BREEDING FOR RESISTANCE TO *FUSARIUM* WILT OF PIGEONPEA (*CAJANUS CAJAN* /L./ MILLSP.) IN KENYA.

by

MICHAEL ANTHONY OKIROR

A thesis submitted in fulfilment for
the degree of
DOCTOR OF PHILOSOPHY IN AGRICULTURE

of the
University of Nairobi
Faculty of Agriculture

1986

DECLARATION

I, Michael Anthony Okiror, hereby declare that the work presented in this thesis is based on the research carried out by me between 1982 and 1986, and that it has not been submitted for a degree in this or any other University.

Signed: _____

M.A. OKIROR

Date: 24-11-86

This thesis has been submitted for examination with our approval as University supervisors.

Signed

V.K. Gupta

Prof. V.K. Gupta

Date 24/11/86

P.M. Kimani

Dr. P.M. Kimani

Date 26/11/86

This work is dedicated to my parents, the late Papa Daniel Abisa Oryokot and Toto Bulandina Kabwire for the sacrifices they made to bring me up; to Jackie and Jack, who are always a source of inspiration to me.

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ACKNOWLEDGEMENTS

Many people have helped in the successful completion of this study. To them all I am grateful. A few however deserve special mention.

First and foremost, Dr. J.F.M. Onim for offering me a scholarship in the Pigeonpea Project. Despite many odds especially after his departure, I continued to benefit from his official guidance. I am further grateful to him for all other help he readily gave whenever I went to consult him at Maseno, Kisumu.

I am indebted also to my supervisors, Prof. V.K. Gupta and Dr. P.M. Kimani for their invaluable guidance during this study. I must also thank Dr. S.P. Singh for his critical review of earlier drafts. His constructive comments resulted in marked improvement of the write up.

While on a study tour at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), I shared the experiences of many scientists involved in pigeonpea improvement and

greatly benefitted from discussions with them. In mind, I have Drs D.G. Faris, M.V. Reddy, M.P. Haware and K.C. Jain. To them all, I say thank you very much.

I am further grateful to Dr. D.G. Faris and the library services at ICRISAT for availing me free of charge useful reprints that made my write-up possible. In this regard too, I am thankful to my friend Gabriel E. Ocholla of Serere Research Station, who while on a training session at ICRISAT spared his valuable time to gather me a few relevant reprints.

This study was made possible through funds to the Pigeonpea Project by the IDRC of Ottawa, Canada for which I am indeed most grateful.

To Joyce, Jackie and Jack, I am thankful to you all for the love you readily showed me and the encouragement obtaining from you especially when things seemed bleak and frustrating. And to all my "mothers", brothers and sisters, thank you for all the sacrifices you made in one way or another to make me what I am now.

Finally, to all friends and colleagues who in one way or the other offered help relevant to this

study, especially the staff of Pigeonpea Project, thank you all.

Last but not least I am grateful also to Jane Njeri Mbugua for typing this thesis.

ABSTRACT

Pigeonpea Cajanus cajan (L.) Millsp.) is one of the main grain legumes grown in Kenya and occupies about 115,000 ha annually. Pigeonpea diseases, especially Fusarium wilt is considered a major factor limiting yields in Kenya and other pigeonpea growing areas of the world.

To boost and stabilise yields, a study to develop Fusarium wilt resistant cultivars was undertaken. It involved developing an effective and reliable technique for evaluating the available germplasm for sources of resistance, testing for variability of the causal fungus, F. udum, and evaluating the potential of electrophoresis in identifying resistance sources in pigeonpea.

Of the five inoculation procedures evaluated, sowing seed into infested soil resulted in fast onset of wilting and high mortalities. It was a simple and repeatable technique and was therefore used for screening the germplasm for sources of resistance. Of the 104 lines of pigeonpea screened in the glasshouse, 51 potentially resistant single plants were harvested. The progenies of these plants (lines) with susceptible checks were tested in the glasshouse and disease nursery. Eleven lines that combined a reasonable degree of resistance to Fusarium wilt with good yield were

finally selected. These were tested at two locations for two seasons (1984 and 1985 short rains). Lines NPP 725 and 726 were resistant to Fusarium wilt but were low yielding. Isolates of F. udum obtained from a wide area of pigeonpea cultivation showed varying levels of pathogenicity suggesting variability in this pathogen. The isolates showed significant differences in growth and morphological characters as well as in biochemical testing involving fungal proteins. Due to the variability in this fungus, testing of promising material across wider areas of cultivation prior to seed distribution is strongly suggested. Genetic studies on the type of resistance in pigeonpea to Fusarium wilt was attempted. The results were inconclusive but indicated a digenic, dominant kind of resistance.

Electrophoretic studies showed consistent differences between resistant and susceptible genotypes based on acid phosphatase, leucine aminopeptidases, and peroxidase enzymes and proteins in nine, 20 and 25 day old plants. The results suggested strongly that it is possible biochemically to screen pigeonpea germplasm for resistance to Fusarium wilt especially at these developmental stages.

1. INTRODUCTION

Pigeonpea (Cajanus cajan (L.) Millsp.) is one of the most widely grown and eaten grain legume in many developing regions of the world. About 90%, is grown in India though there are large areas in East Africa where it is of great importance than commonly recognised.

Kenya is the world's second largest producer after India and grows about 115,000 ha annually (Anon.1980). Pigeonpea in Kenya is second only to food beans (Phaseolus vulgaris L.) as a pulse, and third to food beans and cowpeas (Vigna unguiculata (L) Wallp.) as a food legume in acreage and production. The crop is also important in South East Asia, South and Central America and the Caribbean countries (Table 1).

In Kenya, over 95% of the crop is cultivated in Eastern province in Machakos and Kitui districts, both of which are in the marginal rainfall zone. Production is mainly from peasant farmers who grow it in mixtures especially with maize (Zea mays L.), sorghum (Sorghum bicolor /Linn/ Moench), other legumes such as food beans and cotton (Gossypium

Table 1. World pigeonpea production, 1970-1980

	1970	1974	1979	1980F[a]
WORLD				
Area harvested (000 ha)	2982	2999	3000	2951
Yield (kg/ha)	684	541	703	684
Production (000 tonnes)	2039	1622	2111	2017
AFRICA				
Area harvested (000 ha)	214	241	252	255
Yield (kg/ha)	593	565	589	599
Production (000 ha)	127	136	149	153
NORTH & CENTRAL AMERICA				
Area (000 ha)	24	28	2	9
Yield (kg/ha)	1603	1411	2500	2222
Production (000 tonnes)	38	40	5	20
ASIA				
Area (000 ha)	2723	2723	2718	2656
Yield (kg/ha)	703	530	713	687
Production (000 tonnes)	1913	1442	1938	1824
INDIA				
Area (000 ha)	2655	2646	2663	2600
Yield (kg/ha)	709	532	719	692
Production	1883	1408	1914	1800
THE CARIBBEAN*			1978	
Area (000 ha)	-	-	27	-
Yield (kg/ha)	-	-	691	-
Production (000 tonnes)	-	-	24	-

Source: FAO : 1948 to 1985 world crop and livestock statistics

a - FAO estimates include China

* - Data for 1978 only given

hirsutum L.). The crop is grown mostly for home consumption; however, surpluses are usually sold.

Pigeonpea is a drought tolerant crop and this is attributed to its deep rooting character which enables it to exploit lower soil profiles for residual moisture (Willey et al., 1981). It is often the only crop that gives some grain yield during dry spells when other crops such as maize, and food beans will have wilted and probably dried up.

It is grown for its dry seeds but the green seeds make excellent vegetable. Locally, the seed is cooked whole while in India it is dehulled into 'dhal' and in the Caribbean countries it is popular as a vegetable. It is a rich source of high quality protein (20 to 28%) (Khan, 1973). However, like most other legumes, it is low in sulphur-containing amino acids. Pigeonpea is therefore a very important crop in those marginal rainfall areas where other cheaper sources of protein - especially of animal origin are unavailable or prohibitively expensive for the peasant farmers. In the cereal-based diets of India, pigeonpea is an important protein source, especially for the vegetarian communities.

Pigeonpea is also used as a livestock feed or fodder, as green manure and fuel (Raju, 1981). The rejected seed is compounded with other feeds and fed to poultry, cattle and pigs (Whiteman and Norton, 1981; Onim, 1982). The crop is also important in rotation farming since it helps to restore soil fertility (Kumar Rao et al., 1983) through nitrogen fixation, bringing minerals in the deeper soil horizons to the surface and by improving soil-air ciculation. These activities all contribute to better yields of a following crop (Gooding, 1962).

Although the Kenyan average yields (1300kg/ha) are higher than world average yields (684kg/ha) they are about half (i.e. 2700kg/ha) of what has been realised under research conditions (Onim, 1981). This is attributed to one or several of such factors as use of land races with poor productivity and long duration (8-9 months), lack of or non-use of inputs such as fertilizers and pesticides, poor agronomic practices and lack of pest and disease control. The recent development and release of an early maturing, and high yielding variety dubbed the "miracle pigeonpea" is likely to lead to higher yields and increased returns to the farmers.

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Among the diseases of pigeonpea, Fusarium

wilt is the most important. The disease is also the most important in India (Sen Gupta, 1974) and in other pigeonpea growing regions of Africa (Kannaiyan *et al.*, 1984). Other less important diseases here in Kenya include *Cercospora* leaf spot, *Alternaria* blight and stem canker.

Fusarium wilt of pigeonpea has been identified in all the pigeonpea growing areas of Kenya. A recent survey showed the incidence at between five and 60% with a mean of 12% (Onim, 1981). It increases during wetter years and could greatly undermine efforts directed at developing short maturity varieties with high and stable yields. Although losses directly attributable to wilt were not known, evidence elsewhere indicates they vary from a negligible amount to 100% depending on the stage at which the crop is attacked (Kannaiyan and Nene, 1981).

Breeding *Fusarium* wilt resistant varieties has been conducted since the turn of this century. In most cases it was mainly through routine methods of selection in wilt infested fields and sick soils in glasshouses (Butler, 1908; Deshpande *et al.*,

1963; Mukherjee et al., 1971; Nene and Kannaiyan, 1982). In all cases resistant and/or tolerant cultivars/lines were reportedly identified.

The first priority of the Nairobi University pigeonpea improvement programme was to develop varieties that combined high and stable yields, early maturity and good quality seed. This was done through screening of the germplasm, crossing and selection. At that time disease incidence was very low and of no concern. However, susceptibility of two very promising advanced lines to wilt and the characteristic appearance of wilted plants in most fields (Onim, pers. comm.) prompted a survey to establish the extent and severity of this disease in the country. On the basis of the results obtained it was decided to initiate a breeding programme to develop cultivars with resistance to wilt.

In view of the economic importance of wilt as a constraint to increased yields and the fact that little was reported about the genetics of resistance to Fusarium in pigeonpea, this investigation was commenced with the following objectives:

1. To develop repeatable and effective inoculation techniques that could be used for large scale screening,

2. To identify resistant genotypes from the available germplasm by using the most eff-ctive and suitable screening techniques,

3. To determine if pathotypes of F. udum occur in Kenya, and

4. To investigate the existence of electrophoretic markers, if any, between the Fusarium wilt resistant and susceptible lines of pigeonpea.

2. LITERATURE REVIEW

2.1. Introduction

According to available reports it would seem pigeonpea wilt due to F. udum occurred before the beginning of this 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. However, there are no indications that the causal organism had been identified prior to 1902 nor any investigations made to understand the factors that predisposed plants to attack.

Butler, a British mycologist working in India 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 the genus Fusarium and had no other known host. This fungus was subsequently named Fusarium udum Butl.. Since then this disease has been the subject of many studies in several institutions as well as by individual scientists and attempts to breed wilt resistant varieties made (McRae and Shaw, 1933; Deshpande et al., 1963; Mukherjee et al., 1971;

Nene and Kannaiyan, 1982). Other studies have been directed at disease management by cultural practices (Bose, 1939; Kaiser and Sen Gupta, 1969).

Varieties reported resistant to Fusarium wilt have been obtained through selection in composite land races in farmers' fields, or through hybridisation. For instance, from a badly infested field in Pusa (India), Deshpande et al. (1963) were able to obtain a resistant line. Moderately resistant lines were also obtained through repeated selections in wilt infested soils in wooden boxes in a glass house (Mukherjee et al., 1971).

Fusarium wilt has been reported in most places where pigeonpea is grown both in India and other parts of the world (Mundkur, 1967; Nene, 1977; Onim, 1981; Kannaiyan et al., 1984). Fusarium wilt is considered to be the most important disease of pigeonpea in India (Mundkur, 1967). National mean values for wilt incidence in India vary between 1.12 and 22.61 per cent (Nene, 1977). However, extremely high incidences have also been reported. In Madhya Pradesh, incidences of about 50 per cent have been recorded (Sharma et al., 1977). Mukherjee et al. (1971) reported cases ranging between 93 and 100

per cent in West Bengal. In Kenya, the incidence of Fusarium wilt is estimated at between five and 60 per cent with a mean of 12 per cent (Onim, 1981). However, pockets of wilted plants are usually seen in most pigeonpea fields in Kenya. Like in India, Fusarium wilt is also the most important disease of pigeonpea in Kenya (Onim, 1981). The disease also is of major importance in Malawi, Tanzania, and Zambia (Table 2) (Kannaiyan et al., 1984).

2.2. The Pathogen

2.2.1 Nomenclature

The nomenclature of the causal fungus of Fusarium wilt of pigeonpea has been debateable until it was resolved by the findings of Booth (1971). Prior to 1906 when Butler isolated this fungus, he thought it was an independent species in the genus Fusarium and subsequently named it Fusarium udum Butl. However, snyder and Hansen (1940) in their analyses of a number of isolates of Fusarium including Butler's were not able to find much variation among them and were convinced therefore that this fungus could not be a new and an independent species. They were contented that this fungus was

Table 2. Summary of pigeonpea disease incidence in four African countries (1980).

Country	Total number of locations examined	Wilt		Leaf spot	Powdery mildew	Grey mildew	MSC	R	PSC	RK	AM	AT (gall)
		Average (%)	Range in fields (%)									
Kenya	25	15.9+	0-90	M+	M	L*	-	-	-	-	L*	L*
Malawi	20	36.3+	0-90	M*+	L	-	-	-	L*	L*	-	-
Tanzania	13	20.4+	0-60	L	M*+	L*	L*	L*	-	-	-	-
Zambia	6	-	-	L	M*	-	-	-	*	-	-	-

Source: Kannaiyan et al., 1984

- = Not observed, L = low, M = moderate

* Reported for the first time

+ Diseases of considerable economic importance

MSC = Macrophomina stem canker

R = Rust

PSC = Phoma stem canker

RK = Root knot

AM = A mosaic

AT (gall) = A root tumor (gall).

just a special form of Fusarium oxysporum Schl. which they identified as F. oxysporum f. sp. udum (Butler)Snyd. and Hans.. This confusion has led to the use of both nomenclatures in literature in reference to the fungus causing wilt of pigeonpea. However, Booth (1971) conducted extensive investigations involving analyses of morphological, cultural and physiological characters of this fungus and of F. oxysporum Schl.. His results, presented in his book "the Genus Fusarium" have disproved the views of Snyder and Hansen. According to him, F. udum is an independent and distinct species of Fusarium whose only known host to date is pigeonpea. He therefore upheld the original name as given by Butler in 1906. This too is the nomenclature upheld by the International Crops Research Institute for the Semi Arid Tropics (ICRISAT) (Nene et al., 1981).

2.2.2. Morphology and pigmentation of F. udum colonies in culture.

Morphological and cultural characteristics are important parameters in the identification of any fungus. F. udum cultures often have a deep

purple colouration. When present, aerial mycelia are hyaline and felted with profuse development of pionnote sporodochia.

This fungus produces three types of conidia that are distinct from each other morphologically. These are the macroconidia, microconidia and chlamydospores or the perennating spores (Mundkur, 1967; Booth, 1971). These three spore types are the structures by which this facultative parasite survives in the soil from season to season and year to year. The micro-, and macroconidia remain viable for a considerable time while the chlamydospores do so for even longer periods. According to McRae and Shaw (1933), F. udum can survive in the soil for between eight and 20 years. This is important because the duration of the infectivity of the pathogen in the soil has a bearing on the control measures which should be applied.

2.2.3 Physiologic specialisation of F. udum

Individual genotypes of a fungal pathogen can differ from each other in many inherited characteristics, for example, morphology, physiology, and pathogenicity. All these characteristics together

or individually can be exploited in differentiating the various isolates into distinct strains of the same pathogen. However, to the breeder, it is a quality to which he continuously has to find a lasting soluation. Usually, the new strains that appear are more virulent and destroy what previously was a resistant host, often leading to epidemics. In this regard, breeders are continuously trying to develop varieties that carry new genes for resistance to the new and more virulent strains of the pathogen.

2.2.3.1 Pathogenicity

Of the three attributes - morphology, physiology and pathogenicity - which are already mentioned as possible identification criteria for races, variability is of particular importance to a practical plant breeder. Variability is associated with the development and dissemination of new physiologic races with the ability to attack varieties that previously were resistant. Borges et al. (1976) have pointed out that breeding for disease resistance in plants is a successful enterprise to the degree that the breeder is able to match resistance genes in the host with the new variants generated by the

pathogen.

2.2.3.2. Characterising isolates

Physiologic races are usually distinguished from each other in several ways. However, the most used criterion is the pathogenicity on certain selected differential varieties of a host plant (Allard, 1960; Agrios, 1978; Borges et al., 1976; Russell, 1978). Pathogenicity may be defined as the ability of an organism to cause disease symptoms in a host. Borges et al. (1976) were able to ascertain differences in 17 isolates of Stremphyllium botryosum Wallr. by using several alfalfa (Medicago spp.) differentials. They concluded that the different isolates constituted a group of physiologic races of S. botryosum. Similar results have been reported by Childs (1937) for Polyporus schweinitzii Fr.. Races have also been found to occur in Colletotrichum lindemuthianum (Sacc. and Magn.) Scribner., the fungus causing anthracnose in beans, using pathogenicity tests (Barrus, 1911). Ou et al. (1971) tested fifty isolates of Xanthomonas oryzae (Uyeda and Ishiyama) Dowson, cause of bacterial leaf blight on

24 varieties of rice of varying levels of resistance in an attempt to test if some or all of these isolates constituted distinct races of X. oryzae. No distinct host-race specificity among these isolates was found. However, their levels of virulence on the various varieties were evident suggesting that most of the varieties were resistant to most isolates. Pathogenicity tests, besides elucidating race occurrence, reveal the nature of the host and guide the breeder in deciding what genotypes to select as his breeding material. Ou et al. (1971) found that cultivars with nonspecific or uniform reaction to the strains of X. oryzae are more useful than those with varied reactions, that is, are highly resistant to some strains and completely killed by others.

2.2.3.3. Cultural tests

Besides differences in aggressiveness isolates have also been distinguished on the basis of radial growth rate in artificial media, sporulation and colony or cultural variation (colour of mycellium, size and shape of conidia)(McGee and

Petrie, 1978). Both the cultural characteristics and pathogenicity tests used together ensure reliability of the results, as was found for Ceratocystis ulmi (Schwarz) Buisman which is fungus causing Dutch elm disease in elm (Ulmus spp) (Gibbs and Brasier, 1973).

2.2.3.4. Biochemical analyses

Electrophoretic techniques for analysis of proteins and enzymes have been used extensively in fungal taxonomy as well (Chang et al., 1962; Stipes, 1970; Alfenas et al., 1984). Electrophoresis is a process of forced diffusion in an electrical field. Protein molecules of the test samples are moved through a medium (gel, paper, cellulose) by applying an electrical gradient. Different proteins assume different charges, often with different net sign, at different pHs and their rate of migration through the gradient differ in proportion to their charge and molecular weight. This results in separation of the different proteins into bands which are then resolved by staining or by spectrophotometry to provide visible prints called zymograms. The bands are called isozymes (i.e. molecular forms of an

enzyme).

Usually when genetically similar tissues of the same age are assayed they give identical prints. However, differences will show in the prints if these tissues are genetically unrelated. It is such variation that is of importance in assaying pathogen isolates. Any differences in bands of the isolates will imply that they are not identical and will be a reflection that they carry varying genetic material. This technique has been successfully used to characterise species, cultivars, and varieties of different crops (Bassiri, 1976; Desborough and Peloquin, 1968; Cherry and Ory, 1973; Bassiri and Adams, 1978), to separate potato (Solanum sp.) clones resistant and susceptible to late blight (Umaerus, 1959), to test for homozygosity and purity of genetic material, and to type isolates, races, species and genera of various fungi (Chang, et al., 1962; Durbin, 1966; Stipes, 1970 and Alfenas et al., 1984).

2.2.3.5. Evidence of Variability in F. udum

Variability in F. udum is considered a major drawback in the development of resistant varieties of pigeonpea (Green et al., 1981).

Several reports suggest of the existence of races of F. udum (Sarojini, 1951; Baldev and Amin, 1974; Shit and Sen Gupta, 1978). Sarojini (1951) made six isolates of F. udum and numbered them I to VI. She tested them with several host differentials. Pathogenicity tests showed strains I, II and III to be more virulent than others. She further observed that strains V and VI mainly caused pre-emergence wilt. Mukherjee (1956) evaluated the resistance of a variety of pigeonpea cultivars to Fusarium wilt at several locations. There were varied reactions by the host differentials to tests in the various locations. This indicated that physiologic races of F. udum probably exist. Mukherjee et al. (1971) tested several isolates of F. udum on several lines of pigeonpea. They found significant variation in severity of disease induced by isolates from the different sites, indicating the existence of geographical variation in the frequencies of specific virulent genes. Baldev and Amin (1974) tested 10 isolates of F. udum from various sources on 10 lines of pigeonpea. Only three lines were found to be resistant across all the isolates. Further tests revealed the existence of a number of races of this fungus. Other reports on differences in reaction

of different isolates to host differentials have been obtained in India (Subramanian, 1963). However, like other earlier workers, Subramanian (1963) did not firmly conclude that the various isolates he prepared constituted races of the fungus. Differences in pathogenicity of isolates of this fungus in India have also been reported by Venkataram (1955). If confirmed that physiologic races actually occur for this pathogen, then breeding for wilt resistance has to be diversified to bring together several factors or genes for resistance to develop a broad spectrum resistance and thereby enhance the stability of such resistance. As in several other crops, pigeonpea breeders are aware that breeding for resistance is a continuous process to counter the evolution of often more virulent strains of the pathogen.

2.3 Epidemiological significance of F. udum

In Kenya, pigeonpea is sown at the onset of the short rains in October - November. Between December and January, natural disease incidence is detectable. By March-May, symptomatic plants are more common in pigeonpea fields. Typical of

Fusarium wilt, however, symptoms are first manifested in the pigeonpea plants when they are five to six weeks old. The disease is characterised initially by leaf chlorosis leading to either gradual or sudden withering, wilting and drying of the green parts of the plant, as would occur under drought conditions. Examination of the stem and the roots of such plants with the bark peeled off reveals dark streaks of varying sizes which are more intense in the roots than up the aerial parts. Depending on the age of the plants, these streaks, can be traced up the plant several feet and the earliest branches to wither and wilt being those arising from the blackened parts of the stem. Thus the wilting can be either total - when the whole ring of vascular tissue is blocked - or partial, when only one side - roots and branches - is affected. These symptoms are thought to arise from total or partial blockage of the water vessels in the roots and aerial parts of the plants (Butler, 1918; Kiraly et al., 1974).

Fusarium wilt attacks pigeonpea at the seedling stage, and these die either gradually or rapidly. At this stage it is usually overlooked unless the number of deaths is large. Wilting occurs in spots, with neighbouring plants drying up gradually

but slowly. By the time the crop has attained full height, the dead plants will be found in little groups, in the field, each corresponding to an early case. These groups increase in size by centrifugal spread upto the time of harvest. Fusarium wilt appears to be a disease that can set in at any age between seedling and adult stages.

The fact that this disease may not kill plants immediately, and that a plant can even be wilted partially, and the fact that some plants can be affected at flowering or even at pod filling or as late as only after ratooning, seems to suggest that this disease is of a complex nature.

Variation in wilting is clearly continuous both within varieties and between varieties. Death of the plants is neither sudden nor complete but rather isolated and extends from seedling to adult stages. As was found in rape, Brassica napus L. when infected with Leptosphaeria maculans (Desm.), the causal organism for blackleg, (Thurling and Venn, 1977) resistance was continuous. Variation of this nature is characteristic of polygenic systems. In pigeonpea too, Shaw (1936) characterised the type of resistance to F. udum in pigeonpea as of

a continuous type, although he could not determine the number of genes involved. In pigeonpea, this suggests that this host-pathogen interaction is of a complex type.

2.4 Pigeonpea - F. udum interaction

F. udum is a facultative parasite that has been reported to survive in soil even in the absence of a living host for a period as long as 20 years (McRae and Shaw, 1933). It is a soil-borne pathogen that normally infects the host through its roots, but can also gain entrance through the stem when it is injured with infected objects. Infection is believed to take place through the tender lateral roots and rootlets and possibly root hairs (Butler, 1918; Mundkur, 1967). Nene et al. (1980) established that infection occurs fairly early in the seedlings although symptoms are not visible till later. They isolated the fungus in 15 day old seedlings. Like most soil-borne pathogens (Bilgrami and Dube, 1976) and more so a member of the genus Fusarium which are referred to as weak pathogens (Dickson, 1956) F. udum is greatly aided in its infection of the host by any mechanical damage of

the root system or stem such as that by nematodes, implements and other soil-borne organisms. Like most other species of this genus, F. udum has not been introduced into the host by spraying although infected implements used to ratoon the crop have been suspected to carry inoculum from plant to plant (Onim, personal communication). Phipps and Stipes (1976) attempted to reproduce a Fusarium wilt in Mimosa (Albizia julibrissin Durazz.) by spraying the above-ground parts with a suspension of F. oxysporium f. sp perniciosum. No symptoms appeared. However, root inoculation consistently resulted in disease.

F. udum in an infected plant is restricted to the vascular tissue and is both intra- and intercellular (Mundkur, 1967). 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 for rapid colonisation of the above-ground parts (Phipps and Stipes, 1976).

Two theories have been advanced to explain how the fungus causes wilting in pigeonpea. One is the plugging or occlusion theory, and the other

is the toxin theory (Bilgrami and Dube, 1976). In the occlusion theory, fungal growth within the vessels causes blockage of the water conducting vessels leading to little or no water reaching the aerial parts. The toxin theory explains wilting as resulting from toxins produced by the invading fungus (Gauman, 1951). These toxins interfere with semi permeable membrane of the leaves leading to rapid water loss. Toxins have been detected and isolated in many species of Fusarium (fusaric acid in F. oxysporum f. sp. vasinfectum (Atk.) Snyder and Hansen and lycomarismin in F. oxysporum f. sp. lycopersici (Sacc.) Snyder and Hansen) (Singh, 1968).

Simultaneous with the occlusion theory is the increased incidence of wilted plants as caused by F. udum in fields of pigeonpea that have been ratooned. Ratooning, like the effect of toxins is believed to enhance rapid water loss in the plant and therefore constitute a greater pull of water from the roots. This is said to occur because osmo-regulation is offset in the plant and ratooning, according to ICRISAT scientists (Anon. 1977) is a surer way to screen for resistance. On the contrary, Onim (pers. comm.) suggests that wilting suddenly

appears in ratooned crops probably because of continued use of the ratooning knife which could act as a carrier of inoculum from plant to plant.

2.5. Breeding for resistance to *Fusarium* wilt

Breeding cultivars of crop plants resistant to both pests and disease is perhaps the most spectacular achievement plant breeders have made towards improved and increased food supply. Some of the disease outbreaks of the past, on food crops such as milo disease of sorghum, southern corn leaf blight, blast of rice, rusts of wheat and barley, have been contained largely by growing resistant varieties.

Crop pests and diseases destroy about 50 percent of the world's total crop production annually (Russell, 1978). By developing pest and disease resistant cultivars, plant breeders have helped to avert catastrophes and miseries that accompany famine. Through the use of resistant cultivars losses due to pests and diseases have been greatly reduced and this as well has supplemented other control measures without adding to the costs of production or leading to environmental pollution.

Breeding for disease reistance does not differ fundamentaly from breeding for any other characters (Allard, 1960). However, the most suitable methods depend largely on the mode of pollination of the crop concerned and on the sources of resistance that are available (Russell, 1978). The methods that can be used for pigeonpea, a partially cross-pollinated crop, to obtain resistant genotypes could be mass selection and pureline selection (Butler, 1908; Veheeduddin, 1956; Deshpande et al., 1963).

At the Indian Agricultural Research Institute (IARI), resistant cultivars of pigeonpea were obtained in 1931 by the mass selection procedure from among mixed natural populations (landraces) (Deshpande et al., 1963). The seed of the surviving plants was grown repeatedly in this sickplot till a single plant was obtained that was resistant, multiplied and released as a resistant cultivar, N.P.80. At ICRISAT and at other institutions in India, resistant lines have been developed in sick plots first through both mass

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selection and then by single plant selection (Nene,1977). Hybridisation has also been done to incorporate resistance into promising cultivars (Deshpande et al., 1963).

Shaw (1936) conducted extensive studies into the type and mode of inheritance of resistance to Fusarium wilt in pigeonpea as well as the genetic association of resistance with other morphological characters. His results suggested resistance was dominant to susceptibility with either two (9:7) or three factors (37:27) involved. However, there was no association with any of the phenotypic traits he evaluated. Although disease resistance is a physiological quality, an indirect or even a direct relationship with a desirable morphological character such as stem colour would have been very beneficial as a genetic marker.

2.6 Genetics and sources of resistance to Fusarium wilt.

According to Russell (1978) it is important for the plant breeder to discover at the start of his program the nature of inheritance of resistance he is going to work with and whether it is dominant

or recessive to susceptibility. This too will influence the breeding approach to adopt. However, the identification of plants resistant to wilt requires no advance knowledge of the genetics. For instance, in India, pigeonpea plants resistant to wilt were obtained before breeding and genetic studies were contemplated. This is supported by the fact that despite lack of knowledge on the inheritance of resistance, farmers had developed lines through selection that were often less attacked by the disease.

Plant breeding programs aimed at developing wilt resistant pigeonpea have been underway since early 1900s. This effort, which has been channelled mainly through what are regarded as routine methods of plant breeding, has resulted in the production of wilt resistant cultivars (Butler, 1908; Deshpande et al., 1963; Mukherjee et al., 1971; Nene and Kannaiyan, 1982). However, relatively little work has been reported on the genetics of wilt resistance. The few reports available are conflicting and have failed to provide a complete picture of the genetics of resistance beyond indicating that several genes are involved (McRae

and Shaw, 1933; Green et al., 1981). According to Joshi (1957) the inheritance is controlled by a pair of dominant duplicate genes. Shaw (1936) indicated that several factors could be involved. According to ICRISAT scientists, Haware, M.P. and K.C. Jain (Pers. comm.) resistance is recessive with several factors involved. If this resistance is recessive then it could probably be unstable given the fact that pigeonpea is an often cross-pollinated crop. In the dominant form, it could be easier to maintain with occassional selfings. The fact that it is multigenic could be a useful characteristic since such resistance is usually stable given the fact that F. udum has been reported to occur in other forms (pathotypes). Unfortunately, however, multifactor forms of resistance are usually difficult to handle. It was also shown by Shaw (1936) that this resistance was not associated with any of the phenotypic parameters he studied. Absence of linkage of this type means that transfer of resistance could be done without fear of transfer of deleterious factors.

Complete resistance to wilt has not been found in any of the pigeonpea cultivars grown world-

wide. However, lines showing a high level of resistance have been reported (Butler, 1908; Shaw, 1936; Joshi, 1957; Deshpande et al., 1963; Nene and Kannaiyan, 1982). A number of highly wilt resistant lines such as ICP 8863, ICPL 270, 138 and 8102 have been developed at ICRISAT (Faris, pers. comm.).

2.7. Screening for resistance to Fusarium wilt

The success of a program designed to screen pigeonpea germplasm for sources of resistance to Fusarium wilt depends largely on a rapid inoculation technique that also eliminates escapes.

Resistance to soil-borne pathogens such as F. udum, is best ascertained by testing plants using methods that simulate host-pathogen interaction under natural conditions. Since such pathogens enter their hosts almost universally through the roots, the inoculation techniques to be used should therefore be those that get the pathogen as near as possible to its point of entry.

The introduction of a soil-borne pathogen, such as a member of the genus Fusarium into its host can take the form of dipping seedlings by the

roots into inoculum suspensions and transplanting them in soil in pots (Phipps and Stipes, 1976) by incorporating the inoculum into the soil or by applying it on the soil surface before sowing seed (Russell, 1978). Both these methods have been found effective. For instance, in selecting wheat cultivars for resistance to root diseases such as take-all caused by Ophiobolus graminis (Sacc.) Sacc. inoculation was successful when inoculum was mixed either with seed before drilling or with the soil in which the seed was sown (Russell, 1978). Disease was similarly induced in sugar beet (Beta vulgaris L.) plants using Fusarium pathogens (Armstrong and Armstrong, 1976). Foot rot of peas (Pisum sativum L.) caused by Phoma medicaginis var. pinodella was introduced by several methods (Sakar et al., 1982). However, only immersing seed into inoculum and sowing in sterile soil was found the most effective. The effectiveness of these methods is confirmed also by Mussell and Fay (1971) and Cornway and MacHardy (1978). However, several factors singly or collectively affect the effectiveness of any such methods. These factors include spore concentration, age of plants at inoculation, environmental conditions especially

humidity and temperature (Ribeiro and Hagedorn, 1979).

From these observations, it would appear that dipping of either seed or seedlings in inocula and planting them in pots leads to rapid development of symptoms and is therefore a faster and a more effective method of inducing some of the diseases caused by soil-borne pathogens. This is because they simulate the natural method of pathogen entry into the host. Other methods that have been used successfully include stem injection (Jindal et al. 1982), drenching seedlings with inoculum suspension with or without root injury (Henderson and Winstead, 1961).

In pigeonpea, fast and effective methods have so far not been found by pathologists and breeders in their attempts to screen for wilt resistant genotypes. Since Butler identified the parasite causing wilt in pigeonpea in 1905, evaluation of material has mainly been by harvesting of surviving plants in wilt infested fields and repeating the process many times in effect selection from mixed populations. This approach was also subsequently used (McRae and Shaw, 1933; Shaw, 1936; Deshpande

et al. 1963). At ICRISAT, two methods have been standardised for screening for wilt resistance in pigeonpeas (Nene et al., 1981). These are the sick plot and pot culture methods. The sick plot technique refers to a plot of land being made 'sick' by repeated incorporation of wilted pigeonpea debris and growing a susceptible cultivar to develop the disease in the soil. Screening in the sick plot, however, seems to have been the most widely used technique, probably because it is amenable to screening a large number of germplasm. At IARI breeding for wilt resistance was started in the 1920s exclusively in sick plots.

In Kenya, a local farmer's field at Makueni in Machakos district has been used as a sick plot and all field evaluation of material done there. Repeated planting of pigeonpea in this field and the almost 100 percent destruction of plants each season helped the farmer to obtain from surviving plants a mixed cultivar with some resistance. Although this is a straightforward technique wilting occurs late in the plants. It would therefore not show seedling resistance. Symptoms appear about three months and continue to show until

maturity time. Wilting also occurs in spots corresponding to pockets of high inoculum. This emphasises the difficulty in ensuring even spread of inoculum on a field scale.

Mukherjee et al. (1971) found that testing in the sick plot gave inconsistent reaction and attributed this possibly to the presence of different strains of F. udum in the soil as well as due to the interaction of the diverse microflora present in the soil. The observation that soil microflora affect the pathogenicity of a fungus has been verified (Vasudeva and Roy, 1950; Vasudeva and Govindswamy, 1953; Buxton, 1960). The effect is mainly inhibitory leading to uneven expression of severity of the disease in the plot. However, despite all these shortcomings, the technique has led to the development of several resistant genotypes (Butler, 1908; Shaw et al., 1933; Vaheeduddin, 1956; Deshpande et al., 1963; Nene and Kannaiyan, 1982).

Testing in the glasshouse utilises wilt infested soil in pots or boxes. This approach is rapid with symptoms showing in about a week (Mukherjee et al., 1971; Nene et al., 1980).

Through this technique Mukherjee et al., 1971) were able to identify nine tolerant lines from a population of nearly sixty lines. It is therefore a suitable method for evaluating large collections of germplasm, and/or breeding lines. Also because of controlled conditions in the glasshouse and the fact that sterilised soil is used to grow the inoculum, it is considered a more reliable method to test crosses to obtained the nature and inheritance of resistance (Haware, M. P., pers. comm.).

Another method that has also been used in pigeonpea is the stem inoculation (Sharma et al., 1977). However, it is slow and not amenable to large scale screening. Symptoms often take a month or even longer to appear. In this technique, an incision is made on the stem 10 to 15 cm above the ground. The fungal mycelial mat is applied and absorbent cotton wrapped around this wound and kept in place with cellotape. This is to prevent dessication of the plant.

Resistant and susceptible pigeonpea have also been compared for certain plant compounds (Murthy, 1975). Such compounds included proteins, enzymes, alkaloids and phenols. He found the resistant variety

to have a higher content of total sugars, amino acids and phenols among others but a lower content of phenylalanine. Further bioassay revealed that caffeic and chlorogenic acids and unidentified phenolic compound present only in the resistant variety inhibited spore germination. In conclusion, Murthy suggested that cysteine was effective in counteracting fungal infection by chelating ferric ions and thereby inactivating Fusarium toxin.

In other crop plants higher activities of certain enzymes, e.g. peroxidases, have been reported positively correlated with either resistance or susceptibility to disease (Butler, 1918; Kedar, 1959; Umaerus, 1959; Fehrmann and Diamond, 1967; Okiror et al., 1982). A technique such as the above if found reliable and repeatable would eliminate the necessity for controlled conditions and preparation of inoculum essential in conventional tests. The method would probably also be quicker since tests could be done in very young seedlings. A further advantage would be that the tests could be done without sacrificing the plants since only leaves would be used.

3. MATERIALS AND METHODS

3.1. EXPERIMENT 1: Inoculation methods

3.1.1. Cultivars

Four cultivars of pigeonpea were used in this experiment. This experiment aimed at developing an inoculation method that is rapid, effective and repeatable for identifying sources of resistance in the available germplasm and breeding material. Two of the cultivars NPP* 679 and Munaa are local cultivars. NPP 679 is a single plant selection from a previously selfed population and is apparently resistant to Fusarium wilt. Its selfed seed was used in this study. The cultivar Munaa is a pureline developed from among locally collected germplasm that had reached advanced stages of testing for release as a variety. However, it was not released because it was severely attacked by Fusarium wilt. Both Munaa and NPP 679 are normally late maturing cultivars, and take about nine months to mature. They are green-stemmed and grow to an average height of about two metres. Munaa has white

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*NPP = Nairobi pigeonpea accession number.

flowers and large green pods with brown streaks. NPP 679 has yellow flowers and large green pods. Both cultivars produce large, white/cream seeds. The other two cultivars, ICP 2376 and ICPL 270 were obtained from ICRISAT. They were characterised as susceptible and tolerant to Fusarium wilt, respectively. In our accession records, they are designated NPP 718 and 725. NPP 718 is one of the standard susceptible checks at ICRISAT centre. This was confirmed by preliminary tests here. Both NPP 718 and 725 are dwarf cultivars, with an average height of one metre. They are early maturing, taking about five months to be ripe. They are both green stemmed and have small, green pods. However, NPP 718 has yellow flowers with red veins on the dorsal side of the standard and produces small, cream seeds. NPP 725 has deep yellow flowers and small yellow-brown seeds.

3.1.2. The inoculum

F. udum was isolated from wilted pigeonpea plants obtained from a field at Thika in Kiambu district. The wilted plants were chopped into small pieces and incorporated into forest soil whose particle fractions were: 64% clay, 31% silt, and 5%

sand (Muriuki, 1979). This soil was placed into large wooden boxes which were 94 cm long, 50 cm wide and 34 cm deep (Plate 1). When the soil had been sufficiently infested with inoculum, these boxes were designated "sick". Pure cultures of F. udum were simultaneously grown on potato dextrose agar (PDA) medium. The inoculum was obtained from these cultures by washing the conidia into beakers with sterilised distilled water. The required concentrations were adjusted using a haemacytometer.

3.1.3. Inoculation methods

Five inoculation procedures were investigated to identify a suitable method that could be used for screening germplasm to obtain sources of resistance and/or testing crosses to be made subsequently.

3.1.3.1. Method 1 - Sowing seeds in sick boxes

Ten seeds of each cultivar were sown in separate rows. The seeds were dibbled five centimetres apart within the row and the rows were marked seven centimetres apart. Each cultivar was sown in three rows and replicated three times. Seeds of these cultivars were also sown in uninfested soil as a

Plate 1: Pigeonpea wilt 'Sick boxes' being established. Pigeonpea seeds were planted at the early developmental stage to monitor disease development and provide the pathogen with its host and thereby encourage its spread in the boxes.

control. Plant count was taken two weeks after sowing or when most seeds had germinated. Disease on set and progress was closely monitored and plants wilted recorded every two weeks. A final disease score was recorded three months after taking germination count.

3.1.3.2. Method 2 - Transplanting seedlings into sick boxes.

Three-week-old seedlings of the four cultivars were removed from polythene tubes by washing away soil from their root systems. Twenty vigorous seedlings were selected for each cultivar. Ten of these seedlings had their roots pruned severely (to enhance wilting) with a pair of scissors and the other ten had intact roots. These seedlings were then transplanted into furrows made in the sick boxes. The furrows were marked seven centimetres apart. The seedlings were planted 10 cm apart within the rows with each cultivar transplanted into two furrows. The boxes were watered lightly and regularly to enable the seedlings to get established. Only those seedlings which became established were recorded as the initial plant stand. Any seedlings which then

wilted were uprooted, the skin peeled off from the roots and the stem base to check for the black streaks typical of Fusarium wilt. Number of plants with these symptoms were recorded every two weeks for three months.

3.1.3.3. Method 3 - Soaking seeds in inoculum

Inoculum for soaking seeds was prepared from pure cultures of the fungus grown on PDA. Ten day-old cultures were covered in sterilized distilled water and washed into beakers. These suspensions were then filtered through a double layer of cheese cloth to remove mycelial tissue and pieces of the medium. Four different concentrations - 4.23×10^5, 9.4×10^5, 1.31×10^6, and 1.64×10^6 spores per mℓ were arbitrarily chosen. These concentrations were ascertained by use of a haemacytometer.

Thirty seeds per cultivar were soaked in each inoculum concentration. Ten seeds were taken at intervals of 1, 5, and 7 hours from each inoculum preparation per cultivar and sown in a flat previously filled with sterilized forest soil. The ten seeds were dibbled at five centimeter intervals within a row and the rows were marked seven centimeters apart.

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The control seeds were soaked in sterilized distilled water and sown in shallow boxes filled with the same soil. Germination count was recorded after two weeks and onset of wilt monitored. The number of wilted plants was then recorded after every two weeks for three months.

3.1.3.4. Method 4 - Root dipping

Seedlings used in this test were raised as described earlier in 3.1.3.2. and the inoculum used was prepared as mentioned in 3.1.3.3. Twenty seedlings per cultivar were dipped in each of the four (4.23×10^5, 9.4×10^5, 1.31×10^6, and 1.64×10^6 spores/mℓ) spore concentrations. Ten of these seedlings had their roots pruned with a pair of scissors and the other ten seedlings had their roots intact. These seedlings together were then placed in beakers having inocula of the various concentrations. The seedlings remained in these inocula for 1.5 hours and then were transplanted into disinfected 32 cm plastic pots filled with sterilized Kabete forest soil. Ten seedlings were transplanted into each pot. Control plants were dipped into sterilized distilled water. The pots were watered regularly but lightly to minimise death of plants due to transplanting shock.

Wilted plants were scored every two weeks after transplanting for three months. To ascertain that a seedling died due to wilt, it was uprooted and examined. Only seedlings showing Fusarium wilt symptoms were scored.

3.1.3.5. Method 5 - Stem injection

Four week old seedlings of the four cultivars were used in this study. Two inoculum concentrations, 1.31×10^6 and 1.04×10^6 spores per mℓ, were applied. Using a hypodermic needle, 0.25 mℓ of inoculum was injected into each seedling through the stem four cm above the ground. Ten seedlings per cultivar were injected with inoculum of each of the two concentrations. The control plants were injected with sterilized distilled water. The plants were examined at two week intervals after inoculation upto three months. Any wilted plants were recorded.

Methods 1 and 2 were replicated three times while methods 3, 4 and 5 were replicated four times. All these studies were conducted in the glasshouse, with maximum temperature therein between 38° and $42^{\circ}C$ and minimum between 25° and $29^{\circ}C$. These temperatures may have constituted an unknown influence on the plants.

The final scores were used to compare the five methods. Percent wilt was calculated as the fraction of plants wilted to the total number of plants raised, i.e., recorded at germination or the stand established after transplanting.

3.2. EXPERIMENT 2. Germplasm evaluation

On the basis of the results of experiment 1, the method of sowing seed into wilt infested sick soil in boxes was adopted and used to screen the germplasm and breeding lines for sources of resistance and/or tolerance to F. udum. Field screening was conducted in a sick plot at Makueni, Machakos district.

3.2.1. Sick box screening

Seventeen sick boxes were used to evaluate several accessions of pigeonpea. These boxes were developed as described earlier (Sec. 3.1.2.) with wilted pigeonpea plants collected at four locations: Emali and Kimutwa in Machakos district; Kyangwithya in Kitui district and Thika in Kiambu district. The wilted plants from each site were separately chopped into small pieces and incorporated into soil

in the wooden boxes. Three boxes each were developed with wilted plants from Thika and Kyangwithya, one sick box from Emali isolate and ten sick boxes from Kimutwa isolate. The boxes were watered regularly to enable the pathogen to grow out and infest the soil. Pure fungal cultures were also prepared in the laboratory on potato dextrose agar from pieces of the wilted plants and regularly incorporated into the soil in the respective boxes to increase inoculum load of the soil. After three months a susceptible cultivar, Munaa was sown in all the boxes to test the soil sickness. The degree of wilting obtained (60%) suggested that the boxes were sufficiently infested for use to screen the germplasm.

A hundred and four accessions (Table 3) were tested. They comprised local and exotic collections with diverse characters, such as plant type and height, seed colour and size, and maturity. The local collections were selfed lines selected for yield and other agronomic traits in a breeding programme, while the exotic collections were selfed plants from ICRISAT. Out of the 104 accessions 27 accessions were screened in both the Thika, Kyangwithya and Kimutwa isolates, nine were sown only in Emali isolate and 68 only in the Kimutwa isolate.

Table 3. List of pigeonpea germplasm screened in the sick boxes in the glasshouse.

	Accession number		Origin		Acc. No.		Origin
1.	NPP	675	KENYA	31.	NPP	677	KENYA
2.	"	673	"	32.	"	690	"
3.	"	676	"	33.	"	699	"
4.	"	695	"	34.	"	681	"
5.	"	689	"	35.	"	686	"
6.	"	696	"	36.	No.	20	"
7.	"	702	"	37.	"	2	"
8.	"	674	"	38.	"	5	"
9.	"	679	"	39.	"	53	"
10.	"	694	"	40.	"	51	"
11.	"	678	"	41.	"	13	"
12.	"	672	"	42.	"	34	"
13.	"	683	"	43.	"	21	"
14.	"	684	"	44.	"	7	"
15.	Munaa		"	45.	"	10	"
16.	NPP	671	"	46.	"	33	"
17.	JM*	2192	ICRISAT	47.	"	49	"
18.	"	2381	"	48.	"	14	"
19.	"	2391	"	49.	"	39	"
20.	"	2397	"	50.	"	1	"
21.	"	2388	"	51.	"	18	"
22.	"	2409	"	52.	"	9	"
23.	"	2418	"	53.	"	15	"
24.	"	2419	"	54.	"	42	"
25.	"	2424	"	55.	"	55	"
26.	"	2400	"	56.	NPP	682	"
27.	"	2472	"	57.	"	688	"
28.	NPP	685	KENYA	58.	"	692	"
29.	"	687	"	59.	"	700	"
30.	"	680	"	60.	"	704	"

Table 3 (Contd...)

	Accession number		Origin		Acc. No. number		Origin
61.	NPP	707	KENYA	83.	No.	16	KENYA
62.	"	706	"	84.	"	22	"
63.	"	703	"	85	"	46	"
64.	"	693	"	86	"	30	"
65.	"	697	"	87	"	40	"
66.	"	701	"	88	"	27	"
67.	"	705	"	89	"	48	"
68.	"	698	"	90	"	56	"
69.	"	691	"	91	"	6	"
70.	No	19	"	92	"	52	"
71.	"	8	"	93	"	23	"
72.	"	32	"	94	"	43	"
71.	"	50	"	95	"	25	"
74.	"	41	"	96	"	37	"
75.	"	28	"	97	"	26	"
76.	"	11	"	98	"	3	"
77.	"	35	"	99	"	24	"
78.	"	12	"	100	"	31	"
79.	"	54	"	101	"	4	"
80.	"	47	"	102	"	36	"
81.	"	17	"	103	"	45	"
82.	"	29	"	104	"	38	"

*JM denotes ICRISAT selections made by van der Maesen

Note: NPP 718 is missing because it had not been received from ICRISAT.

Eleven furrows 10 cm apart were marked in each sick box. The first and sixth furrows were planted with Munaa as the susceptible check. The test lines were sown in the other nine furrows. Each furrow received ten seeds placed five cm apart. The boxes were irrigated lightly after every two days to minimise seeds rotting and allow better seed germination. After three weeks a germination count was taken. Wilt incidence was recorded after every two weeks upto 60 days. Plants which did not wilt by flowering time were allowed to form seed and were harvested separately. Such plants were numbered with subscripts of the original line, e.g. 671/1, 671/2,....., the highest subscript indicating the number of plants of the original line that did not wilt and gave seed. Fifty one such plants were harvested from 17 lines (Table 10) and resown in the boxes on 12-7-82. Planting was such that the 51 lines were planted in stages in the four isolates, Emali, Kimutwa, Thika and Kyangwithya. Two more sick boxes were also developed for the Emali isolate. The same plan of planting as used in the preliminary planting of the sick boxes was used. Mortalities of the lines in the different isolates were recorded as before. A disease rating

scale similar to that used at ICRISAT (Nene et al., 1981) but with modifications was used to rate the lines as follows:

Rating	Mortality (%)	Description
1	0-7	Resistant
2	8-16	Moderately resistant
3	17-30	Tolerant
4	31-50	Moderately susceptible
5	51-100	Susceptible

3.2.2. Field screening

A sick plot measuring 60 m by 42 m infested by Kyemole isolate was developed in a farmer's field (Mrs Kaloki's farm) at Makueni. This field had a history of high wilt incidences. The pigeonpea project leased this field and increased the inoculum density by incorporating a lot of chopped wilted pigeonpea plants collected in the area. The seedbed was prepared using hand hoes. This practice was repeated before every planting. The soil in this field is mainly sandy loam. The rainfall pattern is bimodal but since this is a marginal rainfall area, the rains are quite unreliable and usually last about one month in the rainy season.

Pigeonpea is grown mainly in the short rains which begin in October. The crop was dry planted at every planting and ahead of the short rains.

Fifty two lines including NPP 718 and Munaa sown in sick boxes on 12-7-82 were also sown in the sick plot on 26-10-82. The experiment was laid out in a randomised complete block design with three replications. The 51 test lines were sown in single row plots 13 m long. Two seeds were sown per hill at 0.5 m between hills. The susceptible cultivar, Munaa was sown after every ten test lines. Three weeks later the seedlings were thinned to one per hill and germination count taken. The field was visited weekly in the first month and occurrence of wilt recorded. After the first recording of wilted plants subsequent visits were made monthly. A final observation was taken just before the start of harvesting on 8-6-83. Data obtained was statistically analysed and significant differences among pairs of entry mean separated by the Duncan's multiple range test (DMRT) (Gomez and Gomez, 1984). Percent mortalities were used to characterise these lines as indicated earlier (3.2.1.). Data on several phenotypic

characters such as colour of stems, flowers and pods and uniformity in flowering in the lines was recorded in the 1982 planting. Any plant(s) within a line that flowered much earlier than the majority of the plants were tagged and harvested separately. If such plants produced adequate seed and were identical phenotypically to the rest of the plants in the line except for being early maturing, they were sown subsequently instead of the bulk-harvested seed of the particular line.

A second and third plantings of the same entries using the same experimental design were done on 7th and 8th November, 1983 and 15th October 1984. In these plantings, grain yield,(kg/ha). was determined in addition to observations made in the 1982 planting. Yield was determined because of its importance in the final selection of resistant plants or lines for advancement.

3.3. EXPERIMENT 3. Test for variability of F. udum

3.3.1. Collection of isolates

Wilted material was collected from Kitui and Machakos, the principal pigeonpea producing

districts in 1982 and 1983. A total of 12 isolates (Table 4) were collected and from each two to three "sick boxes" developed. The soil in each box was confirmed to be infective by use of a check cultivar, Munaa which is very susceptible to Fusarium wilt. Tests were started only when the boxes had at least 60% wilt of Munaa.

3.3.2. Pathogenicity tests

The pathogenicity of each isolate was determined by inoculating 12 host lines in the sick boxes developed from these isolates. The cultivars used were selfed breeding lines selected from local collections. Twelve furrows 10cm apart were marked in each box. Fifteen seeds of each were sown in a furrow at five cm intervals. The experiment was laid out in a completely randomised design with three replications. The boxes were placed in a glasshouse with a 12-hour photoperiod and day temperatures of 17^{o} to 21^{o}C (minimum) and 34^{o} to 42^{o}C (maximum). The boxes were lightly watered every two days to prevent rotting of seeds. Germination counts were taken three weeks later. The onset of wilting in these isolates was monitored regularly, and the extent of wilting in the lines caused by these isolates recorded every

Table 4: Twelve isolates of F. udum and place of origin.

Isolate	Origin
Emali	Machakos
Wote	Machakos
Tungutu	Kitui
Mulutu	Kitui
Ndumoni	Kitui
Wikililye	Kitui
Thika	Kiambu
Kimutwa	Machakos
Manoni	Machakos
Kyangwithya	Kitui
Kyemole	Machakos
Kyanika	Kitui

two weeks. A final count was done twelve weeks later. Wilt percentage was calculated as the number of plants wilted to the total number of plants raised, i.e. germination count. The final mortalities were used to compare the virulence of these isolates. The data were statistically analysed.

3.3.3. Cultural tests

Besides pathogenicity tests, the isolates were also compared for their cultural characteristics. Characteristics compared were; growth rates, pigmentation and amount of aerial mycelium, and aggressiveness of the isolates to the substrate. All cultural characteristics except growth rate were estimated visually. A comparison was made to determine whether a relationship exists between pathogenicity and cultural characteristics and to determine if isolates could be differentiated on the basis of cultural characteristics alone.

Stem tissues of wilted plants from the twelve sites were surface disinfected with a 5% solution of sodium hypochlorite for two minutes,

plated on PDA treated with streptomycin and incubated at room temperature in the sterile room. When the fungus had grown and sporulated, subcultures were prepared to obtain pure plates of the fungus. Single conidia from pure cultures were plated and studied for cultural characteristics.

Conidial suspensions of these isolates were made in sterilised distilled water using ten-day old cultures and filtered through two layers of cheese cloth to remove excess agar and mycelium. Ten test tubes containing one ml of sterilised water were arranged in a test tube rack. One ml aliquot of the suspension was pipetted into the first test tube and thoroughly mixed. Subsequent dilutions were done serially until the last tube or when the suspension was clear. A clear suspension indicated a very dilute suspension with a low conidial count while a deep pink suspension was usually very concentrated. From the last test tube one ml was pipetted into a plate containing PDA which had been kept overnight in a refrigerator to harden the agar and therefore facilitate easy cutting of agar blocks which bear

conidia. The suspension was spread all over the agar and excess drained off. The plate was then examined under the dissecting binocular microscrope at a magnification of 64 x to locate single conidia. Then with a very thin, sharp glass rod, blocks of agar bearing single conidia were transferred into other plates containing PDA, placing only one conidium into each. Six plates were inoculated with each isolate. A completely randomised design was used in laying the inoculated plates on the bench in the sterile room. The cultures were then incubated under white fluorescent light, 24-hour photoperiod for the entire duration of the experiment. Temperature in the sterile room averaged 22^{o}C.

The plates were examined daily for onset of growth of the conidia and therefore determine intervals at which colony sizes were to be measured. Colony diameters were recorded after two, five, eight and ten days of conidial growth according to the method described by Prasad (1949) on Fusarium solani f. cucurbitae. A line was drawn with a crayon across the plate above the point of inoculation and measuring growth along it. On the

tenth day also, other cultural characteristics were estimated visually and recorded on all three replicates. Data taken on tenth day was statistically analysed to establish if these isolates differed significantly from each other in their growth rates. A statistical test also was conducted to determine the relationship between pathogenicity and growth rates for these isolates.

3.3.4. Biochemical comparison of isolates

Isolates were further characterised by biochemical analysis of intramycelial proteins. This was through electrophoretic techniques.

3.3.4.1. Culture procedure

Eleven of the twelve isolates were compared on the basis of their protein components by electrophoretic analysis. The single spore cultures were grown on PDA. Incubation was in a sterile room at temperatures between 22^{o} to 24^{o}C and a 24 hour daylight supplied by a white fluorescent tube. Ten petri dishes were used for each isolate. The experiment was repeated two times.

3.3.4.2. Protein extraction

Intramycelial proteins were extracted after 14 days of incubation. Figure 1 is a diagrammatic flow chart of the sample preparation, following a technique suggested by McCombs (Stipes, 1970). The collected mycelial mats were recovered by filtration and repeated washing in distilled water. They were then weighed and blended with cold acetone (1 g wet mycelium: 10 mℓ acetone) by grinding in a prechilled mortar and pestle with a pinch of dry, clean acid-washed sand. The resulting slurry (homogenate) was filtered under suction through Whatman no. 1 filter paper on a cold Buchner funnel and washed several times with cold acetone. The mycelial slurry was then worked into a powder. The powders were extracted in a 0.004 M sodium hydrogen carbonate ($NaHCO_3$) solution using 1 mℓ of extracting solution per 100 mg acetone powder for 12 hours at $4^{o}C$. The extracts were then centrifuged at 9,000 x g for 30 minutes to obtain clear supernant solutions. These were then placed into clean, dry vials for immediate use or stored at $4^{o}C$ for later use.

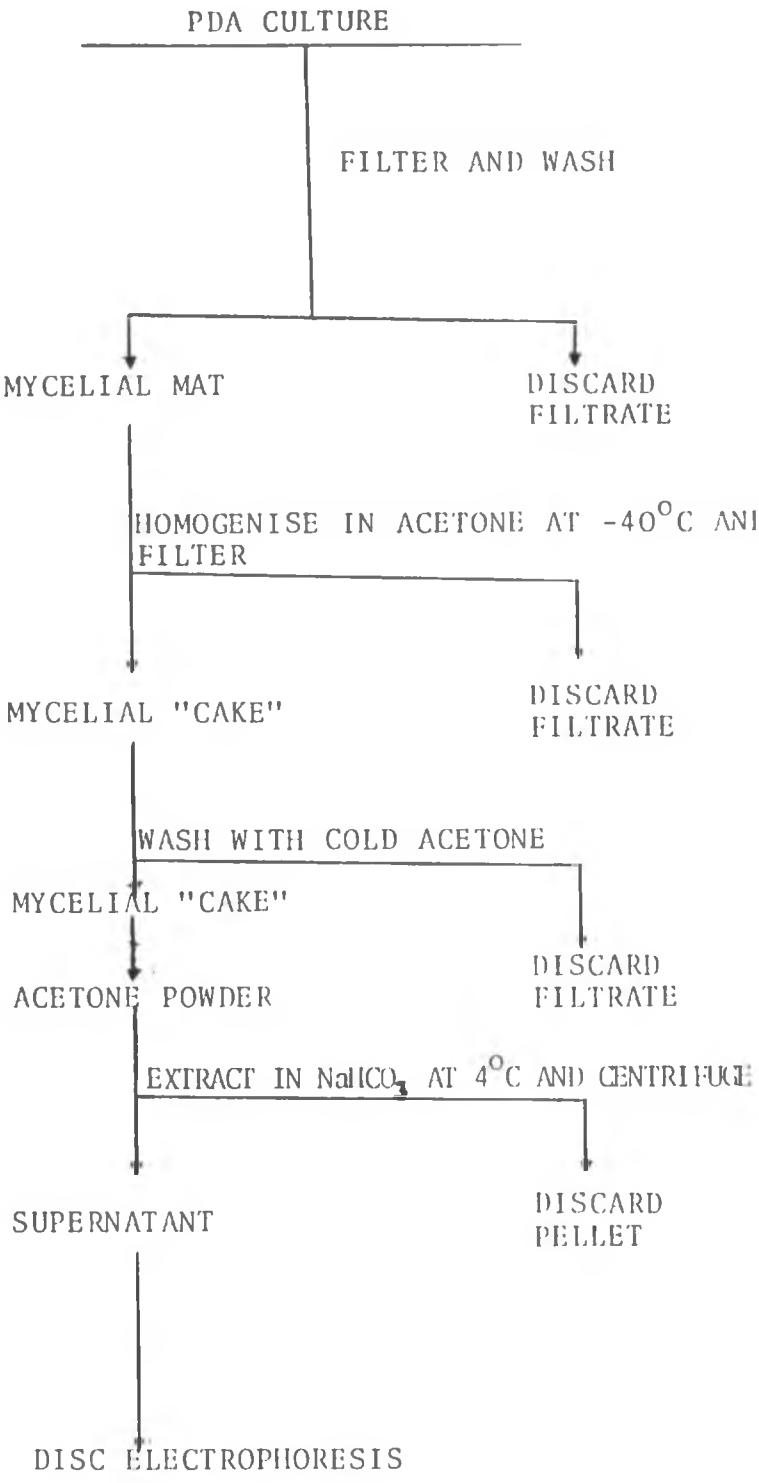

Fig. 1. Diagrammatic scheme of sample preparation of soluble proteins from mycelia of isolates of F. udum.

3.3.4.3. Electrophoresis and visualisation of proteins

The method of disc electrophoresis of Steward et al (1965) was used with some modifications.

The supporting medium was an acrylamide gel. The gel was contained in open ended glass tubes of 6 mm inner diameter and 100 mm long in two layers. Several stock solutions, A to F*, were used to prepare the two gel types (layers). The running gel (lower gel) was made up by mixing component stock solutions as follows:

1 part A
2 parts C
1 part distilled water
8 mℓ ammonium persulphate
2 mℓ E.

The large pore gel (upper gel) was made up in the following composition:

1 part B
2 parts D
1 part E
4 parts F

*The composition and preparation of stock solutions is given in the Appendix.

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The running gel solution was pipetted into each tube to a depth of about 7.5 cm. About 5 mm of distilled water was placed on top of the gel solution using a dropper with a very fine end. The water was added slowly as to avoid mixing with the gel solution. A sharp refractive boundary was visible between the gel solution and water. The test tubes standing upright in a polyethylene rack were then placed before a fluorescent lamp for 30 minutes to polymerise. After polymerisation the water layer was removed by a dropper. The large pore gel solution was then added to each tube to a depth of about 10 mm and similarly layered with distilled water. For a further 30 minutes the tubes were placed before light so that the upper gel also polymerised. The water was drained off and the gel tubes mounted vertically in the electrophoretic chamber to connect each end with the electrolyte in a way that the lower solution was the anode and the upper one the cathode. Equal amount (5 drops) of protein samples (supernatants of isolates) and fresh large pore solution were thoroughly mixed in watch glasses and placed on the top of the upper gel layers using a dropper. After polymerisation, the gel tubes were

topped with the stock buffer (tris-glycine) and subjected to electrophoresis. About 500 ml of this buffer was poured into each of the two compartments of the electrophoretic apparatus. A few drops of bromophenol blue were added to a buffer in the upper compartment and acted as a tracking dye. Protein separation was obtained by using a constant current of 5 mAmp per gel column until the tracking dye was 7 to 10mm from the lower ends of the gel columns. This separation was conducted with the apparatus placed in a refrigerator.

At the end of electrophoresis the gel columns were loosened from their tubes by gently 'rimming' them at their lower ends with a sharp ended four cm long hypodermic needle through which water was flowing. The gels were stained immediately on their removal from the tubes using 1% (w/v) amido black 10B in 7% (v/v) acetic acid for one hour. The gels were then destained in a 7% acetic acid solution. This solution was changed hourly until the bands stood out clearly behind a light blue background. The gels were placed on a gelviewer and photographed.

Diagrammatic representations were also made since the very fine and faint bands would not show well on photographs. Three shades of stippling were used to indicate dense, less dense and weak bands. The bands were drawn on a relative scale (Rf), taking the movement of the front as equal to 100 and drawing the bands accordingly. The mobility of each band was expressed as a percentage of the mobility of the added tracking dye which indicated the moving front.

3.4 EXPERIMENT 4. Electrophoretic comparison of pigeonpea lines resistant and susceptible to *Fusarium* wilt.

Seed of two resistant lines, NPP 725 and 679/1 and two susceptible lines, Munaa and NPP 718 of pigeonpea were obtained from an initial screening trial performed through conventional techniques. Screening was done both in the sick plot and in the six boxes in the glasshouse. The seeds used had been selfed four times and were considered homozygous. Two of the genotypes, Munaa and NPP 679/1, were local materials and the other two, NPP 718 and 725 were from ICRISAT. A detailed description of their characteristrics is given in Section 3.1.

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3.4.1 Growth of seedling and preparation of extracts

Surface sterilized seeds of the four lines were sown in sterilized soil composite (forest soil, sand, farm yard manure and ballast in a 6:2:2:1 ratio) in 37 cm plastic pots. Twenty to thirty seeds of each line were sown in each pot in triplicate. The sowing was staggered to facilitate analysis at different times with limited equipment for electrophoresis. The pots were laid in the glasshouse in a completely randomised design. Temperatures during this study fluctuated between 12^{o} and $25^{o}C$ (minimum) and between 38^{o} and $44^{o}C$ (maximum).

Leaves of three-day old seedlings were harvested from as many seedlings as could yield upto 2 g leaf tissue without mid-ribs. This was then ground in a prechilled mortar and pestle in 2 ml of sodium chloride buffer (0.9%). The resulting slurries (or homogenates) were used directly for starch gel electrophoresis by soaking wicks in them but were centrifuged at 9,000 x g for 25 minutes and the supernatants used for disc electrophoresis. Subsequent leaf extracts were obtained in nine, 13, 20, 25 and 42 days old plants. At each stage only the first true leaves were used to ensure that similar leaf

tissue was compared. The 2 g leaf tissues were obtained from several seedlings at the particular physiological stage. The sampling of several seedlings in each line was done to reduce sampling error and all seedlings whose leaves had been used at a particular stage were usually uprooted and discarded.

3.4.2 Starch gel electrophoresis

For the detection of isozymes, discontinuous gel electrophoresis was carried out according to Gupta and Stebbins (1969).

3.4.2.1 Gel preparation

Preliminary tests showed that a 12% starch gave good band resolution and band separation. In all the runs, this concentration of the hydrolysed starch was therefore used.

The gel was prepared according to the method of Smithies (1955). Twenty four gm of hydrolysed starch was dissolved in 200 ml of Tris-citrate buffer. The buffer was prepared by mixing 15.5 ml of 0.05 m citric acid and 16.0 ml of 0.19 m Tris (Hydroxy-methyl) amino methane and making the volume to 200 ml with distilled water. The starch suspension was heated over a medium-hot bunsen flame with

constant agitation to prevent charring at the bottom of the flask. Heating was stopped when the previously very viscous and opaque suspension started to lose viscosity and became semi-transparent and mobile or when the bubbles began to appear at the bottom of the flask. The gel was then brought to boiling by degassing under suction for 20 to 30 seconds. The gel was subsequently poured evenly in a plexi glass tray (inside dimension 155 x 125 x 60 mm). The tray was filled carefully avoiding trapping air bubbles. To ensure that the gel level was even in the whole tray, the tray was moved forward, backward and sideways on the level table. After cooling for 15 minutes, the gel was covered with a polythene sheet and placed in a refrigerator to set and harden. After 30 minutes the gel was ready for loading.

3.4.2.2. Loading the gel

A slit was made across the gel six cm from one end using a sharp pointed microspatula. The short end became the cathodal end while the longer end the anodal end. The slit, also called the origin was the point of sample application. Enzyme extracts were applied in 6 x 10 mm wicks cut from Whatman No.

3 filter paper. Excess of the sample in the wicks was removed by pressing them between absorbent paper. The wicks were then inserted in a row in the slit.

Electrophoresis was conducted in a refrigerator with the circuit between the gel ends and electrolyte completed using sponge cloths. Electrophoresis was conducted at 165 volts for the first 15 minutes. This was then adjusted to 300 V and each run lasted between three and four hours or until the borate front, visible as dirty-yellow, had moved eight to nine cm into the anodal side of the gel.

After electrophoresis the gel was sliced horizontally and each duplicate half gel stained for one of the four enzyme systems studied. Isozymes were visualised by the methods described by Soren and Gunner (1977), Okiror (1981), Kahler et al. (1981),and Scandalios (1967) for esterase, peroxidase, acid phosphatase (ACP) and leucine amino peptidase (LAP) enzymes respectively.

As soon as distinct bands appeared, the gels were washed in running tap water and placed on a gel viewer. The gels were then viewed, drawn and where necessary photographed. The different band intensities were expressed by different patterns of shading.

3.4.3 Polyacrylamide gel electrophoresis

For the detection of proteins, electrophoresis was carried out in polyacrylamide gel according to the procedure already outlined (3.3.4). Protein assays were done using the same samples used for starch gel electrophoresis.

After electrophoresis and removing the gels from the tubes, the gels were stained in a 1% amido black 10B solution for one hour and destained in 7% acetic acid. The acid was regularly changed till the bands (if any) were visible. The gels were then handled as described in 3.3.4.

4. RESULTS

4.1. Inoculation methods

Wilt symptoms did not appear until about the fourth week after inoculation and in some methods this was delayed upto the nineth week. However, in all cases the symptoms were typical, namely yellowing of leaves gradually leading to wilting and drying and ultimately death of the plant. The seedlings on close examination revealed the characteristic black streaks under the bark of the lower stem and root regions. The appearance and severity of the disease differed for each inoculation method (Table 5).

4.1.1 Direct sowing

A Munaa seedling wilted four weeks after inoculation. Gradually the number rose in this and other lines such that by the seventh week, the level of wilt especially among the susceptible lines was already high. By this technique, 82 and 90% mortalities had been induced in the susceptible lines by the twelveth week. Among the resistant lines, 13% mortality was

Table 5. Effect of inoculation method on Fusarium wilt incidence in four pigeonpea cultivars.

Cultivar	Soil Infestation			Inoculation technique										
	(1) Direct sowing	(2) Seedling transplant		(3) Stem injection spore concentration		(4) Root dipping					(5) Seed soaking				
		Roots intact	Roots pruned	$1.31x10^6$	$1.64x10^6$	Root trt.	spore concentration $4.23x10^5$	$9.4x10^5$	$1.31x10^6$	$1.64x10^6$	Time hrs	Spore concentration $4.23x10^5$	$9.4x10^5$	$1.31x10^6$	$1.64x10^6$
MUNAA	88.9	57.0	58.3	25.0	20.0	intact	46.7	50.9	64.0	90.0	1	5.6	4.8	13.7	14.3
						pruned	43.5	54.8	74.0	85.5	5	12.9	20.0	15.6	21.4
											7	25.0	18.5	25.0	42.9
NPP 679	38.8	48.3	52.3	4.9	6.8	intact	22.2	43.0	47.0	52.8	1	8.8	8.6	5.4	9.1
						Pruned	26.5	45.7	50.1	55.0	5	9.5	23.5	2.7	33.3
											7	16.1	13.2	12.9	27.8
NPP 718	82.0	63.3	68.0	15.3	17.7	intact	50.0	55.5	60.0	83.0	1	10.0	12.5	25.0	30.0
						pruned	50.0	59.5	65.0	79.4	5	22.2	23.2	33.3	33.3
											7	11.1	40.0	43.3	50.0
NPP 725	12.5	16.2	24.4	2.4	3.3	Intact	12.5	43.8	45.0	60.0	1	11.1	3.5	33.3	40.0
						Pruned	15.0	40.0	44.0	65.5	5	12.5	14.0	20.0	10.0
											7	12.9	17.4	40.0	20.0
Mean (of method)	55.7	46.2	50.8	11.9	12.0	intact	32.9	48.3	54.0	71.4	1	8.9	7.4	19.4	23.4
						pruned	33.8	50.0	58.3	71.4	5	14.3	20.2	17.9	24.5
											7	16.3	22.3	30.3	35.2
CV (%)	*30.4*	*12.0*		*40.4*			*4.4*				*4.4*				

recorded for NPP 725 and 39% for NPP 679/1. The unexpected high mortality rate for NPP 679/1 suggested that the line was still segregating for resistance. However, compared to other techniques, this inoculation was considered the simplest to conduct, repeatable and very effective.

4.1.2. Root dipping

In this technique, the first seedling to wilt was that of Munaa and occurred towards the end of the fourth week. This was a seedling that had had its roots pruned and dipped in the most concentrated (1.64×10^6) of the inocula used. Wilt progressively increased with time and like in direct sowing, was very severe by the seventh week especially among the susceptible lines. At the conclusion of the test, the highest mortality for all the tested lines occurred at the highest inoculum concentration (1.64×10^6). Pruning of roots apparently increased mortalities. However, this method led to severe wilting in both the resistant and susceptible lines. This was especially the case at the highest concentration of inoculum. At the inoculum

concentration of 1.31×10^6 mortalities were somewhat lower, being 44 and 50% among the resistant lines and 65 and 74% for the susceptible lines. Since the dead plants on examination showed the characteristics black streaks it was assumed they all died due to Fusarium wilt. Other factors that could have been involved were not established. At the other two concentrations, that is 4.23×10^5, and 9.40×10^5, mortalities were generally much lower (between 12 and 60% respectively). Since the mortalities among the susceptibe lines were low to moderate at these inoculum concentrations, this method was considered less effective and less reliable.

4.1.3 Seedling transplanting

The earliest visible symptoms of wilting occurred in two seedlings each of Munaa and NPP 718 at the end of the fifth week. In NPP 718, it was the seedlings with pruned roots while in Munaa it was those with intact roots. Over the next two weeks a few more plants had wilted and by the end of the seventh week, the incidence was rapidly increasing. Overall, inoculation by this technique

led to moderate mortalities (57 to 68%) among the susceptible lines. Root injury apparently enhanced wilting.

4.1.4. Stem injection

Disease development by this technique was slow. No wilt symptoms appeared until the nineth week and was confined only to Munaa. The first dead plant occurred in NPP 718 in the tenth week. While other plants showed wilt symptoms at much later stages, such as at flowering, this was often preceded by partial wilting. On the whole, this technique induced low mortalities even among the susceptible lines. Upto 25% mortality occurred in Munaa and the figures were lower (2 to 18%) among the other lines. The results were also not consistent. For instance, the 25% deaths in Munaa resulted from the lower of the two inoculum concentrations used. The stronger concentration caused 20% deaths. In addition to this inconsistency, mortalities were generally very low and the procedure cumbersome, and hence this technique was considered ineffective and unsatisfactory.

4.1.5. Seed soaking

Wilting occurred very slowly and was erratic among the inoculum concentrations used. Macroscopic symptoms first showed in the sixth week and the first plant to die due to Fusarium wilt occurred in the seventh week. However, this method induced low mortalities. Even at the highest inoculum concentration, seed soaked for the longest duration (seven hours) had only upto 50% wilt. Mortalities were even much lower at the lower concentrations of inoculum and/or periods of soaking. Evident also from the results was lack of consistency in the results. For instance, it was expected that as inoculum concentration increased so would the mortalities. This was however not always the case. Similarly with longer soaking duration, wilting was expected to rise. This too was not the general pattern. This method, like some of those already described was judged as ineffective, unreliable and therefore unsatisfactory.

Table 6/ shows the average mortalities from each inoculation technique. The highest mortality occurred when seeds were directly sown in infested soil which resulted in a mean mortality of 56%. It

Table 6. Ranking of the inoculation methods used on the basis of mean percent wilt

Inoculation method	% wilt (Mean)		Position relative to other methods
DIRECT SOWING	55.7		1
ROOT DIPPING	52.6		2
(i) Roots pruned		53.4	2^1
(ii) Roots intact		51.7	2^2
SEEDLING TRANSPLANT	48.5		3
(i) Roots pruned		50.8	3^1
(ii) Roots intact		46.2	3^2
SEED SOAKING	20.0		4
STEM INJECTION	12.0		5

Note: This table is a summary of Table 6.

was not only the most effective of the methods tested but also was simple to conduct and was repeatable. Root dipping in inoculum and seedling transplanting into infested soil also were relatively effective methods with mortalities of about 53 and 49% respectively. In both methods, root prunning had the effect of enhancing wilting. Both soaking seed in inoculum suspension and stem injection were less effective methods with 20 and 12% mortalities respectively.

4.2 Germplasm evaluation

Of the twenty seven lines tested simultaneously in the Thika, Kimutwa, and Kyangwithya isolates, 17 lines had some plants surviving on the 60th day in one, two or all the isolates (Table 7). Disease severity rating (DSR) was not considered in this planting. The use of a DSR scale would have meant that only three lines grown in Kimutwa isolate and five lines in Kyangwithya isolate could have been rated and therefore selected as resistant or tolerant (30% or less wilt). No line could have been selected in the Thika isolate. However, in this test emphasis was on the identification of promising

Table 7. Test for resistance to Fusarium wilt in 27 local, and exotic pigeonpea lines with different inocula.

Line*	Inoculum source				%wilt
	Thika	Kimutwa	Kyangwithya	Total	mean
NPP 676	†90.0	80.0	30.0	200.0	66.7
" 695	100.0	80.0	50.0	230.0	76.7
" 889	100.0	100.0	80.0	280.0	93.3
" 702	100.0	100.0	100.0	300.0	100.0
MUNAA	100.0	100.0	90.0	290.0	96.7
NPP 679	90.0	60.0	20.0	170.0	56.7
" 678	100.0	80.0	20.0	200.0	66.7
" 672	90.0	50.0	80.0	220.0	73.3
" 671	100.0	20.0	100.0	220.0	73.3
" 673	100.0	0	90.0	190.0	63.3
" 684	100.0	90.0	30.0	220.0	73.3
" 683	100.0	100.0	20.0	220.0	73.3
" 694	100.0	90.0	70.0	260.0	86.7
" 674	100.0	40.0	100.0	240.0	80.0
" 675	100.0	20.0	100.0	220.0	73.3
" 696	90.0	90.0	100.0	280.0	93.3
JM 2424	100.0	100.0	100.0	300.0	100.0
" 2397	100.0	80.0	90.0	270.0	90.0
" 2381	100.0	100.0	100.0	300.0	100.0
" 2419	100.0	100.0	100.0	300.0	100.0
" 2388	100.0	100.0	100.0	300.0	100.0
" 2472	100.0	100.0	100.0	300.0	100.0
" 2418	100.0	100.0	100.0	300.0	100.0
" 2391	100.0	100.0	100.0	300.0	100.0
" 2400	100.0	90.0	100.0	290.0	96.7
" 2192	100.0	100.0	100.0	300.0	100.0
" 2409	100.0	100.0	100.0	300.0	100.0
Total	2,660.0	2,170.0	2,170.0	7,000.0	
Mean	98.5	80.4	80.4		

†percent wilt

* NPP = Local lines

JM = ICRISAT lines.

material and subjecting it to further evaluation. Of the 77 lines tested in the Kimutwa and Emali isolates, 34 were completely killed (Table 8). In the other 44 lines mortality varied between 10 and 90%. Only four lines -No. 55 and 17 and NPP 688 and 693 -of those listed in Table 8 -could have been selected as resistant. They had 30% or less wilt. Although the number of wilted plants were not recorded after the 60th day, mortalities increased steadily to near 100% for most lines at harvest. However, any plants that survived to give a crop were harvested singly as promising material and subjected to further testing in the sick boxes as well as sick plots in the field. Fifty two such plants were harvested and the progeny of each handled as a line (Plate 2).

The reaction of these 52 lines to the pathogens under glasshouse conditions is given in Table 9 . Of these lines, 20, 17, 25, and 14 showed 30% or less wilt against the Thika, Kyangwithya, Kimutwa and Emali isolates respectively. These lines were considered either resistant, moderately resistant or tolerant to the pathogen. However, the lines that had 30% or less wilt under one isolate were necessarily not all those that reacted similarly in the other isolates. For instance, lines

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Table 8. Percent wilt in 77 lines of pigeonpea tested against Kimutwa and Emali isolates

Line (1)++	Percent wilt(2)	Disease reaction* (3)
NPP 685	100.0	S
No. 20	100.0	S
No. 2	100.0	S
NPP 687	100.0	S
No. 5	90.0	S
MUNAA	100.0	S
No. 53	70.0	S
NPP 680	80.0	S
No. 51	90.0	S
No. 13	90.0	S
No. 34	60.0	S
NPP 677	100.0	S
No. 21	100.0	S
No. 7	100.0	S
NPP 690	40.0	MS
No. 10	100.0	S
No. 33	100.0	S
No. 49	100.0	S
NPP 699	50.0	MS
No. 14	100.0	S
No. 39	100.0	S
NPP 681	100.0	S
No. 1	80.0	S
No. 18	100.0	S
No. 9	100.0	S
No. 15	60.0	S
NPP 686	100.0	S
No. 42	100.0	S

Table 8. (Contd..)

1	2	3
No. 55	10.0	R
NPP 682	80.0	S
No. 19	100.0	S
No. 8	90.0	S
No. 32	60.0	S
No. 50	100.0	S
NPP 688	10.0	R
No. 41	100.0	S
No. 28	90.0	S
No. 11	90.0	S
NPP 692	90.0	S
No. 35	80.0	S
NPP 700	100.0	S
No. 12	80.0	S
No. 54	90.0	S
No. 47	100.0	S
NPP 704	90.0	S
No. 17	20.0	T
No. 29	90.0	S
No. 16	100.0	S
NPP 707	60.0	S
NPP 706+	80.0	S
No. 46+	60.0	S
No. 22+	90.0	S
No. 30+	100.0	S
No. 40+	90.0	S
NPP 703+	90.0	S
No. 27+	100.0	S
No. 48+	50.0	MS

Table 8.(Contd..)

1	2	3
No. 56	90.0	S
NPP 693*	30.0	T
No. 6	80.0	S
NPP 697	100.0	S
No. 52	100.0	S
No. 23	100.0	S
No. 43	60.0	S
No. 25	70.0	S
NPP 701	90.0	S
NPP 705	60.0	S
No. 37	100.0	S
No. 26	100.0	S
NPP 698	90.0	S
No. 3	50.0	MS
No. 24	40.0	MS
No. 31	100.0	S
No. 4	80.0	S
No. 36	80.0	S
No. 45	100.0	S
No. 38	90.0	S
NPP 691	40.0	MS

* S = Susceptible (51-100% mortality)
MS = Moderately susceptible (31-50% mortality)
T = Tolerant (17-30% mortality)
MR = Moderately resistant (8-16% mortality)
R = Resistant (0-7% mortality)
\+ = Lines tested in the Emali isolate

++ Note: i) NPP 718, 725 and 726 had not yet been obtained from ICRISAT.

ii) M.S. was actually selected in this test!

Plate 2: Some of the plants which survived wilt were allowed to grow to maturity and form seed. Such single plants subsequently became lines.

Table 9. Wilt reaction of 52 lines of pigeonpea grown in four isolates of F. udum.

Entry	Isolate and disease reaction				
	Thika	Kyangwithya	Kimutwa	Emali	Mean
1 (1)	(2)	(3)	(4)	(5)	(6)
NPP 671/2	1 (0)*	-	4 (38.9)	4 (50.0)	3 (29.6)
NPP 671/6	2 (13.4)	4 (40.0)	4 (46.7)	4 (46.2)	4 (36.6)
NPP 675/5	1 (9.4)	4 (33.3)	4 (36.4)	5 (63.6)	4 (35.7)
NPP 672/1	3 (22.2)	4 (33.3)	4 (50.0)	5 (69.2)	4 (43.7)
NPP 675/1	3 (25.0)	5 (88.9)	5 (52.1)	-	5 (55.3)
NPP 675/4	2 (10.0)	3 (30.0)	1 (0)	-	2 (13.3)
NPP 725	2 (12.5)	1 (0)	1 (5.6)	2 (9.6)	1 (6.9)
NPP 707/1	1 (0)	2 (10.0)	1 (7.1)	5 (62.5)	3 (19.9)
NPP 696/3	2 (10.0)	-	4 (33.4)	2 (9.1)	3 (17.5)
NPP 695/2	3 (27.8)	5 (85.7)	5 (52.9)	5 (66.7)	5 (58.3)
NPP 675/3	3 (21.7)	4 (45.9)	3 (29.4)	3 (20.0)	3 (29.3)
NPP 691/2	3 (26.8)	-	4 (36.7)	5 (75.0)	4 (46.2)
NPP 690/1	3 (20.0)	2 (14.3)	4 (35.0)	2 (8.4)	3 (19.4)
NPP 671/4	2 (11.1)	4 (50.0)	3 (20.0)	5 (60.0)	4 (35.3)
NPP 688/5	2 (16.8)	-	4 (33.1)	2 (10.0)	3 (20.0)
NPP 673/1	2 (12.5)	1 (0)	4 (36.7)	4 (40.0)	3 (22.3)

Table 9. (Contd..)

(1)	(2)	(3)	(4)	(5)	(6)
NPP 674/2	3 (17.0)	4 (33.3)	4 (45.4)	5 (66.7)	4 (40.6)
NPP 707/2	2 (16.7)	-	1 (5.2)	4 (40.0)	3 (20.6)
NPP 691/5	-	3 (20.0)	4 (49.8)	3 (30.0)	4 (33.3)
NPP 671/3	5 (50.0)	1 (0)	2 (13.3)	1 (0)	3 (15.8)
NPP 691/3	5 (100.0)	1 (0)	4 (34.2)	-	4 (44.7)
NPP 675/6	5 (56.2)	1 (0)	2 (15.6)	3 (25.0)	3 (24.2)
NPP 694/3	5 (66.7)	1 (0)	2 (14.3)	2 (10.0)	3 (22.8)
NPP 695/1	4 (39.0)	3 (20.9)	3 (27.0)	5 (63.6)	4 (37.6)
NPP 673/3	4 (47.2)	3 (30.1)	2 (16.5)	4 (41.3)	4 (33.8)
NPP 699/1	5 (71.4)	2 (10.0)	4 (38.4)	5 (70.0)	4 (47.5)
NPP 688/3	4 (44.4)	2 (16.7)	2 (10.0)	-	3 (23.7)
M.S	4 (33.3)	2 (12.5)	3 (24.2)	4 (50.0)	3 (30.0)
NPP 726	4 (50.8)	3 (23.4)	3 (25.8)	4 (40.0)	4 (35.0)
NPP 674/1	5 (53.8)	3 (21.4)	5 (58.5)	4 (44.4)	4 (44.5)
NPP 688/2	5 (50.0)	5 (70.0)	3 (18.2)	4 (36.4)	4 (43.7)
NPP 671/5	5 (100.0)	5 (58.7)	3 (25.0)	5 (83.3)	5 (66.8)
NPP 671/1	4 (42.9)	5 (66.7)	3 (27.5)	-	4 (45.7)
NPP 688/1	1 (0)	4 (48.2)	3 (30.0)	3 (27.3)	4 (35.2)

Table 9. (Contd...)

(1)	(2)	(3)
NPP 693/3	4 (35.0)	5 (88.9)
NPP 675/2	5 (57.1)	-
NPP 694/1	5 (80.0)	5 (58.3)
NPP 699/2	4 (40.0)	4 (36.8)
NPP 673/2	-	5 (56.3)
NPP 688/4	5 (83.3)	4 (33.3)
NPP 691/1	-	4 (44.6)
NPP 690/2	5 (50.0)	5 (100.0)
NPP 691/4	4 (33.3)	5 (88.9)
NPP 693/1	4 (36.3)	5 (57.1)
NPP 696/1	4 (42.9)	4 (50.0)
NPP 694/2	5 (66.7)	4 (35.7)
NPP 700/1	-	4 (37.5)
NPP 679/1	5 (88.9)	4 (38.8)
NPP 696/2	5 (70.8)	5 (88.9)
NPP 718	5 (100.0)	5 (66.7)

(4)	(5)	(6)
2 (10.0)	2 (16.7)	4 (37.7)
3 (20.0)	2 (10.0)	3 (29.0)
2 (11.1)	4 (66.7)	5 (54.0)
3 (18.4)	-	4 (31.7)
3 (27.0)	5 (71.4)	4 (51.6)
3 (26.3)	2 (8.4)	4 (37.8)
5 (60.0)	1 (0)	4 (34.9)
5 (63.4)	-	5 (71.1)
4 (50.0)	-	5 (57.4)
3 (29.0)	-	4 (40.8)
4 (42.1)	-	4 (45.0)
4 (34.2)	5 (100.0)	5 (59.2)
5 (53.3)	5 (100.0)	5 (63.6)
5 (58.6)	-	5 (62.1)
4 (50.0)	4 (41.7)	5 (62.9)
5 (100.0)	5 (55.0)	5 (80.4)

Table 11 (Contd.)

(1)	(2)	(3)	(4)	(5)	(6)
MUNAA	5 (63.3)	5 (100.0)	5 (95.0)	5 (83.3)	5 (85.4)
NPP 693/2	3 (25.0)	5 (80.0)	5 (75.0)	-	5 (60.0)

* Values in parenthesis are percent mortalities

Rating scale:

Rating	Mortality (%)	Interpretation
1	0 - 7	Resistant
2	8 - 16	Moderately resistant
3	17 - 30	Tolerant
4	31 - 50	Moderately susceptible
5	51 - 100	Susceptible

NPP 671/2, 675/5 and 671/6 were resistant to the Thika isolate but were susceptible to the other three isolates. While a line like NPP 725 was resistant to all the isolates, others were resistant to three, two, one or to no isolate at all. Those that were susceptible to all the four isolates included Munaa and NPP 718, both susceptible checks used in the study. On the basis of means of mortalities of individual lines, only 16 lines had 30% or less wilt. This was less than the number of lines recorded for individual isolates except the Emali isolates. This is because of the non-uniform reaction of the lines in all the isolates. These observations suggest: (1) that the isolates used are of different virulences. This is supported by the fact that a line was usually not wilted to same degree by the four isolates; and (2) that the lines themselves are inherently different in their susceptibilities to these isolates.

If further breeding work was to be confined to Fusarium wilt resistance alone, then only 43 lines would have been of importance (Table 10). Of these, 20 were resistant against one isolate, 14 against two isolates, eight against three isolates and one against all the four isolates. The one line

Table 10 Spectrum of resistance in pigeonpea lines with 30% or less mortalities.

(i) Lines resistant to one isolate:

NPP 671/2, 671/6, 675/5, 672/1, 675/1, 695/2, 691/2, 674/2, 691/3, 688/2, 699/1, 674/1, 671/5, 671/1, 694/1, 699/2, 673/2, 691/1, 693/1, 693/2.

Total = 20

(ii) Lines resistant to two isolates

NPP 696/3, 671/4, 688/5, 673/1, 707/2, 691/5, 695/1, 673/3, 688/3, M.S., NPP 726, 693/3, 675/2, 688/4.

Total = 14

(iii) Lines resistant to three isolates

NPP 707/1, 690/1, 671/3, 675/6, 694/3, 688/1, 675/4, and 675/3.

Total = 8

(iv) Lines resistant to four isolates

NPP 725.

Total = 1

resistant to all four isolates and the eight lines resistant in three isolates were considered more useful than the other lines. The isolates used were obtained at sites far apart in the pigeonpea growing areas of the country. So these nine lines may therefore be considered adapted at least in those areas from where the isolates were obtained because when grown in these areas they would probably not be as prone to wilting as any other lines. The nine lines were however not tested to ascertain if their resistance was mono-, oligo-, or polygenic. However, since the causal fungus is reported to occur in several forms probably multigenic resistance would be more durable.

The reaction of the 52 lines in the sick plot for three seasons is shown in Table 11. In season 1, 49 lines had 30% or less wilt. Of these, 33 were resistant, 13 moderately resistant and three tolerant to F. udum. In the second season, all the 52 lines had 30% or less wilt with 25 rated resistant, 20 moderately resistant and seven tolerant. Although the level of wilting appears relatively low in both seasons and that most of the lines had similar mortalities during the two seasons, the distribution of these lines according to DSR scale shows that the disease level

Table 11 Wilt reaction of 52 lines grown in the sick plot at Makueni

Entry	Season 1 1982/83	Season 2 1983/84	Season 3 1984/85	Mean
(1)	(2)	(3)	(4)	(5)
NPP 688/3	1+ (2.1)*	2 (9.0)	1 (6.2)	1 (5.8)
M.S.	1 (5.3)	2 (10.2)	4 (31.2)	2 (15.6)
NPP 696/3	1 (3.6)	3 (19.0)	2 (10.1)	2 (10.9)
" 695/2	1 (0)	1 (5.2)	2 (14.5)	1 (6.6)
" 694/1	1 (2.9)	1 (3.4)	3 (22.8)	2 (9.7)
" 726	2 (8.1)	3 (24.7)	3 (19.9)	3 (17.6)
" 674/1	1 (5.0)	1 (6.8)	1 (5.4)	1 (5.7)
" 690/1	2 (8.0)	2 (11.5)	2 (12.4)	2 (10.6)
" 699/2	2 (9.1)	1 (6.8)	1 (5.8)	1 (7.2)
" 693/1	2 (12.5)	1 (6.8)	3 (21.5)	2 (13.6)
" 671/4	1 (3.1)	2 (12.3)	2 (16.6)	2 (10.7)
" 673/2	1 (0)	3 (17.9)	2 (13.1)	2 (10.3)
" 688/5	1 (0)	1 (2.4)	1 (5.8)	1 (2.7)
" 673/1	1 (0)	1 (1.2)	1 (3.9)	1 (1.7)
" 679/1	1 (0)	3 (19.3)	2 (12.8)	2 (10.7)
" 688/4	1 (0)	2 (8.7)	1 (3.9)	1 (4.2)
" 693/2	2 (8.6)	1 (4.8)	2 (12.3)	1 (8.5)

Table 11 (Contd...)

(1)	(2)
NPP 674/2	1 (0)
" 707/2	1 (2.7)
" 671/2	1 (0)
" 671/6	1 (0)
" 688/2	1 (0)
" 690/2	1 (0)
" 672/1	1 (0)
" 696/2	1 (0)
" 694/2	1 (5.3)
" 691/5	1 (3.6)
" 671/5	1 (0)
" 675/4	1 (0)
" 691/1	1 (5.7)
" 725	1 (0)
" 671/1	1 (3.2)
" 671/3	1 (1.6)
" 688/1	1 (0.9)
" 693/3	1 (7.5)

(3)	(4)	(5)
2 (9.1)	2 (8.0)	1 (5.7)
2 (13.4)	1 (3.5)	1 (6.5)
2 (11.6)	1 (5.6)	1 (5.7)
2 (8.1)	1 (7.5)	1 (5.2)
1 (5.6)	1 (6.7)	1 (4.1)
1 (5.1)	3 (21.5)	2 (8.9)
2 (12.3)	1 (4.9)	1 (5.7)
2 (9.0)	2 (11.1)	1 (6.7)
2 (11.5)	1 (5.3)	1 (7.4)
1 (1.2)	2 (13.6)	1 (6.1)
1 (2.0)	3 (19.1)	1 (7.0)
1 (3.2)	2 (8.0)	1 (3.7)
1 (4.3)	1 (7.7)	1 (5.9)
2 (12.2)	2 (17.9)	2 (10.0)
1 (6.7)	2 (9.8)	1 (6.6)
1 (0)	2 (13.2)	1 (4.9)
1 (0.8)	1 (6.2)	1 (2.6)
1 (7.1)	2 (9.9)	2 (8.2)

Table 11 (Contd...)

(1)	(2)	(3)	(4)	(5)
NPP 675/6	1 (0)	1 (7.4)	2 (8.3)	1 (5.2)
" 694/3	1 (7.3)	1 (6.0)	1 (5.7)	1 (6.3)
" 673/3	1 (1.5)	1 (2.6)	2 (16.0)	1 (6.7)
" 675/2	2 (9.0)	2 (10.8)	2 (12.2)	2 (10.7)
" 675/1	2 (10.4)	3 (19.2)	2 (11.4)	2 (13.7)
700/1	2 (12.3)	1 (7.2)	4 (31.2)	2 (16.9)
691/3	3 (20.0)	1 (2.4)	3 (21.4)	2 (14.6)
695/1	2 (10.6)	2 (10.9)	1 (3.7)	2 (8.4)
699/1	2 (11.3)	1 (5.8)	3 (18.5)	2 (11.9)
707/1	2 (12.6)	2 (16.4)	1 (5.5)	2 (11.5)
675/3	3 (17.3)	2 (13.4)	2 (11.2)	2 (14.0)
691/2	- -	2 (9.2)	3 (24.6)	2 (16.9)
696/1	2 (14.3)	2 (11.0)	2 (11.1)	2 (12.1)
691/4	3 (27.0)	2 (8.4)	1 (7.1)	2 (14.2)
718	5 (63.5)	3 (29.2)	5 (77.2)	5 (70.4)
Munaa	5 (66.5)	3 (26.8)	5 (54.0)	5 (60.3)
NPP 675/5	5 (80.0)	2 (13.9)	3 (21.4)	3 (17.7)

Table 11 (Contd...)

(1)	(2)	(3)	(4)	(5)
Kioko	- -	- -	2 (12.4)	- -
NPP 670	- -	- -	3 (21.1)	- -
No. of entries under various ratings: 1 = 33		25	18	25
2 = 13		20	20	23
3 = 3		7	10	2
4 = 0		0	2	0
5 = 3		0	2	2

+1 = disease rating , * = Percent mortality = $\left(\frac{\text{No of dead plants in a plot}}{\text{Total number of plants in a plot}} \times 100\right)$

was evidently less severe in the second season. This is also indicated by the fact that even the susceptible lines reacted as tolerant material. The low incidence of wilt during the second season could be attributed to the prevailing conditions during this season, that is short rains, 1983/84. The rainfall received was much below the average and day and soil temperatures were very high. These conditions are considered to have led to low fungal population and activity and therefore the low mortalities. However, in the third season (1984/85) the level of wilting increased slightly over the previous two seasons (Table 11). The susceptible lines, NPP 718 and Munaa were heavily wilted and in some rows, they were completely killed (Plate 3). Even Munaa selection (MS), a single plant selection from Munaa that was apparently resistant succumbed and was heavily wilted (Plate 4). This rise in wilt incidence is probably due to favourable environmental factors especially soil moisture, soil temperature, higher level of inoculum, and soil microflora which favoured increased fungal activity during this season. Consequently during this season, only 38 lines

Plate 3: Severity of wilt during 1984/85 season. The three white labels show from left to right: NPP 688/5, Munaa and NPP 718. Both Munaa and NPP 718 are severely wilted.

Plate 4: The line M.S. was also heavily wilted as can be seen above (Row 2 from left).

including Kioko had 16% or less wilt and could therefore have been selected as either resistant or tolerant lines. Of these, 18 were resistant with 7% or less disease severity (Table 11). However, this increased wilt incidence was not much different from that of the previous seasons. This is confirmed by the highly significant correlations (r = 0.50, 0.67, and 0.69) between the three seasons (Table 12).

On the basis of the means from the three seasons, 50 lines had 30% or less mortalities. All these could therefore be further tested. Of these 25 were resistant, 23 moderately resistant and two tolerant. The susceptible checks, NPP 718 and Munaa had the highest mortalities.

The Kyemole isolate was prepared from wilted plants taken from this sick plot. A comparison of the field and glasshouse (sick boxes) results was therefore made. A positive significant correlation (r = 0.47) was obtained (Table 12). This positive correlation implies reliability of the results and therefore of the two methods. Alternatively it means that either results can be used to confirm results of the other technique. From the means of

Table 12. Correlation coefficients (r) for wilt reaction of the pigeonpea lines in the glasshouse and sick plot. and between the three seasons in the sick plot.

Comparison	Corr. coeff.(r)
1. Kyemole isolate in glasshouse vs mean of three seasons in sick plot at Kyemole	0.47*
2. Season 1 (1982/83) vs season 2 (1983/84)	0.50**
3. Season 1 (1982/82) vs season 3 (1984/85)	0.67**
4. Season 2 (1983/84) vs season 3 (1984/85)	0.59**

* = significant at P <0.05.

** = significant at P <0.01.

sick box tests (Table 9) however, only 16 lines had 30% or less mortalities. This is because the four isolates are of different virulences. However, these 16 lines are adapted to a wider area on the basis of disease reaction.

In the third planting in the sick plot, selection of the most promising lines using yield and its component parameters and disease reaction was attempted. The characteristic features of all the 52 lines with respect to some of the economic characters have been presented in Table 14). Except for lines of Indian origin namely NPP 718, 725 and 726 and the variety NPP 670, all the lines were characteristically tall, with an average height ranging from 1.43 m to 2.35 m. Most of the unimproved cultivars that are grown by farmers are also very tall and late maturing. There were significant differences between the lines for this parameter (Table 13). It was observed that the very tall lines were also late maturing, producing 50% of their flowers after six months. These tall plants were often difficult to harvest since the plants had either to be bent over or the branches had to be cut-off to be harvested while seated.

Table 13. Analysis of variance for phenotypic characters, yield and related traits in 54 pigeon pea lines and cultivars.

Source of variation	Df	Mean squares						
		Plant height	Days to 50% flowering	No. of primary branches	Pods/ plant	Seeds/ pod	100 seed weight	Yield kg/ha
Block	2	0.14**	1283.60**	19.93**	9957.89NS	0.38**	36.10**	185.39NS
Cultivars	53	0.30**	2252.10**	4.54**	11815.29**	0.59**	26.79**	1076.69NS
Error	106	0.03	167.40	1.36	4760.73	0.10	1.82	964.65
Total	161	0.12	867.55	2.65	5934.52	0.27	10.63	992.72
C.V.(%)		9.36	7.99	12.82	76.62+	6.73	7.39	60.18+

+Very high c.v. values were noted for pods/plant and yield (kg/ha). These values are certainly unacceptable and indicate an error. However, even after repeated analysis of the data same values were obtained. The source of error was therefore unidentified.

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		Plant height	Days to 50% flowering	No. of primary branches	Pods/ plant	Seeds/ pod	100 seed weight	Yield kg/ha
Block	2	0.14**	1283.60**	19.93**	9957.89NS	0.38**	36.10**	185.39NS
Cultivars	53	0.30**	2252.10**	4.54**	11815.29**	0.59**	26.79**	1076.69NS
Error	106	0.03	167.40	1.36	4760.73	0.10	1.82	964.65
Total	161	0.12	867.55	2.65	5934.52	0.27	10.63	992.72
C.V.(%)		9.36	7.99	12.82	76.62+	6.73	7.39	60.18+

+Very high c.v. values were noted for pods/plant and yield (kg/ha). These values are certainly unacceptable and indicate an error. However, even after repeated analysis of the data same values were obtained. The source of error was therefore unidentified.

Table 14. Phenotypic characters of pigeonpea lines tested in the sick plot at Makueni

Entry	Plant height (cm)	Days to 50% flowering	No. of primary branches	Seeds/ pod	Pods/ plant	100 seed weight (g)	Yield kg/ha
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
NPP 675/5	200	201	10.0	5.41	98.19	19.84	1660
" 673/2	183	155	8.3	4.58	104.75	17.31	1551
" 674/2	183	143	7.5	4.64	59.91	18.04	996
" 694/3	200	199	9.1	4.35	81.69	19.72	1270
" 726	101	104	8.6	3.81	124.77	8.77	639
" 695/2	192	157	9.8	4.46	101.04	17.80	1613
" 670	86	115	9.1	4.40	38.86	17.33	608
" 696/1	182	145	8.3	5.00	45.58	15.81	1073
" 688/3	182	155	8.4	4.79	29.32	22.48	796
" 693/1	195	157	8.1	4.34	90.19	18.52	940
Munaa	202	208	7.6	4.45	69.67	21.74	1566
NPP 673/1	218	159	10.8	4.56	124.02	17.79	2193
" 691/4	163	157	8.3	4.51	63.32	17.01	1210
" 707/2	221	199	11.8	5.14	94.38	17.65	1634

Table 14.(Contd...)

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
NPP 700/1	166	145	7.7	4.84	67.72	16.25	762
" 725	111	81	8.0	3.67	105.58	11.69	703
" 688/2	227	203	8.5	5.02	74.08	24.50	1881
Kioko	227	206	10.2	5.11	38.18	24.59	1224
NPP 675/2	179	153	11.3	4.27	125.78	16.76	1909
M.S.	168	202	11.3	5.38	60.25	17.49	1282
NPP 671/5	192	154	8.3	4.71	101.97	17.12	1347
" 675/6	194	154	9.8	4.39	108.13	17.09	1544
" 699/1	176	144	7.6	5.04	53.74	19.30	1099
" 707/1	208	199	10.9	5.25	80.33	18.94	2032
" 691/2	235	147	10.7	4.86	51.92	15.87	797
" 694/1	155	199	9.8	4.55	95.74	23.26	2093
" 671/4	211	166	10.7	4.15	110.80	17.44	1462
" 691/1	165	157	9.0	5.21	72.08	16.53	1243
" 688/4	211	160	7.8	5.25	64.78	24.34	1536
" 673/3	188	202	10.8	4.78	98.53	19.09	1792
" 695/1	202	199	11.2	5.73	78.00	21.18	1623
" 690/2	145	145	8.2	3.59	55.73	15.75	1231

Table 14 (Contd...)

(1)		(2)	(3)	(4)
NPP	679/1	213	202	9.6
"	671/1	209	166	8.8
"	671/2	194	154	9.6
"	691/3	176	151	9.4
"	693/2	143	154	9.1
"	694/2	194	165	10.7
"	674/1	156	140	6.9
"	696/2	159	145	7.3
"	718	136	99	10.1
"	688/5	182	157	8.5
"	675/4	182	150	10.1
"	696/3	193	145	7.8
"	675/3	216	199	10.8
"	690/1	158	144	9.0
"	671/3	194	157	9.5
"	675/1	203	157	8.7
"	671/6	181	165	9.3
"	691/5	158	142	8.3

(5)	(6)	(7)	(8)
5.94	72.94	19.60	1786
4.28	106.31	16.72	1618
4.30	73.12	16.63	996
5.17	68.38	18.22	1392
5.12	93.27	15.20	1216
5.27	45.67	20.93	894
5.37	33.19	19.78	937
4.89	46.48	19.64	1450
3.54	172.42	9.57	1271
5.47	71.46	21.87	1711
4.49	73.87	19.60	1218
5.19	56.12	22.00	1159
5.25	70.05	20.96	1317
4.70	90.69	16.91	1043
4.60	117.63	17.67	1146
4.31	123.23	17.18	1338
4.24	113.85	17.64	1393
5.10	99.18	14.53	1403

Table 14 (Contd...)

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
NPP 672/1	198	139	9.7	4.20	68.15	17.52	996
" 699/2	166	140	7.9	5.16	79.56	19.80	1701
" 693/3	217	148	9.1	4.98	73.40	15.50	961
" 688/1	209	200	8.5	5.23	107.10	20.84	2097
Grand Mean	183	161	9.2	4.76	81.95	18.21	1340
S.E. (±)	12.9	10.56	0.95	0.26	55.26	1.10	24.72
C.D.(at 5%)	25.50	20.92	1.89	0.52	109.42	2.18	43.96
C.V.(%)	8.49	7.99	12.82	0.73	16.62	7.39	60.18

The lines/cultivars varied significantly with respect to days to 50% flowering (Table 13). The Indian entries and NPP 670 were generally early maturing. Within 81 and 115 days they had produced 50% of their flowers. The majority of lines however did so after 150 days. Kioko is a very late maturing variety which reaches 50% flowering in about 200 days. This is the case also for Munaa, the susceptible check.

The cultivars/lines also showed significant differences in yield components but did not differ significantly in yield per se (Table 13). The number of seeds in a pod varied between six (NPP 679/1) and four (NPP 718). Some entries were able to produce large quantities of flowers (Table 14). However, much of these flowers usually never developed into pods. This was probably due to the dry conditions at flowering for the late entries. This dry condition led to water stress at flowering which resulted in large floral abortions. Whereas some lines produced many pods (> 100 pods/plant) (e.g. NPP 726, 673/1, 675/2, 671/4, 718 and 675/1), the majority of the lines produced between 30 and 80 pods/plant. It was

further observed that if a plant showed symptoms of water stress at flowering, it not only produced few pods but these pods usually did not fill properly. The seeds in these pods were either vestigial or small and shrivelled. In such lines, this meant that even seed size (100 seed weights) were low. However, in the lines where the pods were fully developed, the seeds were fully formed and mature. This was the case in lines such as NPP 688/3, 694/1, 688/4 and Munaa. All the lines with 100 seed weights of 19.0 g and over usually had only a few seeds that were not fully formed. Most seeds were round and large. The number of pod-bearing branches (primary) per plant varied between eight and 12. There was a significant positive correlation ($r = 0.364$) between the number of primary branches and grain yield. This means that the more the pod bearing branches in a plant the higher its grain yield is likely to be other factors being constant.

Grain yield, a character on which selection of promising lines depended very much on, showed a lot of variability. While some lines had good yields, at least 1600 kg/ha, the majority of the lines had very low yields of between 690 and 1540

kg/ha. The lines with good yields also had good seed sizes, a large number of pods and at least five seeds in a pod. Exceptions did occur however, for instance, in NPP 691/2 and 700/1 (Table 14) where 100-seed weights were rather low. This may partly be due to seeds not attaining full size before the pods dried up. Usually such seeds were under developed and therefore light.

Eleven lines were finally selected as combining a reasonable degree of resistance to Fusarium wilt and a good yield potential (Table 15). All these lines were resistant to wilt in the field. However, based on the means of their mortalities to the four isolates in the glasshouse tests, they were either tolerant or moderately resistant. These lines were also resistant to at least two isolates in the glasshouse test (Table 10). Of all the lines tested, they were the few most promising that combined both a moderate degree of Fusarium wilt resistance and good yields. Except for line 675/4, they yielded over 1,500 kg/ha in the sick plot. The very best among them being 673/1, 707/1, 675/2 and 688/2 (Plates 5, 6 and 7). However, like other local germplasm, these selections are characteristically tall, about 2 m and are late maturing.

Table 15. Performance of eleven promising pigeonpea selections.

Selection number	Disease reaction	% mortality	Yield kg/ha	No.pods/ plant	Number Primary branches/ plant	100 seed weight (g)	Days to 50% flowering	Height (cm)
NPP 673/1	MR	12.3 (2)	2189	124.0	10.8	17.8	159	218
" 707/2	MR	13.2 (2)	1635	94.4	11.8	17.7	199	221
" 675/6	MR	14.8 (2)	1544	108.1	9.8	17.1	154	194
" 707/1	MR	15.0 (2)	2032	80.3	10.9	18.9	199	208
" 688/5	MR	11.8 (2)	1712	71.5	8.5	21.9	157	182
" 675/4	MR	8.5 (2)	1219	73.9	10.1	19.6	150	182
" 688/2	T	24.3 (3)	1880	74.1	8.5	24.5	203	227
" 675/2	T	20.1 (3)	1909	125.8	11.3	16.8	153	179
" 673/3	T	21.5 (3)	1792	98.5	10.8	19.1	202	188
" 695/1	T	22.5 (3)	1624	78.0	11.2	21.2	199	202
" 671/1	T	26.6 (3)	1619	106.3	8.8	16.7	166	209
Munaa	S	72.9 (5)	1565	69.7	7.6	21.7	208	202
Kioko	MR	12.4 (2)*	1224	38.2	10.2	24.6	206	227
NPP 670	T	21.1 (3)*	608	38.9	9.1	17.3	115	86
S.E.(±)		1.68	560.45	25.41	0.91	0.72	11.34	16.41
C.D. (5%)		3.50	1169.10	53.00	1.89	1.50	23.66	34.23
C.V. (%)		11.94	43.18	34.14	11.07	4.56	7.90	8.91

* - One season's data.

Plate 5: A crop of NPP 673/1 nearing full maturity.

Plate 6: One of the eleven selections, NPP 707/1 at full pod set.

Table 7: The row with a label is a crop of NPP 688/2 with pods at various developmental stages.

These selections show a potential equal or even excelling the current commercial varieties such as NPP 670 and Kioko. It is hoped that through an intergrated approach of both production and resistance breeding it will be possible to develop these selection into cultivars which combine these parameters in a desirable way or as very dependable sources of resistance. Although NPP 725 is resistant to wilt, it was excluded because like other accessions of Indian origin it produces small, brown seeds. This seed size and colour is not popular with farmers. In 1973, the Kenya Government imported large quantities of seeds from India with the hope of increasing pigeonpea production in the country. The farmers rejected the seed on the grounds of seed size and colour and the programme was a failure. However, because of its high degree of resistance i is a useful source of resistance and is being used to incorporate this character into the currently grown cultivars and varieties.

4.3. Variation of pathogenicity among isolates of F. udum .

4.3.1 Variation in host-pathogen reaction

The pathogenicity of the twelve isolates is shown in Table 16. Typical symptoms of Fusarium wilt were observed in the plants although the severity of wilt appeared dependent on the cultivar-isolate interaction. Except for a few variations, the reactions were on the whole consistent for the three replicates in a particular cultivar-isolate interaction.

All the cultivars including the standard susceptible check Munaa reacted as resistant against isolate Manoni. The cultivars NPP 707/2 and 671/5 had a similar pattern of reaction except in two isolates, Kimutwa and Thika. The cultivar NPP 694/3 reacted in a similar way to NPP 671/5 except in isolates Emali and Wikililye.

The cultivar NPP 696/3 was resistant to all isolates except Mulutu. There was little similarity between the other lines in their reactions to the test isolates.

Table 16. Reaction of pigeonpea cultivars to twelve isolates of F. udum[a].

Isolate, source and disease reaction[b].

	WOTE			KYEMOLE			THIKA			TUNGUTU			MULUTU			EMALI			KYANIKA			NDUMONI			KIMUTWA			MANONI			WIKILILYE			KYANGWITHYA		
Cultivar	1	2	3[c]	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
NPP 707/2	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R	S	S	S	R	R	R	R	R	R	R	S	S	R	R	R	R	R	R	R	R	R
" 693/3	R	R	R	S	S	R	S	R	R	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S
" 671/3	S	S	R	R	R	R	S	S	R	R	R	R	S	S	R	R	R	R	S	R	S	R	R	R	S	R	S	R	R	S	R	R	R	S	R	S
Munaa	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	S	S	S	R	S	S
NPP 696/3	S	R	R	R	R	R	R	R	R	R	R	R	S	S	S	R	R	R	R	R	R	R	S	R	S	R	R	R	R	R	R	R	R	R	R	R
" 688/5	S	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S	R	R	S	R	R	S	R	S	S	S
" 694/3	R	R	R	R	R	R	S	S	R	R	R	R	R	R	S	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R	S	S	S	R	R	R
" 671/5	R	R	R	R	R	R	S	R	S	R	R	S	R	R	R	S	S	S	R	S	R	R	R	R	R	R	S	R	R	R	R	R	R	S	R	R
" 696/2	S	R	S	R	R	S	S	S	R	R	R	R	R	R	R	S	S	S	S	R	R	S	R	S	S	S	S	R	R	S	R	S	R	S	R	R
" 688/4	R	R	R	S	S	S	S	S	S	R	R	S	S	S	R	R	R	R	R	R	R	S	R	R	R	R	R	R	R	R	S	S	S	S	S	R
" 690/1	R	R	R	R	S	R	R	S	S	S	S	S	R	R	R	R	R	R	R	R	R	S	S	S	S	R	R	R	R	S	R	R	R	R	R	R
" 691/1	S	R	S	S	S	S	R	R	S	S	S	S	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	R	R	R	R	R	S	S	S	S

a Readings taken after 12 weeks are used in this table.

b R = resistant (0-30% wilt), S = susceptible (≥ 31% wilt) to the pathogen.

c The numbers 1 - 3 represent the three replicates carried out with each isolate

Table 16. Reaction of pigeonpea cultivars to twelve isolates of F. udum[a].

Isolate, source and disease reaction[b].

	WOTE			KYEMOLE			THIKA			TUNGUTU			MULUTU			EMALI			KYANIKA			NDUMONI			KIMUTWA			MANONI			WIKILILYE			KYANGWITHYA		
Cultivar	1	2	3[c]	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
NPP 707/2	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R	S	S	S	R	R	R	R	R	R	R	S	S	R	R	R	R	R	R	R	R	R
" 693/3	R	R	R	S	S	R	S	R	R	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S
" 671/3	S	S	R	R	R	R	S	S	R	R	R	R	S	S	R	R	R	R	S	R	S	R	R	R	S	R	S	R	R	S	R	R	R	S	R	S
Munaa	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	S	S	S	R	S	S
NPP 696/3	S	R	R	R	R	R	R	R	R	R	R	R	S	S	S	R	R	R	R	R	R	R	S	R	S	R	R	R	R	R	R	R	R	R	R	R
" 688/5	S	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S	R	R	S	R	R	S	R	S	S	S
" 694/3	R	R	R	R	R	R	S	S	R	R	R	R	R	S	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R	S	S	S	R	R	R
" 671/5	R	R	R	R	R	R	S	R	S	R	R	S	R	R	R	S	S	S	R	S	R	R	R	R	R	R	S	R	R	R	R	R	R	S	R	R
" 696/2	S	R	S	R	R	S	S	S	R	R	R	R	R	R	R	S	S	S	S	R	R	S	R	S	S	S	S	R	R	S	R	S	R	S	R	R
" 688/4	R	R	R	S	S	S	S	S	S	R	R	S	S	S	R	R	R	R	R	R	R	S	R	R	R	R	R	R	R	R	S	S	S	S	S	R
" 690/1	R	R	R	R	S	R	R	S	S	S	S	S	R	R	R	R	R	R	R	R	R	S	S	S	S	R	R	R	R	S	R	R	R	R	R	R
" 691/1	S	R	S	S	S	S	R	R	S	S	S	S	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	R	R	R	R	R	S	S	S	S

a Readings taken after 12 weeks are used in this table.

b R = resistant (0-30% wilt), S = susceptible (≥ 31% wilt) to the pathogen.

c The numbers 1 - 3 represent the three replicates carried out with each isolate

Individual isolates similarly affected the cultivars differently. The number of lines rated resistant or susceptible were usually the same for some isolates. However, the lines were often different. For instance, five lines were susceptible to isolates Wote and Mulutu while four lines were susceptible to isolates Tungutu, Emali and Kyemole. Similarly, three and eight lines each were susceptible to isolates Wikililye and Thika respectively. Two lines were susceptible to the isolates Kyanika and Ndumoni while six were susceptible to isolates Kimutwa and Kyanika.

On the basis of average virulence values, isolates Kyangwithya and Thika were the most virulent (Table 17). They had an average virulence of 3.83 each with no line having a rating of 1.0. Isolate Manoni was the least virulent among the twelve isolates and had an average score of below 2.0. Most of the isolates could however be rated as intermediate with average virulence values between 2.50 and 3.17. Some of the isolates, for instance Kimutwa and Wikililye had the same average virulence value but from both Tables 16 and 17 are not similar. These results suggest that these isolates

Table 17. Virulence of the 12 isolates of F. udum on 12 lines of pigeonpea.

Distribution of 12 lines - number of lines in different disease scales.

Isolates	1	2	3	4	5	Average virulence*
WOTE	1	5	3	1	2	2.83
KYEMOLE	6	3	0	2	1	2.08
THIKA	0	3	1	3	5	3.83
TUNGUTU	1	4	3	1	3	3.08
MULUTU	0	4	4	2	2	3.17
EMALI	2	6	1	1	2	2.58
KYANIKA	4	2	5	0	1	2.33
NDUMONI	1	5	5	1	0	2.50
KIMUTWA	2	4	2	3	1	2.75
MANONI	5	6	0	1	0	1.75
WIKILILYE	2	2	6	1	1	2.75
KYANGWITHYA	0	2	2	4	4	3.83

*Average virulence = Σ(infection rating x freq.)/number of lines.

are different. The results also show that the isolates were not very virulent. This could be their inherent characteristic or that the test cultivars except for cultivar Munaa were resistant to wilt. The differences in average virulence were shown statistically to confirm that these isolates were significantly different from each other (Table 18). Pairwise comparisons of the means of these isolates showed that isolates differed significantly in their virulences (Table 19). On the basis of these observations, the isolates were categorised thus:

Group 1; High virulence isolates: Thika and Kyangwithya;

Group 2; Moderate virulence isolates: Mulutu, Tungutu, Wote, Kimutwa, Emali and Ndumoni.

Group 3; Low virulence isolates: Kyanika, Kyemole and Manoni.

4.3.2. Variation in morphology and growth rate of isolates on culture media.

In addition to the differences in virulences,

Table 18. Analysis of variance for average virulence of the 12 isolates.

Source of variation	df	MS
Replicates	2	0.0038 NS
Isolates	11	1.1787**
Error	22	0.0129
Total	35	0.3788

C.V. = 4.07%, N.S. = Not significant;
** = significant at 1% level.

Table 19. Duncan's Multiple range test for average virulences.

Isolate	Average virulence	
Thika	3.83	a'
Kyangwithya	3.83	a
Mulutu	3.17	b
Tungutu	3.08	b
Wote	2.83	bc
Kimutwa	2.75	bcd
Wikililye	2.75	bcd
Emali	2.58	bcd
Ndumoni	2.50	bcde
Kyanika	2.33	bcdef
Kyemole	2.08	bcdefg
Manoni	1.75	bcdefg

' Any two values having a common letter are not significantly different at 5% level.

these isolates also showed considerable differences in the rate and type of vegetative growth of the mycelium as well as the range in pigmentation in the cultures (Tables 20 to 23). The rate of growth of the single spore colonies was remarkably different among the isolates (Table 20). This variation was evident as from day two of growth. It was also obvious that rapid growth occurred between day five and eight in most isolates. In addition to differences in colony diameters, the isolates grew at different rates. The Emali isolate grew most rapidly (8.15 mm day^{-1}). Five of the isolates had growth rates between 7.0 and 7.8 mm day^{-1} while another lot of five had growth rates between 6.2 and 6.9 mm day^{-1}). Only one isolate, Manoni, grew at a rate below 6.0 mm day^{-1}. These isolates can be ranked from the most rapidly growing to the slowest growing as follows: Emali >Kimutwa >Kyemole> Ndumoni >Kyangwithya >Wote >Thika> Mulutu >Kyanika> Tungutu >Wikililye> Manoni.

Table 21 shows that there were highly significant differences in growth rates among the isolates. Paired comparisons among these isolates showed that isolates with growth rates between 7.07 and 8.15 mm day^{-1} were not significantly different

Table 20. Comparative rates of growth (colony diameters) in mm of 12 isolates of F. udum at room temperature in PDA Plates.

Isolates	Days of inoculation				Average rate of growth per day, mm
	2	5	8	10	
MULUTU	13.3	22.0	49.0	67.0	6.70
KYANIKA	17.3	29.2	54.8	66.0	6.60
THIKA	13.3	31.0	53.7	69.7	6.97
WIKILILYE	18.3	33.7	54.3	62.0	6.20
KYEMOLE	15.3	44.7	65.0	76.7	7.67
EMALI	16.3	36.7	66.7	81.5	8.15
TUNGUTU	21.7	40.3	55.7	65.3	6.53
MANONI	19.3	45.3	53.0	56.0	5.60
KYANGWITHYA	13.8	31.5	56.5	70.8	7.08
WOTE	28.2	48.7	63.7	70.7	7.07
NDUMONI	14.7	37.5	54.8	71.3	7.13
KIMUTWA	15.1	35.6	60.0	77.3	7.73
S.E. ($\pm$)				4.76	
C.D. (at 5%)				9.86	
C.V. (%)				8.37	

Table 21. Analysis of variance for colony sizes on the 10th day of the 12 isolates grown on PDA.

Source of variation	df	MS
Replicates	2	3.45 NS
Isolates	11	146.00**
Error	22	33.93
Total	35	

c.v. % = 8.37; N.S. = Not significant;
** = significant at 1% level.

from each other (Table 22). However, the Emali isolate grew significantly faster than the Kimutwa, Kyemole, Ndumoni, Kyangwithya and Wote isolates. Although it grew slowest of all, the Manoni isolate was not significantly different from isolates with rates of 7.08 mm and less. Such overlaps imply definite classifications could not be made on this parameter. However, the isolates were categorised as follows:

Group 1; Fast growing isolates: Emali, Kimutwa and Kyemole.

Group 2; Moderate growing isolates: Ndumoni, Kyangwithya, Wote, Mulutu, Thika, Kyanika and Tungutu; and

Group 3; Slow growing: Wikililye and Manoni.

There were differences in aerial mycelium and final colour of the medium (Table 23, plate 8). The colour of the colonies varied from pinkish-white or pinkish-brown to deep purple. The mycelial mat was either sparse and less raised (appressed) or fluffy and much raised covering the entire plate. The isolates behaved more or less similarly with respect to their aggressiveness to the substrate. The

Table 22. Duncan's multiple range test for colony diameters in PDA of 12 isolates of F. udum.

Isolate	Average rate of growth (mm)	
EMALI	8.15	a'
KIMUTWA	7.73	ab
KYEMOLE	7.67	ab
NDUMONI	7.13	abc
KYANGWITHYA	7.08	abcd
WOTE	7.07	abcd
THIKA	6.97	bcd
MULUTU	6.70	bcd
KYANIKA	6.60	bcd
TUNGUTU	6.53	cd
WIKILILYE	6.20	d
MANONI	5.60	d

'Any two values having a common letter are not significantly different at 5% level.

Table 23. Descriptive characteristics of 12 isolates of *F. udum* in PDA plates

Isolate (1)	Pigmentation (2)	Growth behaviour (3)	Effect on substrate (4)
MULUTU	Pink	Mycelia appressed and sparse	Turned to a deep pink with brown patches.
KYANIKA	Pinkish-white	Raised, dense mycelia	Turned to a deep pink-brown
WIKILILYE	Whitish-brown	Raised at centre but appressed and sparse at the margin	Turned to a very deep brown
THIKA	Brownish	Less fluffy and restricted mycelium	Turned to light orange with a deeper tint at point of inoculation
KYEMOLE	Reddish-white	Much raised dense mycelia, covering all the substrate	Turned to a light brown to deep brown
TUNGUTU	Pinkish-white	Dense, fluffy mycelium	Turned to a pinkish purple
EMALI	White	Obvious zones of light and dense mycelial growth	Turned to a deep brown
WOTE	Deep purple	Raised, fluffy mycelia	Turned to a very deep purple

Table 23 (Contd...)

(1)	(2)	(3)	(4)
MANONI	Deep purple	Dense and raised at centre, sparse outwards	Turned to purple
KIMUTWA	Whitish-yellow	raised and fluffy	Turned to a deep orange colour
NDUMONI	Light-brown	restricted growth with sparse mycelia	Turned to a light brown, deep at point of inoculation
KYANGWITHYA	Deep brown	fast growing, raised and dense mycelium	Turned to a deep brown

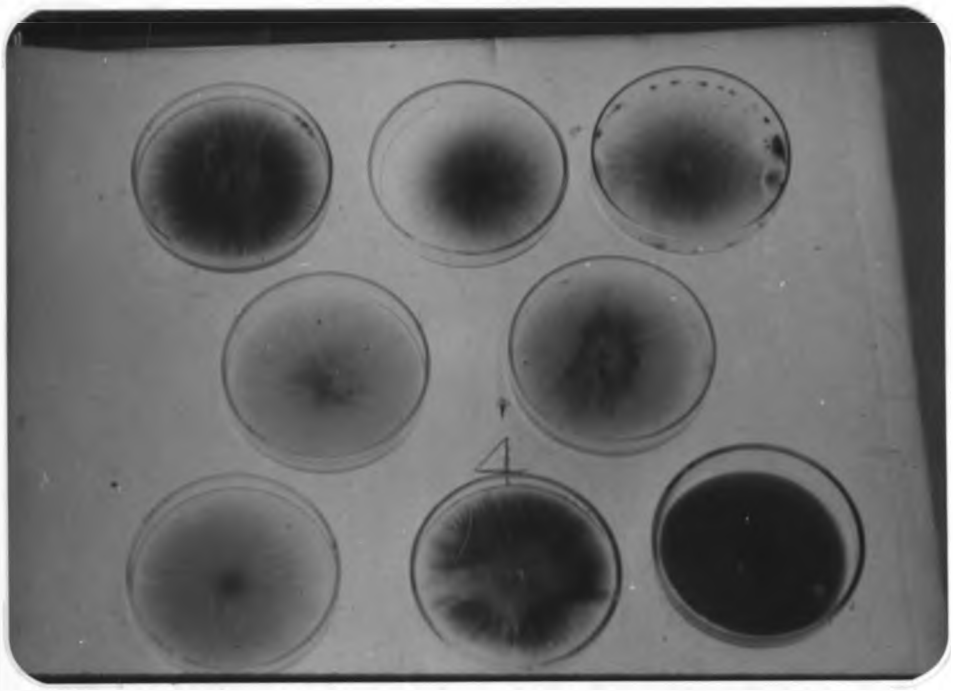

Plate 8: Some of the isolates of F. udum showing variation in pigmentation and type of growth. All cultures on PDA and of the same age (10 days).

From L-R; Top: Wikililye, Emali, and Kyangwithya.

Middle: Tungutu and Kyemole;

Bottom: Kyanika, Mulutu and Wote.

substrate was changed from light cream to either a deep pink or pinkish-brown. The morphology of the conidia in all the isolates was typical. Apparently the rapidity with which the substrate was discoloured depended on the growth rates of the isolates. Fast growing isolates were more aggressive to the substrate and such substrates often had a deeper colour than substrates on which the slow growing isolates were cultured.

A test for any relationship between virulence and growth rates showed a weak association for these characteristics. A non significant correlation coeffifient (r) of 0.15 was found between these two parameters (Table 24). However, the isolate Manoni was the least virulent and also had the lowest growth of all the isolates.

4.3.3. Electrophoretic variation

A range of four to eleven protein bands were observed among the eleven isolates (Fig. 2). Two to five thin bands were present in various isolates towards the origin, except in isolate Emali which did not have any of these bands. Three isolates, viz. Mulutu, Manoni and Thika had only

Table 24 Relationship between average rate of growth in PDA and average virulence of the 12 isolates of F. udum.

Isolates	Average growth rates (mm day^{-1})	Average virulence (Score of virulence)
MULUTU	6.70	3.17
KYANIKA	6.60	2.33
THIKA	6.97	3.83
WIKILILYE	6.20	2.75
KYEMOLE	7.67	2.08
EMALI	8.15	2.58
TUNGUTU	6.53	3.08
MANONI	5.60	1.75
KYANGWITHYA	7.08	3.83
WOTE	7.07	2.83
NDUMONI	7.13	2.50
KIMUTWA	7.73	2.75
MEAN	6.95	2.71
	r_s = 0.1500 NS[1]	

[1] NS = Not significant

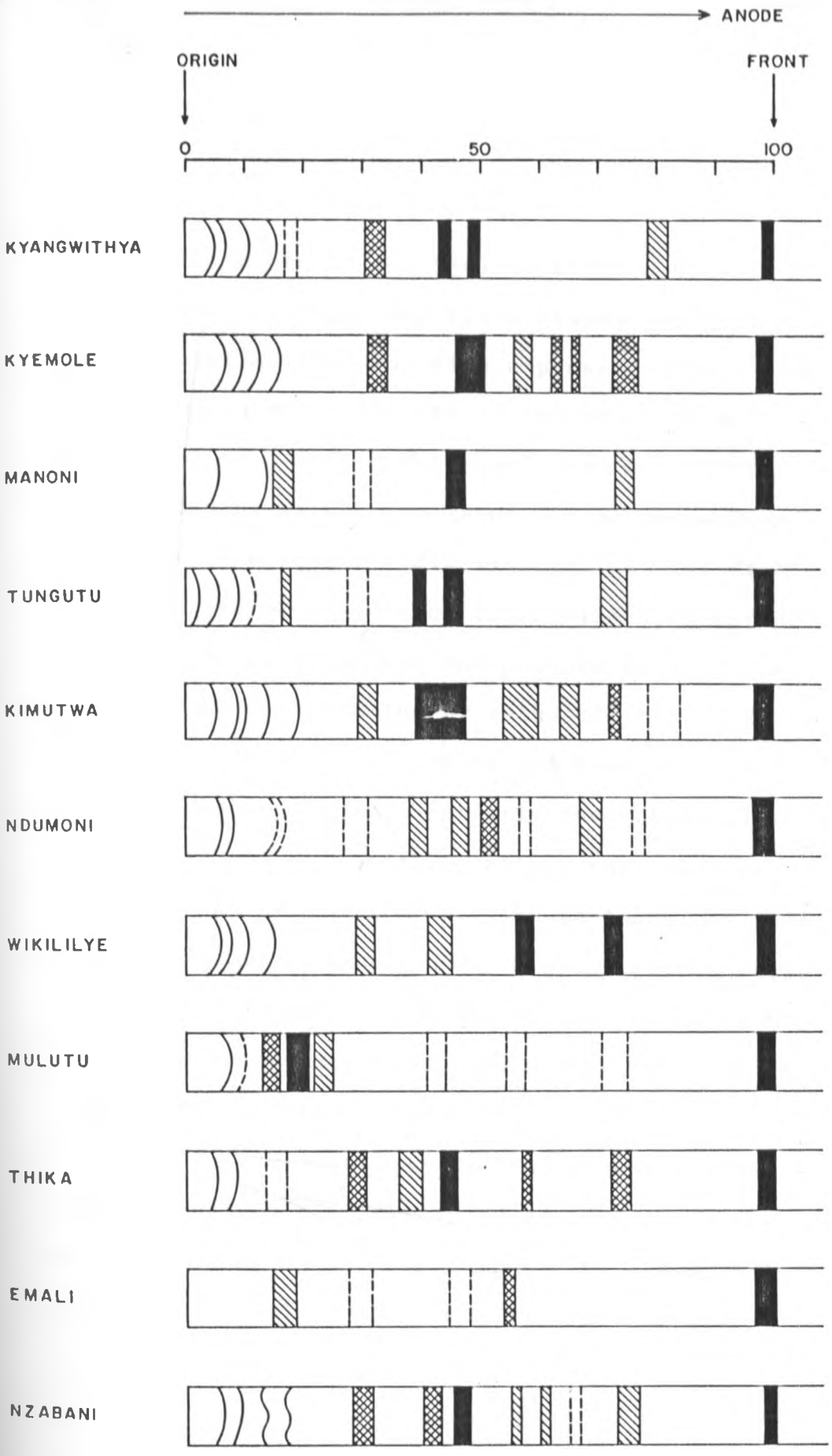

Fig. 2 Electrophoretic separations on acrylamide gel of the soluble proteins of 10 days cultures of F. Udum isolates.

KEY Very Dark Dark Light Faint

two of these bands, the isolate Kimutwa had all the five bands and the other six isolates had four bands. A large variability was also expressed in the number, distribution and intensity of the thick bands among the isolates. The isolate Emali had only four thick bands, two of which were faint (Rf 31 and 48) and the other two were clearly visible. A band of Rf values 28 to 31 was common to all isolates except Mulutu. However, it was not uniform in intensity. Also the band within the Rf values 72 to 77 was common to all the isolates except Emali and Kyangwithya isolates. Similarly the other thick bands were not monomorphic, rather they occurred in some but not all the lines. For instance, isolates Kyemole, Kimutwa, Ndumoni and Nzabani were similar with respect bands at Rfs 34 and 59. Isolates Manoni, Tungutu, Wikililye, Mulutu and Kyangwithya also could be grouped together on the basis of the band between Rf 44 to 50. However, in the two groupings the association could be described as weak since the bands were not of uniform intensities. The isolate Emali with its four bands had its own pattern.

4.4. Electrophoretic comparison of *Fusarium* wilt resistant and susceptible pigeonpea lines

Isozyme patterns observed for various enzymes and fractions of the total soluble protein of the resistant and susceptible pigeonpea lines are illustrated in figures 3 to 10. The patterns of peroxidases, esterases, acid phosphatases and leucine aminopeptidases studied at various plant developmental stages did not show very clear differences with respect to resistance and susceptibility among the lines. However, some of the zymograms especially of protein showed different patterns and therefore were considered useful markers for characterising resistant and susceptible genotypes. Enzyme systems and protein profiles changed with age of the plants. The zymograms that did not reveal any observable differences are not illustrated. In the following paragraphs important zymogram patterns of peroxidases, esterases, leucine aminopeptidases, and acid phosphatases and proteins are described as observed at various stages of plant growth starting from three days upto 42 days stage.

1. Three-day old plants.

The peroxidase zymogram (Fig. 3) showed six

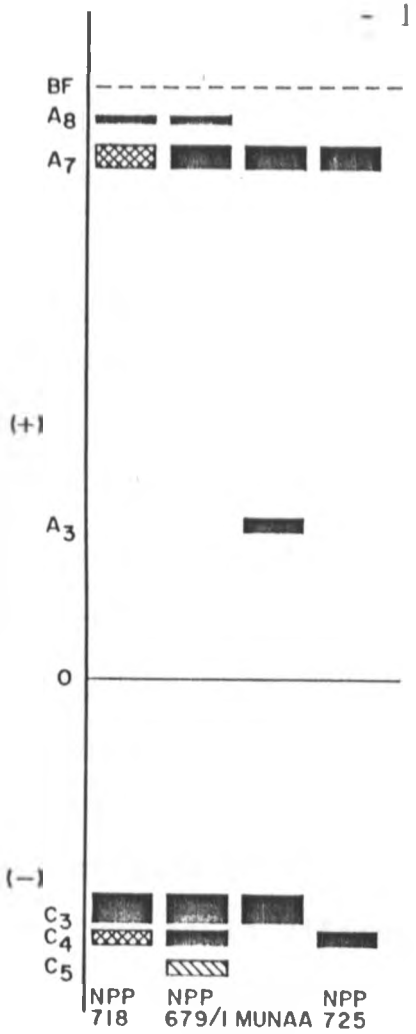

Fig. 3 Schematic diagram of anodic (A) and cathodic (C) peroxidase isozyme bands of four lines 3 days after germination.

K E Y

O	Point of Sample application
(+)	Anodic side
(−)	Cathodic side
- - - -	Borate buffer front
▬	Very dark
▩	Dark
▨	Light
⁚	Faint

bands, three cathodal and three anodal. Only one band was monomorphic (i.e. stained in all the four genotypes). On the basis of both the pattern and intensities of bands, none of the anodal bands were useful for differentiating the two groups of cultivars. Two of the cathodal bands, C_3 and C_5 were also not useful. Even the differences with respect to band C_4 were not consistent. It was very darkly stained in the resistant lines NPP 679/1 and 725 and was darkly stained in NPP 718, but did not appear in Munaa. Thus it is difficult to conclusively link this band with susceptibility or resistance.

2. Nine-day old plants.

Acid phosphatase stained two anodal bands and both were present in all the lines (Fig. 4). The band A1O was characteristically light. However, the other band, A1, depicted three intensities. The band was very dark in resistant lines, NPP 679/1 and 725. In the susceptible lines, Munaa and NPP 718 the band was light and dark respectively. Thus although band A1 was present in all the lines its distinctly higher intensity in resistant lines suggests the association of the isozyme of this band with resistance reaction of lines NPP 679/1 and 725.

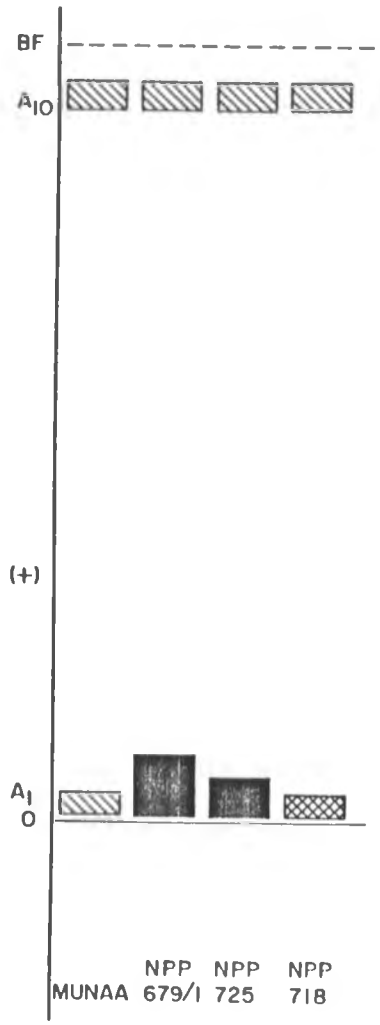

Fig. 4 Schematic diagram of anodic (A) acid phosphatase isozyme bands of four pigeonpea lines 9 days after germination

KEY :— As in Figure 3

There were two bands (Fig. 5) in the leucine aminopeptidase zymogram and both were anodal. Band A10 appeared only in NPP 725 while A9 appeared in the other three lines. The band A9 was very darkly stained in Munaa and NPP 718. It was darkly stained in NPP 679/1 and lacking in NPP 725. On the basis of its intensity, this band was characteristically very dark among the susceptible lines and suggested a possible association between susceptible and this isozyme.

Eight to ten bands were observed in the protein profiles of nine-day old plant (Fig. 6). Many of these bands, with Rf values 4, 5, 33, 51, 62, 80 and 90, were similar in either pattern or intensity among the resistant and susceptible lines. The other bands expressed differences between resistant and susceptible lines. The band with Rf 16 stained in the resistant lines only and that at Rf 62 stained very darkly in NPP 679/1 and 725 but was faint and dark in Munaa and NPP 718 respectively.

3. 13 day-old plants.

There was an increase in the number of peroxidase bands at this stage than at any of the

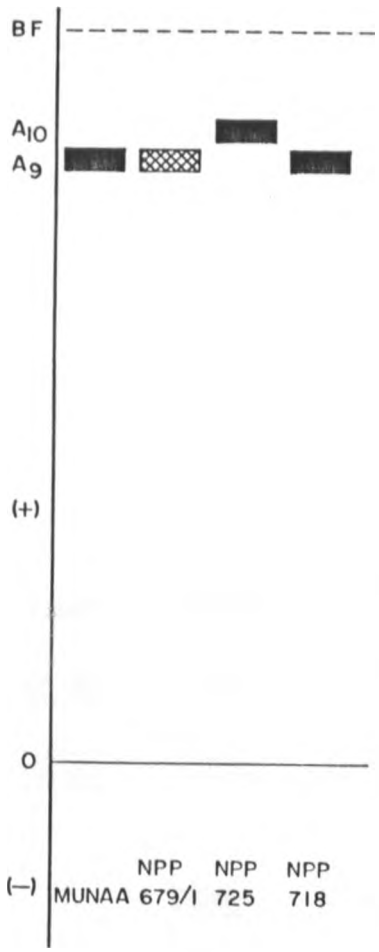

Fig. 5 Schematic diagram of anodic (A) leucine aminopeptidase isozyme bands of four lines 9 days after germination.

KEY :– As in Fig. 3

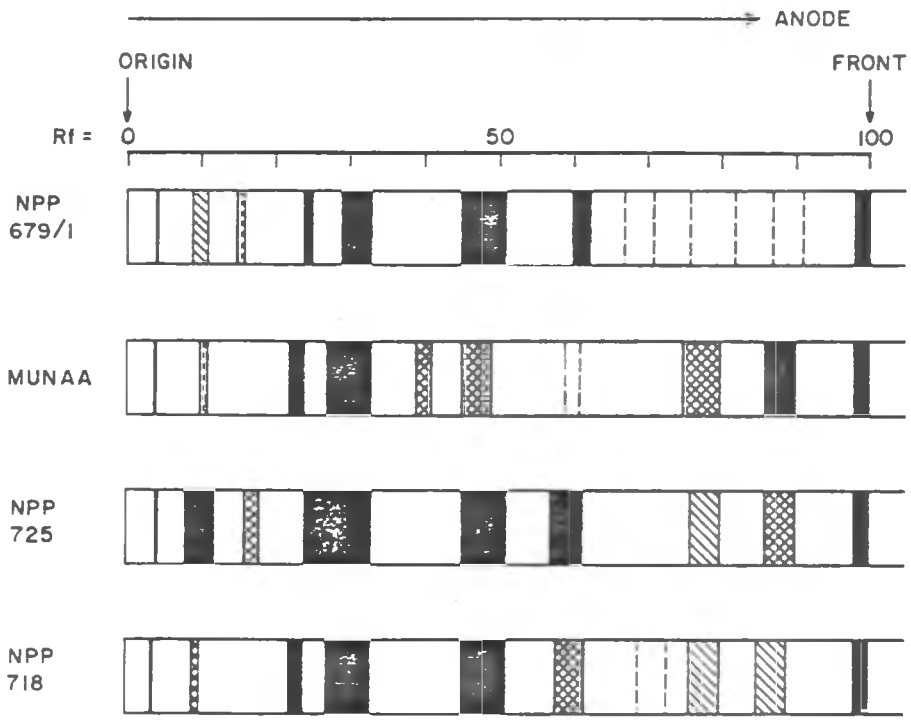

Fig. 6 Diagrammatic interpretation of the electrophoretic separation on acrylamide gel of the soluble proteins of 9-day pigeonpea genotype

KEY :- Band intensities as in Fig. 3

earlier stages (Fig. 7). Many of the bands that were present in three days old plants disappeared at this stage. The increase in number of bands is thought to reflect increased physiological activity in the plants. The disappearance and appearance of bands could be due to differential gene action operating at various developmental stages. All the bands except A_2 and A_3 were monomorphic. The monomorphic bands did not show any consistent differences for either resistance or susceptibility. For instance, band A_5 was darkly stained in the resistant lines (NPP 679/1 and 725) but was light in one susceptible line Munaa and very dark in the other susceptible line (NPP 718). Beside this band, none of the other bands expressed any differences that could be useful in the identification/classification of resistant and susceptible cultivars.

4. Five to eight protein bands appeared in the 20 day-old plants of the various lines (Fig. 8). However, only three bands showed some differences which could be used to characterise resistant and susceptible cultivars of pigeonpea. The band with - in Rf values 35 to 43 stained very darkly in the susceptible lines but was dark in NPP 725 and light

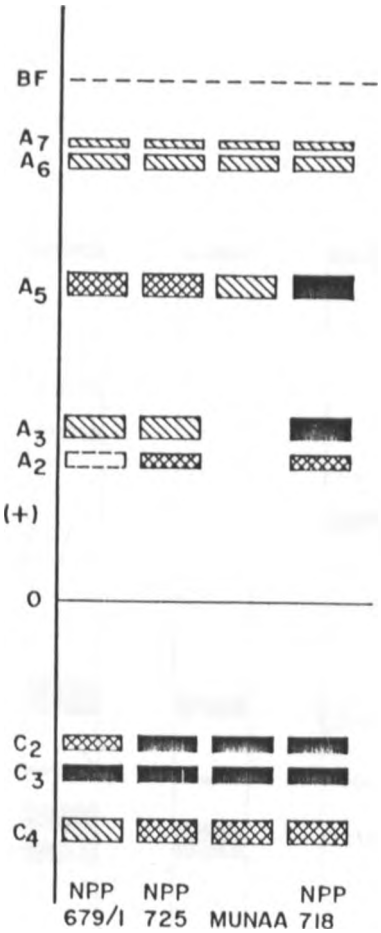

Fig. 7 Schematic diagram of anodic (A) and cathodic (C) peroxidase isozyme bands of four pigeonpea lines 13 days after germination

KEY :— As in Fig. 3

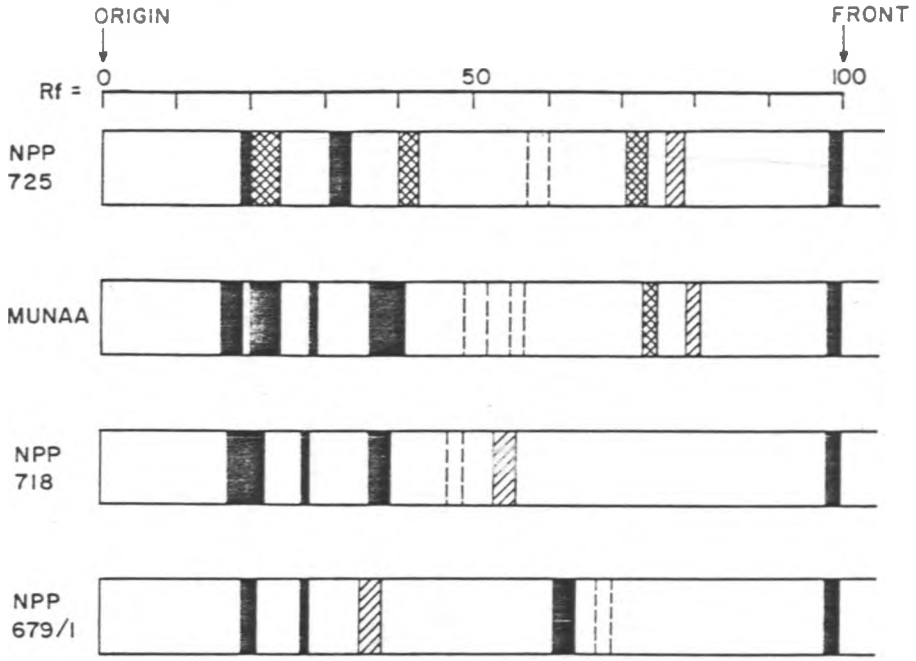

Fig. 8 Diagrammatic interpretation of the electrophoretic separation on acrylamide gel of the soluble proteins of 20-day-old seedlings of four pigeonpea cultivars

KEY :- As in Fig. 3

in NPP 679/1, both resistant lines. The two bands within Rf values 47 to 56 appeared only in the susceptible lines.

5. 25 days-old plants.

A total of nine peroxidase bands were present in the zymogram of 25 day-old plants (Fig. 9). Five of these bands, A2, A3, A5, C3 and C4 appeared in all the lines, A6 and C1 only in NPP 718 and A4 and C2 in all the lines except Munaa. Among the resistant lines, NPP 679/1 and 725, the intensities of bands A5, A4 and C2 were consistent. However, this was not the case for the susceptible lines. The only band of importance at this stage was C3. It was dark to very dark among the resistant lines and was uniformly light in the susceptible lines.

6. 42 days-old plants.

Examination of the zymograms of the various enzymes and proteins for 42 day-old plants did not show any important differences. This stage was therefore considered not suitable for electrophoretic study.

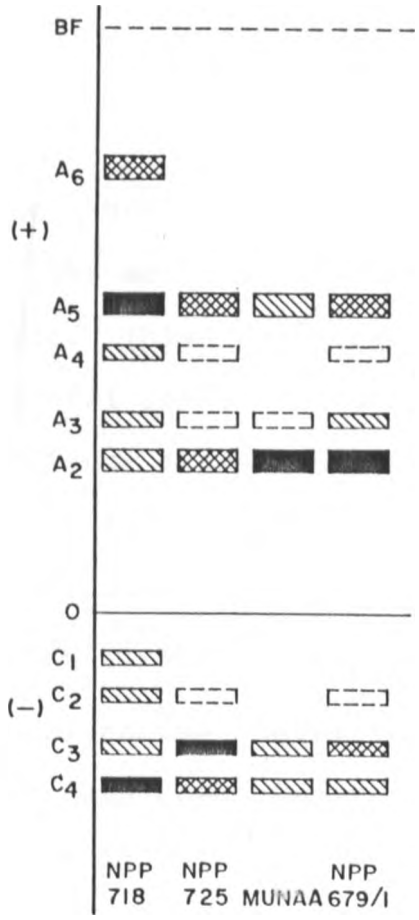

Fig. 9 Schematic diagram of anodic (A) and cathodic (C) peroxidase isozyme bands of four pigeonpea lines 25 days after germination.

KEY :— As in Fig. 3

5. DISCUSSSION

The inherent quality of resistance to disease is one of the most important factors in crop improvement. This character is conditioned by one, two or several genes, the degree of complexity in its inheritance increasing with the number of genes. Disease resistance is also considered a complex character because its expression is known after a host-pathogen interaction.

In pigeonpea, several Fusarium wilt resistant selections have been reported (Deshpande et al., 1963; Mukherjee et al., 1971; Nene and Kannaiyan, 1982). However, it has often occurred that what was first reported as resistant turns out to be susceptible when tested later. This is especially true of selections made in sick plots because such lines are often not entirely homozygous. Pigeonpea is an often-outcrossing crop and this makes it very difficult to obtain pure lines in the field. The difficulty in ensuring absolute purity of material has hindered the determination of factors conferring resistance to Fusarium wilt. It is therefore not surprising that resistance is reported as complex with two and

possibly three factors involved (Shaw, 1936; Joshi, 1957). Reports are conflicting also on the nature of this resistance. Some indicate that resistance is dominant, others suggest that it is recessive (Shaw, 1936; Joshi, 1957; Haware and Jain, pers. comm.).

F. udum is a soil-borne pathogen and is not easily controlled by chemicals. The cost of chemical control is prohibitive for most Kenyan farmers. Several cultural methods such as intercropping with tobacco and sorghum, rotation and fallowing have been tried. All result in marked reduction in pigeonpea mortalities. However,complete control appears unlikely by any of these methods. Breeding wilt resistant cultivars appears the only sure way for effective control of Fusarium wilt in pigeonpea.

In an effort to develop pigeonpea cultivars resistant to Fusarium wilt, we conducted several investigations. These included the development of a reliable inoculation technique for Fusarium wilt, using such a technique to search for resistance in pigeonpea germplasm, testing for variability in F. udum, and determining the number of genes involved in resistance and how they are inherited.

5.1 Inoculation methods

Provided the necessary genes for wilt resistance exist in a pigeonpea population, success in producing selections of superior resistance will depend on the effectiveness of the inoculation technique used to screen the material. Several inoculation methods were evaluated in this study. The results showed the root dip method as the most dependable of the five inoculation methods (Table 5). At the lowest and highest conidia concentrations, differences between resistant and susceptible genotypes were clear cut. At the lowest inoculum concentration (4.23×10^5) mortalities in the resistant genotypes were 12.5 and 26.5% and 43.5 and 60.0% among the susceptible genotypes. At the highest concentration (1.64×10^6) mortalities were very high both in the resistant and susceptible genotypes. These were 52.8 and 65.5% among the resistant lines and 79.4 and 90% in the susceptible lines. However, at the intermediate concentrations, 9.4×10^5 and 1.31×10^6 spores/mℓ, there was no clear cut difference between resistant and susceptible genotypes. Despite the clear cut differences between resistant and susceptible genotypes given at the lowest and highest inoculum concentrations,

there was evidence of genotype-inoculum interactions. This is likely to affect the suitability of root dip as an inoculation method. It is a very tedious, time consuming method especially when large numbers of plants are involved. Also, a lot of seedlings die after resetting (transplanting) apparently due to transplanting shock. Such loss of seedlings not only reduces plant populations but dead plants can be a source of error as they can be assumed to have died due to wilt. As was evident at the highest inoculum concentration, this method can also result in severe symptom expression even in resistant plants. This phenomenon was observed also in cotton inoculated by root dip with various concentrations of F. oxysporum f. vasinfectum (Miller and Cooper, 1967). Despite these drawbacks, the method is considered very effective. This effectiveness is apparently enhanced by root pruning. In most genotypes pruning led to higher mortalities irrespective of inoculum concentrations. This role of pruning was however not evident when onset of wilting was considered. Root dip method also was found very effective for inoculating cotton genotypes with F. oxysporum f. vasinfectum (Wiles 1963; Miller and Cooper, 1967). The root dip method is also being used at ICRISAT

(Faris, pers. comm.). Among the methods evaluated in this study, it was ranked second on the basis of the mean of mortalities of the four genotypes (Table 6). However, more work is needed to establish how dependable and repeatable this technique is. This can be done by taking more lines and/or concentrations.

Sowing seeds into wilt infested soil also gave clear-cut differences between the resistant and susceptible genotypes. The susceptible genotypes recorded 82.0 and 88.9% mortalities. Among the resistant genotypes, these were 12.5 and 38.8%. The large difference in mortalities among the resistant genotypes is due probably to varying degrees of resistance in these genotypes and strongly points to the need to use a large number of lines. However, because of its clear cut separation of resistant and susceptible genotypes, its simplicity and its effectiveness, this method was used to screen pigeonpea germplasm for resistance to Fusarium wilt. It is also amenable to large scale screening and it is also perhaps the only method which best simulates the natural method of pathogen-host interaction. F. udum is soil-borne and invades the host through fine roots after seed has germinated. On the basis of mean

mortalities, (55.7%), it was considered the best inoculation method. A similar method involving mixing inoculum of F. oxysporum f. vasinfectum before sowing seed of cotton strains was found to give the best comparison with results of field screening in wilt infested soils (Perry, 1962; Rao and Rao, 1966; Hillocks, 1984). This method has one major limitation. This is lack of control of inoculum in the soil. However, unlike in root dip where inoculum concentrations are calibrated before use, in infested soils it is very difficult to estimate the inoculum load of the soil. If the soil is not sufficiently infested, mortalities will be low and escapes many. When inoculum is very high, symptoms will be very severe even in resistant genotypes.

There were no clear cut differences between resistant and susceptible lines inoculated by transplanting seedlings in wilt infested soil. The susceptible lines suffered moderate mortalities (57.0 and 68.0%). However, the resistant lines showed large differences, 16.2 and 48.3% for intact roots and 24.4 and 52.3% when roots were pruned. In all the four lines, root injury led to more

mortalities. On the basis of the mean of mortality of the four lines, this method caused 50.8% deaths and was ranked third (Table 6). A comparable technique (sick pot technique) was used at ICRISAT (Nene et al., 1980) and was reported fast and very effective. All the seedlings were wilted by the second week after transplanting. In the results of this study, wilting did not start until the fifth week and even by the twelfth week between 16.2 and 68.0% mortalities were obtained. The difference in the two tests could be attributed to differences in genotypes and method of inoculum preparation. This method, however, has several drawbacks. Inoculum load of the soil is difficult to quantify. Due to transplanting shock and root injury, many seedlings die. This not only reduces initial plant stand but can be a source of error especially when scoring for wilted plants. Without examining plants for the black streaks at the bark all dead plants could be recorded as dead due to wilt. Examining each seedling makes the method tedious and time consuming.

Inoculation by soaking seed in inoculum suspension prior to sowing led to very low

mortalities even among susceptible plants (Table 5). Symptom development occurred so slowly that the first wilted plants were recorded seven weeks after inoculation. Mortalities were generally low, between 5.6 to 42.9% in Munaa and between 10.0 and 50.0% in NPP 718. Among the resistant lines mortalities were 2.7 to 27.8% in NPP 679/1 and 10.0 to 40.0% in NPP 725. Besides being low, these mortalities also showed no clear cut differences between resistant and susceptible lines thereby making the method ineffective. The results were also characterised by inconsistencies. For instance one would have expected a linear relationship between both inoculum concentration and duration of soaking and mortalities. This was not apparent from the results obtained. This observation also implies that this method is unreliable. The low mortalities resulting from this inoculation technique could be due mainly to the fact that seed soaking does not provide appropriate site for penetration by the pathogen and its further development. Apparently soil-borne pathogens are not often successfully introduced into their hosts by aerial inoculation techniques (Phipps and Stipes, 1976). In normal host-pathogen interaction, root stimuli, believed

to be root exudates made up of amino acids and carbohydrates cause the spores to germinate in root vicinity and grow towards the roots to cause infection. At least no evidence of such stimuli being produced by seed on soaking has been reported and even if the fungus did get into the seed it will probably have lost part of its infectivity by the time it is ready to establish itself in the host. However, in other crop-pathogen situations, this technique is very successful causing very severe symptoms. This is especially so with seed borne pathogens. For instance, severe symptoms of halo blight were induced in peas by soaking seed in a suspension of Pseudomonas syringae pv. phaseolicola (Zaiter and Coyne, 1984).

Stem injection, like seed soaking resulted in very low mortalities (2.4 to 25.0%) among the lines (Table 6). Macro symptoms were often slow to develop and were less severe even in the susceptible lines. The first wilted plant was recorded in the tenth week. On the basis of the mean of mortalities of the four lines, this method caused 12.0% wilt. The technique was therefore

rated ineffective, and less amenable to large scale screening. The technique is also tedious especially when large numbers of plants are involved. However, by using the syringe, there is greater control of the inoculum dose. The results of this study, however, differ from those reported by Sharma et al. (1977). They reported wilting to occur within a month of inoculation. However, they did not indicate inoculum concentrations used and the level of susceptibility of the genotypes they used in their study. The results of this study also differ from those observed in cotton where the most effective inoculation technique with F. oxysporum f. vasinfectum was by stem inoculation (Bugbee and Sappenfield, 1972, Kappelman, 1975). They all found that stem inoculation gave results which compared well with results of field screening in wilt infested soil.

All the inoculation techniques studied except sowing seed or transplanting seedlings in sick soils, use inoculum preparations. This means therefore that the fungus is maintained in pure cultures. Keeping the fungus in artificial medium for long periods and at low temperatures has been

reported to result in loss of virulence and even mutation (Russell, 1978) thereby changing the pathogenicity of inocula in use.

5.2. Germplasm evaluation

In the preliminary tests, all the ICRISAT lines tested (JM prefix) were found susceptible to Fusarium wilt (Table 7). These lines had been reported as resistant at ICRISAT. The behaviour of these ICRISAT lines suggested that the isolates used at ICRISAT are different from those used in this test. This also could be due to host-pathogen specificity, characteristic of gene-for-gene interaction.

Overall, two, six and 12 lines of pigeonpea could have been selected as resistant against isolates Emali, Kyangwithya, and Kimutwa respectively. However, no line was resistant to Thika isolate. Whereas the results point out the differences in virulence of these isolates, the results also imply that little of our germplasm was resistant to Fusarium wilt. From all the sick-boxes, only 50 plants (subsequently grown as lines) grew to maturity and yielded sizeable quantities

of seed. However, in subsequent tests these lines also continued to segregate for susceptibility and in the process resistance was being selected for through death of the susceptible plants.

When selection of promising material was extended to include all lines with 30% or less wilt, 43 lines were found to be resistant in one, two, three or all the four isolates. Of these, one was resistant in all four isolates, eight in three isolates, 14 in two isolates and 20 in one isolate (Table 10). Line NPP 725, resistant in all the four isolates and the eight lines resistant in three isolates were considered more useful than the other lines. The isolates used were obtained at sites widely apart in the pigeonpea growing areas of the country. Therefore the nine lines are likely to be more adapted to areas where these isolates are prevalent. The use of a large number of isolates (representative inoculum of the pathogen) to screen for resistance to any disease is strongly preferred so as to obtain a host genotype which can safely be grown over a large area.

Screening in the sick plot resulted in more lines than in the glasshouse being rated resistant

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(Table 11). Thirty three were rated resistant during the first year, 25 during the second year, and 18 during the third year. Seven lines were resistant for all three seasons. It was observed, however, that disease generally was lower in the field than in the glasshouse. This is attributed to differences in inoculum load and environmental variations. Usually if inoculum of high concentration is used to screen resistant cultivars, the latter will elicit a susceptible response. The inoculum load in the boxes was much higher than in the field. There is usually no control over environmental factors in the field and therefore such factors as low soil moisture and high soil temperatures can impair fungal activity and therefore result in low mortalities. However, a positive significant correlation ($r = 0.47$) obtained between glasshouse and field results implied that both results and therefore the methods were reliable. These results also suggested that either technique could be used to confirm results of the other.

Previous pigeonpea improvement programmes in Kenya have emphasised the economic attributes

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Previous pigeonpea improvement programmes in Kenya have emphasised the economic attributes

such as high yield, early maturity, medium height and acceptable seed qualities but resistance to diseases like Fusarium wilt did not receive adequate attention. However, with the increase in the severity of Fusarium wilt in recent past it was realised that in any pigeonpea breeding programme, resistance to this disease should be given priority.

In the tests conducted already, satisfactorily resistant cultivars were identified among the germplasm. Such sources of resistance could be deployed in the improvement of the current varieties that are susceptible to wilt.

Since very resistant lines are necessarily not high yielding, great care was exercised during selection to avoid sacrificing one character for the other. In selecting promising material to advance emphasis was therefore placed on those moderately resistant and even tolerant lines that had acceptable yield levels. Eleven lines (Table 15, plates 5 to 7) were identified and selected following repeated screening of 50 different lines in the sick plot at Makueni and in the sick boxes in the glasshouse.

It is important to realise that in developing Fusarium wilt resistant lines we are aiming at overcoming one of those factors that affect yield. Simultaneous improvement of these characters is therefore important. Improvements for disease resistance should therefore be run concurrently with the improvement of yield and yield-related characters (Hawtin et al., 1981). Where high yield and resistance to Fusarium wilt do not occur together, it is possible to bring the two traits together into a common background by hybridisation. One other useful attribute in these lines is that most of them were resistant against at least two isolates.

Preliminary results of studies for disease resistance involving late maturing susceptible lines and NPP 725 have produced some F_2 segregants that are early maturing, short and apparently resistant to Fusarium wilt. Thus the three attributes could be brought together into a single variety. The variety NPP 670, for instance is a selection from a cross between a local line and one of Indian origin. When the variety NPP 670 was grown in Makueni, it grew to a height of only 87 cm and flowered in about 100 days. Improvement in the eleven lines on such traits as earliness,

and medium height can be done through hybridisation and selection by adopting appropriate breeding approaches.

Evaluation of pigeonpea germplasm for resistance to Fusarium wilt in both the sick plot and in the sick boxes are essential even if tests under controlled conditions of the glasshouse appear more reliable. The results of either of the tests confirm those of the other. Disease expression in the field is usually not as high as in the glasshouse, but the field provides natural environment for testing and rating of the host genotypes. This is the only environment where the genetic potential of the crop is determined. In the field, the plant is exposed to an interaction of fluctuating natural conditions and a variety of microorganisms both pathogenic and non pathogenic, favourable and antagonistic.

5.3 Variability in F. udum

Genetic diversity in any population, plant or animal is extremely important for its survival and genetic advancement. Under extremely harsh environmental conditions it is often only those

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Genetic diversity in any population, plant or animal is extremely important for its survival and genetic advancement. Under extremely harsh environmental conditions it is often only those

plants with the resistance/tolerance, usually inherent, that are able to survive. Such early theories as "survival of the fittest" stemmed from this fundamental principle. It is the tool that plant breeders have judiciously used to improve and stabilise yields either by exploiting the genetic potential of such a crop or by breeding for resistance or tolerance to factors that affect yield. The property of genetic diversity unfortunately extends also to such organisms as plant parasitic pathogens. Literature is extensive about the failures by breeders to develop durable and stable resistance in plants due to diversity in the pathogens that initially caused the disease.

Variants of plant pathogens today exist in such diverse forms (*forma speciales*) that it is not possible now to be sure of developing varieties that will stay for long without succumbing to new and often more virulent races. In *F. udum* it has also been widely suspected that it occurs in diverse forms and each with its own degree of virulence. Pathogenicity studies conducted in present investigation further confirm this observation. In

this study, an attempt was made to differentiate 12 F. udum isolates on the basis of susceptibility/ resistance reaction of host cultivars.

According to Table 16, the isolate Manoni is easily separable from the others because it was the only isolate to which all the lines including Munaa were resistant. The Mulutu isolate is distinguished by line NPP 696/3. This line was resistant against all isolates except Mulutu. The Wote isolate is distinguished from Kimutwa isolate using line NPP 707/2. These two isolates affected all the other lines similarly. However, NPP 707/2 was resistant to the Wote isolate but susceptible to the Kimutwa isolate. The lines NPP 688/4 and 690/1 can distinguish isolates Kyemole and Tungutu. These two isolates caused same reactions to the other lines except these two. The line NPP 688/4 was susceptible to the Kyemole isolate but was resistant to isolate Tungutu. The pattern was reversed for NPP 690/1. The Kyanika isolate was similar to the Manoni isolate except in two lines, NPP 671/3 and Munaa. In the Manoni isolate, both lines behaved as resistant while in Kyanika isolate they were susceptible. There was little, if any,

relationship among the other isolates, viz. Thika, Emali, Ndumoni, Wikililye and Kyangwithya in the way they affected the test lines. None of the lines used could be used to differentiate these isolates. There are no standard pigeonpea host differentials yet reported for characterising isolates of F. udum as has been done in beans (Barrus, 1911) and chickpea (Haware and Nene, 1982). Since the lines used in this study had not been used before for similar studies, it was found incomplete to consider isolates as races. Further studies on host differentials would be necessary so they could be used in studies of this kind.

The distribution of the test lines in the disease severity scale and the divergent average virulences among these isolates further confirmed their pathogenic differences (Table 17). The Kyangwithya and Thika isolates were the most virulent of the twelve isolates. They each had an average virulence of 3.83. The two isolates could however be distinguished from one another on the basis of individual genotype rating. The Manoni isolate was the least virulent with an average value of 1.75.

Differences in virulence were also evident among the other isolates. The results of pathogenicity tests thus obtained demonstrate distinct variabilities among these isolates and could be indicators of the possible occurrences of "races" of F. udum in Kenya. These results further serve as proof to the already reported occurrence of races of this pathogen (Sarojini, 1951; Booth, 1971; Mukherjee et al., 1971; Baldev and Amin, 1974).

These isolates also showed variations in cultural characters such as growth rate, pigmentation, amount and kind of aerial mycelium. Colony sizes of these isolates were different from the second to the tenth days of growth (Table 20). The Emali isolate was the fastest growing while the Manoni isolate was the slowest. Growth rates of the other isolates were between those of the isolates mentioned above. The colour of the colonies varied from pinkish-white or pinkish-brown to deep purple and the aerial mycelium was either sparse and less raised or was abundant and fluffy (cottony) (Table 23, plate 8). Variations in cultural characters of isolates of F. udum have also been reported by Chattopadhyay and Sen Gupta (1967), Booth (1971),

Baldev and Amin (1974), and Shit and Sen Gupta (1978).

Attempts to establish a relationship between growth rate and virulence in these isolates were unsuccessful. Contrary to a general opinion that very virulent or aggressive isolates are also very fast growing, no such relationship was found in this study. A positive, non significant relationship ($r = 0.15$) was found between the two parameters. However, the isolate Manoni was both the least virulent and the slowest growing. The isolates Thika and Kyangwithya were the most virulent but were not the fastest growing. Such inconsistencies have also been reported in isolates of F. oxysporum f. cucurbitae (Prasad, 1949). However, a strong association between growth rate and virulence has been shown by isolates of Ceratocystis ulmi (Gibbs and Brasier, 1973).

Further characterisation of these isolates on the basis of their protein profiles revealed no striking differences among the isolates except for a few minor qualitative and quantitative variations in the bands. The bands that appeared

in all or most of the isolates were either of same mobilities or intensities. However, where differences were depicted, these usually related to either number of bands found or their intensities. For instance, the fine bands (Rf 0 - 20) differed in number and/or intensity among the isolates (Fig. 2). The Kimutwa isolate had five while isolates Kyangwithya, Kyemole, Tungutu, Ndumoni, and Nzabani had four each. However, in isolates Ndumoni, two of these bands were faint and in isolate Tungutu, one was faint and in isolate Nzabani, the fastest two of these bands were wavy. The isolates Mulutu, Thika and Manoni each had only two of these bands. However, in Mulutu, one of the bands was faint. In isolate Emali, these fine bands were lacking. Differences were also shown between isolates by the bands at Rfs 20, 30 to 35, and 40 to 50. From present-day ideas on gene-enzyme relationships and gene-protein relationships and on the mechanism of protein synthesis it is possible to account for electrophoretic variations such as among these isolates. Since proteins are under genetic control, the patterns could be a reflection of differences in

the genetic make-ups of these isolates and visibly demonstrates differences between these isolates. The differences thus shown by the zymogram (Fig. 2) are another indication that these isolates are different.

However, there were no bands that were characteristic of either the very virulent or the less virulent isolates. A positive relationship between virulence and protein components of the isolates could therefore not be derived from these results. However, there could be an indirect relationship whereby the genes involved in the biochemical pathway for virulence are related to those for synthesis of such proteins as are resolved at electrophoresis.

The value of protein profiles for characterising physiological races or isolates of fungi, however, appears to depend on the group of organisms studied. Macko et al. (1967) differentiated races of Puccinia graminis f. sp. tritici and Jeng and Hubbes (1983) distinguished between aggressive and non-aggressive isolates of Ceratocystis ulmi. Chang et al. (1962) also found small but significant differences between patterns obtained from strains of Neurospora crassa.

However, Shipton and Fleishman (1969) and Gill and Powell (1968) could not separate races of Puccinia coronata f. sp. avenae and Phytophthora fragariae respectively on the basis of protein components. Some scientists, (Reddy, M.V., pers. comm.) suggest that electrophoresis cannot show differences between virulent and avirulent isolates of a pathogen because virulence is triggered when there is a host-pathogen interaction. In the absence of a host, the proteins conditioning virulence are dormant just like in a non-virulent isolate. According to them, the proteins that are resolved at electrophoresis are usually structural proteins, which may bear no relationship to the potential of the isolate to attack its host. However, where morphological characters can be used to characterise virulence and non virulence as in pneumonia-causing bacterium, Streptococcus pneumoniae (Griffith, 1928) some relationship can be said to exist between virulence and structural proteins. In the above bacterium, the wild (virulent) form had a smooth polysaccharide capsule while the mutant (non-virulent) form had a rough capsule. At electrophoresis, the two capsules would certainly demonstrate differences in bands.

Ultimately, it may be said that these studies suggest that the 12 isolates are indeed different from each other and that they are a few of the different forms of F. udum obtaining here in Kenya. This is an important fact as it determines the strategies to develop and adopt in an effort to breed wilt resistant pigeonpea varieties. For instance, it is now imperative to screen material with as divergent an inoculum as can be obtained, that is, all promising material will have to be grown and tested in as wide an environment as possible so as to end up only with high yielding material that carries with it a broad spectrum of resistance. Such material is usually expected to be more stable than material distinctly resistant to one strain of the pathogen. Secondly, this variability in F. udum now means that work on the inheritance of resistance to this pathogen is likely to be more complicated since the crosses will react differently to each isolate.

In conclusion, it may be re-emphasised that the observations made above are important to pigeonpea breeders worldwide in general and those in Kenya in particular because they serve as a warning to

them that the resistance that may have been identified now and any other that may subsequently be identified is not durable nor is it universally effective. Breeding for Fusarium wilt resistance, therefore has to be continued to keep pace with the evolution of new and often more virulent strains of this pathogen.

5.4 Electrophoretic comparison of pigeonpea lines resistant and susceptible to Fusarium wilt.

Crop improvement in the past few years has included biotechnological approaches to supplement the conventional methods. Techniques such as genetic engineering, tissue culture, etc are nowadays widely being used to introduce desirable genes into traditional crops. Even in plant pathology, an approach that would overcome the need for controlled environments and frequent preparation of inocula to evaluate plant material for disease resistance would find much appeal. Where morphological characteristics of plants have been used exclusively to categorise crop species, varieties and even fungal species, the results have been found incomplete (Chang et al., 1962; Gupta, 1974 and Bassiri and Adams, 1978).

In this study, investigations were conducted to establish if a biochemical approach through electrophoresis could yield biochemical markers that are repeatable and dependable for the identification of resistant and susceptible plants. This technique has been demonstrated to be both sensitive and reproducible (Chang et al., 1962; Steward et al., 1965).

The results of this study to a large extent demonstrated no difference between the resistant and susceptible plants at most developmental stages. However, these enzymes and proteins did show some difference which could assist in the identification of resistant and susceptible cultivars of pigeonpea. Lack of qualitative and/or quantitative differences in these enzymes and proteins demonstrated the fact that not all enzymes or proteins play a direct role in disease resistance of plant. The role of enzymes and protein in resistance to diseases in plants has been debateable (Kedar, 1959; Umaerus,1959; Hunt and Barnes, 1982; Johnson and Lee, 1978).

Screening plants at the nine days stage gave sufficiently variable results that could be used to identify resistant and susceptible plants. The A1 band of acid phosphate (Fig. 4) had a distinctly

higher intensity in resistant lines implying that the higher the intensity of this band the more will be the resistance of the host. Similarly, the band A9 of Leucine aminopeptidase (Fig. 5) could also be used in differentiating resistant and susceptible genotypes. In the resistant genotypes this band was very dark staining whereas in one of the susceptible lines it was dark and in the other it was completely absent. Even the protein zymogram (Fig. 6) demonstrated strong differences in bands with Rf values 16 and 62 between resistant and susceptible plants. This developmental stage is therefore considered a very important stage for biochemical comparison of resistant and susceptible genotypes of pigeonpea.

Useful differences were also expressed in 20 day old plants (Fig. 8). In this zymogram, protein bands within the Rf value 35 to 43 and 47 to 56 were important. The band within Rf 35 to 43 was very dark in the susceptible lines and was light to dark in the resistant lines. The two bands within Rf 47 to 56 appeared only in the susceptible lines.

In the peroxidase zymogram of 25 day old plants (Fig. 9) only band C_3 expressed differences between resistant and susceptible plants. This

higher intensity in resistant lines implying that the higher the intensity of this band the more will be the resistance of the host. Similarly, the band A9 of Leucine aminopeptidase (Fig. 5) could also be used in differentiating resistant and susceptible genotypes. In the resistant genotypes this band was very dark staining whereas in one of the susceptible lines it was dark and in the other it was completely absent. Even the protein zymogram (Fig. 6) demonstrated strong differences in bands with Rf values 16 and 62 between resistant and susceptible plants. This developmental stage is therefore considered a very important stage for biochemical comparison of resistant and susceptible genotypes of pigeonpea.

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In the peroxidase zymogram of 25 day old plants (Fig. 9) only band C_3 expressed differences between resistant and susceptible plants. This

band was dark and very dark among the resistant lines but was uniformly light among the susceptible lines.

The results discussed above suggest strongly that it is possible biochemically to screen pigeon-pea germplasm for Fusarium wilt resistance. This will involve using different enzymes such as ACP, LAP and peroxidase and proteins using nine, 20 and 25 day old plants.

This technique would not necessarily replace the traditional techniques but would supplement them. The electrophoretic technique eliminates the need to make artificial inoculations and field observations. In addition the plant is not sacrificed since only leaves are used during the test.

This approach has been widely used with varying degrees of success depending on the crop type and the enzyme systems studied. Similar results as discussed above were also reported in beans (Okiror et al., 1982), and in peas (Hunt and Barnes, 1982). The technique also gave positive results in lucerne (Gupta, 1974), barley (Orlob and Arny, 1961) and in wheat (Seevers et al., 1971).

However, the electrophoretic study of peroxidases, glucose-6-phosphodehydrogenase, acid

phosphatase, malate dehydrogenase and proteins in Trifolium pratense (Stavely and Hansen, 1967) and peroxidase in Tobacco (Veech, 1969) did not reveal any differences between healthy resistant and susceptible plants. Also, on the basis of protein profiles, no differences between healthy resistant and susceptible plants were observed in wheat (Barrett and McLaughlin, 1954) and soybean (Hilty and Schmitthenner, 1966).

Apparently the success of the method is expected to vary with crop, enzyme, and age of the plants under study.

CONCLUSIONS

1. These results indicate that sowing seed in wilt infested soil in large wooden boxes is not only an effective technique but also simple, repeatable and can be used to screen large numbers of entries rapidly for resistance to wilt.

2. The evaluation of pigeonpea material for resistance to Fusarium wilt in the sick plot as well as in the glasshouse is necessary to confirm the reaction of the plants. Single plant selections are effectively made in the glasshouse but not in the sick plot. Because of the difficulty in establishing uniform infestation over the whole area only line selection is effective and therefore reliable under sick plot conditions. The results of both sick plot and glasshouse gave a strong, positive correlation suggesting that not only were the results consistent but the two methods were also reliable as screening techniques.

3. The results obtained in this study strongly suggest that different forms ("races"?) of F. udum exist in Kenya. This fact necessitates the development and release to farmers for cultivation only those varieties with proven resistance, that is broad based and stable. Continuous search for other sources of resistance is therefore essential. However, since no standard host differentials have been developed for testing isolates of F. udum, further studies in this direction are essential to make the characterisation of isolates into races easy and more accurate.

4. Since resistance is dominant to susceptibility, its maintenance in pigeonpea, an often out-crossing crop, should be less costly. Through occasional selfing it should be possible to recover it. Its digenic nature is a further advantage since it should be more stable than the monogenic forms. Broad base and more reliable forms of resistance can be built up by pyramiding of alleles from several backgrounds.

5. Electrophoretic studies showed consistent difference between resistant and susceptible pigeonpea genotypes based on enzymes (ACP, LAP, Peroxidases) and proteins in nine, 20 and 25 day-old plants. These results suggested strongly that it is possible biochemically to screen pigeonpea germplasm for resistance to Fusarium wilt especially at these plant developmental stages.

APPENDIX: Stock solutions used in disc electrophoresis

Stock solution A. (pH 8.9)

IN Hydrochloric acid	48.0 mℓ
Tris (hydroxymethyl) amino methane (Tris)	36.6 g
N,N,N',N' - Tetra methylethylene diamine (Temed)	0.23 mℓ
Distilled water to	100 mℓ

Stock solution B (pH 6.7)

IN Hydrochloric acid	48.0 mℓ
Tris (hydroxylmethyl) amino methane (Tris)	5.98 g
Temed	0.46 mℓ
Distilled water to	100 mℓ

Stock solution C (11.2%)

Acrylamide	28.0 g
Crystallized N,N' - methylene-bisacrylamide (Bis)	0.735 g
Distilled water to	100 mℓ

Stock solution D

Acrylamide	10 g
Bisacrylamide	2.5 g
Distilled water to	100 mℓ

APPENDIX (Contd...)

Stock solution E

Riboflavin	4.0 mg
Distilled water to	100 mℓ

Stock solution F

Sucrose	40 g
Distilled water to	100 mℓ

Stain (fixitive stock solution)

Amido black 10^B	0.7 g
7% acetic acid to	100 mℓ

Destain (wash solution)

Acetic acid	70 mℓ
Distilled water to make vol. to	1000 mℓ

Stock buffer (electrolyte) (pH 8.3)

Tris (hydroxylmethyl) amino methane (Tris)	6.0 g
Glycine	28.8 g
Distilled water to make vol. to	1000 mℓ

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