieces of stem from one plant were surface sterilized for 2 to 3 minutes in 2.5% soidum hypochlorite, washed in sterile distilled water and transferred asceptically to petri plates having PDA medium with 133 mg of streptomycin. The plates were incubated at 25°C in a 12-hour light/ dark cycle and examined periodically for the presence of fungal growth characteristic of *F. udum* from the stem pieces.

3.10.2.7 Data collection and analysis

The fungal cultures obtained on the petri plates from the infected seedlings were identified by comparing them with the stock cultures of the various *F. udum* isolates and also examining them under light microscope. The average relative presence (%) of the *F. udum* at various heights of the stem of pigeonpea plants was determined. The wilt incidence data was analysed by ANOVA procedure using the SAS system computer package release 6.12 (SAS Institute Inc., Cary, USA). Means separation was by LSD test. Pearson correlation coefficient was determined between relative presence (%) of the *F. udum* at various heights of the stem of pigeonpea plants and wilt incidence. The experiment was repeated once.

4.0 RESULTS

4.1 Distribution and incidence of *Fusarium* wilt of pigeonpea in Kenya

4.1.1 Pigeonpea types and cropping systems

The average hectarage of pigeonpea per farmer was 0.6 hectares (1.5 acres). Most farmers grew local varieties of pigeonpea and mainly as an intercrop. The improved varieties observed were in pure stand or intercropped with the local varieties of pigeonpea. Fiftyfive (64.0%) pigeonpea farms had local varieties, 26 (30.2%) farms had improved varieties, and 5 (5.8%) farms had both types. The improved varieties of pigeonpea were more prevalent in Makueni, Machakos and Mbeere districts as compared to the other districts namely Meru, Nyambene, Tharaka-Nithi, Taita-Taveta, Kilifi and Malindi, where mainly local pigeonpea varieties were grown. Pigeonpea was mainly intercropped with maize (*Zea mays* L.). Other intercrops noted include sorghum (*Sorghum bicolor* (Linn.) Moench), cotton (*Gossypium hirsutum* L.), common bean (*Phaseolus vulgaris* L.), green grams (*Vigna radiata* (L.) R. Wilczek), cowpeas (*Vigna unguiculata* (L.) Walp.), cassava (*Manihot esculenta* Crantz), hyacinth bean (*Lablab purpureus* (L.) Sweet), sweet potatoes (*Ipomoea batatas* (L.) Lam.) and citrus (*Citrus sinensis* (L.) Osbeck, *Citrus limon* (L.) Burm.f.). The ratio of pigeonpea in the various intercrop combinations ranged from 1:1 to 1:10, with an average of 1:2. The farms with pigeonpea monocrop (sole crop) were 28 (33%), and were mostly in Makueni, Machakos and Mbeere districts, while farms with pigeonpea as an intercrop were 58 (67%).

4.1.2 Field symptoms of *Fusarium* wilt

Patches of dead pigeonpea plants characterized *Fusarium* wilt symptoms in the field especially during flowering and podding stages (Plate 1). Partial wilting of the plant was a distinct feature characterized by a purple band extending upwards from the base of the main stem (Plate 2). In some cases, the purple band had turned whitish

Plate 1. A farmer with her pigeonpea crop (variety NPP 670) showing severe *Fusarium* wilt in Mbeere district.

Plate 2. Wilt due to *Fusarium* showing the characteristic purple band (Pb) on one side, extending the full length of the stem of a pigeonpea plant.

to greyish-white due to the presence of masses of conidia. These bands extended to one or more lateral roots. Other symptoms included the browning or blackening of the xylem tissue; drying of some branches, especially the lower ones, without any visible band on the main stem; die-back symptoms on the dead branches; young plants die as if from drought but with obvious browning and blackening in the xylem; and the leaves showed loss of turgidity, interveinal clearing, chlorosis and yellowing.

4.1.3 *Fusarium* wilt occurrence, prevalence and incidence

Out of the 86 pigeonpea farms surveyed, 55 (64%) had *Fusarium* wilt while 31 (36%) did not have any wilt symptom. There were significant differences ($P \leq 0.05$) in wilt incidence among pigeonpea farms (plots) surveyed (Table 1, Appendix 8.5.1). The *Fusarium* wilt incidence ranged from 0.0 to 96.1% with a mean of 8.4% (mean for untransformed data was 14.3%). Sixty four percent of pigeonpea farms with *Fusarium* wilt had a mean wilt incidence of 22.3%. Eleven pigeonpea farms had over 31% wilt incidence; 9 farms with 21-30% wilt; 13 farms with 11-20% wilt; 22 farms with 1-10% wilt; and 31 farms with no wilt incidence. The highest wilt incidence was 96.1% from farm number 12, which is a *Fusarium* wilt sick plot maintained by ICRISAT at Kiboko Experimental Station in Makueni district. Pigeonpea farm numbers 13 and 14 with 50.2% and 66.7% wilt, respectively, were *Fusarium* wilt sick plots maintained by KARI at NDFRC-Katumani in Machakos district. The highest wilt incidence in the farmer's plots was 87.6% in farm number 48 in Chuka division, Tharaka-Nithi district followed by 75.3% in farm number 59 in Taveta division, Taita-Taveta district.

Fusarium wilt of pigeonpea occurred in 12 districts out of 13 districts covered during the survey (Table 2, Figure 1). *Fusarium* wilt was highly prevalent in Machakos (100%), Meru (86%), Nyambene (83%), Kitui (80%) and Makueni (75%) districts. The disease was moderately prevalent in Taita-Taveta (67%), Tharaka-Nithi (57%), Mbeere (56%) and Thika (50%) districts, with low prevalence in Malindi (20%) district and was not observed in Kilifi district. The disease prevalence from two farms in Kirinyaga district was 100%. Four pigeonpea farms were sampled from the experimental plots

Table 1. *Fusarium* wilt incidence in various pigeonpea farms in Kenya (June to September 1997)

Pigeonpea farm	District	Division	AEZ[1]	Altitude (m.a.s.l)	Mean % wilt incidence[2]	*F. udum* isolate(s)[3]
1	Makueni	Makueni	LM5	1190	6.0 (14.1)	MK01
2	Makueni	Makueni	LM5	1155	1.1 (5.2)	MK02
3	Makueni	Makueni	LM5	1080	5.0 (12.6)	MK03
4	Makueni	Makueni	LM5	1140	16.8 (24.2)	MK04
5	Makueni	Makueni	LM5	1210	5.9 (13.5)	MK05
6	Makueni	Makueni	LM5	1120	7.3 (15.4)	MK06
7	Makueni	Kathonzweni	LM5	1050	21.0 (27.2)	MK07
8	Makueni	Kathonzweni	LM5	1075	0.0 (0.0)	-
9	Makueni	Kathonzweni	LM5	1050	0.0 (0.0)	-
10	Makueni	Kathonzweni	LM5	1060	0.0 (0.0)	-
11	Makueni	Makindu	LM6	975	8.9 (17.3)	MK08, MK09
12	Makueni	Makindu	LM6	975	96.1 (80.2)	MK10
13	Machakos	Central	UM4	1600	50.2 (45.1)	MS01, MS02
14	Machakos	Central	UM4	1600	66.7 (58.8)	MS03, MS04
15	Machakos	Mwala	LM3	1280	19.7 (25.9)	MS05
16	Machakos	Mwala	LM4	1190	7.2 (14.1)	MS06
17	Machakos	Tathui	LM5	1045	25.4 (30.2)	MS07, MS08, MS09
18	Machakos	Yathui	LM5	1050	1.9 (6.8)	MS10
19	Kitui	Central	UM4	1420	9.5 (17.5)	KT01, KT02
20	Kitui	Central	LM4	1175	16.3 (22.9)	KT03
21	Kitui	Chuluni	LM4	1150	28.8 (32.3)	KT04
22	Kitui	Chuluni	LM4	1130	0.0 (0.0)	-
23	Kitui	Kabati	LM5	900	20.6 (26.5)	KT05
24	Mbeere	Mwea	LM4	1120	8.5 (16.8)	MB01
25	Mbeere	Mwea	LM5	1100	17.8 (24.3)	MB02
26	Mbeere	Mwea	LM5	1100	5.7 (13.4)	MB03
27	Mbeere	Mwea	LM5	940	39.1 (38.7)	MB04, MB05
28	Mbeere	Mwea	LM5	940	8.4 (16.3)	MB06, MB07
29	Mbeere	Mwea	LM4	1125	0.0 (0.0)	-
30	Mbeere	Mwea	LM4	1120	0.0 (0.0)	-
31	Mbeere	Mwea	LM5	970	0.0 (0.0)	-
32	Mbeere	Mwea	LM5	925	0.0 (0.0)	-
33	Meru	Miriga-Meru West	UM3	1465	13.7 (21.4)	MR01
34	Meru	Miriga-Meru West	UM3	1465	43.6 (41.3)	MR02
35	Meru	Miriga-Meru West	LM4	1415	11.4 19.5	MR03
36	Meru	Miriga-Meru West	UM3	1470	0.0 (0.0)	-
37	Meru	Miriga-Meru East	LM3	1120	9.2 (14.9)	MR04
38	Meru	Miriga-Meru East	UM3	1200	30.8 (33.2)	MR05, MR06
39	Meru	Abothochi	LM3	1120	22.4 (28.2)	MR07
40	Nyambene	Tigania West	UM3	1395	20.7 (27.0)	NY01
41	Nyambene	Tigania West	UM3	1395	8.1 (16.4)	NY02, NY03
42	Nyambene	Tigania West	UM4	1400	18.7 (25.3)	NY04
43	Nyambene	Tigania North	UM4	1590	4.2 (11.4)	NY05, NY06
44	Nyambene	Tigania North	LM4	1435	4.7 (10.7)	NY07, NY08
45	Nyambene	Tigania North	LM4	1415	0.0 (0.0)	-
46	Tharaka-Nithi	Chuka	UM3	1390	0.0 (0.0)	-
47	Tharaka-Nithi	Chuka	LM3	1300	24.6 (29.7)	TN01, TN02
48	Tharaka-Nithi	Chuka	LM3	1300	87.6 (72.5)	TN03
49	Tharaka-Nithi	Chuka	LM5	920	0.0 (0.0)	-
50	Tharaka-Nithi	Chiagarika	LM5	820	26.4 (30.8)	TN04
51	Tharaka-Nithi	Chiagarika	LM5	820	17.5 (24.6)	TN05
52	Tharaka-Nithi	Chiagarika	LM5	810	0.0 (0.0)	-

53	Taita-Taveta	Mwatate	LM5	800	0.0 (0.0)	-
54	Taita-Taveta	Mwatate	LM5	720	0.0 (0.0)	-
55	Taita-Taveta	Taveta	LM5	880	9.6 (18.0)	TT01
56	Taita-Taveta	Taveta	LM5	945	9.1 (17.5)	TT02
57	Taita-Taveta	Taveta	LM5	945	20.3 (26.7)	TT03
58	Taita-Taveta	Taveta	LM5	950	69.6 (56.8)	TT04, TT05
59	Taita-Taveta	Taveta	LM5	855	75.3 (60.4)	TT06
60	Taita-Taveta	Taveta	LM5	895	0.0 (0.0)	-
61	Taita-Taveta	Tausa	L5	650	0.0 (0.0)	-
62	Taita-Taveta	Tausa	L5	655	17.7 (24.7)	TT07
63	Taita-Taveta	Tausa	L5	705	19.0 (25.3)	TT08
64	Taita-Taveta	Tausa	L5	700	27.5 (31.5)	TT09
65	Kilifi	Bahari	L3	10	0.0 (0.0)	-
66	Kilifi	Bahari	L3	15	0.0 (0.0)	-
67	Kilifi	Bahari	L3	50	0.0 (0.0)	-
68	Kilifi	Ganze	L4	165	0.0 (0.0)	-
69	Kilifi	Ganze	L4	220	0.0 (0.0)	-
70	Malindi	Magarini	L3	40	1.0 (4.9)	ML01
71	Malindi	Magarini	L3	25	0.0 (0.0)	-
72	Malindi	Magarini	L3	60	0.0 (0.0)	-
73	Malindi	Marafu	L4	130	0.0 (0.0)	-
74	Malindi	Marafu	L4	145	0.0 (0.0)	-
75	Kirinyaga	Mwea	LM4	1125	7.5 (15.9)	KR01
76	Kirinyaga	Mwea	LM4	1130	2.3 (8.5)	KR02, KR03
77	Thika	Kakuzi	UM4	1430	10.6 (18.8)	TK01, TK02
78	Thika	Kakuzi	UM4	1490	9.3 (17.6)	TK03
79	Thika	Kakuzi	UM4	1500	13.0 (20.1)	TK04, TK05, TK06
80	Thika	Thika	UM4	1410	0.0 (0.0)	-
81	Thika	Thika	UM4	1430	0.0 (0.0)	-
82	Thika	Thika	UM4	1420	0.0 (0.0)	-
83	Nairobi	Westlands	UM3	1820	63.2 (54.3)	NB01, NB02
84	Nairobi	Westlands	UM3	1820	34.6 (36.0)	NB03, NB04
85	Nairobi	Westlands	UM3	1820	0.0 (0.0)	-
86	Nairobi	Westlands	UM3	1820	0.0 (0.0)	-
Mean					8.4 (16.8)	
SE (±)					27.2	
CV (%)					31.1	
LSD (0.05)					7.3	

[1]Belts of zones: UM= Upper Midland Zones (annual mean temperature 18-21°C, mean minimum temperature 11-14°C); LM= Lower Midland Zones (annual mean temperature 21-24°C, mean minimum temperature >14°C); L= Lowland Zones (annual mean temperature >24°C, mean maximum temperature <31°C) (Jaetzold and Schmidt, 1983).
Main zones: 3= semi-humid, 4= transitional, 5= semi-arid and 6= arid.
UM3= marginal coffee zone, UM4= sunflower-maize zone, LM3= lower midland cotton zone, LM4= marginal cotton zone/lower midland sunflower-maize zone, LM5= lower midland livestock-millet zone, LM6= lower midland ranching zone, L3= coconut-cassava zone, L4= cashewnut-cassava zone and L5= lowland livestock-millet zone.
[2]Values in parenthesis are means obtained from transformed percent *Fusarium* wilt incidence data using arc sine transformation. Mean before transformation was 14.3%
[3]*F. udum* isolates that appear together were obtained from different infected plants from the same pigeonpea farm (site). However, isolates MS08 and MS09 from pigeonpea farm number 17 in Machakos district, and TK04 and TK05 from pigeonpea farm number 79 in Thika district were obtained from the same infected plant in the respective sites.

in the Field Station, Kabete Campus (University of Nairobi) in Nairobi district with a prevalence of 50%. There were significant differences ($P \leq 0.05$) in *Fusarium* wilt incidence among the districts surveyed (Table 2, Appendix 8.5.2). High mean *Fusarium* wilt incidence (>10% wilt) in the farmer's plots was observed in Tharaka-Nithi (22.3% wilt), Taita-Taveta (20.7% wilt), Meru (18.7% wilt), Kitui (15.0% wilt) and Machakos (13.6% wilt, excluding 2 farms with 58.5% wilt which were sick plots) districts. Moderate mean wilt incidence (5-10% wilt) was observed in Mbeere (8.8% wilt), Nyambene (9.4% wilt), Makueni (6.5% wilt, excluding one sick plot with 96.1% wilt) and Thika (5.5% wilt) districts, while minimal wilt was found in Malindi district (0.2% wilt). The mean wilt incidence in Nairobi district was 24.5%, while it was 4.9% in Kirinyaga district. A Pearson correlation coefficient of 0.52 ($P = 0.07$) was found between prevalence and incidence of *Fusarium* wilt among the districts surveyed.

Fusarium wilt occurred in 8 AEZs out of 9 AEZs covered during the survey (Table 3). Higher wilt prevalence was observed in LM3 (100%), LM6 (100%), UM4 (72.7%), LM5 (67.7%), LM4 (66.7%) and UM3 (63.6%) zones as compared to L3 (16.7%) and L4 (0.0%) zones. There were significant differences ($P \leq 0.05$) among AEZs in the *Fusarium* wilt incidence on pigeonpea (Table 3, Appendix 8.5.3). Zone LM6 with 2 farms sampled had the highest wilt incidence of 52.5%. Zone LM3 had 32.7% wilt from 5 farms, while zone LM5 with 31 farms had 13.2% wilt. The wilt incidence for the other zones were 19.5%, 16.6%, 16.1%, 7.2%, 0.2% and 0.0% for UM3, UM4, L5, LM4, L3, and L4 zones, respectively. A Pearson correlation coefficient of 0.82 ($P = 0.01$) was found between prevalence and incidence of *Fusarium* wilt among the AEZs surveyed.

Fusarium wilt was observed from 40 to 1820 meters above sea level (m.a.s.l) (Table 1). Wilt was highly prevalent at altitude ranges of 1201-1400 m.a.s.l (87.5%), 1001-1200 m.a.s.l (76.0%), 801-1000 m.a.s.l (70.6%) and 1401-1600 m.a.s.l (68.8%). Wilt prevalence was moderate at 601-800 m.a.s.l (50.0%) and 1801-2000 m.a.s.l (50.0%), low prevalence at below 600 m.a.s.l. There were significant differences ($P \leq 0.05$) among altitude ranges in the *Fusarium* wilt incidence on pigeonpea (Table 4,

Table 2. Prevalence and incidence of *Fusarium* wilt in various districts in Kenya

District	Pigeonpea farms	Farms with *Fusarium* wilt	Wilt prevalence (%)	Wilt incidence (%)[1]
Makueni	12	9	75	14.0 (17.5) cd
Machakos	6	6	100	28.5 (30.2) a
Kitui	5	4	80	15.0 (19.8) bc
Mbeere	9	5	56	8.8 (12.1) ef
Meru	7	6	86	18.7 (22.6) b
Nyambene	6	5	83	9.4 (15.1) de
Tharaka-Nithi	7	4	57	22.3 (22.5) b
Taita-Taveta	12	8	67	20.7 (21.7) b
Kilifi	5	0	0	0.0 (0.0) g
Malindi	5	1	20	0.2 (1.0) g
Kirinyaga	2	2	100	4.9 (12.2) ef
Thika	6	3	50	5.5 (9.4) f
Nairobi	4	2	50	24.5 (22.6) b
Mean			64.0	8.4 (16.8)
SE (±)				27.2
CV (%)				31.1

[1]Means with the same letter down the column are not significantly different at 5% level according to Student-Newman-Keuls (SNK) test. Values in parenthesis were transformed from percent *Fusarium* wilt incidence data using arc sine transformation.

Table 3. Prevalence and incidence of *Fusarium* wilt in various agro-ecological zones (AEZs) in Kenya

AEZ	Pigeonpea farms	Farms with *Fusarium* wilt	Wilt prevalence (%)	Wilt incidence (%)[1]
UM3	11	7	63.6	19.5 (20.9) c
UM4	11	8	72.7	16.6 (19.5) c
LM3	5	5	100.0	32.7 (34.2) b
LM4	12	8	66.7	7.2 (11.7) e
LM5	31	21	67.7	13.2 (16.2) d
LM6	2	2	100.0	52.5 (48.8) a
L3	6	1	16.7	0.2 (0.8) f
L4	4	0	0.0	0.0 (0.0) f
L5	4	3	75.0	16.1 (20.4) c
Mean			62.5	8.4 (16.8)
SE (±)				27.2
CV (%)				31.1

[1]Means with the same letter down the column are not significantly different at 5% level according to SNK test. Values in parenthesis were transformed from percent *Fusarium* wilt incidence data using arc sine transformation.

Appendix 8.5.4). High wilt incidence was observed at the altitude ranges of 801-1000 m.a.s.l (23.6%), 1201-1400 m.a.s.l (23.2%), and 1801-2000 m.a.s.l (24.5%). Wilt incidence was moderate at 1401-1600 m.a.s.l (14.8%) and 601-800 m.a.s.l (10.7%), and low incidence at below 600 m.a.s.l. A Pearson correlation coefficient of 0.77 ($P = 0.02$) was found between *Fusarium* wilt prevalence and incidence among the altitude ranges surveyed.

Prevalence of *Fusarium* wilt on local and improved pigeonpea varieties were 63.6% and 61.5%, respectively (Table 5). There were no significant differences ($P \leq 0.05$) among pigeonpea types in the *Fusarium* wilt incidence on pigeonpea (Table 5, Appendix 8.5.5). Wilt incidence were 17.1% and 13.4% on improved and local varieties, respectively. A Pearson correlation coefficient of -0.92 ($P = 0.26$) was found between *Fusarium* wilt prevalence and incidence among the pigeonpea types surveyed.

The kind of pigeonpea cropping system practiced by the farmers had an effect on *Fusarium* wilt prevalence and incidence (Table 6). The wilt prevalence on the sole crop and intercrop were 67.9% and 62.1%, respectively. There were significant differences ($P \leq 0.05$) among cropping systems in the *Fusarium* wilt incidence on pigeonpea (Appendix 8.5.6). The wilt incidence on the sole crop and intercrop were 15.4% and 13.7%, respectively. A Pearson correlation coefficient of 0.98 ($P = 0.14$) was found between *Fusarium* wilt prevalence and incidence among the pigeonpea cropping systems surveyed.

Table 4. Prevalence and incidence of *Fusarium* wilt at various altitudes in Kenya

Altitude range (m.a.s.l)	Pigeonpea farms	Farms with *Fusarium* wilt	Wilt prevalence (%)	Wilt incidence (%)[1]
0-200	9	1	11.1	0.1 (0.5) c
201-400	1	0	0.0	0.0 (0.0) c
601-800	6	3	50.0	10.7 (13.6) b
801-1000	17	12	70.6	23.6 (24.3) a
1001-1200	25	19	76.0	9.6 (14.4) b
1201-1400	8	7	87.5	23.2 (26.3) a
1401-1600	16	11	68.8	14.8 (17.6) b
1801-2000	4	2	50.0	24.5 (22.6) a
Mean			51.8	8.4 (16.8)
SE (±)				27.2
CV (%)				31.1

[1]Means with the same letter down the column are not significantly different at 5% level according to SNK test. Values in parenthesis were transformed from percent *Fusarium* wilt incidence data using arc sine transformation.

Table 5. Prevalence and incidence of *Fusarium* wilt on pigeonpea types in Kenya

Pigeonpea type	Pigeonpea farms	Farms with *Fusarium* wilt	Wilt prevalence (%)	Wilt incidence (%)[1]
Improved	26	16	61.5	17.1 (18.3) a
Local	55	35	63.6	13.4 (16.3) ab
Improved and local	5	4	80.0	9.5 (14.3) b
Mean			68.4	8.4 (16.8)
SE (±)				27.2
CV (%)				31.1

[1]Means with the same letter down the column are not significantly different at 5% level according to SNK test. Values in parenthesis were transformed from percent *Fusarium* wilt incidence data using arc sine transformation.

Table 6. Prevalence and incidence of *Fusarium* wilt on pigeonpea cropping systems in Kenya

Pigeonpea cropping system	Pigeonpea farms	Farms with *Fusarium* wilt	Wilt prevalence (%)	Wilt incidence (%)[1]
Sole crop	28	19	67.9	15.4 (18.0) a
Intercrop	58	36	62.1	13.7 (16.2) b
Mean			65.0	8.4 (16.8)
SE (±)				27.2
CV (%)				31.1

[1]Means with the same letter down the column are not significantly different at 5% level according to SNK test. Values in parenthesis were transformed from percent *Fusarium* wilt incidence data using arc sine transformation.

4.2 *F. udum* isolation and identification

The colonies of *F. udum* isolates covered a 9 cm (diameter) PDA plate in 9 to 12 days at 25°C. The cultures had luxuriant to scanty aerial mycelium, fluffy or fibrous mycelium texture, and white, buff to luteous, lilac to dark purple, rose or mauve and light blue colours. The hyphae were highly branched, slender, septate and hyaline, and produced conidiophores, and frequently sporodochia and chlamydospores. Microcinidia were hyaline, straight to curved, 0-1 septate and measured 3.4-18.7 µm x 1.7-4.2 µm. Macroconidia were hyaline, falcate with a distinct foot cell, hooked apices, 2-7 septate and measured 13.6-55.9 µm x 2.5-5.1 µm. All the isolates were pathogenic to *Fusarium* wilt susceptible pigeonpea variety KAT 60/8. When re-isolated onto PDA medium, they produced cultural and morphological characteristics similar to those of the original isolates.

4.3 *Fusarium* wilt inoculation techniques

4.3.1 Incubation time to initial wilt symptoms

The initial symptoms of *Fusarium* wilt on pigeonpea plants were epinasty, interveinal yellowing of lower leaves and drooping of leaves. *Fusarium* wilt symptoms appeared from 8 to 10 days and 4 weeks on plants of wilt susceptible pigeonpea varieties KAT 60/8 and NPP 670 inoculated with the isolates MR01 and TT01 using root-dip and colonized whole rice techniques, respectively. Wilt resistant pigeonpea variety ICP 9174 developed symptoms after 2 weeks of incubation when inoculated with both isolates using root-dip technique while isolate MR01 induced symptoms after 5 weeks using colonized whole rice technique. No wilt symptoms developed on variety ICP 9174 eight weeks after inoculation with isolate TT01 using colonized whole rice technique. Wilt resistant pigeonpea variety ICP 8863 succumbed to infection and developed symptoms 2 weeks after incubation with isolate TT01 using root-dip technique with no symptoms when inoculated using colonized whole rice technique. Variety ICP 8863 did not develop wilt symptoms when inoculated with isolate MR01 using root-dip or colonized whole rice technique. Re-isolation of

F. udum from plants with wilt symptoms was possible at 2 weeks and 5 weeks after root-dip and colonized whole rice inoculation, respectively.

4.3.2 *Fusarium* wilt incidence

Fusarium wilt incidence on pigeonpea plants was significantly different ($P \leq 0.05$) between the inoculation techniques, and among pigeonpea varieties and incubation time, but not significantly different among *F. udum* isolates (Tables 7 and 8, Appendix 8.5.7). Significant differences ($P \leq 0.05$) in wilt incidence occurred between the interactions of inoculation technique-variety, inoculation technique-isolate, variety-isolate, variety-incubation time and inoculation technique-variety-isolate, but not significant differences ($P \leq 0.05$) were observed between the inoculation technique-incubation time, isolate-incubation time, inoculation technique-variety-incubation time, variety-isolate-incubation time and inoculation technique-variety-isolate-incubation time. The wilt incidences varied from 0 to 100% with a mean of 26.3%. High wilt incidence of 44.9% was recorded when plants were inoculated using root-dip technique when compared to colonized whole rice technique which caused 7.7% wilt. Wilt incidences observed on pigeonpea varieties KAT 60/8, NPP 670, ICP 9174 and ICP 8863 were 53.6%, 46.5%, 2.9% and 2.1%, respectively *Fusarium* wilt incidences between resistant varieties ICP 8863 and ICP 9174 were not significantly different ($P \leq 0.05$) while incidences between susceptible varieties KAT 60/8 and NPP 670 were significantly different. Wilt incidences due to *F. udum* isolates TT01 and MR01 were 26.8% and 25.8%, respectively and not significantly different ($P \leq 0.05$).

Table 7. Wilt incidence (%) over time (weeks) of four pigeonpea varieties inoculated with two *F. udum* isolates using two inoculation techniques in a glasshouse experiment

Incubation time (weeks)	Pigeonpea variety	*F. udum* isolate	Inoculation technique		Mean
			Root-dip	Colonized rice	
3	KAT 60/8	MR01	85	0	42.5
		TT01	80	0	40.0
	NPP 670	MR01	70	0	35.0
		TT01	75	0	37.5
	ICP 8863	MR01	0	0	0.0
		TT01	5	0	2.5
	ICP 9174	MR01	0	0	0.0
		TT01	5	0	2.5
		Mean	40.0	0.0	20.0
4	KAT 60/8	MR01	80	5	42.5
		TT01	80	5	42.5
	NPP 670	MR01	65	5	35.0
		TT01	80	10	45.0
	ICP 8863	MR01	0	0	0.0
		TT01	10	0	5.0
	ICP 9174	MR01	5	0	2.5
		TT01	5	0	2.5
		Mean	40.6	3.1	21.9
5	KAT 60/8	MR01	100	15	57.5
		TT01	90	15	52.5
	NPP 670	MR01	75	20	47.5
		TT01	85	10	47.5
	ICP 8863	MR01	0	0	0.0
		TT01	5	0	2.5
	ICP 9174	MR01	5	0	2.5
		TT01	5	0	2.5
		Mean	45.6	7.5	26.6
6	KAT 60/8	MR01	100	20	60.0
		TT01	90	15	52.5
	NPP 670	MR01	75	15	45.0
		TT01	90	15	52.5
	ICP 8863	MR01	0	0	0.0
		TT01	10	0	5.0
	ICP 9174	MR01	5	5	5.0
		TT01	5	0	2.5
		Mean	46.9	8.8	27.9
7	KAT 60/8	MR01	100	30	65.0
		TT01	95	25	60.0
	NPP 670	MR01	80	25	52.5
		TT01	90	15	52.5
	ICP 8863	MR01	0	0	0.0
		TT01	10	0	5.0
	ICP 9174	MR01	5	5	5.0
		TT01	5	0	2.5
		Mean	48.1	12.5	30.3

8	KAT 60/8	MR01	100	30	65.0
		TT01	95	35	65.0
	NPP 670	MR01	80	25	52.5
		TT01	90	20	55.0
	ICP 8863	MR01	0	0	0.0
		TT01	10	0	5.0
	ICP 9174	MR01	5	5	5.0
		TT01	5	0	2.5
		Mean	48.1	14.4	31.3
		CV (%)	95.2	158.7	
		SE	1828.2	149.6	
		LSD (p=0.05)	21.1	6.0	

Table 8. Overall *Fusarium* wilt incidence on pigeonpea plants for the inoculation techniques, varieties, isolates and incubation time

Variable	Wilt incidence (%)	LSD ($P \leq 0.05$)
Inoculation technique:		
Root-dip	44.9	2.0
Colonized whole rice	7.7	
Pigeonpea variety:		
KAT 60/8	53.6	2.9
NPP 670	46.5	
ICP 9174	2.9	
ICP 8863	2.1	
F. udum isolate:		
TT01	26.8	2.0
MR01	25.8	
Incubation time:		
Week 3	20.0	3.5
Week 4	21.9	
Week 5	26.6	
Week 6	27.8	
Week 7	30.3	
Week 8	31.2	
Mean	26.3	
CV (%)	38.5	
SE	102.4	

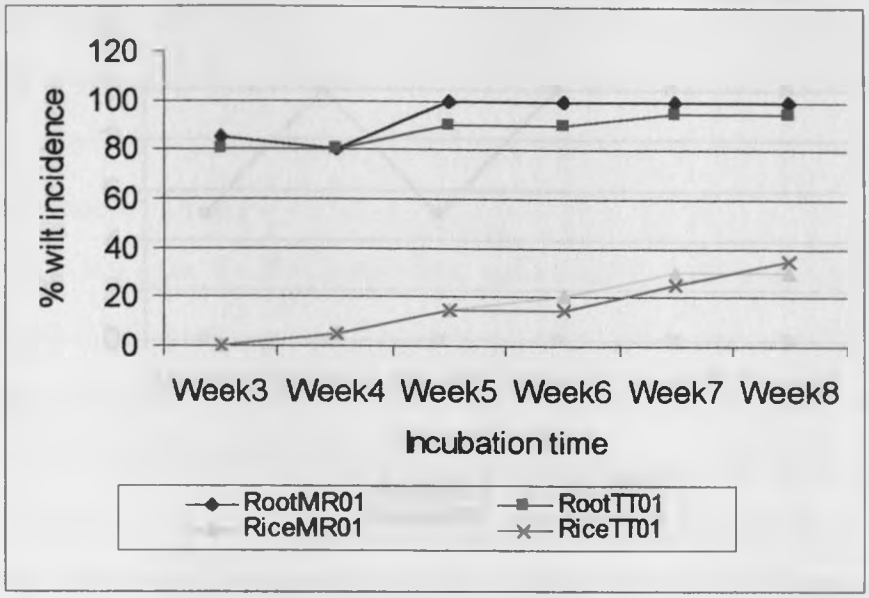


Figure 2. *Fusarium* wilt incidence (%) over incubation time (weeks) on pigeonpea variety KAT 60/8 inoculated with isolates MR01 and TT01 using root-dip (RootMR01 and RootTT01) and colonized whole rice (RiceMR01 and RiceTT01) techniques

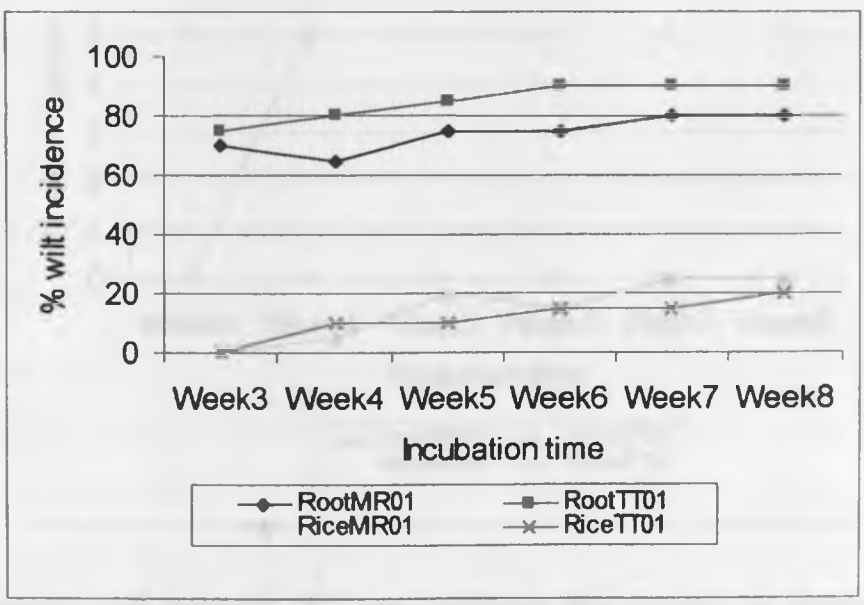


Figure 3. *Fusarium* wilt incidence (%) over incubation time (weeks) on pigeonpea variety NPP 670 inoculated with isolates MR01 and TT01 using root-dip (RootMR01 and RootTT01) and colonized whole rice (RiceMR01 and RiceTT01) techniques

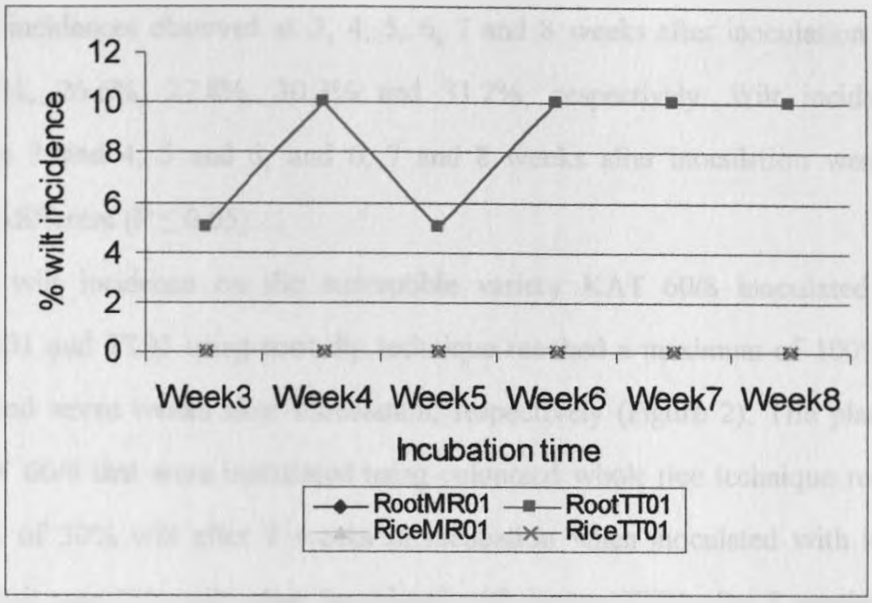


Figure 4. *Fusarium* wilt incidence (%) over incubation time (weeks) on pigeonpea variety ICP 8863 inoculated with isolates MR01 and TT01 using root-dip (RootMR01 and RootTT01) and colonized whole rice (RiceMR01 and RiceTT01) techniques

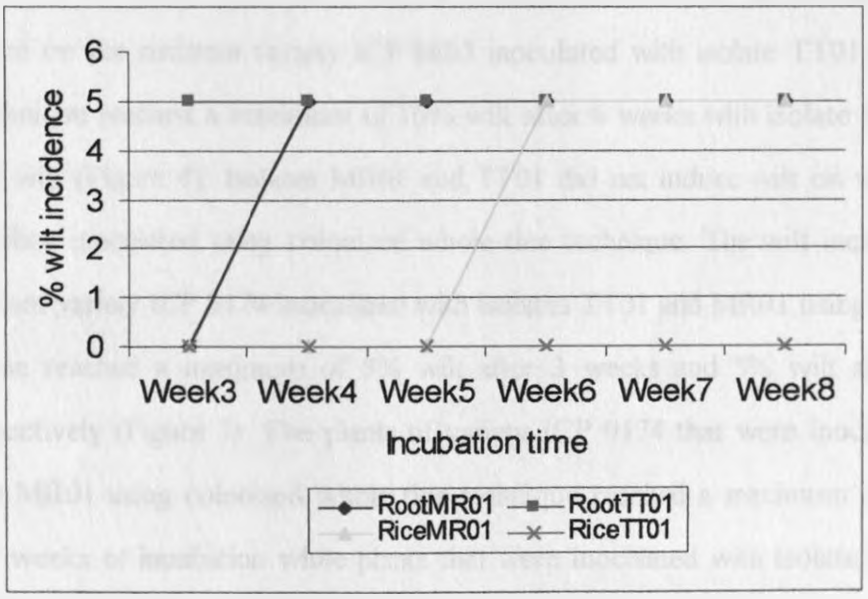


Figure 5. *Fusarium* wilt incidence (%) over incubation time (weeks) on pigeonpea variety ICP 9174 inoculated with isolates MR01 and TT01 using root-dip (RootMR01 and RootTT01) and colonized whole rice (RiceMR01 and RiceTT01) techniques

Wilt incidences observed at 3, 4, 5, 6, 7 and 8 weeks after inoculation were 2.0%, 21.9%, 26.6%, 27.8%, 30.3% and 31.2%, respectively. Wilt incidences, however, on 3 and 4, 5 and 6, and 6, 7 and 8 weeks after inoculation were not significantly different ($P \leq 0.05$).

The wilt incidence on the susceptible variety KAT 60/8 inoculated with isolates MR01 and TT01 using root-dip technique reached a maximum of 100% and 95% four and seven weeks after inoculation, respectively (Figure 2). The plants of variety KAT 60/8 that were inoculated using colonized whole rice technique reached a maximum of 30% wilt after 7 weeks of incubation when inoculated with isolate MR01 while it was 35% wilt when inoculated with isolate TT01 after 8 weeks. The wilt incidence on the susceptible variety NPP 670 inoculated with isolates TT01 and MR01 using root-dip technique reached a maximum of 90% wilt after 6 weeks and 80% wilt after 7 weeks, respectively (Figure 3). Variety NPP 670 inoculated with MR01 and TT01 isolates using colonized whole rice technique showed an incidence of 25% wilt after 7 weeks and 20% wilt after 8 weeks of incubation, respectively. The wilt incidence on the resistant variety ICP 8863 inoculated with isolate TT01 using root-dip technique reached a maximum of 10% wilt after 6 weeks with isolate MR01 inducing no wilt (Figure 4). Isolates MR01 and TT01 did not induce wilt on variety ICP 8863 when inoculated using colonized whole rice technique. The wilt incidence on the resistant variety ICP 9174 inoculated with isolates TT01 and MR01 using root-dip technique reached a maximum of 5% wilt after 3 weeks and 5% wilt after 4 weeks, respectively (Figure 5). The plants of variety ICP 9174 that were inoculated with isolate MR01 using colonized whole rice technique reached a maximum of 5% wilt after 6 weeks of incubation while plants that were inoculated with isolate TT01 did not develop wilt.

4.4 Cultural characteristics of *F. udum* isolates

Various cultural characteristics were used to classify 79 isolates of *F. udum* into various groups. These characteristics included growth of aerial mycelia, mycelial

texture, radial mycelial growth (colony diameter), sporulation and pigmentation on PDA medium after 8 days of incubation at 25°C.

4.4.1 Growth of aerial mycelia

Three types of aerial mycelial growth were identified and these included luxuriant, moderately luxuriant and scanty growths (Table 9, Plates 3, 4, 5 and 6). Luxuriant, moderately luxuriant and scanty mycelia were designated as group 1, 2 and 3, respectively. The isolates which exhibited growth in groups 1, 2 and 3 were 27 (34.2%), 29 (36.7%) and 23 (29.1%), respectively. The mycelia of the three groups of isolates were raised, uniform or suppressed around the disc. Isolates from Malawi (MAL01a and MAL01b) and India (IND01a and IND01b) belonged to groups 1 and 3, respectively. Isolates from various districts in Kenya had similar or different aerial mycelial growths. The composition of isolates from districts with five or more isolates was mostly in group 1 with 83.3%, 60.0%, 44.4% and 37.5% isolates in Thika, Kitui, Taita-Taveta and Nyambene districts, respectively (Tables 1 and 9). Group 1 was not found in Mbeere district. The composition of group 2 was 57.1%, 50.0%, 50.0%, 44.4% and 40.0% isolates in Meru, Machakos, Makueni, Taita-Taveta and Tharaka-Nithi districts, respectively. Group 2 was absent in Kitui district. The composition of group 3 was 57.1%, 40.0%, 40.0% and 37.5% isolates in Mbeere, Kitui, Tharaka-Nithi and Nyambene districts, respectively. Group 3 was absent in Thika district.

F. udum isolates from various AEZs in Kenya varied in their aerial mycelial growth (Tables 1 and 9). Considering AEZs with five or more isolates, the composition of group 1 was 46.7%, 42.9%, 23% and 20.0% isolates in UM4, UM3, LM5 and LM4 zones, respectively. The composition of group 2 was 46.2%, 42.9%, 26.7% and 10.0% isolates in LM5, UM3, UM4 and LM4 zones, respectively. The composition of group 3 was 70.0%, 30.8%, 26.7% and 8.4% isolates in LM4, LM5, UM4 and UM3 zones, respectively. There were no marked differences in aerial mycelial growths among the isolates at various altitudes in Kenya. Group 1, 2 and 3 appeared in almost equal proportion of 33.3% isolates at the altitude range of

Table 9. Cultural characteristics[1] of *F. udum* isolates on PDA medium and wilt incidence (%)[2] on susceptible pigeonpea variety KAT 60/8

Isolate	Aerial mycelia	Mycelial texture	Mycelial colour[3]	Substrate colour[3]	Colony diameter[4] (mm)	Spores[5] (x 10^5)	% wilt[6]
MK01	Moderately luxuriant, raised	Fibrous	Buff	Buff	81.3	9.4	75
MK02	Scanty, suppressed[7]	Fibrous	Dark purple	Purple	81.9	13.4	90
MK03	Moderately luxuriant, raised	Fibrous	Buff	Buff	81.2	9.6	75
MK04	Moderately luxuriant, raised	Fibrous	White	Lilac	78.8	7.1	85
MK05	Scanty, raised	Fibrous	Buff	Pale luteous	80.7	11.0	80
MK06	Luxuriant, suppressed	Fluffy	Rose	Purple	80.4	11.5	55
MK07	Luxuriant, suppressed	Fluffy	White	Pale luteous	80.2	5.8	75
MK08	Moderately luxuriant, suppressed	Fluffy	Dark purple	Purple	77.3	17.9	40
MK09	Moderately luxuriant, suppressed	Fibrous	Purple	Lilac	77.8	11.2	60
MK10	Luxuriant, uniform	Fluffy	White	Lilac	84.2	6.5	80
MS01	Moderately luxuriant, suppressed	Fluffy	Purple	Lilac	80.8	11.2	90
MS02	Luxuriant, uniform	Fluffy	White	Pale luteous	82.0	3.8	65
MS03	Scanty, suppressed	Fibrous	Lilac	Lilac	80.8	7.3	75
MS04	Scanty, uniform	Fibrous	Dark purple	Purple	80.8	15.3	85
MS05	Moderately luxuriant, raised	Fluffy	Purple	Purple	77.3	5.7	85
MS06	Luxuriant, suppressed	Fluffy	White	Lilac	79.8	8.9	65
MS07	Scanty, uniform	Fibrous	Dark purple	Dark purple	77.5	17.7	65
MS08	Moderately luxuriant, raised	Fluffy	White	Buff	79.5	9.2	55
MS09	Moderately luxuriant, uniform	Fluffy	Dark purple	Mauve	79.5	2.7	55
MS10	Moderately luxuriant, uniform	Fibrous	Buff	Buff	81.4	3.6	85
KT01	Luxuriant, uniform	Fluffy	White	Buff	80.2	6.4	95
KT02	Scanty, uniform	Fluffy	Dark purple	Purple	78.7	24.6	90
KT03	Scanty, uniform	Fibrous	Buff	Pale luteous	82.9	6.2	65
KT04	Luxuriant, raised	Fluffy	White	Pale luteous	76.7	8.9	70
KT05	Luxuriant, raised	Fluffy	Flax blue	Purple	83.3	3.8	80
MI301	Scanty, suppressed	Fibrous	Purple	Purple	82.6	5.0	55
MI302	Moderately luxuriant, uniform	Fibrous	Purple	Purple	84.5	8.6	80
MI303	Moderately luxuriant, raised	Fibrous	Rose	Lilac	78.9	5.6	90
MI304	Scanty, suppressed	Fibrous	Buff	Purple	78.3	11.1	85
MI305	Scanty, suppressed	Fibrous	Dark purple	Purple	72.7	12.8	95
MI306	Scanty, suppressed	Fibrous	Purple	Purple	81.9	11.0	70
MI307	Moderately luxuriant, suppressed	Fibrous	Buff	Lilac	80.9	11.1	85
MR01	Scanty, suppressed	Fibrous	Flax blue	Dark purple	84.1	7.1	100
MR02	Moderately luxuriant, suppressed	Fibrous	Buff	Lilac	82.6	6.7	55
MR03	Moderately luxuriant, suppressed	Fibrous	Flax blue	Purple	83.9	4.8	70
MR04	Luxuriant, suppressed	Fluffy	Rose	Purple	79.5	6.9	65
MR05	Luxuriant, uniform	Fluffy	Purple	Lilac	83.0	5.2	55
MR06	Moderately luxuriant, uniform	Fibrous	Buff	Purple	84.3	6.5	55
MR07	Moderately luxuriant, suppressed	Fibrous	Flax blue	Dark purple	84.9	9.8	55
NY01	Luxuriant, uniform	Fibrous	White	Luteous	84.2	4.0	85
NY02	Luxuriant, suppressed	Fluffy	Buff	Vinaceous buff	84.3	4.8	95
NY03	Luxuriant, suppressed	Fluffy	Buff	Pale luteous	85.7	6.3	75
NY04	Scanty, suppressed	Fibrous	Buff	Lilac	82.7	11.9	70
NY05	Moderately luxuriant, uniform	Fibrous	Flax blue	Lilac	79.6	6.1	70
NY06	Moderately luxuriant, uniform	Fluffy	Dark purple	Purple	81.5	7.8	70
NY07	Scanty, little visible mycelia	Fibrous	Purple	Purple	78.4	21.1	100
NY08	Scanty, uniform	Fibrous	Purple	Purple	81.6	5.8	100
TN01	Scanty, uniform	Fibrous	Flax blue	Lilac	80.2	9.4	65
TN02	Moderately luxuriant, uniform	Fibrous	Dark purple	Dark purple	75.1	5.8	70
TN03	Moderately luxuriant, suppressed	Fluffy	White	Lilac	81.5	3.6	90
TN04	Scanty, suppressed	Fibrous	Glaucos sky blue	Purple	85.4	4.0	95
TN05	Luxuriant, suppressed	Fluffy	Purple	Purple	76.6	3.5	55
TT01	Moderately luxuriant, suppressed	Fibrous	Luteous	Pale luteous	78.6	16.9	100
TT02	Moderately luxuriant, raised	Fibrous	Buff	Lilac	81.5	6.0	60
TT03	Moderately luxuriant, uniform	Fluffy	Buff	Buff	86.3	9.0	75

TT04	Luxuriant, raised	Fluffy	White	Purple	80.3	8.2	90
TT05	Luxuriant, raised	Fluffy	Purple	Luteous	81.8	4.4	100
TT06	Scanty, suppressed	Fibrous	Buff	Luteous	82.5	17.5	100
TT07	Luxuriant, suppressed	Fluffy	Lilac	Purple	81.2	6.2	85
TT08	Moderately luxuriant, suppressed	Fluffy	Purple	Purple	71.4	21.4	80
TT09	Luxuriant, suppressed	Fluffy	Buff	Pale luteous	82.0	4.0	85
ML01	Moderately luxuriant, suppressed	Fibrous	Purple	Purple	83.5	4.8	75
KR01	Scanty, uniform	Fibrous	Buff	Pale luteous	83.5	17.1	60
KR02	Scanty, uniform	Fibrous	Rose	Pale luteous	84.6	1.4	65
KR03	Scanty, uniform	Fibrous	Lilac	Lilac	81.9	3.8	55
TK01	Luxuriant, uniform	Fluffy	Purple	Lilac	88.8	5.0	80
TK02	Luxuriant, suppressed	Fluffy	Dark purple	Lilac	87.7	4.6	65
TK03	Luxuriant, raised	Fluffy	Dark purple	Purple	88.5	3.1	90
TK04	Luxuriant, uniform	Fibrous	Purple	Lilac	85.4	3.8	45
TK05	Moderately luxuriant, uniform	Fluffy	Lilac	Purple	84.3	8.8	35
TK06	Luxuriant, uniform	Fluffy	Lilac	Lilac	85.7	6.1	55
NB01	Luxuriant, raised	Fluffy	Mauve	Purple	83.9	4.8	75
NB02	Moderately luxuriant, raised	Fluffy	White	Pale luteous	82.6	4.4	90
NB03	Luxuriant, suppressed	Fluffy	White	Pale luteous	81.2	6.8	80
NB04	Moderately luxuriant, uniform	Fluffy	Rose	Purple	73.6	7.6	75
MAL01a	Luxuriant, uniform	Fluffy	Purple	Purple	80.1	5.1	80
MAL01b	Luxuriant, uniform	Fluffy	Luteous	Pale luteous	80.4	5.4	90
IND01a	Scanty, uniform	Fibrous	Lilac	Lilac	73.6	7.5	70
IND01b	Scanty, uniform	Fibrous	Lilac	Lilac	73.3	8.8	65
Mean					81.2	8.2	74.9
CV (%)					0.8	22.4	15.7
SE					0.4	0.0	138.4
LSD (p=0.05)					1.0	0.3	16.4

[1]Data on cultural characteristics was taken at 8 days after incubation on PDA at 25°C

[2]Data on per cent wilt was taken at 6 weeks after root-dip inoculation

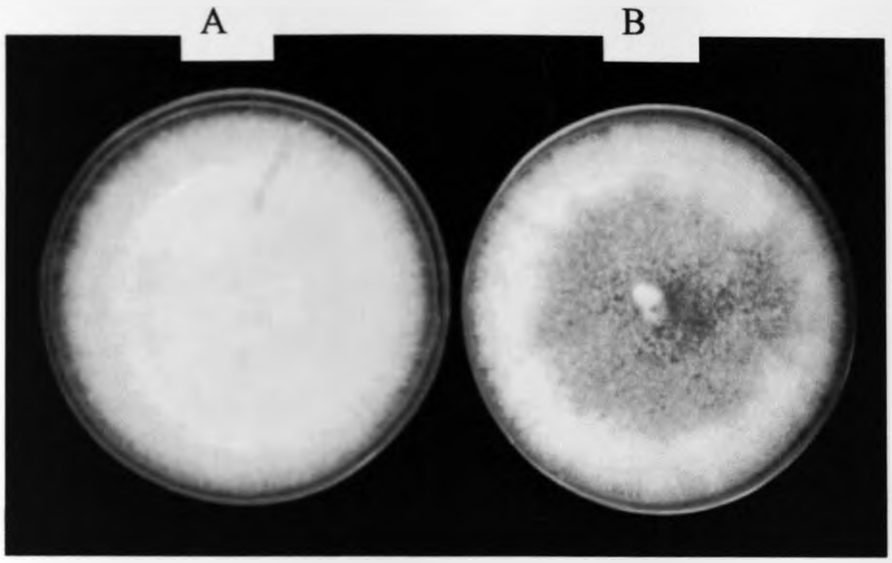
[3]Mycelial and substrate colour were determined according to the mycological colour chart (Rayner, 1970) (Appendix 8.2)

[4]Colony diameter was taken as radial mycelial growth after 8 days of incubation.

[5]Spores was the sporulation (conidia/ml)

[6]Wilt incidence (%) was on pigeonpea variety KAT 60/8 at 6 after weeks of incubation

[7]Suppressed around the disc



3

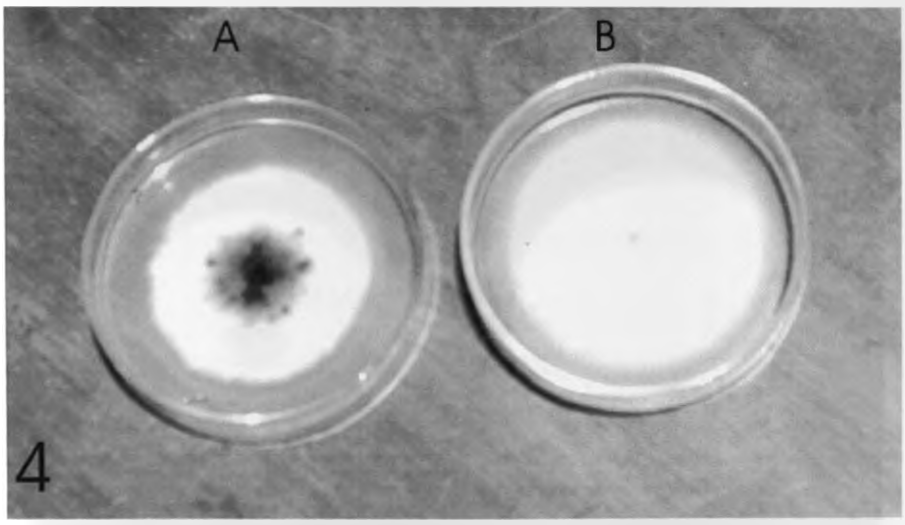


Plate 3. Cultural characteristics of *F. udum* isolates NY02 and MR06 on PDA eight days after incubation at 25°C. NY02 (A) has fluffy luxuriant aerial mycelia while isolate MR06 (B) has fibrous moderately luxuriant aerial mycelia.

Plate 4. Cultural characteristics of *F. udum* isolates TT08 and MK07 on PDA eight days after incubation at 25°C. TT08 (A) has luxuriant aerial mycelia with moderate radial growth while isolate MK07 (B) has moderately luxuriant mycelia with slow radial growth.

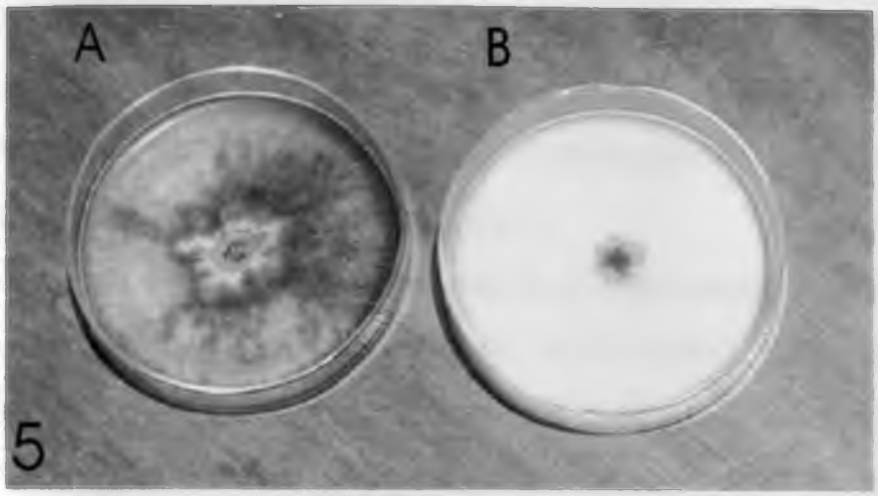


Plate 5. Cultural characteristics of *F. udum* isolates TN02 and MR05 on PDA eight days after incubation at 25°C. TN02 (A) has purple colour on moderately luxuriant aerial mycelia while isolate MR05 (B) has dark purple colour on scanty aerial mycelia.

Plate 6. Cultural characteristics of *F. udum* isolates MR04 and MS02 on PDA eight days after incubation at 25°C. MR04 (A) has purplish pink colour on luxuriant aerial mycelia while isolate MS02 (B) has white colour luxuriant aerial mycelia.

1201-1400 m.a.s.l while they had 36.8%, 31.6% and 31.6% isolates, respectively at 1401-1600 m.a.s.l (Tables 1 and 9). Groups 1, 2 and 3 had a composition of 31.3%, 37.5% and 31.3% isolates, respectively at 801-1000 m.a.s.l and 26.1%, 43.5% and 30.4% isolates, respectively at 1001-1200 m.a.s.l.

In some cases isolates showed variation in aerial mycelial growth within the same farm and even on the same plant. For instance isolates MS07 and MS08 or MS09 obtained from different infected plants from the same pigeonpea farm (farm number 17) in Machakos district showed differences in aerial mycelial growth. This was the case with isolates MB06 and MB07 from farm number 28 in Mbeere district, and isolates TN01 and TN02 from farm number 47 in Tharaka-Nithi district. Isolates TK04 and TK05 obtained from the same farm (farm number 79) and from the same infected pigeonpea plant from Thika district showed variation in growth of aerial mycelia.

4.4.2 Mycelial texture

Two types of mycelial texture that were identified on *F. udum* isolates were fluffy (cottony) and fibrous (woolly), and they were designated as group 1 and 2, respectively (Table 9, Plate 3). Group 1 had 38 isolates (48.1%) while group 2 had 41 isolates (51.9%). Isolates from Malawi (MAL01a and MAL01b) and India (IND01a and IND01b) belonged to group 1 and 2, respectively. Isolates from various districts in Kenya showed differences in mycelial texture. Considering districts with five or more isolates, the composition of group 1 was 75.0%, 70.0%, 66.7%, 60.0%, 44.4%, 42.9%, 40.0%, 40.0% and 0.0% isolates in Nyambene, Machakos, Thika, Tharaka-Nithi, Taita-Taveta, Mbeere Makueni, Kitui and Meru districts, respectively (Tables 1 and 9). The composition of group 2 was 100.0%, 60.0%, 60.0%, 57.1%, 55.6%, 40.0%, 33.3%, 30.0% and 25.0% isolates in Meru, Kitui, Makueni, Mbeere, Taita-Taveta, Tharaka-Nithi, Thika, Machakos and Nyambene, respectively.

The isolates from various AEZs showed differences in mycelial texture. Considering AEZs with five or more isolates, the composition of group 1 was 66.7%,

57.1%, 34.6% and 20.0% isolates at UM4, UM3, LM5 and LM4 zones, respectively (Tables 1 and 9). The composition of group 2 was 80.0%, 65.4%, 42.9% and 33.3% isolates at LM4, LM5, UM3 and UM4 zones, respectively. Isolates from different altitudes showed different mycelial texture. The composition of group 1 was 52.6%, 44.4%, 43.8% and 34.8% isolates at 1401-1600, 1201-1400, 801-1000 and 1001-1200 m.a.s.l, respectively (Tables 1 and 9). The composition of group 2 was 65.2%, 56.3%, 55.6% and 47.4% isolates at 1001-1200, 801-1000, 1201-1400 and 1401-1600 m.a.s.l, respectively.

In some cases isolates showed variation in mycelial texture within the same farm and even on the same plant. For instance isolates MK08 and MK09 obtained from different infected plants from the same pigeonpea farm (farm number 11) in Makueni district showed differences in mycelial texture. This was the case with isolates NY05 and NY06 from farm number 43 in Nyambene district, and isolates MR05 and MR06 from farm number 38 in Meru district. Isolates TK04 and TK05 obtained from the same farm (farm number 79) and from the same infected pigeonpea plant from Thika district showed variation in mycelial texture.

4.4.3 Radial mycelial growth (colony diameter)

The radial mycelial growth was significantly different ($P \leq 0.05$) among *F. udum* isolates with a mean of 81.2 mm and a range of 71.4 mm (isolate TT08) to 88.8 mm (isolate TK01) colony diameter (Table 9, Appendix 8.5.8, Plate 4). Based on the inter-quartile ranges of the colony diameter the isolates were grouped into three radial mycelial growths namely fast growth with above 83.9 mm, moderate growth with 78.0-83.9 mm and slow growth with below 78.0 mm. Isolates with fast, moderate and slow growths were designated as group 1, 2 and 3, respectively. The isolates in groups 1, 2 and 3 were 17 (21.5%), 50 (63.3%) and 12 (15.2%), respectively. Isolates MAL01a and MAL01b from Malawi fitted in group 2, while isolates IND01a and IND01b from India fitted in group 3.

The radial mycelial growths of 75 isolates of *F. udum* from Kenya were significantly different ($P \leq 0.05$) among various districts with a mean of 81.4 mm and a range of 76.6 mm in Tharaka-Nithi district to 86.7 mm in Thika district (Table 10, Appendix 8.5.9). Isolates from Thika (86.7 mm), Meru (83.2 mm) and Nyambene (82.2 mm) districts, which had five or more isolates, showed significant differences ($P \leq 0.05$) in colony diameter. Isolates from Taita-Taveta (80.6 mm) were significantly different ($P \leq 0.05$) from isolates from Tharaka-Nithi (79.6 mm) districts but not significant ($P \leq 0.05$) with isolates from Makueni (80.4 mm), Kitui (80.4 mm), Mbeere (80.0 mm) and Machakos (79.9 mm) districts. The composition of group 1 was 100.0% and 42.9% isolates in Thika and Meru districts, respectively but absent from Machakos and Kitui districts (Tables 1 and 9). The composition of group 2 was 100.0%, 80.0%, 77.8%, 71.4%, 70.0%, 62.5% and 57.1% isolates in Machakos, Kitui, Taita-Taveta, Mbeere, Makueni, Nyambene and Meru districts, respectively but absent in Thika district. While the composition of group 3 was 40.0%, 20.0% and 20.0% isolates in Tharaka-Nithi, Makueni and Kitui ditricts, respectively with total absence in Nyambene, Meru, Thika and Machakos districts.

There were significant differences ($P \leq 0.05$) in radial mycelial growth among *F. udum* isolates from various AEZs in Kenya (Table 11, Appendix 8.5.10). The mean colony diameter was 81.4 mm with a range of 78.2 mm in L5 to 83.5 mm in L3. Isolates from UM4 (83.1 mm) and LM5 (80.6 mm) zones, which had five or more isolates, showed significant difference ($P \leq 0.05$) in colony diameter while isolates from UM3 (81.9 mm) and LM4 (81.6 mm) were not significantly different ($P \leq 0.05$). The composition of group 1 was 40.0%, 35.7%, 11.5% and 10.0% isolates in UM4, UM3, LM5 and LM4 zones, respectively. The composition of group 2 was 80.8%, 80.0%, 60.0% and 50.0% isolates in LM5, LM4, UM4 and UM3 zones, respectively. While the composition of group 3 was 8.4%, 10.0%, 7.7% and 0.0% isolates in UM3, LM4, LM5 and UM4 zones, respectively.

There were significant differences ($P \leq 0.05$) in radial mycelial growth among *F. udum* isolates from various altitude ranges in Kenya (Table 12, Appendix 8.5.11).

Table 12. Radial mycelial growth (colony diameter), sporulation and wilt incidence (%) (on pigeonpea variety KAT 60/8) of *F. udum* isolates from various altitude ranges in Kenya

Altitude range (m.a.s.l)	Number of isolates	Colony diameter (mm)	Sporulation (conidia/ml x 10^5)	Wilt incidence (%
0-200	1	83.5 a	4.8 e	75.0 ab
601-800	3	78.2 e	10.5 a	83.3 a
801-1000	16	80.6 d	9.7 ab	79.4 a
1001-1200	23	81.3 c	8.0 bc	67.4 b
1201-1400	9	81.3 c	6.9 cd	79.4 a
1401-1600	19	82.9 b	8.4 bc	75.5 ab
1801-2000	4	80.3 d	5.9 de	80.0 a
Mean		81.4	8.3	74.9
CV (%)		0.8	22.6	15.7
SE		0.4	0.0	137.3

Means with the same letter down the column are not significantly different at 5% level according to SNK test.

Table 10. Radial mycelial growth (colony diameter), sporulation and wilt incidence (%) (on pigeonpea variety KAT 60/8) of *F. udum* isolates from various districts in Kenya

District	Number of isolates	Colony diameter (mm)	Sporulation (conidia/ml x 10^5)	Wilt incidence (%
Thika	6	86.7 a	5.2 e	61.7 d
Malindi	1	83.5 b	4.8 e	75.0 abc
Kirinyaga	3	83.3 b	7.4 bcd	60.0 d
Meru	7	83.2 b	6.7 cde	65.0 cd
Nyambene	8	82.2 c	8.5 abc	83.1 ab
Taita-Taveta	9	80.6 d	10.4 a	86.1 a
Makueni	10	80.4 de	10.3 a	71.5 bc
Kitui	5	80.4 de	10.0 a	80.0 ab
Nairobi	4	80.3 de	5.9 de	80.0 ab
Mbeere	7	80.0 de	9.3 ab	80.0 ab
Machakos	10	79.9 de	8.5 abc	72.5 bc
Tharaka-Nithi	5	79.6 e	5.3 e	75.0 abc
Mean		81.4	8.3	74.9
CV (%)		0.8	22.6	15.7
SE		0.4	0.0	137.3

Means with the same letter down the column are not significantly different at 5% level according to SNK test.

Table 11. Radial mycelial growth (colony diameter), sporulation and wilt incidence (%) (on pigeonpea variety KAT 60/8) of *F. udum* isolates from various AEZs in Kenya

AEZ	Number of isolates	Colony diameter (mm)	Sporulation (conidia/ml x 10^5)	Wilt incidence (%
L3	1	83.5 a	4.8 e	75.0 ab
UM4	15	83.1 a	8.4 c	72.0 ab
UM3	14	81.9 b	5.9 de	76.1 ab
LM4	10	81.6 b	8.3 c	70.5 b
LM5	26	80.6 c	9.0 bc	79.0 ab
LM3	3	80.6 c	7.5 cd	68.3 bc
LM6	3	79.8 d	11.9 a	60.0 c
L5	3	78.2 e	10.5 ab	83.3 a
Mean		81.4	8.3	74.9
CV (%)		0.8	22.6	15.7
SE		0.4	0.0	137.3

Means with the same letter down the column are not significantly different at 5% level according to SNK test.

Table 12. Radial mycelial growth (colony diameter), sporulation and wilt incidence (%) (on pigeonpea variety KAT 60/8) of *F. udum* isolates from various altitude ranges in Kenya

Altitude range (m.a.s.l)	Number of isolates	Colony diameter (mm)	Sporulation (conidia/ml x 10^5)	Wilt incidence (%
0-200	1	83.5 a	4.8 e	75.0 ab
601-800	3	78.2 e	10.5 a	83.3 a
801-1000	16	80.6 d	9.7 ab	79.4 a
1001-1200	23	81.3 c	8.0 bc	67.4 b
1201-1400	9	81.3 c	6.9 cd	79.4 a
1401-1600	19	82.9 b	8.4 bc	75.5 ab
1801-2000	4	80.3 d	5.9 de	80.0 a
Mean		81.4	8.3	74.9
CV (%)		0.8	22.6	15.7
SE		0.4	0.0	137.3

Means with the same letter down the column are not significantly different at 5% level according to SNK test.

The mean colony diameter was 81.4 mm with a range of 78.2 mm at 601-800 m.a.s.l to 83.5 mm at 0-200 m.a.s.l. Isolates at altitudes 801-1000 m.a.s.l (80.6 mm) and 1401-1600 m.a.s.l (82.9 mm), which had five or more isolates, showed significant difference ($P \leq 0.05$) in colony diameter while isolates at 1001-1200 m.a.s.l (81.3 mm) and 1201-1400 m.a.s.l (81.3 mm) showed no significant difference ($P \leq 0.05$). The composition of group 1 36.8%, 33.3%, 18.8% and 17.4% isolates at 1401-1600, 1201-1400, 801-1000 and 1001-1200 m.a.s.l, respectively (Tables 1 and 9). The composition of group 2 was 78.3%, 63.2%, 56.3% and 44.4% isolates at 1001-1200, 1401-1600, 801-1000 and 1201-1400 m.a.s.l, respectively. While the composition of group 3 was 25.0%, 22.2%, 4.3% and 0.0% isolates 801-1000, 1201-1400, 1001-1200 and 1401-1600 m.a.s.l, respectively.

In some cases isolates showed variation in radial mycelial growth within the same farm. For instance isolates TN01 and TN02 obtained from different infected plants from the same pigeonpea farm (farm number 47) in Tharaka-Nithi district district showed differences in colony diameter. This was the case with isolates KR02 and KR03 from farm number 76 in Kirinyaga district, and isolates NB03 and NB04 from farm number 84 in Nairobi district.

4.4.4 Sporulation

Sporulation was significantly different ($P \leq 0.05$) among *F. udum* isolates with a mean of 8.19×10^5 conidia/ml and a range of 1.4×10^5 conidia/ml (isolate KR02) to 24.6×10^5 conidia/ml (isolate KT02) (Table 9, Appendix 8.5.12). Based on the inter-quartile ranges of the conidial concentration the isolates were grouped into four types of sporulation namely very high sporulation with above 15.9×10^5 conidia/ml, high sporulation with $8.0\text{-}15.9 \times 10^5$ conidia/ml, moderate sporulation $5.0\text{-}7.9 \times 10^5$ conidia/ml and low sporulation with below 5.0×10^5 conidia/ml. Isolates with very high, high, moderate and low sporulation were designated as group 1, 2, 3 and 4, respectively. The composition of group 1, 2, 3 and 4 were 8 (10.1%), 23 (29.1%), 28 (35.4%) and 20 (25.3%) isolates, respectively. Isolates MAL01a and MAL01b from

Malawi fitted in group3, while isolates IND01a and IND01b from India fitted in group 3 and 2, respectively.

Sporulation of 75 isolates of *F. udum* from Kenya were significantly different ($P \leq 0.05$) among various districts with a mean of 8.3×10^5 conidia/ml and a range of 4.8×10^5 conidia/ml in Malindi district to 10.4×10^5 conidia/ml in Taita-Taveta district (Table 10, Appendix 8.5.13). Isolates from Taita-Taveta (10.4×10^5 conidia/ml), Makueni (10.3×10^5 conidia/ml), Kitui (10.0×10^5 conidia/ml), Nyambene (8.5×10^5 conidia/ml), Mbeere (9.3×10^5 conidia/ml) and Machakos (8.5×10^5 conidia/ml) districts, which had five or more isolates, did not show significant difference ($P \leq 0.05$) in sporulation. Isolates from Tharaka-Nithi (5.3×10^5 conidia/ml), Thika (5.2×10^5 conidia/ml) and Meru (6.7×10^5 conidia/ml) districts showed no significant difference ($P \leq 0.05$). Similarly isolates from Machakos, Meru and Nyambene districts showed no significant difference ($P \leq 0.05$). The composition of group 1 was 33.3% isolates in Taita-Taveta district, and 20.0% isolates in Kitui, Machakos and Makueni districts (Tables 1 and 9). The composition of group 2 was 71.4%, 60.0% and 40.0% isolates in Mbeere, Makueni and Machakos districts, respectively. The composition of group 3 was 72.4%, 50.0%, 40.0% and 33.3% isolates in Meru, Nyambene, Kitui and Thika districts, respectively. While the composition of group 4 was 60.0%, 50.0% and 30.0% isolates in Tharaka-Nithi, Thika and Machakos districts, respectively.

There were significant differences ($P \leq 0.05$) in sporulation among *F. udum* isolates from various AEZs in Kenya (Table 11, Appendix 8.5.14). The mean conidial concentration was 8.3×10^5 conidia/ml with a range of 4.8×10^5 conidia/ml for one isolate in L3 to 11.9×10^5 conidia/ml for isolates in LM6. Isolates from LM5 (9.0×10^5 conidia/ml), LM4 (8.3×10^5 conidia/ml) and UM4 (8.4×10^5 conidia/ml) zones, which had five or more isolates, showed no significant difference ($P \leq 0.05$) in sporulation but showed significant difference ($P \leq 0.05$) with isolates from UM3 (5.9×10^5 conidia/ml). The composition of group 1 was 20.0% isolates in LM4 zone and 11.5% from LM5 zone. The composition of group 2 was 50.0%, 26.7% and 20.0%

isolates in LM5, UM4 and LM4 zones, respectively (Tables 1 and 9). While the composition of group 3 57.1%, 40.0% and 30.0% isolates in UM3, UM4 and LM4 zones, respectively.

There were significant differences ($P \leq 0.05$) in sporulation among *F. udum* isolates from various altitude ranges in Kenya (Table 12, Appendix 8.5.15). The mean conidial concentration was 8.3 x 10^5 conidia/ml with a range of 4.8 x 10^5 conidia/ml at 0-200 m.a.s.l to 10.5 x 10^5 conidia/ml at 601-800 m.a.s.l. Isolates from altitudes 801-1000 m.a.s.l (9.7 x 10^5 conidia/ml), 1401-1600 m.a.s.l (8.4 x 10^5 conidia/ml) and 1001-1200 m.a.s.l (8.0 x 10^5 conidia/ml), which had five or more isolates, showed no significant difference ($P \leq 0.05$) in sporulation. Isolates from 1201-1400 m.a.s.l (6.9 x 10^5 conidia/ml) did not show significant difference ($P \leq 0.05$) with the isolates from 1001-1200 m.a.s.l and 1401-1600 m.a.s.l. The composition of group 1 was 18.8%, 10.5% and 8.7% isolates at 801-1000, 1401-1600 and 1001-1200 m.a.s.l, respectively (Tables 1 and 9). The composition of group 2 was 43.8% isolates at 801-1000 m.a.s.l, 39.1% isolates at 1001-1200 m.a.s.l and 33.3% isolates at 1201-1400 m.a.s.l. The composition of group 3 was 47.4%, 34.8% and 33.3% isolates at 1401-1600, 1001-1200 and 1201-1400 m.a.s.l, respectively. While the composition of group 4 was 33.3%, 26.3% and 25.0% isolates at 1201-1400, 1401-1600 and 801-1000 m.a.s.l, respectively.

In some cases isolates showed variation in sporulation within the same farm and even on the same plant. For instance isolates TN01 and TN02 obtained from different infected plants from the same pigeonpea farm (farm number 47) in Tharaka-Nithi district showed differences in sporulation. This was the case with isolates NY07 and NY08 from farm number 44 in Nyambene district, isolates TT04 and TT05 from farm number 58 in Taita-Taveta district, and isolates MS07, MS08 and MS09 from pigeonpea farm 17 in Machakos district. Isolates MS08 and MS09 obtained from the same farm (farm number 17) and from the same infected pigeonpea plant from Machakos district showed variation in sporulation.

4.4.5 Pigmentation

4.4.5.1 Mycelial pigments

Five groups of mycelial pigments were identified on *F. udum* isolates (Table 9, Appendix 8.2). The pigment groups include white, yellow (buff, vinaceous buff, pale luteous or luteous), purple (lilac, purple or dark purple), pink (rose or mauve) and light blue (flax blue or glaucous sky blue), and were designated as group 1, 2, 3, 4 and 5, respectively. The isolates in groups 1, 2, 3, 4 and 5 were 13 (16.5%), 19 (24.1%), 34 (43.0%), 6 (7.6%) and 7 (8.9%), respectively. Isolates from India had similar mycelial pigment and were placed in group 3. Isolates MAL01a and MAL01b from Malawi belonged to group 3 and group 2 mycelial pigments, respectively. Isolates from various districts in Kenya had varying mycelial pigments. Considering districts with five or more isolates, the composition of group 1 was 40.0% isolates in Kitui district, and 30.0% isolates in Machakos and Makueni districts (Tables 1 and 9). The composition of group 2 was 55.6%, 37.5%, 30.0%, 28.6% and 28.6% isolates in Taita-Taveta, Nyambene, Makueni, Mbeere and Meru districts, respectively. The composition of group 3 was 100.0%, 60.0%, 57.1%, 40.0% and 37.5% isolates in Thika, Machakos, Mbeere, Tharaka-Nithi and Nyambene districts, respectively. The composition of group 4 was 8.4% isolates in Meru district and 10.0% isolates in Makueni district, while the composition of group 5 was 42.9% isolates Meru district and 40.0% from Tharaka-Nithi district.

Isolates from various AEZs in Kenya varied in mycelial pigmentation. Considering AEZs with five or more isolates, the composition of group 1 was 28.6% and 20.0% isolates in UM3 and LM4 zones, respectively (Tables 1 and 9). The composition of group 2 was 38.5% isolates in LM5 zone and 28.6% isolates in UM3 zone, while the composition of group 3 was 73.3%, 40.0% and 30.8% isolates in UM4, LM4 and LM5 zones, respectively. The composition of group 4 and 5 was 8.4% and 10.0% isolates in UM3 and LM4 zones, respectively. Isolates from various altitudes also varied in mycelial pigmentaion. The composition of group 1 was 22.2% isolates at 1201-1400 m.a.s.l and 21.7% isolates at 1001-1200 m.a.s.l. The

composition of group 2 was 44.4% isolates at 1201-1400 m.a.s.l and 37.5% isolates at 801-1000 m.a.s.l, while the composition of group 3 was 68.4%, 37.5% and 30.4% isolates at 1401-1600, 801-1000 and 1001-1200 m.a.s.l, respectively (Tables 1 and 9). The composition of group 4 was 17.4% isolates at 1001-1200 m.a.s.l, while the composition of group 5 was 15.8% isolates at 1401-1600 m.a.s.l.

In some cases isolates showed variation in mycelial pigmentation within the same farm and even on the same plant. For instance isolates MB04 and MB05 obtained from different infected plants from the same pigeonpea farm (farm number 27) in Mbeere district showed differences in mycelial pigmentation. This was the case with isolates MR05 and MR06 from farm number 38 in Meru district, isolates TT04 and TT05 from farm number 58 in Taita-Taveta district, and isolates NB01 and NB02 from pigeonpea farm 83 in Nairobi district. Isolates MS08 and MS09 obtained from the same farm (farm number 17) and from the same infected pigeonpea plant from Machakos district showed variation in mycelial pigmentation. Isolates obtained from the same culture that showed differences in mycelia pigment were MAL01a and MAL01b from Malawi.

4.4.5.2 Substrate pigments

Three groups of substrate pigments were identified on *F. udum* isolates (Table 9, Appendix 8.2). The pigment groups include yellow (buff, vinaceous buff, pale luteous or luteous), purple (lilac, purple or dark purple) and pink (mauve), and were designated as group 1, 2 and 3, respectively. Groups 1, 2 and 3 comprised of 23 (29.1%), 55 (69.6%) and 1 (1.3%) isolates, respectively. Isolates from Malawi and India had similar mycelial pigment and were fitted in group 2. Isolates from various districts in Kenya had varying substrate pigments. Considering districts with five or more isolates, the composition of group 1 was 60.0%, 55.6%, 40.0%, 37.5% and 30.0% isolates in Kitui, Taita-Taveta, Makueni, Nyambene and Machakos ditricts, respectively (Tables 1 and 9). The composition of group 2 was 100.0% isolates in Mbeere, Meru, Tharaka-Nithi and Thika districts, and 62.5%, 60.0%, 60.0%, 44.4%

and 40.0% isolates in Nyambene, Makueni, Machakos, Taita-Taveta and Kitui districts, respectively. Group 3 had one isolate from Machakos district.

Isolates from various AEZs and altitudes in Kenya showed variation in substrate pigmentation. Considering AEZs with five or more isolates, the composition of group 1 was 40.0%, 38.5 and 35.7% isolates in LM4, LM5 and UM3 zones, respectively. The composition of group 2 was 86.7%, 64.3%, 60.0% and 57.7% isolates in UM4, UM3, LM4 and LM5 zones, respectively while group 3 was 3.8% isolates in LM5 zone (Tables 1 and 9). The composition of group 1 was 44.4% isolates at 1201-1400 m.a.s.l and 39.1% isolates at 1001-1200 m.a.s.l. While the composition of group 2 was 89.5%, 75.0%, 56.5% and 55.6% isolates at 1401-1600, 801-1000, 1001-1200 and 1201-1400 m.a.s.l, respectively (Tables 1 and 9).

In some cases isolates showed variation in substrate pigmentation within the same farm and even on the same plant. For instance isolates KR02 and KR03 obtained from different infected plants from the same pigeonpea farm (farm number 76) in Kirinyaga district showed differences in substrate pigmentation. This was the case with isolates TT04 and TT05 from farm number 58 in Taita-Taveta district, and isolates NB01 and NB02 from pigeonpea farm 83 in Nairobi district. Isolates MS08 and MS09 obtained from the same farm (farm number 17) and from the same infected pigeonpea plant from Machakos district showed variation in substrate pigmentation. Isolates obtained from the same culture that showed differences in substrate pigment were MAL01a and MAL01b from Malawi.

4.5 Conidial measurements of *F. udum* isolates

4.5.1 Hyphae

The mycelium of *F. udum* isolates was septate, hyaline, slender and highly branched hyphae (Plate 7). Conidiophores appeared from the hyphae and were either single or branched. Monophialides were produced at the tip of the conidiophores. Chlamydospores were produced on hyphae of 3 to 4 week-old cultures of all the

isolates and were terminally or intercalary, single or in chains, globose or oval in shape (Plates 7, 9 and 10).

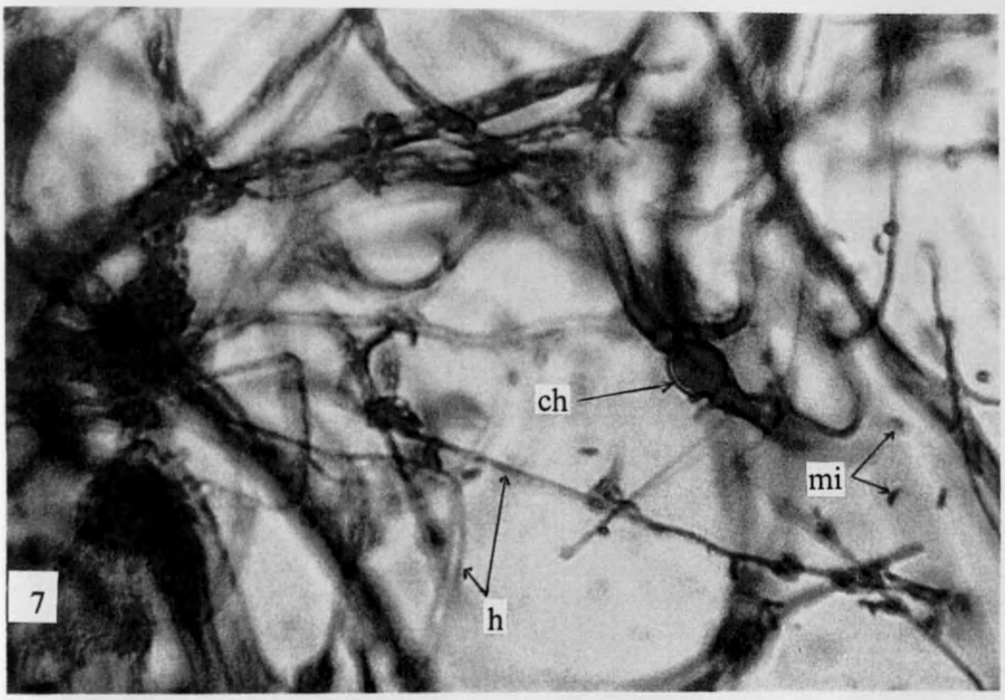
4.5.2 Conidia

All the 79 isolates of *F. udum* produced both microconidia and macroconidia with more of the former than the latter (Plate 8). Microcinidia were borne on microconidiophores. Microcinidia were thin-walled, hyaline, 0-1 septate (mostly 0 septate), straight to curved, ovoid to fusoid, scattered. Macroconidia were produced on macroconidiophores. Macroconidia were thin-walled, hyaline, falcate with a distinct foot cell, apical cell decreases in width towards the tip and with strongly curved or hooked apices. The macroconidia were 2-7 septate with mostly 3 septate, less frequently 4-5 septate, very rarely 7 septate.

F. udum isolates also varied in conidial length and width. The mean length and width of microconidia were 9.5 µm and 2.7 µm, respectively (Table 13). The range of microconidia was 3.4-18.7 µm x 1.7-4.2 µm. The means of the longest microconidia were 11.6 µm, 11.5 µm and 11.2 µm on isolates TT08, NY07 and TT09, respectively. The mean of the shortest microconidia was 6.5 µm as shown by isolate TT03. The means of the largest and the least width of microconidia were 3.2 µm and 2.3 µm as exhibited by isolates MR05 and NB03, respectively.

The mean length and width of macroconidia were 27.0 µm and 3.4 µm, respectively (Table 13). The range of macroconidia was 13.6-55.9 µm x 2.5-5.1 µm. The means of the longest macroconidia were 37.2 µm and 32.5 µm on isolates NY07 and MR01, respectively. The means of the shortest macroconidia were 23.1 µm, 23.2 µm and 23.5 µm on isolates NY06, TT03 and NY04, respectively. Isolates with the largest macroconidial width were MS02 and KR03 with 4.4 µm and 3.9 µm, respectively, while those with the least width included isolates NY02 and MS04 with 3.2 µm.

For the purpose of comparisons, and based on the inter-quartile ranges the lengths of macroconidia were used to categorize the isolates into three groups.



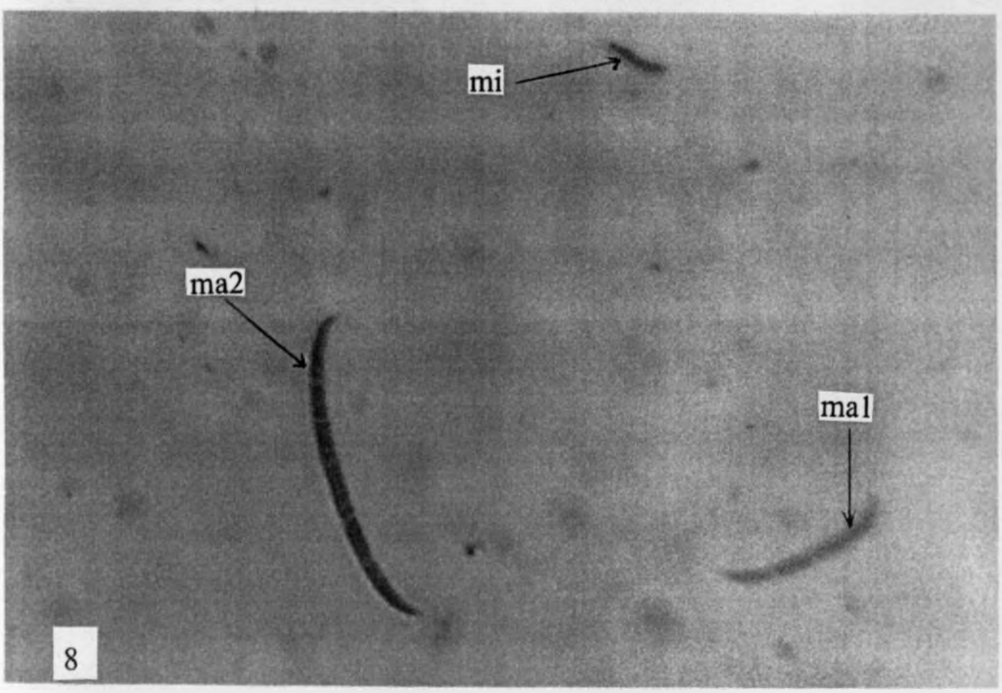
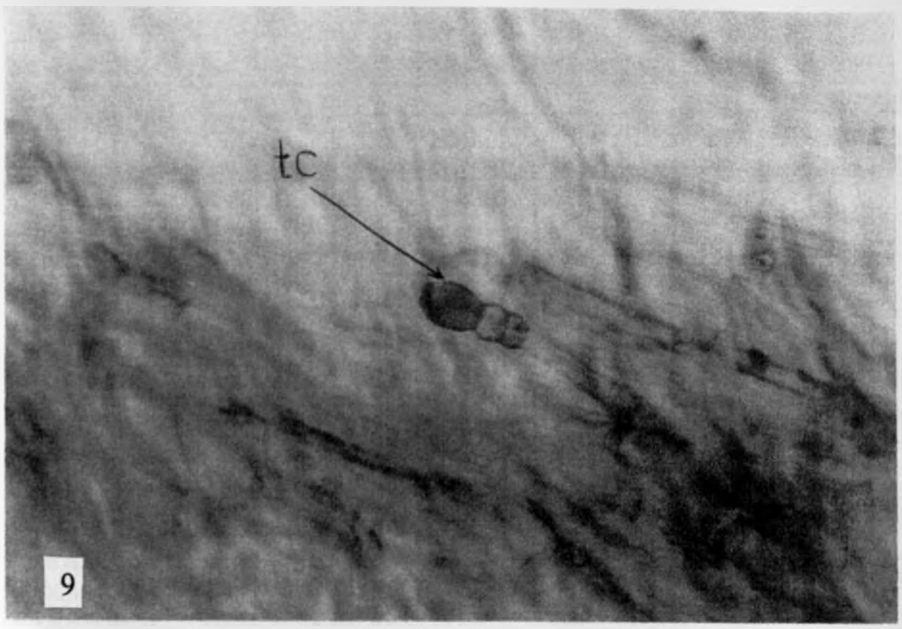


Plate 7. Light micrograph of mycelia of *F. udum* isolate MAL01a obtained from PDA culture three weeks after incubation at 25°C showing slender hyphae (h), microconidia (mi) and a single intercallery chlamydospore (ch) (x 500).
Plate 8. Light micrograph of conidia of *F. udum* isolate NY07 obtained from PDA culture ten days after incubation at 25°C showing microconidia (mi) with 1 septum, macroconidia (ma1) with 3 septa, and macroconidia (ma2) with 5 septa (x 1,000).



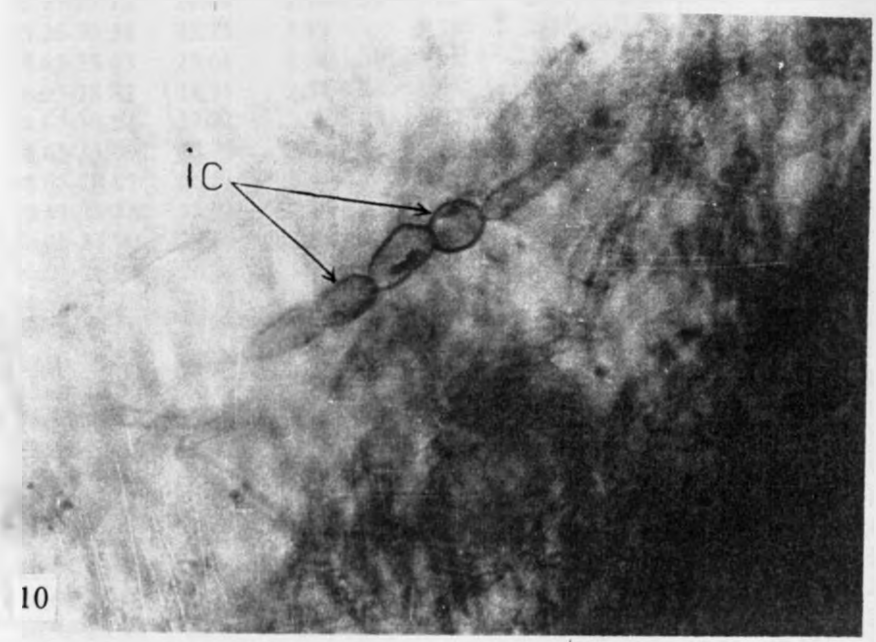


Plate 9. Light micrograph of *F. udum* isolate NB02 obtained from PDA culture three weeks after incubation at 25°C showing 3 terminal chlamydospores (tc) appearing in chain (x 600).

Plate 10. Light micrograph of *F. udum* isolate ML01 obtained from PDA culture three weeks after incubation at 25°C showing 4 intercallery chlamydopsres (ic) appearing in chain (x 600).

Table 13. Conidial measurements of 79 isolates of *F. udum* on PDA medium ten days after incubation at 25°C.

Isolate	Macroconidia					Microconidia			
	Length (µm)		Width (µm)		Septa	Length (µm)		Width (µm)	
	Range	Mean	Range	Mean		Range	Mean	Range	Mean
IND01a	20.34-45.77	29.06	3.39-5.09	3.56	2-4	6.78-18.65	9.17	1.70-3.39	2.49
IND01b	20.34-42.38	27.36	3.39-5.09	3.56	2-4	6.78-16.95	10.19	1.70-3.39	2.73
KR01	16.95-23.73	24.62	2.54-4.24	3.53	2-3	5.09-15.26	9.49	2.54-4.24	2.97
KR02	20.34-25.43	26.52	2.54-4.24	3.39	2-3	5.09-18.65	10.36	1.70-3.39	2.54
KR03	15.26-23.73	24.72	3.39-5.09	3.93	2-3	5.09-15.26	9.18	2.54-3.39	2.75
KT01	18.65-37.29	31.76	2.54-4.24	3.39	2-4	5.09-18.65	10.66	1.70-3.39	2.77
KT02	18.65-33.90	26.88	2.54-4.24	3.32	2-4	5.09-16.95	9.65	1.70-3.39	2.54
KT03	13.56-27.12	25.24	3.39-4.24	3.50	2-3	5.09-18.65	9.71	1.70-3.39	2.62
KT04	16.95-32.21	28.96	3.39	3.39	2-4	5.09-16.95	10.74	1.70-3.39	2.70
KT05	18.65-30.51	26.60	3.39	3.39	2-3	5.09-15.26	9.13	1.70-3.39	2.67
MAL01a	18.65-35.60	27.13	3.39-4.24	3.50	2-3	5.09-18.65	9.00	1.70-3.39	2.54
MAL01b	18.65-33.90	26.28	3.39-4.24	3.53	2-3	5.09-18.65	9.63	1.70-3.39	2.63
MB01	18.65-30.51	27.29	3.39	3.39	2-3	3.39-18.65	9.58	1.70-3.39	2.61
MB02	18.65-30.51	27.94	3.39	3.39	3	5.09-18.65	9.98	1.70-3.39	2.64
MB03	18.65-30.51	26.32	2.54-3.39	3.30	2-3	5.09-16.95	9.66	1.70-3.39	3.05
MB04	16.95-27.12	26.04	2.54-3.39	3.28	2-3	5.09-16.95	10.74	1.70-3.39	2.42
MB05	15.26-30.51	25.25	3.39	3.39	2-3	5.09-16.95	9.28	1.70-3.39	2.60
MB06	18.65-25.43	25.61	2.54-3.39	3.32	2-3	5.09-18.65	9.27	1.70-3.39	2.69
MB07	16.95-28.82	24.91	2.54-4.24	3.45	2-3	5.09-16.95	9.08	1.70-3.39	2.72
MK01	18.65-30.51	27.31	3.39-4.24	3.67	2-3	3.39-16.95	9.59	1.70-3.39	3.06
MK02	18.65-35.60	28.79	3.39	3.39	2-4	5.09-16.95	9.32	1.70-3.39	2.68
MK03	18.65-28.82	26.81	2.54-4.24	3.31	2-3	5.09-13.56	8.23	1.70-3.39	2.54
MK04	19.11-35.28	31.23	2.54-4.24	3.56	2-4	5.88-16.17	10.07	2.54-3.39	3.15
MK05	18.65-33.90	29.67	2.54-4.24	3.39	2-3	5.09-10.17	7.55	1.70-2.54	2.39
MK06	16.95-28.82	26.76	1.70-4.24	3.19	2-3	5.09-13.56	8.64	1.70-4.24	2.75
MK07	16.95-37.29	30.22	2.54-3.39	3.25	2-3	5.09-18.65	10.58	1.70-3.39	2.67
MK08	18.65-35.60	27.99	2.54-4.19	3.46	2-3	5.09-13.56	7.63	1.70-3.39	2.33
MK09	16.95-30.51	27.54	3.39	3.39	2-3	5.09-16.95	9.42	1.70-3.39	2.97
MK10	15.26-30.51	26.67	2.54-3.39	3.25	2-4	5.09-16.95	10.65	1.70-3.39	2.35
ML01	13.56-25.43	24.91	2.54-4.24	3.30	2-3	6.78-16.95	10.02	1.70-3.39	2.78
MR01	18.65-47.46	32.45	3.39-4.24	3.45	2-7	5.09-16.95	8.94	1.70-3.39	2.62
MR02	13.56-25.43	25.36	2.54-4.24	3.39	2-3	3.39-13.56	8.63	1.70-3.39	2.54
MR03	15.26-28.82	25.73	2.54-3.39	3.24	2-3	5.09-13.56	8.84	1.70-3.39	2.48
MR04	15.26-32.21	27.05	2.54-4.24	3.39	2-3	5.09-15.26	8.05	1.70-3.39	2.61
MR05	16.95-30.51	26.28	2.54-4.24	3.51	2-3	5.09-15.26	10.05	1.70-3.39	3.21
MR06	18.65-28.82	27.05	3.39	3.39	3	5.09-16.95	9.26	2.54-3.39	2.80
MR07	16.95-25.43	24.00	3.39	3.39	3	5.09-13.56	8.05	2.54-3.39	2.75
MS01	16.95-32.21	26.65	2.54-5.09	3.50	2-3	5.09-16.95	9.36	1.70-3.39	2.80
MS02	18.65-40.68	27.73	3.39-4.24	4.44	2-6	5.09-16.95	9.15	1.70-3.39	2.71
MS03	18.65-28.82	27.30	3.39-4.24	3.58	2-4	3.39-18.65	10.10	1.70-3.39	2.66
MS04	18.65-30.24	27.05	2.54-3.39	3.15	2-3	5.09-16.95	8.63	1.70-3.39	2.54
MS05	20.34-27.12	26.54	2.54-3.39	3.22	3	3.39-16.95	10.17	1.70-3.39	2.63
MS06	13.56-23.73	24.10	3.39	3.39	2-3	5.09-16.95	8.31	1.70-3.39	2.46
MS07	18.65-33.90	26.83	2.54-4.24	3.45	2-4	5.09-15.26	9.32	1.70-3.39	2.60
MS08	18.65-42.38	31.56	3.39-4.24	3.59	2-5	5.09-18.65	10.87	1.70-3.39	2.88
MS09	20.34-35.60	27.17	2.54-4.24	3.30	2-3	5.09-16.95	9.32	1.70-3.39	2.54
MS10	13.56-30.51	25.70	2.54-5.09	3.32	2-3	5.09-16.95	9.93	1.70-3.39	2.61
NB01	15.26-32.21	27.50	2.54-4.24	3.39	2-3	3.39-16.95	10.06	1.70-3.39	2.59
NB02	16.95-33.90	27.24	2.54-4.24	3.46	2-3	5.09-18.65	10.23	1.70-3.39	2.74
NB03	16.95-30.51	25.78	2.54-3.39	3.24	2-3	5.09-16.95	9.20	1.70-3.39	2.26
NB04	16.95-27.12	24.72	2.54-4.24	3.30	2-3	4.24-15.26	8.99	1.70-3.39	2.61

NY01	20.34-27.12	27.00	3.39-4.24	3.54	2-3	5.09-16.95	8.96	2.54-3.39	2.78
NY02	18.65-23.73	24.82	2.54-3.39	3.15	2-3	5.09-16.95	8.61	1.70-3.39	2.61
NY03	16.95-25.43	24.10	3.39	3.39	2-3	3.39-15.26	8.63	1.70-3.39	2.47
NY04	13.56-27.12	23.50	2.54-4.24	3.39	2-3	5.09-18.65	9.65	1.70-3.39	2.74
NY05	18.65-22.04	24.12	3.39	3.39	2-3	5.09-13.56	9.08	1.70-3.39	2.66
NY06	15.26-23.73	23.13	3.39-4.24	3.63	2-3	5.09-16.95	8.34	1.70-3.39	2.61
NY07	22.04-55.94	37.20	2.54-5.09	3.59	2-7	6.78-18.65	11.48	1.70-3.39	2.77
NY08	20.34-45.77	31.69	3.39-5.09	3.43	2-5	5.09-15.26	9.00	1.70-3.39	2.48
TK01	18.65-27.12	24.98	3.39-4.24	3.60	2-3	5.09-18.65	10.17	1.70-3.39	2.77
TK02	15.26-33.90	26.64	3.39-4.24	3.63	2-4	5.09-15.26	9.55	1.70-3.39	2.85
TK03	16.95-27.12	26.04	3.39-4.24	3.56	2-3	5.09-15.26	9.63	1.70-3.39	2.63
TK04	18.65-28.82	27.30	3.39-4.24	3.50	2-4	5.09-15.26	8.76	1.70-3.39	2.75
TK05	16.95-30.51	26.42	3.39-4.24	3.71	2-3	5.09-18.65	9.61	1.70-3.39	2.26
TK06	18.65-27.12	26.18	3.39-4.24	3.53	2-3	5.09-18.65	9.49	1.70-3.39	2.71
TN01	16.95-25.43	24.58	3.39	3.39	2-3	5.09-16.95	8.87	2.54-3.39	2.80
TN02	20.34-28.82	27.31	3.39	3.39	3	5.09-18.65	8.93	1.70-3.39	2.45
TN03	16.95-25.43	28.58	2.54-3.39	3.28	2-3	5.09-16.95	10.63	1.70-3.39	2.39
TN04	16.95-30.51	25.90	3.39-4.24	3.46	2-3	5.09-15.26	7.87	1.70-3.39	2.42
TN05	15.26-25.43	24.51	3.39	3.39	2-3	5.09-16.95	9.56	2.54-3.39	2.91
TT01	16.95-28.82	25.90	2.54-4.24	3.19	2-3	5.09-16.95	10.82	1.70-3.39	2.73
TT02	18.65-40.68	29.21	3.3-4.24	3.55	2-4	5.09-11.87	7.80	1.70-3.39	2.37
TT03	16.95-23.73	23.24	3.39	3.39	3	3.39-10.19	6.50	2.54	2.54
TT04	18.65-37.29	28.52	3.39-4.24	3.39	2-3	5.09-18.65	10.69	1.70-3.39	2.80
TT05	20.34-35.60	31.23	3.39-4.24	3.50	2-4	5.09-15.26	10.17	1.70-3.39	2.73
TT06	16.95-25.43	26.04	3.39-4.24	3.47	2-3	5.09-15.26	9.46	2.54-3.39	2.83
TT07	18.65-28.82	28.15	3.39	3.39	2-3	5.09-18.65	10.17	1.70-3.39	2.49
TT08	18.65-33.90	29.69	3.39-4.24	3.72	2-4	6.78-18.65	11.62	2.54-3.39	2.92
TT09	16.95-33.90	28.54	2.54-4.24	3.67	2-4	6.78-15.26	11.19	2.54-3.39	2.97
Mean		27.02		3.44			9.45		2.66
SD		2.36		0.18			0.94		0.19
CV		0.09		0.05			0.10		0.07
SE		0.27		0.02			0.11		0.02

Group 1 isolates had long macroconidia of 30.1 µm and above, group 2 isolates had medium macroconidia of 25.1 to 30.0 µm, and group 3 isolates had short macroconidia of 25.0 µm and below. The number of isolates in group 1, 2 and 3 were 8 (10.1%), 55 (69.6%) and 16 (20.3%), respectively (Table 13). Isolates from Malawi and India belonged to group 2.

There were variations in conidial length of isolates from various districts, AEZs and altitudes in Kenya (Tables 1 and 13). The composition of group 1 was 25.0% and 20.0% isolates in Nyambene and Makueni districts, respectively with absence in Mbeere, Thika and Tharaka-Nithi districts. The composition of group 2 was 85.7%, 83.3%, 80.0%, 80.0% and 80.0% isolates in Mbeere, Thika, Kitui, Makueni and Machakos districts, respectively. The composition of group 3 was 62.5% and 40.0% isolates in Nyambene and Tharaka-Nithi districts, respectively with total absence in Makueni and Kitui districts. The composition of group 1 was 20.0% and 15.4% isolates in LM4 and LM5 zones, respectively while group 2 composed of 73.1%, 66.7% and 64.3% isolates in LM5, UM4 and UM3 zones, respectively. The composition of group 3 was 30.0%, 28.6% and 26.7% isolates in LM4, UM3 and UM4 zones, respectively. The composition of group 1 was 21.1% and 13.0% isolates at 1401-1600and 1001-1200 m.a.s.l, respectively with absence at 1201-1400 m.a.s.l. The composition of group 2 was 75.0%, 69.6%, 63.2% and 55.6% isolates at 801-1000, 1001-1200, 1401-1600 and 1201-1400 m.a.s.l, respectively while group 3 composed of 44.4% isolates at 1201-1400 m.a.s.l.

In some cases isolates showed variation in macroconidial length within the same farm and even on the same plant. For instance isolates TN01 and TN02 obtained from different infected plants from the same pigeonpea farm (farm number 47) in Tharaka-Nithi district showed differences in macroconidial length. This was the case with isolates TT04 and TT05 from farm number 58 in Taita-Taveta district. Isolates MS08 and MS09 obtained from the same farm (farm number 17) and from the same infected pigeonpea plant from Machakos district showed variation in macroconidial length.

4.6 Virulence of *F. udum* isolates on wilt susceptible pigeonpea variety KAT 60/8

The wilt symptoms observed in the glasshouse included epinasty, interveinal yellowing of lower leaves after 8 to 10 days of root-dip inoculation, followed by drooping of the leaves. All the isolates initiated visible wilt symptoms on KAT 60/8 seedlings at 2 weeks after inoculation. *F. udum* was readily isolated onto PDA medium from pigeonpea seedlings that had wilt symptoms at 2 weeks after inoculation. The earliest symptoms were observed 8 days after inoculation of seedlings using isolates MB05, MR01, TT01 and MAL01a while seedlings inoculated with isolates TK05, KR01, MK08, TN05 and MB01 took 2 weeks for the appearance of first symptoms. Ultimately the leaves dried and defoliation occurred from 2 to 3 weeks after inoculation. Browning and sometimes blackening of the vascular tissue was also observed. Most infected plants had wilted and died six weeks of inoculation, while the control plants remained healthy throughout (Plates 11 and 12).

Pathogenicity results of 79 isolates of *F. udum* used to inoculate variety KAT 60/8 showed significantly differences ($P \leq 0.05$) in wilt incidence with a mean of 74.9% wilt (Table 9, Appendix 8.5.16). All the isolates were pathogenic to variety KAT 60/8 but they differed in their levels of virulence. The wilt incidence on variety KAT 60/8 ranged from 35% when inoculated with isolate TK05 to 100% when inoculated with isolates MR01, NY07, NY08, TT01, TT05 and TT06 (Table 9). The isolates were grouped into two pathogenic groups namely group 1 and 2. Group 1 were rated as highly virulent, and the isolates in this group induced 61-100% wilt on variety KAT 60/8 and constituted 62 (78.5%) isolates. Group 2 with of 17 (21.5%) isolates, were moderately virulent and induced 31-60% wilt on the same variety. Isolates MAL01a and MAL01b from Malawi, and IND01a and IND01b from India fitted in group 1.

There was significant difference ($P \leq 0.05$) of variation in virulence on variety KAT 60/8 among 75 isolates of *F. udum* from various districts in Kenya (Table 10,

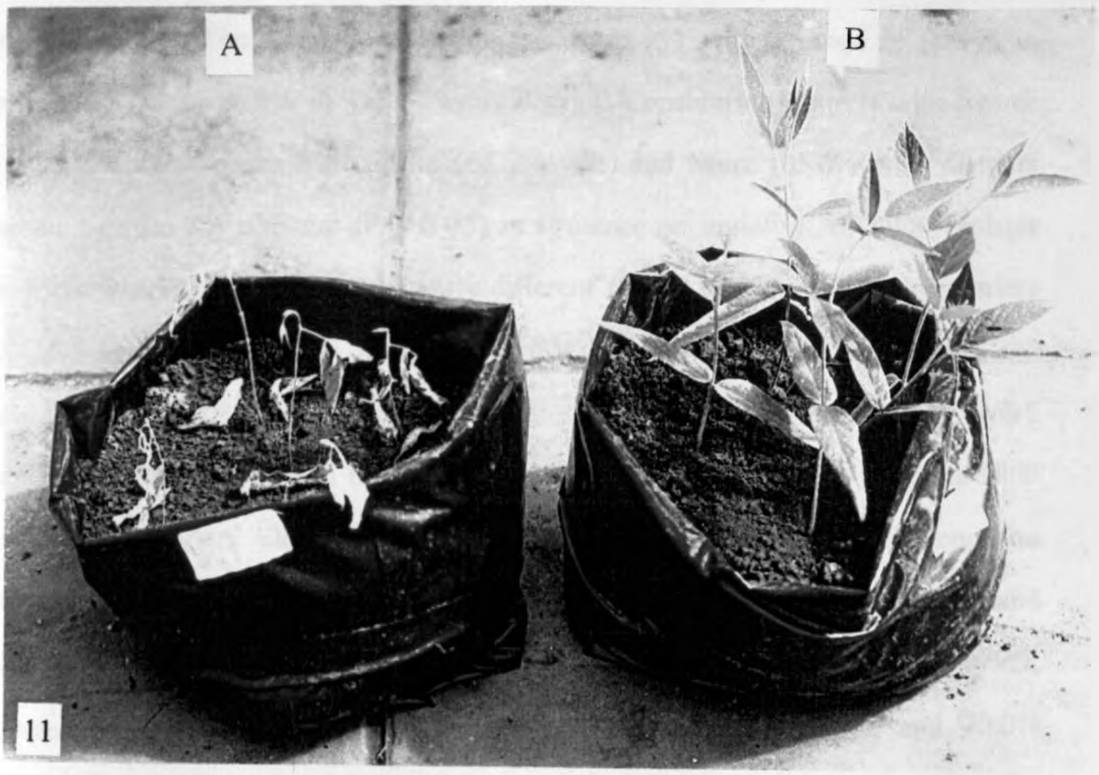


Plate 11. Reaction of wilt susceptible pigeonpea variety KAT 60/8 to *F. udum* isolate MS04 six weeks after root-dip inoculation showing inoculated pigeonpea plants (A) and control pigeonpea plants (B).

Plate 12. Reaction of wilt susceptible pigeonpea variety KAT 60/8 to isolates MAL01a (A), MS04 (B), TN05 (C) and control (D) twelve weeks after root-dip inoculation.

Appendix 8.5.17). The mean wilt incidence was 74.9% with a range of 60.0% in Kirinyaga district to 86.1% in Taita-Taveta district. Considering districts with five or more isolates, the isolates from Thika (61.7% wilt) and Meru (65.0% wilt) districts were not significantly different ($P \leq 0.05$) in virulence on variety KAT 60/8. Isolates from Meru district were not significantly different ($P \leq 0.05$) in virulence on variety KAT 60/8 with isolates from Makueni (71.5% wilt), Machakos (72.5% wilt) and Tharaka-Nithi (75.0% wilt) districts. Isolates from Taita-Taveta (86.1% wilt), Nyambene (83.1% wilt), Kitui (80.0% wilt), Mbeere (80.0% wilt), and Tharaka-Nithi (75.0% wilt) districts were not significantly different ($P \leq 0.05$) in virulence on variety KAT 60/8. The composition of group 1 was 100.0% isolates in Kitui and Nyambene districts, 88.9% and 85.7% isolates in Taita-Taveta and Mbeere districts, respectively, 80.0% isolates in Machakos and Tharaka-Nithi districts, and 70.0% isolates in Makueni district (Tables 1 and 9). The composition of group 2 was 57.1%, 50.0%, 30.0% and 20.0% isolates in Meru, Thika, Makueni and Machakos districts, respectively.

There was significant difference ($P \leq 0.05$) of variation in virulence on variety KAT 60/8 among 75 isolates of *F. udum* from various AEZs in Kenya (Table 11, Appendix 8.5.18). The mean wilt incidence was 74.9% with a range of 60.0% in LM6 zone to 83.3% in L5 zone. However, when the AEZs with five or more isolates were considered, there were no significant differences ($P \leq 0.05$) in virulence on variety KAT 60/8 of the isolates from LM5 (79.0% wilt), UM3 (76.1% wilt), UM4 (72.0% wilt) and LM4 (70.5% wilt) zones. The composition of group 1 was 80.8%, 80.0%, 78.6% and 70.0% isolates in LM5, UM4, UM3 and LM4 zones, respectively (Tables 1 and 9). The composition of group 2 was 30.0%, 21.4%, 20.0% and 19.2% isolates in LM4, UM3, UM4 and LM5 zones, respectively.

There was significant difference ($P \leq 0.05$) of variation in virulence on variety KAT 60/8 among 75 isolates of *F. udum* from various altitudes in Kenya (Table 12, Appendix 8.5.19). The mean wilt incidence was 74.9% with a range of 67.4% wilt at 1001-1200 m.a.s.l to 83.3% wilt at 601-800 m.a.s.l. When altitude ranges with five or

more isolates were considered, isolates at 801-1000 m.a.s.l (79.4% wilt), 1201-1400 m.a.s.l (79.4% wilt) and 1401-1600 m.a.s.l (75.5% wilt) were not significantly different ($P \leq 0.05$) in virulence on variety KAT 60/8. Isolates at 1401-1600 m.a.s.l were not also significantly different ($P \leq 0.05$) in virulence on variety KAT 60/8 from isolates at 1001-1200 m.a.s.l. The composition of group 1 was 100.0%, 75.0%, 78.9% and 60.9% isolates at 1201-1400, 1401-1600, 801-1000 and 1001-1200 m.a.s.l, respectively. The composition of group 2 was 39.1%, 25.0% and 21.1% isolates at 1001-1200, 801-1000 and 1401-1600 m.a.s.l, respectively.

In some cases isolates showed variation in virulence on variety KAT 60/8 within the same farm. For instance isolates MK08 and MK09 obtained from different infected plants from the same pigeonpea farm (farm number 11) in Makueni district showed differences in virulence on variety KAT 60/8. This was the case with isolates NY02 and NY03 from farm number 41 in Nyambene district, and TK05 and TK06 from farm number 79 in Thika district.

4.7 Physiological race typing of *F. udum* isolates

Twenty-one isolates of *F. udum* induced significantly different ($P \leq 0.05$) wilt incidences on pigeonpea varieties (Table 14, Appendix 8.5.20). The response of pigeonpea varieties to various isolates of *F. udum* was significantly different ($P \leq 0.05$). The various interactions among 7 pigeonpea varieties and 21 isolates of *F. udum* were significantly different ($P \leq 0.05$). The wilt incidence varied from 0 to 100% with a mean of 24.9%. Although variety-isolate interactions were significant ($P \leq 0.05$) for wilt incidence, their contribution to total variation was lower than that of varieties and isolates separately. The significant difference ($P \leq 0.05$) was much higher for varieties than for isolates, which meant that there was more variation among pigeonpea varieties (8.6 to 77.8% willt) than among *F. udum* isolates (16.2 to 34.3% wilt).

The pigeonpea varieties responded differentially to different *F. udum* isolates in some cases. The highest level of mean disease incidence was observed six weeks

Table 14. Wilt incidence (%) of 7 pigeonpea varieties inoculated with 21 isolates of *F. udum* six after root-dip inoculation

Isolate	Wilt incidence (%)[1] on pigeonpea varieties							Mean[3]	CV (%)	SE	LSD (5%)
	KAT 60/8[2]	C-11	ICPL 87105	ICEAP 00040	ICP 8858	ICP 9174	ICP 8863				
TT08	100.0	53.3	40.0	26.7	20.0	0.0	0.0	34.3 a	24.5	95.2	17.1
ML01	86.7	53.3	33.3	13.3	33.3	6.7	6.7	33.3 ab	41.4	190.5	24.2
KT05	80.0	40.0	26.7	26.7	13.3	6.7	20.0	30.5 abc	51.6	247.6	27.6
NB03	86.7	33.3	20.0	26.7	6.7	26.7	6.7	29.5 abcd	51.2	228.6	26.5
NB01	86.7	40.0	33.3	13.3	6.7	0.0	13.3	27.6 abcde	35.3	95.2	17.1
MAL01a	93.3	13.3	33.3	33.3	13.3	0.0	6.7	27.6 abcde	54.7	228.6	26.5
MS04	93.3	13.3	53.3	26.7	0.0	6.7	0.0	27.6 abcde	35.3	95.2	17.1
NY07	80.0	33.3	26.7	33.3	6.7	0.0	6.7	26.7 abcde	54.3	209.5	25.4
MB05	80.0	26.7	40.0	13.3	0.0	20.0	0.0	25.7 bcdef	38.0	95.2	17.1
MK02	86.7	26.7	33.3	13.3	0.0	6.7	6.7	24.8 cdef	61.1	228.6	26.5
MK10	100.0	13.3	13.3	13.3	20.0	6.7	6.7	24.8 cdef	49.9	152.4	21.6
NY02	86.7	26.7	13.3	20.0	6.7	13.3	6.7	24.8 cdef	52.9	171.4	22.9
TK02	53.3	46.7	13.3	6.7	6.7	13.3	26.7	23.8 cdefg	48.5	133.3	20.2
MS10	73.3	20.0	13.3	0.0	20.0	13.3	20.0	22.8 cdefg	57.3	171.4	22.9
MR04	66.7	13.3	26.7	6.7	13.3	13.3	13.3	21.9 defg	63.0	190.5	24.5
MR02	66.7	20.0	26.7	13.3	13.3	13.3	0.0	21.9 defg	74.6	266.7	28.6
TN05	53.3	33.3	20.0	13.3	0.0	26.7	6.7	21.9 defg	56.4	152.4	21.6
TN01	73.3	13.3	20.0	26.7	6.7	0.0	0.0	20.0 efg	43.6	76.2	15.3
TT02	60.0	6.7	20.0	6.7	0.0	20.0	13.3	18.1 efg	72.4	171.4	22.9
IND01b	66.7	13.3	13.3	6.7	13.3	6.7	6.7	18.1 efg	63.8	133.3	20.2
KR03	60.0	20.0	13.3	0.0	6.7	0.0	13.3	16.2 g	80.9	171.4	22.9
Mean[4]	77.8 a	26.7 b	25.4 b	16.2 c	9.8 d	9.5 d	8.6 d	24.9			
CV (%)	17.5	52.6	62.0	85.2	120.1	105.8	121.2	52.0			
SE	184.1	196.8	247.6	190.5	139.7	101.6	107.9	166.9			
LSD (5%)	22.4	23.1	25.9	22.7	19.5	16.6	17.1	7.9			

[1]Disease scale: resistant (R) = 0-10% wilt, moderately resistant (MR) = 11-20% wilt, moderately susceptible (MS) = 21-30% wilt, and susceptible (S) = 31-100% wilt (Reddy and Raju, 1997).

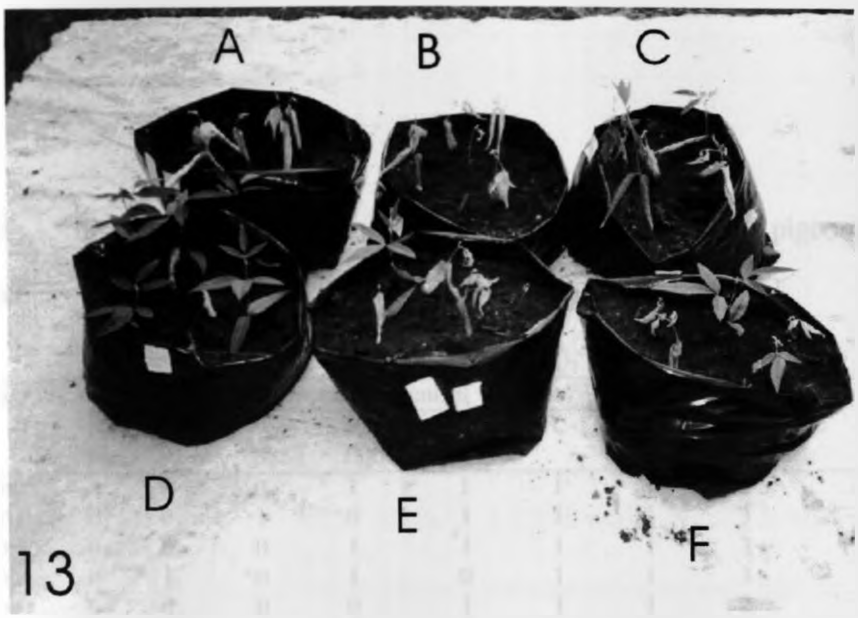
[2]Wilt susceptible variety as control.

[3]Means with the same letter down the column are not significantly different at 5% level using LSD test.

[4]Means with the same letter across the row are not significantly different at 5% level using LSD test.

after root-dip inoculation on the susceptible control KAT 60/8 at 77.8% wilt, followed by C-11 and ICPL 87105 at 26.7% and 25.4% wilt, respectively (Table 14, Plate 13). Pigeonpea varieties with least mean wilt incidence were ICP 8863, ICP 9174 and ICP 8858 with 8.6%, 9.5% and 9.8% wilt, respectively (Table 14, Plate 14). Pigeonpea varieties ICP 8863, ICP 9174 and ICP 8858 did not show consistent differences in their reaction against the 21 isolates tested. Varieties C-11 and ICPL 87105 showed a consistently different reaction (susceptibility) to the isolates followed by variety ICEAP 00040. Varieties C-11 and ICPL 87105 had resistant reaction (R or MR) to 10 isolates and had susceptible reaction (MS or S) to 11 isolates, with both having a maximum of 53.3% wilt incidence (Tables 14 and 15, Figure 7). The minimum wilt incidence on these varieties was 6.7% on C-11 and 13.3% on ICPL 87105. Variety ICEAP 00040 had resistant and susceptible reaction to 14 and 7 isolates, respectively (Table 15). The maximum and minimum wilt incidence on this variety was respectively 33.3% and 0.0%. Varieties ICP 8858, ICP 9174 and ICP 8863 showed resistance to 20, 19 and 20 isolates, respectively (Table 15). The maximum and minimum wilt incidence were 33.3% and 0.0%, and 26.7% and 0.0% on ICP 8858, and ICP 9174 and ICP 8863, respectively.

All *F. udum* isolates were virulent to wilt susceptible pigeonpea variety KAT 60/8 (control) but reacted differently with the varieties C-11, ICPL 87105, ICEAP 00040, ICP 8858, ICP 9174 and ICP 8863 (Tables 14 and 15). Isolates TT02, IND01b, KR03, MS10 and MK10 had low virulence (plants were resistant or moderately resistant) in 6 differential varieties. Isolates with low virulence in 5 differential varieties were MR02, MR04 and TN01, while isolates that showed high virulence to 5 differential varieties were TT08, ML01, KT05, NB03 and NY07.



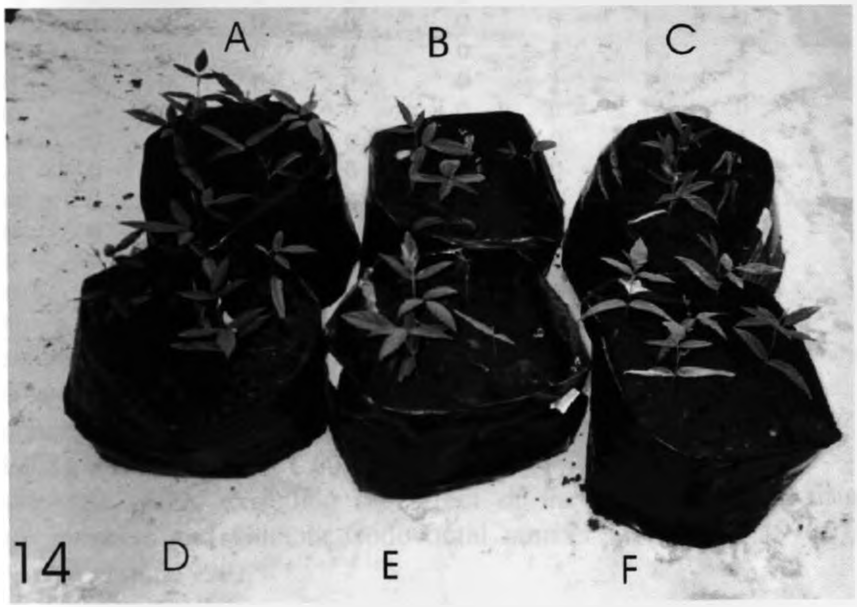


Plate 13. Reaction of wilt susceptible variety KAT 60/8 to *F. udum* isolates MAL01a (A), TK02 (B), NB03 (C), control (D), MB05 (E) and MS10 (F) six weeks after root-dip inoculation.

Plate 14. Reaction of wilt resistant variety ICP 9174 to *F. udum* isolates MAL01a (A), TN01 (B), MK10 (C), control (D), MB05 (E) and MS10 (F) six weeks after root-dip inoculation.

Table 15. Presence (1)[1] or absence (0) of *Fusarium* wilt among pigeonpea differential varieties and physiologic races of *F. udum*

Isolate	Pigeonpea differential varieties							Disease presence[3]	Physiologic race[4]	
	ICP 8863	ICP 9174	ICP 8858	ICEAP 00040	ICPL 87105	C-11	KAT 60/8[2]		Habgood (binary)	Gilmour (octal)
TT08	0	0	0	1	1	1	1	3	56	7
ML01	0	0	1	0	1	1	1	3	52	46
KT05	0	0	0	1	1	1	1	3	56	7
NB03	0	1	0	1	0	1	1	3	42	25
NB01	0	0	0	0	1	1	1	2	48	6
MAL01a	0	0	0	1	1	0	1	2	24	3
MS04	0	0	0	1	1	0	1	2	24	3
NY07	0	0	0	1	1	1	1	3	56	7
MB05	0	0	0	0	1	1	1	2	48	6
MK02	0	0	0	0	1	1	1	2	48	6
MK10	0	0	0	0	0	0	1	0	0	0
NY02	0	0	0	0	0	1	1	1	32	4
TK02	1	0	0	0	0	1	1	2	33	14
MS10	0	0	0	0	0	0	1	0	0	0
MR04	0	0	0	0	1	0	1	1	16	2
MR02	0	0	0	0	1	0	1	1	16	2
TN05	0	1	0	0	0	1	1	2	34	24
TN01	0	0	0	1	0	0	1	1	8	1
TT02	0	0	0	0	0	0	1	0	0	0
IND01b	0	0	0	0	0	0	1	0	0	0
KR03	0	0	0	0	0	0	1	0	0	0

[1]Presence of disease: 1 = 21-100% wilt; absence of disease: 0 = 0-20% wilt

[2]Wilt susceptible pigeonpea variety as control

[3]Number of pigeonpea differential varieties with wilt disease per *F. udum* isolate excluding the control (KAT 60/8)

[4]Physiologic races, excluding the effect of the control variety, using di-Habgood binary numbers and Gilmour-Code octal number (Habgood, 1970; Gilmour, 1973; Herrmann *et al.*, 1999).

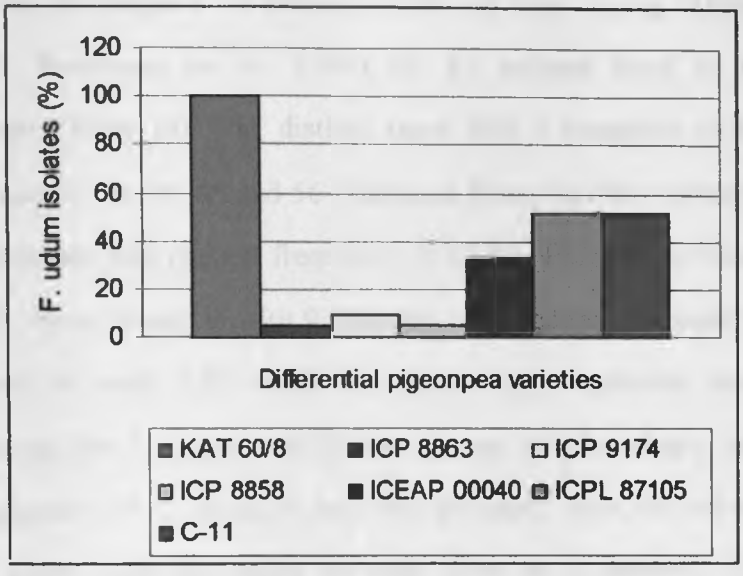


Figure 6. Bar chart showing the distribution of 21 isolates of *F. udum* causing susceptible reaction (>20% wilt) among seven pigeonpea differentials.

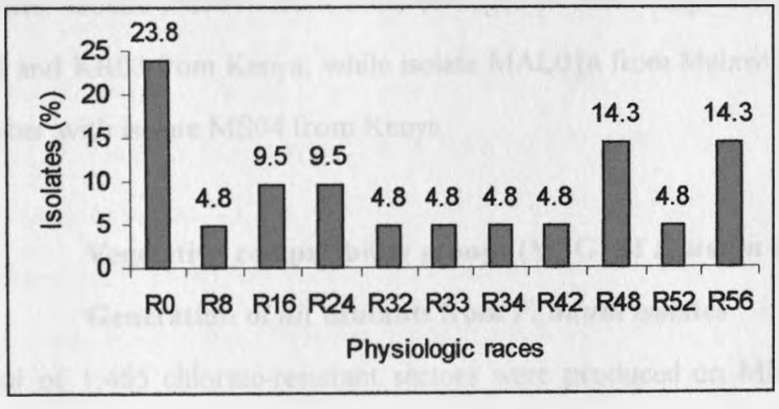


Figure 7. Bar chart showing the distribution of 21 isolates of *F. udum* among the physiologic races. Races were based on Habgood binary number method (Habgood, 1970). R = physiologic race.

Using the di-Habgood or Gilmour-Code of race typing (Habgood, 1970; Gilmour, 1973; Herrmann *et al.*, 1999) the 21 isolates fitted in 11 *F. udum* physiologic races (Table 15). The distinct races with a frequency of two or more isolates were races 0, 16, 24, 48 and 56 (Habgood binary number method) (Figure 7). Race 0 was dominant with percent frequency of 23.8%, followed by races 48 and 56 with 8.4% each, races 16 and 24 with 9.5% each, and the least dominant were races 8, 32, 33, 34 and 42 with 4.8% each. By performing a pairwise similarity (Dice coefficient) among the 11 races, two distinct groups of physiologic races could be identified (Appendix 8.9.1). Race 0 had 0% similarity with the other races. The remaining 10 races could be linked to each other at a similarity of over 65%. Therefore race 0 appeared to be independent while the other 10 races could be closely related. There was no dominant race of *F. udum* in the districts, AEZ or altitudes, although the isolates that were subjected to race typing were about a quarter of the total number available. The distinct races were found in L, LM and UM, and from low to high altitudes. Isolate IND01b from India belonged to race 0 together with MK10, MS10, TT02 and KR03 from Kenya, while isolate MAL01a from Malawi belonged to race 24 together with isolate MS04 from Kenya.

4.8 Vegetative compatibility groups (VCG) of *F. udum* isolates

4.8.1 Generation of *nit* mutants from *F. udum* isolates

A total of 1,465 chlorate-resistant sectors were produced on MMC from 79 single-spore isolates of *F. udum* with a mean of 18.5 sectors per isolate from three replicates (colonies) (Table 18, Plates 15 and 16). The isolates differed considerably in their sectoring frequency on MMC. Sectors produced per colony ranged from 1.2 sectors as observed in culture plate of isolate IND01a to 3.0 sectors for isolate TK06 with an overall mean of 1.9 sectors. Majority of the sectors appeared in the first and second weeks of incubation, and a few in the third week. The chlorate-resistant sectors were recovered at a mean frequency of between 0.14 and 0.68 sectors per colony on MMC, with an overall mean of 0.35 sectors per colony and 6.4 sectors per

isolate (Table 18). These chlorate resistant sectors were unable to utilize nitrate as the sole source of nitrogen and consequently grew as thin expansive colonies with no aerial mycelium on MM. These sectors were designated *nit* mutants.

4.8.2 Phenotypic classification of *nit* mutants

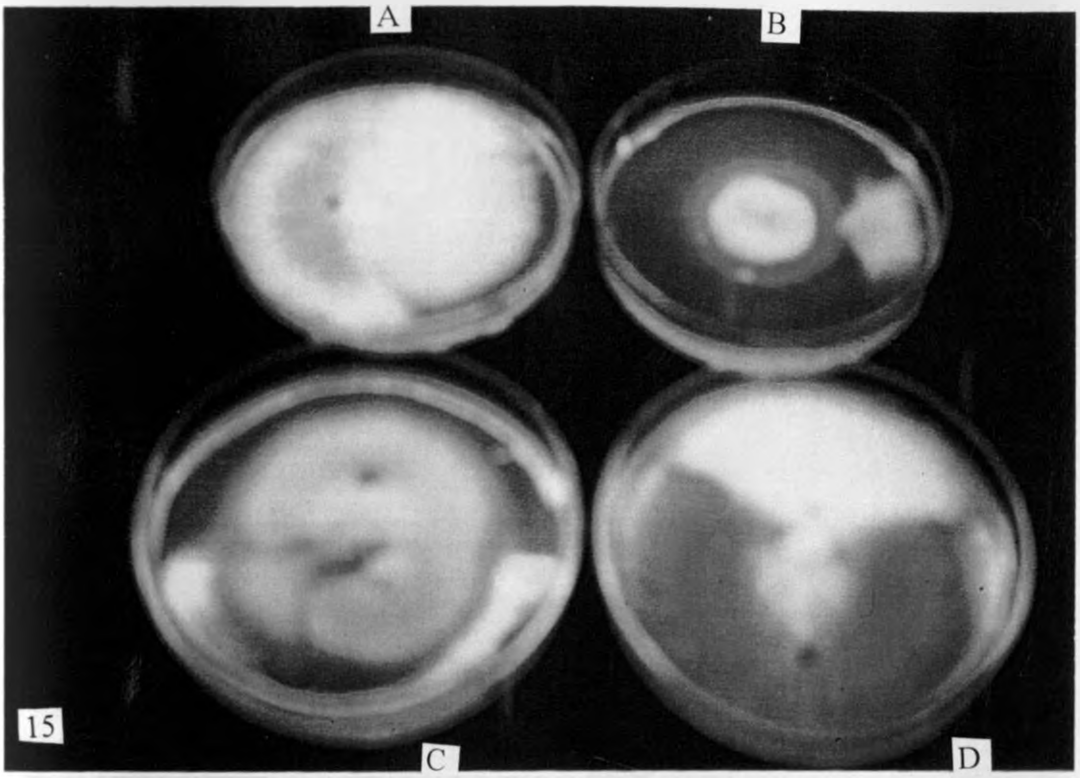
The *nit* mutants that did not utilize nitrate but utilized nitrite and hypoxanthine on the respective media as the sole source of nitrogen were designated as *nit*1; *nit* mutants that did not utilize nitrite were designated as *nit*3; and *nit* mutants that did not utilize hypoxanthine were designated as NitM (Table 16; Plate 17). All *nit* mutants utilized ammnonium and uric acid as sole sources of nitrogen in the media (Plate 18). The original isolates (wild-type) utilized nitrate, nitrite, ammonoium, hypoxanthine and uric acid as sole nitrogen sources in respective media. The *nit* mutants that utilized the various nitrogen sources had a wild-type growth on the respective media while a thin expansive growth with no aerial mycelium appeared for *nit* mutants that did not utilize the nitrogen sources in the media. The *nit* mutants showed a wild-type growth, typical of the original isolates, on PDA medium. Five hundred and five sectors were *nit* mutants, majority of which were *nit*1 constituting 399 (79.0%) of *nit* mutants with a mean of 5.1 per isolate (Table 18). Ninety-two (18.2%) of the *nit* mutants were *nit*3 with a mean of 1.2 per isolate while 14 (2.8%) were NitM having a mean of 0.2 per isolate. Forty (50.6%) isolates had only *nit*1, 1 isolate (1.3%) had *nit*3, 29 isolates (36.7%) had *nit*1 and *nit*3, 4 isolates (5.1%) had *nit*1 and NitM, 5 isolates (6.3%) had *nit*1, *nit*3 and NitM, with no isolate having only either NitM or *nit*3 and NitM.

Table 16. Identification of nitrate non-utilizing (*nit*) mutants from *F. udum* by growth on different nitrogen sources

Mutation[1]	Mutant designation	Growth on nitrogen sources[2]				
		Nitrate	Nitrite	Ammonium	Hypoxantine	Uric acid
None	Wild-type	+	+	+	+	+
Nitrate reductase structural locus	*nit*1	-	+	+	+	+
Pathway-specific regulatory locus	*nit*3	-	-	+	+	+
Molybdenum cofactor loci	NitM	-	+	+	-	+

[1]Adopted from Garret and Amy (1978) and Marzluf (1981) on the basis of analysis of mutants from *Aspergillus nidulans Neurospora crassa*, respectively; and as used by Correll *et al.* (1987) for *Fusarium oxysporum*.

[2]Growth on basal medium with various nitrogen sources; + = typical wild-type growth, - = thin growth with no aerial mycelium.



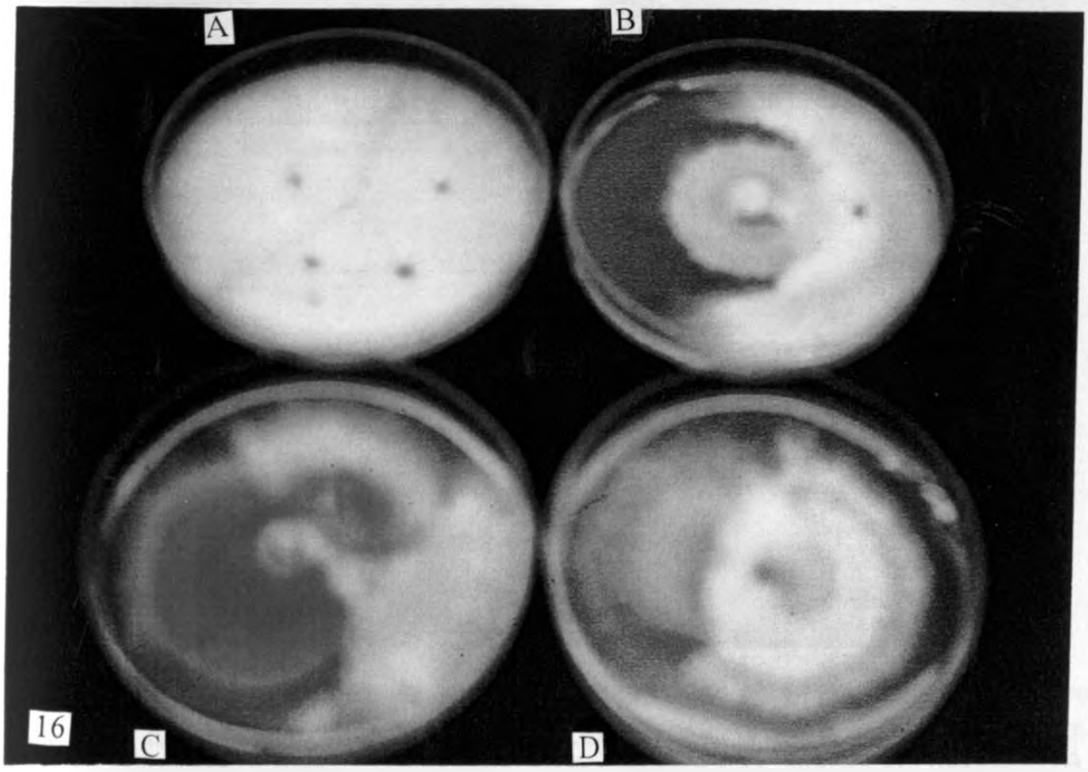
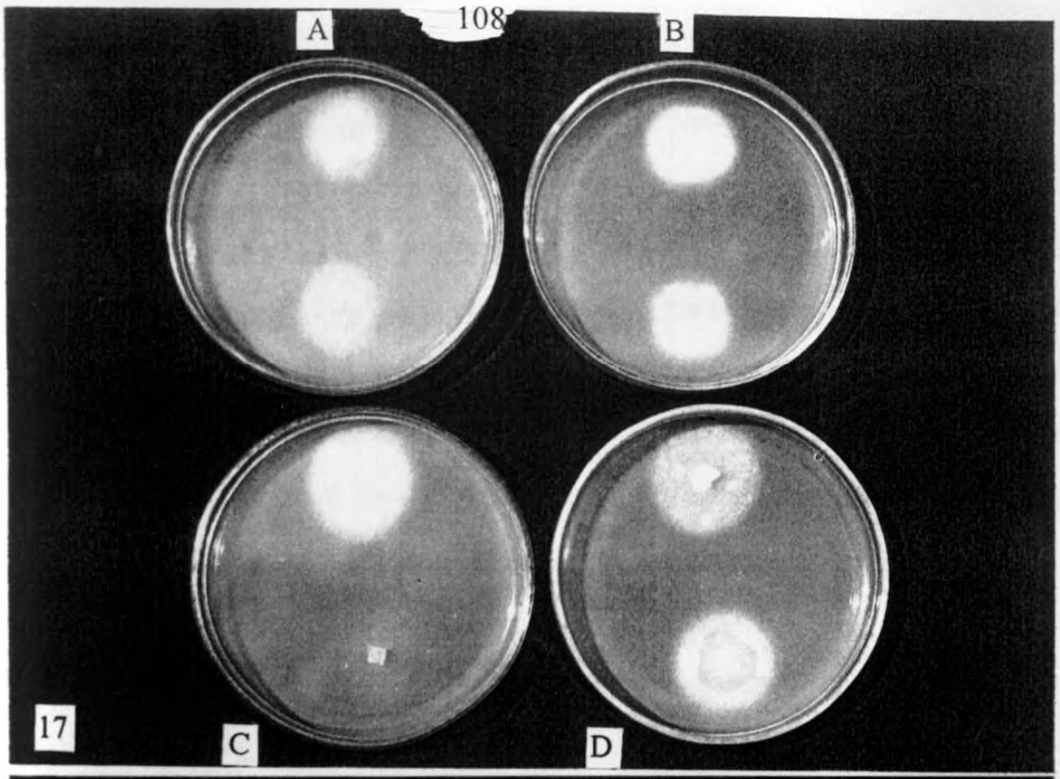


Plate 15. Variation in sectoring among *F. udum* isolates MK10, MK07, MK02 and MK04 on MMC medium three weeks after incubation at 25°C. Isolates MK10 (A), MK07 (B) and MK04 (D) have 2 sectors while isolate MK02 (C) has 3 sectors.
Plate 16. Variations in sectoring of *F. udum* isolate KT01 on MMC medium three weeks after incubation at 25°C. Plate A has 4 sectors; plates B and D have 2 sectors while plate C has 3 sectors.



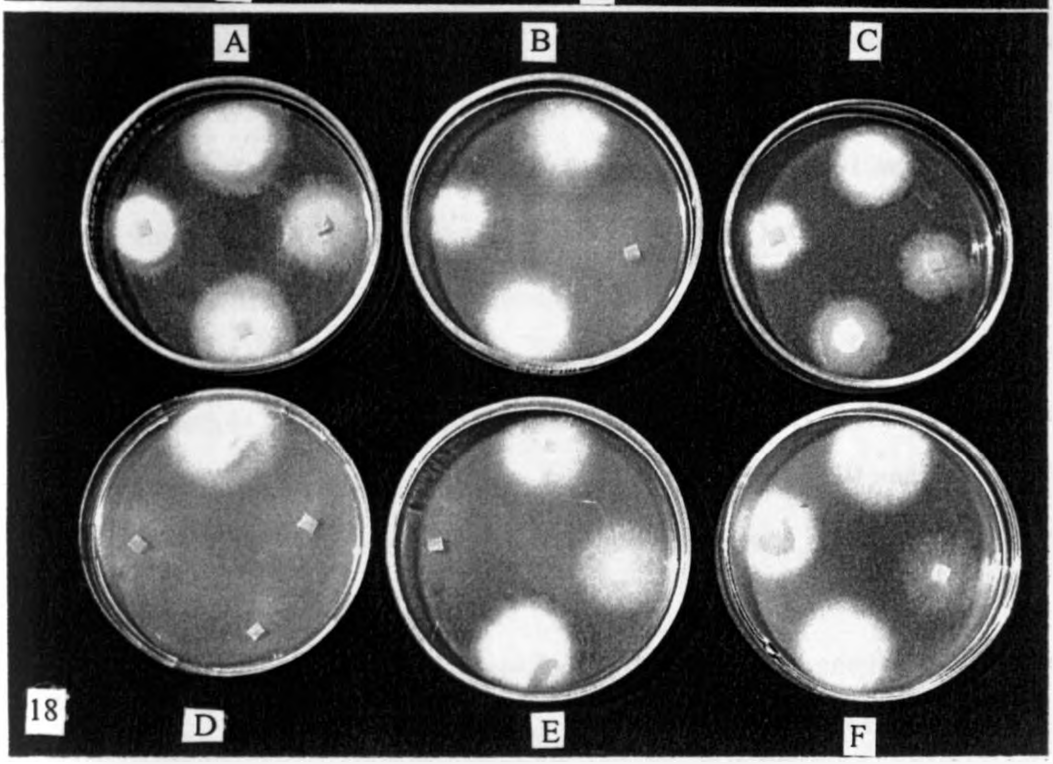


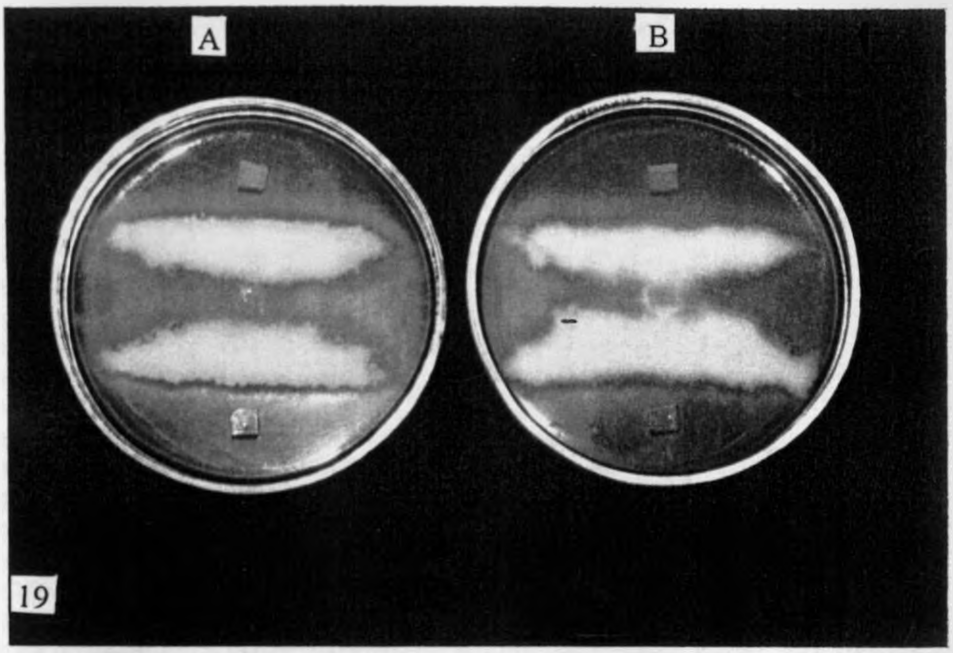
Plate 17. Phynotypic identification of *nit*1 and NitM of *F. udum* isolate MS05 five days after incubation at 25°C. The arrangement of colonies on each plate was: upper, *nit*1; lower, NitM. Nitrogen sources are: nitrite medium (A), ammonium medium (B), hypoxanthine medium (C) and uric acid medium (D).

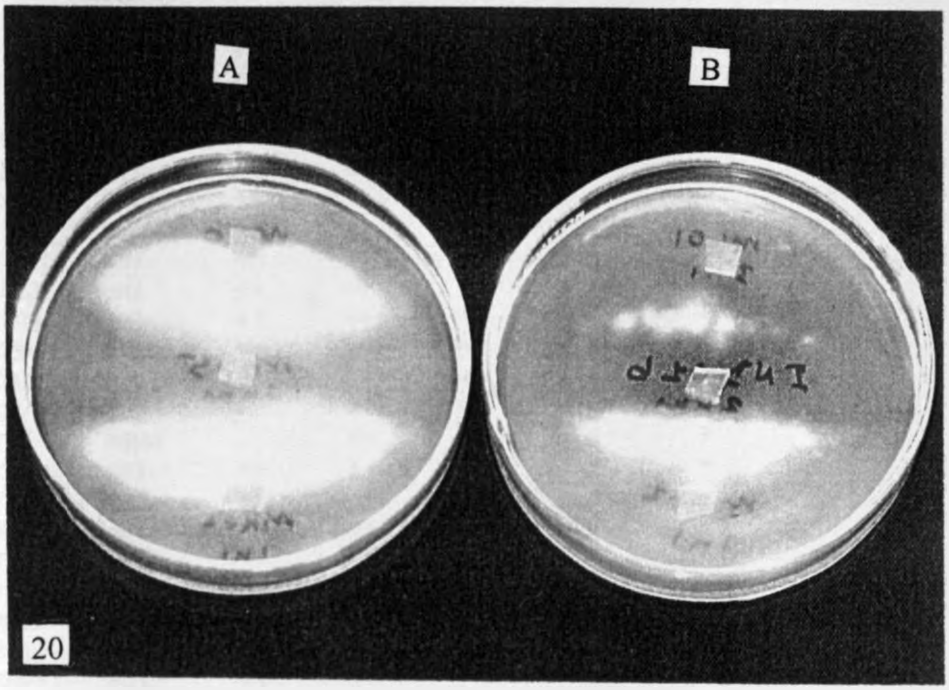
Plate 18. Growth of wild-type and *nit* mutants of isolate TK03 on six different media five days after incubation at 25°C. The arrangement of colonies on each plate was: upper, wild-type; lower, *nit*1; left, NitM; right, *nit*3. The media are: PDA medium (A), nitrite medium (B), ammonium medium (C), minimal medium (D), hypoxanthine medium (E) and uric acid medium (F).

4.8.3 Complementation tests between *nit* mutants

Phenotypically similar and complementary *nit* mutants of the same isolate were tested for vegetative compatibility reaction. Of the 38 isolates (48.1%) with complementary *nit* mutants, 10 isolates (28%) were found to be self-incompatible, although not all possible combinations were tested because few isolates produced NitM mutants. NitM mutants were generated from only 9 isolates out of 79, while 40 isolates had only *nit*1 mutant (Table 18). All the isolates were tested for vegetative compatibility reaction using similar *nit* mutant phenotypes of *nit*1 or *nit*3 or NitM from the same isolate and non formed heterokaryosis where the *nit* mutants converge. Vegetative compatibility reaction was a more robust wild-type with dense aerial mycelial growth observed between *nit*1 and NitM phenotypes of different isolates than that between *nit*3 and NitM. Compatibility reaction between *nit*1 and *nit*3 mutants of different isolates was moderate to weak. A total of 1,248 vegetative compatibility reactions between 8 NitM tester mutants and *nit*1 and/or *nit*3 mutants of different *F. udum* isolates were performed (Table 17). One to two *nit*1 and/or *nit*3 were used for complementation reaction per isolate. The number of isolates with strong wild-type growth (++) were 411 (32.9%), those with moderate to weak wild-type growth (+) were 607 (48 6%), those with no reaction (-) were 209 (16.8%), while those with uncertain reaction (+-) were 21 (1.7%) (Plates 19 and 20). Wild-type or positive reaction (++ and/or +) was therefore observed in 1,018 (81.6%) reactions (Table 17).

All the isolates formed heterokaryons with at least one tester and all could be linked to the other testers (Table 17). Twenty four (30%) isolates formed heterokaryons with between 2 and 7 NitM testers while 55 (70%) isolates formed heterokaryons with all the 8 NitM testers. Isolate MR05 developed heterokaryosis with testers IND01a/1 and TK03/1, and could easily be linked to other isolates such as MR06 that developed heterokaryosis with all the 8 testers. Isolate NB01 developed heterokaryosis with testers MS05/1, MR06/1 and TK03/1, and could easily be linked to other isolates such as NB03 that developed heterokaryosis with all the 8 testers





Plates 19 and 20. Complementation tests among *nit*1 mutants of some isolates of *Fusarium udum* and NitM mutants of different isolates on minimal medium ten days after incubation at 25°C.

Plate 19. (A) A strong complementation between *nit*1 of TT09 (centre block) with NitMs of IND01a (upper block) and IND01b (lower block); (B) A strong complementation between *nit*1 of KR02 (centre block) with NitMs of IND01a (upper block) and IND01b (lower block).

Plate 20. (A) A strong complementation between *nit*1 of ML01 (upper block) and *nit*1 of MR02 (lower block) with NitM of MS05 (centre block); (B) A weak complementation between *nit*1 of ML01 (upper block) with NitM of IND01b (centre block) and a moderate complementation between *nit*1 of MR02 (lower block) with NitM of IND01b (centre block).

Table 17. Complementation tests[1] between *nit* mutants of *F. udum* isolates

Isolate/ mutant	Mutant phenotype	NitM Testers							
		MS05/1	IND-01a/1	MB01/3	KR03/1	TT04/1	MR06/1	MS07/4	TK03/1
MK01/1	*nit*1	++	++	++	++	+	+	+	-
MK01/4	*nit*1	++	++	++	++	+	++	+	+
MK02/2	*nit*1	++	++	++	++	+	+	+	-
MK02/6	*nit*1	++	++	++	++	++	+	+	+-
MK03/3	*nit*1	++	++	++	++	++	+	+	+-
MK03/4	*nit*1	++	++	++	++	++	++	+	+
MK04/2	*nit*3	+	+	+	+	+	-	-	+
MK04/7	*nit*1	+	++	+	++	+	+	+	+
MK05/1	*nit*1	++	++	++	++	++	+	-	-
MK05/8	*nit*3	++	++	++	++	+	++	-	+
MK06/1	*nit*1	++	++	++	++	++	++	+	-
MK06/2	*nit*1	++	++	++	++	-	+	-	-
MK07/2	*nit*1	++	++	++	++	+	++	-	+
MK07/3	*nit*1	++	++	++	++	-	+	-	-
MK07/4	*nit*3	++	+	+	+	-	-	+	-
MK08/4	*nit*1	+	++	+	+	+	+	-	+
MK08/8	*nit*1	++	++	++	++	+	-	-	-
MK09/1	*nit*1	++	++	++	+	-	+	-	+
MK09/2	*nit*1	++	++	+	++	-	+-	-	+
MK10/3	*nit*1	+	++	+	+	+	+	+	+
MK10/4	*nit*1	++	++	++	+	+	+	+	+
MS01/1	*nit*1	++	+	++	+	+	++	+	-
MS01/3	*nit*1	++	++	++	++	+	+	+	+
MS02/1	*nit*1	+	++	++	++	+	+	+	-
MS02/2	*nit*1	++	++	++	++	+	+	+	+
MS03/3	*nit*3	+	++	+	+	-	+	+	-
MS03/4	*nit*1	++	++	++	++	+	+	-	-
MS04/2	*nit*1	++	++	+	-	-	+	+	-
MS04/7	*nit*3	-	-	+	-	-	-	-	-
MS05/1	NitM	-	++	++	+	+	++	-	++
MS05/2	*nit*1	++	+	+	+	+	+	+	+
MS05/3	*nit*1	++	++	+	+	-	+	+	-
MS06/1	*nit*1	++	++	++	+	+-	+	+	+-
MS06/2	*nit*1	++	++	++	+	-	++	+	-
MS07/1	*nit*1	+	+	+	-	+	+	+	+
MS07/3	*nit*3	++	++	++	++	+	+	++	+
MS07/4	NitM	-	-	-	-	-	-	-	-
MS08/1	*nit*1	++	++	-	++	+	+	+	-
MS08/2	*nit*3	++	++	++	++	+	+	+	-
MS09/1	*nit*1	++	++	++	++	+	+	+	+-
MS10/1	*nit*1	++	++	++	++	+	+	+	+
MS10/2	*nit*1	++	++	++	++	++	++	+	+
KT01/2	*nit*3	++	+	++	+	+	++	+	+
KT01/12	*nit*1	++	++	++	+	+	+	+	+
KT01/16	*nit*1	+	+	+	+	+	+	+	-
KT02/5	*nit*1	++	++	++	++	++	+	+	+
KT02/6	*nit*1	++	++	++	+	+	++	+	+
KT03/1	*nit*1	++	+	+	+	+	-	+	+
KT04/1	*nit*3	+	+	++	-	-	+	+	-
KT04/5	*nit*1	+	++	+	+	+	+	+	+
KT05/1	*nit*3	++	-	+	-	-	+	+	-
KT05/2	*nit*1	++	++	++	++	++	+	+	-
MB01/2	*nit*1	++	++	++	+	+	+-	-	+

MB01/3	NitM	++	+	+	+	+	+	-	-
MB02/1	*nit*1	+	+	-	-	-	-	+	-
MB02/2	*nit*1	++	++	++	+	+	+	-	-
MB03/1	*nit*1	+	++	++	++	++	++	+	+
MB03/2	*nit*1	+	++	+	+	+	+	+	+
MB04/1	*nit*1	+	++	++	+	+	+	+	+
MB05/1	*nit*1	++	++	+	+	+	+-	+	+-
MB06/2	*nit*3	-	-	+	-	-	+	-	+
MB06/3	*nit*1	++	++	+	+	+	-	+	+
MB07/1	*nit*1	++	++	-	-	-	-	+	+
MB07/2	*nit*1	++	++	++	+	+	+	+	+
MR01/2	*nit*1	++	++	++	+	+	+	+	+
MR01/3	*nit*1	++	++	-	-	-	-	-	-
MR02/1	*nit*1	++	++	++	++	+	-	+	+
MR02/2	*nit*1	++	++	++	+	++	+	+	+
MR03/3	*nit*1	++	++	++	+	++	+	+	+
MR03/4	*nit*1	+	++	+	+	+	-	+	+
MR04/1	*nit*3	+	+	+	-	-	-	-	-
MR04/2	*nit*1	++	+	++	++	+	+	-	-
MR05/1	*nit*1	+-	+	+-	-	-	+-	-	+
MR06/1	NitM	++	++	-	-	-	-	-	-
MR06/4	*nit*1	++	+	++	+	+	+	+	+
MR06/5	*nit*1	++	+	++	++	+	+	+	+
MR07/1	*nit*1	+	+	++	+	+	+	-	+
MR07/3	*nit*1	-	+	-	-	-	-	+	-
NY01/1	*nit*1	++	+	++	++	+	+	+	+
NY01/2	*nit*1	++	+	+	+	+	+	+	+
NY02/2	*nit*1	++	++	++	+	+	++	+	+
NY02/3	*nit*3	+	-	+	-	-	+	-	+
NY03/1	*nit*1	++	++	++	+	+	++	+	+
NY04/1	*nit*3	+	-	+	+	-	-	+-	+
NY04/3	*nit*1	+	++	++	+	+	+	+	+
NY05/1	*nit*1	++	++	++	+	+	++	+	+
NY05/4	*nit*1	++	+	+	+	+	+	+	+
NY06/2	*nit*3	-	+	+	-	+	+	-	-
NY06/5	*nit*1	+	++	+	+	+	+	+	+
NY07/1	*nit*1	++	++	+	+	+	+	+	+
NY07/2	*nit*3	++	++	++	++	+	+	+	+
NY08/1	*nit*1	++	++	++	++	++	++	+	+-
TN01/1	*nit*1	+	+	+	-	+	+-	-	-
TN02/1	*nit*1	+	-	+	-	-	+	-	-
TN02/2	*nit*1	+	+	+	+	+	+	+	+
TN03/1	*nit*3	+	+	+	+	+	-	+	+
TN03/4	*nit*1	+	++	+	+	+	+	-	-
TN04/2	*nit*1	++	++	+	++	++	+	+	+
TN04/6	*nit*1	++	++	++	+	+	+	+	-
TN05/3	*nit*1	++	++	+	++	++	+	-	+
TN05/8	*nit*1	+	++	++	+	+	+	+-	-
TT01/2	*nit*1	++	++	++	++	++	+	+	+
TT01/4	*nit*3	+	+	+	+	-	-	+	-
TT02/1	*nit*1	++	++	+	+	++	-	+	+
TT02/2	*nit*1	-	++	++	+	+	+	-	-
TT03/1	*nit*1	+	++	+	++	++	+	+	+
TT04/1	NitM	+	+	+	+	-	-	-	-
TT04/2	*nit*1	++	++	++	++	++	+	-	-
TT04/3	*nit*1	++	++	++	++	+	+	+	+
TT05/1	*nit*1	++	++	++	++	+	+	+	+
TT05/4	*nit*1	++	++	++	+	+	+	+	+
TT06/1	*nit*1	++	++	++	++	+	+	+	+
TT06/2	*nit*1	++	++	++	+	+	+	+	+

TT07/1	*nit*1	++	++	+	++	+	+	-	+
TT07/2	*nit*1	++	++	+	+	+	+	+	+
TT08/2	*nit*1	++	+	+	+	+	+	+	+
TT08/3	*nit*1	++	++	-	++	-	-	-	+
TT09/1	*nit*1	++	++	++	++	++	++	+	+
TT09/2	*nit*1	++	++	++	++	++	++	+	+
ML01/1	*nit*1	++	++	++	++	+	-	+	+
ML01/3	*nit*1	++	++	+	+	+	+	+	+
KR01/1	*nit*1	++	++	++	++	+	+	+	+
KR01/2	*nit*1	++	++	++	+	+	+	+	+
KR02/3	*nit*1	++	++	++	++	++	+	+	+
KR02/4	*nit*1	++	++	++	++	++	++	+	+
KR03/1	NitM	+	+	-	-	-	-	-	-
KR03/4	*nit*1	++	++	+	++	+	+	+	-
KR03/7	*nit*1	++	++	++	++	+	+	+	+
TK01/1	*nit*1	++	++	++	++	+	++	+	+
TK01/2	*nit*1	++	++	+	+	+	+	+	+
TK02/1	*nit*1	++	+	++	+	-	+	-	+
TK03/1	NitM	++	++	-	-	-	-	-	-
TK03/2	*nit*3	+	-	+	-	+	-	+	+
TK03/6	*nit*1	++	++	++	+	+	+	+	+
TK04/2	*nit*1	+	++	+	+	+	+	+	+
TK04/7	*nit*3	++	++	++	+	+	+	+	+
TK05/1	*nit*1	+	++	+	+	+	+	+	+
TK05/2	*nit*1	++	++	++	++	+	-	-	-
TK06/1	*nit*3	+	++	+	-	+	+	+	+
TK06/6	*nit*1	++	++	+	++	+	-	+	+
NB01/1	*nit*1	+	+-	+-	+-	+-	+	+-	+
NB02/2	*nit*1	+	-	+	-	-	-	-	-
NB02/3	*nit*1	+	+	+	+	-	+	+	+
NB03/1	*nit*1	+	++	++	++	+	+	-	+
NB03/2	*nit*1	++	++	++	+	+	+	+	+
NB04/2	*nit*1	+	+	++	+	+	+	+	+
NB04/3	*nit*1	+	++	++	++	+	+	-	-
MAL01a/2	*nit*1	++	++	++	++	+	+	+	+
MAL01b/1	*nit*3	++	++	++	++	++	++	-	+
MAL01b/2	*nit*3	+	+	++	+	+	+	-	+
IND01a/1	NitM	++	-	+	+	+	++	-	+
IND01a/4	*nit*1	++	++	++	++	+	+	+	+
IND01a/5	*nit*1	+	+	+	+	+	-	+	-
IND01b/2	NitM	+	+	+	-	+	+	-	+
IND01b/4	*nit*1	++	++	+	++	+	+	+	+
IND01b/6	*nit*1	-	++	+	+	+	-	+	-
Heterokaryons									
++		104	111	86	62	24	22	1	1
+		43	34	58	69	98	99	106	100
-		8	10	10	24	32	30	46	49
+-		1	1	2	1	2	5	3	6
Total		156	156	156	156	156	156	156	156

[1]Complementation tests = types of heterokaryons where the two mutants converge

++ = Wild-type growth of aerial mycelium after 4-7 days (strong reaction)

+ = Wild-type growth of aerial mycelium after 8-20 days (moderate to weak reaction)

- = No growth of aerial mycelium after 20 days (no reaction)

+- = Uncertain

Table 18. Sectors on MMC medium, *nit* mutants, heterokaryons and vegetative compatibility (% VC) of *F. udum* isolates

Isolate	Sectors per colony	Total *nit* mutants	*Nit* mutant phenotypes			Heterokaryons[1]					% VC[2]
			*nit*1	*nit*3	NitM	++	+	-	+-	Total	
MK01	1.8	7	7	0	0	9	6	1	0	16	94
MK02	2.1	8	6	2	0	9	5	1	1	16	87
MK03	1.8	7	7	0	0	11	4	0	1	16	94
MK04	2.5	7	6	1	0	2	12	2	0	16	87
MK05	2.4	10	6	4	0	10	3	3	0	16	81
MK06	1.8	7	7	0	0	10	2	4	0	16	75
MK07	1.4	7	4	3	0	10	7	7	0	24	71
MK08	1.3	2	2	0	0	5	7	4	0	16	75
MK09	1.8	7	6	1	0	6	5	4	1	16	69
MK10	2.1	7	5	2	0	4	12	0	0	16	100
MS01	2.0	5	5	0	0	7	8	1	0	16	94
MS02	1.8	5	5	0	0	7	8	1	0	16	94
MS03	2.6	11	8	3	0	5	7	4	0	16	75
MS04	2.6	11	4	7	0	2	4	10	0	16	47
MS05	1.4	5	4	0	1	7	13	4	0	24	83
MS06	1.3	3	3	0	0	7	5	2	2	16	75
MS07	2.0	6	3	2	1	5	10	9	0	24	62
MS08	1.9	8	4	4	0	7	6	3	0	16	81
MS09	1.4	2	2	0	0	4	3	0	1	8	87
MS10	1.8	4	4	0	0	10	6	0	0	16	100
KT01	2.7	18	12	6	0	11	12	1	0	24	96
KT02	2.3	10	10	0	0	9	7	0	0	16	100
KT03	1.8	6	6	0	0	1	6	1	0	8	87
KT04	2.4	10	8	2	0	2	11	3	0	16	81
KT05	1.4	3	2	1	0	6	5	5	0	16	69
MB01	2.1	6	3	0	3	4	8	3	1	16	75
MB02	1.8	4	4	0	0	3	6	7	0	16	56
MB03	1.9	5	3	2	0	6	10	0	0	16	100
MB04	1.7	4	4	0	0	2	6	0	0	8	100
MB05	1.9	4	4	0	0	2	4	0	2	8	75
MB06	1.9	13	9	4	0	2	8	6	0	16	62
MB07	1.7	7	7	0	0	5	7	4	0	16	75
MR01	2.1	7	4	3	0	5	5	6	0	16	62
MR02	1.4	3	3	0	0	8	7	1	0	16	94
MR03	2.7	6	6	0	0	5	10	1	0	16	94
MR04	2.1	9	8	1	0	3	6	7	0	16	56
MR05	1.3	2	2	0	0	0	2	3	3	8	25
MR06	1.7	8	4	2	2	7	11	6	0	24	75
MR07	1.8	5	5	0	0	1	8	7	0	16	56
NY01	1.3	5	5	0	0	4	12	0	0	16	100
NY02	1.4	3	0	3	0	4	8	4	0	16	75
NY03	1.4	2	2	0	0	4	4	0	0	8	100
NY04	2.3	5	3	2	0	2	10	3	1	16	75
NY05	1.7	6	6	0	0	5	11	0	0	16	100
NY06	1.8	9	7	2	0	1	11	4	0	16	75
NY07	1.3	7	5	2	0	6	10	0	0	16	100
NY08	1.4	2	2	0	0	6	1	0	1	8	87
TN01	1.7	3	3	0	0	0	4	3	1	8	50
TN02	1.3	3	3	0	0	0	11	5	0	16	69
TN03	2.6	10	7	3	0	1	12	3	0	16	81
TN04	2.5	9	7	2	0	7	8	1	0	16	94

TN05	2.9	15	12	3	0	6	7	2	1	16	81
TT01	1.7	6	5	1	0	5	8	3	0	16	81
TT02	1.8	9	7	2	0	5	7	4	0	16	75
TT03	1.8	4	4	0	0	3	5	0	0	8	100
TT04	1.8	5	4	0	1	9	9	6	0	24	75
TT05	2.0	10	7	3	0	7	9	0	0	16	100
TT06	1.4	4	4	0	0	7	9	0	0	16	100
TT07	1.7	5	5	0	0	5	10	1	0	16	94
TT08	1.4	4	4	0	0	4	8	4	0	16	75
TT09	1.9	6	6	0	0	12	4	0	0	16	100
ML01	1.2	4	4	0	0	6	9	1	0	16	94
KR01	1.8	5	5	0	0	7	9	0	0	16	100
KR02	1.4	5	5	0	0	11	5	0	0	16	100
KR03	2.6	12	6	5	1	7	10	7	0	24	71
TK01	1.8	4	4	0	0	7	9	0	0	16	100
TK02	1.7	3	3	0	0	2	4	2	0	8	75
TK03	2.1	9	6	2	1	5	10	9	0	24	62
TK04	2.1	9	6	3	0	4	12	0	0	16	100
TK05	2.1	5	5	0	0	5	8	3	0	16	81
TK06	3.0	14	9	5	0	4	10	2	0	16	87
NB01	1.4	2	2	0	0	0	3	0	5	8	37
NB02	2.1	8	8	0	0	0	9	7	0	16	56
NB03	1.7	4	4	0	0	6	9	1	0	16	94
NB04	1.4	4	4	0	0	4	10	2	0	16	87
MAL01a	1.7	5	5	0	0	4	4	0	0	8	100
MAL01b	2.3	5	2	3	0	7	7	2	0	16	87
IND01a	1.2	8	6	0	2	6	14	4	0	24	83
IND01b	1.3	8	5	1	2	4	15	5	0	24	79
Total	(1465)	505	399	92	14	411	607	209	21	1248	
Mean	1.9	6.4	5.1	1.2	0.2	5.2	7.7	2.7	0.3	15.8	80.5
%		34.5	79.0	18.2	2.8	32.9	48.6	16.8	1.7		

[1]Heterokaryons: ++ = Wild-type growth of aerial mycelium after 4-7 days
+ = Wild-type growth of aerial mycelium after 8-20 days
- = No growth of aerial mycelium after 20 days
+- = Uncertain

[2]%VC: Percent (%) vegetative compatibility/heterokaryosis: ++ or + reactions

(Table 17). Isolate MS04 developed heterokaryosis with testers MS05/1, IND01a/1, MB01/3, MR06/1 and MS07/4, and could easily be linked to other isolates such as MS05 that developed heterokaryosis with all the 8 testers. Using similar criterion, the 24 isolates that did not develop heterokaryons with all NitM testers were linked to the other 55 isolates that showed wild-type mycelial growth with all testers. Using this criterion, the *F. udum* isolates from Kenya, and one strain each from Malawi and India were grouped into one vegetative compatibility group, VCG 1.

Differences in complementation reaction were observed between NitM tester mutants and *nit*1 or *nit*3 mutants of *F. udum* isolates. NitM tester MS05/1 had 94% wild-type growth (++ or +) with *nit*1 and/or *nit*3 or NitM of the isolates, the others being 93% for IND01a/1, 92% for MB01/3, 84% for KR03/1, 78% for TT04/1 and MR06/1, 69% for MS07/4 and 65% for TK03/1 (Table 17). Strong reactions that formed heterokaryons readily in 4-7 days were observed between the *nit* mutants of the isolates and NitM testers MS05/1, IND01a/1 and MB01/3, while moderate reactions that formed heterokaryons mainly in 8-14 days were observed between *nit* mutants of isolates and NitM testers KR03/1, TT04/1 and MR06/1. Weak reactions formed heterokaryons mainly in 12-20 days between *nit* mutants of the isolates and NitM testers MS07/4 and TK03/1. Seventy-six (96%) isolates had 50% and above wild-type growth with the NitM testers while 3 (4%) isolates (MS04, MR05 and NB01) had below 50% (Table 18).

F. udum isolates from various districts, AEZs and altitudes in Kenya exhibited variation in vegetative compatibility (heterokaryosis). Considering districts with five or more isolates, vegetative compatibility (heterokaryosis) was 89.0%, 88.9%, 86.6%, 84.2%, 79.8%, 77.6%, 75.0%, 74.3% and 66.0% among *F. udum* isolates in Nyambene, Taita-Taveta, Kitui, Thika, Machakos, Mbeere, Tharaka-Nithi, Makueni and Meru districts, respectively (Tables 1 and 18). Vegetative compatibility among isolates from various AEZs with five or more isolates was 87.0%, 84.1%, 83.2% and 71.8% in LM4, UM4, LM5 and UM3 zones, respectively (Tables 1 and 18). Vegetative compatibility of isolates from various altitude ranges with five or more

isolates was 85.4%, 79.3%, 78.0% and 77.6% at 1401-1600, 1201-1400, 1001-1200 and 801-1000 m a.s.l, respectively (Tables 1 and 18).

Differences among isolates from the same site (farm) or same pigeonpea plant or same strain in vegetative compatibility were low. However, isolates obtained from the same farm with a difference of ≥25% heterokaryosis included MR05 (25%) and MR06 (75%) from farm number 38 in Meru district, KR02 (100%) and KR03 (71%) from farm number 76 in Kirinyaga district, and NY05 (100%) and NY06 (75%) from farm number 43 in Nyambene district (Tables 1, 17 and 18).

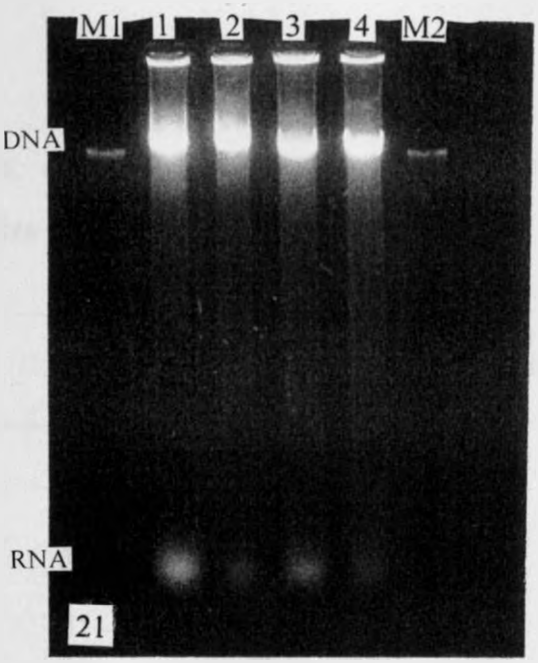
4.9 Amplified fragment length polymorphism (AFLP) of *F. udum* isolates

4.9.1 Extraction of fungal DNA

The amounts of mycelia of *F. udum* harvested from 200 ml of Czapek Dox plus AZ liquid medium (CDAZ) ranged from 5 to 12 g. The 2.5 g mycelial powder used for the DNA extraction using CTAB method yielded between 40 and 200 ng DNA/µl with most of the isolates yielding 80 to 120 ng DNA/µl. The use of undigested λ *Hind* III DNA having 200 ng DNA/µl provided a good estimate of the concentration of DNA present in the DNA samples of the isolates (Plate 21). The method of CTAB was however slightly modified due to high amounts of RNA present in the DNA samples (Plate 22). The method of DNA extraction was modified by using higher concentration of RNase A to digest RNA, and by performing phenol:chloroform:isoamyl alcohol and a second chloroform: isoamyl alcohol extractions.

4.9.1 AFLP analysis

A total of 326 bands were amplified from seven primer combinations with 121 being polymorphic (Table 19). Polymorphism was 37% with an average of 17 polymorphic bands per primer combination. Polymorphism was highest among isolates with primer combination *Eco*R I (E)+TA/*Mse* I (M)+CAT (67%), followed



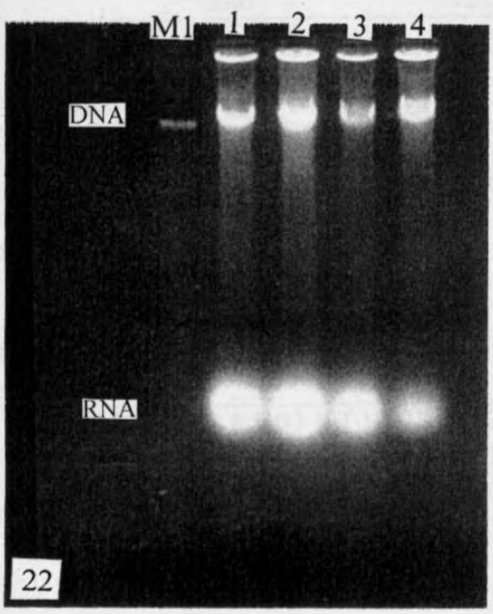


Plate 21. Estimation of the amount of DNA extracted by comparing the intensity of undigested λ *Hind III* DNA containing 200 ng DNA/µl (M1) or 100 ng DNA/µl (M2) with the intensities of DNA of four *F. udum* samples. The DNA samples were from isolates MK10 (1), MS01 (2), NB02 (3) and TT01 (4) extracted using CTAB method and RNA digested by 5 µl of 20 mg/ml RNase A.

Plate 22. Four DNA samples from different *F. udum* isolates showing large quantities of RNA before digestion with 10 µl of 20 mg/ml RNase A. The DNA samples were from isolates MB07 (1), TN01 (2), KT03 (3) and MK05 (4).

Table 19. Primers, amplified bands and polymorphic bands obtained by AFLP analysis of 56 isolates of *F. udum*

Primer combination (*Eco*R I/*Mse* I)	Number of isolates with bands	Amplified bands	Polymorphic bands	Polymorphism (%)
TA/CAT	47	30	20	67
AA/CAG	40	72	28	39
AG/CAA	47	46	17	37
AG/CAG	44	45	11	24
AC/CAG	47	55	15	27
TG/CAC	45	50	22	44
AG/CAC	44	28	8	29
Total		326	121	37

Table 20. AFLP groups of *F. udum* isolates obtained using dendrograms of two to three primer combinations

AFLP group	No. of isolates	*F. udum* isolates
I	40	KR01 KR02 KR03 KT01 KT02 KT03 MAL01a MB01 MB03 MB04 MB07 MK01 MK02 MK03 MK05 MK08 MK10 MR05 MS01 MS03 MS06 MS07 MS08 NB01 NB03 NB04 NY01 NY02 NY04 NY07 NY08 TK02 TK03 TK05 TN03 TN04 TN05 TT01 TT02 TT06
II	9	MR03 MR04 MS09 NY03 TT05 NB02 TK04 MS04 TT08
III	7	MB05 MR02 MR06 MR07 MS05 MS10 TK06

by E+TG/M+CAC (44%), E+AA/M+CAG (39%) and E+AG/M+CAA (37%). Lower polymorphism was observed among isolates with primer combinations E+AG/M+CAC (29%), E+AC/M+CAG (27%) and E+AG/M+CAG (24%). Typical banding patterns are shown in Figures 8 and 9.

The dendrograms (trees) obtained from binary data of each primer combination by UPGMA method showed a consistent cluster pattern, although the clusters ranged from 4 to 6. Dendrograms from combined binary data were generated for two primer combinations (Figure 10) and three primer combinations (Figures 11 and 12) with almost similar clustering pattern as observed for individual primer combinations. In Figure 10, cluster 1 isolates were similar at 94.9%, cluster 2 at 91.1% and cluster 4 at 87.3%. In Figure 11, cluster 1 isolates were similar at 95.7% and cluster 2 at 95.1%; while in Figure 12, cluster 1 isolates were similar at 97.1%. The remainder of the clusters in the three dendrograms was represented by single isolates. Dendrograms that were obtained from 2 to 3 primer combinations were used to classify the 56 *F. udum* isolates into three AFLP groups, namely group I, II and III (Table 20, Figures 10, 11 and 12, other dendrograms not shown). AFLP group I comprised of 40 isolates (71.4%), with 9 (16.1%) and 7 (12.5%) isolates in group II and group III, respectively.

Analysis of the binary banding data from all the seven primer combinations, however, showed that the 56 isolates of *F. udum* were genetically related (Figure 13, Table 20, Appendices 8.9.2). Isolate MAL01a from Malawi was used as the root of the dendrogram in order to determine the relationships among the isolates from Kenya. All the isolates from Kenya were not significantly distant (bootstrap value 1,000) genetically with the isolate from Malawi (Figure 13). Isolates from Kenya were represented by three main clusters (cluster 1, 2 and 3) and isolates TT06 and NB01. Due to the absence of a bootstrap value of 500 or greater at the fork (F) where these clusters meet, the isolates were considered to be genetically related. However, among the three clusters, there were isolates that were more closely related than the others. These include isolates that belong to AFLP group III, some isolates in group I, but not with isolates in group II.

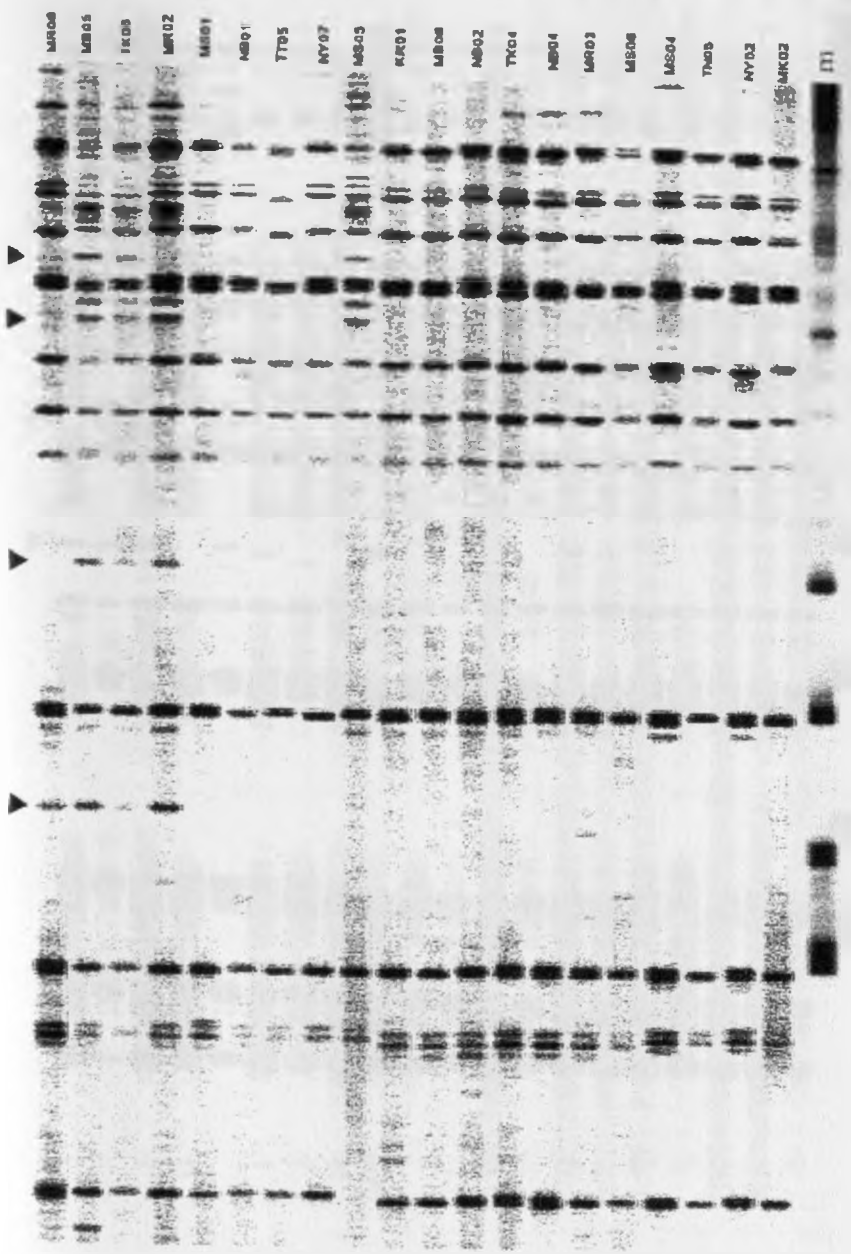


Figure 8. Typical banding pattern of AFLP fingerprint obtained by AFLP analysis with primer combination *Eco*R I + TA/*Mse* I + CAT. Some polymorphic bands are indicated with an arrow; m 100bP = digested λ *Hind III* DNA molecular weight marker.

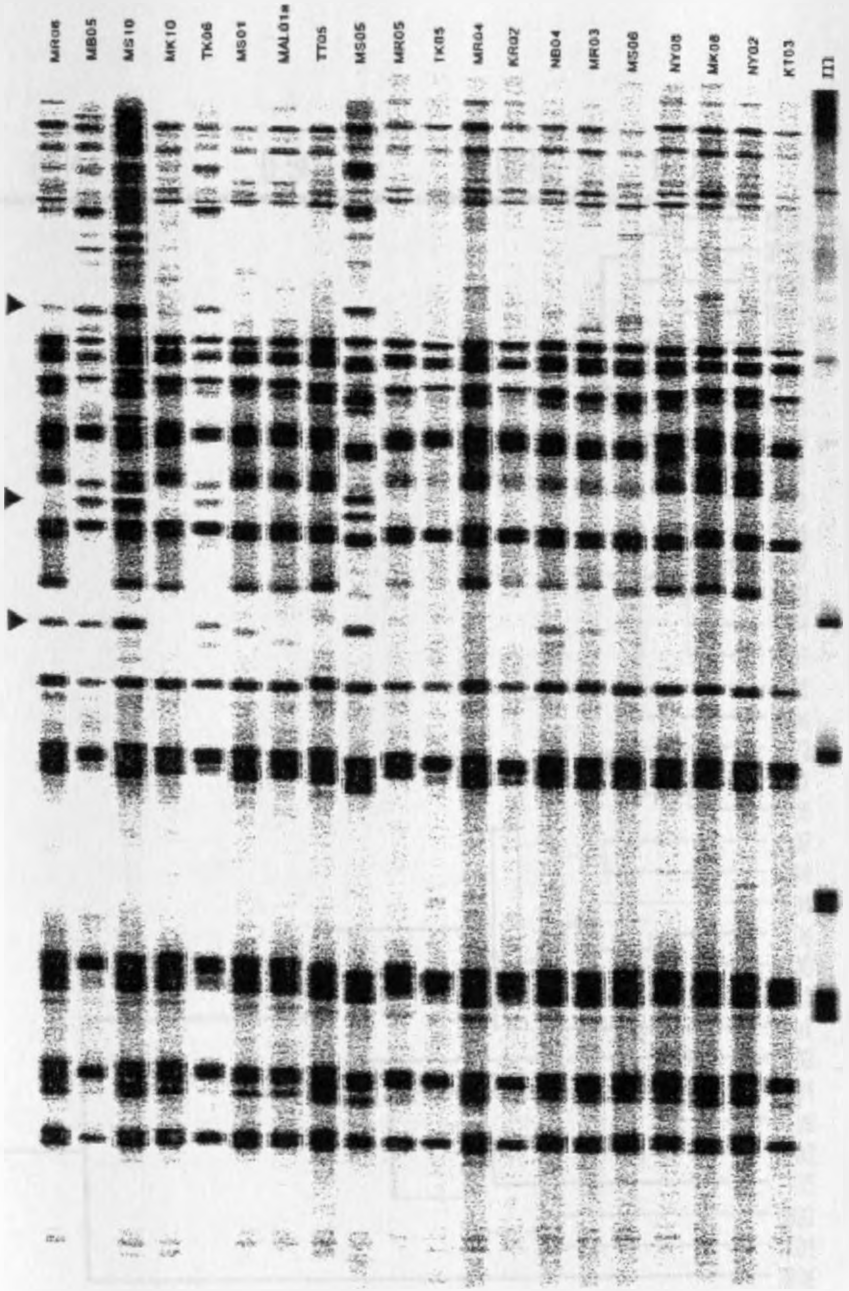


Figure 9. Typical banding pattern of AFLP fingerprint obtained by AFLP analysis with primer combination *Eco*R I +AG/*Mse* I + CAA. Some polymorphic bands are indicated with an arrow; m = 100bp digested λ *Hind III* DNA molecular weight marker.

Figure 10. Dendrogram derived from AFLP analysis of 39 isolates of *F. udum* using a combined matrix from 2 primer combinations (TA/CAT and TG/CAC) by UPGMA method with Dice similarity coefficient using NTSYS 1.8 program

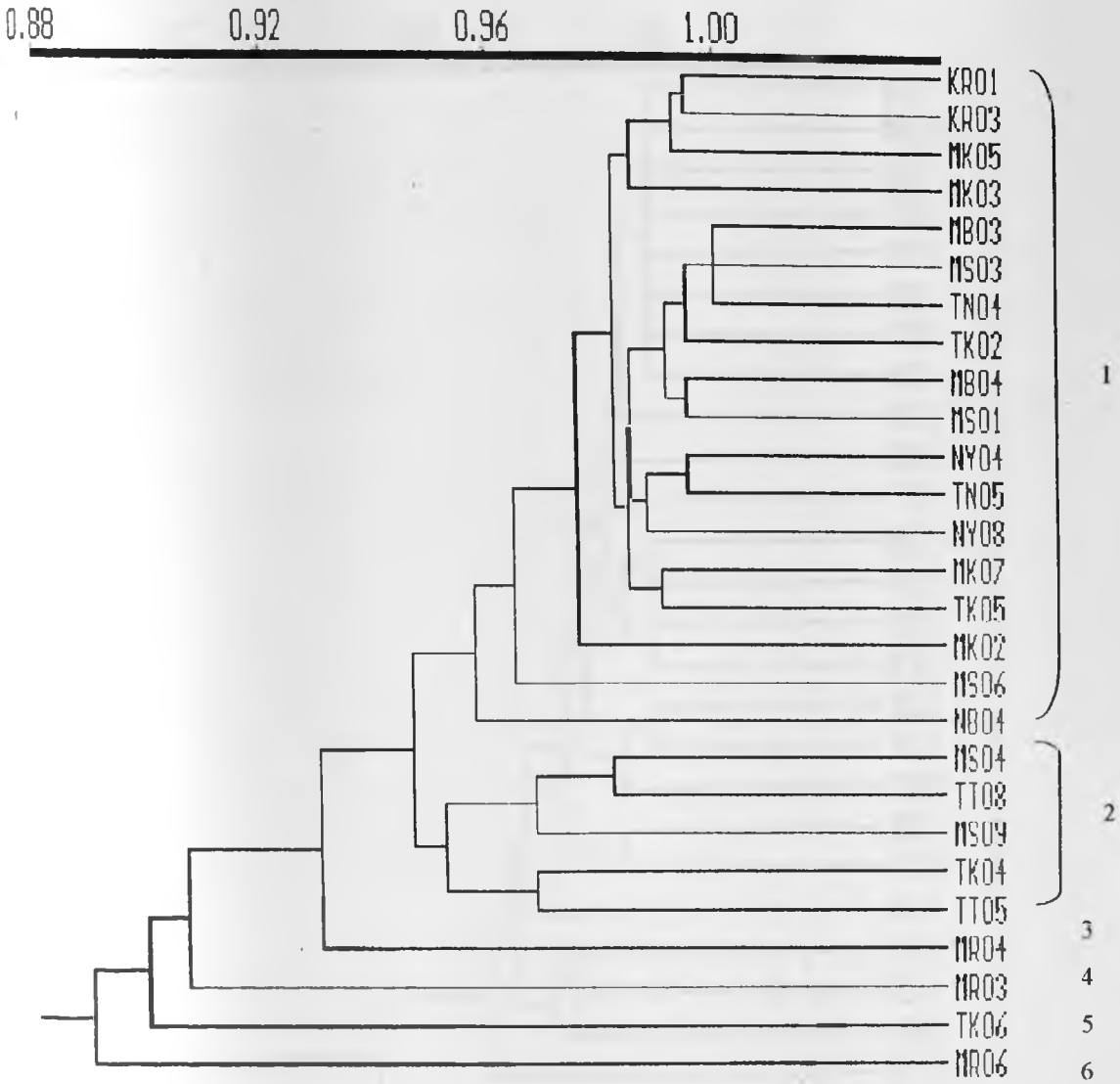


Figure 11. Dendrogram derived from AFLP analysis of 27 isolates of *F. udum* using a combined matrix from 3 primer combinations (TA/CAT, AA/CAG and TG/CAC) by UPGMA method with Dice similarity coefficient using NTSYS 1.8 program

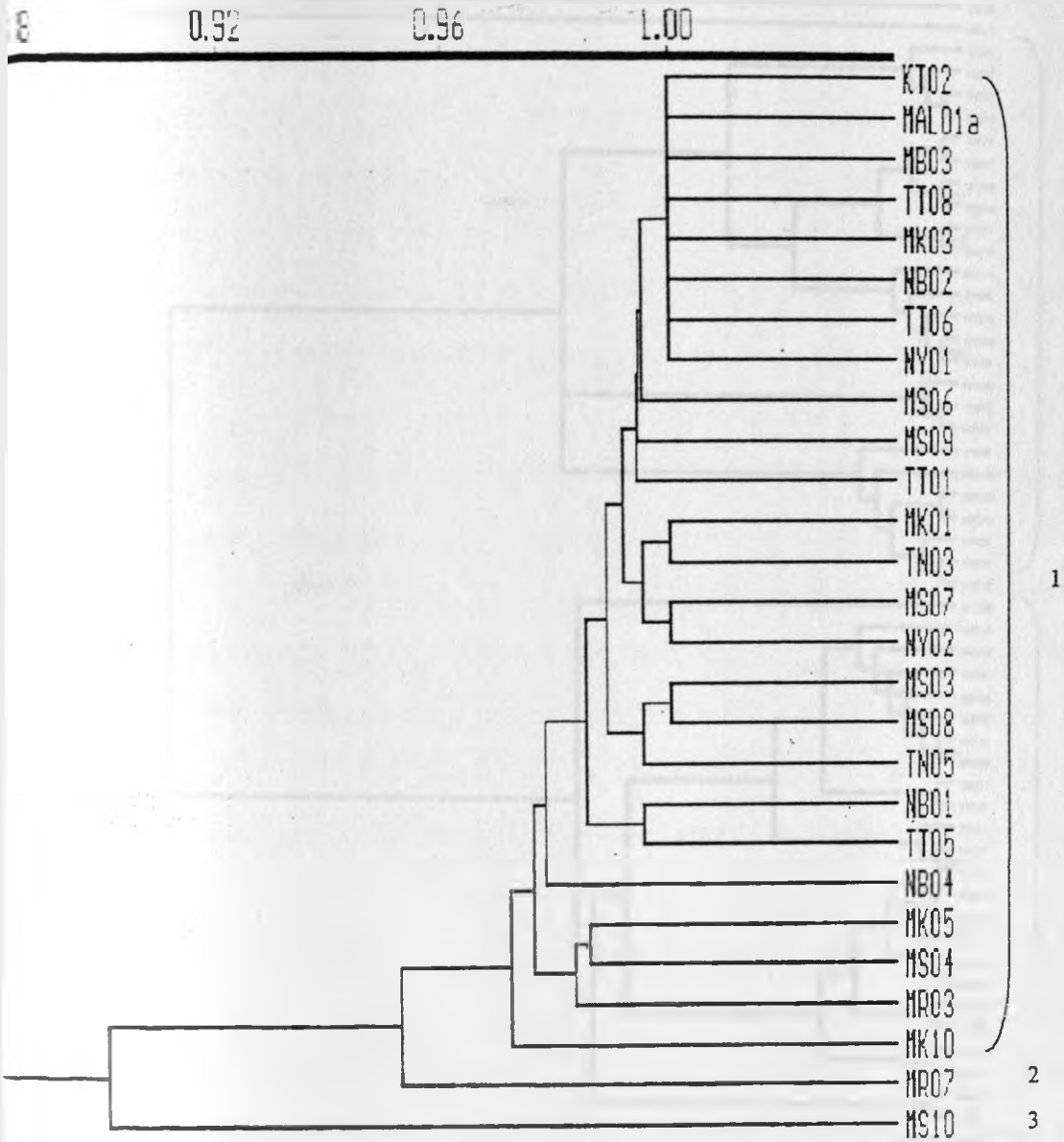


Figure 12. Dendrogram derived from AFLP analysis of 27 isolates of *F. udum* using a combined matrix from 3 primer combinations (AG/CAG, AC/CAG and AG/CAC) by UPGMA method with Dice similarity coefficient using NTSYS 1.8 program

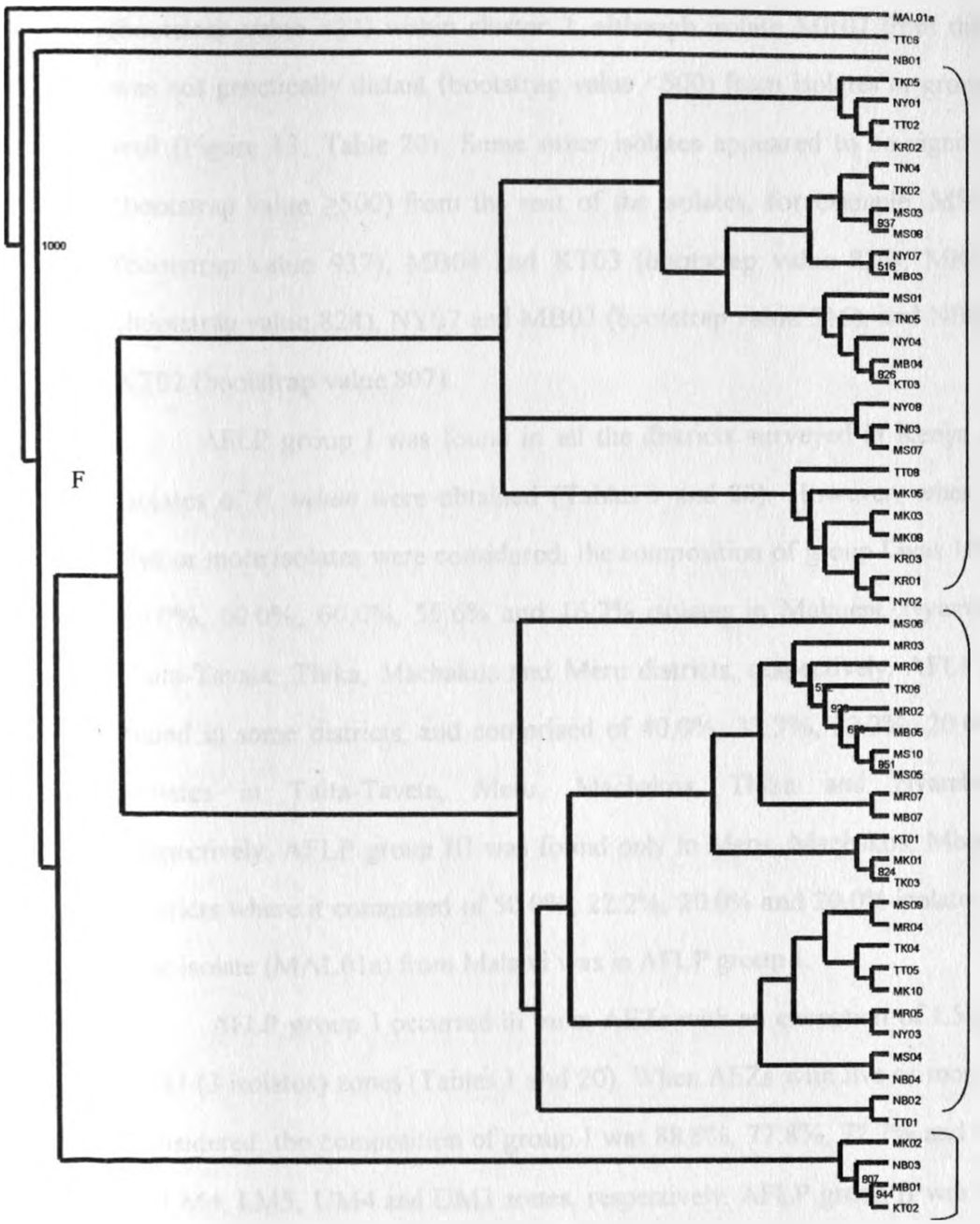


Figure 13. Dendrogram showing genetic relationships of 56 isolates of *F. udum* was obtained by the strict consensus tree (rooted) using UPGMA method with Nei and Li (1979) distance index from 7 primer combinations in the PHYLIP 3.5c program. Numbers on the branches (only values ≥500 are indicated) were generated by bootstrapping with 1,000 replications are indicated.

Bootstrapping supported the separation of AFLP group III as genetically distant (bootstrap value 522) within cluster 2, although isolate MR07 from the same group was not genetically distant (bootstrap value <500) from isolates in groups I and II as well (Figure 13, Table 20). Some other isolates appeared to be significantly distant (bootstrap value ≥500) from the rest of the isolates, for example, MS03 and MS08 (bootstrap value 937), MB04 and KT03 (bootstrap value 826), MK01 and TK03 (bootstrap value 824), NY07 and MB03 (bootstrap value 516), and NB03, MB01 and KT02 (bootstrap value 807).

AFLP group I was found in all the districts surveyed in Kenya where the 56 isolates of *F. udum* were obtained (Tables 1 and 20). However, when districts with five or more isolates were considered, the composition of group I was 100.0%, 83.3%, 80.0%, 60.0%, 60.0%, 55.6% and 16.7% isolates in Makueni, Nyambene, Mbeere, Taita-Tavata, Thika, Machakos and Meru districts, respectively. AFLP group II was found in some districts, and comprised of 40.0%, 33.3%, 22.2%, 20.0% and 16.7% isolates in Taita-Taveta, Meru, Machakos, Thika and Nyambene districts, respectively. AFLP group III was found only in Meru, Machakos, Mbeere and Thika districts where it comprised of 50.0%, 22.2%, 20.0% and 20.0% isolates, respectively. The isolate (MAL01a) from Malawi was in AFLP group I.

AFLP group I occurred in most AEZs with an exception of L5 (1 isolate) and LM3 (3 isolates) zones (Tables 1 and 20). When AEZs with five or more isolates were considered, the composition of group I was 88.9%, 77.8%, 72.7% and 33.6% isolates in LM4, LM5, UM4 and UM3 zones, respectively. AFLP group II was found in some AEZs, and it comprised of 18.2% isolates in UM3 and UM4 zones, 11.1% isolates in LM4 and LM5 zones. AFLP group III was not found in LM4, L5 and L6 zones, but it comprised of 18.2%, 11.1% and 9.1% isolates in UM3, LM5 and UM4 zones, respectively.

AFLP group I was found at altitudes over 800 m.a.s.l (Tables 1 and 20). When AEZs with five or more isolates were considered, the composition of group I was 81.8%, 72.2%, 71.4% and 64.3% isolates at 801-1000, 1001-1200, 1201-1400, 1401-

1600 m.a.s.l, respectively. AFLP group II was found at 700 to 1820 m.a.s.l, and comprised of 21.4%, 8.4%, 11.1% and 9.1% isolates at 1401-1600, 1201-1400, 1001-1200 and 801-1000 m.a.s.l, respectively. AFLP group III was found at 940 to 1500 m.a.s.l, and comprised of 16.7%, 8.4%, 8.4% and 9.1% isolates at 1001-1200, 1201-1400, 1401-1600 and 801-1000 m.a.s.l, respectively.

Some isolates from the same site (farm) that were placed in different AFLP groups include TK06 (group III), TK04 (group II) and TK05 (group I) from farm number 79 in Thika district; MS09 (group II), MS07 (group I) and MS08 (group I) from farm number 17 in Machakos district; MR05 (group I) and MR06 (group III) from farm number 38 in Meru district; and NB01 (group I) and NB02 (group II) from farm number 83 in Nairobi district (Tables 1 and 20). Isolates from the same pigeonpea plant and placed in different AFLP groups were MS08 (group I) and MS09 (group II) from farm number 17 in Machakos district, and TK05 (group I) and TK04 (group II) from farm number 79 in Thika district.

4.10 Relationships among various techniques used to characterize *F. udum* isolates

F. udum isolates with luxuriant aerial mycelial growth that had fluffy and fibrous mycelia were 25 (31.6%) and 2 (2.5%), respectively (Table 9). Isolates with moderately luxuriant aerial mycelial growth that had fluffy and fibrous mycelia were 12 (15.2%) and 17 (21.5%), respectively while isolates with scanty aerial mycelial growth that had fluffy and fibrous mycelia were 1 (1.3%) and 22 (27.8%), respectively. Isolates with luxuriant aerial mycelial growth that had fast, moderate and slow radial mycelial growth were 9 (11.4%), 16 (20.3%) and 2 (2.5%), respectively. Isolates with moderately luxuriant aerial mycelial growth that had fast, moderate and slow radial mycelial growth were 5 (6.3%), 18 (22.8%) and 6 (7.6%), respectively while isolates with scanty aerial mycelial growth that had fast, moderate and slow radial mycelial growth were 3 (3.8%), 17 (21.5%) and 3 (3.8%), respectively. Isolates with luxuriant aerial mycelial growth that had very high, high, moderate and low

sporulation were 0 (0.0%), 4 (5.1%), 12 (15.2%) and 11 (13.9%), respectively. Isolates with moderately luxuriant aerial mycelial growth that had very high, high, moderate and low sporulation were 3 (3.8%), 10 (12.7%), 10 (12.7%) and 6 (7.6%), respectively while isolates with scanty aerial mycelial growth that had very high, high, moderate and low sporulation were 5 (6.3%), 9 (11.4%), 6 (7.6%) and 3 (3.8%), respectively.

Isolates of *F. udum* with fluffy mycelia that had fast, moderate and slow radial mycelial growth were 9 (11.4%), 23 (29.1%) and 6 (7.6%), respectively while isolates with fibrous mycelia that had fast, moderate and slow radial mycelial growth were 8 (10.1%), 28 (35.4%) and 5 (6.3%), respectively (Table 9). Isolates with fluffy mycelia that had very high, high, moderate and low sporulation were 3 (3.8%), 8 (10.1%), 15 (19.0%) and 12 (15.2%), respectively while isolates with fibrous mycelia that had very high, high, moderate and low sporulation were 5 (6.3%), 15 (19.0%), 13 (16.5%) and 8 (10.1%), respectively. Isolates with fast radial mycelial growth that had very high, high, moderate and low sporulation were 0 (0.0%), 4 (5.1%), 6 (7.6%) and 7 (8.9%), respectively. Isolates with moderate radial mycelial growth that had very high, high, moderate and low sporulation were 6 (7.6%), 15 (19.0%), 18 (22.8%) and 12 (15.2%), respectively while isolates with slow radial mycelial growth that had very high, high, moderate and low sporulation were 2 (2.5%), 4 (5.1%), 4 (5.1%) and 1 (1.3%), respectively. A Pearson correlation coefficient of –0.40 ($P = 0.01$) was found between radial mycelial growth and sporulation. The various groups of aerial mycelial growth, mycelial texture, radial mycelial growth and sporulation had majority of the isolates with purple group pigments followed by the yellow group pigments (Table 9).

F. udum isolates with luxuriant aerial mycelial growth that had long, medium and short macroconidia were 3 (3.8%), 19 (24.1%) and 5 (6.3%), respectively (Tables 9 and 13). Isolates with moderately luxuriant aerial mycelial growth that had long, medium and short macroconidia were 2 (2.5%), 20 (25.3%) and 7 (8.9%), respectively while isolates with scanty aerial mycelial growth that had long, medium and short macroconidia were 3 (3.8%), 16 (20.3%) and 4 (5.1%), respectively.

Isolates with fluffy mycelia that had long, medium and short macroconidia were 4 (5.1%), 26 (32.9%) and 8 (10.1%), respectively while isolates with fibrous mycelia that had long, medium and short macroconidia were 4 (5.1%), 29 (36.7%) and 8 (10.1%), respectively. Isolates with fast radial mycelial growth that had long, medium and short macroconidia were 1 (1.3%), 11 (13.9%) and 5 (6.3%), respectively. Isolates with moderate radial mycelial growth that had long, medium and short macroconidia were 7 (8.9%), 35 (44.3%) and 9 (11.4%), respectively while isolates with slow radial mycelial growth that had long, medium and short macroconidia were (0.0%), 9 (11.4.9%) and 2 (2.5%), respectively. Isolates with very high sporulation that had long, medium and short macroconidia were 1 (1.3%), 6 (7.6%) and 1 (1.3%), respectively while isolates with high sporulation that had long, medium and short macroconidia were 1 (1.3%), 16 (20.3%) and 6 (7.6%), respectively. Isolates with moderate sporulation that had long, medium and short macroconidia were 5 (6.3%), 18 (22.8%) and 5 (6.3%), respectively while isolates with low sporulation that had long, medium and short macroconidia were 1 (1.3%), 15 (19.0%) and 4 (4.1%), respectively. The isolates with medium and short macroconidia had purple group as the main pigment followed by the yellow group pigment. Isolates with long macroconidia had similar proportion of purple and yellow pigments on the substrate while white colour was dominant on the mycelia.

The correlation coefficients (Pearson) were 0.15 (P = 0.09) for length and width of macroconidia, 0.41 (P = 0.01) for lengths of macroconidia and microconidia, 0.09 (P = 0.45) for length of macroconidia and width of microconidia, 0.07 (P = 0.52) for width of macroconidia and length of microconidia, 0.25 (P = 0.02) for widths of macroconidia and microconidia, and 0.36 (P = 0.01) for length and width of microconidia. The correlation coefficients between macroconidial length and radial mycelial growth, and between macroconidial length and sporulation were –0.22 (P = 0.05) and 0.14 (P = 0.21), respectively.

F. udum isolates with luxuriant aerial mycelial growth that were highly and moderately virulent were 22 (27.8%) and 5 (6.3%), respectively (Table 9). Isolates

with moderately luxuriant aerial mycelial growth that were highly and moderately virulent were 20 (25.3%) and 9 (11.4%), respectively while isolates with scanty aerial mycelial growth that were highly and moderately virulent were 20 (25.3%) and 3 (3.8%), respectively. Isolates with fluffy mycelia that were highly and moderately virulent were 30 (38.0%) and 8 (10.1%), respectively while isolates with fibrous mycelia that were highly and moderately virulent were 32 (40.5%) and 9 (11.4%), respectively. Isolates with fast radial mycelial growth that were highly and moderately virulent were 12 (15.2%) and 5 (6.3%), respectively. Isolates with moderate radial mycelial growth highly and moderately virulent were 30 (38.0%) and 8 (10.1%), respectively while isolates with slow radial mycelial growth that were highly and moderately virulent were 8 (10.1%) and 3 (3.8%), respectively. Isolates with very high sporulation that were highly and moderately virulent were 6 (7.6%) and 2 (2.5%), respectively while isolates with high sporulation that were highly and moderately virulent were 18 (22.8%) and 5 (6.3%), respectively. Isolates with moderate sporulation that were highly and moderately virulent were 22 (27.8%) and 6 (7.9%), respectively while Isolates with low sporulation that were highly and moderately virulent were 16 (20.3%) and 4 (5.1%), respectively. Isolates with long macroconidia that were highly and moderately virulent were 7 (8.9%) and 1 (1.3%), respectively (Tables 9 and 13). Isolates with medium macroconidia that were highly and moderately virulent were 43 (54.5%) and 12 (15.2%), respectively while isolates with short macroconidia that were highly and moderately virulent were 12 (15.2%) and 4 (5.1%), respectively.

The Pearson correlation coefficient between virulence and radial mycelial growth was –0.05 (P = 0.67), while the correlation coefficient between virulence and sporulation was 0.11 (P = 0.33). The correlation between virulence and macroconidia length of *F. udum* isolates was 0.30 (P = 0.01).

AFLP group I isolates of *F. udum* with luxuriant, moderately luxuriant and scanty aerial mycelial growth were 12 (21.4%), 12 (21.4%) and 16 (28.6%), respectively (Tables 9 and 20). Isolates in group II with luxuriant, moderately

luxuriant and scanty aerial mycelial growth were 4 (7.1%), 4 (7.1%) and 1 (1.8%), respectively while isolates in group III with luxuriant, moderately luxuriant and scanty aerial mycelial growth were 1 (1.8%), 5 (8.9%) and 1 (1.8%), respectively. AFLP group I isolates with fluffy and fibrous mycelial texture were 18 (32.1%) and 22 (39.3%), respectively. Isolates in group II with fluffy and fibrous mycelial texture were 6 (10.7%) and 3 (5.4%), respectively while isolates in group III with fluffy and fibrous mycelial texture were 2 (3.6%) and 5 (8.9%), respectively. AFLP group I isolates with fast, moderate and slow radial mycelial growth were 8 (8.4%), 28 (50.0%) and 4 (7.1%), respectively. Isolates in group II with fast, moderate and slow radial mycelial growth were 2 (3.6%), 6 (10.7%) and 1 (1.8%), respectively while isolates in group III with fast, moderate and slow radial mycelial growth were 3 (5.4%), 2 (3.6%) and 2 (3.6%), respectively. AFLP group I isolates with very high, high, moderate and low sporulation were 7 (12.5%), 11 (19.6%), 12 (21.4%) and 10 (17.9%), respectively. Isolates in group II with very high, high, moderate and low sporulation were 1 (1.8%), 1 (1.8%), 2 (3.6%) and 5 (8.9%), respectively while isolates in group III with very high, high, moderate and low sporulation were 0 (0.0%), 2 (3.6%), 4 (7.1%) and 1 (1.8%), respectively. AFLP group I isolates that had long, medium and short macroconidia were 4 (7.1%), 28 (50.0%) and 8 (8.4%), respectively (Tables 13 and 20). Isolates in group II that had long, medium and short macroconidia were 1 (1.8%), 7 (12.5%) and 1 (1.8%), respectively while isolates in group III that had long, medium and short macroconidia were 0 (0.0%), 6 (10.7%) and 1 (1.8%), respectively.

AFLP group I isolates that were highly and moderately virulent on wilt susceptible pigeonpea variety KAT 60/8 were 31 (55.4%) and 9 (16.1%), respectively (Tables 9 and 20). Isolates in group II that were highly and moderately virulent were 7 (12.5%) and 2 (3.6%), respectively while isolates in group III that were highly and moderately virulent were 3 (5.4%) and 4 (7.1%), respectively. Individual groups of AFLP comprised of different physiologic races of *F. udum*, for example, AFLP group I had physiologic races 0, 24, 32, 33, 34, 42 and 48; group II had races 16, 24 and 56;

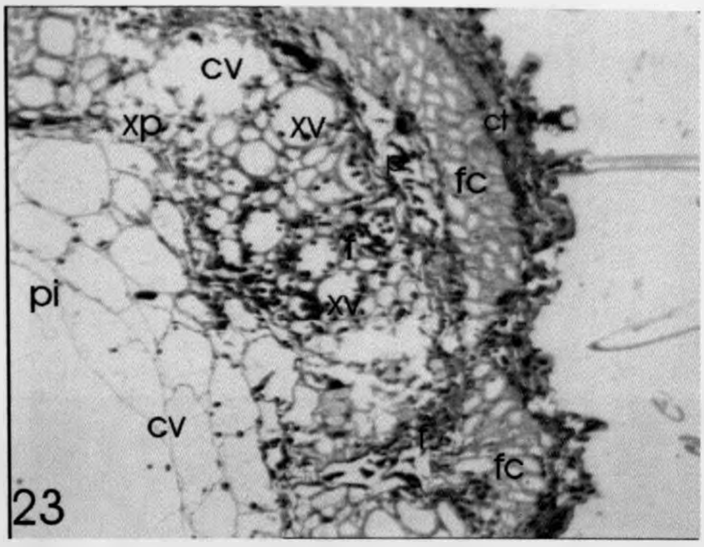
and group III had races 0, 16 and 48 (Tables 15 and 20). AFLP groups belonged to a single VCG, VCG 1 (Tables 17, 18 and 20).

4.11 Colonization of pigeonpea by *F. udum*

4.11.1 Histology of inoculated and non-inoculated pigeonpea plants

F. udum was not observed in the xylem vessels at 1 and 3 days after root-dip inoculation, but appeared to be confined to only a few vessels after 7 days in the susceptible variety (KAT 60/8). Masses or a few conidia were observed in some xylem vessels of both resistant (variety ICP 8863 and C-11) and susceptible (variety KAT 60/8) pigeonpea varieties from day 7 and thereafter. The fungus continued to grow and spread to adjacent vessel elements, and eventually established in a large number of xylem vessels after 14 days of inoculation. The growth of the fungus was both inter- and intra-cellular in resistant and susceptible plants. The colonization of the xylem vessels in the root and the stem by *F. udum* was scanty in the resistant plants (ICP 8863 and C-11), while it was highly prolific in susceptible plants (KAT 60/8) (Plates 23, 24 and 25). Once the fungus had established itself in susceptible plants, cavities appeared between the xylem cylinder and the pith, and also between the xylem and phloem (Plate 23). After fully colonizing the xylem vessels, the fungus spread to xylem parenchyma, the pith, vascular cambium, phloem and cortex. The initial small cavities eventually enlarged to cover the entire transverse area of the infected vascular tissue in both the root and stem after 21 days of inoculation of the susceptible (KAT 60/8). The xylem parenchyma cells and the pith disintegrated in some areas of susceptible plants leading to isolated xylem vessel elements. Cavities were generally not observed in the vascular bundle and the pith of the stem of resistant plants, but occasionally small cavities could be observed in their roots.

The colonization of the cortex of the root and stem by *F. udum* was rated as scanty, scanty to moderate and dense in varieties ICP 8863 (resistant), C-11 (resistant) and KAT 60/8 (susceptible), respectively. Many cortex cells of susceptible plants were filled with mycelia and some disintegrated leading to the formation of cavities



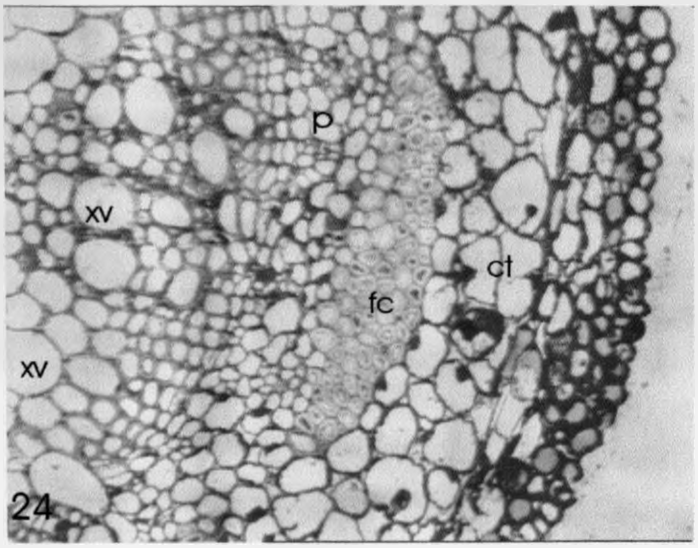


Plate 23. Light micrograph of transverse section of the stem of wilt susceptible variety KAT 60/8 twenty one days after inoculation with isolate MS04 showing high fungal colonization (x 200). Xylem vessels (xv), xylem parenchyma cells (xp), pith (pi), phloem (p), and the cortex (ct) are heavily colonized by the fungus (f); and some areas disintegrated leading to large cavity formation (cv). The fibre cells (fc) were less affected.

Plate 24. Light micrograph of transverse section of the stem of wilt resistant variety ICP 8863 twenty one days after inoculation with isolate TN05 showing low fungal colonization (x 200). Xylem vessels (xv) are less colonized by the fungus, and the pith, phloem (p), fibre cells (fc) and cortex (ct) appears unaffected.

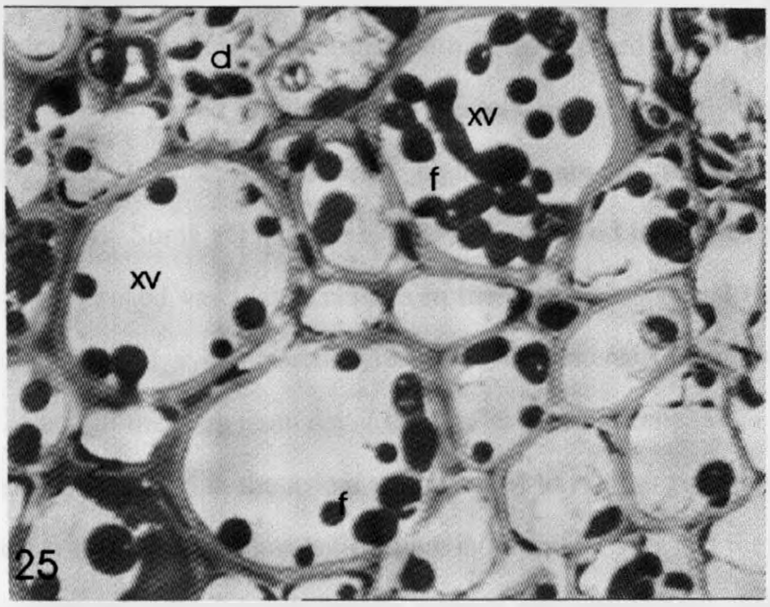
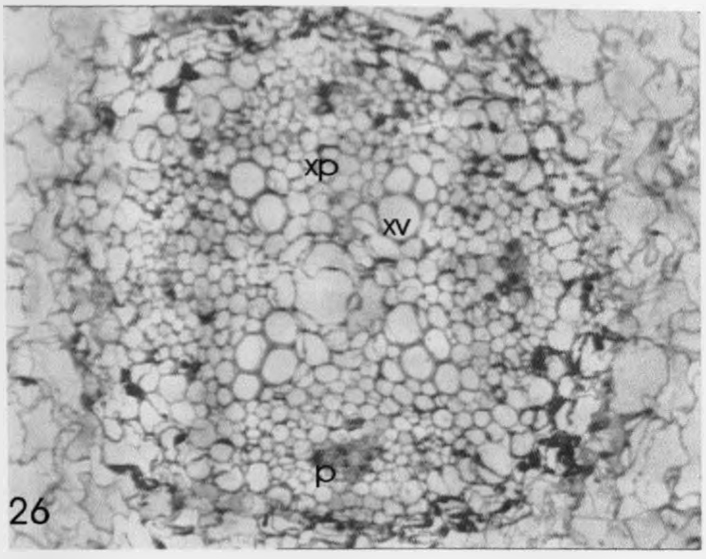


Plate 25. Light micrograph of transverse section of the stem of wilt susceptible variety KAT 60/8 twenty one days after inoculation with isolate MS04 showing intense fungal (f) colonization (x 1,000). Heavily colonized xylem vessels (xv) with some beginning to disintegrate (d) can be observed.

Fungal colonization of the taproot epidermis was scanty to moderate in resistant plants, while dense colonization was observed in the epidermis of the root and stem of susceptible plants. Fibre cells had scanty fungal colonization in both resistant and susceptible plants. No fungal colonization was observed in control plants.

Generally, there were no host responses observed that could obstruct *F. udum* spread in xylem vessels one day after inoculation, although some gel material could be observed in some vessels of the resistant plants (Plate 26). Gels appeared as light or dark coloured secretions (materials) with undetermined origin (Plates 27, 28 and 32). Gels were observed in the xylem vessels more clearly 3 to 21 days after inoculation, and they appeared with high intensity in resistant plants than in susceptible plants. Some gel materials were also observed in control plants, but at low intensity. Gels caused vascular occlusion in the xylem vessels and they also appeared in some phloem and xylem parenchyma cells. Gel intensity increased in roots from day 3 to 21 and in the stem from day 7 to 21 after inoculation.

Tyloses appeared in the xylem vessels three days after root-dip inoculation, and it increased with increase in incubation time in both the roots and the stem (Table 21). The percentage of xylem vessels having tyloses was significantly different ($P \leq 0.05$) among the pigeonpea varieties, *F. udum* isolates, site of sectioning (root or stem), and days after inoculation (Appendix 8.5.21). Variability in tylose formation in xylem vessels was high with time (days) after inoculation, followed by the pigeonpea varieties, and least on *F. udum* isolates and site of sectioning. The effect of interactions of variety-isolate, variety-days, isolate-days, and section-days on tylose formation was significantly different ($P \leq 0.05$) while the interaction between variety-site of sectioning and isolate-site of sectioning had no significant difference ($P \leq 0.05$) on tylose formation. Tyloses were not observed one day after inoculation. However, thereafter they were observed in varying intensities such as few (0.2-1.4%) in the root at 3 days after inoculation, followed by a steady increase in both the root (2.7-16.0%) and stem (0.4-13.7%) from 7 to 14 days after inoculation. Tylose



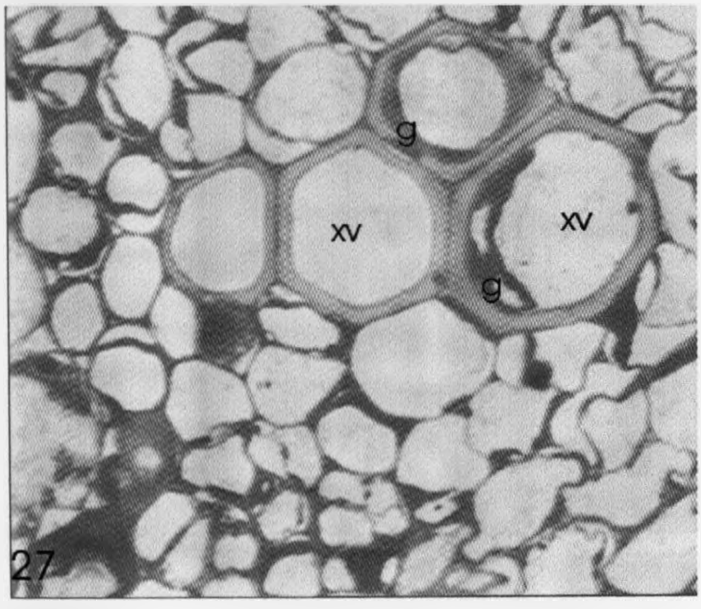
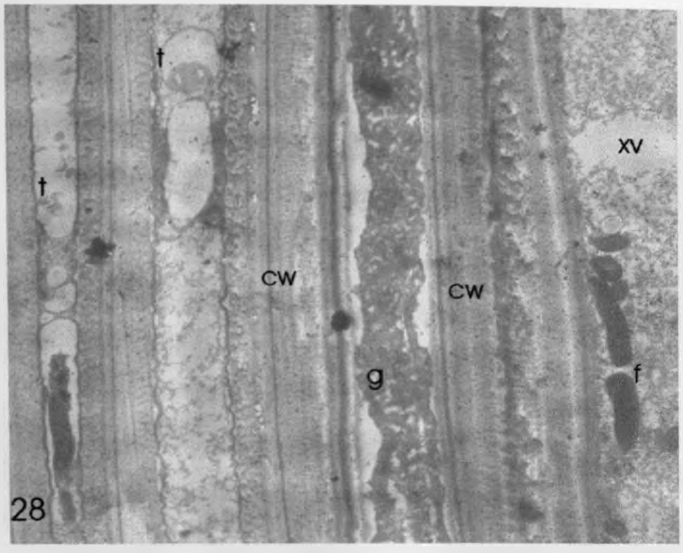


Plate 26. Light micrograph of transverse section of the root of wilt susceptible variety KAT 60/8 one day after inoculation with isolate MS04 (x 200). No sign of fungal infection was observed in the xylem vessels (xv), xylem parenchyma cells (xp) and phloem (p).

Plate 27. Light micrograph of transverse section of the root of wilt resistant variety ICP 8863 one day after inoculation with isolate TN05 (x 1,000). Gel material (g) could be observed in the some xylem vessels (xv).



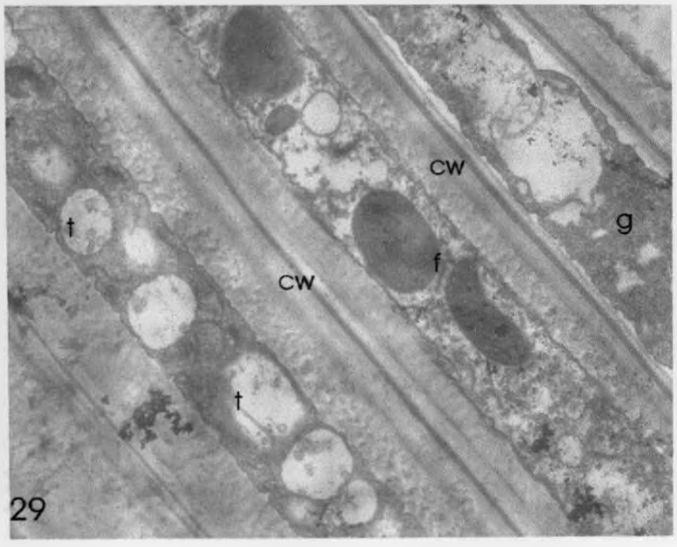
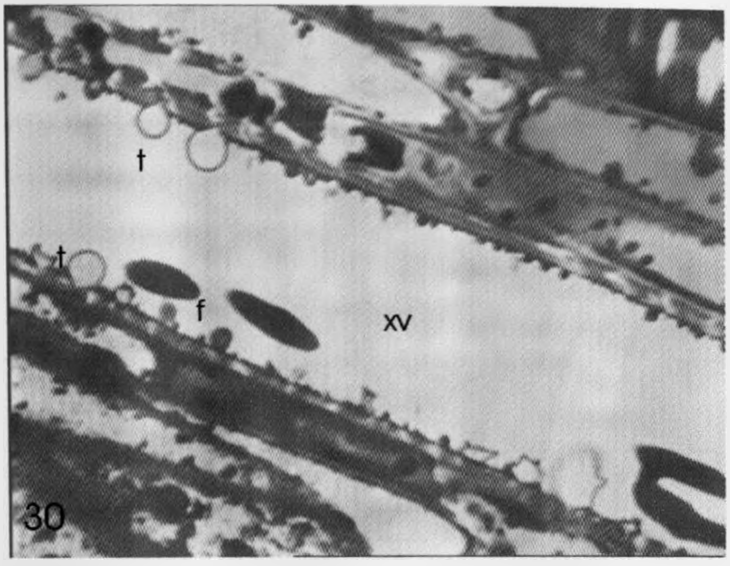


Plate 28. Transmission electron micrograph (TEM) of longitudinal section of the root of wilt resistant variety ICP 8863 seven days after inoculation with isolate TN05 (x 1,500). Xylem vessels (xv) occluded with tyloses (t) and gel (g) material, with presence of the fungus (f) in one vessel while the cell wall (cw) remain unaffected.

Plate 29. TEM of longitudinal section of the root of wilt resistant variety C-11 seven days after inoculation with isolate TN05 showing occlusion of the xylem vessels (xv) and presence of the fungus (f) (x 2,500). Xylem vessels are also occluded by tyloses (t) and gel material (g) while the cell wall (cw) appears unaffected.



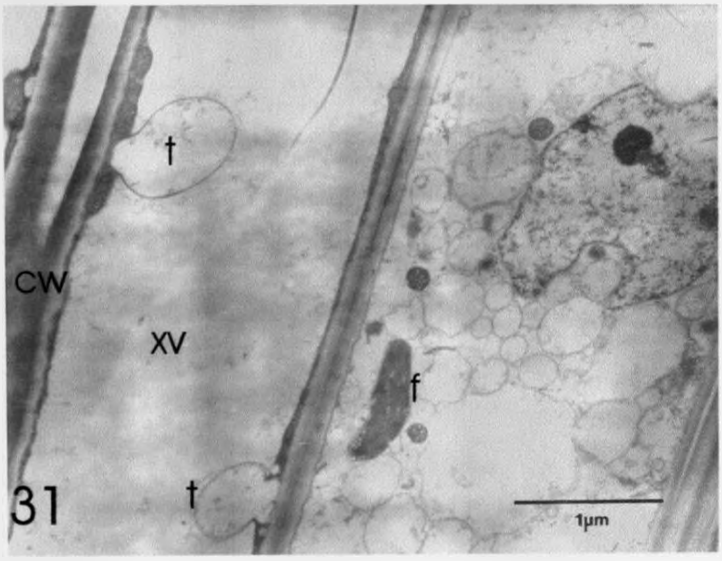
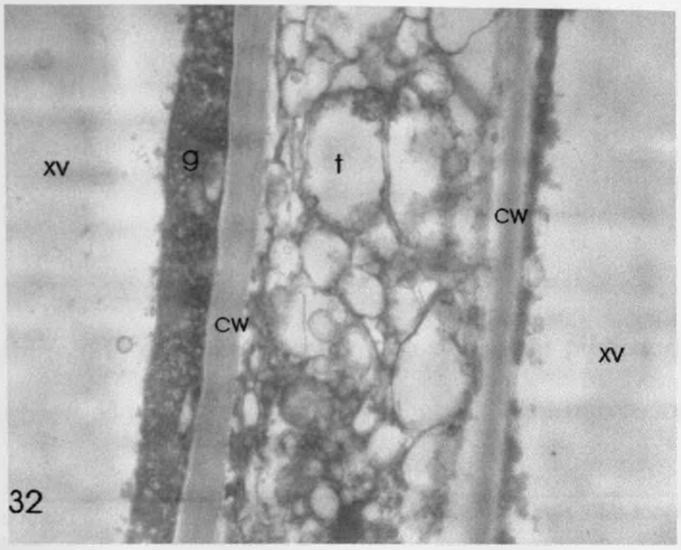


Plate 30. Light micrograph of transverse section of the stem of wilt resistant variety C-11 fourteen days after inoculation with isolate TN05 (x 1,000) showing the initial stage of tylose (t) formation in the xylem vessels (xv) from the cell walls (cw), and the presence of the fungus (f) in the vessel.

Plate 31. TEM of longitudinal section of the stem of wilt resistant variety ICP 8863 fourteen days after inoculation with isolate TN05 showing tylose (t) from the cell walls (cw) and presence of the fungus (f) in the xylem vessels (xv) (x 1,200).



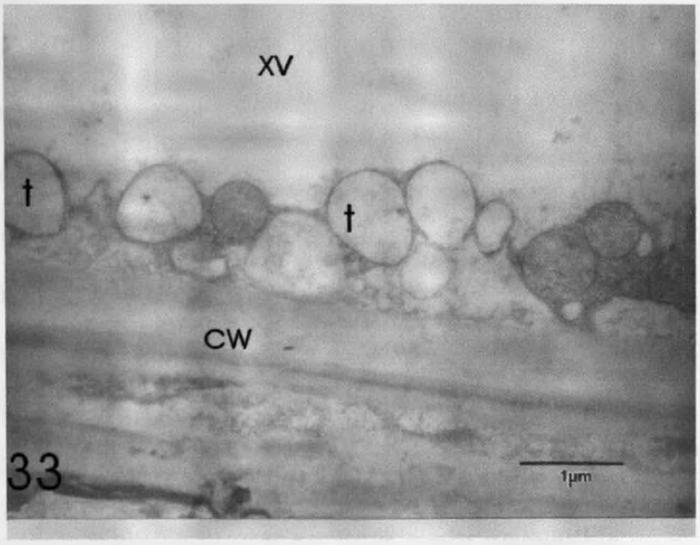


Plate 32. TEM of longitudinal section of the stem of wilt resistant variety ICP 8863 twenty one days after inoculation with isolate TN05 (x 6,000) showing xylem vessel (xv) completely blocked by tyloses (t) with the next vessel having gel material (g) while the cell wall (cw) remain unaffected.

Plate 33. TEM of longitudinal section of the stem of wilt resistant variety C-11 fourteen days after inoculation with isolate TN05 (x 5,000) showing a group of developing tyloses (t) from the cell wall (cw) expanding into the xylem vessel (xv).

Table 21. Percentage (%) of xylem vessels with tyloses in pigeonpea plants infected with *F. udum* as observed using a Nikon Microphot FX light microscope

Pigeonpea variety[1]	*F. udum* isolate	Site of sectioning[2]	Days after root-dip inoculation				
			1	3	7	14	21
C-11	TN05	Root	0.0	0.7	9.1	15.7	16.2
		Stem	0.0	0.0	3.1	10.1	17.5
	MS04	Root	0.0	0.0	4.4	10.0	13.0
		Stem	0.0	0.0	0.0	4.2	8.4
	Control[3]	Root	0.0	0.0	0.0	0.0	0.0
		Stem	0.0	0.0	0.0	0.0	0.0
ICP 8863	TN05	Root	0.0	1.4	14.8	16.0	15.1
		Stem	0.0	0.0	5.5	13.7	22.5
	MS04	Root	0.0	0.2	5.6	15.8	11.8
		Stem	0.0	0.0	1.7	7.9	12.3
	Control	Root	0.0	0.0	0.0	0.0	0.0
		Stem	0.0	0.0	0.0	0.0	0.0
KAT 60/8	TN05	Root	0.0	0.2	2.5	5.5	6.2
		Stem	0.0	0.0	0.0	2.8	3.2
	MS04	Root	0.0	0.0	2.7	5.0	8.0
		Stem	0.0	0.0	0.4	2.0	1.6
	Control	Root	0.0	0.0	0.0	0.0	0.0
		Stem	0.0	0.0	0.0	0.0	0.0

Mean = 6.2; SE = 6.0; CV (%) = 39.8; LSD ($P \leq 0.05$) = 0.9 (variety); 0.7 (isolate); and 1.0 (time in days).

[1]ICP 8863 and C-11 = wilt resistant, KAT 60/8 = wilt susceptible.

[2]Root = tap root at 2 cm from point of inoculation, stem = stem at 2 cm above the collar region.

[3]Control = seedlings roots were trimmed and dipped in sterile distilled water.

formation seems to be higher in the stem tissues of both resistant varieties ICP 8863 (22.5%) and C-11 (17.5%) at 21 days after inoculation.

There were large significant differences ($P \leq 0.05$) in tylose formation between resistant and susceptible plants, with more vessels containing tyloses in the former than the latter. There were few xylem vessels occluded by tyloses in the susceptible plants with most occlusion occurring in the root system (8.0% in variety KAT 60/8).

F. udum isolate TN05 initiated more tyloses in xylem vessels than isolate MS04 in both resistant and susceptible plants. The highest tylose formation was 22.5% in the stem of variety ICP 8863 inoculated with isolate TN05, followed by 17.5% in the stem of variety C-11 inoculated with TN05 at 21 days after inoculation. The maximum tylose formation initiated by isolate MS04 was 16.2%, in the root of variety C-11 at day 21 of inoculation, followed by 16.0% in the root of variety ICP 8863 at day 14 after inoculation.

Occlusion of xylem vessels by tyloses was normally effected by both individual tyloses and multi-tyloses (Plates 28, 29, 30, 31, 32 and 33). In resistant plants, high levels of tylose formation corresponded to low levels of fungal colonization. Tyloses and gels appeared either together or separately in the xylem vessels. Tylose formation was not observed in control plants of resistant varieties namely ICP 8863 and C-11, and susceptible variety KAT 60/8.

4.11.2 Presence of fungal hyphae of *F. udum* at different height levels of pigeonpea plants

Fusarium wilt symptoms were observed 8 to 10 days after root-dip inoculation on susceptible variety (KAT 60/8) using *F. udum* isolates MS04, KT05, MAL01a, MK10 and TN05. Early wilt symptoms were observed on some plants of the resistant variety C-11 two weeks after inoculation, but with no symptoms observed on the resistant variety ICP 8863. There were significant differences ($P \leq 0.05$) in *Fusarium* wilt incidence among pigeonpea varieties, and between the interaction of variety-isolate, while there was no significant difference ($P \leq 0.05$) among *F. udum* isolates

(Table 22, Appendix 8.5.22). The mean wilt incidence was 38.2% with a range of 0.0-100.0%. At eight weeks after inoculation, most plants (84.0%) of susceptible KAT 60/8 had wilt symptoms and were either fallen or remained standing, while 16.0% had no visible wilt symptoms. Variety C-11 had 22.7% wilt incidence with most of the plants showing wilt symptoms but remained standing, while 77.3% plants remained without external wilt symptoms. Few (8.0%) plants of variety ICP 8863 had shown wilt symptoms 8 weeks after inoculation, while 92.0% plants were visibly healthy. *Fusarium* wilt incidence was significantly different ($P \leq 0.05$) among isolates KT05 (46.7%) and TN05 (31.1%), while there was no significant difference ($P \leq 0.05$) between each of these isolates (KT05 and TN05) and isolates MK10 (40.0%), MAL01a (37.8%) or MS04 (35.6%). Control plants appeared healthy with no visible symptoms throughout the experimental period.

The isolates of *F. udum* were re-isolated along the full length of the stem measured in the three pigeonpea varieties eight weeks after inoculation (Table 22). The five isolates were re-isolated along the entire length of stem measured from all four plants of the wilt susceptible variety (KAT 60/8) with the mean relative presence being 100% from 0 to 25 cm of the stem. In the resistant plants (varieties ICP 8863 and C-11), the rate of fungal spread upward the main stem decreased with height. The mean relative presence of the fungus on variety C-11 was 90% at 0 cm and 65% at 25 cm, while variety ICP 8863 had means of 75% and 40% at 0 cm and at 25 cm, respectively.

The correlation coefficient (Pearson) between *Fusarium* wilt incidence (%) and relative presence (%) of *F. udum* along the pigeonpea stem at 0 cm, 5 cm, 10 cm, 15 cm, 20 cm and 25 cm were 0.66 ($P = 0.01$), 0.71 ($P = 0.01$), 0.77 ($P = 0.01$), 0.77 ($P = 0.01$), 0.81 ($P = 0.01$) and 0.84 ($P = 0.01$), respectively.

Table 22. Vertical spread of *F. udum* isolates in wilt susceptible and resistant pigeonpea plants, and wilt incidence 8 weeks after root-dip inoculation

Pigeonpea variety	*F. udum* isolate	Relative presence (%) of fungal hyphae[1]						Wilt incidence (%)[1]
		Plant height (cm)						
		0[2]	5	10	15	20	25	
ICP 8863	MS04	75	75	50	75	50	50	0.0
	TN05	50	50	75	50	25	25	6.7
	MAL01a	75	50	50	25	25	25	6.7
	MK10	75	75	75	75	50	50	6.7
	KT05	100	50	75	50	50	50	20.0
	Mean	75	60	65	55	40	40	8.0
C-11	MS04	75	75	75	50	75	50	13.3
	TN05	100	75	50	75	50	50	33.3
	MAL01a	100	100	75	75	75	75	13.3
	MK10	75	75	75	50	50	50	13.3
	KT05	100	100	100	100	100	100	40.0
	Mean	90	85	75	75	70	65	22.7
KAT 60/8	MS04	100	100	100	100	100	100	93.3
	TN05	100	100	100	100	100	100	53.3
	MAL01a	100	100	100	100	100	100	93.3
	MK10	100	100	100	100	100	100	100.0
	KT05	100	100	100	100	100	100	80.0
	Mean	100	100	100	100	100	100	84.0
Mean								38.2
CV								31.2
SE								142.2

[1]Relative presence (%) of fungal hyphae from 4 plants that were selected at random.
[2]Collar region
[3]LSD ($P \leq 0.05$) = 8.9 (variety) and 11.5 (isolate).

5.0 DISCUSSION

5.1 Distribution and incidence of *Fusarium* wilt of pigeonpea in Kenya

The survey established that the local (long-duration) varieties of pigeonpea are more widely cultivated than the improved (mainly short- and medium-duration) varieties. The local varieties were more prevalent in Meru, Nyambene, Tharaka-Nithi, Taita-Taveta, Kilifi and Malindi districts. However, farmers in Makueni, Machakos and Mbeere districts cultivated more improved than local pigeonpea varieties. This could be due to the fact that pigeonpea improvement and technology transfer programmes are traditionally based in these districts (S.N. Silim of ICRISAT, personal communication). This can partially account for the high proportion of the improved varieties, and the high adoption rate in Makueni, Machakos and Mbeere when compared to the other districts. The local varieties were mainly grown as an intercrop or mixed with other crops while the improved varieties were grown mainly as monocrop. In traditional cropping systems, pigeonpea is often intercropped or mixed with other crops (Acland, 1971; Osiru and Kibira, 1981). This is primarily because long-duration, tall varieties with slow initial growth rates are grown at wide row spacing. In addition to this, the long-duration varieties take two cropping seasons to mature thus necessitating the need for an intercrop to meet family food needs. As a sole crop, long-duration pigeonpea has limited scope in low rainfall, non-irrigated areas. The present study noted that pigeonpea was intercropped with many different crops namely maize, sorghum, cotton, common bean, green gram, cowpea, cassava, hyacinth bean, sweet potato and citrus. The intercrops of pigeonpea previously reported in Kenya include maize, cowpea, sorghum, sweet potato, pearl millet and banana (Hillocks and Songa, 1993). Acland (1971) reported that in Africa, pigeonpea is commonly intercropped with maize, sorghum, cowpea and cassava in the first year, but thereafter it is allowed to perennate as a sole crop in subsequent years. Published reports on cropping systems involving pigeonpea in Africa are, however, scanty (Ali, 1990).

Various symptoms of *Fusarium* wilt on pigeonpea plants were observed during the survey, and these included patches of dead plants especially during flowering and podding stages, partial wilting of the plant characterized by, a purple band extending upwards from the base of the main stem, browning or blackening of the xylem tissue, the leaves showed loss of turgidity, interveinal clearing, chlorosis and yellowing, drying of some branches without any visible band on the main stem and die-back symptoms on the dead branches. These wilt symptoms were similar to the ones reported on pigeonpea by Butler (1906), Mohanty (1946), Booth (1971), Nene (1980), Mehrotra (1980) and Allen (1983).

The prevalence of *Fusarium* wilt among the 86 pigeonpea farms visited was 64.0%. The disease was observed in 12 out of 13 districts covered during the survey. However, wilt prevalence varied among the districts with Machakos district recording the highest prevalence (100%), followed by Meru (86%), Nyambene (83%), Kitui (80%) and Makueni (75%) districts. The prevalence of *Fusarium* wilt was low in Malindi (20%) district with 0% in Kilifi district. Songa *et al.* (1991) reported 100% wilt prevalence in Machakos (8 farms) and Kitui (2 farms) in Kenya during a survey of pigeonpea diseases in the two districts. During *Fusarium* wilt/root knot nematode of pigeonpea survey in Eastern Kenya, Hillocks and Songa (1993) reported 30% wilt prevalence for 42 sites sampled in four districts. They noted wilt prevalences of 38%, 35%, 25% and 0% in Meru, Machakos, Kitui and Embu districts, respectively, which are lower than that reported in the present study, and the findings of Songa *et al.* (1991).

The mean *Fusarium* wilt incidence was 8.4% with a range of 0 to 96.1%. *Fusarium* wilt incidences in the farmer's plots varied considerably with the higher levels observed in Tharaka-Nithi (22.3%), Taita-Taveta (20.7%), Meru (18.7%), Kitui (15.0%) and Machakos (13.6%) districts, followed by moderate wilt incidences in Mbeere (8.8%), Nyambene (9.4%), Makueni (6.5%), Thika (5.5%) and Kirinyaga (4.9%) districts. The least wilt incidence of 0.2% wilt incidence was observed in Malindi district, Kilifi district recording no wilt incidence. Songa *et al.* (1991) reported 11.4%

and 5.5% *Fusarium* wilt incidences in Machakos and Kitui districts, respectively. In the present study higher wilt incidence was observed in Machakos and Kitui districts. Songa *et al.* (1991) reported 55% wilt incidence in Katumani wilt sick plot in Machakos district, which was 58.5% wilt (two sick plots) in the present study. The present survey included Taita-Taveta, Kilifi and Malindi districts in Coast province for the first time. The disease is fully established in Taita-Taveta with scanty incidence in Malindi and none in Kilifi.

The correlation between *Fusarium* wilt prevalence and incidence among the districts was moderate (r = 0.52) and significantly different. Districts with high wilt prevalence and incidence included Machakos, Meru, Kitui, Makueni and Taita-Taveta. Districts with moderate wilt prevalence and with high wilt incidence include Tharaka-Nithi and Nairobi. It is important to note that high prevalence of *Fusarium* wilt did not necessarily lead to high incidence.

The differences among pigeonpea farms in *Fusarium* wilt incidence could have been due to differences in pigeonpea varieties grown, the cropping system practiced, the stage of plant growth during the survey and control measures undertaken by farmers to contain the wilt. Pigeonpea varieties differ in susceptibility to *Fusarium* wilt (Butler, 1908; Shit and Sen Gupta, 1978; Sheldrake *et al.*, 1978; Nene, 1980; Nene and Kannaiyan, 1982; Kimani *et al.*, 1994; Songa *et al.*, 1995;). Wilt incidence generally increases when the crop is ratooned or retained as a perennial. Wilting is more common during the crop's reproductive phase (Mundkur, 1935). The spread of *Fusarium* wilt between pigeonpea farms could be due to planting of seed contaminated with the pathogen. Most farmers buy pigeonpea seed for planting from the local markets or plant their own seed. Until recently there were no certified pigeonpea seed in Kenya and even when available it is in limited supply and very costly for the small scale farmer. *F. udum* may be transmitted as carry-over as a contaminant of pigeonpea seed (Mohanty, 1946; Nene, 1980).

There were variations in wilt prevalence and incidence among various AEZs and altitudes. Lower midlands (LM) (LM3, LM4, LM5 and LM6) had higher wilt

prevalence and incidence, followed by upper midlands (UM) (UM3 and UM4), and least in lowlands (L) (L3, L4 and L5). The correlation of wilt prevalence and incidence was high (r = 0.82) yet not significantly different among various AEZs in Kenya. AEZs with high wilt prevalence had high wilt incidence and vice versa. Wilt prevalence was 83.6%, 68.2% and 30.6% in LM, UM and L, respectively, while wilt incidence was 26.4%, 18.1% and 5.4% in LM, UM and L, respectively. Moderate altitudes (801-1400 m.a.s.l) had high prevalence (78.0%), followed by high altitudes (1401-2000 m.a.s.l) (59.4%) and least at low altitudes (0-800 m.a.s.l) (20.4%). Moderate (18.8% wilt) and high (19.6%) altitudes had high wilt incidence compared with low (3.6%) altitudes. The correlation of wilt prevalence and incidence was moderately high (r = 0.77) and not significantly different among various altitudes in Kenya. Altitudes with high wilt prevalence generally had high wilt incidence and vice versa. Altitude ranges with high wilt prevalence and high incidence were 1201-1400 m.a.s.l and 801-1000 m.a.s.l with 87.5% and 70.6% prevalence, and 23.2% and 23.6% incidence, respectively.

Different AEZs and altitudes in Kenya have varying soil types, temperatures and rainfall. *Fusarium* wilt prevalence and incidence could be affected by these climatic factors. The zone groups (UM, LM and L) are temperature belts defined according to the maximum temperature limits within which the main crops in Kenya can flourish, while the main zones (UM3-4, LM3-6 and L3-5) are based on their probability of meeting the temperature and water requirements of the main leading crops (Jaetzold and Schmidt, 1983). *F. udum* can withstand widely varying conditions, including a pH of 4.6-9.0 and soil temperatures as high as 35°C, although temperatures of 17 to 30°C are conducive for disease development (Mundkur, 1935; Singh and Bhargava, 1981). A higher number of *F. udum* populations have been reported on sandy soil (94%) than on heavy black soil (18%) (Shukla, 1975), and at 30% soil water-holding capacity (Singh and Bhargava, 1981). The present study has reported for the first time the relationship of prevalence and incidence of *Fusarium* wilt of pigeonpea with respect to AEZs and altitudes in Kenya.

Slight differences were observed in *Fusarium* wilt prevalence but no significant differences noted in wilt incidence among pigeonpea types. Wilt prevalence on farms with local pigeonpea varieties was slightly higher (63.6%) than on farms with improved varieties (61.5%). Wilt incidence on pigeonpea farms with improved and local varieties were 17.1% and 13.4%, respectively. High significantly different correlation (r = -0.92) was observed between wilt prevalence and incidence among pigeonpea types. High wilt incidence was associated with lower wilt prevalence and vice versa. No differences were observed in *Fusarium* wilt prevalence yet significant differences were noted in wilt incidences among pigeonpea cropping systems. Wilt prevalence on farms with sole crop system was slightly higher (67.9%) than on farms with intercrop system (62.1%). However, significantly higher wilt incidence was noted on farms with sole crop system (15.4%) than on farms with intercrop system (13.7%). High significantly different correlation (r = 0.98) was observed between wilt prevalence and incidence among pigeonpea cropping systems. High wilt incidence was associated with lower wilt prevalence and vice versa. It is important to note that improved pigeonpea was cultivated mainly as sole crop while the local long duration was mainly grown as intercrops. Hillocks and Songa (1993) noted low (<10%) to high (>10%) wilt incidence on both sole crop and intercrop systems from pigeonpea farms in eastern Kenya. It appears that in Kenya farmers normally intercrop pigeonpea, especially local type (long-duration), in order to spread risk and maximize land utilization per unit area, and not necessarily as a control measure for *Fusarium* wilt and yet this approach seems to contain the disease as observed during this survey. The prevalence and incidence of wilt under various pigeonpea types and cropping systems could also be affected by the prevailing soil factors. Work at ICRISAT Centre (ICRISAT, 1993) has shown that while most production (cropping) systems reduced the population of *F. udum* to below the economic threshold level in Alfisol, none did so in the Vertisol. The sole perennial pigeonpea systems had 50% wilt incidence in both Vertisol and Alfisol (ICRISAT, 1993).

5.2 *Fusarium* wilt inoculation techniques

The root-dip inoculation technique was found to be very effective and reliable for virulence studies of *F. udum* and screening pigeonpea germplasm under the glasshouse for *Fusarium* wilt resistance when compared to the colonized whole rice grain technique. Such reliable results could be obtained 3 weeks after root-dip inoculation using *Fusarium* wilt incidence as the parameter of assessment. The pathogen could be re-isolated from plants that showed wilt symptoms onto PDA medium 14 days after root-dip inoculation. Pigeonpea varieties were separated clearly into their respective susceptibility classes while *F. udum* isolates did not show any significant variability in virulence. The root-dip inoculation technique has been found to be most dependable and effective than other techniques developed when inoculating pigeonpea genotypes with *F. udum* under the glasshouse. Out of five inoculation techniques studied, Okiror (1986) found root-dip method to be more effective and reliable in inducing *Fusarium* wilt of pigeonpea than either direct sowing on sick soil, transplanting on sick soil, soaking seed in the inoculum or stem injection with inoculum. Workers at ICRISAT are currently using root-dip inoculation technique for screening germplasm for *Fusarium* wilt resistance in glasshouse experiments (ICRISAT, 1990). The findings of the present study correlated with the findings of Reddy and Raju (1993), and Changaya-Banda *et al.* (1996) who also used root-dip method with an inoculum concentration of 1 x 10^6 conidia/ml to study the reaction of pigeonpea genotypes to *F. udum* isolates. Wiles (1963), and Miller and Cooper (1967) found this technique to be very effective for the inoculation of cotton genotypes with *F. oxysporum* f.sp. *vasinfectum*. The disadvantages of this technique, however, are that it is tedious and time consuming, some seedlings could die after transplanting due to transplanting shock, and at very high inoculum concentrations some resistant pigeonpea genotypes show high wilt incidence. It is necessary therefore to establish the optimum inoculum concentration before starting an experiment using root-dip method, and to check for the dark/brown strip inside the

stem of wilted plants and/ or plate stem pieces on PDA medium in order to confirm that the plants actually died due to *F. udum*.

Planting pigeonpea seed with colonized (with *F. udum*) whole rice grains induced low disease incidence even with the most wilt susceptible pigeonpea variety (KAT 60/8). Variety KAT 60/8 showed wilt incidence of 35% and 30% eight weeks after inoculation using colonized whole rice grain with *F. udum* isolates TT01 and MR01, respectively. The pathogen was re-isolated from wilted plants onto PDA medium six weeks after planting. This technique has been found to be very effective for screening bean germplasm for resistance against *Macrophomina phaseolina*, the causal agent of charcoal rot of common bean (Songa, 1995).

Root-dip inoculation technique was therefore a more adequate method for inoculating *F. udum* pathogen into pigeonpea plants under the glasshouse in the present study when compared to colonized whole rice grain technique. It was found to be fast and reliable although it was tedious to inoculate and transplant seedlings. Trimming roots of the pigeonpea seedlings to about 4 cm in length from the collar region and transplanting them after inoculation did not led to any noticeable death of plants due to transplanting shock.

5.3 Cultural characteristics of *F. udum* isolates

Single spore isolates of *F. udum* from Kenya (75 isolates), Malawi (2 isolates) and India (2 isolates) showed variation in cultural characteristics. In this study, aerial mycelial growth, mycelial texture, mycelial colour, substrate colour, radial mycelial growth (colony diameter) and sporulation were used to group 79 isolates of *F. udum* on PDA medium incubated at 25°C for 8 days into 3, 2, 5, 3, 3 and 4 groups, respectively. Okiror (1986) found variation among 12 isolates of *F. udum* obtained from Machakos, Makueni and Thika districts (Kenya) in mycelial growth, pigmentation and colony diameter on PDA medium incubated at room temperature for 10 days. He observed higher variability in mycelial colour than substrate colour as noted in the present study. He also reported dense (luxuriant) and sparse (scanty)

mycelial growth with a colony diameter of 56.0 to 81.5 mm. The difference in the colony diameter in the present study with that observed by Okiror (1986) could be due to the differences in the isolates and culturing conditions, but they appear to be correlated. Changaya-Banda *et al.* (1996) reported 25 pathotype groups from 75 isolates of *F. udum* from Malawi on the basis of cultural characteristics on Nash-Snyder (pentachloronitrobenzine) medium incubated at 25°C for 10 days. Like in the present study, they noted differences of isolates in sporulation, colony diameter, mycelial texture, aerial mycelial growth and pigmentation. In their study using 7 isolates of *F. udum* from Rajasthan State (India), Gaur and Sharma (1989) classified these isolates into 4 groups by mycelial growth and 3 groups by sporulation on PDA medium incubated at 28°C, indicating variability of this pathogen with respect to these parameters as observed in the present study. They observed a colony diameter of 50.0 to 90.0 mm on 12-day-old cultures and higher variability in substrate pigment than mycelial pigment. The present findings revealed more variation in mycelial pigment than substrate pigment and a colony diameter of 71.4 to 88.8 mm on 8-day-old cultures. Gupta *et al.* (1988) classified 71 isolates of *F. udum* from 30 districts of Madhya Pradesh (India) into 7 groups on the basis of cultural characteristics on PDA medium incubated at 25±2°C. They observed variability in mycelial growth, colony diameter, sporulation, pigmentation and mycelial dry mass. They identified 4 groups of sporulation and a colony diameter of 53.0 to 90.0 mm on 6-day-old cultures. Shit and Sen Gupta (1978) identified 3 groups of *F. udum* on 7 isolates from India on the basis of aerial mycelia and mycelial texture on different media that included PDA, oatmeal agar, Richard's synthetic agar and sucrose casamino acid medium. They also found variation in pigmentation and sporulation on these media. Gerlach and Nirenberg, (1982) reported that the colonies of *F. udum* are fast growing, reaching 7.8-8.2 cm diameter in 8 days at 25°C on PDA medium, which correlates with the findings of the present study.

The various districts in Kenya had isolates that were placed in the same or different groups of cultural characteristics, although in some districts certain groups

were dominant over the others. Each group of aerial mycelial growth, mycelial texture, radial mycelial growth and sporulation showed dominance in some districts. However, groups 1, 2 and 3 of mycelial pigment showed dominance in Kitui, Taita-Taveta and Thika districts, respectively. Group 1 of substrate pigment showed dominance in Kitui and Taita-Taveta while group 2 showed dominance in Mbeere, Meru, Tharaka-Nithi and thika districts. The remaining groups of pigments (mycelial and substrate) did not show similar trend. On the basis of cultural characteristics, one district could have more than one group and a single group could be found in more than one district. On the basis of cultural characteristics Gupta *et al.* (1988) observed that the distribution of *F. udum* isolates were irregular, one type of isolate was obtained from several districts in India and one district had more than one type of isolate which correlated with the present findings. Gupta *et al.* (1986) observed similar trend in the distribution of *F. oxysporum* f.sp. *ciceri* isolates causing vascular wilt of chickpea (*Cicer arietinum*) in India.

Cultural characteristics of some isolates of *F. udum* were associated with a particular agro-ecological zone. For instance, isolates with luxuriant aerial mycelial growth, fluffy mycelial texture and fast radial mycelial growth and moderate sporulation were dominant in UM (UM3 and UM4), while isolates with scanty aerial mycelial growth, fluffy mycelial texture, moderate radial mycelial growth and high sporulation were mainly found in LM (LM4 and LM5). Thus, using cultural characteristics it could be possible to associate certain groups of *F. udum* with their geographical origin (AEZ). Only isolates with the purple group of pigments on both mycelia and substrate showed dominance in UM zone. Using cultural characteristics some groups of *F. udum* were also associated to a particular altitude range, mainly moderate altitudes (801-1400 m.a.s.l) or high altitudes (1401-2000 m.a.s.l). Isolates with fluffy mycelial texture were dominant at high altitudes, while isolates with fibrous mycelial texture were dominant at moderate altitudes. Isolates with fast radial mycelial growth were dominant at high altitudes, while isolates with moderate and slow radial mycelial growth were dominant at moderate altitudes. Correlation of

cultural characteristics of *F. udum* isolates with AEZs and altitude was reported for the first time in Kenya.

Some isolates of *F. udum* from the same site such as MK08 and MK09 or the same pigeonpea plant such as TK04 and TK05 or the same strain (culture) such as MAL01a and MAL01b were placed in different groups on the basis of cultural characteristics. Single spore isolates from single strains of *F. udum* have been found to vary among themselves with regard to growth pattern, segmentation, and capacity to secrete metabolic products (Upadhyay and Rai, 1992).

Some cultural characteristics of *F. udum* were found to be correlated. Isolates with luxuriant aerial mycelial growth such as MK07 and KT04 were fluffy, had slow to fast radial mycelial growth, and low to high sporulation. Isolates with scanty aerial mycelial growth such as MB05 and MS07 were fibrous, had slow to moderate radial mycelial growth, and low to very high sporulation. A Pearson correlation coefficient of -0.40 ($P = 0.01$) was observed between radial mycelial growth and sporulation, implying that isolates with fast radial mycelial growth had lower sporulation than isolates with slow radial mycelial growth. Gupta *et al.* (1988) observed that *F. udum* isolates with slow growth had medium to abundant sporulation while those with fast growth had poor to medium sporulation, an observation made in the current study.

5.4 Conidial measurements of *F. udum* isolates

The descriptions of hyphae, chlamydospores and conidia of *F. udum* grown on PDA medium incubated at 25°C in the present study were similar to that reported by other workers (Butler, 1910; Wollenweber, 1931; Wollenweber and Reinking, 1935; Booth, 1971; Subramanian, 1971; Booth, 1978; Mehrotra, 1980; Gerlach and Nirenberg, 1982; Rai and Upadhyay, 1982; Ahmed and Reddy, 1983). Microconidia, macroconidia and chlamydospores were observed in all the 79 isolates of *F. udum*. Microconidia were, however, more abundant than macroconidia.

The range of microconidia and macroconidia measurements of *F. udum* on PDA incubated at 25°C in the present study were 3.4-18.7 x 1.7-4.2 µm and 13.6-55.9

x 2.5-5.1 µm, respectively, and were within the range of that reported by other workers. The range of microconidia and macroconidia measurements of *F. udum* reported by other workers include respectively 3.9-13.8 x 2.1-3.6 µm and 9.2-46.4 x 3.7-4.8 µm on PDA medium incubated at 28°C (Gaur and Sharma, 1989); 7-12 x 1.5-3.5 µm and 8.5-45 x 1.5-4.0 µm on PDA medium incubated at 25±2°C (Rai and Upadhyay, 1982); 5-15 x 2-4 µm and 15-50 x 3-5 µm (media and incubation temperature not indicated) (Mehrotra, 1980); and 6-8 x 3-3.5 µm and up to 30-40 x 3-3.5 µm on PDA medium incubated at 25°C (Booth, 1978). Gerlach and Nirenberg, (1982) reported 0-septum conidia, 1-septum, 3-septa conidia and 4-5 septa conidia of *F. udum* on PDA medium incubated at 25°C measuring 5-15 x 1.5-3.5 µm, 11-20 x 1.5-4.0 µm, 17-46 x 2.0-4.2 µm and 31-62 x 2.5-4.4 µm, respectively. Septation for microconidia and macroconidia ranged from 0-1 and 2-7, respectively, as reported also by Gerlach and Nirenberg, (1982), and Ahmed and Reddy (1983). It is evident from this study that variability exists in *F. udum* isolates in terms of morphological characteristics. Sarojini (1951) and Subramanian (1955) found variation in morphological and colony characteristics of *F. udum*. Padwick (1940) reported that *F. udum* is a highly variable species with regard to its ability to produce chlamydospores. Gupta *et al.* (1986) observed variability in conidial length on 6 isolates of *F. oxysporum* f.sp. *ciceri* causing vascular wilt of chickpea.

There were variations in conidial length of *F. udum* isolates from various pigeonpea farms, districts, AEZs and altitudes in Kenya. *F. udum* isolates were grouped into 3 groups on the basis of the length of macroconidia. Isolates with long macroconidia were dominant in Nyambene and Makueni districts, those with medium macroconidia were dominant in Mbeere, Thika, Kitui, Makueni and Machakos districts, and those with short macroconidia were dominant in Nyambene and Tharaka-Nithi districts. The 3 groups of isolates were more common in lower midlands (LM) than in upper midlands (UM). Isolates with long macroconidia were more common at high altitudes, while medium and short macroconidia were more common at moderate altitudes. Conidial measurements, and hence the morphology, of

F. udum appear to vary according to the geographical origin (districts/AEZs) of the isolates. Changaya-Banda *et al.* (1996) did not find much variation in macroconidial lengths and widths of *F. udum* isolates, with an exception of one isolate, collected from various districts and AEZs in Malawi.

Variations in the length of macroconidia were observed in some isolates from the same farm (site) for example TN01 and TN02, and TT04 and TT05, and those obtained from the same pigeonpea plant, for example MS08 and MS09.

The correlation of the measurements of macroconidia and microconidia of *F. udum* in the present study were low with an exception of moderate relations between the lengths of macroconidia and microconidia ($r = 0.41$, $P = 0.01$), and also between the lengths and widths of microconidia ($r = 0.36$, $P = 0.01$), which meant that these measurements could be used to predict the other using a linear equation. The lengths of either the macroconidia or microconidia could therefore be used to classify *F. udum* isolates into distinct groups. The relationships of the length of macroconidia with cultural characteristics were low. For example, the correlation coefficients between the lengths of macroconidia with radial mycelial growth ($r = -0.22$, $P \leq 0.05$) and sporulation ($r = 0.14$, $P \leq 0.05$) were low, and hence these characteristics could not be predicted using a linear equation.

5.5 Virulence of *F. udum* isolates on wilt susceptible pigeonpea variety KAT 60/8

A wilt susceptible variety KAT 60/8 was used to confirm the ability of the 79 isolates of *F. udum* to induce wilt. The initial symptoms of *Fusarium* wilt on variety KAT 60/8 appeared in the second week, and the pathogen could be re-isolated onto PDA medium from wilted plants after 14 days. Nene *et al.* (1979) retrieved *F. udum* from the roots of a susceptible pigeonpea cultivar within 15-30 days of sowing in 'wilt sick' soil, but symptoms developed two weeks later. These two findings are similar with respect to fungal re-isolation although different inoculation techniques were used. Other wilt symptoms noted on pigeonpea plants in the present study

included drying and defoliation of leaves from 2 to 3 weeks, browning and blackening of the xylem tissue, and death of most of the wilted plants after 6 weeks of inoculation. The wilt symptoms observed in the glasshouse were similar to those observed in the field.

Variations in the ability of *F. udum* to cause wilt on variety KAT 60/8 were used to classify isolates into two virulent groups namely the highly virulent group (group 1) and the moderately virulent group (group 2). There were differences in virulence of isolates from the same or different districts. All the isolates (100%) from Kitui and Nyambene districts were highly virulent, while 88.9%, 85.7%, 80.0%, 80.0% and 70.0% isolates from Taita-Taveta, Mbeere, Machakos, Tharaka-Nithi, and Makueni districts, respectively were highly virulent. Moderately virulent isolates were dominant in Meru district (57.1%) and moderate in Thika district (50.0%). Although differences in virulence were observed at various AEZs, there was no dominant virulent group at LM and UM zones. Both virulent groups were dominant at moderate altitudes when compared to higher altitudes. These observations indicate that the virulence of *F. udum* could vary depending on the geographical origin of the isolates. Variability in virulence of *F. udum* isolates from different geographical origins have also been observed elsewhere (Shit and Sen Gupta, 1978; Okiror, 1986; Gupta *et al.*, 1988; Gaur and Sharma, 1989). Variability in virulence on KAT 60/8 was also observed on isolates from the same pigeonpea farm, for example, MK08 and MK09, NY02 and NY03, and TK05 and TK06. Thus *F. udum* isolates from the same locality or geographical origin could also vary in virulence.

The correlation between virulence and sporulation was low ($r = 0.11$, $P \leq 0.05$), and hence the two variables could not be predicted using a linear equation. This was true for Meru and Thika districts that had isolates with low virulence. It is important to note that most isolates that had high sporulation did not necessarily have high virulence. The correlation between radial mycelial growth and virulence was low ($r = -0.05$, $P \leq 0.05$), and hence the two variables could not be predicted using a linear equation. Okiror (1986) found no relationship between radial mycelial growth of 12

F. udum isolates from Kenya and their virulence as confirmed in this study using 79 isolates of the pathogen. The present findings were also supported by the work of Gaur and Sharma (1989) who did not observe any correlation between colony diameter and sporulation, and virulence. Relationship between sporulation and virulence has been observed with other *Fusarium* species. Wellman and Blainsdell (1941) observed that isolates of *F. oxysporum* f.sp. *lycopersici* causing tomato wilt produced abundant mycelia and were more virulent than those producing scanty mycelia. This phenomenon was also observed by Prasad (1949) while studying *F. oxysporum* f.sp. *cucurbitae* causing cucurbit root rot. A strong association between radial mycelial growth and virulence has been shown by isolates of *Ceratocystis ulmi* (Gibbs and Brasier, 1973). Highly virulent isolates had near similar proportion of luxuriant, moderately luxuriant and scanty aerial mycelial growth. Moderately virulent isolates were, however, mainly moderately luxuriant in aerial mycelial growth. Shit and Sen Gupta (1978) also observed correlation between aerial mycelial growth and pathogenicity on *F. udum* isolates. They found that isolates producing luxuriant mycelial growth were weakly to moderately pathogenic. Gaur and Sharma (1989) did not observe any correlation between aerial mycelial growth and virulence contrary to the present findings and that of Shit and Sen Gupta (1978). There was no correlation between mycelial texture and virulence, and pigmentation and virulence in the present study.

The correlation between virulence and macroconidia length of *F. udum* isolates was moderate ($r = 0.30$, $P = 0.01$). The implication is that conidial length could have an effect on the virulence of *F. udum*.

5.6 Physiological race typing of *F. udum* isolates

Seven pigeonpea varieties namely KAT 60/8 (control), C-11, ICPL 87105, ICP 8863, ICP 8858, ICP 9174 and ICEAP 00040 were used to assess virulence variability of *F. udum* isolates. The differences in responses of the seven pigeonpea varieties to 19 isolates of *F. udum* from Kenya and 1 isolate each from Malawi and India

confirmed virulence variability in virulence of 79 isolates. Okiror (1986) reported variation in virulence of 12 isolates of *F. udum* from Kenya using 6 pigeonpea lines (Munaa and 5 NPP lines). Gaur and Sharma (1989) made similar observations on variability in virulence of 7 isolates of *F. udum* from India using 18 pigeonpea varieties. Studies using other pigeonpea varieties against *F. udum* isolates have been reported by Mukherjee *et al.* (1971), Sarojini (1951), Baldev and Amin (1974), Shit and Sen Gupta (1978), and Gupta *et al.* (1988).

The pigeonpea varieties responded differentially when inoculated with 21 isolates of *F. udum*. For example, varieties C-11 and ICPL 87105 showed a consistently different reaction to the isolates, followed by variety ICEAP 00040. Pigeonpea varieties ICP 8863, ICP 9174 and ICP 8858 did not show consistent differences in their reaction to 21 isolates of *F. udum*. In this study the pigeonpea varieties viz ICP 8863, ICP 9174 and ICP 8858 were found to be resistant to 21 isolates of *F. udum*. Pigeonpea variety ICEAP 00040 gave a moderately resistant type of reaction to the 21 isolates, while varieties C-11 and ICPL 87105 gave a moderately susceptible reaction to the same isolates. The present findings correlate with other reports from India especially resistance observed in varieties ICP 8863, ICP 8858 and ICP 9174. However, differences or similarities in the resistance of variety C-11 to *F. udum* isolates do exist among different reports. Reddy and Raju (1993) found pigeonpea varieties ICP 8863 (Maruti), ICP 9174 and C-11 to be resistant to *Fusarium* wilt. Gaur and Sharma (1989) found varieties ICP 8858 and C-11 to be resistant to wilt while variety ICP 8863 was moderately susceptible (30-70% wilt). Nene and Kannaiyan (1982) found varieties ICP 8858, ICP 8863 and C-11 (ICP 7118) to be resistant to wilt. Although Baldev and Amin (1974), Shit and Sen Gupta (1978), Gupta *et al.* (1988), and Nene and Kannaiyan (1982) found pigeonpea variety C-11 to be resistant to *Fusarium* wilt, the ranges of wilt incidence were 0-25%, 0-55%, 0-50%, and 2.2-70%, respectively. Singh and Mishra (1976) however found variety C-11 to be susceptible to *Fusarium* wilt. Reddy *et al.* (1990) have indicated that variety

C-11 does not have high level of resistance but variety ICP 8863 has high and stable resistance to *Fusarium* wilt.

Pigeonpea varieties reported to be resistant to *Fusarium* wilt in Kenya include ICP 8863 (0.0% wilt), ICP 87051 (9.6% wilt) and BDN 1 (3.3% wilt) (Songa *et al.*, 1995). *Fusarium* wilt susceptible varieties in Kenya include KAT 60/8 (44-63.1% wilt), ICPL 87105 (18.9-32.9% wilt) and ICP 2376 (21.3-95.8% wilt), while variety ICP 9145 was found to be resistant (0.0% wilt) at Katumani wilt 'sick' plot but susceptible (71.0% wilt) at Kiboko wilt 'sick' plot (Songa *et al.*, 1995). The present findings showed ICP 8863 and ICPL 87105 to be resistant (8.6% wilt) and susceptible (25.4% wilt), respectively to wilt. In the present study pigeonpea varieties KAT 60/8, C-11, ICPL 87105, ICEAP 00040, ICP 9174, ICP 8863 and ICP 8858 were resistant to 0, 10, 10, 14, 19, 20 and 20 isolates of *F. udum*, respectively. Changaya-Banda *et al* (1996) has also reported differential reactions of 7 pigeonpea differential lines namely ICP 2376, ICP 9145, ICP 8858, ICP 8859, ICP 8862, ICP 8863 and ICP 9174 to 75 isolates of *F. udum* from Malawi.

Eleven physiologic races of *F. udum* were identified when 21 isolates were used to inoculate 7 pigeonpea differential varieties. The distinct races were race 0, 16, 24, 48 and 56. The least distinct races were race 8, 32, 33, 34 and 42. Race 0 was most dominant and appeared to be independent from the rest while the remaining 10 races were closely related. The methods of Habgood (1970) and Gilmour (1973) have been used elsewhere to identify physiologic races of various plant pathogenic fungi. Habgood method has been used to identify races of, for example, *Puccinia striiformis* of wheat (Kema and Lange, 1992) and *Erysiphe graminis* of rye (Kast and Geiger, 1982). The present study has confirmed the possible existence of physiologic races of *F. udum*. Earlier findings have suggested a possible existence of physiologic races of *F. udum* but no specific studies were undertaken to document their existence (Mukherjee *et al.*, 1971; Baldev and Amin, 1974; Shit and Sen Gupta; 1978; Okiror, 1986; ICRISAT, 1987; Changaya-Banda *et al.* 1996). Physiologic races have been identified in other plant pathogens such as tomato wilt fungus *F. oxysporum f.sp.*

lycopersici (Alexander and Tucker, 1945) and *Colletotrichum lindemuthianum* causing anthracnose of common bean (Menezes and Dianese, 1988).

Physiologic races of *F. udum* appeared to be independent of the geographical origin (districts or AEZs) of the isolates, and cultural, conidial and virulent characteristics. There was no geographic pattern evident of 41 physiologic races identified from 138 isolates of *C. lindemuthianum* from South, Central and North America (Balardin *et al.*, 1997).

5.7 Vegetative compatibility groups (VCG) of *F. udum* isolates

The recovery of chlorate resistant sectors from *F. udum* isolates on MMC medium was 35%. These sectors were unable to utilize nitrate as a sole source of nitrogen and consequently grew as thin expansive colonies with no aerial mycelium on MM. They were designated *nit* mutants. The recovery of these sectors differed considerably among the isolates with a mean frequency of 0.14 and 0.68 sectors per colony. Clark *et al.* (1995) recovered 55.8% and 65.8% chlorate resistant sectors of *F. lateritium* isolates on KPS (potato sucrose agar containing chlorate) and KMM (similar to MMC), respectively. Correll *et al.* (1987) recovered 78-98% chlorate resistant sectors of *F. oxysporum* isolates, some of which utilized nitrate, at a mean frequency between 0.33 and 0.96 sectors per colony on MMC and between 0.92 and 1.65 sectors per colony on PDC (potato dextrose agar containing chlorate). Sunder and Satyavir (1998) found sectoring frequency of *F. moniliforme* isolates on KPS with a range of 0.88 and 3.17 sectors per colony. These findings indicate that the sectoring of individual isolates of a particular fungal species differs and hence the number of chlorate resistant sectors recovered. Although the recovery frequency of chlorate resistant sectors in the present study was lower when compared with that of other *Fusarium* species, the results are correlated and the differences could be due to the type of fungal species, type of medium used for sectoring and culturing conditions. *Nit* mutants have also been recovered from a number of other fungi, including *Aspergillus nidulans* (Cove, 1976), *Neurospora crassa* (Marzluf, 1981),

Fusarium solani (Correll, 1986), *Aspergillus flavus* (Papa, 1986), *Verticillium dahliae* (Elena and Paplomatas, 1998), and *Stagonospora nodorum* (Caten and Newton, 2000).

The *nit* mutants recovered from *F. udum* could be divided into three distinct phenotypic classes namely *nit*1, *nit*3 and NitM. These classes presumably reflect mutations at a nitrate reductase structural locus (*nit*1), a nitrate-assimilation pathway-specific regulatory locus (*nit*3), and loci (at least five) that affect the assembly of a molybdenum-containing cofactor necessary for nitrate reductase activity (NitM) (Klittich *et al.*, 1986; Correll *et al.*, 1987). The majority of the *nit* mutants recovered on MMC were *nit*1 mutants (79%), followed by *nit*3 mutants (18%), with the least being NitM mutants (3%). Correll *et al.* (1987) recovered *nit* mutants of *F. oxysporum* at frequencies of 59-66% for *nit*1, 10-28% for *nit*3 and 10-25% for NitM on MMC. However, they obtained higher recovery of *nit*1 (82-95%) using PDC as compared to MMC, but lower recovery of *nit*3 (0-12%) and NitM (2-10%). Clark *et al.* (1995) recovered *nit* mutants of *F. lateritium* at frequencies of 77.2% for *nit*1, 7.4% for *nit*3 and 15.4% for NitM on KPS as compared to 77.7% for *nit*1, 9.7% for *nit*3 and 12.6% for NitM on KMM. Thus it seems according to other findings as quoted above and the present study that majority of *nit* mutants recovered in fungal populations are usually *nit*1. The present results and that of Correll *et al.* (1987) indicate that *nit*3 are more than NitM mutants. However, Clark *et al.* (1995) found NitM to be more than *nit*3. Apart from using KPS and KMM amended with 1.5% $KClO_3$ as in the present study and the work done by Correll *et al.* (1987), higher $KClO_3$ could be increased in the media to 3.0, 4.5 or 6.0% in order to generate chlorate resistant sectors of some isolates (Clark *et al.*, 1995). Most studies on the generation of chlorate resistant sectors have used 1.5% $KClO_3$ as an amendment to potato dextrose agar or minimum medium (Puhalla, 1985; Katan *et al.*, 1991; Sunder and Satyavir, 1998). Varying the amount of chlorate in the medium therefore could increase or decrease the number of NitM and *nit*3 mutants generated. Leslie (1990) has indicated that some strains of *Fusarium* species such as *F. oxysporum* and *F. moniliforme* are normally resistant to

1.5% $KClO_3$, and many of these strains can be restricted on media containing 3.0 or 4.5% $KClO_3$. The differences in the number of NitM and *nit*3 mutants generated could also be due to the type of fungal species and the medium used for sectoring, and the culturing conditions. It is necessary therefore to choose a medium containing chlorate that could yield higher frequency of NitM mutants since they are used as testers during *nit* mutant complementation reactions for the purpose of determining vegetative compatibility groups. The NitM mutants have been reported to be very reliable *nit* mutant tester strains in vegetative compatibility tests (Correl *et al.*, 1987).

Generally, *F. udum* isolates were self-compatible but a few of them were self-incompatible especially between *nit*1 and *nit*3 mutants. NitM mutants were obtained from only 9 isolates out of 79, and 40 isolates had only *nit*1 mutant. It is important to note that not all isolates were tested for self-compatibility. Other work done on different *Fusarium* species correlate with the findings of the present study. Clark *et al.* (1995) reported that all the isolates of *F. latericium* were self-compatible, although a small number of *nit* mutants did not form heterokaryons with other *nit* mutants from the same isolate. In other studies, some pairings of *nit*1 with *nit*3 mutants recovered from the same parental strain of *F. oxysporum* did not result in complementation (Correll *et al.*, 1987). In the present study vegetative compatibility reaction was evidenced by more robust wild-type accompanied by dense aerial mycelial growth between *nit*1 and NitM mutants from different isolates than between *nit*3 and NitM mutants which concur with the findings of Correll *et al.* (1987). Complementations between *nit*1 and *nit*3 mutants from different isolates were moderate to weak. Correll *et al.* (1987) found that complementation between *nit*1 and *nit*3 mutants was not evident 2 to 3 weeks after incubation and at 3 weeks, the reaction was weak.

When 8 NitM tester mutants were paired with *nit*1 and/or *nit*3 mutants of different *F. udum* isolates for vegetative compatibility reactions, 81.6% had wild-type mycelial growth or positive reaction (++ or +), 16.8% showed no reaction (-), and 1.7% showed uncertain reaction (+-). All the isolates formed heterokaryons with at least one tester and all could be linked to most of the other testers. Fifty five (70%)

isolates formed heterokaryons with all 8 NitM testers while 24 (30%) isolates formed heterokaryons with some NitM testers with no complementation with others. Isolate MR05 for example formed a moderate to weak reaction with NitM testers IND01a/1 and TK03/1 but no reaction with the remaining 6 testers. This isolate could be linked to the other NitM testers through isolates MR06 or NY07 or any of the 55 isolates that formed heterokaryons between their *nit* mutants and all the NitM testers. Using this criterion, 24 isolates that formed heterokayons with some NitM testers were linked together with the other NitM testers and hence to the 55 isolates. All the *F. udum* isolates from Kenya, and each strain from Malawi and India, could therefore be grouped into one vegetative compatibility group, VCG 1. Plyler *et al.* (2000) used similar criterion to group 71% of 72 isolates of *F. oxysporum* f.sp. *canariensis* causing vascular wilt disease of *Phoenix canariensis* (date palm) into a single vegetative compatibility group (VCG 0240). Studies with populations of *F. oxysporum* f.sp. *vasinfectum* and *F. oxysporum* f.sp. *dianthi* in Israel revealed one VCG for each pathogen (Katan and Katan, 1988; Katan *et al.*, 1989). Studies on other fungal populations that have shown more than one VCG include *F. oxysporum* f.sp. *radicis-lycopersici* with 8 VCGs (Katan *et al.*, 1991; Katan and Katan, 1999); *Verticillium dahliae* isolates from Greece with 3 VCGs (Elena and Paplomatas, 1998); and *F. moniliforme* isolates from India with 10 VCGs (Sunder and Satyavir, 1998).

In asexually reproducing fungi, vegetative compatible strains are much more likely to be genetically similar than vegetative incompatible strains yet similar with respect to other traits. Vegetative compatibility groups therefore could be good predictors of genetic similarity, clonal lineage, or both. In the present study a single VCG (VCG 1) of *F. udum* was found to have more than one pathotype group based on cultural characteristics, conidial measurements, virulence on a wilt susceptible variety and physiological race typing. Relationship between heterokaryosis (vegetative compatibility) of isolates with their cultural characteristics and races could not be determined conclusively. However, heterokaryosis and virulence, and heterokaryosis and geographical origin (districts) of the isolates appear to be closely

related as observed in the isolates from Meru district. Other findings using different pathogenic fungi made similar observations as in the present study. Sunder and Satyavir (1998) observed that isolates of *F. moniliforme* from different VCGs and also within the same VCG varied considerably in virulence and gibberrelic acid (GA_3) production. Katan *et al.* (1994) found VCG 0135 of *F. oxysporum* f.sp. *melonis* in Israel that contained races 0 and 2, and VCG 0138 that contained races 0, 1, and 1-2. Correl *et al.* (1986) found correlation between VCG and colony size, and virulence of *F. oxysporum* f.sp. *apii* isolates. However, Elena and Paplomates (1998) found that the VCGs of *Verticillium dahliae* from different hosts in Greece were not correlated with the geographical origin, and did not appear to be related through pathogenicity. The differences in heterokaryosis of *F. udum* isolates among some districts and to some extent agro-ecological zones demonstrates that there is a possibility of the existence of different clonal lineages of this pathogen.

5.8 Amplified fragment length polymorphism (AFLP) of *F. udum* isolates

The Czapek Dox plus AZ liquid medium used for growing *F. udum* isolates for DNA extraction appeared to be a good medium for mycelial growth. In this study no other media was investigated for mycelial growth for DNA extraction. However, it is important to note that this medium has been used successfully by Coddington and Gould (1992) for mycelial production of *F. oxysporum* f.sp. *pisi*. Another liquid medium found to yield high amounts of fungal mycelia for DNA extraction is potato dextrose broth (Edel *et al.*, 1995; Koenig *et al.*, 1997). The CTAB method used for DNA extraction from fresh plant tissue (Doyle and Doyle, 1990), and herbarium and mummified plant tissues (Rogers and Bendich, 1985) was used successfully to extract DNA from *F. udum* isolates in the present study. The CTAB method has been used to extract DNA in other fungi and these include *F. oxysporum* species (Edel *et al.*, 1995), *Pyrenopeziza brassicae* (Majer *et al.*, 1996), *F. oxysporum* f.sp. *cubense*

(Koenig *et al.*, 1997), *Colletotrichum lindemuthianum* (Balardin *et al*, 1997), and *Mycosphaerella pinodes* and *Phoma medicaginis* var. *pinodella* (Onfroy *et al.*, 1999).

The modification of CTAB method for DNA extraction from *F. udum* was necessary because the DNA samples had high amounts of RNA. It was necessary to use high concentration of RNase A (20 mg/ml) in order to digest RNA. Doyle and Doyle (1990) used a concentration of 10µg/ml RNase A in order to digest RNA from DNA samples of plant tissue. High concentrations of RNase A have been used in other studies to digest RNA from fungal DNA samples and these include 20 µg/µl RNase A, 10 µg/µl RNase A and 20 mg/ml RNase A for DNA samples of filamentous fungi (*Phanerochaete chrysosporium*, *Coprinus cinereus*, *Aspergillus nidulans*) (Raeder and Broda, 1985), *F. oxysporum* f.sp. *pisi* (Coddington and Gould, 1992) and *F. oxysporum* f.sp. *cubense* (Koenig *et al.*, 1997), respectively. By performing phenol:chloroform:isoamyl alcohol extraction after RNA digestion followed by a second chloroform:isoamyl alcohol extraction, a high quality DNA was obtained. The purity of genomic DNA affects the restriction digestion during AFLP analysis (Life Technologies, 1995).

Molecular markers such as RAPD and RFLP have proved extremely useful in the study of populations of plant pathogenic fungi. Recent reports, however, have suggested that AFLP technique is more efficient in rapidly generating genotype data for large number of individuals including fungi (Majer *et al.*, 1996; Gonzalez *et al.*, 1998), bacteria (Lin and Kuo, 1995; Janssen *et al.*, 1996), and plants (Lin and Kuo, 1995). The present study also demonstrated that AFLP markers are useful in the study of genetic variation of *F. udum* isolates. Using seven primer combinations with *Eco*R I (E) + 2 and *Mse* I (M) + 3 selective nucleotides at the 3'-end of the primers, a total of 327 bands were amplified with 124 polymorphic bands. However, the number of amplified bands and polymorphism of individual primer combinations varied, with primers having more Ts and As namely E + TA/M + CAT, E + TG/M + CAC and E + AA/M + CAG showing higher polymorphism and generally more bands amplified than primers having more Gs and Cs namely E + AG/M + CAA, E + AG/M + CAC, E

+ AC/M + CAG and E + AG/M + CAG. An exception was observed with primer combination E + TA/M + CAT which showed the highest polymorphism (67%) but with the second lowest number of amplified bands (30 bands). Janssen *et al.* (1996) showed that the choice of the restriction enzymes (endonucleases), and the length and composition of the selective nucleotide would determine the complexity of the final AFLP fingerprint. The present findings are consistent with the work of Majer *et al* (1996) who also showed that the number of polymorphisms varied with primer combination in AFLP analysis of pathogenic fungi *Cladosporium fulvum* and *Pyrenopeziza brassicae* isolates. They however used E + 2 and M + 2 nucleotides, but indicated that for fungi with larger genomes, it may be necessary to use + 3 primer combinations while for some small genomes + 1 primers may be required. Gonzalez *et al.* (1998) used 2 instead of 3 selective nucleotides (E + 2/M + 2) in order to generate adequate number of bands for AFLP analysis of *Colletotricum lindemuthianum* isolates. The number of amplified fragments depends on C and G composition of the selective nucleotide (Life Technologies, 1995). In general, the more Cs and Gs used as selective nucleotides in the amplification primers, the fewer DNA fingerprints amplified. For highly complex genomes, such as those from plants or animals, at least 3 selective nucleotides at the 3'-end of both primers are required to obtain suitable AFLP fingerprints (Vos *et al.*, 1995). Primer selectivity is good for primers with 1 or 2 selective nucleotides in simple genomes such as fungi, bacteria and some plants. Selectivity is still acceptable with primers having 3 selective nucleotides, but this is lost with the addition of the fourth nucleotide (Vos *et al.*, 1995).

Statistical analysis of AFLP data enabled the division of *P. ultimum* isolates into three AFLP groups namely groups I, II and III, although they appeared to originate from a single genetic lineage. By performing bootstrap of 1,000 replications, there was no significant difference (bootstrap value >500) in the genetic distances between the three AFLP groups. However, some isolates in AFLP groups III and I were genetically distant (bootstrap value >500) from other isolates in the same groups or in

group II. AFLP group I was dominant in Makueni, Nyambene and Mbeere districts, group II was dominant in Taita-Taveta and Meru districts, and group III was dominant in Meru district. AFLP group I was more common in LM zones than UM zones while groups II and III were more common in UM zones than LM zones. AFLP groups III and I were more common at moderate altitudes while group II was more common at higher altitudes. *F. udum* is a deuteromycete and therefore natural populations of the pathogen may consist of clonal lineages produced by asexual reproduction. The present study has shown that distinct genetic variation in *F. udum* could exist and that the AFLP groups could depend on their geographic origin. Gonzalez *et al.* (1998) has classified *C. lindemuthianum* isolates from Mexico using AFLP analysis into two major groups according to the type of common bean cultivar or system of cultivation from which they originated, and into smaller subgroups generally associated with the geographical location from which they were obtained. Koenig *et al.* (1997) has identified ten clonal lineages of *F. oxysporum* f.sp. *cubense* using RFLP analysis, and the two largest lineages had pantropical distribution while the minor lineages were found only in limited geographical regions. In this study, however, there were some isolates from the same site that were genetically distant (bootstrap value >500) and were placed in different AFLP groups, and these include TK04and TK06, TK05 and TK06, MS07 and MS08, MS08 and MS09, MR05 and MR06. Still other isolates namely MS08 and MS09 from the same pigeonpea plant were placed in different groups and were genetically distant (bootstrap value >500). The genetic variation observed in *F. udum* isolates from the same site and even from the same plant demonstrates the superior discriminative power of AFLP toward the differentiation of related fungal isolates that belong to the same species. Janssen *et al.* (1996) has differentiated highly related bacterial strains of *Xanthomonas* that belong to the same species or biovar using AFLP analysis, highlighting the potential of this fingerprinting method in epidemiological and evolutionary studies.

AFLP groups of *F. udum* appeared to be independent of cultural characteristics, conidial measurements, virulence, physiologic races and VCG.

However, AFLP group III had high proportion of its isolates (57%) in moderately virulent group. AFLP groups II and I appeared to represent highly pathogenic isolates while group III represent moderate pathogenic isolates. An AFLP group could have more than one group based on cultural characteristics and conidial measurements, and more than one physiologic race. In the present study, a single VCG (VCG 1) was found to have more than one AFLP group although isolates that showed low heterokarysis from Meru district belong to AFLP group III. Probably a clear relationship between VCG and AFLP could be obtained if large number of *F. udum* isolates from many countries or diverse geographical origins were studied. Okiror (1986) found low variation in protein profiles of 12 *F. udum* isolates from Kenya, but indicated that their differences could be observed at genetic level. Although he used few isolates, the present study has confirmed that differences in *F. udum* strains exist at molecular level. He however found no relationship between the virulence of the isolates and their protein components. Woo *et al.* (1996) did not find any correspondence between RFLP or RAPD markers and races of *F. oxyxporum* f.sp. *phaseoli* isolates but found that the molecular markers corresponded to VCGs. Molecular polymorphism generated by RAPD analysis of *C. lindemuthianum* isolates from the Americas showed no obvious patterns correlated with virulence (races), and no geographical pattern was evident (Balardin *et al.*, 1997).

5.9 Colonization of pigeonpea by *F. udum*

The presence of *F. udum* in the wilt susceptible pigeonpea plants was characterized by mycelia and conidia in the xylem vessels, plugging on some xylem vessels, disintegration of the xylem parenchyma cells in the infected areas, and the formation of cavities in the vascular bundle, the pith and the cortex. Similar observations have been reported in other hosts infected by *Fusarium* wilt fungi, for example carnation plants infected by *F. oxysporum* f.sp. *dianthi* (Pennypacker and Nelson, 1972). The exact mode of *F. udum* entry into pigeonpea plant has not been determined, although reports indicate that the infection takes place through the fine

lateral branches of the roots and the fungus continues to grow in the xylem vessels (Mehrotra, 1980; Upadhyay and Rai, 1992). Booth (1978) has indicated that pathogenesis of *F. udum* follows a similar pattern to infection by *F. oxysporum* in infecting the vascular tissue. Contrary to the situation in banana, where conidia are reported to be abundant in the xylem vessels from the beginning of pathogenesis (Beckman, 1964), conidia were not observed in the vessels of pigeonpea plants infected with *F. udum* until the mycelium was well established 7 days after inoculation. Conidia were then formed abundantly in susceptible plants and less in resistant plants. Similar results were also observed in carnation plants infected by *F. oxysporum* f.sp. *dianthi* (Pennypacker and Nelson, 1972) when conidia could not be observed until abundant mycelia had formed. Masses of conidia and mycelial plugs occur in tomato infected by *Fusarium* wilt fungus (Chambers and Gorden, 1963). Masses of conidia and mycelial plugs could cause a reduction in water flow through the vessel elements, and thus may contribute to the wilt syndrome (Saaltink and Diamond, 1964).

The formation of gels, and especially at higher intensity in resistant than susceptible plants, could contribute to resistance of plants to *F. udum* pathogen. The formation of gels as a host response to vascular infection (Beckman, 1964) occurs in a wide variety of hosts including banana (Beckman and Zarrogian, 1967), cotton (Bell, 1974), sunflower (Robb *et al.*, 1975), and chrysanthemum (Robb *et al.*, 1975). Some gel materials were also observed in control plants, but at low levels in the present study. Gels result from a response of plants to a wide variety of host-specific and non-host-specific pathogens and from non-pathogenic soil microflora (Beckman and Halmos, 1962; Van der Molen *et al.*, 1977). Gels appeared as early as day 3 after inoculation, and this correlated with the findings of Beckman *et al.* (1962) who observed gels in *Fusarium* infected banana in the region of infection 2 days after inoculation. Resistant pigeonpea plants had low hyphal colonization and high gel formation. The formation of gels seemed to localize the pathogen, at least temporarily, and as such it could represent a significant defence response of the host

preventing rapid colonization of the upper portions of infected plants. Mace (1978) considered that the absence of significant levels of occlusion during the first 48 hours after infection of the stem xylem precludes gelation from having a role in resistance of cotton to *Verticillium* wilt. Marley and Hillocks (1993) observed that 15 days after pigeonpea was inoculated with *F. udum*, most of the vessels in susceptible plants (Malawi local) had become occluded while there were low numbers of occluded vessels in the resistant cultivar (ICP 9145). These observations were based on the proportion of dysfunctional vessel's failure to take up basic fuchsin dye. Their conclusion was that vascular occlusion is less important for wilt resistance in pigeonpea than in other hosts of vascular wilt diseases.

Resistance to *F. udum* in the roots and stems of wilt resistant pigeonpea plants was associated with lower levels of hyphal colonization, relatively higher occlusion due to gels, and more tyloses than in the susceptible plants. Similar results on hyphal colonization and tylose formation have been reported in tomato infected by *Verticillium* (Tjamos and Smith, 1975), and also in tomato infected by *Fusarium* and *Verticillium* (Hutson and Smith, 1980). The larger number of vessels with tyloses in resistant plants than in susceptible plants in this study suggests that tyloses were associated with resistance. Blockage of the transpiration flow by tyloses may prevent the upward movement of conidia and create a static environment within the vessels in which phytoalexins and lytic enzymes can operate effectively (Tjamos and Smith, 1975). Marley and Hillocks (1993) observed the formation of tyloses in very low numbers in wilt resistant plants of pigeonpea inoculated with *F. udum* 15 days after inoculation. This contrasts with the current observations where tylose formation in pigeonpea occurred in low numbers 3 days after inoculation but increased from 7 days onwards, and that they occurred in reasonable numbers and could therefore have contributed to resistance to invading *F. udum*. The maximum occlusion of vessels by tyloses in resistant plants of variety ICP 8863 and C-11 were 22.5% and 17.5%, respectively, and 8.0% in susceptible variety (KAT 60/8) 21 days after root-dip inoculation. Beckman *et al.* (1972), however, reported higher (48%) vessel occlusion

by tyloses in tomato plants 48 hours after inoculation when compared to the findings of the present study. Hutson and Smith (1980) observed occlusion of tomato plants due to *Fusarium* at a maximum of 32.8%, 31.4%, 28.3% and 39.8% vessels containing tyloses at 7, 14, 21 and 28 days after inoculation, respectively. Blackhurst and Wood (1963) reported that the percentage of vessels occluded by tyloses in tomato, 19 days after root inoculation with *Verticillium albo-atrum*, was higher in wilt resistant variety Loran Blood (36%) than in the susceptible variety Ailsa Craig (23%).

Differences in tylose formation were observed between *F. udum* isolates MS04 and TN05. Pennypacker and Nelson (1972) also observed differences in isolates of *F. oxysporum* f.sp. *dianthi* in pathogenesis in carnation plants. It was found in the present study that isolate MS04 was more virulent than isolate TN05. It is possible that different isolates of *F. udum* synthesize cell material or other metaboloites at different concentrations that could affect pathogenesis in pigeonpea plants.

Clear differences in tylose formation were observed between resistant and susceptible varieties of pigeonpea. Resistant varieties had higher tylose formation, with ICP 8863 having more tyloses between 3 and 14 days after inoculation. Differences between resistant and susceptible varieties were observed in the vertical spread of the pathogen when five *F. udum* isolates were tested. In the present study, pigeonpea varieties ICP 8863, C-11 and KAT 60/8 were categorized as resistant, moderately susceptible, and susceptible, respectively. Marley and Hillocks (1993) found that *F. udum* pathogen spread in the full length of the stem measured (up to 20.8 cm) in both resistant (ICP 9145) and susceptible (Malawi local) plants of pigeonpea. They also observed that although the pathogen was found in the full length of the stem measured in the resistant variety, the plants did not show any wilt symptoms, which is similar to the current findings where variety ICP 8863 was found to be resistant to wilt and yet the pathogen was isolated at up to 25 cm upward the stem. Tyloses and gels in resistant pigeonpea plants could play a role in allowing the accumulation of phytoalexins that could then prevent *F. udum* from producing secondary conidia in xylem vessels or reduce the fungal activity. This could explain

the reason why the fungus was isolated at upto 25 cm in resistant plants and yet no visible wilt symptoms could be observed. Mace (1978) observed that the combined effects of rapid tylosis and terpenoid aldehyde phytoalexin synthesis are needed for the resistance of cotton plants to *Verticillium* wilt. Rapid occlusion of vessels in resistant plants prevents systemic distribution of secondary conidia and allows subsequent accumulation of phytoalexins within the localized infection sites. Once the pathogen is effectively localized in wilt resistant plants, formation of new, non-infected xylem tissue enables the plants to compensate for vessels occluded by responses to the primary infections.

Pathogenesis in diseases caused by vascular wilt fungi depends upon the entry of the organism into the xylem of the host, and its continued spread through the vascular system, although other conditions may have to be fulfilled before symptoms are expressed (Talboys, 1972). The present study has shown that vascular occlusion due to gels and tylose formation in wilt resistant pigeonpea plants inoculated with *F. udum* may serve to complement the antifungal effect of cajanol and other phytoalexins in inducing resistance to wilt as proposed by Marley and Hillocks (1993), and as reported by Tjamos and Smith (1975) in tomato plants inoculated with *Verticillium*.

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Fusarium wilt caused by *F. udum* is the most common disease of pigeonpea in Kenya. The disease was widely distributed in the pigeonpea growing districts in Kenya with wilt prevalence, incidence and incidence range of 64%, 8.4% and 0-96.1%, respectively. According to the present study *Fusarium* wilt was not found in Kilifi district. The presence of *F. udum* has been documented in Taita-Taveta and Malindi districts for the first time. *Fusarium* wilt is more common in lower midlands (LM) than upper midlands (UM) or lowlands (L), and can be found from 40 to 1820 m.a.s.l. Sole cropping system supported more wilt than intercrop system while improved and local pigeonpea varieties showed no differences in wilt incidence. Multilocational trials of improved pigeonpea varieties for wilt resistance should be a priority in the management of the disease. Where possible, farmers should be encouraged to intercrop local varieties of pigeonpea with cereals such as sorghum in order to reduce wilt incidence.

The root-dip inoculation technique was found to be a better method of inoculating *F. udum* pathogen into pigeonpea plants in the glasshouse experiments than colonized whole rice grain technique. Screening of pigeonpea germplasm and breeding material in the glasshouse using root-dip inoculation technique at optimum inoculum concentration for wilt resistance should be conducted before field evaluation.

The combined strategy of classifying isolates of *F. udum* by cultural characteristics, conidial measurements, virulence on wilt susceptible pigeonpea varieties, physiological race typing, VCG and AFLP analysis is extremely useful in the understanding of the variability that exist in this economically important pathogen. More than one distinct group of pathogenic isolates of *F. udum* was identified using these techniques, with an exception of VCG with one group. Physiologic races and VCG appeared to be independent of the other techniques. Only AFLP group III could be linked to moderate virulence, otherwise, AFLP appeared independent of the other

techniques. There was geographic (district and/or AEZs) pattern among some groups of isolates of *F. udum* using cultural characteristics, conidial measurements, virulence and AFLP analysis, and not with, physiologic races and VCG. However, differences in isolates of *F. udum* from the same locality and even from the same pigeonpea plant are common. Genetic variation of *F. udum* was revealed using AFLP analysis and physiological race typing, and not with VCG. However, to draw more conclusions on genetic variability in *F. udum*, more work involving large samples of isolates from various countries should be subjected to VCG, AFLP analysis and physiological race typing using standard differentials. In pigeonpea improvement programmes aimed at breeding for *Fusarium* wilt resistance, testing of pigeonpea germplasm should be done in wilt 'sick' soils at different locations. Laboratory and glasshouse experiments should utilize *F. udum* isolates with varying virulence levels from different geographical origin.

Fusarium wilt susceptible pigeonpea plants inoculated with *F. udum* show more hyphal colonization in the root and stem tissues, and low xylem vessel occlusion by tyloses and gels. Wilt resistant pigeonpea plants inoculated with *F. udum*, however, had low hyphal colonization in the root and stem tissues, and high occlusion of the xylem vessels by tyloses and gels. It can be concluded that tyloses and gels, formed in wilt resistant plants due to infection by *F. udum* could possibly be contributing to resistance in pigeonpea. They may also serve to complement the antifungal effect of phytoalexins in inducing resistance in pigeonpea to *F. udum*. Pigeonpea germpasm that readily produce tyloses and phytoalexins upon infection with *F. udum* are potential sources of wilt resistance.

6.2 Recommendations

1 Work to be initiated on the role of soil types and soil pH, on the distribution of *F. udum* in the lower Coastal areas where no or low incidence of *Fusarium* wilt were recorded.

2. Based on the low incidence of *Fusarium* wilt at altitudes below 400 m above sea level, intensification of pigeonpea growing is highly recommended to improve on the country's output and food security.

3. More intensive surveys could be initiated at locational level within the districts in order to clearly capture the role of pigeonpea types and cropping system in *Fusarium* wilt prevalence and incidence, and to determine the economic losses incurred by the farmers due to this disease.

4. Future studies on the characterization of *F. udum* using physiological race typing, VCG and AFLP analysis should focus on variability at farm level within pigeonpea growing districts using larger sample size of pathogenic and non-pathogenic isolates for systematic mapping of genetic variability of this fungus.

5. Development and/or standardization of pigeonpea differential cultivars and identification of resistance genes for *Fusarium* wilt in the host should be one of the priority research areas.

6. The use of other chlorate media and varying the percentage of potassium chlorate to generate a large number of NitM tester mutants of *F. udum* for VCG should be investigated.

7. AFLP analysis of *F. udum* isolates could be modified in order to generate more fragments and possibly higher polymorphism by using primer pairs *Eco*R I + 1 (or +2) and *Mse* I + 2 selective nucleotides. The optimisation of DNA extraction technique for *F. udum* should also be investigated since high quality DNA is necessary for AFLP analysis.

8. Studies are required to determine the relationship between the mechanical barriers (tyloses, gels) and the chemical barriers (phytoalexins), and especially the localization of the latter, in inducing resistance to *Fusarium* wilt in resistant pigeonpea plants.

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8.0 APPENDICES

Appendix 8.1. Hactarage and yield of four major grain legumes in Kenya

Crop	Acreage (Ha)	Yield (Tones)	Yield/ha
Common bean	589,800	466,057	0.8
Cowpea	119,420	64,640	0.5
Pigeonpea	87,530	90,430	1.0
Grams	62,670	33,470	0.5

Source: Ministry of Agriculture, Government of Kenya, Annual Report, 1990.

Appendix 8.2. Mycological colour key for pigment identification on cultures of *F. udum*

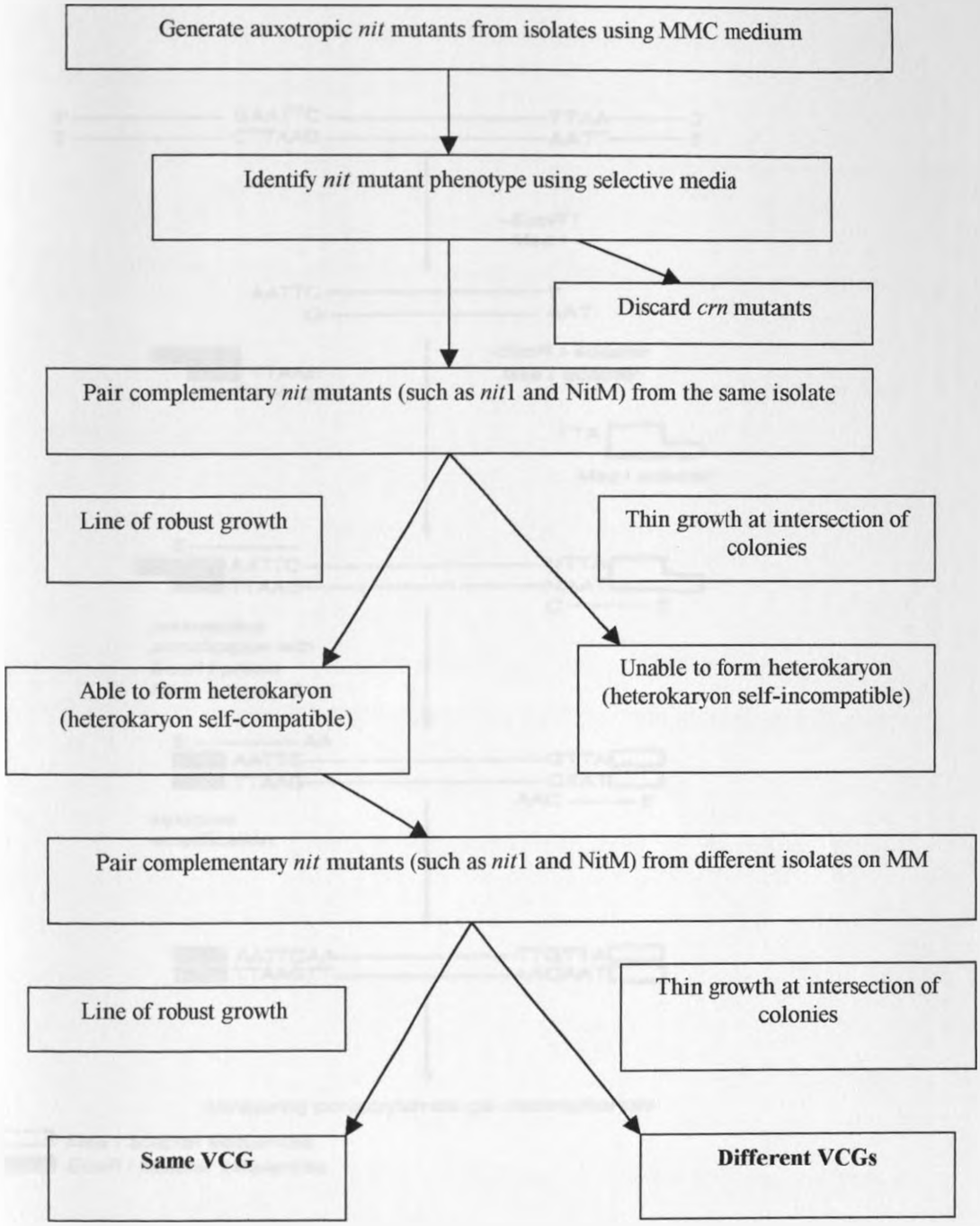
Colour Name	Number	ISCC-NBS Name
Lilac	-	Light purple
Pale luteous	11	Light yellow
Buff	-	Pale yellow ("cream")
Dark purple	36	Dark deep reddish purple
White	-	White
Purple	35	Strong reddish purple
Rose	1	Purplish pink
Luteous	12	Vivid yellow
Mauve	34	Brilliant purple
Flax blue	52	Light purplish blue
Vinaceous buff	86	Light yellowish brown
Glaucous sky blue	93	Pale blue

ISCC = Inter-Society Colour Council of America

NBS = National Bureau of Standards

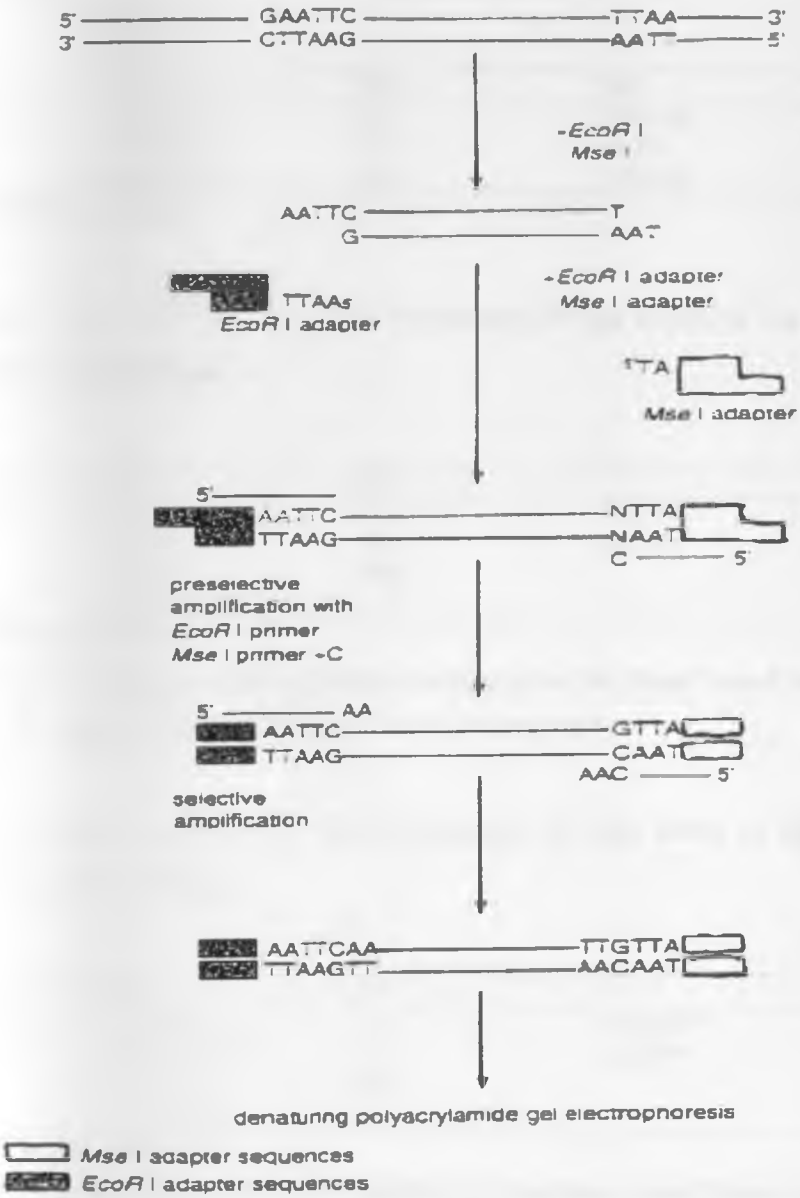
Source: Rayner, 1970.

Appendix 8.3. Vegetative compatibility group (VCG) identification procedure



Source: Adopted from VCG procedure of Leslie (1990). *Crn* mutants are mutants from sectors that are resistant to chlorate but still able to utilize nitrate as a sole nitrogen source.

Appendix 8.4. An example of the AFLP procedure using one primer pair



N = Selective nucleotide

Appendix 8.5. Analysis of variance (ANOVA) tables

Appendix 8.5.1. ANOVA for field *Fusarium* wilt incidence of pigeonpea in Kenya, June-September 1997

Source	SS	DF	MS	F
Farm	110813.34	85	1303.69	47.89**
Error	7023.21	258	27.22	
Total	117836.55	343	1330.91	

**Significant at 1% level

Appendix 8.5.2. ANOVA by GLM procedure for the effect of the district on field *Fusarium* wilt incidence

Source[1]	SS	DF	F
District	21026.42	12	64.37**
Farm	89786.92	73	45.18**
Error	7023.21	258	
Total	117836.55	343	

**Significant at 1% level

[1]Values for the interaction between district and farm, and the Mean Sum of Squares are missing because the number of farms sampled per district were not equal.

Appendix 8.5.3. ANOVA by GLM procedure for the effect of the AEZ on field *Fusarium* wilt incidence

Source[1]	SS	DF	F
AEZ	27429.54	8	125.96**
Farm	83383.80	77	39.78**
Error	7023.21	258	
Total	117836.55	343	

**Significant at 1% level

[1]Values for the interaction between AEZ and farm, and the Mean Sum of Squares are missing because the number of farms sampled per AEZ were not equal.

Appendix 8.5.4. ANOVA by GLM procedure for the effect of altitude on field *Fusarium* wilt incidence

Source[1]	SS	DF	F
Altitude	18775.59	7	98.53**
Farm	92037.75	78	43.35**
Error	7023.21	258	
Total	117836.55	343	

**Significant at 1% level

[1]Values for the interaction between altitude and farm, and the Mean Sum of Squares are missing because the number of farms sampled per altitude range were not equal.

Appendix 8.5.5. ANOVA by GLM procedure for the effect of pigeonpea type (variety) on field *Fusarium* wilt incidence

Source[1]	SS	DF	F
Pigeonpea	432.21	2	7.94**
Farm	110381.13	83	48.85**
Error	7023.21	258	
Total	117836.55	343	

**Significant at 1% level

[1]Values for the interaction between pigeonpea types (varieties) and farm, and the Mean Sum of Squares are missing because the number of farms sampled per pigeonpea type were not equal

Appendix 8.5.6. ANOVA by GLM procedure for the effect of cropping system on field *Fusarium* wilt incidence

Source[1]	SS	DF	F
Cropping system	243.39	1	8.94**
Farm	110569.95	84	48.36**
Error	7023.21	258	
Total	117836.55	343	

**Significant at 1% level

[1]Values for the interaction between cropping system and farm, and the Mean Sum of Squares are missing because the number of farms sampled per cropping system were not equal.

Appendix 8.5.7. ANOVA for wilt incidence on pigeonpea varieties inoculated with *F. udum* using two techniques

Source	SS	DF	MS	F
Technique	132759.38	1	132759.38	1296.09**
Variety	220136.46	3	73378.82	716.38**
Isolate	84.38	1	84.38	0.82^{ns}
Time	6542.71	5	1308.54	12.77**
Technique x variety	108261.46	3	36087.15	352.31**
Technique x isolate	651.04	1	651.04	6.36**
Technique x time	384.38	5	76.88	0.75^{ns}
Variety x isolate	953.13	3	317.71	3.10*
Variety x time	5344.79	15	356.32	3.48**
Isolate x time	209.38	5	41.88	0.41^{ns}
Technique x variety x isolate	1186.46	3	395.49	3.86**
Technique x variety x time	769.79	15	51.32	0.50^{ns}
Variety x isolate x time	428.13	15	28.54	0.28^{ns}
Technique x variety x isolate x time	337.50	20	16.88	0.16^{ns}
Error	29500.00	288	102.43	
Total	507548.96	383	1325.19	

*Significant at 5% level.

**Significant at 1% level.

^{ns}Not significant at 5% level.

Appendix 8.5.8. ANOVA for colony diameter of *F. udum* isolates after 8 days of incubation on PDA medium

Source	SS	DF	MS	F
Isolates	2799.40	78	35.89	90.49**
Error	62.67	158	0.40	
Total	2862.07	236	36.29	

**Significant at 1% level

Appendix 8.5.9. ANOVA by GLM procedure for the effect of the district on colony diameter of *F. udum* isolates from Kenya

Source[1]	SS	DF	F
District	868.44	11	201.10**
Isolate	1558.03	63	63.00**
Error	58.89	150	
Total	2485.35	224	

**Significant at 1% level

[1]Values for the interaction between district and isolate, and the Mean Sum of Squares are missing because the number of isolates sampled per district were not equal.

Appendix 8.5.10. ANOVA by GLM procedure for the effect of the AEZ on colony diameter of *F. udum* isolates from Kenya

Source[1]	SS	DF	F
AEZ	329.31	7	119.84**
Isolate	2097.15	67	79.73**
Error	58.89	150	
Total	2485.35	224	

**Significant at 1% level

[1]Values for the interaction between AEZ and isolate, and the Mean Sum of Squares are missing because the number of isolates sampled per AEZ were not equal.

Appendix 8.5.11. ANOVA by GLM procedure for the effect of altitude on colony diameter of *F. udum* isolates from Kenya

Source[1]	SS	DF	F
Altitude	278.53	6	118.25**
Isolate	2147.94	68	80.46**
Error	58.89	150	
Total	2485.35	224	

**Significant at 1% level

[1]Values for the interaction between altitude and isolate, and the Mean Sum of Squares are missing because the number of isolates sampled per altitude range were not equal.

Appendix 8.5.12. ANOVA for sporulation of *F. udum* isolates after 8 days of incubation on PDA medium

Source	SS	DF	MS	F
Isolates	51.83	78	0.66	19.75**
Error	5.32	158	0.03	
Total	57.14	236	0.70	

**Significant at 1% level

Appendix 8.5.13. ANOVA by GLM procedure for the effect of the district on sporulation of *F. udum* isolates from Kenya

Source[1]	SS	DF	F
District	7.83	11	20.29**
Isolate	43.43	63	19.66**
Error	5.26	150	
Total	56.52	224	

**Significant at 1% level

[1]Values for the interaction between district and isolate, and the Mean Sum of Squares are missing because the number of isolates sampled per district were not equal.

Appendix 8.5.14. ANOVA by GLM procedure for the effect of the AEZ on sporulation of *F. udum* isolates from Kenya

Source[1]	SS	DF	F
AEZ	4.76	7	19.41**
Isolate	46.50	67	19.79**
Error	5.26	150	
Total	56.52	224	

**Significant at 1% level

[1]Values for the interaction between AEZ and isolate, and the Mean Sum of Squares are missing because the number of isolates sampled per AEZ were not equal.

Appendix 8.5.15. ANOVA by GLM procedure for the effect of altitude on sporulation of *F. udum* isolates from Kenya

Source[1]	SS	DF	F
Altitude	2.98	6	14.18**
Isolate	48.27	68	20.25**
Error	5.26	150	
Total	56.52	224	

**Significant at 1% level

[1]Values for the interaction between altitude and isolate, and the Mean Sum of Squares are missing because the number of isolates sampled per altitude range were not equal.

Appendix 8.5.16. ANOVA for virulence (% wilt) of *F. udum* isolates on wilt susceptible pigeonpea variety KAT 60/8 six weeks after root-dip inoculation

Source	SS	DF	MS	F
Isolates	73498.73	78	942.29	6.81**
Error	32800.00	237	138.40	
Total	106298.73	315	1080.69	

**Significant at 1% level

Appendix 8.5.17. ANOVA by GLM procedure for the effect of the district on virulence (% wilt) of *F. udum* isolates from Kenya on variety KAT 60/8

Source[1]	SS	DF	F
District	18658.28	11	12.35**
Isolate	53336.39	63	6.16**
Error	30900.00	225	
Total	102894.67	299	

**Significant at 1% level

[1]Values for the interaction between district and isolate, and the Mean Sum of Squares are missing because the number of isolates sampled per district were not equal.

Appendix 8.5.18. ANOVA by GLM procedure for the effect of the AEZ on virulence (% wilt) of *F. udum* isolates from Kenya on variety KAT 60/8

Source[1]	SS	DF	F
AEZ	7171.78	7	7.46**
Isolate	64822.89	67	7.04**
Error	30900.00	225	
Total	102894.67	299	

**Significant at 1% level

[1]Values for the interaction between AEZ and isolate, and the Mean Sum of Squares are missing because the number of isolates sampled per AEZ were not equal.

Appendix 8.5.19. ANOVA by GLM procedure for the effect of altitude on virulence (% wilt) of *F. udum* isolates from Kenya on variety KAT 60/8

Source[1]	SS	DF	F
Altitude	8511.25	6	10.33**
Isolate	63483.42	68	6.80**
Error	30900.00	225	
Total	102894.67	299	

**Significant at 1% level

[1]Values for the interaction between altitude and isolate, and the Mean Sum of Squares are missing because the number of isolates sampled per altitude range were not equal.

Appendix 8.5.20. ANOVA for virulence (% wilt) of 21 isolates of *F. udum* on 7 pigeonpea varieties six weeks after root-dip inoculation

Source	SS	DF	MS	F
Isolate	9710.66	20	485.53	2.91**
Variety	227120.18	6	37853.36	226.81**
Isolate x variety	40117.91	120	334.32	2.00**
Error	49066.67	294	166.89	
Total	326015.42	440	38840.11	

**Significant at 1% level

Appendix 8.5.21. ANOVA for percentage (%) of xylem vessels having tyloses in resistant and susceptible pigeonpea varieties inoculated with *F. udum*

Source	SS	DF	MS	F
Variety	1422.21	2	711.11	118.06**
Isolate	371.58	1	371.58	61.69**
Variety x isolate	175.70	2	87.85	14.58**
Section	327.87	1	327.87	54.43**
Variety x section	10.19	2	5.10	0.85^{ns}
Isolate x section	14.36	1	14.36	2.38^{ns}
Variety x isolate x section	1.05	2	0.53	0.09^{ns}
Days	3563.99	3	1188.00	197.24**
Variety x days	621.07	6	103.51	17.19**
Isolate x days	103.79	3	34.60	5.74**
Variety x isolate x days	89.51	6	14.92	2.48**
Section x days	197.69	3	65.90	10.94**
Variety x section x days	200.72	6	33.45	5.55**
Isolate x section x days	104.35	3	34.78	5.77**
Variety x isolate x section x days	39.26	6	6.54	1.09^{ns}
Error	867.34	144	6.02	
Total	8110.65	191	42.46	

**Significant at 1% level

^{ns}Not significantly different at 5% level.

Appendix 8.5.22. ANOVA for virulence (% wilt) of 5 isolates of *F. udum* on 3 pigeonpea varieties eight weeks after root-dip inoculation

Source	SS	DF	MS	F
Variety	48764.44	2	24382.22	171.44**
Isolate	1191.11	4	297.78	2.09^{ns}
Variety x isolate	5635.56	8	704.45	4.95**
Error	4266.67	30	142.22	
Total	59857.78	44	1360.40	

**Significant at 1% level

^{ns}Not significant at 5% level

Appendix 8.6 Media

Appendix 8.6.1 Potato dextrose agar (PDA)

PDA (Difco)	39 g
Distilled water	1 l

Appendix 8. 6.2 PDA + streptomycin

PDA (Difco)	39 g
Streptomycin	133 mg
Distilled water	1 l

Appendix 8. 6.3 Tap Water agar

Agar	20 g
Chlorine (Cl)	6 g
Tap water	1 l

Appendix 8.6.4 SNA medium

KH_2PO_4	1.0 g
KNO_3	1.0 g
$MgSO_4.7H_2O$	0.5 g
KCl	0.5 g
Glucose	0.2 g
Sucrose	0.2 g
Agar	20 g
Distilled water	1 l

Appendix 8. 6.5 Czapek Dox plus AZ liquid medium (CDAZ)

Czapek Dox salts (Oxoid)	33.4 g
AZ solution	10 ml
Distilled water	1 l

AZ mineral salts solutions:

$CuS0_4.5H_20$	22 mg
$MnCl_2.4H_20$	0.1 g
$ZnCl_2$	0.1 g
$Ca(N0_3)_26H_20$	0.1 g
$BaCl_2.2H_20$	20 mg
$(NH4)_6Mo_70_{24}.4H_20$	20 mg
Distilled Water	1 l

Appendix 8. 6.6 Basal Medium

Sucrose	30 g
KH_2PO_4	1 g
$MgSO_4.7H_2O$	0.5 g
KCl	0.5 g
$FeSO_4.7H_2O$	0.01 g
Agar	20 g
Trace element solution	0.2 ml
Distilled Water	1 l

Trace Element Solution:

Citric acid	5 g
$ZnSO_4.7H_2O$	5 g
$Fe(NH_4)_2(SO_4)_2.6H_2O$	1 g
$CuSO_4.5H_2O$	0.25 g
$MnSO_4.H_2O$	0.05 g
H_3BO_4	0.05 g
$NaMoO_4.2H_2O$	0.05 g
Distilled Water	95 ml

Appendix 8. 6.7 Minimal agar medium with chlorate (MMC)

Basal medium	1 l
L-asparagine	1.6 g
$NaNO_3$	2 g
$KClO_3$	15 g

Appendix 8. 6.8 Minimal Medium (MM) (= nitrate medium)

Basal medium	1 l
$NaNO_3$	2 g

Appendix 8. 6.9 Nitrite ($NaNO_2$) Medium

Basal medium	1 l
$NaNO_2$	0.5 g

Appendix 8. 6.10 Hypoxanthine Medium

Basal medium	1 l
Hypoxanthine	0.2 g

Appendix 8. 6.11 Ammonium Medium

Basal medium	1 l
Ammonium tartrate	1 g

Appendix 8. 6.12 Uric acid Medium

Basal medium	1 l
Uric acid	0.2 g

Appendix 8.7 Reagents and solutions/buffers for DNA extraction and AFLP analysis

Appendix 8.7.1 Reagents

(i) Liquid nitrogen

(ii) Isopropanol

(iii) Ethanol

(iv) RNase A (Roche Diagnostics Gmbh, Mannheim, Germany)

(v) Sodium acetate

(vi) Undigested *Hind* III of λ DNA 20 ng/µl (Roche Diagnostics Gmbh, Mannheim, Germany)

(vii) Sodium hydroxide (NaOH)

(viii) *Taq* DNA polymerase (Roche Diagnostics Gmbh, Mannheim, Germany)

(ix) [γ-^{32}P]ATP (Amersham International plc, Buckinghamshire, UK)

(x) Silicone oil

(xi) Kodak liquid X-ray developer (Eastman Kodak Co.; USA)

(xii) Kodak rapid fixer (Eastman Kodak Co.; USA)

Appendix 8.7.2 Solutions/buffers

Appendix 8.7.2.1 CTAB extraction buffer (100 ml):

2% (w/v) 3-D-cetyltrimethylammonium bromide (CTAB)	2 g
1.4 M Sodium chloride	8.2 g
0.2% (w/v) Beta-mercaptoethanol	740 mg
20 mM EDTA	1.2 g
100 mM Tris-HCl (pH 8.0)	1 g
1% Polyvinylpyrodilone (PVP-25)	200 µl

Appendix 8.7.2.2 Chloroform:isoamyl alcohol (24:1 v/v) (250 ml):

Chloroform	240 ml
Isoamyl alcohol	10 ml

Appendix 8.7.2.3 Phenol: Chloroform:isoamyl alcohol (25:24:1 v/v/v) (500 ml):

Phenol	250 ml
Chloroform	240 ml
Isoamyl alcohol	10 ml

Appendix 8.7.2.4 Washing buffer (100 ml):

76% (v/v) Ethanol	100 ml
10 mM Ammoniumacetate	0.08 g

Appendix 8.7.2.5 TE buffer (500 ml):

10 mM Tris-HCl (pH 7.4)	0.61 g
1 mM EDTA	0.19 g
Distilled deionised water	500 ml

Appendix 8.7.2.6 RNase A (1 ml):

RNase A	20 mg
TE buffer	900 µl

Appendix 8.7.2.7 Ehtidium bromide (100 ml):

Ethidium bromide	1 g
Distilled deionised water	100 ml

Appendix 8.7.2.8 TAE 50x (500 ml):

Tris	242 g
Distilled deionised water	700 ml
Glacial acetic acid	57.1 ml
0.5 M EDTA pH 8.0	100 ml

Appendix 8.7.2.9 TAE 1x (500 ml):

TAE 50x	10 ml
Distilled deionised water	490 ml

Appendix 8.7.2.10 Agarose gel (50 ml):

1% Agarose	0.5 g
TAE 1x	50 ml
Ethidium bromide	5 µl

Appendix 8.7.2.11 Loading dye (5 ml):

0.25% Bromophenol blue	12.5 mg
50% Glycerol	2.5 mg
0.5 M EDTA	1000 µl

Appendix 8.7.2.12 Polyacrylamide (sequencing) gel 6% (60 ml):

Urea	25.2 g
40% Acrylamide	9 ml
TBE 10X	6 ml
Sterile distilled water	60 ml
10% Ammonium persulfate (APS)	250 µl
TEMED (Pharmacia, Uppsala, Sweden)	50 µl

Appendix 8.7.2.13 Acrylamide 40%:

Acrylamide (Sigma, St. Louis, MO, USA)	38 g
Bisacrylamide (Sigma, St. Louis, MO, USA)	2 g
Distilled water	100 ml

Appendix 8.7.2.14 TBE 10X (1 litre):

0.83 M Boric acid	51.35 g
1 M TrisHCl	121.10 g
10 mM EDTA	3.72 g
Distilled water	1 l

Appendix 8.7.2.15 Primer labelling with ^{32}P (50 µl):

*Eco*R I (select one)	18 µl
5X kinase buffer	10 µl
[γ-^{32}P]ATP (3,000 Ci/mmol)	20 µl
T4 kinase	2 µl

Appendix 8.7.2.16 Radioactive λ *Hind* III DNA (100 bp) marker (30 µl):

Buffer H (Roche Diagnostics Gmbh, Mannheim, Germany)	3 µl
λ *Hind* III DNA (250 µg/ml)	16 µl
Mix dA, dC, dTTP 5 mM	1 µl
80 µCi [α-^{32}P]dGTP 3,000 mCi/mmol (Ammersham International plc, Buckinghamshire, UK)	8 µl
1 U Klenow (1 U/µl) (Roche Diagnostics Gmbh, Mannheim, Germany)	1 µl
Distilled water	1 µl

Appendix 8.7.2.17 Repel silane (10 ml):

Dimethyldichlorosilane	200 µl
Chloroform	9.8 ml

Appendix 8.7.2.18 Bind silane (6.26 ml):

Ethanol (absolute)	5 ml
Bind silane	15 µl
Acetic acid	1.25 ml

Appendix 8.7.2.19 Formamide dye:

98% (w/v) formamide

10 mM EDTA

0.1% (w/v) bromophenol blue

0.1% (w/v) xyline cyanol

Appendix 8.7.2.20 Gel fixing solution (2000 ml):

10% acetic acid	200 ml
Distilled water	1800 ml

Appendix 8.7.3 Reagents and solutions provided in AFLP system II kit

(i) *Eco*R I/*Mse* I [1.25 units/µl each in 10 mM Tris-HCl (pH 7.5), 50 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.1 mg/ml BSA, 50% (v/v) glycerol, 0.1% Triton X-100]

(ii) 5X reaction buffer [50 mM Tris-HCl (pH 7.5), 50 mM Mg-acetae, 250 mM K-acetate]

(iii) Distilled water

(iv) Adapter/ligation solution [*Eco*R I/*Mse* I adapters, 0.4 mM ATP, 10 mM Tris-HCl (pH 7.5), 10 mM Mg-acetate, 50 mM K-acetate]

(v) T4 DNA ligase [1 unit/µl in 10 mM Tris-HCl (pH 7.5), 1 mM DTT, 50 mM KCl, 50% glycerol (v/v)]

(vi) TE buffer [10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA]

(vii) Pre-amp primer mix

(viii) T4 kinase [10 units/µl in 50 mM Tris-HCl (pH 7.6), 25 mM KCl, 1 mM 2-mercaptoethanol, 0.1 µM ATP, 50% (v/v) glycerol]

(ix) 5X kinase buffer [350 mM Tris-HCl (pH 7.6), 50 mM $MgCl_2$, 500 mM KCl, 5 mM 2-mercaptoethanol]

(x) *Eco*R I primers (27.8 ng/µl): E-AA, E-AC, E-AG, E-AT, E-TA, E-TC, E-TG, E-TT

(xi) *Mse* I primers (6.7 ng/µl, dNTPs): M-CAA, M-CAC, M-CAG, M-CAT, M-CTA, M-CTC, M-CTG, M-CTT

(xii) 10X PCR buffer plus Mg [200 mM Tris-HCl (pH 8.4), 15 mM $MgCl_2$, 500 mM KCl]

Appendix 8.8 Solutions/buffers for microscopy

Appendix 8.8.1 Primary fixative:

2% sucrose

5% glutaraldehyde

0.1 M piperazine-N,N'-bis (2-ethane-sulfonic acid) (PIPES) pH 8.0

Appendix 8.8.2 Washing buffer:

2% sucrose

0.1 M PIPES pH 8.0

Appendix 8.8.3 Secondary fixative:

2% osmium tetraoxide (OsO_4)

0.2 M PIPES pH 6.8

Appendix 8.8.4 Resin (450 ml):

Araldite CY212 (Sigma, St. Louis, MO, USA)	100 ml
Agar 100 resin	100 ml
Dodecenyl succinic anhydride (DDSA)	250 ml

Appendix 8.8.5 LM stain solution:

1% toluene blue O

1% borax

Appendix 8.8.6 Urenyl acetate stain for TEM:

Urenyl acetate

50% ethanol

Appendix 8.9 Matrices

Appendix 8.9.1. Pairwise similarity matrix (Dice coefficient) among physiologic races of *F. udum*

Race	0	8	16	24	32	48	56	33	34	42	52
0											
8	0										
16	0	0									
24	0	67	67								
32	0	0	0	0							
48	0	0	67	50	67						
56	0	50	50	80	50	80					
33	0	0	0	0	67	50	40				
34	0	0	0	0	67	50	40	50			
42	0	50	0	40	50	40	67	40	80		
52	0	0	50	40	50	80	67	40	40	33	

Similarity average = 28

Sample standard deviation = 34.

Appendix 8.9.2 Matrix of similarity among 56 isolates of *F. udum* generated from binary banding data of 7 primer combinations using Nei and Li (1979) distance index using RFLPfrag program

	MAL01a	MB01	MB03	MB04	MB05	MB07	MK01	MK02	MK03	MK05	MK08	MK10	KR01	KR02	KR03	KT01	KT02	KT03	MR02	MR03	MR04
MB01	0.997																				
MB03	0.996	0.996																			
MB04	0.997	0.996	0.998																		
MB05	0.980	0.967	0.972	0.974																	
MB07	0.989	0.994	0.991	0.993	0.980																
MK01	0.998	0.994	0.995	0.996	0.979	0.991															
MK02	0.997	0.995	0.995	0.997	0.977	0.992	0.996														
MK03	0.996	0.994	0.998	0.997	0.974	0.990	0.996	0.996													
MK05	0.995	0.992	0.997	0.997	0.975	0.991	0.996	0.995	0.998												
MK08	0.996	0.991	0.999	0.997	0.971	0.990	0.995	0.995	0.999	0.998											
MK10	0.996	0.993	0.996	0.996	0.981	0.986	0.994	0.995	0.996	0.996	0.996										
KR01	0.995	0.991	0.998	0.997	0.975	0.991	0.996	0.995	0.999	0.999	0.999	0.998									
KR02	0.995	0.994	0.999	0.996	0.949	0.990	0.991	0.992	0.996	0.996	0.998	0.995	0.996								
KR03	0.996	0.993	0.999	0.997	0.971	0.991	0.995	0.995	0.998	0.998	0.999	0.996	1.000	0.998							
KT01	0.997	0.996	0.994	0.995	0.967	0.992	0.993	0.995	0.992	0.990	0.992	0.991	0.989	0.994	0.992						
KT02	0.998	1.000	0.998	0.999	0.981	0.994	0.999	0.999	0.998	0.996	0.997	0.994	0.997	0.995	0.998	0.998					
KT03	0.997	0.999	0.997	1.000	0.975	0.992	0.999	0.997	0.997	0.997	0.995	0.993	0.997	0.995	0.997	0.996	0.999				
MR02	0.976	0.967	0.970	0.965	0.985	0.977	0.980	0.964	0.972	0.975	0.972	0.979	0.969	0.967	0.967	0.967	0.975	0.976			
MR03	0.988	0.985	0.989	0.987	0.979	0.982	0.992	0.987	0.990	0.991	0.991	0.991	0.989	0.987	0.988	0.987	0.989	0.986	0.979		
MR04	0.992	0.985	0.994	0.991	0.976	0.985	0.993	0.989	0.993	0.992	0.998	0.994	0.991	0.998	0.993	0.992	0.989	0.985	0.970	0.986	
MR05	0.995	0.992	0.998	0.997	0.963	0.990	0.993	0.992	0.996	0.998	0.997	0.995	0.999	0.997	0.998	0.990	0.994	0.995	0.979	0.991	0.996
MR06	0.990	0.978	0.986	0.985	0.976	0.980	0.985	0.985	0.986	0.986	0.987	0.993	0.985	0.985	0.985	0.980	0.985	0.982	0.977	0.988	0.983
MR07	0.992	0.991	0.992	0.995	0.984	0.993	0.991	0.993	0.992	0.992	0.991	0.989	0.995	0.986	0.991	0.990	0.992	0.990	0.985	0.989	0.984
MS01	0.997	0.995	0.997	0.999	0.973	0.992	0.995	0.996	0.996	0.996	0.997	0.995	0.996	0.996	0.997	0.995	0.998	0.998	0.965	0.986	0.992
MS03	0.996	0.998	0.998	0.998	0.973	0.993	0.996	0.996	0.996	0.995	0.996	0.993	0.995	0.997	0.996	0.998	0.999	0.999	0.970	0.986	0.992
MS04	0.995	0.989	0.994	0.992	0.977	0.988	0.994	0.993	0.995	0.995	0.995	0.994	0.995	0.992	0.995	0.989	0.993	0.992	0.974	0.990	0.991
MS05	0.970	0.957	0.968	0.966	0.988	0.971	0.973	0.969	0.970	0.969	0.974	0.978	0.971	0.960	0.969	0.968	0.971	0.962	0.988	0.979	0.973
MS06	0.994	0.994	0.997	0.995	0.973	0.988	0.994	0.993	0.996	0.995	0.996	0.993	0.995	0.996	0.996	0.992	0.996	0.996	0.971	0.989	0.992
MS07	0.997	0.994	0.998	0.998	0.975	0.991	0.998	0.997	0.998	0.997	0.998	0.995	0.997	0.996	0.996	0.994	0.998	0.998	0.972	0.990	0.991
MS08	0.996	0.998	0.997	0.998	0.973	0.992	0.996	0.995	0.995	0.994	0.996	0.993	0.995	0.997	0.996	0.998	0.999	0.998	0.976	0.988	0.996
MS09	0.992	0.987	0.992	0.989	0.980	0.983	0.994	0.989	0.994	0.992	0.995	0.994	0.991	0.991	0.991	0.989	0.991	0.988	0.974	0.992	0.994
MS10	0.978	0.968	0.976	0.974	0.990	0.975	0.976	0.977	0.977	0.978	0.980	0.981	0.976	0.976	0.977	0.974	0.973	0.968	0.988	0.983	0.981

Appendix 8.9.2 Matrix of similarity among 56 isolates of *F. udum* generated from binary banding data of 7 primer combinations using Nei and Li (1979) distance index using RFLPfrag program - continued

	MAL01a	MB01	MB03	MB04	MB05	MB07	MK01	MK02	MK03	MK05	MK08	MK10	KR01	KR02	KR03	KT01	KT02	KT03	MR02	MR03	MR04
NB01	0.998	0.997	0.997	0.996	0.975	0.990	0.996	0.994	0.996	0.995	0.996	0.996	0.994	0.997	0.995	0.993	0.997	0.997	0.971	0.989	0.991
NB02	0.996	0.995	0.996	0.996	0.978	0.992	0.998	0.995	0.996	0.994	0.996	0.994	0.995	0.993	0.996	0.995	0.998	0.996	0.975	0.990	0.991
NB03	0.998	1.000	0.995	0.997	0.982	0.990	0.998	0.999	0.996	0.994	0.996	0.994	0.995	0.994	0.996	0.998	1.000	0.998	0.979	0.990	0.994
NB04	0.993	0.991	0.994	0.992	0.977	0.986	0.993	0.993	0.995	0.994	0.995	0.994	0.994	0.992	0.995	0.990	0.994	0.993	0.970	0.992	0.989
NY01	0.996	0.998	0.999	0.999	0.975	0.991	0.996	0.996	0.997	0.997	0.998	0.995	0.997	0.998	0.998	0.996	1.000	0.999	0.969	0.989	0.993
NY02	0.996	0.993	0.998	0.996	0.975	0.991	0.997	0.995	0.999	0.998	0.999	0.996	1.000	0.995	0.999	0.991	0.997	0.998	0.974	0.991	0.991
NY03	0.993	0.988	0.992	0.988	0.971	0.986	0.994	0.986	0.994	0.994	0.998	0.995	0.992	0.995	0.991	0.989	0.990	0.990	0.977	0.993	0.993
NY04	0.995	0.995	0.997	0.999	0.968	0.992	0.996	0.996	0.996	0.997	0.996	0.994	0.997	0.994	0.995	0.992	0.999	1.000	0.966	0.986	0.987
NY07	0.997	0.998	1.000	1.000	0.967	0.993	0.996	0.996	0.998	0.996	0.997	0.995	0.996	0.998	0.998	0.995	1.000	0.999	0.970	0.989	0.989
NY08	0.994	0.993	0.998	0.997	0.965	0.989	0.995	0.994	0.997	0.998	0.999	0.995	0.998	0.998	0.997	0.991	0.995	0.997	0.972	0.987	0.992
TK02	0.996	0.996	0.998	0.999	0.972	0.993	0.997	0.996	0.997	0.996	0.998	0.994	0.997	0.997	0.998	0.995	0.999	0.999	0.966	0.987	0.993
TK03	0.988	0.984	0.986	0.988	0.978	0.981	1.000	0.987	0.987	0.986	0.994	0.994	0.986	0.993	0.984	1.000	0.984	0.983	0.984	0.986	0.990
TK04	0.995	0.986	0.993	0.992	0.979	0.986	0.997	0.995	0.994	0.992	0.996	0.995	0.994	0.992	0.994	0.993	0.994	0.989	0.975	0.989	0.992
TK05	0.994	0.996	0.998	0.997	0.964	0.989	0.994	0.993	0.997	0.997	0.997	0.995	0.997	0.998	0.998	0.993	0.996	0.998	0.970	0.987	0.991
TK06	0.984	0.977	0.981	0.981	0.986	0.983	0.981	0.982	0.983	0.982	0.982	0.982	0.982	0.979	0.982	0.976	0.984	0.985	0.983	0.977	0.978
TN03	0.997	0.995	0.996	0.995	0.978	0.986	0.998	0.996	0.995	0.995	0.994	0.995	0.994	0.993	0.994	0.994	0.998	1.000	0.977	0.993	0.995
TN04	0.996	0.998	0.998	0.999	0.972	0.993	0.997	0.997	0.996	0.996	0.997	0.993	0.996	0.997	0.997	0.997	1.000	0.998	0.964	0.986	0.993
TN05	0.996	0.997	0.999	0.999	0.976	0.992	0.996	0.997	0.998	0.997	0.999	0.995	0.998	0.998	0.998	0.995	0.999	0.999	0.971	0.989	0.993
TT01	0.993	0.998	0.994	0.994	0.974	0.992	0.997	0.991	0.992	0.991	0.992	0.990	0.991	0.992	0.992	0.996	0.999	0.998	0.976	0.985	0.993
TT02	0.995	0.995	0.999	0.996	0.968	0.991	0.994	0.994	0.997	0.996	0.998	0.995	0.996	0.999	0.998	0.995	0.998	0.994	0.967	0.989	0.998
TT05	0.996	0.991	0.995	0.993	0.974	0.986	0.994	0.993	0.995	0.994	0.997	0.996	0.993	0.997	0.994	0.992	0.993	0.992	0.974	0.990	0.994
TT06	0.999	0.996	0.997	0.997	0.975	0.993	0.998	0.997	0.997	0.995	0.997	0.994	0.995	0.994	0.995	0.996	0.999	0.997	0.973	0.988	0.991
TT08	0.997	0.993	0.997	0.996	0.974	0.989	0.996	0.994	0.998	0.997	0.999	0.997	0.997	0.996	0.997	0.992	0.997	0.996	0.974	0.990	0.993

Appendix 8.9.2 Matrix of similarity among 56 isolates of *F. udum* generated from binary banding data of 7 primer combinations using Nei and Li (1979) distance index using RFLPfrag program - continued

MR05	MR05	MR06	MR07	MS01	MS03	MS04	MS05	MS06	MS07	MS08	MS09	MS10	NB01	NB02	NB03
MR06	0.987														
MR07	0.993	0.992													
MS01	0.997	0.985	0.995												
MS03	0.996	0.983	0.991	0.997											
MS04	0.995	0.988	0.991	0.992	0.991										
MS05	0.965	0.977	0.978	0.965	0.965	0.972									
MS06	0.995	0.987	0.994	0.994	0.995	0.991	0.971								
MS07	0.995	0.986	0.992	0.996	0.996	0.994	0.969	0.997							
MS08	0.996	0.985	0.991	0.997	1.000	0.991	0.969	0.995	0.997						
MS09	0.992	0.985	0.988	0.989	0.990	0.995	0.975	0.989	0.994	0.992					
MS10	0.978	0.982	0.983	0.975	0.973	0.981	0.994	0.978	0.976	0.976	0.983				
NB01	0.996	0.986	0.991	0.996	0.996	0.994	0.968	0.996	0.995	0.996	0.994	0.980			
NB02	0.995	0.981	0.990	0.995	0.997	0.992	0.970	0.994	0.995	0.998	0.993	0.975	0.994		
NB03	0.993	0.989	0.991	0.997	0.996	0.993	0.977	0.993	0.999	0.996	0.993	0.980	0.998	1.000	
NB04	0.991	0.985	0.990	0.992	0.992	0.995	0.972	0.992	0.995	0.990	0.993	0.981	0.995	0.992	0.993

TK02	TK02	TK03	TK04	TK05	TK06	TN03	TN04	TN05	TT01	TT02	TT05	TT06
TK03	0.985											
TK04	0.993	0.985										
TK05	0.996	0.987	0.991									
TK06	0.983	0.979	0.987	0.981								
TN03	0.994	0.996	0.995	0.996	0.984							
TN04	0.999	0.986	0.993	0.996	0.983	0.995						
TN05	0.998	0.986	0.993	0.998	0.985	0.995	0.999					
TT01	0.995	0.990	0.989	0.991	0.976	0.991	0.996	0.994				
TT02	0.997	0.994	0.994	0.998	0.980	0.994	0.998	0.998	0.994			
TT05	0.993	0.989	0.995	0.994	0.981	0.997	0.993	0.994	0.991	0.996		
TT06	0.997	0.984	0.995	0.994	0.979	0.998	0.998	0.998	0.999	0.997	0.994	
TT08	0.996	0.987	0.995	0.997	0.980	0.996	0.995	0.997	0.992	0.996	0.995	0.998

Appendix 8.9.2 Matrix of similarity among 56 isolates of *F. udum* generated from binary banding data of 7 primer combinations using Nei and Li (1979) distance index using RFLPfrag program - continued

	MR05	MR06	MR07	MS01	MS03	MS04	MS05	MS06	MS07	MS08	MS09	MS10	NB01	NB02	NB03	NB04	NY01	NY02	NY03	NY04	NY07	NY08
NY01	0.997	0.985	0.992	0.998	0.998	0.992	0.969	0.996	0.997	0.997	0.992	0.978	0.996	0.996	0.997	0.995						
NY02	0.996	0.984	0.992	0.995	0.995	0.995	0.971	0.997	0.998	0.994	0.992	0.975	0.995	0.996	0.996	0.996	0.997					
NY03	0.998	0.982	0.989	0.988	0.990	0.994	0.968	0.991	0.992	0.994	0.995	0.978	0.991	0.992	0.993	0.990	0.989	0.992				
NY04	0.996	0.982	0.995	0.998	0.998	0.992	0.959	0.993	0.997	0.997	0.988	0.971	0.995	0.994	0.996	0.991	0.997	0.996	0.989			
NY07	0.997	0.980	0.991	0.997	1.000	0.992	0.957	0.999	0.997	1.000	0.992	0.971	0.998	0.996	1.000	0.996	0.999	0.997	0.990	0.999		
NY08	0.997	0.986	0.990	0.996	0.996	0.992	0.962	0.995	0.998	0.996	0.990	0.974	0.994	0.992	0.994	0.993	0.997	0.998	0.992	0.997	0.997	
TK02	0.995	0.983	0.991	0.998	0.998	0.993	0.965	0.996	0.997	0.998	0.990	0.975	0.996	0.997	0.997	0.994	0.998	0.997	0.989	0.997	0.999	0.996
TK03	0.995	0.983	0.973	0.988	0.988	0.985	0.973	0.982	0.975	0.998	0.988	0.974	0.975	0.987	0.998	0.982	0.985	0.980	0.986	0.989	0.974	0.987
TK04	0.993	0.985	0.990	0.993	0.991	0.993	0.975	0.991	0.993	0.993	0.994	0.983	0.992	0.996	0.999	0.993	0.992	0.994	0.993	0.991	0.989	0.991
TK05	0.997	0.984	0.990	0.996	0.996	0.993	0.961	0.996	0.997	0.996	0.991	0.974	0.996	0.993	0.995	0.993	0.998	0.998	0.991	0.997	0.998	0.998
TK06	0.980	0.975	0.983	0.981	0.983	0.980	0.978	0.979	0.982	0.982	0.980	0.979	0.976	0.983	0.986	0.981	0.983	0.980	0.977	0.981	0.979	0.983
TN03	0.994	0.991	0.991	0.995	0.995	0.993	0.973	0.994	0.999	0.995	0.995	0.984	0.998	0.997	0.998	0.994	0.995	0.997	0.995	0.995	0.999	0.996
TN04	0.995	0.983	0.991	0.998	0.999	0.992	0.965	0.996	0.997	0.999	0.990	0.974	0.997	0.997	0.997	0.993	0.999	0.996	0.988	0.997	1.000	0.996
TN05	0.997	0.985	0.992	0.998	0.999	0.993	0.968	0.996	0.998	0.998	0.993	0.977	0.996	0.996	0.997	0.995	0.998	0.998	0.991	0.999	0.999	0.998
TT01	0.991	0.981	0.992	0.993	0.995	0.988	0.970	0.992	0.998	0.995	0.989	0.972	0.997	0.998	0.992	0.987	0.994	0.995	0.994	0.991	1.000	0.992
TT02	0.996	0.986	0.991	0.996	0.997	0.993	0.973	0.996	0.997	0.997	0.993	0.979	0.996	0.996	0.996	0.993	0.999	0.996	0.994	0.993	0.998	0.997
TT05	0.996	0.988	0.987	0.993	0.993	0.993	0.971	0.993	0.993	0.994	0.993	0.981	0.996	0.994	0.996	0.992	0.994	0.993	0.995	0.991	0.994	0.994
TT06	0.994	0.984	0.992	0.997	0.998	0.994	0.968	0.995	0.997	0.998	0.993	0.974	0.998	0.996	1.000	0.993	0.998	0.996	0.992	0.997	0.998	0.994
TT08	0.996	0.987	0.992	0.996	0.995	0.996	0.970	0.995	0.997	0.995	0.995	0.979	0.997	0.995	0.996	0.995	0.996	0.998	0.995	0.996	0.997	0.996