Development of high yielding pigeonpea (*Cajanus cajan*) germplasm with resistance to Fusarium wilt (*Fusarium udum*) in Malawi

by

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General abstract

Pigeonpea [*Cajanus cajan* (L.) Millspaugh] is a very important grain legume crop for food, cash and firewood in Malawi. However, its production is affected by Fusarium wilt (*Fusarium udum* Butler), which causes up to 100% yield loss. The deployment of resistant varieties would be an economical way to manage the disease, and for this, more information is needed on farmers' preferences for local landraces, how farmers and consumers can be involved in developing new varieties resistant to wilt disease, and the genetics of inheritance of resistance. This information would be used to devise a breeding strategy.

A participatory rural appraisal was used in the southern region of Malawi to identify pigeonpea production and marketing constraints. Results showed that Fusarium wilt was the most prevalent and destructive disease of pigeonpea in the area. Other constraints included pests, flower abortion, low yields, and low soil fertility. Local landraces accounted for 84% of the pigeonpea production in Malawi. Local landraces were preferred due to their fast cooking time, taste, and the high prices they earn the farmer. Participatory variety selection was used to identify landraces with desirable traits that could be used in the breeding programme. Farmers and buyers selected ten local landraces which were used in the genetic improvement programme.

Pigeonpea local landraces and International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) genotypes were evaluated for wilt resistance, yield, and secondary traits at three sites over three seasons. Most of the landraces were susceptible to wilt and late maturing. However, AP10, a local landrace, was high yielding and resistant to wilt and outperformed ICRISAT varieties. This local landrace showed promise for use as a source material for Fusarium wilt resistance in other locally adapted farmer-preferred varieties lacking resistance. The local landraces needed genetic improvement in wilt resistance, yield, early maturity, number of branches and seeds pod^{-1}.

Laboratory and screenhouse studies were performed to develop a new Fusarium wilt screening technique. Grains of finger millet, sorghum, and wheat were tested as media for multiplying *F. udum* isolates. Pathogenicity tests were done on Bunda College and Bvumbwe Research Station isolates. The Bunda isolate was then used in an infested-seed inoculation technique against eight differential cultivars. The results showed that

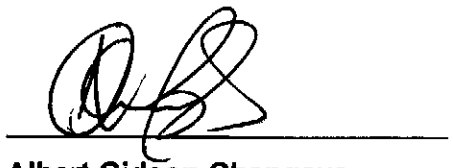
finger millet, sorghum and wheat were equally effective for rapid multiplication of *F. udum* isolates. Wheat grain showed the best results for pathogen multiplication and inoculation, due to the large seed size for easy handling. The inoculation process involved placing infested wheat grain on bruised pigeonpea roots and transplanting into soil in pots. The infested seed inoculation technique, which is the first of its kind for pigeonpea, was effective in screening pigeonpea for wilt resistance.

The selected landraces were crossed with wilt resistant testers in a 12 lines x 4 testers mating scheme, and 48 F_1 crosses were generated. These F_1 crosses were evaluated for wilt resistance, yield, and secondary traits. The variations among F_1 crosses for wilt and secondary traits were due to additive gene action in both parents and the dominance effects arising from the interactions of parents. Parental lines, with good combining ability effects for wilt resistance (AP2, AP3, and AP4), days to 50% flowering, seed pod^{-1}, plant height, stem diameter, and number of primary and secondary branches were identified, while ICEAP00554 (tester) was a good general combiner for wilt resistance and days to 50% flowering. These lines would be useful in breeding for Fusarium wilt resistance in farmer-preferred pigeonpea genotypes in Malawi or similar environments. Specific F_1 crosses were identified with significant SCAs for wilt resistance, days to 50% flowering, and secondary branches. The significance of GCA and SCA effects, which indicated importance of both additive and non-additive gene effects, respectively, suggested that both selection and hybridisation would be useful to improve the resistance in farmer-preferred varieties.

Segregation analyses were conducted on F_2 populations to determine the resistance to susceptibility phenotypic ratios. The Chi-square analyses showed that resistance to wilt was dominant over susceptibility in most F_2 populations. The segregation ratios of 3:1, 13:3, 15:1, and 9:7 (R:S) indicated that either one dominant gene, or two inhibitory genes, or two independent dominant genes, or two complementary genes, respectively, were conferring wilt resistance in these crosses. Involvement of only a few genes governing wilt resistance suggested few complications, if any, in breeding for this trait in these locally adapted pigeonpeas. The Pedigree breeding method would be recommended for incorporating these traits.

Declaration

I, **Albert Gideon Changaya**, hereby declare that this thesis, submitted for the degree of Doctor of Philosophy in Plant Breeding in the Faculty of Science and Agriculture, University of KwaZulu-Natal, Pietermaritzburg, except where the work of others is acknowledged, is the result of my own investigation. This thesis has not been submitted for any degree or examination at any other university.



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14/04/2008

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Supervisors' Approval

The University of KwaZulu-Natal approves this thesis of **Albert Gideon Changaya** in fulfillment of the requirements for the degree of Doctor of Philosophy in Plant Breeding.

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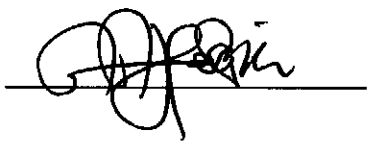
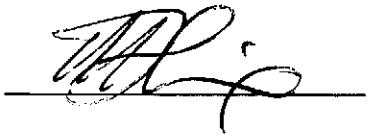
Dr John Derera		04-10-2008
Professor Mark D. Laing		11/04/08

Dedication

To my dear wife, Eunice, beloved children, Sally and Shaun, for their sacrifice, perseverance, understanding, love and moral support.

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I would like to thank God Almighty for providing me with such an opportunity to attain a PhD qualification. To Him be the glory forever.

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Introduction to thesis

Pigeonpea production in Malawi

Pigeonpea [*Cajanus cajan* (L.) Millspaugh] is one of the major grain legume (pulse) crops of the tropics and subtropics (Saxena et al., 2002[1]). It ranks sixth worldwide in area and production in comparison to other grain legumes such as dry beans (*Phaseolus vulgaris* L.), peas (*Pisum sativum* L.), groundnuts (*Arachis hypogaea* L.), soybeans (*Glycine max* L.) and chickpeas (*Cicer arietinum* L.) (Nene and Sheila, 1990). In Malawi, it comes second in production to groundnuts (Figure 1), despite the hectarage being third after beans and groundnuts (Figure 2). The average yields have been second to groundnuts (Figure 3) (Ministry of Agriculture and Food Security, 2007).

The crop is grown by smallholder farmers in all three political regions of Malawi (northern, central and southern) (Figure 4) as an intercrop with staple food crops such as maize, sorghum and cassava (Sakala, 1992). Most of the pigeonpea is grown in the southern region (99.4%) and very little in the central region (0.1%) and northern region (0.5%) (Ministry of Agriculture and Food Security, 2007).

Pigeonpea offers many benefits to subsistence farmers as a food and cash crop (Nene and Sheila, 1990). It is also used as fodder for domestic animals (Arya et al., 2002). It improves soil fertility and benefits subsequent cereal crops through nitrogen fixation when grown in a mixture with maize and sorghum (Sakala, 1992; Yun et al., 2001). In addition, it is one of the crops that contributes significantly to fuel wood for many households in the southern region of Malawi (Edje, 1984).

Pigeonpea yields can reach up to 2 t ha^{-1} (Chauhan, 1990). However, there are many constraints limiting actual production; these constraints have resulted in low yields of about 600 to 700 kg ha^{-1} (Chauhan, 1990; Ministry of Agriculture and Food Security, 2007). The constraints include diseases, pests, agronomic and abiotic factors. Other constraints are of minor importance but Fusarium wilt is the most important limiting factor in pigeonpea production in Malawi (Subrahmanyam et al., 1992; Gwata et al., 2006; ICRISAT, 2006).

[1] Referencing format in this thesis is for American Crop Science Journal (with some modifications)

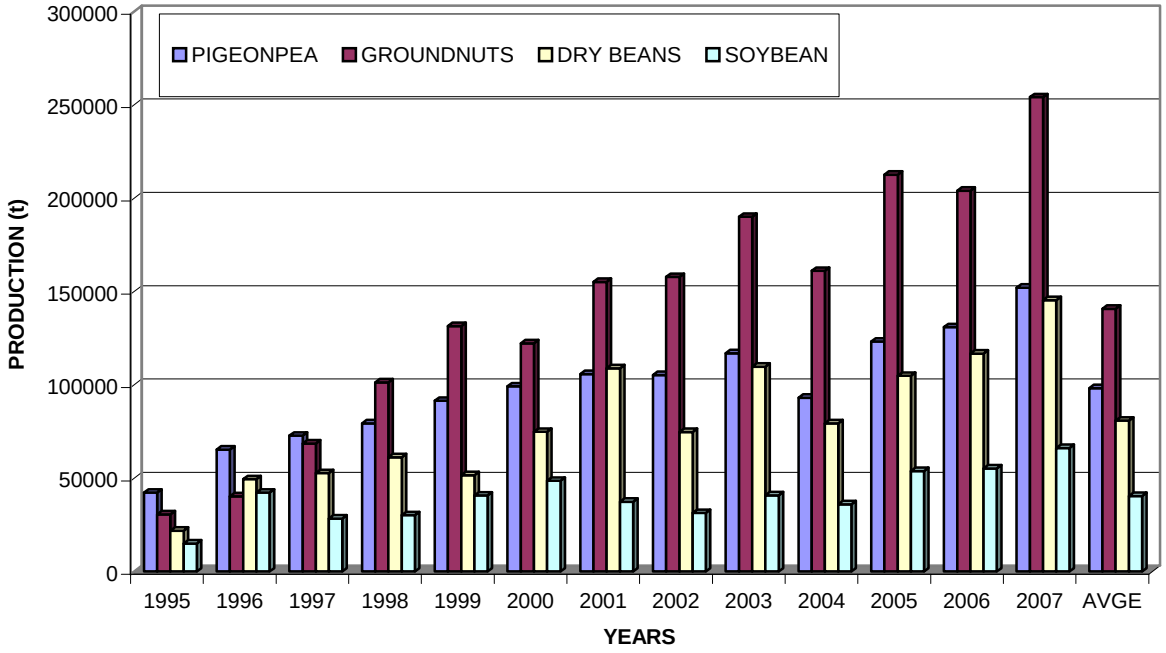

Figure 1: Legume production in Malawi from 1995 to 2007 (MOAFS, 2007)

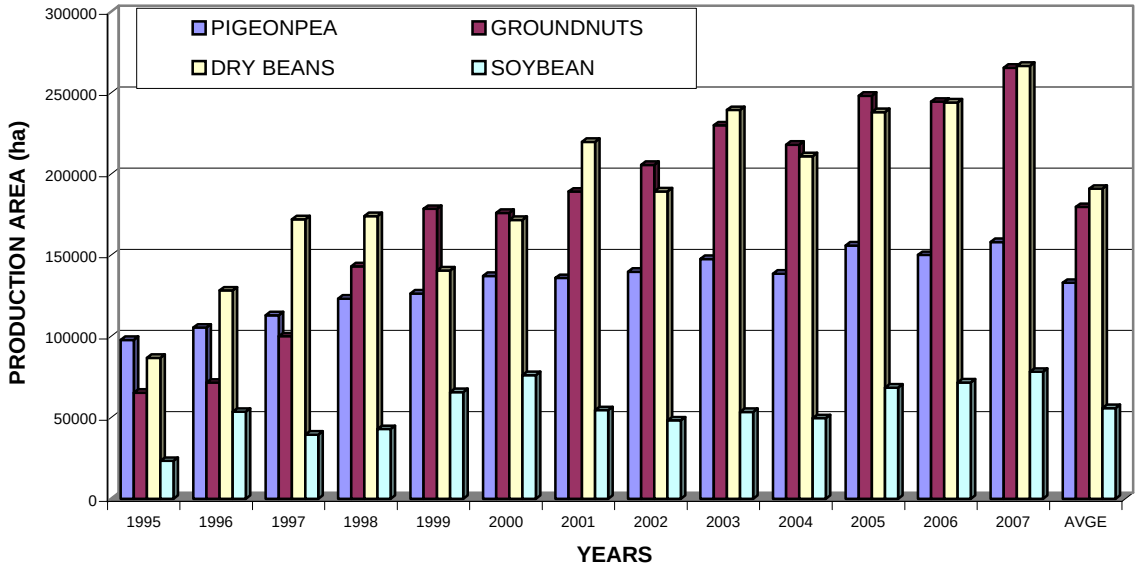

Figure 2: Area under legume production in Malawi from 1995 to 2007 (MOAFS, 2007)

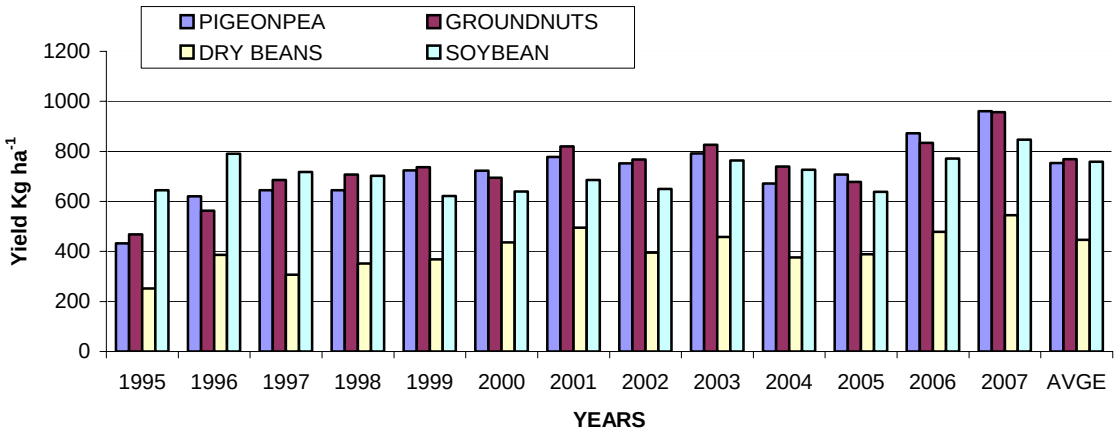

Figure 3: National average yields of selected legumes in Malawi from 1995 to 2007 (MOAFS, 2007)

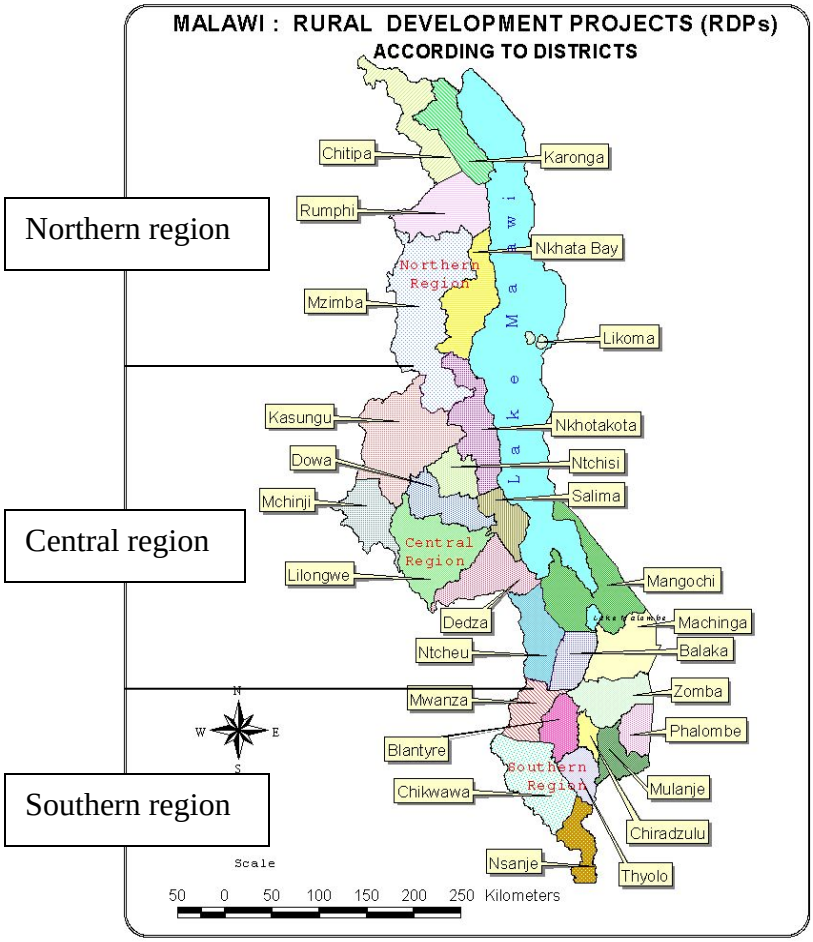

Figure 4: Map of Malawi showing three political regions (Malawi Survey Department, 2007)

Fusarium wilt disease in Malawi

In Malawi, diseases are major constraints to pigeonpea production (Subrahmanyam et al., 1992; Gwata et al., 2006). Fusarium wilt (*Fusarium udum* Butler) is the most prevalent and destructive disease of pigeonpea, causing yield losses as high as 50-100% in susceptible cultivars (Soko, 1992). If branches or the whole plant wilt, seed does not form, especially when wilting occurs before or during flowering. In some genotypes, the disease is characterised by the wilting of the plant on one side, while the other side is healthy. The disease is present in the southern and northern regions of the country, but is most widespread in the southern region where most of the pigeonpea is grown. Continuous cropping due to small land holding size is one of the factors responsible for the disease in farmers' fields. Although chemical and biological control measures exist (Singh et al., 2002), use of resistant varieties is a practical and economical strategy to manage the disease (Reddy et al., 1990a), especially for subsistence farmers, who cannot afford chemical or biological control measures.

Due to the high incidence of wilt in Malawi, as reported by Kannaiyan et al. (1984), the Department of Agricultural Research in the Ministry of Agriculture released Fusarium wilt resistant cultivars with the aim of increasing pigeonpea production. In 1987, a high yielding, Fusarium wilt resistant cultivar, ICP9145, was released (Reddy et al., 1990b; Reddy et al., 1995). ICP 9145 is also drought tolerant, large seeded (15.6 g 100 seed^{-1}), and comparatively early maturing relative to the local landraces (Kawonga, 1992). ICP9145 is a landrace collected from Kiboko, Kenya, by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in 1976. The plant was identified as being disease-free in a Fusarium wilt sick plot. It was screened for several seasons at ICRISAT stations in India, Kenya, and Malawi, before being released (Reddy et al, 1995). The variety is therefore an introduction to Malawi from Kenya. The plants of ICP9145 are compact, of indeterminate growth habit, and tall (215 cm), making them suitable for mixed cropping systems (Reddy et al., 1995). The release of ICP9145 not only reduced the incidence of wilt nationally from 36.3% in 1980 (Kannaiyan et al., 1984) to 6.3% in 1991 (Reddy et al., 1992) but also increased the area under pigeonpea production from 19,904 ha in the 1985/86 season to 87,758 ha in the 1994/95 season (Ministry of Agriculture and Livestock Development, 1995/96). Average yields increased from 432 to 630 kg ha^{-1} (Babu et al., 1992).

In 2000, another Fusarium wilt resistant variety called *Kachangu* (ICEAP00040), developed by ICRISAT Nairobi (Kenya), was released in Malawi following screening in multi-locational sites (Silim et al*.,* 2005). The variety was released in Malawi after screening in multi-locational sites. Unlike ICP9145, *Kachangu* was bred using the pedigree breeding method (Late Dr H.N. Soko[2], personal communication, 2003). The variety has further reduced the incidence of wilt and risk of crop failure. The traders and farmers prefer this variety to ICP9145 because of preferred seed colour (whitish - cream), large seeds (more than 18 g 100 seeds^{-1}), and milling quality for split peas (Late Dr H.N. Soko, personal communication, 2003). Plants of *Kachangu* are compact, of indeterminate growth habit, with branches inclined to the main stem, and tall enough for use in a mixed cropping system (Silim et al., 2005).

It is assumed that the two Fusarium wilt resistant varieties increased the market volume. However, an impact assessment of the two varieties needs to be conducted to confirm this assumption. So far, no pigeonpea variety has been bred in Malawi with resistance to Fusarium wilt. Dr H.N. Soko (personal communication, 2003) developed pigeonpea lines up to an F7 generation, but had to suspend this pigeonpea-breeding programme due to lack of funding.

Five years after the release of ICP9145, Kawonga (1992) observed Fusarium wilt in fields planted to ICP9145 in the Blantyre Agricultural Development Division (BADD), suggesting loss of resistance in the variety. The loss of resistance in the variety could have been due to the development of a new virulent race of the pathogen (Borojevic, 1990) or due to outcrossing between local landraces and ICP9145. Studies carried out by several researchers worldwide, including Malawi (Changaya-Banda et al., 1996; Sivaramckrishnan et al., 2002), have indicated the existence of pathogenic variation in *F. udum*. This variation may be due to the sexual process, mutation, heterokaryosis, parasexualism, or heteroploidy (Borojevic, 1990; Agrios, 2005). There is a very strong possibility of new virulence in *F. udum*.

In addition to variability in the pathogen, several other reasons for the recent susceptibility of ICP9145 could exist. Outcrossing in pigeonpea ranges from 0 - 70% (Reddy, 1990). Many farmers still grow local landraces, and most Malawian landraces

[2] Late Dr H. N. Soko, Chitedze Research Station, P.O. Box 158, Lilongwe, Malawi

are susceptible to wilt. Outcrossing of ICP9145 with landraces would have resulted in progenies becoming susceptible because more susceptible genes were being brought into the population. Farmers recycle seed and the probability is high that the recycled seed would have become susceptible. There is no selection pressure on the part of the farmer because the seed for the next planting is obtained from the highly mixed seed lot. Commercial seed companies avoid multiplying seed for low-income crops such as pigeonpea, sorghum, millet, and cowpeas because they perceive them as non-profitable (Dr Richard Jones[3], personal communication, 2004). With the pathogenic variability of *F. udum* and local farmers' preferences, there is a need to develop resistant varieties from a local breeding programme with the traits farmers prefer.

Research approach

Most Malawian farmers still plant local landraces which are susceptible to the disease, despite the release of the two Fusarium wilt resistant varieties. It thus seems likely that local landraces may possess certain desirable traits that the two wilt resistant varieties do not have. This speculation needs to be confirmed, through participatory rural appraisal. Why are local landraces popular among farmers and can these farmers and other consumers be involved in the selection of pigeonpea materials with desirable traits? The germplasm selected by this process would serve as parental materials, in the pigeonpea genetic improvement programme, to breed new varieties which are resistant to the disease, but also have desirable traits (Sharma and Duveiller, 2006; Danial et al., 2007).

Screening pigeonpea germplasm for resistance to wilt disease, during the breeding process, has relied heavily on natural inoculation in a sick plot (Reddy et al., 1995; Infantino et al., 2006). The method has, however, produced inconsistent results due to poor distribution of the inocula in the soil and varying environmental conditions (Nene et al., 1981; Burgess et al., 1994). Several artificial inoculation methods have also been tried to screen pigeonpea germplasm for Fusarium wilt resistance: root dip and transplanting technique (Roberts and Kraft, 1971), water culture technique (Nene and Kannaiyan, 1982), and stem injection (Katsantonis et al., 2003). However, all these methods have shortfalls as their successes depend on spore concentration, age of the

[3] Dr Richard Jones, Program Leader, SCOSA, ICRISAT, Nairobi, Kenya

plant, and environmental conditions (Ribeiro and Hagedorn, 1979). Therefore, there is a need to develop new, simple screening methods for wilt resistance in pigeonpea.

Some scientists have reported that multiple genes control pigeonpea resistance to Fusarium wilt, others have suggested two complementary genes, and yet others reported duplicate dominant genes, a single dominant gene, and a single recessive gene (Singh et al., 1988; Singh et al., 1990; Reddy et al., 1998; Okiror, 2002). The different findings were predictable because they worked in separate environments with different germplasm. Therefore, the germplasm from Malawi is also most probably likely to behave differently. Determining the number and nature of genes governing wilt resistance and the nature of inheritance of that resistance in pigeonpea was thus considered important. Genetic studies, through analysis of segregating ratios, have been used to determine the number and nature of genes governing certain traits in pigeonpea and other crops (Bahadur et al., 2002, Aher et al., 2003).

Study objectives

The overall objectives of this research were to identify the new sources of Fusarium wilt resistance in pigeonpea and to study the nature of inheritance of the resistance. The long-term goal of the research was to breed for high yielding pigeonpea varieties that are resistant to Fusarium wilt and have characters desirable to the consumer.

Specific objectives

The specific objectives of the study were to:

1. Evaluate farmers' attitudes and/or perceptions about Fusarium wilt disease in pigeonpea;
2. Identify pigeonpea cultivar preferences by the farmers and the trade;
3. Select local landraces, with desirable attributes, through stakeholders' participatory variety selection (PVS) for use in the development of new resistant varieties;
4. Develop a Fusarium wilt screening technique;
5. Screen the selected local landraces against Fusarium wilt disease;
6. Identify new sources of resistance and study the nature of inheritance of resistance to Fusarium wilt disease, yield and secondary traits in pigeonpea; and

7. Develop new Fusarium wilt resistant pigeonpea lines by crossing local landraces with Fusarium wilt resistant tester lines.

Thesis structure

The thesis has five chapters. Chapter 1 reviews key topics in the literature: the description of the crop, the constraints affecting pigeonpea production, Fusarium wilt disease, and breeding methods used in pigeonpea improvement. This chapter also outlines the gaps that exist in pigeonpea research. Chapter 2 investigates why farmers still grow local landraces despite the release of two wilt resistant varieties. It also explains how participatory variety selection (PVS) was used to select parental materials among local germplasm that were used in the breeding programme. The evaluation of local landraces and other imported materials for yield, yield components and disease resistance is covered in Chapter 3. Chapter 4 describes a novel method that was developed to screen pigeonpea germplasm and filial generations for resistance to Fusarium wilt. The type of resistance and nature of inheritance of the resistance to Fusarium wilt disease, and the genetics of yield and secondary traits are covered under genetic studies in Chapter 5. The thesis ends with overview of how overall and specific objectives were met, and the implications of the research findings to pigeonpea breeding. The referencing format followed in this thesis is that of American Crop Science Journal with some modifications.

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Chapter 1: Literature review

1.1 Introduction

The literature is reviewed in three different sections. The first section covers pigeonpea as a crop, its role among other legumes, its taxonomy, its origin, its worldwide distribution, its importance and its production constraints. The second section focuses on the most important production constraint that limits pigeonpea production in Malawi – Fusarium wilt disease. This is done by examining the distribution, importance and symptoms of the disease: an in-depth description of the disease's causative agent – *Fusarium udum* (Butler) and an identification of control measures of the disease. The third section covers the various aspects of breeding such as the role of participatory rural appraisal in pigeonpea breeding, breeding techniques, the genetics of breeding, inheritance studies of Fusarium wilt resistance and the role of biotechnology tools such as marker-assisted selection in pigeonpea breeding.

1.2 Taxonomy of *Cajanus*

Pigeonpea (*Cajanus cajan* (L.) Millspaugh) is the only cultivated food crop of the Cajaninae sub-tribe of the economically important leguminous tribe, Phaseoleae, which contains many bean genera consumed by man (e.g. *Phaseolus, Vigna, Cajanus, Lablab* and *Macrotyloma*). Within the tribe Phaseoleae, the sub-tribe Cajaninae is well distinguished by the presence of vesicular glands on the leaves, calyx, and pods (van der Maesen, 1990). Eleven other genera remain in the Cajaninae, the larger ones being *Rhynchosia, Eriosema*, *Dunbaria* and *Flemingia*.

The cultivated pigeonpea stands alone as a crop species in the sub-tribe; most species of this sub-tribe belong outside the pigeonpea gene pool, or at most, in its tertiary gene pool, although several *Cajanus* species can be placed in the secondary gene pool (van der Maesen, 1990). The primary gene pool includes cultivar collections of *Cajanus cajan*. The secondary gene pool includes *C. acutofolius, C. albicans, C. cajanifolius, C. lanceolatus, C. latisepalus, C. lineatus, C. reticulatus, C. scarabaeoides* var. *scarabaeoides, C. sericeus,* and *C. trinervius*. The tertiary gene pool contains *C. goensis, C. heynei, C. kerstingii, C. mollis, C. platycarpus, C. rugosus* and *C. volubilis* (van der Maesen, 1990). There are little, if any, chances of species crossing between

different gene pools under natural conditions because of incompatibility. It would be very difficult, therefore, to use resistance genes for resistance to diseases, pests, and abiotic stresses from the wild species into the cultivated pigeonpea. However, some scientists have succeeded in crossing pigeonpea with wild related species (Dundas, 1990; Singh et al., 1990; Aruna et al., 2005; Mallikarjuna and Saxena, 2005; Saxena et al., 2005).

With the recent revision of the taxonomy of *Cajanus* (van der Maesen, 1990), the *Atylosia* species do not appear to differ sufficiently from *Cajanus* to warrant generic status. Morphological, cytological, chemical, and hybridization data support this merger, even if the needed taxonomic changes are for convenience. The genus *Atylosia* forms the secondary gene pool of 34 known species. *Atylosia* and *Cajanus* are mainly distinguished by a persistent aril, or strophiole, on the seeds of *Atylosia*. The character is simply inherited and has occurred in some cultivars in *Cajanus*. It is reported that 22 out of 34 *Atylosia* species occur in India, Sri Lanka, and Burma, one in Mauritius, seven in Australia, one in Malasyia, two in China, and one in Thailand (Sharma and Green, 1980). *Cajanus*, as recognized now, has 32 species and the genus *Cajanus* is distributed in the old world with 18 species in Asia, 15 in Australia and one in Africa (van der Maesen, 1990). The only distinct difference between the two genera is that *Cajanus* is found only under cultivation, while *Atylosia* spp are all uncultivated, wild, weedy forms (Sharma and Green, 1980). However, it is not known how far *Atylosia* is from domestication.

In 1956, Deodikar and Thakar observed close affinity between *Cajanus* and some erect species of *Atylosia* and suggested the possibility of transferring Fusarium wilt resistance from *Atylosia* to *Cajanus*. Three species, *A. lineata, A. sericea,* and *A. scarabaeoides,* were successfully crossed with *C. cajan*. Cytological studies showed that all were close to *Cajanus*, but *A. lineata* is the closest (Sharma and Green, 1980). However, more recent crosses revealed *A. cajanifolia* to be the closest relative of *C. cajan*. Except for pod and seed characters, it is difficult to distinguish *Atylosia* from *Cajanus* (Sharma and Green, 1980). There are thus increased possibilities of transferring some desirable genes from the wild relatives into cultivated pigeonpea for disease and pest resistance and yield. Reddy (2004) suggested several appropriate methods for exploitation of the genes. He proposed traditional breeding methods (TBM) and marker-assisted selection (MAS) for crossing *C. cajan* with weedy counterparts or progenitors of cultivated

pigeonpea in the primary gene pool (*C. cajanifolius*). Reddy (2004) also proposed TBM, MAS, and introgression through backcross (BC) programmes between pigeonpea and cross-compatible species producing more or less fertile hybrids in the primary and secondary gene pool. For crossing with the tertiary gene pool, embryo rescue, chromosome doubling and BC + MAS would be appropriate for crossing between pigeonpea and cross-compatible species, producing viable but sterile hybrids. Reddy (2004) also proposed that protoplast fusion, aided by molecular markers, would be suitable for crossing pigeonpea with cross-compatible species, producing non-viable hybrids in the same tertiary gene pool and other gene pools.

1.3 Origin and distribution of pigeonpea

Most of the evidence points to India as the place where pigeonpea originated because of the presence of several wild relatives, the large diversity of the crop gene pool, ample linguistic evidence, a few archaeological remains, and the wide usage in daily cuisine (van der Maesen, 1990). The diversity of the crop in India is much larger than in Africa, and this made Vavilov, in 1951, list pigeonpea to be of Indian origin (van der Maesen, 1990). However, it spread quite early to the rest of the world. India and Myanmar account for 16 related wild species, one of which, *C. cajanifolius*, could be considered as a progenitor (van der Maesen, 1990). Many authors: Purseglove (1968), Rachie and Roberts (1974) considered Eastern Africa as the centre of origin, as pigeonpea seems to occur wild in Africa (van der Maesen, 1990). The scarce, but often cited, archaeological evidence of one seed in an ancient Egyptian tomb, and the wild occurrences in Africa, further favoured speculation that pigeonpea had an African origin (van der Maesen, 1990). However, Africa harbors only one close wild relative of pigeonpea, *C. kerstingii* (van der Maesen, 1990).

Pigeonpea is widely grown on the Indian subcontinent. It is also grown in Southeast Asia, Africa, and the Americas. There is a substantial area under pigeonpea production in Kenya, Malawi, Mozambique, Tanzania, and Uganda in Africa, and in the Dominican Republic and Puerto Rico in Central America. In most other countries, pigeonpea is grown in small areas and as a backyard crop (Nene and Sheila, 1990). India has dominated production of pigeonpea (91.3% of world production) during the last decade (Muller et al., 1990). The other countries with a notable pigeonpea production are Malawi

(3.5%), Eastern Africa (2.6%), Nepal and Myanmar in Asia (1.5%) and the Americas (1.1%) (Muller et al., 1990).

1.4 Importance of pigeonpea

Pigeonpea offers many benefits to subsistence farmers as a food and cash crop and also ensures stable crop yields in times of drought (Nene and Sheila, 1990). As a food source, pigeonpea offers a cheap source of valuable protein to people. Its protein content averages 21%, although some high-protein lines are being bred with up to 30% protein (Sharma and Green, 1980; Gupta et al., 2001; Saxena et al., 2002). Pigeonpea has more minerals, ten times more fat, five times more vitamin A, and three times more vitamin C than ordinary peas (Madeley, 1995). Pigeonpea yields more energy, protein, and beta-carotene ha^{-1} than other important pulse crops (Muller et al., 1990). The World Health Organization (WHO) recommends 0.75 g of protein daily for each kg of body weight to meet the needs of most of the general world population (Shils et al., 1994; Garrison and Somer, 1995). In the southern region of Malawi, pigeonpea forms an integral part of the diet of small-scale farmers, and, more especially, it forms part of the ration provided to workers on large-scale farms. Pigeonpea is consumed as dhal (split cotyledons), whole cooked seed, and cooked green pods. The recommended daily intake of nutrients for populations in Africa is outlined in Table 1.1.

Table 1.1: World Health Organization (WHO) recommended daily intake of protein for populations in Africa

Population group	Protein (g)	Population group	Protein (g)
Adult man (55 kg)		**Children**	
Sedentary	31	Below 1 year	14
Active	31	1 – 3 years	16
Very Active	31	4 – 6 years	20
		7 – 9 years	25
Adult Woman (47 kg)			
Sedentary	24	Boys 10 – 19 years	30
Active	24	Girls 10 – 19 years	29
Very Active	24		
Pregnant	33		
Lactating	41		

Source: Latham (1979)

The seed husks and pod walls of pigeonpea are commonly fed to cattle, and green leaves are used as cattle fodder. After the pods are harvested, plants are often left in the

field for cattle to graze the new green leaves such plants produce (Nene and Sheila, 1990; Shiying et al., 2001). Pigeonpea is not only used as fodder for domestic animals (Shiying et al., 2001; Arya et al., 2002), but also has the potential to fill the late summer/fall (off-season) gap in forage availability (Arnold, 2002). In one experiment, scientists showed that pigeonpea cultivar ICPL 93047 produced 54 t ha^{-1} of green fodder and 29 t ha^{-1} of dry fodder in five cuttings (Shiying et al., 2001).

Pigeonpea is grown mainly for home consumption. However, varying quantities of surplus grain are sold for cash (Muller et al., 1990). In Malawi and the other Eastern African countries, pigeonpea is processed into dhal and exported to India, Europe and South Africa.

Pigeonpea, as a legume, improves soil fertility through biological nitrogen fixation. It is reported to contribute approximately 40 kg N ha^{-1} (Kumar Rao et al., 1990). Leaf fall at maturity not only adds to the organic matter in the soil, but also provides additional nitrogen. This also benefits subsequent cereal crops when grown in a mixture with maize and sorghum (Sakala, 1992; Yun et al., 2001; Arya et al., 2002).

The deep root system of pigeonpea is reported to break plough pans, thus improving the soil structure (Nene and Sheila, 1990). The deep rooting system also enables the plant to be drought tolerant (Nene and Sheila, 1990; Johansen, 2003) and among the legumes, pigeonpea has a relatively high level of dehydration tolerance (Johansen, 2003). The extensive ground cover provided by pigeonpea prevents soil erosion by wind and water, encourages infiltration, minimizes sedimentation, and smothers weeds (Nene and Sheila, 1990).

Pigeonpea is a perennial, but is most often cultivated as an annual crop. Because of the long maturity period of pigeonpea, the landraces and the traditional cultivars are almost always grown as intercrops or in mixed cropping systems with shorter-duration crops. In Africa, pigeonpea is commonly intercropped with maize, sorghum, cowpeas and cassava (Nene and Sheila, 1990). The mixed cropping systems have advantages to the farmer, especially in optimizing land utilization.

Pigeonpea is used as a green manure crop in some countries. The tall perennial plants can serve as windbreak hedges, and occasionally pigeonpea plants are used as shade for tree crops or vanilla (Nene and Sheila, 1990). Pigeonpea is also grown as a perennial to mark field boundaries.

Pigeonpea is one of the crops that contribute significantly to fuel wood for many households. The dry stems of pigeonpea are an important household fuel wood (Chatarvedi et al., 2001; Shiying et al., 2001). Ten t ha^{-1} of dry sticks can routinely be obtained from pigeonpea to serve as fuel wood (Nene and Sheila, 1990). In an agroforestry trial at Bunda College in Malawi, Edje (1984) reported that at the end of the second year, the pigeonpea crop grown as 5000, 10000 and 20000 plants ha^{-1} produced 10.1, 11.7 and 12.5 t ha^{-1} of fuel wood, respectively. Faris and Singh (1990) reported fuel wood of 57.6 t ha^{-1} in Colombia and 51 t ha^{-1} in Western Australia in two cuttings within one year. After eight months in India, an actual wood yield of 32 t ha^{-1} was obtained in one cutting. In India, the pigeonpea sticks are also used to make field fences, huts, and baskets (Nene and Sheila, 1990).

Pigeonpea has many traditional medicinal uses. Dry roots, leaves, flowers, and seeds are used in different countries to treat a wide range of ailments of the skin, liver, lungs, and kidney (Nene and Sheila, 1990). The roots are used to treat febrile diseases and relieve fever, constrict tissue for controlling bleeding, and destroy internal worms. The leaves can be used to treat jaundice, trauma, cough, burn infection, and bedsores, (Shiying et al., 2001).

Pigeonpea has many other potential uses, one of which is serving as an important host for the scale insect that produces lac (Nene and Sheila, 1990). High yields of up to 750 kg ha^{-1} of lac of superior quality have been reported (Shiying et al., 2001). Lac is processed into shellac, which is used as a dye for wool, silk, leather goods and synthetic dyes. It is used in medicine as hepatoprotective and antiobesity drugs. Shellac is also used in several industrial applications, such as surface coatings, textiles, printing, cosmetics, pharmaceuticals, and adhesives. It is even used in the electrical industry ([http://en.wikipedia.org/wiki/Lac] 2007/09/17). Pigeonpea leaves are also used to feed silkworms; starch is used to produce noodles; for fermented food such as *tempe,* which was previously made from soybeans but can now be made from pigeonpea; preparing a

soft drink/mild liquor called “Chicha” in Venezuela and production of paper pulp (Nene and Sheila, 1990).

1.5 Production constraints on pigeonpea

The incidence of diseases is a major cause of unstable yields of pigeonpea, particularly in intensively managed systems (Chauhan, 1990). Pigeonpea is attacked by more than 210 pathogens (Nene et al., 1996). These include fungi, bacteria, viruses, nematodes, and mycoplasma-like organisms. Fortunately, only a few of these pathogens cause economic losses (Kannaiyan et al., 1984). The diseases of economic importance include Fusarium wilt (*F. udum*), sterility mosaic (virus), Phytophthora blight *(Phytophthora drechsleri* Tucker f.sp *cajani* Pal et al. Kannaiyan et al., 1984), Macrophomina root rot (*Macrophomina phaseolina* (Tassi) Goid.), stem canker (*Phoma cajani* (Rangel) Khune and Kapoor), Alternaria leaf spot *(Alternaria tenuissima* (Kuze ex. Pers.) Wilthire), Cercospora leaf spot (*Cercospora cajani* Hennings) and witches’ broom *(Mycoplasma/Virus)*.

In Malawi, diseases are major constraints to pigeonpea production (Subrahmanyam et al., 1992). Most diseases are of relatively minor importance, but Fusarium wilt, caused by *F. udum,* is the most common and destructive disease of pigeonpea (Changaya-Banda, 1997; Hillocks et al., 2000; Gwata et al., 2006), and can cause yield losses as high as 50-100% in susceptible cultivars (Soko, 1992). The disease is more prevalent in the southern region where most of the pigeonpea is grown. The disease is favoured by a continuous cropping system with minimal crop rotation and use of susceptible cultivars. Though dependent on the stage at which plants wilt, yield loss can approach 100% when wilt occurs at the pre-pod stage (Reddy et al., 1990).

Pigeonpea is host to over 200 species of insects (Reed and Lateef, 1990). Some of these insects cause sufficient crop losses to be regarded as major pests, but the majority are seldom abundant enough to cause much damage, or are of sporadic or localized importance, and may be regarded as minor pests. Insects are found chewing or sucking pigeonpea plants from seedling to harvest, and no part of the plant is immune to attack. The pod-damaging insects, pod borer (*Helicoverpa armigera* Hub), pod borer (*Maruca testulalis* Geyer), larvae of blue butterfly *(Lampides boeticus* L. *and Catochrysops strado* Fab.), plume moth *(Exelastis atomosa* Wals.), thrips

(*Megalurothrips uitatus* Bagnall), blister beetles (*Mylbris pustulata* Thunberg), pod fly (*Melanagromyza obtusa* Malloch.) and sucking bugs (*Nezara viridula* L.), are the most important pests on this crop. Pod damage can greatly reduce crop yield, as the pigeonpea's potential to compensate for pod damage is limited. The pests include rats; birds such as pigeons attacking at sowing; adults of some weevils *(Myllocerus* spp / *Phyllobiu*s spp); beetles that feed on the cotyledons; and cutworms (*Agrotis* spp), that attack during the seedling stages. Jassids (*Empoasca kerri* Pruthi), aphids *(Aphis craccivora* Koch), mites (*Aceria cajani* Channabasavanna), red spider mite (*Schizotetranychus cajani* Gupta), whitefly *(Bemisia tabaci* Genn), leaf webbers *(Gepholita critica* Meyr), stem fly *(Ophiomyia centrosematis* de Meijere), scales (*Icerya purchasi* Maskell), nematodes (*Meloidogyne* spp) and cow bugs (*Oxyrhachis* spp) are important pests of the vegetative growth stage of pigeonpea. Termites *(Microtermes* spp), white grubs (*Lachnosterna consanguinea* Blanchard), and small larvae of the nodule-damaging fly mostly affect roots (Reed and Lateef, 1990). The diversity in the range of pigeonpea insect pests is a challenge to plant breeders, to breed for tolerance to all these insect pests. Currently, a few pest tolerant cultivars have been developed in India but are susceptible to wilt (Singh et al., 1990).

Other production constraints are of minor importance, but they range from production factors to climatic conditions. These are abiotic constraints such as soil pH lower than 5.0 or higher than pH 8.0, poor nutrient status of the soil, drought stress, conditions conducive to water logging, and low light intensity (Chauhan, 1990). It is thus imperative to breed for specific environmental conditions. Work is underway at ICRISAT in Kenya on breeding pigeonpea for specific environments such as high latitude areas (ICRISAT, 2007).

1.6 Fusarium wilt disease

Fusarium wilt, caused by *F. udum* Butler*,* is the most important soil borne disease of pigeonpea, and has been reported to be the major cause for the declining trend in pigeonpea production in Malawi (Subrahmanyam et al., 1992; Gwata et al., 2006). The disease can appear in young seedlings but the highest mortality occurs at the flowering and podding stages. Even if the disease appears in patches in the early years, it can extend to the entire field if pigeonpea is grown continuously in the same field year after year. The wilt incidence increases when the crop is ratooned or retained as a perennial

(Reddy et al., 1990). If branches or the whole plant wilt, seed does not form, resulting in total crop failure. In some situations, farmers have either to abandon pigeonpea production or rent a piece of disease-free land elsewhere for pigeonpea cultivation.

Fusarium wilt disease was first described in 1906 in Bihar state, India, and has been reported in 15 countries, including Malawi, but it is more important in India and Eastern Africa (Reddy et al., 1990). In Malawi, yield losses of more than 50% have been reported in Thyolo and Mulanje districts (Soko, 1992). The annual pigeonpea crop losses due to wilt in Eastern Africa has been estimated at US $5 million, while in India the annual crop loss was estimated at US $36 million (Kannaiyan et al., 1984). This is a very significant loss that could be reduced by the use of resistant varieties in integrated disease management programme.

1.6.1 Disease symptoms

The initial visible symptoms of Fusarium wilt disease are the loss of turgidity in leaves (drooping) and slight interveinal chlorosis (Figure 1.1). The foliage shows slight chlorosis, and sometimes becomes bright yellow before wilting. Leaves are retained on wilted plants. The initial internal characteristic symptom of wilt is the browning of the xylem vessels from the root system to the stems. The xylem gradually develops black streaks, and brown or black purple bands appear on the stem surface of partially wilted plants, extending upwards from the base. When the bark of such bands is peeled off, browning or blackening of the wood beneath can be seen (Reddy et al., 1990). When young (1-2 mo old) plants die from wilt, they usually do not show external banding (purple band symptom), but have obvious internal browning or blackening (Reddy et al., 1998) (Figure 1.2). Wilt is caused by both physical blockage of the xylem by the fungus and the production of toxins (Parry, 1993).

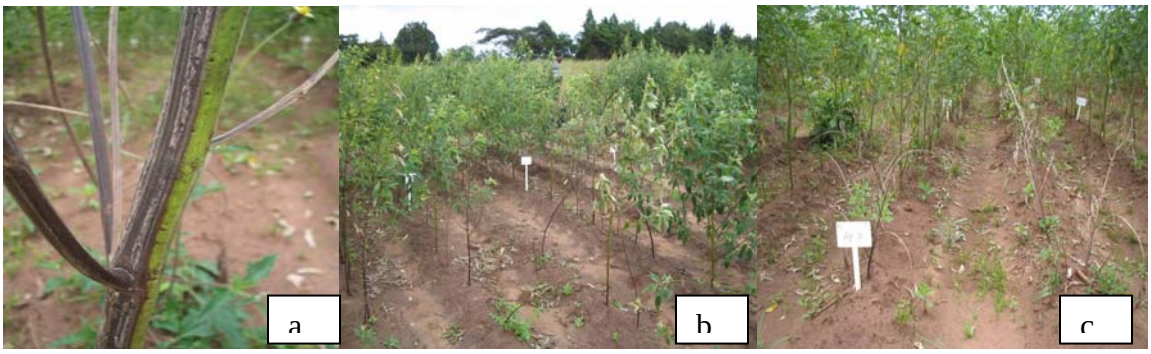

Figure 1.1: Symptoms of Fusarium wilt in big plants in the field: a) dried stem on one side; b) wilting plants, and c) dried plants and field damage

Figure 1.2: Symptoms of Fusarium wilt in seedlings: a) yellowing of a leaf on one side; b) yellowing of leaves on one side of the plant; c) death of the seedling while green

1.6.2 Disease epidemiology

The disease is not only more prevalent in early planted than late-planted crops, but is also more prevalent in irrigated than rain-fed crops (Chaudhary et al., 2001). Early sowing, weed management, and vigorous crop growth favour wilt development (Reddy et al., 1998) because of less intense competition while the pathogen concentrates on one host. More wilt inocula are found in sand (94%) than in heavy black soil (18%), and the fungal population is found to be highest at 30% soil water-holding capacity and at soil temperatures between 20 and 30^0 C (Reddy et al., 1990). Recent work has indicated that the fungus can survive in the soil up to 120 cm in depth (Reddy et al., 1998). Root-knot nematode (*Meloidogyne* spp) infection increases wilt-incidence in both wilt-susceptible and wilt-resistant pigeonpea cultivars, while cyst nematodes (*Heterodera cajani* Koshy) enhance the pathogenicity of *F. udum* in wilt-susceptible genotypes (Reddy et al., 1990; Reddy et al., 1998). This knowledge helps understand the integrated pest and disease management (IPM) strategies that can be used either to prevent or control the disease.

1.6.3 The fungus *Fusarium udum* (Butler)

Fusarium udum is a soilborne fungus. It survives in the tissues that it colonises as a parasite, or in the soil as a saprophyte. It spreads about 3 m through the soil in one season, apparently along roots (Allen, 1983; Reddy et al., 1990). It survives as spores, hyphae, and chlamydospores, and it spreads through wind and running water during rain (erosion). The fungus can survive in infected plant stubble for 2.5 yr in vertisols, and 3 yr in alfisols. The perfect state, or the sexual stage of *F. udum,* is reported to be *Gibberella indica* (Reddy et al., 1990), but not much work has been done on its sexual stage, and little effort has been done on characterizing the perithecia, asci, or ascospores of the species of this genus (Samuels et al., 2001). Sexual reproduction, especially of the heterozygotic conidia or mycelia, can bring about a great deal of variability (Agrios, 2005). Several workers have reported the occurrence of physiologic races of *F. udum* (Gupta et al., 1988; Reddy and Raju, 1993; Bakshia et al., 2001; Mudhukeshwara and Seshadri, 2001; Kiprop et al., 2002; Sivaramckrishnan et al., 2002). The physiologic races are characterized by increased virulence or change in cultural and morphological characteristics. Figure 1.3 shows the colonies of *F. udum* growing on PDA media. With increased virulence, the pathogens are able to cause disease in resistant cultivars.

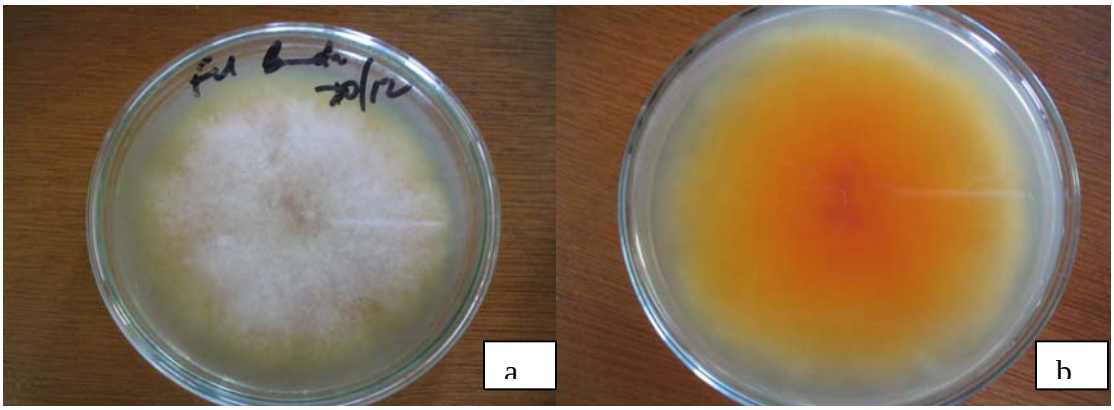

Figure 1.3: Colonies of *Fusarium udum* growing on Potato Dextrose Agar: a) front; and b) back of the Petri dishes

Variability in fungus can be caused by sexual genetic changes. This is true for fungi which produce ascospores, oospores, and basidiospores. In the sexual cycle, variation can be caused by segregation and recombination of genes during meiotic division of the zygote as a result of genetic crossovers. In genetic crossover, parts of chromatids (and the genes they carry) of one chromosome in a pair, are exchanged with parts of

chromatids of the other chromosome (Agrios, 2005). Variability is also caused by mutation, which is a change in the genetic material of an organism, which is then transferred in a hereditary fashion to the progeny. Mutation occurs spontaneously in nature in all living organisms (Borojevic, 1990; Agrios, 2005). Variability can also be caused by heterokaryosis, where cells of the fungal hyphae, or parts of hyphae contain two or more nuclei that are genetically different as a result of fertilization or anastomosis. Anastomosis is the union of hypha with another, resulting in intercommunication of their genetic material (Agrios, 2005). The process of parasexualism can also bring about variability where recombinations occur within fungal heterokaryons. This comes about by the occasional fusion of the two nuclei and the formation of a diploid nucleus, which cannot revert to its haploid state. The level of the genetic variability through parasexualism may equal or surpass that brought about by sexual reproduction. Heteroploidy can also bring about variability. This is the existence of cells, tissues, or whole organisms, with numbers of chromosomes per nucleus that are different from the normal 1n or 2n complement for the particular organism (Agrios, 2005). The pathogenic variability calls for more work on breeding for resistance, and other disease management strategies, to keep pace with ever-changing pathogens. Work on breeding for resistance should also include all the known and unknown races of the particular pathogen to avoid minor races from becoming major ones later.

Variability in *F. udum* may be morphological in its colony characteristics or pathogenic (Allen, 1983). Morphological variability may appear as sectors or patches in the parent colony, loss of aerial mycelium, an increase in macroconidial production, or an increase in pigmentation. Isolates capable of profuse mycelial growth are often less pathogenic than those producing scanty mycelium (Allen, 1983). Pathogenic variability may result in isolates with increased virulence (Puhalla, 1981; Kiprop et al., 2002; Infantino et al., 2006). Multilocation testing of promising materials in the Indian Council of Agricultural Research (ICAR)-ICRISAT uniform trials for pigeonpea wilt resistance, in collaboration with the All India Coordinated Pulse Improvement Project (AICPIP), has confirmed that it is common for genotypes to react differently in different sites, but the extent to which pathogenic variation, rather than other factors, is responsible for the apparent loss of wilt resistance remains unclear (Allen, 1983). The site differential reactions are due to differential cultivars and microbial activities affecting the pathogen. Studies carried out at ICRISAT Centre (Patancheru, India), and multi-locational testing of resistant genotypes

in India, also pointed to the presence of physiological races in *F. udum* (Reddy et al., 1990). However, further standardization of the inoculation technique, differential varieties, and the rating scale, are needed to fully understand the nature of the pathogenic variability present in *F. udum* (Reddy et al., 1990). This implies that if durable resistance has to be achieved, there is a need to first standardize the procedures for the above-mentioned variables.

1.6.4 Disease control measures

Fusarium wilt is a soilborne disease and, as such, any farming practice or cultural operation that reduces the soil population of the pathogen can help to reduce the wilt incidence. These practices include fallowing the fields and rotation with crops such as sorghum, tobacco, or crotolaria (Reddy et al., 1990). Pigeonpea intercropped with castor oil plants, sorghum, maize, and groundnut is less affected by wilt than mono-cropped plants (Reddy et al., 1998). Application of zinc, and heavy application of nitrogen in the form of farmyard manure, have been reported to retard colonization of pigeonpea by *F. udum* and hasten the disappearance of the fungus from the soil (Reddy et al., 1990). It has also been reported that the bacterium, *Bacillus subtilis,* produces the antibiotic, bulboformin, which inhibits the growth of *F. udum,* resulting in reduced incidence of wilt (Allen, 1983). Field fumigation with chemicals, and biological control measures such as the application of *Trichoderma,* have been successful, but they are usually expensive; out of the reach of small-scale farmers. Crop rotation may not work in Malawi due to small landholding size, especially in the southern region, where 98% of the crop is grown (see Chapter 2). Although cultural, chemical and biological control measures exist (Patel et al., 2001; Singh et al., 2002), use of resistant varieties is a practical and economical strategy to manage the disease (Reddy et al., 1990; Reddy et al., 1998; Ruckenbauer et al., 2001), especially for subsistence farmers who cannot afford expensive chemicals. This use can only be achieved through breeding for resistance to the disease. However, use of resistant varieties can only be successful if it is used as an integral part of the whole IPM programme (Infantino et al., 2006).

1.7 Breeding pigeonpea

Breeding pigeonpea is a challenge because the objectives and methods chosen in the breeding programme depend on the nature and magnitude of genetic variation, the reproductive behaviour, usage, adaptation to the environments and cropping systems

involving the crop. High stable yield, with acceptable grain quality, is the major breeding objective. Stable yield is sought by incorporating resistance to biotic stresses such as diseases (wilt, sterility mosaic, phytophthora blight), pests, and abiotic stresses (waterlogging, drought, acidity, and salinity) (Singh et al., 1990). It is essential to breed for a range of resistances to pathogenic organisms in order to reduce the need for chemical controls to a minimum, and thus lower production costs, increase the nutritional value of agricultural products, and improve the environment (Borojevic, 1990). Other objectives have focused on breeding pigeonpea for specific production systems; special traits such as suitability for vegetable products and fodder: high protein content for the animal feed industry, suitability for processing for canning; the milling quality for split peas (dhal); and market preferences (Singh et al., 1990). Breeding programmes in Malawi have focused on high stable yields, fitting into the intercropping system, ratoonability (Sakala, 1992), appropriate maturity period, and market preferences (seed size and colour) (ICRISAT, 2006). Short and extra short-duration, short-statured pigeonpeas, with comparatively low sensitivity to photoperiod and temperature interactions, have been bred by ICRISAT. Medium and long-duration pigeonpeas are principally grown as intercrops with tall cereals (maize, sorghum, and millets), and a variety of other crops. However, selection for competitiveness and high productivity from early generations in mixed cropping systems is not practical (Singh et al., 1990), because selections at early generations are done in pure stands instead of interrows. A participatory rural appraisal was conducted in the current study (see Chapter 2) to identify and set the breeding priorities for Malawi.

1.7.1 Breeding techniques in pigeonpea

Since the beginning of pigeonpea cultivation, farmers have exercised selection for specific traits suitable for their cropping systems and this led to the development of landraces which are still popular today (Singh et al., 1990). Early breeding efforts were aimed at improving yield and acceptability for specific uses and production systems, and selections were made from landraces. Most of the early improvement work in pigeonpea was confined to selection and pedigree evaluation from landraces adapted to the region in which such selection was exercised (Singh et al., 1990).

Diallel and line x tester mating schemes using three or more well-adapted cultivars as testers have been used (Singh et al., 1990). The commonly used breeding methods for a

self-pollinated crop are applicable to pigeonpea, even though a considerable amount of outcrossing occurs in the species. Bulk hybrid advance by single-pod descent, and single-seed descent, have proven successful in breeding for high-yielding lines. Stratified mass selection and mass selection with progeny testing have been tried in Kenya for yield gains in pigeonpea (Singh et al., 1990), and in India to estimate heritability and genetic advance (Singh et al., 2003). Mass selection, selfed progeny selection, and half-sib progeny selection, have been used to estimate genotypic and phenotypic variance, heritability, and genetic advance for some yield traits in pigeonpea (Singh et al., 2003). Singh et al. (1990) suggested recurrent selection and population breeding methods as ways to accumulate desirable genes and facilitate the breaking of linkages in pigeonpea and other self-pollinated species. Pigeonpea has a substantial amount of non-additive genetic variance and hybrid vigour for yield (Singh et al., 1990).

The discovery of stable genetic male sterility, coupled with its outcrossing nature, has opened the possibility of commercial use of the heterosis in pigeonpea, enabling the economic production of hybrid seed. Successful hybrids are produced from those combinations where specific combining ability effects result in considerable heterosis in the F_1 generation. A number of mutants, both induced and spontaneous, for various qualitative characters, have been reported in pigeonpea and a few cultivars have been developed through the use of induced quantitative variability (Singh et al., 1990).

In pigeonpea, and in several self-pollinated species, varietal improvement methods based on pedigree, bulk pedigree, backcross- and multiple-crossing techniques have been useful in recombining simply inherited characters such as disease resistance, seed size and colour, and maturity (Singh et al., 1990). Pedigree selection has been useful in breeding for highly heritable traits such as disease resistance, seed size, seed colour, growth habit, and seed number per pod (Green et al., 1981). There are wide ranges of options to choose from when embarking on breeding in pigeonpea. However, the choice of the best technique depends on the objectives, time involved, and the technical know-how of the breeder.

1.7.2 Breeding for disease resistance in pigeonpea

Fusarium wilt (*F. udum*) is a major disease of pigeonpea. Other important pigeonpea diseases in Africa include Cercospora leaf spot (*Cercospora cajani*) and powdery mildew

(*Oidiopsis taurica*) (Reddy et al., 1990). In India, sterility mosaic and Fusarium wilt were found to be major diseases of pigeonpea. With the availability of effective screening methods, breeding for resistant genotypes started with the identification of resistant germplasm accessions (Gupta et al., 1988; Okiror, 1998; Reddy et al., 1998). Emphasis was put on understanding the inheritance of resistance for each disease: nature and number of genes involved in governing the resistance (Singh et al., 1990). At ICRISAT, the disease resistance breeding programmes have aimed at breeding varieties with multiple disease resistance to Fusarium wilt, sterility mosaic, and phytophthora blight, using a multiple disease screening nursery. Some sources with combined resistance have been identified (Singh et al., 1990).

1.7.3 Breeding for Fusarium wilt resistance in pigeonpea

Breeding for wilt-resistant genotypes started as early as 1906 in India when the Fusarium wilt pathogen was identified (Reddy et al., 1990). However, the results of studies in the mechanism of inheritance of wilt resistance are still contradictory and not fully understood.

Breeding for Fusarium wilt resistance is usually done by pedigree or mass-pedigree selection, although in some cases, backcrossing has also been successful (Singh et al., 1990). Some resistant varieties, which were bred using various methods, include ICP 9145 (Reddy et al., 1995) and ICEAP 00040 (Silim et al., 2005). In India, the resistance in the cultivars is site-specific, depending on the *F. udum* races prevalent in the area.

1.8 Mechanisms of host plant disease resistance

Resistance can be physical or biochemical or a combination of the two (Agrios, 2005). Plants have pre-existing structural characteristics (thick cuticles, waxes on leaves and fruit surfaces, thick cell walls, and late opening of the stomata) that act as physical barriers inhibiting the pathogen from gaining entrance and spreading through the plant. Chemically, plant cells and tissues produce substances (fungitoxic exudates from the leaves, phenolic compounds, tannins, lectins, peroxidase, glucanases, chitinases, and phytoalexins) which either are toxic to the pathogen or create conditions that inhibit the growth of the pathogen in the plant (Chakravorty and Scott, 1991; Agrios, 2005). Fusarium wilt resistant cultivars of pigeonpea produce cajanol (Marley and Hillocks, 1993), chlorogenic acid, caffeic acid, and an unknown phenolic acid (Reddy et al., 1998),

which are known to inhibit the germination and germ tube growth of conidia of *F. udum***.** Breeding for chemical resistance might thus be more advantageous than mechanical resistance because of the inhibitive properties of the chemicals on the pathogen advancement in the host. There are many factors that are responsible for the breakdown of the physical barriers to entry into a host plant, such as bruising of the roots during cultivation and nematode infestation. These create avenues for pathogen entry into the host plant.

Resistance in a host plant can be lost due to the ability of some pathogens to produce chemicals that can detoxify the phytoalexins. For instance, the bean pathogen, *Fusarium solani* f. sp. *phaseoli*, can detoxify at least four of the major phytoalexins of beans, namely kievitone, phaseolin, phaseollidin, and phaseollinisoflavan. Although the genes conferring phytoalexin detoxification are often linked to pathogenicity, there are several fungi which are sensitive to phytoalexins even though they can metabolize them (Chakravorty and Scott, 1991). For durable resistance it would be advisable to breed for both mechanical and chemical resistances.

1.9 Genetics of Fusarium wilt resistance in pigeonpea

Infectious plant diseases are the result of interaction between the host plant and the pathogen. Genetic materials (DNA) govern the properties of each of these two organisms (Agrios, 2005). Studies of the inheritance of resistance versus susceptibility in plants prove that single genes control resistance and their absence allows susceptibility. Studies of the host/pathogen interactions prove that resistant genes in the plant are specific for avirulent genes in the pathogen (Agrios, 2005).

Under favourable environmental conditions, therefore, the outcome – infection (susceptibility) or noninfection (resistance) – in each host/pathogen combination is predetermined by the genetic material of the host and of the pathogen. The number of genes determining resistance or susceptibility varies from plant to plant. However, in most host/pathogen combinations, the number of genes involved, and what they control, is not yet known (Agrios, 2005). Therefore, understanding the genetic systems operating in a given host/plant environment is the basis for disease management.

More than 210 pigeonpea diseases have been documented (Nene et al., 1996). Fusarium wilt, sterility mosaic, and phytophthora blight (in descending order of importance) are the most important diseases that cause serious economic losses (Saxena and Sharma, 1990). Current studies on the genetics of disease resistance in pigeonpea are limited.

Several scientists have reported that resistance to wilt in pigeonpea is controlled by a varying number of genes, starting from multiple factors to two complimentary genes to a single dominant gene. Sharma (1986) (cited by Saxena and Sharma, 1990) confirmed the dominance of resistance over susceptibility, and, in one trial, suggested that resistant parents had major genes for wilt while susceptible parents had minor or polygenes for wilt resistance. This type of genetic system is likely to influence the proportion of resistant and susceptible plants in segregating populations in a random fashion, and consequently complicates the genetic ratios and their interpretations.

Shaw (1936), as reported by Reddy et al. (1990b), observed a segregating ratio of 9:7 (2 complementary genes) in the F_2 generation of a cross between T5 and T80 with the resistant parent being dominant. Reddy et al. (1990) cited a report by IARI (1946) which reported that in the mutant, “Cawnpore”, duplicate genes were found to govern resistance. Joshi (1957) (as cited by Reddy et al., 1990) suggested that a pair of dominant duplicate genes governed wilt resistance. Pawar and Mayee (1986) (cited by Reddy et al., 1990) reported that resistance in 15-3-3 and C11 was dominant over susceptibility. Clearly, there is a need to develop a better understanding of the inheritance of resistance, particularly in view of the fact that genotypes show different levels of resistance under field conditions (Reddy et al., 1990).

1.10 Screening techniques of pigeonpea for Fusarium wilt disease

Pigeonpea cultivars are screened for their resistances to Fusarium wilt through natural or artificial inoculation (Infantino et al., 2006). The basic objective of the screening exercise is to test whether the pigeonpea cultivars are resistant or susceptible to the available races of *F. udum* prevalent in an area.

A natural screening process relies on planting various pigeonpea cultivars in sick plots. The susceptible cultivars can succumb to the disease at different growth stages, while

the resistant cultivars remain healthy during the entire growing season. However, this technique has its shortfalls. The distribution of the pathogen in the soil is not even and this leads to disease escape in some cultivars. Secondly, the expression of the disease varies from season to season, depending on the environmental conditions in the soil such as moisture content, soil temperature and soil structure (Burgess et al., 1994).

Due to these shortfalls in the natural screening process, scientists have reverted to an artificial inoculation method as a way of ensuring the success of the screening. In this technique, pigeonpeas are inoculated with a known isolate of *F. udum* at a given concentration. ICRISAT (1986) reported the use of root-dip and transplanting methods to inoculate seven-day-old seedlings of pigeonpea with *F. udum*. Reddy and Raju (1993) reported the inoculum concentration of 1×10^6 colony forming units (cfu) ml^{-1} in sterile distilled water as ideal for inoculations. The seedlings are raised in sterile sandy soils and are stressed by bruising the roots during inoculations. The bruised roots are dipped in the inoculum for one minute and transplanted into pots containing sterile soil (ICRISAT, 1986).

Burgess et al. (1994) and Tuite (1969) reported the use of infested soils with a known concentration of propagules for inoculations. This is similar to the natural method or sick plot technique, but the difference is that this method uses a known inoculum concentration. Lindell et al. (1986) reported the use of wheat, barley, oats, and corn chaff (with or without grain) as suitable substrates for *F. graminearum, F. culmorum, F. avenaceum and F. moniliforme*. The chaff is spread evenly in a known amount of soil in pots. The seedlings are then planted into the “sick” soil. For root parasitizing and wilt organisms, use of infested sterile soil with pure culture in inoculations could work. However, sterile soil may not be suitable because of the exclusion of the microflora as the most important factor of a soil environment (Tuite, 1969). For instance, the positive interaction between Fusarium wilt and nematode infestation increases the severity of wilt. For soilborne pathogens, the following experimental elements should be considered: seedlings instead of older plants; non-sterile soil free of pathogens; glass or other moisture proof containers; and controlled soil moisture and soil and air temperature (Burgess et al., 1994).

Artificial inoculation methods have some potential problems. In using 1 x 10^6 cfu ml^{-1}, it is not certain whether the entire colony (spore) forming units will germinate and be infectious. Okiror (1998) reported a late start of wilting where pigeonpea seeds were soaked in a spore suspension, showing slow germination of the spores. Dilution of the inoculum concentration to the required level is difficult to achieve. It requires special skills in the use of a haemacytometer. In spite of the method (rotary shaker or chaff) used to multiply the inoculum, the fungus develops various forms of reproductive propagules (micro- or macroconidia, chlamydospores and hyphae) (Tuite, 1969), which would make it difficult to predict whether they will all germinate and infect the plants.

All the methods used in screening pigeonpea for wilt reaction, so far, require the application of stress to the inoculated plants. The stress comes in the form of bruising the roots, reducing the amount of water, and exposing the plants to high temperatures (Burgess et al., 1994). The stress mechanisms are aimed at allowing invasion and colonisation to take place or to make the plant more vulnerable to attack. Chapter 4 describes the screening method that was used (developed) in the present study that took into account the shortfalls outlined above.

1.11 Marker-assisted selection of host plant resistance

Molecular marker-assisted selection (MAS) can be used in advancing the progenies from one filial generation to the other in the absence of the disease, thereby reducing the need for field evaluation (Sagers et al., 1994; Miklas et al., 2006). Many markers have been developed to assist in the selection of genotypes for resistance to Fusarium wilt in chickpeas, field peas, and beans (Fall et al., 2001; Sharma et al., 2004; Okubara et al., 2005; Millan et al., 2006). In one experiment, Njiti and Lightfoot (2006) used marker-assisted selection in selecting soybean seedlings for resistance to Fusarium wilt in the greenhouse. Bell-Johnson et al. (1998) were able to select against some soybean parental materials with deleterious phenotypes through the use of satellite markers. Quantitative trait loci (QTLs) were used to select maize genotypes with resistance to *F. moniliforme* (Fan et al., 2007). Therefore, the use of molecular marker assisted selection is quite possible in pigeonpea breeding and can assist in advancing the filial generations in the Fusarium wilt-breeding programme. However, very little work, if any, has been done on the use of MAS in pigeonpea breeding.

1.12 Summary

A great deal of research has been done on pigeonpea and its production constraints. Pigeonpea is the only cultivated food crop of the Cajaninae sub-tribe of the Phaseoleae. It belongs to the primary gene pool. This is disadvantageous to the crop's improvement because it is so distinct that it is incompatible with all *Cajanus* species in the secondary and tertiary gene pools. However, there are many desirable traits in the secondary and tertiary gene pools that can be transferred into cultivated pigeonpeas. Such traits include disease resistance to wilt, annuality, high pod set, and early maturity. Any crossing between species can only occur through modern biotechnology techniques such as embryo rescue, chromosome doubling and protoplast fusion. All these techniques require sophisticated and expensive equipment that cannot easily be found. Future research should focus on how useful genes in the secondary and tertiary gene pools can be introgressed into *C. cajan*.

Pigeonpea has many uses but the obvious ones are that it provides a cheap source of valuable protein and a good source of firewood. Pigeonpea production in Malawi is affected by many factors but Fusarium wilt disease (*F*. *udum*) is the most important constraint. It can cause up to 100% yield loss in pigeonpea especially in susceptible cultivars. It attacks pigeonpea plants at all growth stages. Breeding for Fusarium wilt resistance is the best approach towards solving the problem. However, the two resistant varieties that have been released in Malawi have not been widely adopted by farmers; probably because of a lack of seed and desirable traits that farmers prefer.

Pigeonpea breeding for resistance to Fusarium wilt can be enhanced if proper disease-screening techniques are developed, this will speed up the advancement of the filial generations under artificial inoculations. Most of the screening techniques in use now have shortfalls. Future research should focus on developing other simpler screening methods. There is also the need to explore biotechnology techniques in pigeonpea breeding such as marker-assisted selection (MAS) and quantitative trait loci (QTL) to introgress desirable genes from the other gene pools to improve yield, quality and resistance to diseases and pests. However, most of these biotechnology approaches have limited use because not much has been achieved so far in their applications. Therefore, researchers still have much work to do in the genetic improvement of pigeonpea using conventional breeding techniques.

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Chapter 2: Participatory study of pigeonpea farming system, landraces, and the preferred traits for consumption and marketing in Malawi

Abstract

Despite the release of two high yielding and Fusarium wilt resistant varieties, most of the smallholder farmers in Malawi still grow local landraces, which are low yielding due to their susceptibility to Fusarium wilt disease. A survey was conducted in the southern region of Malawi to evaluate farmers' perceptions of the disease; to assess pigeonpea production and marketing constraints; to establish why local landraces are preferred; and to identify landraces with desirable traits that can be used in the genetic improvement of the crop. A participatory rural appraisal was used to identify production and marketing constraints as well as traits that farmers value in local landraces. Farmer- and buyer-participatory variety selection was used to identify local landraces with desirable traits. These landraces were to be used in the pigeonpea improvement programme to develop genotypes that are not only resistant to Fusarium wilt disease, but also maintain desirable traits. Ten farmers in each of the six major pigeonpea growing districts participated in the study. The results of the survey showed that farmers perceive Fusarium wilt as the main problem in pigeonpea production. Despite the fact that most of the local landraces were susceptible to Fusarium wilt, they accounted for 84% of the total pigeonpea production in Malawi. Most farmers do not deploy any control measures due to small land holding size. The majority of the farmers recycled seed or shared it among themselves to maintain the desirable traits. Other pigeonpea production constraints included pests, low soil fertility, flower abortion, and weather factors. Pigeonpea is mainly consumed as cooked dry seeds, and forms part of the ration for the workers in large-scale estates. Traders export pigeonpea as dhal or whole grain seeds to Asia, Europe and South Africa. Farmers prefer local landraces for such traits as fast cooking, taste, large seed size, and high prices. The trade, on the other hand, prefers large white seeded varieties for ease of processing into dhal and/or whole grain export markets. There is a need to improve the resistance in the local landraces to Fusarium wilt, while maintaining the desirable traits. Farmers and buyers selected ten local landraces, coded AP1 - AP10, from a collection of 43 germplasm accessions that were used as parental materials in the breeding programme.

2.1 Introduction

Pigeonpea (*Cajanus cajan* (L.) Millsphaugh) has a potential yield of over 2 t ha^{-1}. However, the average yields have stagnated to about 600 – 700 kg ha^{-1} worldwide (Chauhan, 1990; Ministry of Agriculture and Food Security, 2007). This is a result of many constraints that limit pigeonpea production, not only in Malawi, but worldwide. Such constraints include diseases and pests, poor cropping systems, lack of improved varieties, small landholding sizes, erratic climatic conditions, poor soil conditions, and lack of crop nutrition.

Participatory rural appraisal (PRA) has been used extensively in the identification of the production constraints of many crops (Debrah et al*.,* 1998; Tenywa et al., 1999; Ebregt et al*.,* 2004; Erkossa et al., 2004; Singh, 2004; Dorward et al., 2007). In addition, plant breeders have taken advantage of PRA methods and participatory plant breeding (PPB) to understand the desirable traits that farmers prefer. Participatory plant breeding can be defined as a collaborative process, where professional plant breeders and farmers share decisions in several steps of the plant breeding cycle (Ceccarelli et al., 2003). Farmers' participation in technology development is an important factor increasing the probability of success of the technology (Ceccarelli et al., 2003). Farmers can be involved at various stages of technology development: participatory crop improvement (PCI), participatory variety selection (PVS), and participatory plant breeding (PPB). The PCI is the involvement of farmers in all stages of the breeding process. Participatory variety selection contributes to the identification of materials that have traits that correspond to farmers' preferences (Danial et al., 2007). Participatory variety selection is a logical phase before PPB. It is selection of specific varieties or lines from amongst advanced or genetically stable populations and lines, while PPB is the selection within a segregating population. In the PVS phase, important attention is paid to characters other than yield that are typically of importance to small-scale farmers, such as seed colour, taste, cooking time and secondary uses. Identification and selection of materials through farmers' collaboration will presumably increase the adoption rates of those varieties (Almekinders and Elings, 2001; Ceccarelli et al., 2003; Danial et al., 2007).

One of the rationales for engaging in PPB is to address traits that are not being effectively selected for in traditional, centralised breeding programmes (Smith et al., 2001). Through farmers' participation, breeders realize how important and diverse

farmers' preferences are to the whole breeding process (Danial et al., 2007). In recent years, there has been an increasing consensus that users' participation in technology development is an important factor in increasing the probability of success for the technology (Ceccarelli et al., 2003). Without the close participation of the farmers, local crop improvement programmes are likely to be unsuccessful (Elings et al., 2001). Participatory crop improvement aims at more effectively addressing the needs of farmers in marginal areas in developing countries (Almekinders and Elings, 2001; Smith et al., 2001; McElhinny et al., 2007), because it defines selection criteria that are important to the local community (Almekinders and Elings, 2001; Elings et al., 2001). In some experiments, results showed that breeders' and farmers' selection differed for a number of agronomic traits, depending on whether the selection was done on the station or farmers' fields (Ceccarelli et al., 2001; McElhinny et al., 2007). Small-scale farmers have been growing local landraces for a long time and, hence, they can be involved in deriving adapted lines for use in breeding programmes (Singh et al., 1990). Much success, in terms of genetic improvement and adoption, has been reported where local landraces have been used as parental materials in the breeding programme (Sharma and Duveiller, 2006; Danial et al., 2007). Local varieties remain, in most situations, the primary source of germplasm for the majority of the small-scale farmers (Almekinders and Elings, 2001; Manu-Aduening et al., 2006).

Pigeonpea is grown in a wide range of cropping systems in Malawi, the most common being intercropping or mixed cropping with maize, sorghum and cassava (Sakala, 1992; Gwata et al., 2006). Intercropping results in a low plant population, which affects yields. The lack of availability of improved genotypes has been cited as one of the reasons for low yields (Chauhan, 1990). Most of the pigeonpea production in the country comes from the use of local landraces. The majority of small-scale farmers in developing countries, including Malawi, recycle seed saved from the previous harvest, or may use seeds locally procured from family members and friends. Certified seed is not available for the farmers. Local landraces of pigeonpea, cowpeas, finger millet, and pumpkins, are the primary source of germplasm for the majority of small-scale farmers (Almekinders and Elings, 2001). The selection of the recycled seed is often based on post harvest quality traits. Therefore, it is important that farmers are involved in the crop improvement strategies at all stages.

A survey was therefore conducted among pigeonpea farmers in the country to investigate the reasons for low pigeonpea production. The PRA was used to identify production constraints, while PVS was used to select local landraces with desirable attributes to the farmers and consumers. These would be the parent materials that could be used to breed new pigeonpea varieties. Therefore, the objectives of the PRA were to:

1. Evaluate farmers' perceptions of Fusarium wilt disease in all major pigeonpea growing areas;
2. Assess pigeonpea production and marketing constraints;
3. Assess why local landraces are preferred over released varieties and identify farmer-preferred traits; and
4. Identify and collect local pigeonpea landraces with desirable attributes that could be used in a breeding programme to develop new Fusarium wilt resistant varieties acceptable to end users.

2.2 Materials and methods

2.2.1 Description of the study area

The survey and germplasm collection were both conducted in the southern region of Malawi. The region is densely populated, with approximately 146 people km^{-2} (National Statistics Office, 1998), with an average farming family of five people (National Statistics Office, 2005). Pigeonpea forms part of the cropping system. The crop is regarded as a minor crop and is intercropped with major crops such as maize, cassava, and sorghum. The crop is grown under diverse environmental conditions, from hot to cool areas, with altitude ranging from as low as 52 m above sea level (masl) along the Shire River, to as high as 1190 masl in hilly areas. Fusarium wilt is observed in almost all the fields with varying incidence levels.

2.2.2 Survey of pigeonpea production and marketing

In August-September of 2005, a survey was conducted in the southern region of Malawi because the region produces most of the nation's pigeonpea. Using a purposive sampling procedure (Bailey, 1978; Peil, 1982; Kerlinger, 1985), a list of six major pigeonpea-growing districts was drawn up, namely, Balaka, Phalombe, Machinga, Mangochi, Mulanje, and Nsanje. One extension planning area (EPA) $district^{-1}$ was

randomly chosen, for interviewing farmers (Salant and Dillman, 1994; Sapsford, 1999). An EPA is the smallest agricultural unit in a district. Ten farmers were selected EPA^{-1}, using a systematic sampling method. Farmers, every 5 km along accessible roads, were interviewed (Alreck and Settle, 1995; Sapsford, 1999). The survey was done in liaison with the plant pathology section of Bvumbwe Research Station of the Department of Agricultural Research and Technical Services, as well as the Department of Extension of the Ministry of Agriculture and Food Security (MOAFS). The survey team consisted of the principal researcher (plant breeder), the plant pathologist from MOAFS, and an extension agent from the EPA. Three separate questionnaires (Peil, 1982; Salant and Dillman, 1994) were designed to capture information from the different stakeholders, small-scale farmers, buyers and large-scale farmers (estates). A total of 62 farmers, (24 men and 38 women), were interviewed. The location of each farmer was recorded using a geographical positioning system (GPS) (Appendix 2.1). A structured questionnaire served as a guide to obtain consistent information on the problem of Fusarium wilt, pigeonpea production and usage.

Ten pigeonpea buyers, based in Blantyre, were interviewed to assess pigeonpea marketing constraints. The buyers were selected using the cluster sampling method (Peil, 1982; Kerlinger, 1985). All the ten buying companies were interviewed. Marketing managers or owners of the pigeonpea buying companies were interviewed on market preferences, limitations, and processing of pigeonpea.

Fourteen estates, where pigeonpea is used as part of the ration for the workers, were selected using a simple random sampling method (Gupta and Saxena, 2002; Sharma and Kumar, 2003). The questionnaire focussed on pigeonpea production, marketing, and use at estate level.

2.2.3 Pigeonpea germplasm collection

Germplasm collection was done in November 2004, just before the onset of the rains. The survey was done in the six major pigeonpea growing districts of Chikwawa, Chiradzulu, Mwanza, Nsanje, Thyolo, and Zomba. The districts were drawn up using a simple random sampling procedure (Bailey, 1978; Peil, 1982; Kerlinger, 1985). Ten farmers were targeted, using a systematic sampling method, where one farmer was interviewed (Figure 2.1) every 5 km along accessible roads. In total, 61 farmers (both

men and women selected at random) were interviewed. The location of each farmer was properly recorded, using a GPS. The germplasm collection team remained the same survey team as described in Section 2.2.2.

Figure 2.1: Interviewing a pigeonpea farmer in Mwanza district, Malawi

The germplasm collection involved designing guiding questions which involved some agronomic and pathology aspects of the landraces which could not be captured at that time of the year through a field visit. Information on the reaction of the cultivar to Fusarium wilt was needed to establish the reaction of the landrace, and also to make sure that already released varieties such as Sauma (ICP9145) and Kachangu (ICEAP00040) were not collected. At least 0.5 kg of seed was collected from the farmers during the survey. The seed (germplasm) was carefully labelled with a district code, germplasm number, and coordinates from the GPS. To avoid mixtures, no pigeonpea seed was bought from the market. Forty-three (43) pigeonpea landraces (germplasm) were collected from the 61 farmers. The germplasm was categorised into 15 groups by the PRA team, based on seed size and colour.

2.2.4 Farmers' and buyers' participatory variety selection (PVS)

Farmers were also involved in the variety selection following the germplasm collection using a spatial sampling method (Bailey, 1978; Peil, 1982; Kerlinger, 1985; Alreck and Settle, 1995). Focus groups comprising 30 farmers EPA^{-1} (Peil, 1982; Kerlinger, 1985; Alreck and Settle, 1995) (Figure 2.2), were asked to rank the germplasm groups from the best to the worst and to select the best ten landraces from the collection, on a scale

of one to ten, giving reasons for their choices (Lourette and Smale, 2000). These landraces were coded AP, meaning ARET Pigeonpea. ARET stands for Agricultural Research and Extension Trust, the author's employer. This exercise was repeated with farmers at three different areas, Dwale EPA in Thyolo, Dzaone EPA in Zomba and Mombezi EPA in Chiradzulu. Eighty-nine farmers recorded their data on a simple data sheet.

Figure 2.2: Some members of a focus group at the Extension Planning Area for participatory variety selection

Buyers play a crucial role in the marketing of pigeonpea, hence their involvement in the variety selection. Marketing managers or owners of buying companies (Section 2.2) were also interviewed on market preferences. They were requested to choose the best 10 landraces from the 15 germplasm groups collected from the farmers, giving reasons for their selections. The exercise thus compared and contrasted the preferences of the farmers and the trade. The guiding questions also looked into aspects of pigeonpea processing and marketing.

2.2.5 Data analysis

The statistical package for social scientists (SPSS) computer programme was used to analyse the data from the survey on pigeonpea production and marketing and germplasm collection. Cross-tabulations were used in the analysis and the percentages

of the respondents were calculated. Frequencies attached to the ranking of each local landrace (germplasm group), during farmer participatory variety selection, were recorded. Ten landraces, with the best frequencies, were selected for the breeding programme.

2.3 Results

2.3.1 Pigeonpea cropping system

The results of the survey showed that 69% of the farmers in the southern region of Malawi have a landholding size of less than 0.8 ha family^{-1} (Table 2.1). It is also clear from the same table that the area (hectares) under pigeonpea production is relatively small because the majority (42%) of the farmers have less than 0.2 ha under pigeonpeas. Some farmers even go to the extent of borrowing land to increase maize and pigeonpea production. Due to the small landholding size, the majority of the farmers (82%) produce less than 100 kg year^{-1} of pigeonpea (Figure 2.3).

Table 2.1: Land holding size per farming family and area under pigeonpea production in southern region of Malawi in 2005

Land holding size (ha)	% respondents	Area under pigeonpea (ha)	% respondents
0 - 0.4	29	0.1 – 0.2	42
0.4 - 0.8	40	0.2 - 0.4	18
0.8 - 1.2	19	0.4 - 0.8	19
1.2 - 1.6	2	0.8 - 1.6	13
>2	10	> 1.6	8

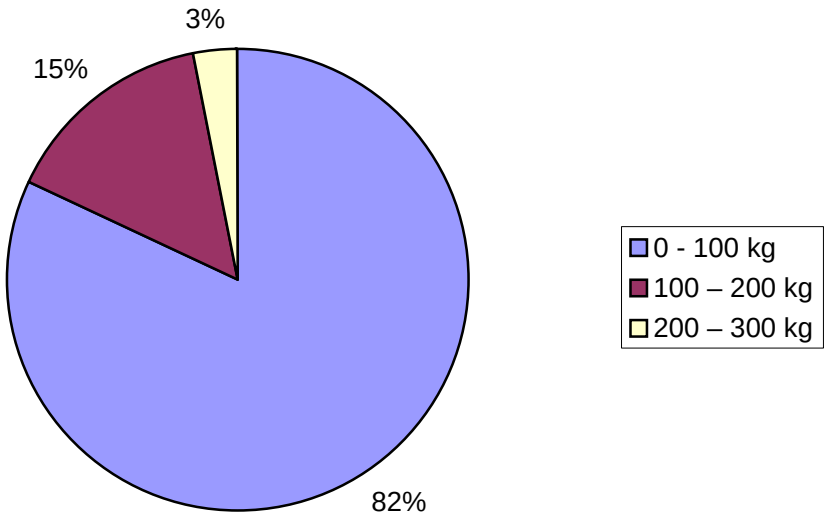

Figure 2.3: Pigeonpea production per farming family in southern region of Malawi in 2005

The bulk (84%) of the pigeonpea crop produced in Malawi is grown under a mixed cropping system. Only a few farmers practise sole, strip, and alley cropping systems to demarcate boundaries or to separate one crop field from the other (Figure 2.4).

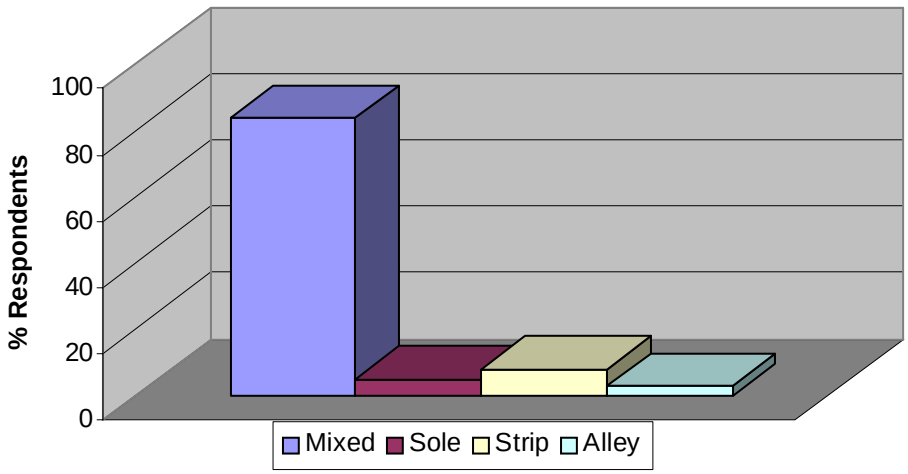

Figure 2.4: Pigeonpea cropping system in southern region of Malawi in 2005

Local landraces dominate the production of pigeonpea because 84% of the farmers use local landraces, while a small proportion of the farmers grow released varieties (Table

2.2). The landraces vary in seed colour and size (Figure 2.5). The results also show that most of the farmers grow late maturing cultivars, with no farmer growing short duration cultivars (Table 2.2).

Table 2.2: Local name given to the variety and maturation period of pigeonpea local landraces in southern region of Malawi in 2005

Variety name	% respondents	Maturation period	% respondents
Local	84	Medium maturing	36
Released	16	Late maturing	64

It was observed that farmers give various vernacular names to these local landraces depending on the characteristics of the germplasm – be it early maturing, taste or seed colour. The names include *Wamakolo* or *Nandolo* (simply local landrace), White, Hybrid or Research (released varieties), *Mthawa June* (matures before winter in June – medium maturing), *Nazombe* (mixed white and brown colour), *Ndewelewe* (ten seeds pod^{-1}), Chinese (from China), *Chilinga* (red in colour), *41* (named after an early maturing maize variety) and *Cham'masala* (does well in old farms with low fertility).

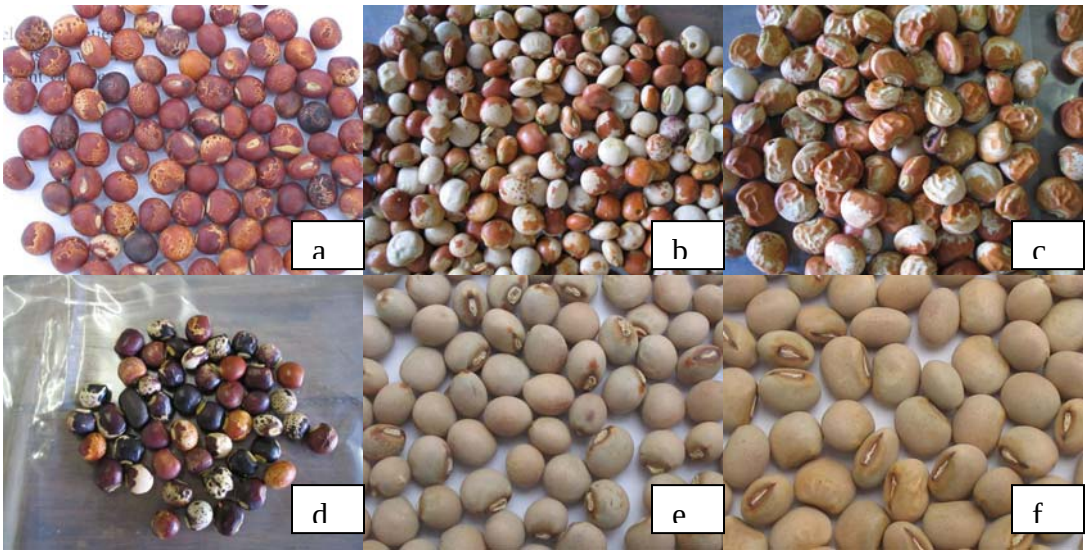

Figure 2.5: Seed colour variation among the pigeonpea germplasm collected in southern region of Malawi in 2004: a) Red mottled – AP8; b) Cream and brown – AP6; c) Cream and brown – AP9; d) Speckled, mottled, black and white - AP15; and released varieties: cream - e) ICP9145; and f) ICEAP00040

A high proportion of the farmers either bought seed for planting from the market (37.7%) or planted their own seed (34.4%). The Government and non-governmental

organisations (NGOs) (11.5%) also played a role in the distribution of seed for the improved varieties. The rest of the farmers obtained seed from their friends or relatives (Figure 2.6).

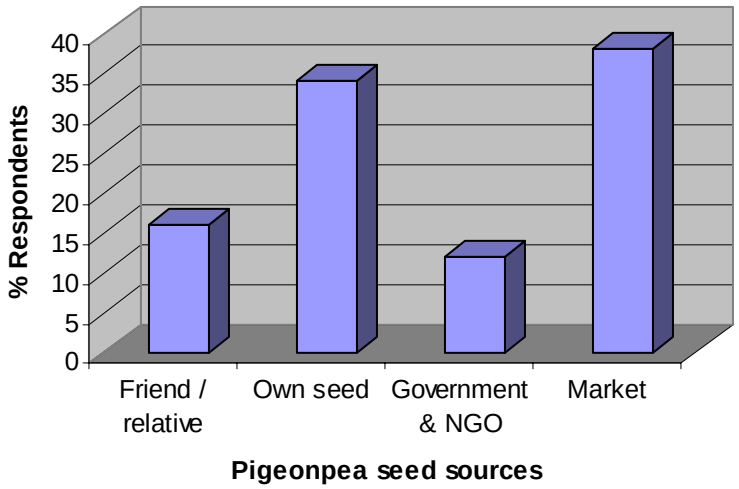

Figure 2.6: Sources of pigeonpea seed in southern region of Malawi in 2004

The results also indicated that most of the farmers (57%) had not changed their pigeonpea variety for more than five years. About 7% of the farmers had not changed seed for four to five years, while the rest had been growing the same pigeonpea variety for less than three years. The majority of the farmers (67%) selected their seed for the next planting from their seed lot after harvesting (Table 2.3). A few farmers selected their seed from the field by looking at yield attributes (number of branches, number of pods branch^{-1} or number of seeds pod^{-1}) (Figure 2.7).

Table 2.3: Production period under the same variety and farmers' seed selection criteria in southern region of Malawi in 2004

Production period under the same variety (yr)	% respondents	Criteria for seed selection	% respondents
1 - 3	36	Strong plant	15
4 – 5	7	Harvested seed lot	67
> 5	57	Others	18

Figure 2.7: Pigeonpea plant showing extensive podding at a farmer's field in Machinga district in Malawi

The results indicated that 34% of the farmers did not store pigeonpea (Figure 2.8). However, out of the 66% of the farmers who managed to harvest their pigeonpea, many farmers used chemicals and ash to protect their stored pigeonpea against weevils. A few farmers stored the pigeonpea as unwinnowed (Figure 2.9) or hung their pigeonpea in a bag by their cooking fire.

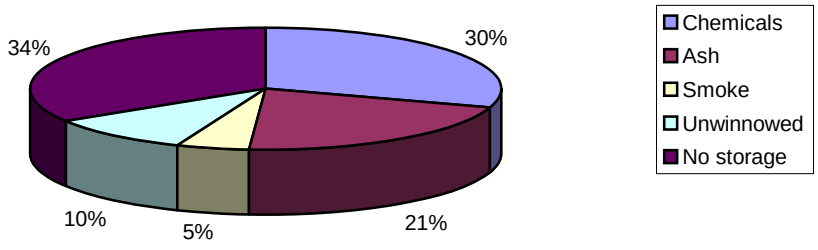

Figure 2.8: Framer techniques of pigeonpea seed storage in southern region of Malawi

Figure 2.9: Seed stored in crushed pigeonpea leaves

2.3.2 Pigeonpea uses and marketing

Pigeonpea was mainly (74%) consumed as cooked dry seeds, while a few consumers cooked pigeonpea as fresh green seed (Figure 2.10). Farmers reported that fresh green seeds are consumed in four forms: peeled green seeds cooked as beans; cooked mashed green seeds (*chipere* – Chewa language); unpeeled pods cooked and eaten as a snack between meals; and peeled pods mixed with dried cassava and cooked (*makata* – Chewa language).

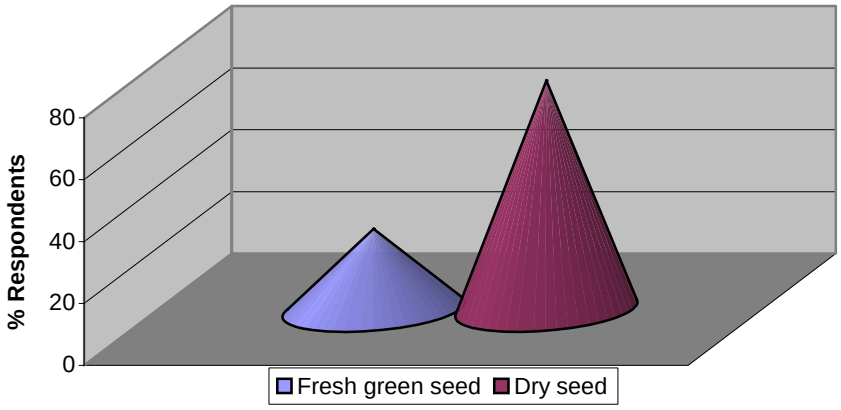

Figure 2.10: Forms of pigeonpea consumption in Malawi

A high proportion of the farmers interviewed ate pigeonpea as part of their ration every other day, once a week, or twice a week. A few farmers ate pigeonpea once fortnightly (3%) or monthly, the rest eat pigeonpea daily (Figure 2.11).

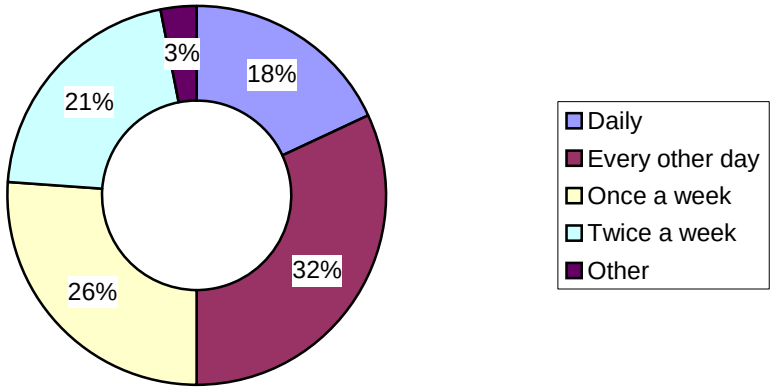

Figure 2.11: Frequency of pigeonpea consumption at household level in southern region of Malawi in 2005.

Due to low pigeonpea production in 2004, 66% of the farmers did not sell their pigeonpea; they kept all that they harvested for food and seed for the next growing season. The rest kept varying quantities of what they harvested, while only 3% of the farmers harvested nothing due to drought (Figure 2.12).

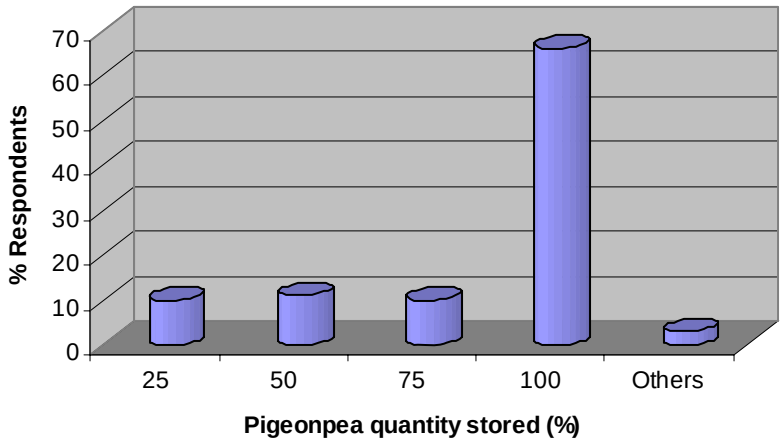

Figure 2.12: Proportion of pigeonpea harvest that was stored in southern region of Malawi in 2004

Fourteen large-scale farmers were interviewed and only 7.1% grew pigeonpea for consumption using released varieties; the rest depended on buying from the small-scale farmers around them or from open markets (Table 2.4). Most (85.7%) of the estates provided rations of pigeonpea and beans to their workers throughout the year. A few estates provided rations to their workers during the critical period of tobacco production (October to July). The majority (78.6%) of the estates used a cup (100 g uncooked) to ration pigeonpea to their workers, while a few used a plate (Table 2.4).

Table 2.4: Pigeonpea production at estate level, period over which pigeonpea is rationed to workers and measure of pigeonpea ration to workers in southern region of Malawi in 2005

Pigeonpea production at estate level	% respondents
Yes	7.1
No	92.9
Period of rationing pigeonpea to workers	% respondents
October- April	7.1
October-July	7.1
All year round	85.7
Measure of pigeonpea ration to workers	% respondents
Cup	78.6
Plate	21.4

About 80% of the pigeonpea buying companies in Malawi bought less than 20000 t of pigeonpea each in 2003/04, 10% bought between 60000 and 80000 t each, while the rest bought above 80000 t each of pigeonpea from the farmers (Figure 2.13). Forty percent of the companies sold all the processed pigeonpea locally, but 60% of the companies targeted both local and outside markets (Table 2.5). Pigeonpea products were exported to such markets as India, Pakistan, Indonesia, Malaysia, Singapore, Britain, Belgium, Germany, Trinidad and Tobago, and the Republic of South Africa. Of the companies that sold pigeonpea locally, 50% sold it as whole grain, while the rest sold it as dhal (Figure 2.14). Malawians of Asian origin normally consume pigeonpea as dhal, while most indigenous smallholder farmers use whole grain seed for food.

Half of the pigeonpea export products was dhal and the other half was exported as whole grain (Table 2.5).

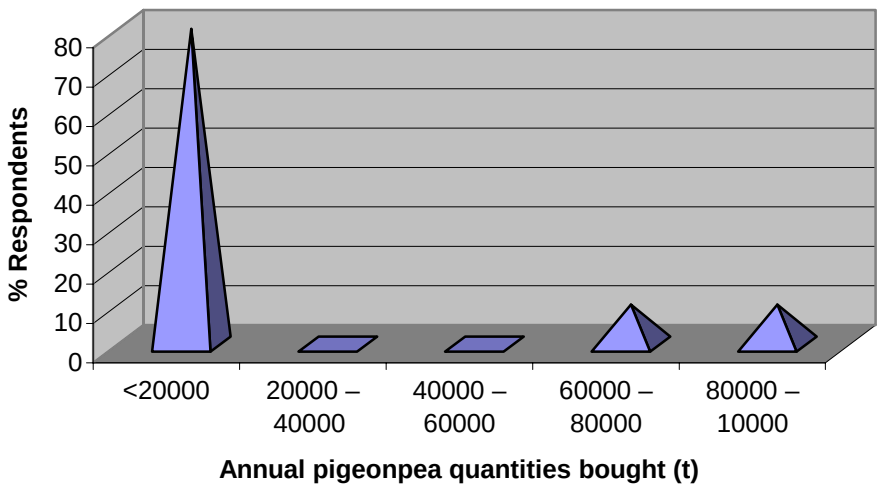

Figure 2.13: Quantities of pigeonpea bought annually by companies in southern region of Malawi

Figure 2.14: a) Well processed; b) Poorly processed pigeonpea in the factory; and c) dhal in commercial shops in southern region of Malawi

Table 2.5: Percentage of buyers on local and export sales and type of pigeonpea product sold

Percentage of buyers, local and export sales	% respondents	Type of pigeonpea product sold	% respondents
Local	40	Whole grain	50
Both local and overseas	60	Dhal	50

Very few buyers looked at seed size, seed colour and volume of the product to determine the buying price, while prevailing market forces, based on available supply, determined the price at which pigeonpea was bought from the farmers (Figure 2.15).

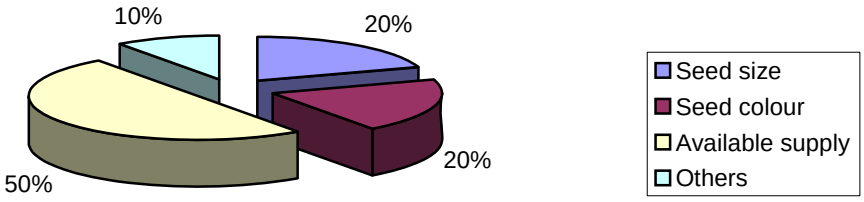

Figure 2.15: Determinants of price at which pigeonpea was bought from farmers in southern region of Malawi in 2005

2.3.3 Pigeonpea production constraints

Pigeonpea production is affected by several constraints. Forty two percent of the farmers indicated pod borers as a big constraint, especially for those farmers growing medium maturing varieties. Aphids and pod sucking insects were minor problems. However, other insects such as white insects (identified as scale insects - Figure 2.16), beetles, elegant grasshoppers, stem maggots, cutworms, and white grubs, caused significant damage (37%) to pigeonpea (Figure 2.17).

Figure 2.16: Scale insects – *Icerya purchasi*

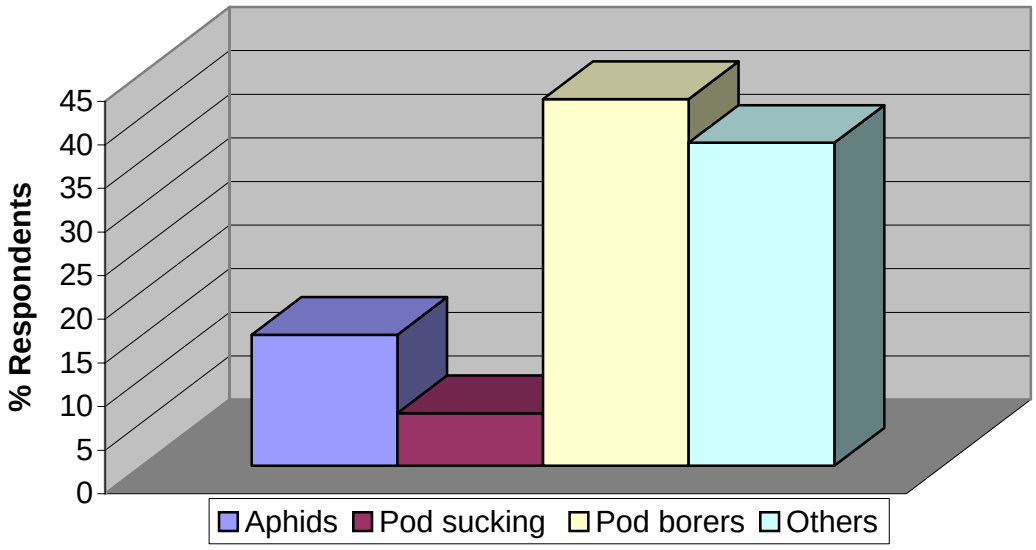

Figure 2.17: Insect pests affecting pigeonpea production in southern region of Malawi in 2005

The majority of the farmers (94%) mentioned weevils as the main storage problem, while the remaining farmers did not have any storage problem because they did not store any pigeonpea due to low production (Figure 2.18).

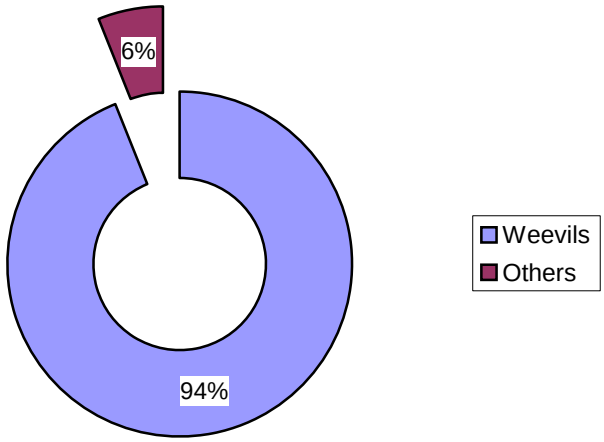

Figure 2.18: Storage pests of pigeonpea in southern region of Malawi

The majority (67.8%) of the farmers indicated flower abortion as another big production constraint. Soil fertility and weeds were regarded as minor production constraints. However, other constraints (29%) that played a significant role in pigeonpea production included drought, lack of a proper market for the crop, low prices, low potential yields, browsing animals (goats), inefficient labour, late maturity, late germination, and unavailability of certified seed (Figure 2.19).

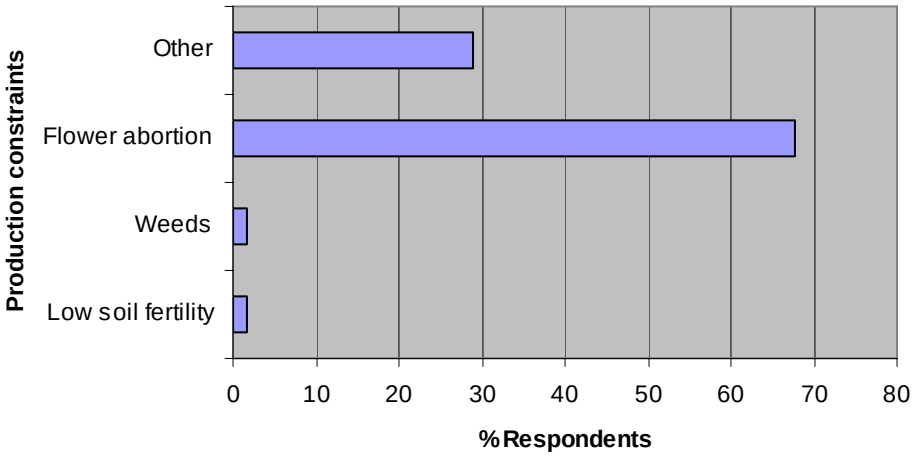

Figure 2.19: Other pigeonpea production constraints in southern region of Malawi

Ninety three percent of the farmers interviewed indicated that Fusarium wilt was not only the most prevalent disease in their fields but also the main constraint affecting pigeonpea production in their area. Of the remaining 7%, 2% of the farmers complained of nematode infection, while 5% claimed that they never experienced any disease problems (Figure 2.20).

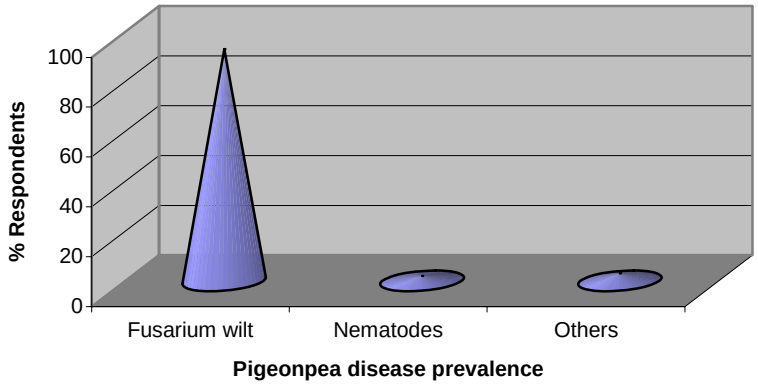

Figure 2.20: Prevalence of pigeonpea diseases in farmers' fields in southern region of Malawi in 2005

2.3.4. Fusarium wilt disease in pigeonpea

Most of the farmers (83.6%) reported that they grew pigeonpea cultivars which were susceptible to Fusarium wilt disease (Figure 2.21). Some farmers (14.8%) reported that their varieties were tolerant to wilt, while the rest reported that they grew resistant varieties (Figure 2.22).

Figure 2.21: Symptoms of Fusarium wilt disease: a) stem wilting on one side; and b) wilting of plants and disease damage in the field at Bvumbwe Research Station sick plot

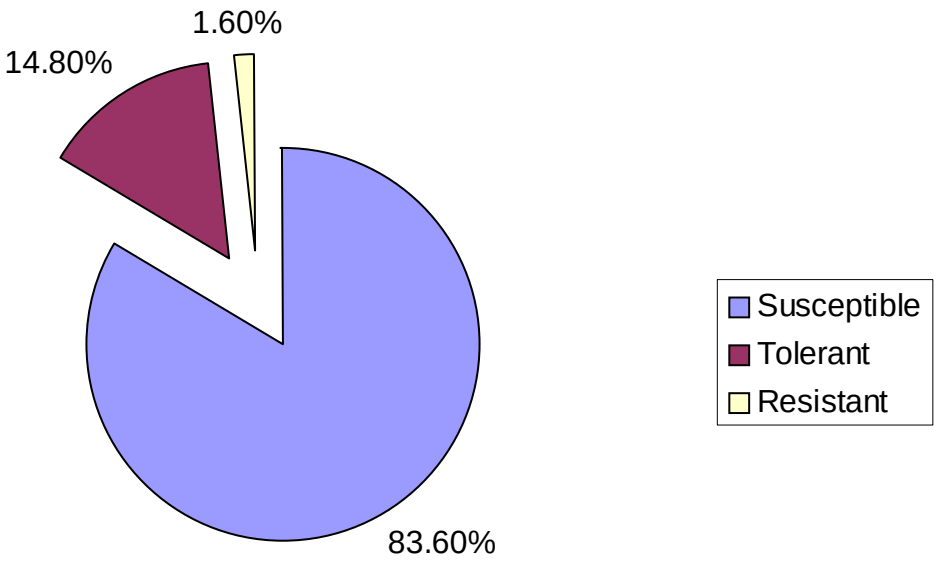

Figure 2.22: Farmers' perception of pigeonpea landraces reaction to Fusarium wilt disease in southern region of Malawi

The survey also showed that Fusarium wilt disease was the most serious disease of pigeonpea (84% of farmers) that is contributing greatly to the reduction in pigeonpea yields. The rest of the farmers indicated that the disease existed in the area, but was not severe (Table 2.6).

Table 2.6: Farmers' understanding of Fusarium wilt disease in southern region of Malawi

Farmers' perceptions	Categories	% respondents
Severity of Fusarium wilt	Very severe	84
	Not severe	16
Prevalence of Fusarium wilt	Very prevalent	79
	Prevalent	11
	Not prevalent	10
Control measures used for Fusarium wilt	Fallowing	2
	No control	98
Willingness of farmers to buy improved seed	Yes	97
	No	3

Seventy nine percent (79%) of the farmers indicated that wilt was most prevalent in most of their pigeonpea growing areas. About 11% of the farmers indicated that wilt was still prevalent in their area; while the rest claimed that the disease was present in farmers' fields but was not prevalent (Table 2.6). Most farmers were aware that wilt was the most destructive disease of pigeonpea but they did not deploy any control measures. Only 2% of the farmers indicated that they tried fallowing their fields to control the disease. The majority (97%) of the farmers were willing to buy improved seed as a way of increasing pigeonpea production. Only a few farmers gave a negative response - that they would not wish to buy improved pigeonpea seed (Table 2.6).

2.3.5. Pigeonpea cultivar preferences

About 39% of the farmers reported that landraces cooked faster than released varieties, and this was the main reason for preferring local landraces. About 15% of the farmers indicated that taste was the main reason, while an equal number of farmers mentioned yield. Ten percent (10%) of the farmers preferred local varieties because of large seed size, while only 3% indicated seed colour as a reason for their preference of the local landraces. The rest of the farmers had other reasons for preferring their local varieties: the high prices that they get when selling a particular local landrace, drought tolerance, improvement of soil fertility, compatibility in the intercropping system, pest resistance,

early maturation, high expansion rate after cooking, and big stems for firewood (Figure 2.23).

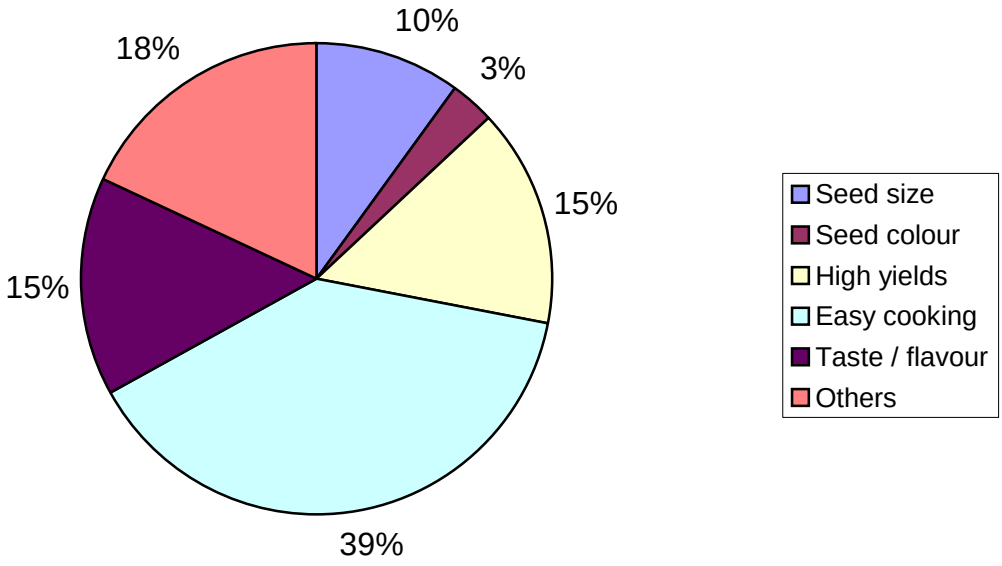

Figure 2.23: Reasons for farmers' preferences for pigeonpea local landraces in southern region of Malawi

The majority of the farmers preferred large seeded pigeonpea. About 20% preferred medium sized seed, while the rest preferred small sized pigeonpea seeds (Table 2.7). Most farmers (52.5%) preferred white (cream) coloured pigeonpea, 11.5% preferred the mixture of white and brown or mottled colour, and the rest preferred a red mottled colour. The results also showed that the majority of the farmers preferred tall plants, 26% preferred medium plants in the field, while very few farmers wanted dwarf varieties (Figure 2.24).

Table 2.7: Farmers' preferences on seed colour and seed size in southern region of Malawi

Seed colour	% respondents	Seed size	% respondents
Large	69	White (cream)	52.5
Medium	20	Mixed (white & brown)	11.5
Small	11	Red	36.0

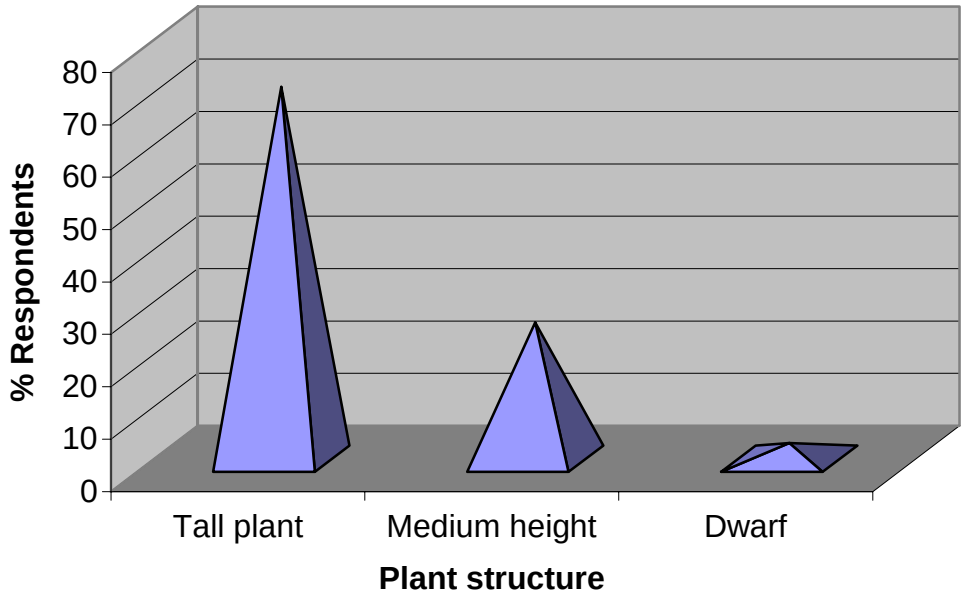

Figure 2.24: Farmers' preferences for pigeonpea plant structure in southern region of Malawi

Most pigeonpea buying companies preferred large seeded pigeonpea. Only one company (10%) indicated medium seed size as a preference. The majority (70%) of the companies preferred white colour compared to a mixed colour (Table 2.8).

Table 2.8: Buyers' preferences for seed colour and seed size in Blantyre, Malawi

Seed size	% respondents	Seed colour	% respondents
Large	90	White (cream)	70
Medium	10	Mixed (white & brown)	30

Half of the pigeonpea buyers looked at seed colour of pigeonpea before buying, while 30% of the buyers looked for good quality seed, irrespective of seed colour and seed size. About 10% of the buyers were particular about seed size, while the remaining 10% preferred a large quantity without looking at specific traits (Figure 2.25).

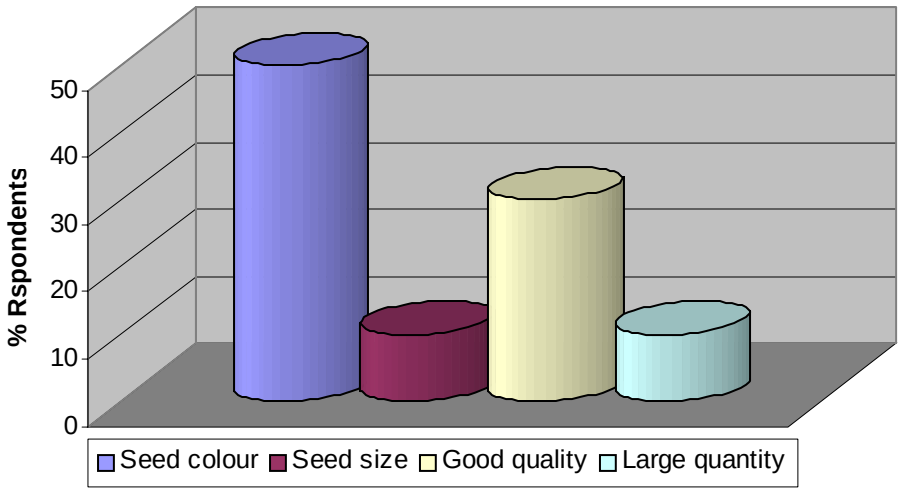

Figure 2.25: Buyers' preferences for pigeonpea cultivars, Blantyre, Malawi in 2005

Farmers' expectations from breeders were very diverse, for traits such as high yields, disease resistance, pest resistance and good quality. However, most farmers (85.2%) expected plant breeders to develop varieties that give answers to diverse problems (Figure 2.26). Farmers' expectations include large round seeds, fast cooking time, adaptable to local conditions (low soil fertility, heavy rainfall and no flower abortion in winter), early maturing, good flavour/taste, drought tolerant, ease of field management, double flowering and bushy plants for firewood. The expectations of the buyers were mainly for good quality seed (80% of buyers) and high yielding varieties for farmers (20%) (Figure 2.27).

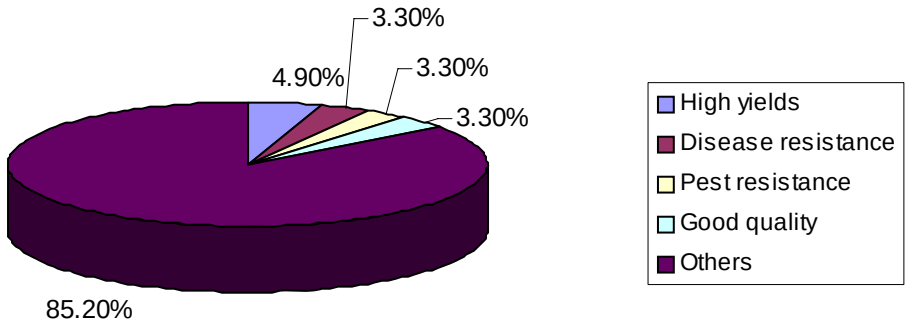

Figure 2.26: Farmers' expectations from the plant breeders in southern region of Malawi

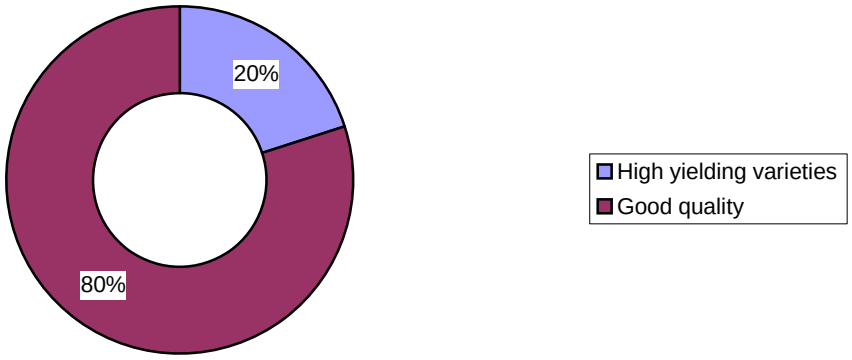

Figure 2.27: Buyers' expectations from the plant breeders, Blantyre, Malawi

2.3.6. Participatory variety selection

The survey team characterized the 43 genotypes to 15 groups across all the districts according to seed colour and size (Table 2.9). Most of the germplasm accessions were similar. Many farmers indicated that germplasm was shared among farmers, despite the distances from which the germplasm were collected. Farmers travel long distances visiting their relatives and, along the way, they share seeds of various crops including pigeonpea. It is very likely, therefore, that some of the germplasm accessions had similar origin.

Table 2.9: Initial grouping of pigeonpea germplasm per district based on seed size and colour by the survey team

District	Samples collected	Seed size			Seed colour			
		Large	Medium	Small	White	Mottled	Black & White	Red
Zomba	11	6	5	0	5	4	1	1
Thyolo	5	1	4	0	1	4	0	0
Chikwawa	6	0	5	1	0	4	0	2
Mwanza	8	3	5	0	3	5	0	0
Nsanje	4	0	4	0	1	3	0	0
Chiradzulu	9	2	6	1	0	6	1	2
Total	43	12	29	2	10	26	2	5

The 15 representative groups of germplasm were taken back to the farmers and the buyers for participatory variety selection, to use focus groups (clubs or associations) taking advantage of farmer meetings. From the farmers' choices, 10 local landraces were selected for breeding work to develop new varieties. Seed colour that is associated with good flavour (taste) and large seed size featured highly on their selection criteria.

The buyers also selected local landraces and released varieties, depending on the export demands. AP 14 was dropped from the list due to a small seed sample and could not be used in the trials (Table 2.10). Initially ten local landraces - coded AP1 to AP10 – were selected for genetic improvement. Two other landraces, AP23 and AP29, were added to the list from the 15 groups due to geographical misrepresentation. This brought the total entries for genetic improvement to twelve. These 12 were crossed with four Fusarium wilt resistant varieties from ICRISAT, Kenya in order to introgress resistant genes into the preferred local landraces while maintaining the desirable attributes.

Table 2.10: Farmer participatory variety selection and reasons for selection

Number of local landrace	Number of respondents	Reasons for Selection
AP1	7	Seed colour associated with flavour
AP2	5	Seed colour (flavour)
AP3	9	Seed colour (flavour)
AP4	5	Seed colour (flavour)
AP5	6	Seed colour (flavour)
AP6	7	Seed colour (flavour)
AP7	5	Seed colour (flavour)
AP8	13	Early maturing and large seeded
AP9	5	Seed colour and large seed size
AP10	10	Early maturing (escape drought) and large seeded
AP11	4	Flavour
AP12	4	Flavour
AP13	1	Flavour
AP14	7	Early maturing
AP15	2	Liked by the trade – easy marketing
Total	89	

2.4 Discussion and conclusion

The survey and germplasm collection were conducted in typical pigeonpea growing areas ranging from low altitude to high altitude areas. Most farmers were very receptive, interactive and knowledgeable of the pigeonpea crop. The results have brought new perceptions and challenges to pigeonpea breeding in Malawi.

Pigeonpea forms part of the cropping system for small-scale farmers in the southern region of Malawi. The crop is mostly intercropped with such crops as maize, beans, pumpkins, cassava, groundnuts, cowpeas, pearl millet, and sorghum. Sakala (1992) and Gwata et al. (2006) reported similar results. Most farmers intercrop pigeonpea because

of small landholding size (0.4 - 0.8 ha) in relation to the average farming family of 5 people as reported by the National Statistics Office (2005). This is why many farmers put only 0.2 ha of land to pigeonpea production, leading to low total output. There is a need, therefore, to breed for high yielding varieties that can do well in an intercropping system.

Local landraces, which are predominantly late maturing, account for a high proportion of the pigeonpea production in the country. The low adoption of the released wilt-resistant varieties may be attributed to lack of farmer-preferred traits in the improved varieties. These landraces vary in many features, among which are seed colour, size, plant height, and maturity periods. The genetic variations among local landraces show potential for further selection.

There are varying sources of pigeonpea seed for planting. Use of own seed and sharing among friends and relatives indicated that farmers were particular about certain traits which they wanted to maintain. The fact that most farmers had been growing the same cultivar for more than five years supports the importance of maintaining the desirable traits. The number of farmers who buy seed from the market suggests the potential market for the improved (certified) seed. Currently, the two improved varieties give limited options to the farmers on the desirable traits. Therefore, the release of more improved pigeonpea varieties would give farmers better options.

The majority of the farmers chose seed from the harvested seed lot. The implication is that farmers want to maintain and preserve the desirable traits in their cultivar, such traits including taste, colour, and fast cooking. It can be concluded, therefore, that yield and other attributes are less important (Smith et al., 2001) than the desirable traits, and selection pressure for yield is almost non-existent. This is despite the fact that some farmers could exert selection pressure by choosing plants in the field with good yield attributes (plant vigour, more number of branches, greater number of pods branch^{-1} and greater number of seeds pod^{-1}). Participatory plant breeding should involve such farmers, who choose good plants in the field, in improving pigeonpea genetically.

Pigeonpea is mostly consumed as cooked dry peas to be used with the main Malawian dish of *Nsima* (maize based). It is also consumed as cooked green peas and green

pods. Most of the tobacco estates in the southern region of the country use pigeonpea as an integral part of the ration for the workers. Most estates provide 150 g of cooked pigeonpea per person per meal, which is enough to provide 19.5 g of actual protein (Latham, 1979). Pigeonpea genotypes with high protein content would thus benefit estate workers and farmers.

Most of the pigeonpea produced in Malawi is consumed locally. The quantity of pigeonpea that is bought annually is limited by the availability of the export markets and the quantity that farmers offer on the market because most farmers keep the greater part of the pigeonpea harvest for food. The prevailing market forces/trends based on available supply, determine the prices at which buyers purchase pigeonpea from farmers. Both dhal and whole grain are available on the local market for consumers. This ensures their availability for those who can access pigeonpea in shops. The ICRISAT released varieties are preferred for whole grain export markets because of their white colour. Some companies indicated that ICRISAT varieties are more difficult to process into dhal than local pigeonpea because they have a hard seed coat, while the local cultivars peel off easily (Singh and Jambunathan, 1990). ICEAP 0040 was specifically developed for its disease resistance, fast cooking and ease of dehulling ([1]Dr Said Silim, personal communication, 2008). However, it was not widely adopted by farmers due to lack of seed. The implication of this is that breeders should breed cultivars with a loose seed coat for the desired milling quality.

Farmers perceived pests, small landholding size, lack of suitable varieties, flower abortion, adverse weather, and low soil fertility as minor problems but regarded diseases as major problems. Among the diseases, Fusarium wilt was regarded as a major production constraint in Malawi, reducing pigeonpea yield. Most farmers reported that they grew pigeonpea cultivars that were susceptible to Fusarium wilt.

Fusarium wilt was reported to be the most serious disease of pigeonpea. This confirms earlier reports by Soko (1992), Subrahmanyam et al. (1992), Changaya-Banda (1997), Hillocks et al. (2000) and Gwata et al. (2006). The severity of the disease may be attributed to lack of proper rotation or other control measures due to small land holding

[1] Dr Said N. Silim, ICRISAT, Nairobi, Kenya

size. If pigeonpea production is to improve in Malawi, resistant cultivars should be developed to control the wilt disease.

A high proportion of farmers preferred local landraces due to their short cooking time. The southern region of Malawi is heavily deforested, and pigeonpea stems are the reliable sources of firewood (Edje, 1984; Arya et al., 2002). Therefore, fast cooking cultivars save firewood. Taste (flavour) also ranked highly in the farmers' preferences. The released varieties (Sauma and Kachangu) are not popular among farmers for food because they take long to cook and they are not as tasty as the local landraces. For these farmers, seed colour is an indication of flavour/taste and shorter cooking time, while size is an indication of weight for marketing purposes. Tall plants are preferred for compatibility with maize in the cropping system and for firewood, though most of the tall plants are late maturing. Other reasons for preferring local varieties include their high prices, drought tolerance, capacity to improve soil fertility, pest tolerance, early maturation, high expansion rate after cooking, and big stems for firewood.

Almost all the buying companies prefer large seeded pigeonpea for processing into dhal (split cotyledons). This is based on the machine calibration. One company indicated that they prefer buying local landraces because their seed coats (testas) are easier to remove than the released varieties; seed colour also plays a role for whole grain or dhal markets. Seed size may not be an issue for companies that usually export pigeonpea as whole grain. It was clear from the survey that buyers' requirements were similar to farmers' preferences in terms of seed size and colour. Therefore, selection for large seed size and proper colour, during breeding, would cater for both farmers and buyers.

Most farmers expect plant breeders to develop varieties that have such attributes as whiteness, roundness and largeness of seeds, their fast cooking rates, adaptability to local climatic conditions, early maturation, good flavour/taste, drought tolerance, double flowering in a season, and the bushiness of plants for firewood. This list of preferences is an indication of the many challenges that exist in pigeonpea production, calling for more concerted interventions from scientists in various disciplines to address the farmers' expectations. The expectations of the buyers are mainly good quality seed which can easily be processed into dhal and/or exported as whole grain, and high yielding varieties to boost the pigeonpea production.

The PRA showed that Fusarium wilt was the main production constraint and yet farmers used susceptible cultivars due to their desirable traits. Deployment of resistant varieties, an integral part of integrated disease management strategy, could be the viable option for managing the disease. The involvement of farmers and the trade, through PVS, helped to select suitable local landraces, with desirable attributes, for use in the breeding programme. The selected landraces would be crossed with Fusarium wilt resistant varieties from ICRISAT, Kenya to introgress wilt resistant genes, while maintaining the farmer-preferred traits.

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Appendix

Appendix 2.1: Details of the germplasm collection

District	Farmer name	Farmer number	Altitude (masl)	Coordinates / location		Germplasm collected
				Southings	Eastings	
Mwanza	L. Masitala	3	830	15.51300	34.46906	2
	F. Kapalamula	5	678	15.61446	34.53190	1
	M. L. Zembere	6	647	15.62258	34.52381	1
	M. Butawo	7	760	15.55632	34.48927	1
	Maganga	8	775	15.55625	34.48919	1
	M. Edwin	9	821	15. 53083	34.46427	1
	D. Maliseni	10	813	15.52640	34.46514	1
Chiradzulu	H. Kalilombe	1	1054	15.72099	35.15593	1
	K. Gilinjala	2	1041	15.71986	35.15474	1
	E. Payinodi	4	1064	15.71327	35.14309	3
	E. Mbatamila	5	1073	15.71573	35.13675	1
	G. Nazombe	8	1013	15.73076	35.15788	1
	R. Kamwendo	9	1054	15.73512	35.14176	1
	D. Kachere	10	1066	15.73494	35.13950	1
Chikwawa	M. James	1	89	16.31257	35.09915	3
	G. Damiano	2	89	16.10764	34.90425	2
	B. Katayika	7	93	16.17971	34.99729	1
	Taimu	10	91	16.28840	35.05920	1
Nsanje	R. Yonasi	1	80	16.31257	35.09915	1
	J. Kunti	2	53	16.38554	35.14034	2
	F. Lapozo	4	84	16.41925	35.14648	1
Zomba	Moloka	1	886	15.53855	35.29282	1
	Asima	3	876	15.56356	35.29428	1
	Jackson	4	844	15.58532	35.29119	4
	Mohomed	5	866	15.56293	35.30164	1
	Haliana	9	845	15.56147	35.30973	1
	Kachala	10	870	15.54170	35.29896	2
Thyolo	K. Ndawanje	1	1134	15.99383	35.0663	1
	Nsuza	2	1166	16.00172	35.04124	1
	Kastomu	3	1151	16.03688	35.02969	1
	Mpezeni	6	1173	15.97968	35.07168	1
	Mdeule	7	1182	15.97586	35.08029	1
	Lodzani	9	1156	15.96747	35.07471	1

Chapter 3. Evaluation of pigeonpea germplasm for yield, yield components, and resistance to Fusarium wilt disease in Malawi

Abstract

Farmers and buyers, through participatory variety selection, selected 10 local pigeonpea landraces with desirable traits as parental materials for a genetic improvement programme. These landraces, together with cultivars from ICRISAT and from the Department of Research in Malawi, were evaluated for yield and yield components and assessed for their level of wilt resistance. Yield and yield components were evaluated in a randomized complete block design (RCBD) at Kandiya for three seasons and at Bvumbwe for one season. Data collection involved yield, days to 50% flowering, number of primary, secondary and tertiary branches, number of seeds pod^{-1}, and seed dry weight. The assessment of wilt resistance was done at a sick plot at Bunda College for two seasons and at a sick plot at Bvumbwe Research Station for one season. The results showed significant variations among genotypes for such traits as yield, yield components, and reaction to Fusarium wilt. The variation showed potential for selection for genetic improvement. AP10, a local landrace, yielded better than the local and ICRISAT genotypes, and was also resistant to wilt, indicating that answers to the farmers' problems are possible; and that farmers' germplasm should be exploited for answers before looking at external sources. Most of the landraces, except for AP3, AP4, and AP10, were highly susceptible to wilt, confirming the farmers' claims. Generally, local landraces were medium to late maturing. All the ICRISAT genotypes were resistant except for ICEAP00068 and KAT60/8, which were susceptible. From the evaluation results, these landraces needed to be improved through breeding. The recommendation was, therefore, to breed for Fusarium wilt resistance, early maturation, high yield, more seeds pod^{-1}, large seeds, and medium plant height in the selected local landraces while maintaining the farmer- and consumer-preferred traits.

3.1 Introduction

Pigeonpea (*Cajanus cajan* (L.) Millspaugh) is predominantly a smallholder crop in Malawi, mostly cultivated in intercropping systems with maize, cassava, sorghum, and groundnuts. The southern region of Malawi accounts for more than 99% of the total pigeonpea production in the country (Ministry of Agriculture and Food Security, 2007). The cultivation of pigeonpea is dominated by local landraces and yields have remained low, at about 600 – 700 kg ha^{-1} (Ministry of Agriculture and Food Security, 2007). The agronomy and the genetics of the crop need to be improved to increase yields.

In pigeonpea, yield is a function of several factors such as the number of primary branches, number of pods $plant^{-1}$, number of seeds pod^{-1}, and size of seed. Therefore, the compatibility of the cultivars in the intercropping system is crucial for the expression of the yield components (Singh et al., 1995). There are several factors that are responsible for low yields in pigeonpea, and these include lack of improved genotypes, lack of resistance to diseases and pests, low soil fertility, and terminal drought. Not all these factors play a major role at the same time; some of them may be seasonally significant (Chauhan, 1990).

A lack of improved genotypes is often cited as one of the reasons for low yields of pigeonpea (Chauhan, 1990). The majority of small-scale farmers in Malawi grow local landraces with varying genetic potentials for yield. Most of the landraces are late maturing: 8 – 10 mo (ICRISAT, 2006) compared to 5 mo for some improved genotypes. These local landraces incur greater risks of being exposed to abiotic stress such as frost or drought (Chauhan, 1990).

The most important limiting factor in pigeonpea production is Fusarium wilt (Subrahmanyam et al., 1992; Gwata et al., 2006; ICRISAT, 2006). Yield losses range from 50 – 100%. The disease contributes significantly to low yields in Malawi as most farmers' fields are infested with the pathogen. The disease is soil-borne, and attacks the seedlings through the root system, eventually causing stem wilting followed by death of the plant at the flowering or podding stage (ICRISAT, 2005). The use of resistant cultivars is the most effective way of controlling the disease (Gwata et al., 2006; ICRISAT, 2006) and to improving yields.

Several descriptors are used in the characterization of pigeonpea to estimate the extent of variation in the collection, among many objectives of characterization (Remanandan, 1990). These descriptors include days to 50% flowering, days to 75% maturity; base flower colour; second flower colour; pattern of streaks on petals (sparse, medium, dense streaks, and union coverage of second colour); flowering pattern; growth habit (determinate, semi determinate and indeterminate); plant habit (erectness or compactness, semi spreading, spreading or trailing); plant height at maturity; number of primary; secondary and tertiary branches; stem colour (green, sun red, purple, and dark purple); number of racemes; raceme length; number of pods plant^{-1}; number of seeds pod^{-1}; pod length; pod colour (green, purple, mixed, green and purple, and dark purple); pod shape; seed colour (cream, mottled, cream and brown, black and white); seed shape; seed mass (100 seed-mass); harvest index (HI); shelling ration; and protein percentage. There are more than 18 secondary descriptors which may also be used in the characterization of the genotypes, including vigor at 50% flowering, leaf colour, leaf size, stem thickness, pod texture, and seed eye colour (IBPGR/ICRISAT, 1981; Reddy, 1990; Remanandan, 1990; ICRISAT, 2006). The growth habit determines the suitability of the genotypes for the cropping system. The use of these descriptors depends on the interest of the researcher. For instance, Santos et al*.* (1995) and Rehman et al*.* (1999) reported variation in germplasm in terms of days to first harvest, plant height at maturity, dry matter yield, and grain yield. Days to 50% flowering, days to 75% maturity, plant height, 100-seed mass and yield were used to characterize Sri Lankan germplasm (Saxena et al., 1998). Patel and Patel (1998) added number of clusters plant^{-1}, pod thickness, methionine content (%) and total soluble sugar content (%) in their characterization of the pigeonpea germplasm.

Molecular characterization of pigeonpea germplasm could be useful since it is independent of environmental effects. Developing molecular markers for the various traits could enhance molecular characterization; of particular interest is the characterization of the resistance genes for Fusarium wilt, which is presently the most devastating pigeonpea disease in Eastern and Southern Africa (ESA) (ICRISAT, 2006). Some work is being done on the development of molecular markers for Fusarium wilt disease (Odeny[1], 2004, personal communication).

[1] Odeny, ICRISAT, India

The assessment of the diversity among the landraces, by measuring the variation in phenotypic traits, is a pre-requisite for designing an efficient breeding programme (ICRISAT 2007). The evaluation of the landraces could help establish the potential and weakness of this germplasm; from which strategies could be put in place for the breeding programme. The landraces can form an important component of a pigeonpea breeding programme. Therefore, the objective of this research was to evaluate the Malawian pigeonpea germplasm and ICRISAT cultivars for yield and yield components and resistance to Fusarium wilt disease.

3.2 Materials and methods

3.2.1 Selection of genotypes used in the evaluation trials

Twenty-nine genotypes were used in both yield and disease evaluation trials. These came from three different sources (Table 3.1). Fifteen cultivars came from ICRISAT, Kenya, varying in their resistance to Fusarium wilt disease. Three local accessions (that were apparently resistant to wilt), originated from the Legume Breeding Section of the Department of Research in the Ministry of Agriculture and Food Security in Malawi (Source: Late H.N. Soko, Chitedze Research Station). The remainder were local landraces of unknown reaction to F. wilt. These landraces included ten that were selected by farmers and traders during the participatory variety selection (Chapter 2). The two released varieties in Malawi served as controls for wilt resistance, yield and yield components. KAT60/8 and ICEAP00068 were susceptible controls for Fusarium wilt.

Table 3.1: Pigeonpea genotypes used in evaluation trials

Item No.	Genotype	Source	Resistance status to Fusarium wilt	Maturity duration
1	AP1	Malawi local landrace	Unknown	Unknown
2	AP2	Malawi local landrace	Unknown	Unknown
3	AP3	Malawi local landrace	Unknown	Unknown
4	AP4	Malawi local landrace	Unknown	Unknown
5	AP5	Malawi local landrace	Unknown	Unknown
6	AP6	Malawi local landrace	Unknown	Unknown
7	AP7	Malawi local landrace	Unknown	Unknown
8	AP8	Malawi local landrace	Unknown	Unknown
9	AP9	Malawi local landrace	Unknown	Unknown
10	AP10	Malawi local landrace	Unknown	Unknown
11	ACC2253	Government (Mw)	Tolerant	Late maturing
12	ACC2291	Government (Mw)	Tolerant	Late maturing
13	ACC2298	Government (Mw)	Tolerant	Late maturing
14	ICP9145	ICRISAT Kenya	Resistant (check) in Malawi, susceptible in Kenya	Late maturing
15	ICEAP00040	ICRISAT Kenya	Resistant (check)	Late maturing
16	ICEAP00068	ICRISAT Kenya	Susceptible (check)	Medium maturing
17	KAT60/8	ICRISAT Kenya	Susceptible (check)	Early maturing
18	ICEAP00020	ICRISAT Kenya	Relatively resistant	Late maturing
19	AP19	Malawi local landrace	Unknown	Unknown
20	ICEAP00554	ICRISAT Kenya	Resistant	Medium maturing
21	ICEAP00540	ICRISAT Kenya	Resistant	Medium maturing
22	ICPL87051	ICRISAT Kenya	Resistant	Medium maturing
23	AP23	Malawi local landrace	Unknown	Unknown
24	ICEAP00557	ICRISAT Kenya	Unknown	Medium maturing
25	ICEAP00053	ICRISAT Kenya	Relatively resistant	Late maturing
26	ICEAP00932	ICRISAT Kenya	Relatively resistant	Late maturing
27	ICEAP00933	ICRISAT Kenya	Relatively resistant	Late maturing
28	ICEAP00936	ICRISAT Kenya	Relatively resistant	Late maturing
29	AP29	Malawi local landrace	Unknown	Unknown

3.2.2 Evaluations of yield and plant descriptors of local landraces and ICRISAT cultivars

The yield evaluation trial, comprising 29 genotypes, was conducted at Kandiya Research Station in the central region of Malawi (Figure 3.1), about 10 km from Chitedze Research Station. Kandiya Research Station is 1108 masl, 13.97379^0S, and 033.73085^0E. The site is devoid of Fusarium wilt disease. The trial was laid out in a randomised complete block design (RCBD). Each plot comprised two rows (ridges) of 3.3 m long, spaced at 0.9 m apart. The pigeonpea plants were spaced at 300 mm within the row. The trial was planted with the onset of the first rains and no fertilizers were applied to simulate

farmers' practices. The plot was constantly scouted for insect pests, and Rogor (Dimethoate) at 6.5g / 10 litres water or Acephate (Orthene) at 38ml / 10 litres water was sprayed when the need arose. Weeds were controlled by hand. The trial was repeated over three seasons, 2004/05, 2005/06 and 2006/07. There was drought in the first season and the distribution of the rain was poor, especially towards the end of the season. In the second season, the rainfall was less than in the first season but the distribution was better towards the end of the season than in the first season (Figure 3.2). The trial at Bvumbwe Research Station in 2004/05 was converted from a disease- to a yield-evaluation trial in the first year due to low Fusarium wilt disease pressure.

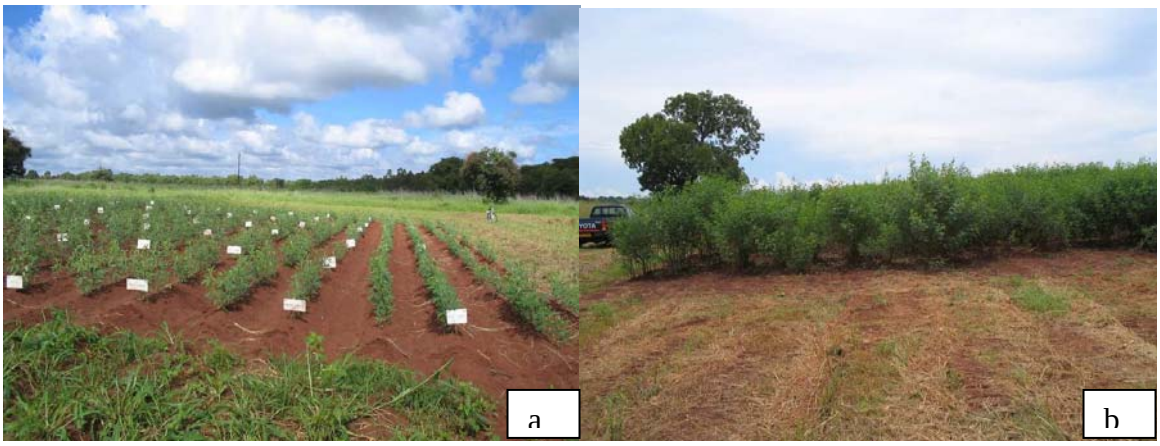


Figure 3.1: Pigeonpea yield trial at Kandiya Research Station, Lilongwe, Malawi: a) Trial establishment at 3 wk; b) Vegetative growth at 4 mo.

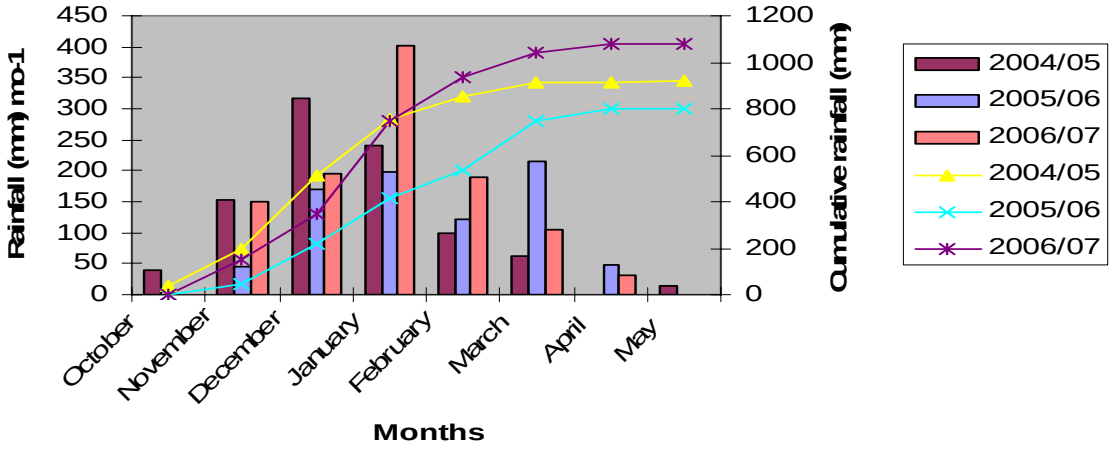


Figure 3.2: Distribution of rainfall during the season at Kandiya Research Station, Lilongwe, Malawi from 2004 to 2007 (Note: Lines are cumulative while bars are monthly rainfall)

Data collection in these trials included days to 50% flowering, number of primary, secondary and tertiary branches, plant height at maturity, stem diameter, number of seeds pod^{-1}, yield and 100-seed mass. Observations were also made on flower colour, pod colour, stem colour, pattern of flower streaks, and growth habit (Remanandan, 1990; ICRISAT, 2006).

3.2.3 Screening of pigeonpea local landraces and ICRISAT cultivars for Fusarium wilt resistance through natural inoculation

Screening of pigeonpea genotypes for their resistances to Fusarium wilt was done over two seasons (2004/05 and 2005/06) at Bunda College and Bvumbwe Research Station sick plots (Figure 3.3). The 29 genotypes were planted in an RCBD. Each plot consisted of two rows of 3.3 m long spaced at 0.9 m apart with pigeonpea plants spaced at 300 mm within the row. The trial was planted with the first rains and hand weeding was done whenever weeds appeared. Chemicals and fertilizers were not applied to pigeonpea to simulate farmers' conditions and for fear of affecting the impact of the pathogen.

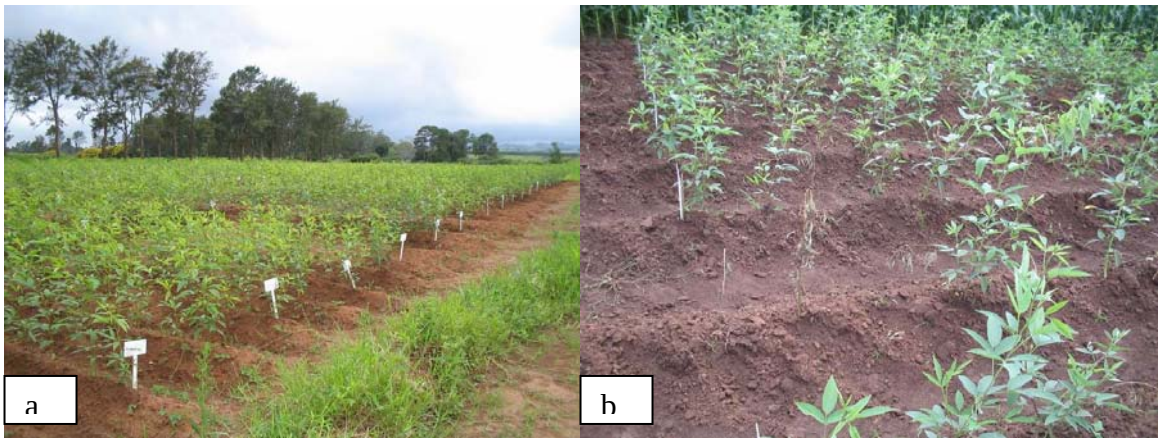


Figure 3.3. Fusarium wilt disease screening trial in the sick plot: a) Trial establishment, b) Infection and damage by Fusarium wilt disease

Data collection in these trials concentrated on the number of pigeonpea plants dying, wilting, or showing typical symptoms of Fusarium wilt as described by Reddy et al. (1990). Data collection commenced one week after germination, starting with plot stand and later counting the number of diseased and dead plants in each plot. Genotypes with less than 20% wilt, based on stand count plot^{-1}, were considered resistant to the disease. The disease assessment was based on the following scale by Nene and Kannaiyan, (1982):

0 - 20% infection – resistant;

21 - 40% infection – moderately resistant/tolerant;

41 - 60% infection - susceptible;

61 - 80% infection – moderately susceptible;

81 - 100% highly susceptible

3.2.4 Data analysis

The data on both yield and yield components, and diseased/dead plants were analyzed, using analysis of variance (ANOVA) of the Genstat statistical package. Data transformations were done where coefficients of variations were high. Log base e (Log_e (x + c) was used in data transformation, and the value of c was arbitrarily taken as 10. F_{max} (Fujino, 1979) was used to calculate the homogeneity of the data across sites and over seasons. $F_{max} = S^2_{max} / S^2_{min}$. Accept H_o (homogeneity of data) if $F_{max} < F\alpha$ (k, n – 1).

Only homogenous data were combined in the analysis over sites and/or seasons. Correlations were also done between Fusarium wilt incidences and other characteristics such as yield, days to 50% flowering, plant height, stem thickness, seed pod^{-1} and 100-seed mass.

3.3 Results

3.3.1 Evaluations of pigeonpea yield and yield components

There were significant ($P < 0.05$) yield variations among the genotypes at both sites over three seasons. In the 2004/05 season, AP10, a local landrace, out yielded all the other entries (Figure 3.4, Appendix 3.2). The medium maturing genotypes yielded better than late maturing genotypes and escaped the drought. Most of the local landraces did not do well because they matured late and were affected by the drought. All the medium duration cultivars from ICRISAT, Kenya, performed well. These included ICEAP00068, ICEAP00540, ICEAP00554, ICEAP00557, and KAT60/8. However, ICPL87051, which is also medium duration, yielded poorly. Among the local germplasm, ACC2298 from the government research department yielded 2.5 t ha^{-1}, which was better than ACC2253 and ACC2291. AP8, which resembles AP10 in many aspects such as flower and seed colour, yielded 1.1 t ha^{-1}. AP19 and AP23 did not yield anything at Kandiya in the first

year because they were planted late as replacements for ICP7035 and ICP11298, respectively, both with very low germination.

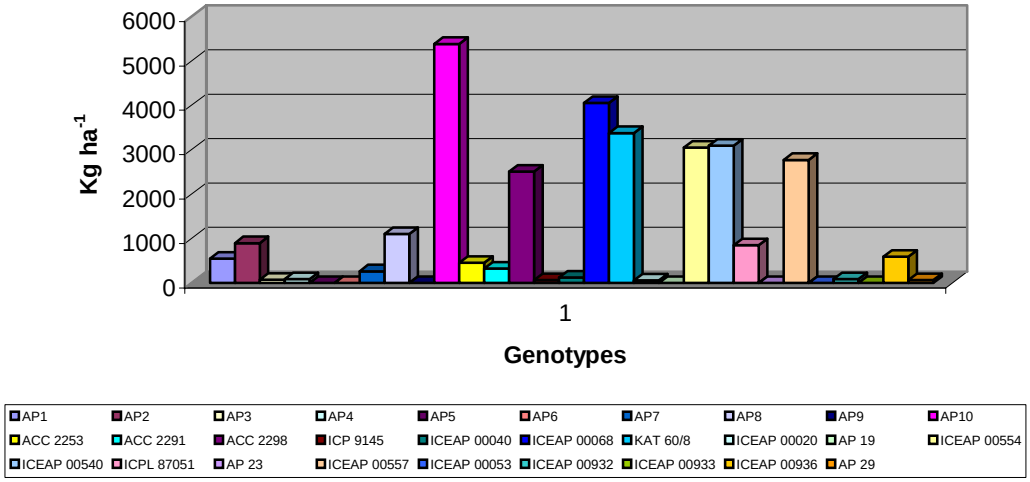


Figure 3.4: Yield performance of pigeonpea genotypes at Kandiya Research Station in 2004/05 season

The second year (2005/06) at Kandiya was very different. The yields were lower than in the first year, with AP4 (local landrace) being the highest yielder at 2.883 t ha^{-1} (Figure 3.5, Appendix 3.2). AP4 is one of the late duration local landraces. Most of the genotypes which yielded well in the second year (> 2 t ha^{-1}) were late duration types, with only ICEAP00540 a medium maturing genotype, giving a yield of just above 2 t ha^{-1}. Nine genotypes, of which six were local landraces, yielded less than 1 t ha^{-1} in the second year.

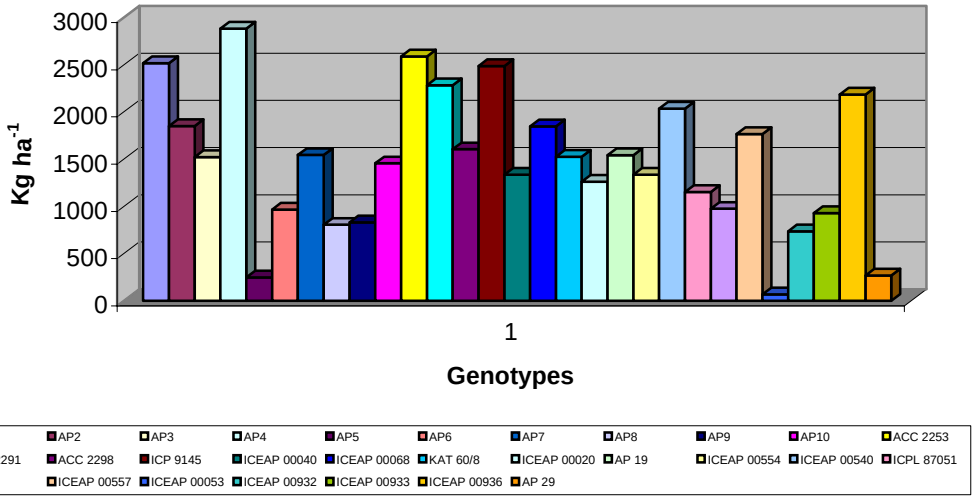


Figure 3.5: Yield performance of pigeonpea genotypes at Kandiya Research Station in 2005/06 season

In the third year (2006/07), the amount of rainfall was the highest and the yields were the lowest among the three seasons, with no cultivar yielding above 1 t ha^{-1}. Five cultivars barely yielded above 0.5 t ha^{-1}. In descending order of yield these were KAT60/8, AP10, ICEAP00540, ICEAP00557 and AP2, (Figure 3.6, Appendix 3.2). Apart from AP2, all the others were medium maturing cultivars.

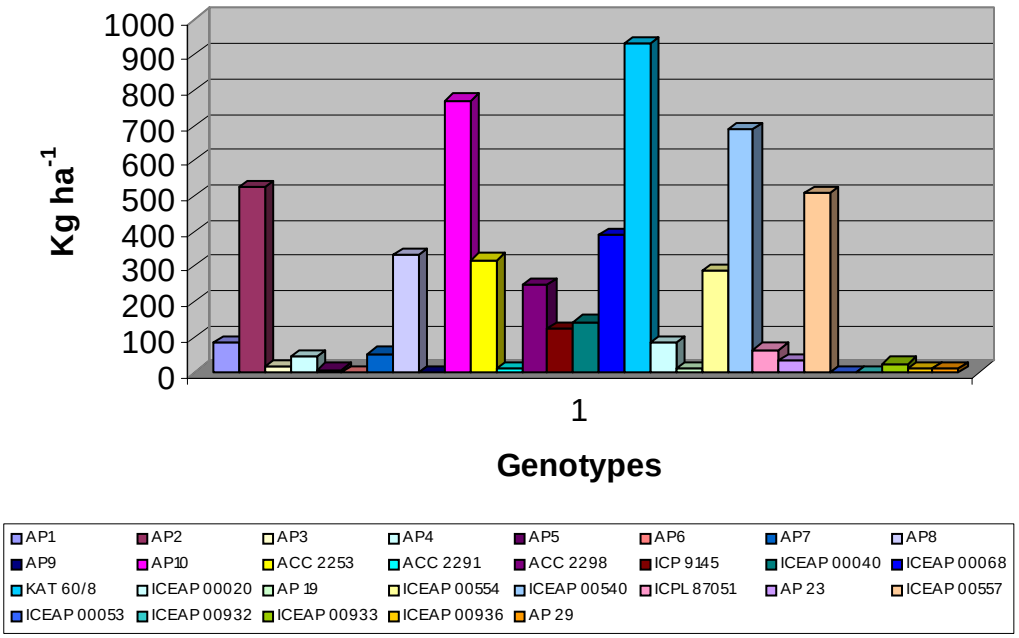


Figure 3.6: Yield performance of pigeonpea genotypes at Kandiya Research Station in 2006/07 season

ICEAP00936 was the highest yielder (1.8 t ha^{-1}), followed by ICEAP00040 and ICEAP00932 at Bvumbwe in the first year (2004/05) (Figure 3.7, Appendix 3.2), while most of the other genotypes yielded less. However, there were significant variations in yields among the genotypes. Both medium and late duration genotypes performed well. Five out of 13 local landraces yielded above 1 t ha^{-1}, with AP4 and AP10 yielding 1.515 and 1.258 t ha^{-1}, respectively. Most of the ICRISAT genotypes had yields of more than 1 t ha^{-1}.

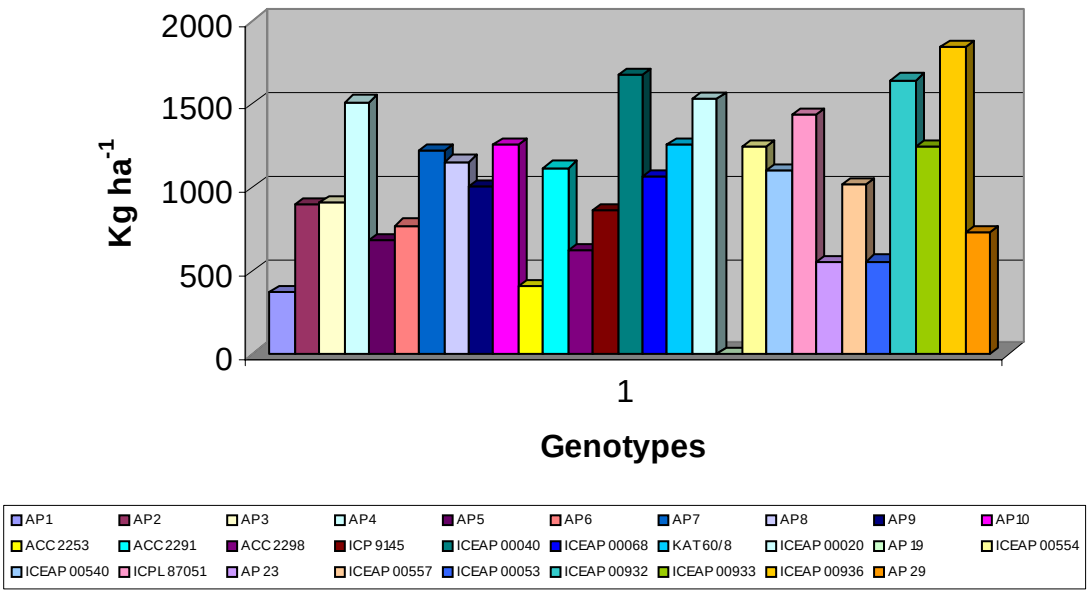


Figure 3.7: Yield performance of pigeonpea genotypes at Bvumbwe Research Station in 2004/05 season

Combined data analysis was done for two seasons (2004/05 and 2005/06) at Kandiya Research Station, while the Bvumbwe Research Station's (2004/05) data were combined with Kandiya Research Station's in 2006/07 through calculation of the F_{max} test. The results of the combined yield analysis over the two seasons at Kandiya Research Station showed AP10 and ICEAP00068 to be consistently high yielders (Figure 3.8a), while KAT60/8 and AP 10 were high yielders for Bvumbwe Research Station (2004/05) and Kandiya Research Station (2006/07) (Figure 3.8b). Over two seasons at Kandiya Research Station, more than 50% of the genotypes, of which six were landraces, yielded more than 1 t ha^{-1}. At Bvumbwe Research Station in 2004/05 and Kandiya Research Station in 2006/07, most of the genotypes yielded less than 800 kg ha^{-1} (Figure 3.8b).

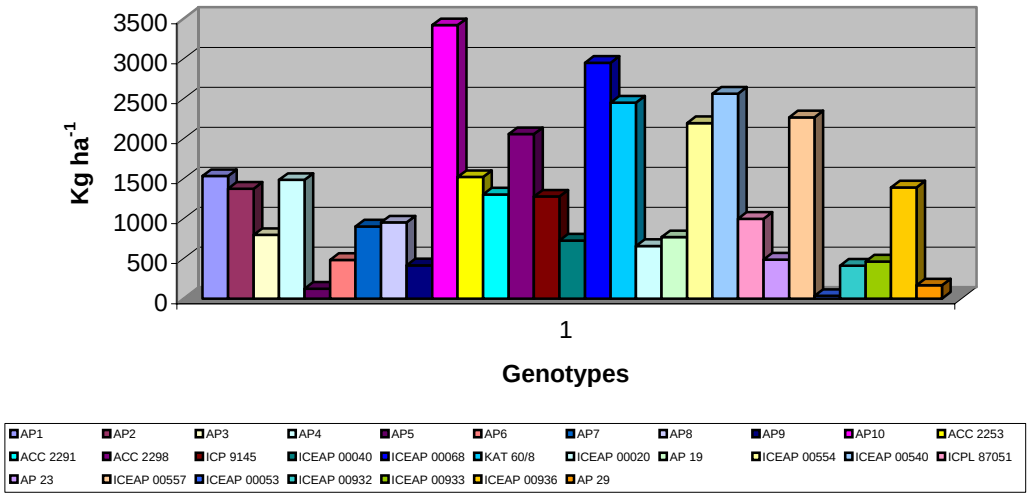


Figure 3.8a: Combined yield performance of pigeonpea genotypes at Kandiya Research Station in 2004/05 and 2005/06 seasons

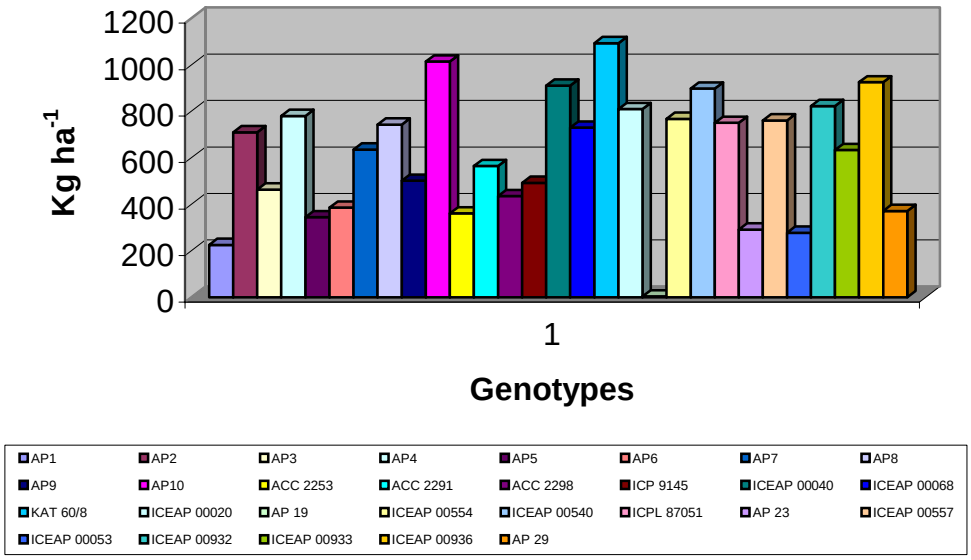
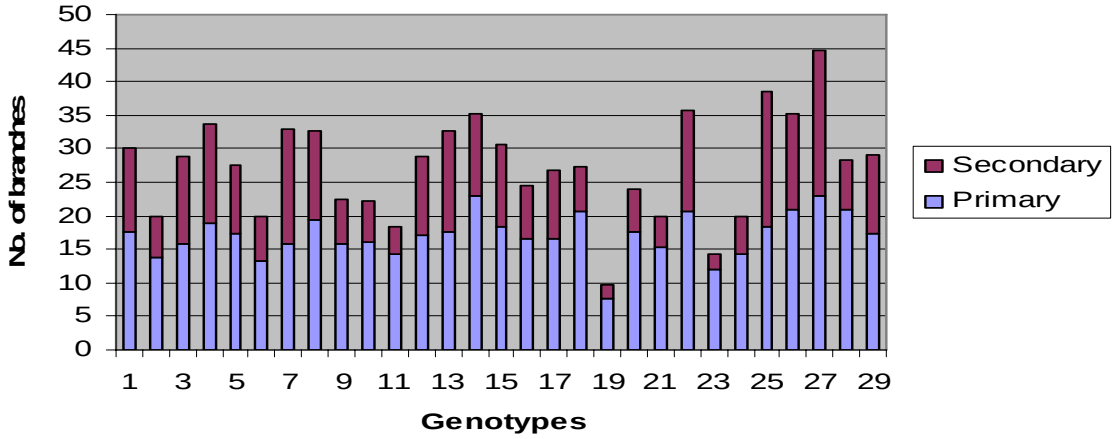


Figure 3.8b: Combined yield performance of pigeonpea genotypes at Bvumbwe Research Station in 2004/05 and Kandiya Research Station in 2006/07

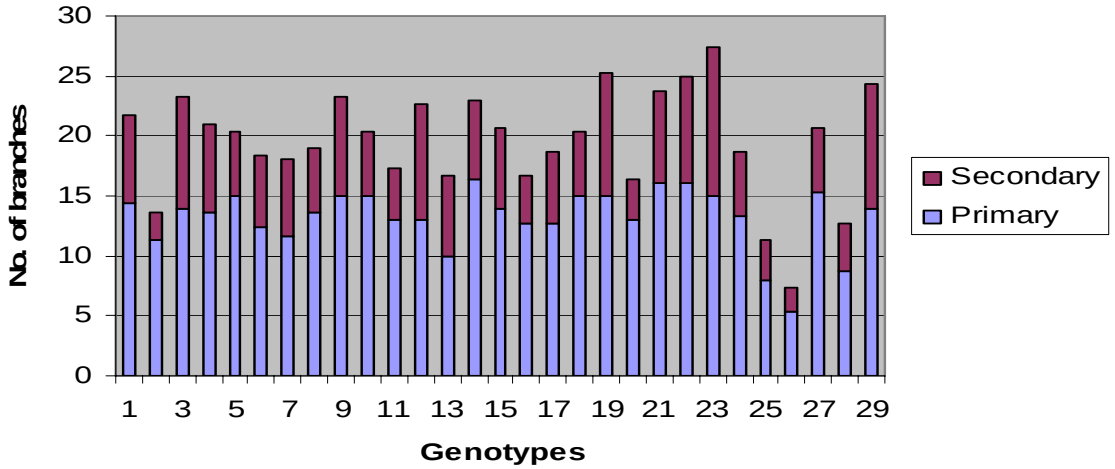
The number of primary and secondary branches varied significantly ($P < 0.05$) among the genotypes (Figure 3.9, Appendix 3.3). The number of primary branches plant^{-1} among genotypes ranged from 13.7 to 23. There was no specific trend between the medium and the long duration genotypes. The secondary branches ranged from 4 to 21.7 branches plant^{-1}. Most of the genotypes had a high number of both primary and secondary branches. Almost all the genotypes did not produce the tertiary branches.

Very few genotypes (less than 5) produced an insignificant number of tertiary branches whose data were not included in the analysis.



Legend: ***1****=AP1,* ***2****=AP2,* ***3****=AP3,* ***4****=AP4,* ***5****=AP5,* ***6****=AP6,* ***7****=AP7,* ***8****=AP8,* ***9****=AP9,* ***10****=AP10,* ***11****=ACC2253,* ***12****=ACC2291,* ***13****=ACC2298,* ***14****=ICP9145,* ***15****=ICEAP00040,* ***16****=ICEAP00068,* ***17****=KAT60/8, 18=ICEAP00020,* ***19****=AP19,* ***20****=ICEAP00554,* ***21****=ICEAP00540,* ***22****=ICPL87051,* ***23****=AP23,* ***24****=ICEAP00557,* ***25****=ICEAP00053,* ***26****=ICEAP00932,* ***27****=ICEAP00933,* ***28****=ICEAP00936,* ***29****=AP29.*

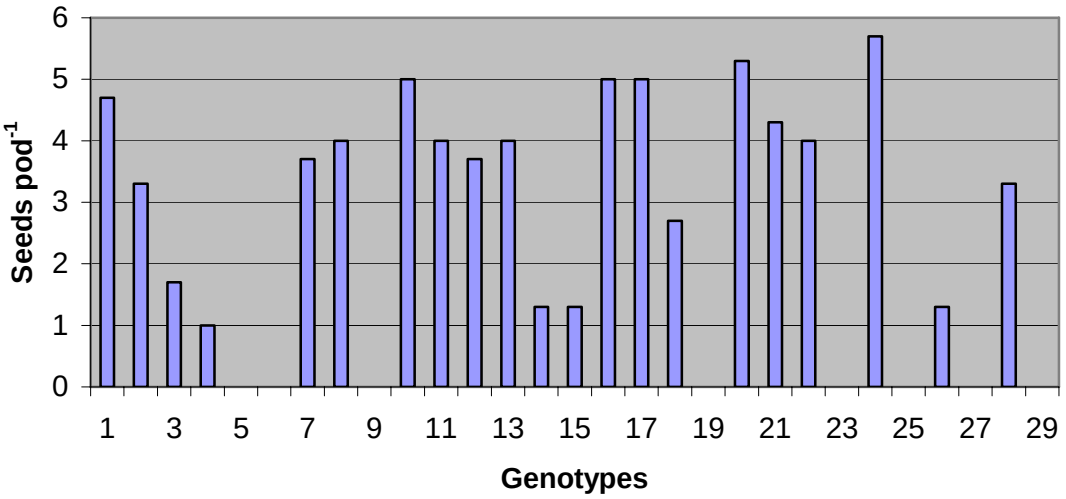
Figure 3.9a: Number of primary and secondary branches for pigeonpea genotypes at Kandiya during 2004/05



Legend: ***1****=AP1,* ***2****=AP2,* ***3****=AP3,* ***4****=AP4,* ***5****=AP5,* ***6****=AP6,* ***7****=AP7,* ***8****=AP8,* ***9****=AP9,* ***10****=AP10,* ***11****=ACC2253,* ***12****=ACC2291,* ***13****=ACC2298,* ***14****=ICP9145,* ***15****=ICEAP00040,* ***16****=ICEAP00068,* ***17****=KAT60/8, 18=ICEAP00020,* ***19****=AP19,* ***20****=ICEAP00554,* ***21****=ICEAP00540,* ***22****=ICPL87051,* ***23****=AP23,* ***24****=ICEAP00557,* ***25****=ICEAP00053,* ***26****=ICEAP00932,* ***27****=ICEAP00933,* ***28****=ICEAP00936,* ***29****=AP29.*

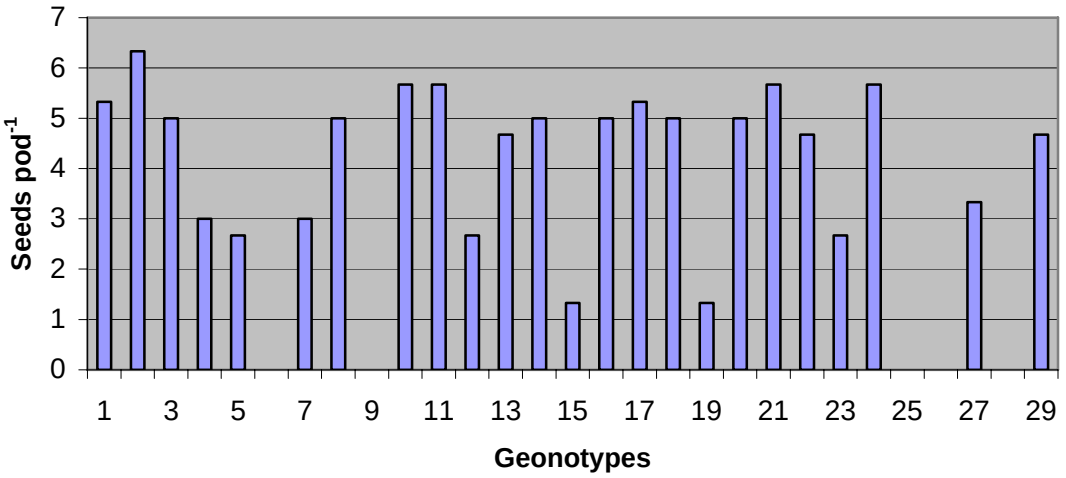
Figure 3.9b: Number of primary and secondary branches for pigeonpea genotypes at Kandiya during 2006/07

Variations were highly significant ($P < 0.001$) for the number of seeds pod^{-1} among the genotypes with a range of 1 to 5.7 seeds pod^{-1} (Figure 3.10, Appendix 3.4). In the first year, the following genotypes, in descending order, had a high number of seeds pod^{-1}: ICEAP00557, ICEAP00554, AP10, ICEAP00068, KAT60/8, and AP1. In the third year, AP2, AP10, ACC2253, ICEAP00540, and ICEAP00557 produced more seeds pod^{-1} than the rest. The combined analysis showed that ICEAP00557 had the highest number of seeds pod^{-1}. However, AP1, AP2, AP8 and AP10 were the only local landraces with more than 4 seeds pod^{-1}. Most of the medium duration genotypes from ICRISAT had a high number of seeds pod^{-1} (>5). Almost all the late duration genotypes had less than 4 seeds pod^{-1}.



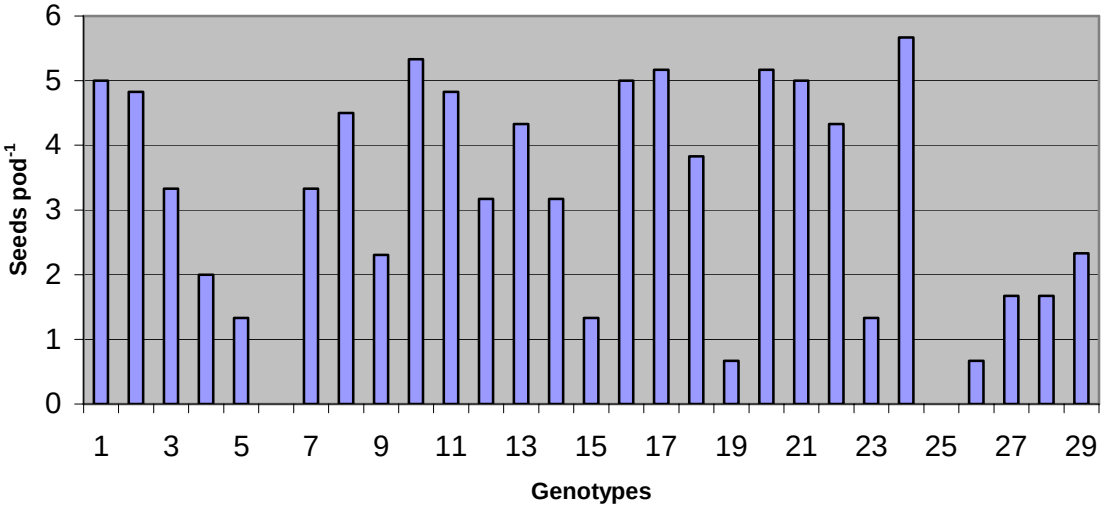
Legend: ***1****=AP1,* ***2****=AP2,* ***3****=AP3,* ***4****=AP4,* ***5****=AP5,* ***6****=AP6,* ***7****=AP7,* ***8****=AP8,* ***9****=AP9,* ***10****=AP10,* ***11****=ACC2253,* ***12****=ACC2291,* ***13****=ACC2298,* ***14****=ICP9145,* ***15****=ICEAP00040,* ***16****=ICEAP00068,* ***17****=KAT60/8, 18=ICEAP00020,* ***19****=AP19,* ***20****=ICEAP00554,* ***21****=ICEAP00540,* ***22****=ICPL87051,* ***23****=AP23,* ***24****=ICEAP00557,* ***25****=ICEAP00053,* ***26****=ICEAP00932,* ***27****=ICEAP00933,* ***28****=ICEAP00936,* ***29****=AP29.*

Figure 3.10a: Number of seeds per pod for pigeonpea genotypes at Kandiya Research Station in 2004/05 season



Legend: ***1****=AP1,* ***2****=AP2,* ***3****=AP3,* ***4****=AP4,* ***5****=AP5,* ***6****=AP6,* ***7****=AP7,* ***8****=AP8,* ***9****=AP9,* ***10****=AP10,* ***11****=ACC2253,* ***12****=ACC2291,* ***13****=ACC2298,* ***14****=ICP9145,* ***15****=ICEAP00040,* ***16****=ICEAP00068,* ***17****=KAT60/8,* *18=ICEAP00020,* ***19****=AP19,* ***20****=ICEAP00554,* ***21****=ICEAP00540,* ***22****=ICPL87051,* ***23****=AP23,* ***24****=ICEAP00557,* ***25****=ICEAP00053,* ***26****=ICEAP00932,* ***27****=ICEAP00933,* ***28****=ICEAP00936,* ***29****=AP29.*

Figure 3.10b: Number of seeds per pod for pigeonpea genotypes at Kandiya Research Station in 2006/07 season



Legend: ***1****=AP1,* ***2****=AP2,* ***3****=AP3,* ***4****=AP4,* ***5****=AP5,* ***6****=AP6,* ***7****=AP7,* ***8****=AP8,* ***9****=AP9,* ***10****=AP10,* ***11****=ACC2253,* ***12****=ACC2291,* ***13****=ACC2298,* ***14****=ICP9145,* ***15****=ICEAP00040,* ***16****=ICEAP00068,* ***17****=KAT60/8,* *18=ICEAP00020,* ***19****=AP19,* ***20****=ICEAP00554,* ***21****=ICEAP00540,* ***22****=ICPL87051,* ***23****=AP23,* ***24****=ICEAP00557,* ***25****=ICEAP00053,* ***26****=ICEAP00932,* ***27****=ICEAP00933,* ***28****=ICEAP00936,* ***29****=AP29.*

Figure 3.10c: Combined analysis of number of seeds per pod for pigeonpea genotypes at Kandiya Research Station over two seasons (2004/05 and 2006/07)

There were significant ($P < 0.001$) variations among the genotypes for 100-seed mass (g) (Figure 3.11, Appendix 3.4). In 2004/05 and 2005/06, the seed sizes ranged from 14.1 g to 23.4 g 100 seeds^{-1}. Except for three genotypes, ACC2298, ICPL87051, and ICEAP00933, all the genotypes registered seed size of >15 g 100 seeds^{-1}. AP9, one of the local landraces, registered the highest seed size of 23.4 g 100 seeds^{-1}. The second was ICEAP00040 (Kachangu) with 21.3 g 100 seeds^{-1}. AP8, ICEAP00932, and AP10 have equally large seed with 20.5 – 20.7 g 100 seeds^{-1} (Figures 3.11a and 3.11b). The results for 2006/07 have not been included because seed weight values were variable and very low due to missing plots.

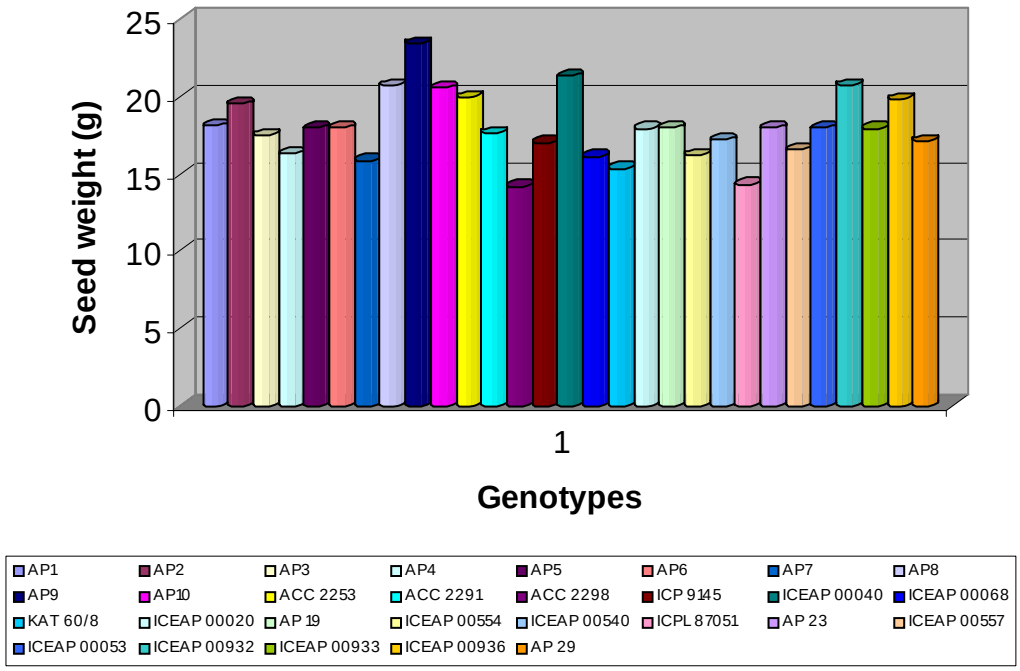


Figure 3.11a: 100-seed mass (g) for pigeonpea genotypes at Kandiya Research Station during 2004/05 season

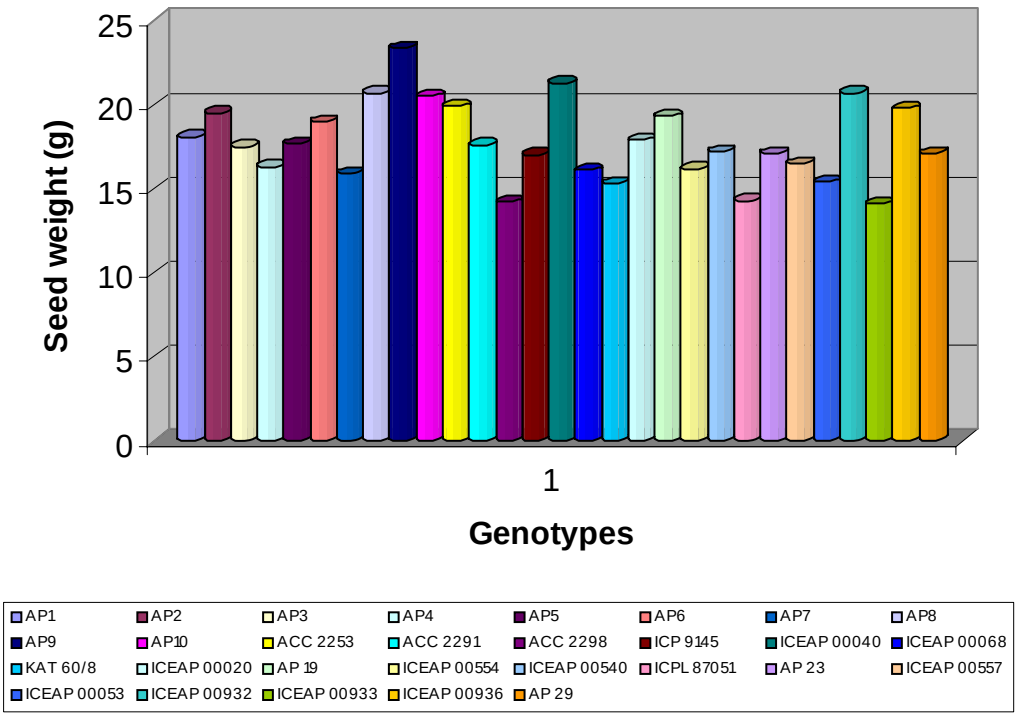


Figure 3.11b: 100-seed mass (g) for pigeonpea genotypes at Kandiya Research Station during 2006/07 season

The genotypes varied significantly ($P < 0.001$) for days to 50% flowering (Figure 3.12, Appendix 3.5) with a range of 79 to 164 d at Kandiya Research Station in Lilongwe. Most of the local landraces, with the exception of AP10 and AP8, took more than 120 d to reach 50% flowering. All the six medium duration genotypes from ICRISAT took less than 100 d to reach 50% flowering during the first season (Figure 3.12a). The trend was similar in the second and third years but the duration reduced by about 20 d (Figures 3.12b and 3.12c). In all three years, AP6, AP9, AP23, and AP29 flowered late. The pattern for days to 50% flowering is similarly shown in the combined analysis, with the addition of ICEAP00053 from ICRISAT (Figure 3.12d).

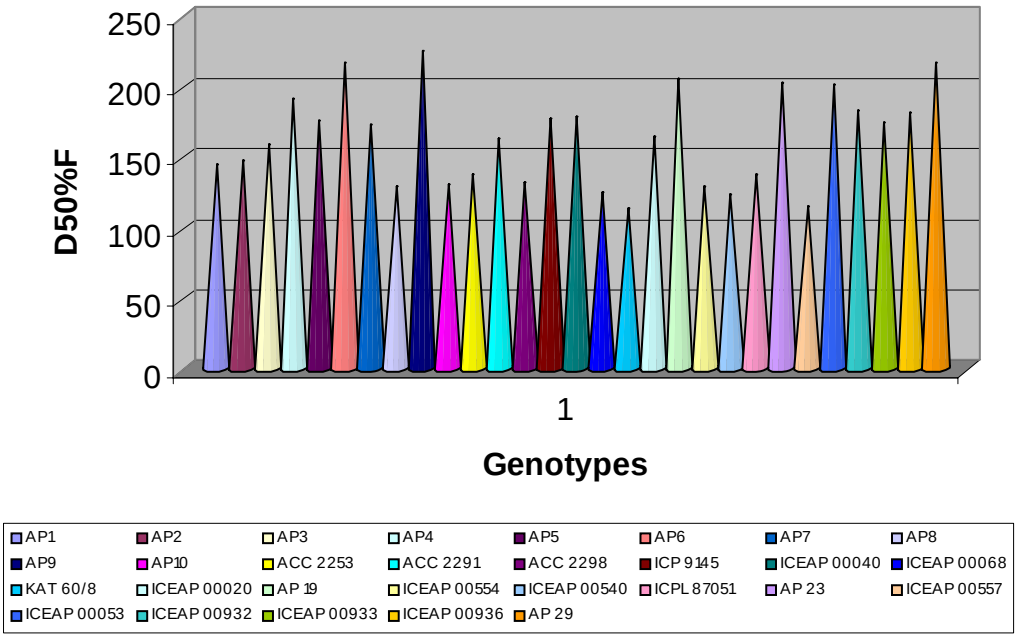


Figure 3.12a: Days to 50% flowering (D50%F) for pigeonpea genotypes at Kandiya Research Station during 2004/05 season

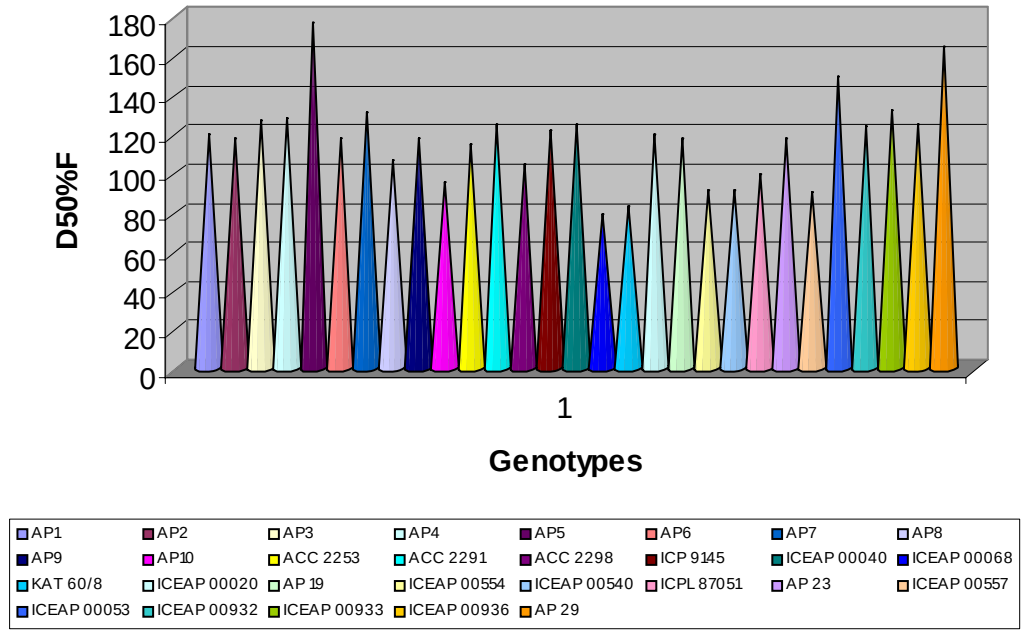


Figure 3.12b: Days to 50% flowering (D50%F) for pigeonpea genotypes at Kandiya Research Station during 2005/06 season

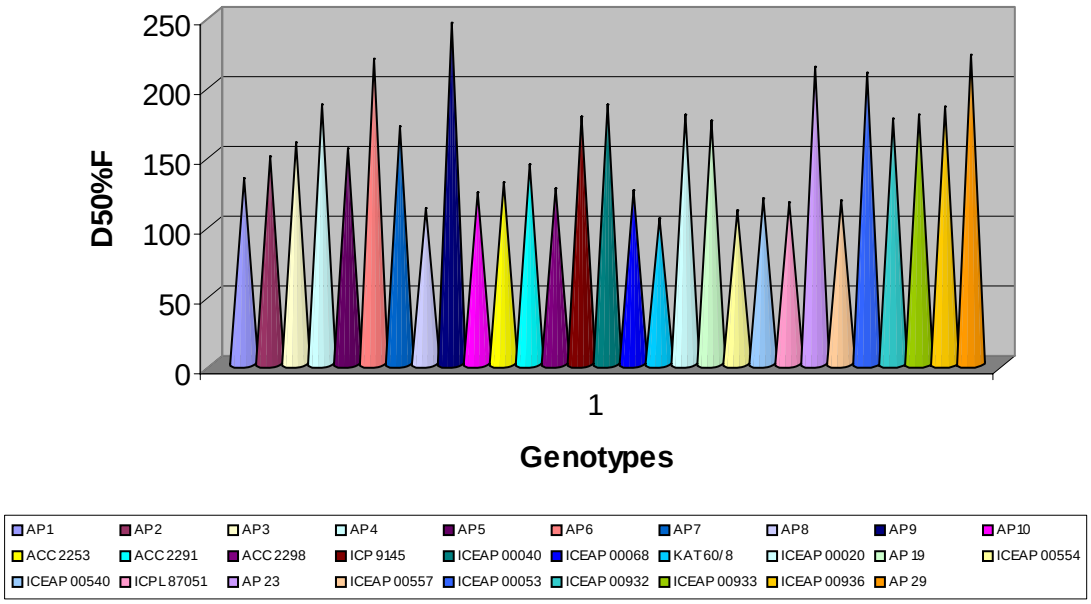


Figure 3.12c: Days to 50% flowering (D50%F) for pigeonpea genotypes at Kandiya Research Station during 2006/07 season

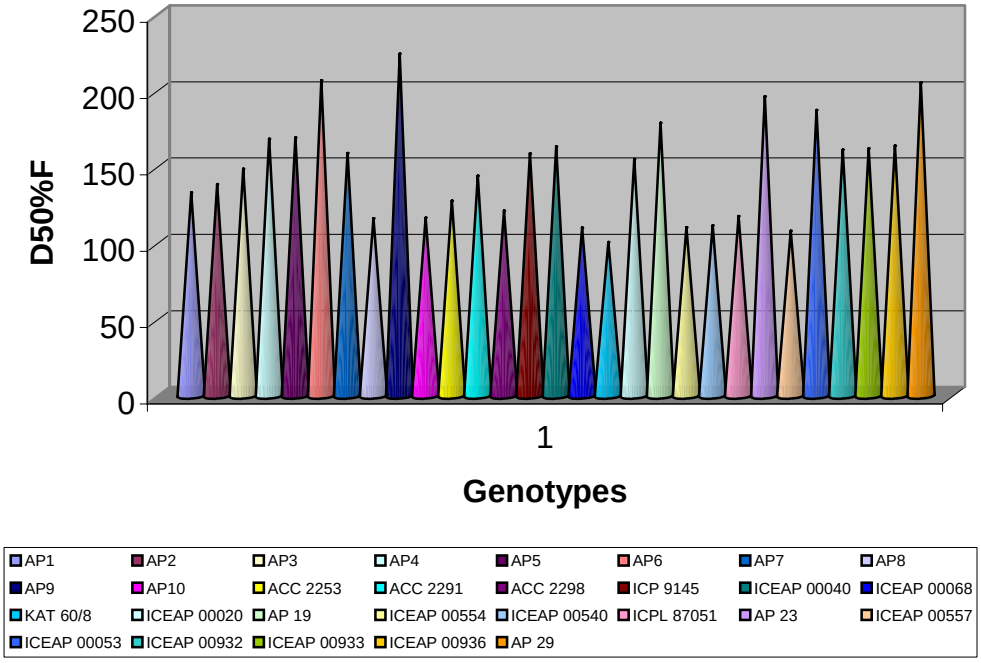


Figure 3.12d: Combined analysis of days to 50% flowering (D50%F) for pigeonpea genotypes at Kandiya Research Station during over three seasons (2004/05, 2005/06 and 2006/07) seasons

The genotypes significantly ($P < 0.001$) differed in their plant heights (Figure 3.13, Appendix 3.6). In 2004/05, ICP9145 produced the tallest plants at Kandiya Research

Station (Figure 3.13a), AP2 produced the tallest plants in 2006/07 (Figure 3.13b). At Bvumbwe Research Station in 2004/05, several genotypes produced tall plants, such as ICEAP00040, ICEAP00020, ICEAP00932, ICEAP00936, and AP29 (Figure 3.13c). The combined analysis over the two sites and three seasons showed that about nine genotypes produced tall plants (>2 m) (Figure 3.13c). The medium maturing genotypes from ICRISAT produced shorter plants than the late maturing plants. The trend was not clear among the local landraces.

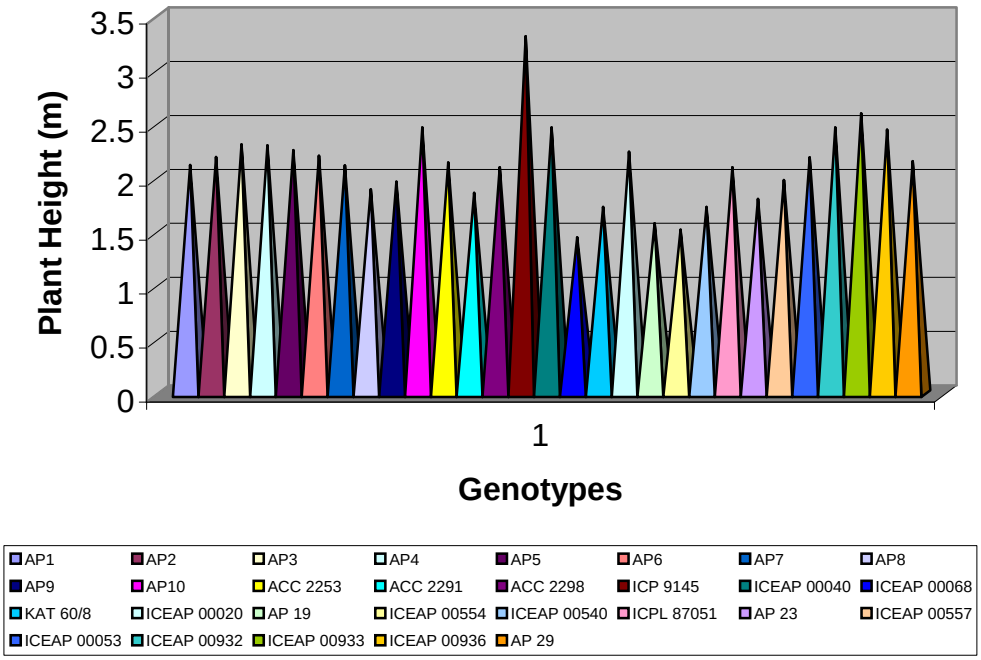


Figure 3.13a: Plant heights (m) for pigeonpea genotypes at Kandiya during 2004/05 season

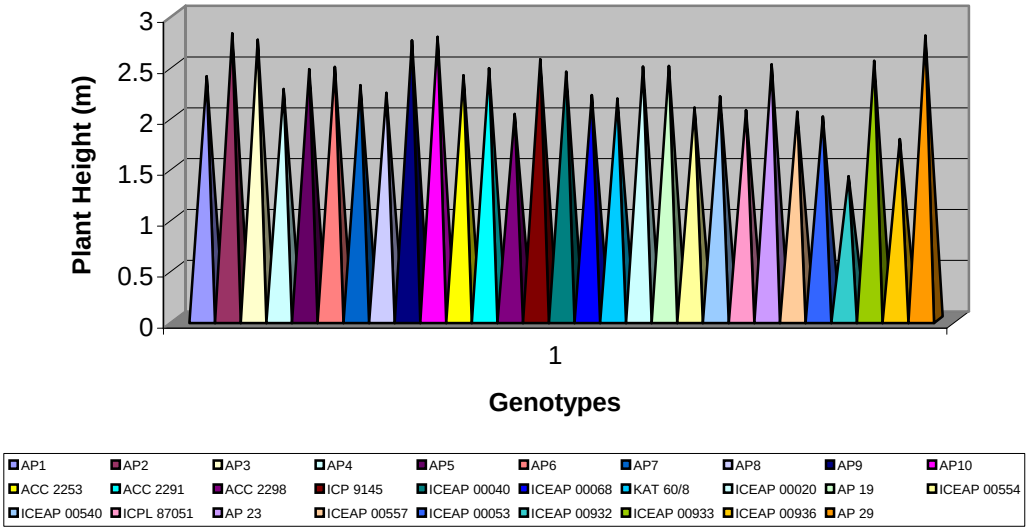


Figure 3.13b: Plant heights (m) for pigeonpea genotypes at Kandiya during 2006/07 season

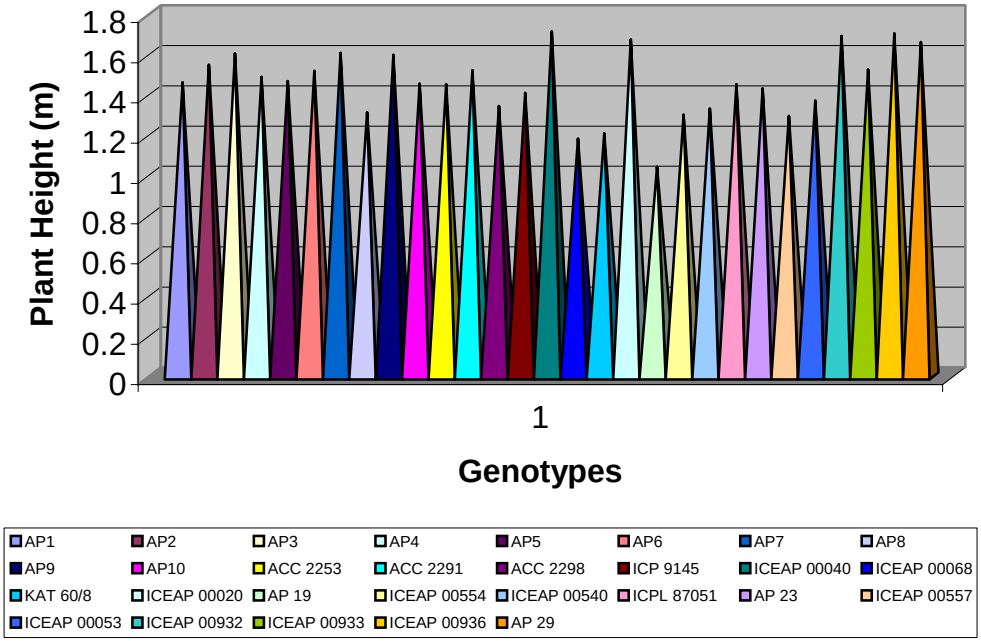


Figure 3.13c: Plant heights (m) for pigeonpea genotypes at Bvumbwe during 2004/05 season

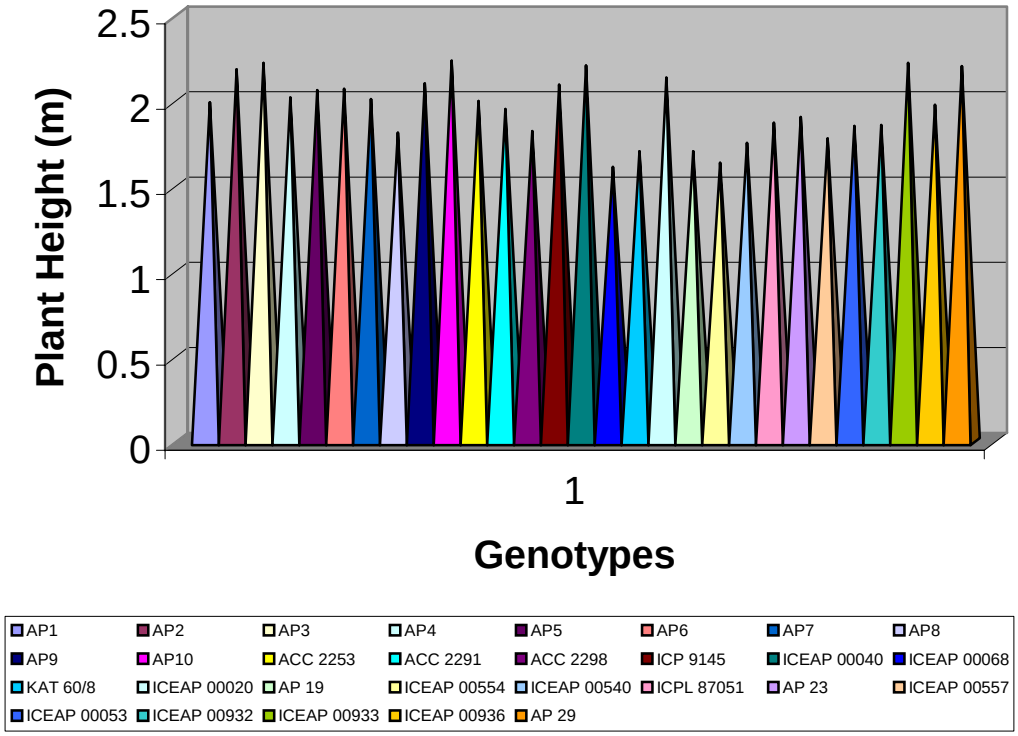


Figure 3.13d: Combined analysis of plant heights (m) for pigeonpea genotypes at Kandiya during 2004/05 and 2006/07 and at Bvumbwe during 2004/05 seasons

The genotypes differed significantly ($P < 0.001$) in stem thickness (diameter), which reflected plant vigour both at Kandiya and Bvumbwe Research Stations (Figure 3.14, Appendix 3.6). At Kandiya Research Station, stems ranged from 7 to 25 mm in diameter in 2004/05, with ICEAP00933 producing the thickest stem. Most of the genotypes, including all the local landraces, produced thick stems of more than 20 mm (Figure 3.14a). In 2006/07, the trend was similar to the first season except for a few landraces that produced less than 20 mm (Figure 3.14b). At Bvumbwe Research Station, the stems ranged from 11 to 15 mm in thickness (Figure 3.14c). Most of the landraces produced thick stems on average.

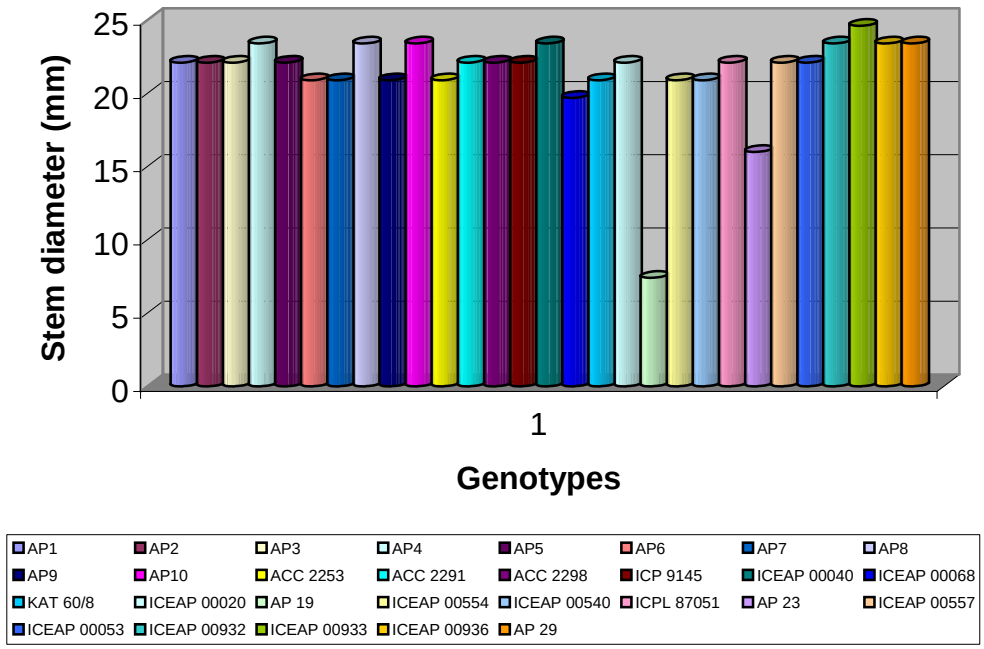


Figure 3.14: Stem thickness (mm) among pigeonpea genotypes at Kandiya Research Station during a) 2004/05; b) 2006/07; c) and at Bvumbwe Research Station during 2004/05

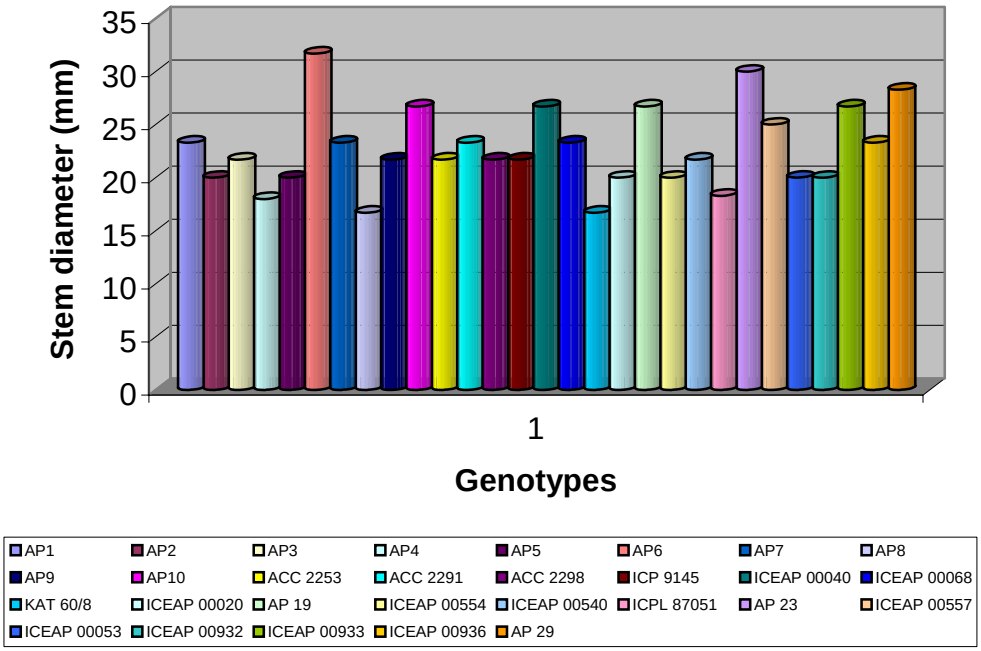


Figure 3.14: Stem thickness (mm) among pigeonpea genotypes at Kandiya Research Station during a) 2004/05; b) 2006/07; c) and at Bvumbwe Research Station during 2004/05

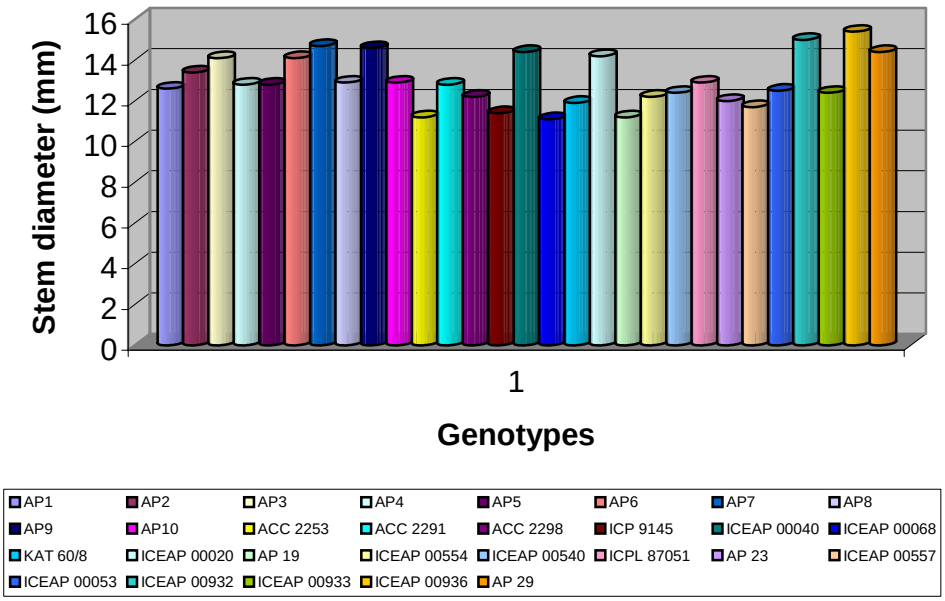


Figure 3.14: Stem thickness (mm) among pigeonpea genotypes at Kandiya Research Station during a) 2004/05; b) 2006/07; c) and at Bvumbwe Research Station during 2004/05

The local landraces also varied in their growth habit, flower, and stem colour (Table 3.2). All the landraces were semi-spreading, except for AP6 and AP7, which were spreading in their plant type. This shows that all the landraces were extensively branching. The stems were mostly green in colour, with a few having sun red colour (Figure 3.15). ICEAP00020 was the only genotype from ICRISAT which had a distinct purple stem with dark green leaves. The most common flower colour was yellow on the petals. The petals were variable in the density of the streaks, on the dorsal side of the flag, ranging from no streaks (plain) through dense streaks to union coverage of the second colour. AP4, AP8, and AP10 had red petals with union coverage on the dorsal side and yellow petals, while the rest had yellow petals with different densities and colours of the streaks (Figure 3.16). The pod colours were also variable, with pure green, mixed green and blackish, mixed green and brown, mixed green, and dark purple, purple, mixed green and red to dark purple. The colour and thickness of the stripes on the pods also varied among the genotypes (Figure 3.17).

Table 3.2: Phenotypic characteristics of the 29 pigeonpea genotypes

Genotype	**Phenotypic characteristics of the pigeonpea genotypes**			
	Plant habit	**Stem colour**	**Flower colour**	**Pod colour**
AP1	Semi spreading	Green and	Light green,	Green, dark purple

Genotype	Phenotypic characteristics of the pigeonpea genotypes			
	Plant habit	**Stem colour**	**Flower colour**	**Pod colour**
		sun red	medium purple streaks	stripes
AP2	Semi spreading	Green	Yellow, dense red streaks	Green, black stripes
AP3	Semi spreading	Green	Ivory	Green and dark purple
AP4	Semi spreading	Green	Yellow, sparse streaks	Green and purple
AP5	Semi spreading	Green	Yellow, medium purple streaks	Green and dark purple
AP6	Spreading	Green	Ivory	Green and brown stripes
AP7	Spreading	Green	Yellow, medium purple streaks	Green and dark purple pods
AP8	Semi spreading	Green	Deep yellow, union coverage	Brown
AP9	Semi spreading	Green	Yellow, sparse streaks	Green and brown stripes
AP10	Semi spreading	Green	Deep yellow, union coverage	Purple
ACC2253	Semi spreading	Green and sun red	Deep yellow, dense streaks	Green
ACC2291	Semi spreading	Green and sun red	Yellow, medium streaks	Green
ACC2298	Semi spreading	Sun red to purple	Ivory	Green
ICP9145	Semi spreading	Green and sun red	Ivory	Green
ICEAP00040	Erect and compact	Green	Ivory	Green
ICEAP00068	Semi spreading	Green	Ivory	Green
KAT60/8	Spreading	Green and sun red	Ivory	Light green
ICEA 00020	Erect and compact	Dark purple	Orange, dense red streaks	Green and dark purple
AP19	Semi spreading	Green and sun red	Yellow, union coverage	Green and dark purple
ICEAP00554	Semi spreading	Green	Ivory	Green
ICEAP00540	Spreading	Green	Ivory	Light green
ICPL87051	Spreading (unique)	Green	Yellow,	Green and black stripes
AP23	Semi spreading	Green	Yellow, medium red streaks	Green and purple
ICEAP00557	Semi spreading	Green and sun red	Yellow, medium purple streaks	Green and purple stripes
ICEAP00053	Erect and compact	Green	Ivory	Green
ICEAP00932	Erect and compact	Green	Ivory	Green and dark purple
ICEAP00933	Erect and compact	Green	Ivory	Green
ICEAP00936	Erect and compact	Green	Ivory	Green
AP29	Semi spreading	Green and sun red	Deep yellow, dense red streaks	Green and brown stripes

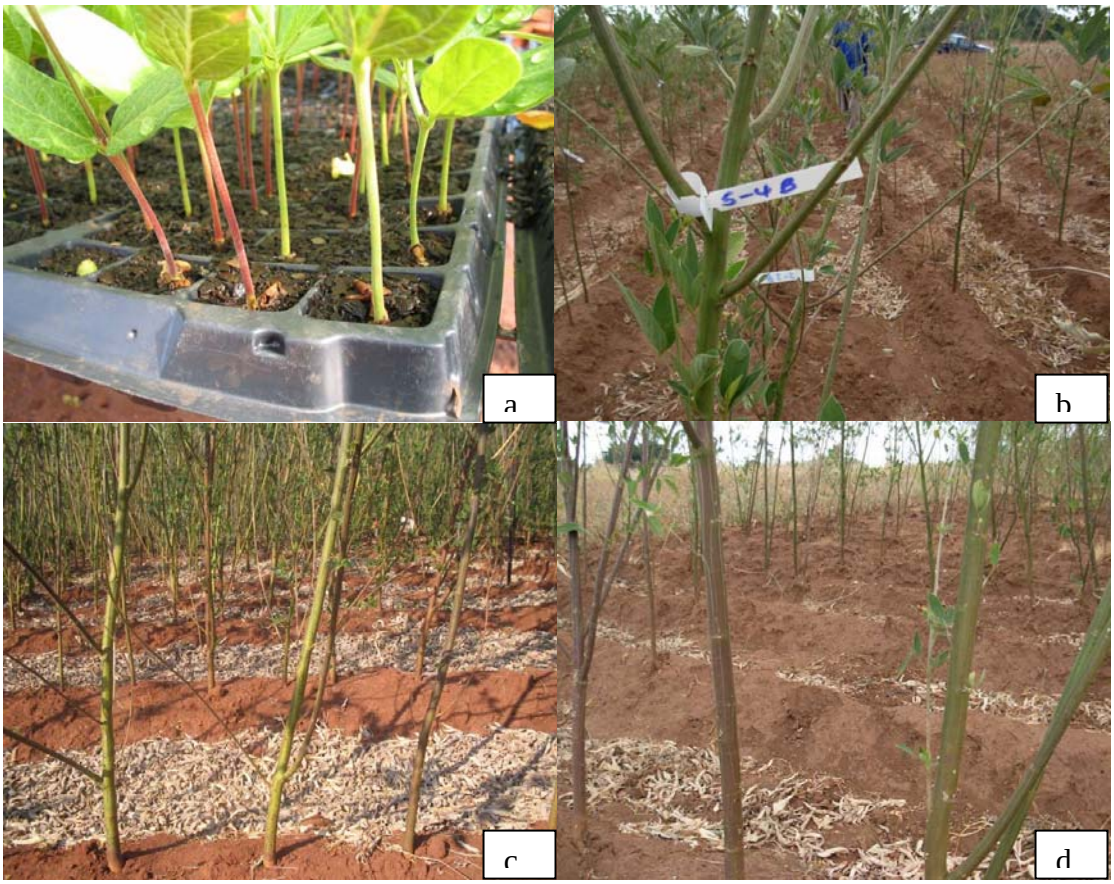


Figure 3.15 Pigeonpea stem colours a Bunda sick plot in 2005: a) Purple and green; b) Green; c) Green and sun red in the background; d) Sun red and green

Figure 3.16: Pigeonpea flower colours at Kandiya Research Station screen house, Malawi in 2005: a) Yellow and ivory with no streaks; b) Contrast between plain light yellow and with sparse streaks; c) Contrast between medium and dense streaks; d) Union coverage

Figure 3.17a: Pigeonpea pod colours at Kandiya Research Station screen house, Malawi in 2005: a) Green with black stripes, dark purple, brown, green, and black; b) Green with black stripes, dried, plain green and green with brown stripes

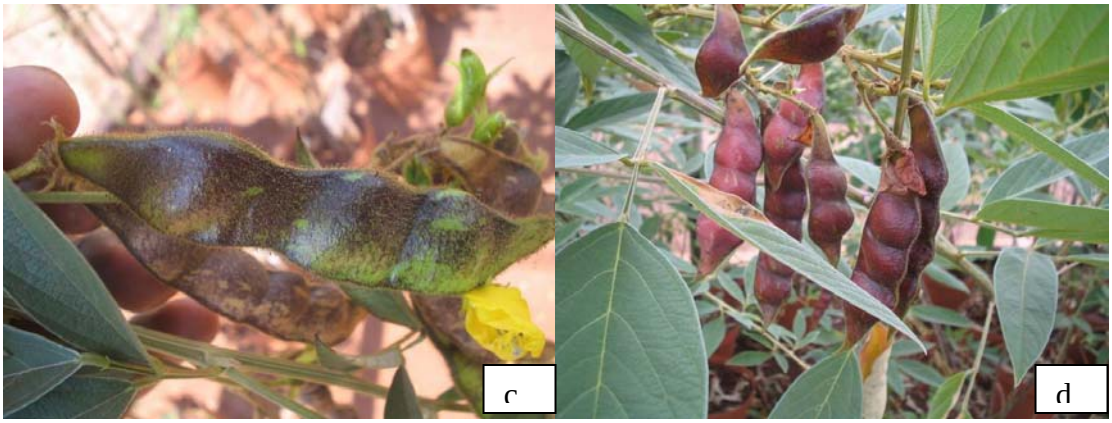


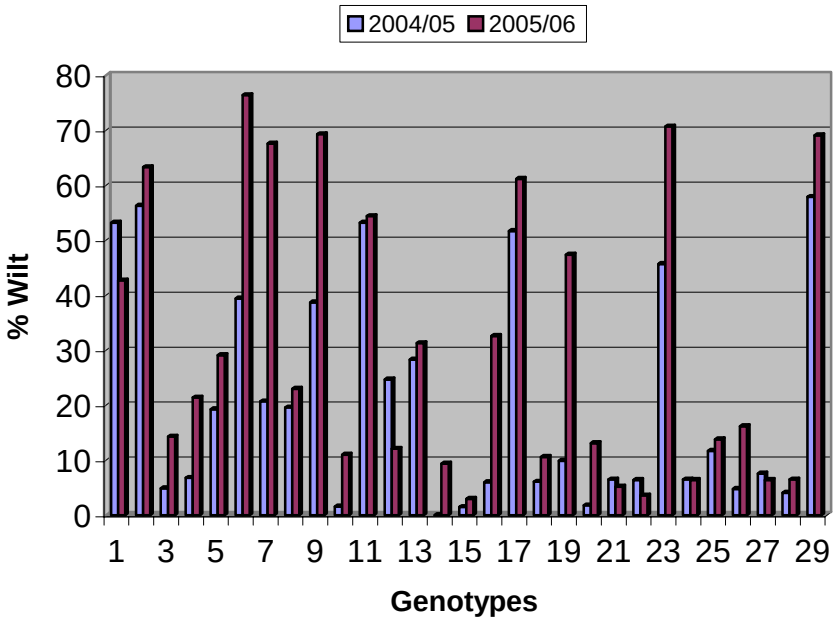
Figure 3.17b: Pigeonpea pod colours: c) Green black; d) Reddish brown

3.3.2 Screening of pigeonpea germplasm for Fusarium wilt disease resistance through natural infection

The results showed that genotypes varied significantly ($P < 0.001$) in their reaction to Fusarium wilt under natural infection, both at Bunda College and Bvumbwe Research Station sick plots (Figure 3.18, Appendix 3.7).

In the first year (2004/05), AP3, AP4, AP5, AP7, AP8, AP10, and AP19 showed resistance to Fusarium wilt (<20% wilt); AP6 and AP9 were moderately resistant (21 – 40% wilt); while the rest of the local landraces showed susceptibility (Figure 3.18). All three genotypes from the Department of Research (Malawian Government), were susceptible to the disease but the genotypes from ICRISAT showed a high level of resistance (<10% infection). However, KAT60/8 showed a high level of susceptibility to wilt, while ICEAP00068 was resistant to wilt.

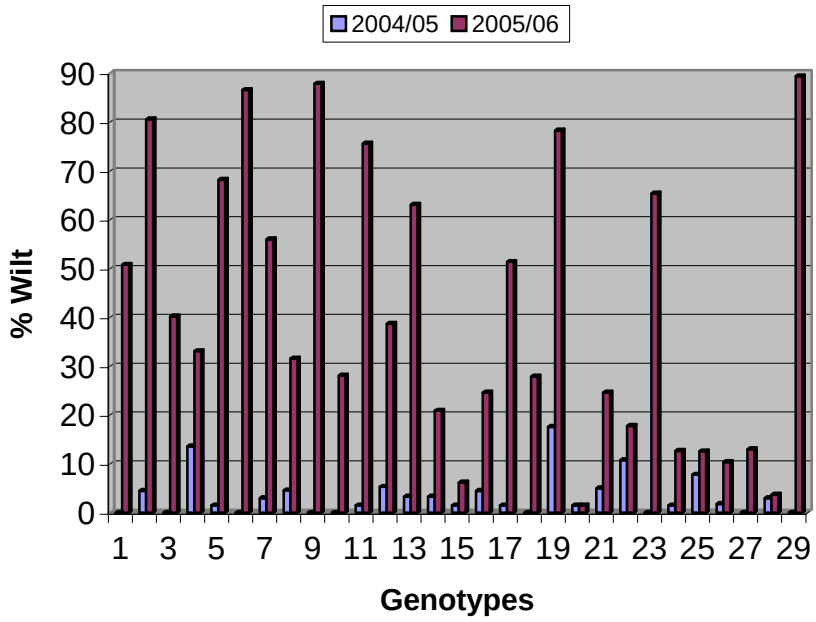
In the second year (2005/06), the disease pressure was higher than the first year, and most of the local landraces succumbed to the disease, with only AP3 and AP10 showing resistance (<20% wilt), while AP4, AP5, and AP8 were moderately resistant (21 – 40% wilt). ACC2291 from Department of Research also showed some degree of resistance. All the genotypes from ICRISAT with designated resistance showed resistance to the disease (< 20% wilt) (Nene and Kannaiyan, 1982). Both ICEAP00068 and KAT60/8 showed susceptibility to the disease. Except for AP1, all the genotypes showed higher disease incidence in the second year (2005/06) than in the first year (Figure 3.18).



Legend: ***1****=AP1,* ***2****=AP2,* ***3****=AP3,* ***4****=AP4,* ***5****=AP5,* ***6****=AP6,* ***7****=AP7,* ***8****=AP8,* ***9****=AP9,* ***10****=AP10,* ***11****=ACC2253,* ***12****=ACC2291,* ***13****=ACC2298,* ***14****=ICP9145,* ***15****=ICEAP00040,* ***16****=ICEAP00068,* ***17****=KAT60/8, 18=ICEAP00020,* ***19****=AP19,* ***20****=ICEAP00554,* ***21****=ICEAP00540,* ***22****=ICPL87051,* ***23****=AP23,* ***24****=ICEAP00557,* ***25****=ICEAP00053,* ***26****=ICEAP00932,* ***27****=ICEAP00933,* ***28****=ICEAP00936,* ***29****=AP29.*

Figure 3.18: Comparison in Fusarium wilt incidences of pigeonpea cultivars at the end of trial at Bunda sick plot between 2004/05 and 2005/06 seasons

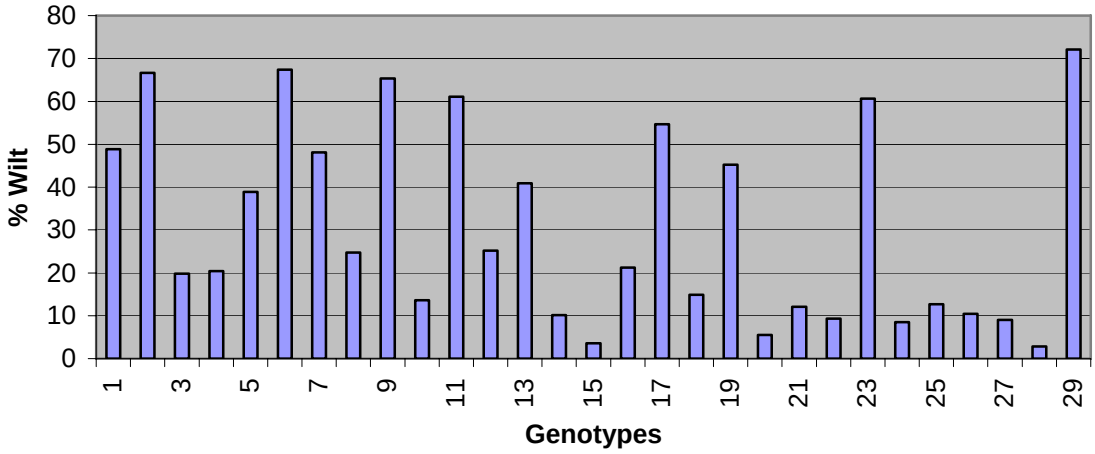
The disease pressure was very low in the first year at the Bvumbwe Research Station sick plot as evident in the reaction of the susceptible cultivars (KAT 60/8 and ICEAP 00068). Hence, there were no significant differences ($P > 0.05$) among the genotypes in their reaction to the disease. All genotypes seemed to be resistant to the disease (Figure 3.19). Eventually, the trial was converted to a yield evaluation trial. However, in the second year, significant variations ($P < 0.001$) were observed among the genotypes in their reaction to Fusarium wilt disease. The disease pressure was high and most of the local landraces showed high susceptibility to the disease. AP3, AP5, AP8, and AP10 showed moderate resistance to the disease. Most of the resistant genotypes from ICRISAT were resistant, except for ICP9145, ICEAP00020 and ICEAP00540, which succumbed to the disease. ICEAP00068 and KAT60/8 also showed susceptibility to wilt.



Legend: ***1****=AP1,* ***2****=AP2,* ***3****=AP3,* ***4****=AP4,* ***5****=AP5,* ***6****=AP6,* ***7****=AP7,* ***8****=AP8,* ***9****=AP9,* ***10****=AP10,* ***11****=ACC2253,* ***12****=ACC2291,* ***13****=ACC2298,* ***14****=ICP9145,* ***15****=ICEAP00040,* ***16****=ICEAP00068,* ***17****=KAT60/8,* *18=ICEAP00020,* ***19****=AP19,* ***20****=ICEAP00554,* ***21****=ICEAP00540,* ***22****=ICPL87051,* ***23****=AP23,* ***24****=ICEAP00557,* ***25****=ICEAP00053,* ***26****=ICEAP00932,* ***27****=ICEAP00933,* ***28****=ICEAP00936,* ***29****=AP29.*

Figure 3.19: Fusarium wilt incidences of pigeonpea genotypes at the end of trial at Bvumbwe sick plot in 2004/05 and 2005/06 seasons

The combined analysis for both the Bunda College and Bvumbwe Research Station sick plots over two seasons showed that genotypes varied significantly ($P < 0.001$) in their reaction to Fusarium wilt (Figure 3.20). The combined analysis excluded Bvumbwe in 2004/05 due to the low disease pressure at the sick plot. The results showed that among the local landraces AP3, AP4, and AP10 were resistant (<20% infection), AP5 and AP8 were moderately resistant (21 – 40%), while the rest were susceptible (>41% infection). The three genotypes from the Department of Research (Malawian Government) were susceptible to wilt, with the exception of ACC2291, which showed moderate resistance. Among ICRISAT genotypes, all were resistant to wilt, except for ICEAP00068 and KAT60/8

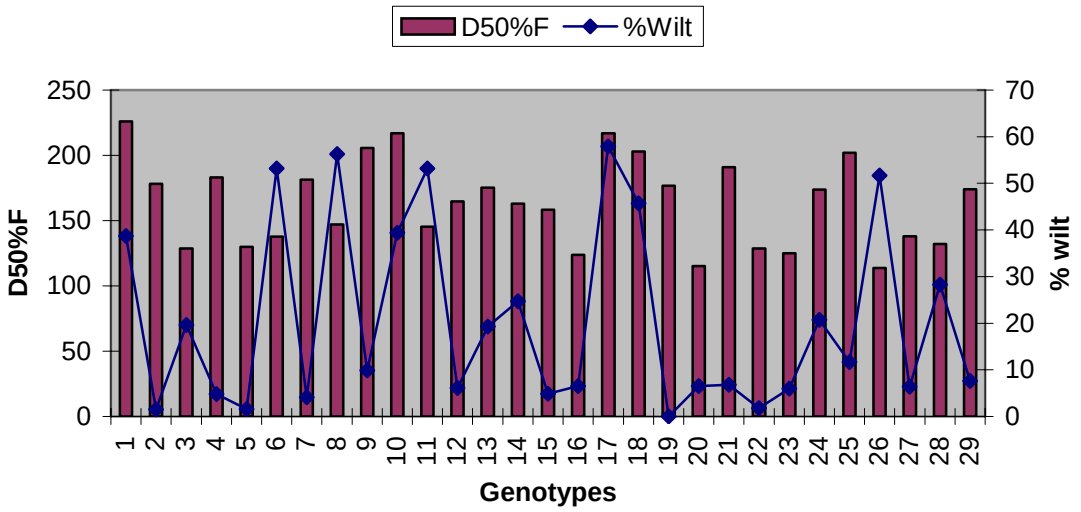


Legend: ***1****=AP1,* ***2****=AP2,* ***3****=AP3,* ***4****=AP4,* ***5****=AP5,* ***6****=AP6,* ***7****=AP7,* ***8****=AP8,* ***9****=AP9,* ***10****=AP10,* ***11****=ACC2253,* ***12****=ACC2291,* ***13****=ACC2298,* ***14****=ICP9145,* ***15****=ICEAP00040,* ***16****=ICEAP00068,* ***17****=KAT60/8, 18=ICEAP00020,* ***19****=AP19,* ***20****=ICEAP00554,* ***21****=ICEAP00540,* ***22****=ICPL87051,* ***23****=AP23,* ***24****=ICEAP00557,* ***25****=ICEAP00053,* ***26****=ICEAP00932,* ***27****=ICEAP00933,* ***28****=ICEAP00936,* ***29****=AP29.*

Figure 3.20: Combined analysis of Fusarium wilt reaction of pigeonpea genotypes over two sites and two seasons (excluding Bvumbwe in 2004/05)

There were no correlations between Fusarium wilt incidences and other characteristics of the pigeonpea genotypes. Figure 3.21 (Appendix 3.8) shows that there were no definite trends in the way pigeonpea genotypes reacted to Fusarium wilt in relation to maturity period (d to 50% flowering), yield, plant height, stem thickness, seed pod^{-1}, and seed mass. The genotypic reaction to wilt was not influenced by the pigeonpea descriptor and descriptor states.

The disease incidence varied among pigeonpea genotypes, depending on the inherent genetic resistance to Fusarium wilt (Appendix 3.9). The trend among susceptible cultivars was that the incidence increased sharply during the first three months then stabilized from the fourth month. In susceptible cultivars, more plants died during the early months (Figure 3.22a). In resistant cultivars, two trends were observed: either only a few plants died with time (Figure 3.22b), or a few plants died during the first 3 mo, and then increased from 4 mo up to harvesting (Figure 3.22c). The results showed that there were no pigeonpea cultivars which were immune to Fusarium wilt disease.



Legend: ***1****=AP 1,* ***2****=AP 2,* ***3****=AP 3,* ***4****=AP 4,* ***5****=AP 5,* ***6****=AP 6,* ***7****=AP 7,* ***8****=AP 8,* ***9****=AP 9,* ***10****=AP 10,* ***11****=ACC 2253,* ***12****=ACC 2291,* ***13****=ACC 2298,* ***14****=ICP 9145,* ***15****=ICEAP 00040,* ***16****=ICEAP 00068,* ***17****=KAT 60/8, 18=ICEAP 00020,* ***19****=AP 19,* ***20****=ICEAP 00554,* ***21****=ICEAP 00540,* ***22****=ICPL 87051,* ***23****=AP 23,* ***24****=ICEAP 00557,* ***25****=ICEAP 00053,* ***26****=ICEAP 00932,* ***27****=ICEAP 00933,* ***28****=ICEAP 00936,* ***29****=AP 29.*

Figure 3.21: Correlations between Fusarium wilt and days to 50% flowering in pigeonpea genotypes. (Other correlations are in Appendix 3.8)

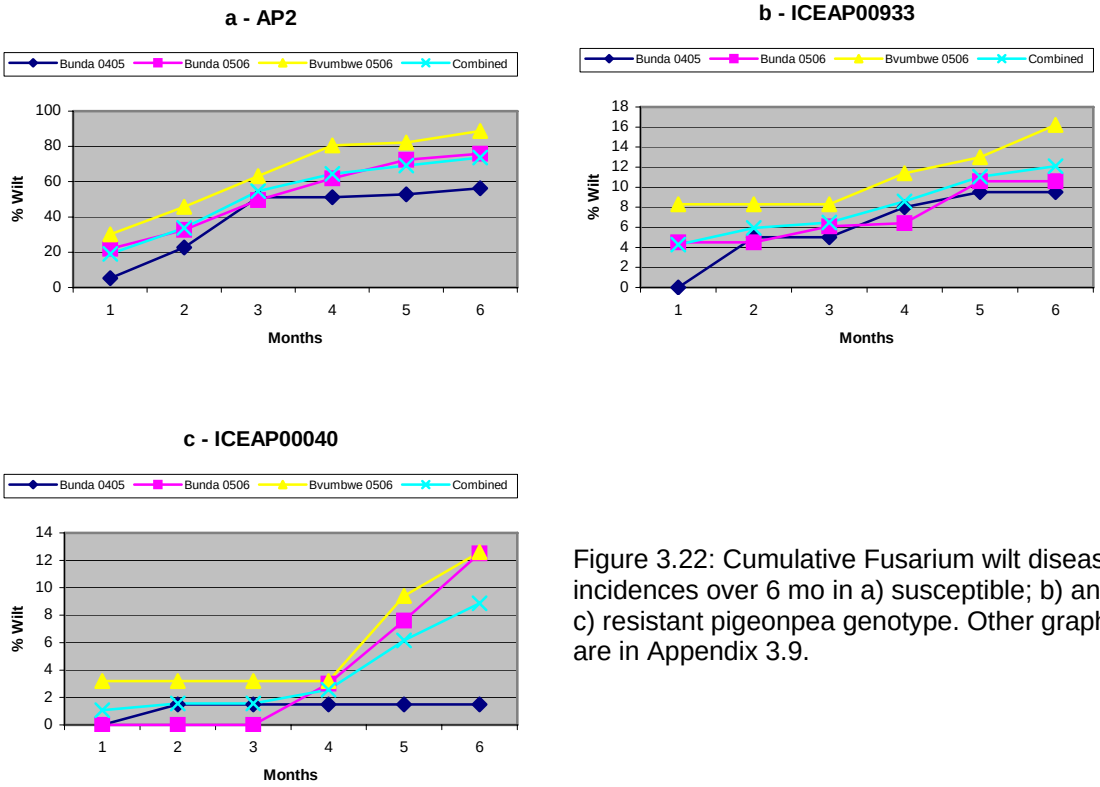


Figure 3.22: Cumulative Fusarium wilt disease incidences over 6 mo in a) susceptible; b) and c) resistant pigeonpea genotype. Other graphs are in Appendix 3.9.

3.4 Discussion and conclusion

The purpose of this research was to evaluate the Malawian pigeonpea germplasm for wilt resistance, performance of yield, and yield components. The assessment of wilt was done in sick plots with optimum levels of inoculum; the reaction of the germplasm was a true reflection of their resistance levels (except at Bvumbwe Research Station sick plot in the first season). The evaluation of yield and yield components was conducted in a disease-free environment. The results of the evaluation trials showed traits that can be improved through breeding.

The drought in 2004/05 affected the outcome of the yield trials, both at Kandiya and Bvumbwe Research Stations. However, the season favoured medium duration genotypes and these were the only ones that did better than the late duration genotypes, with higher yield levels than those in the year that followed. The medium duration genotypes escaped the drought. AP10, a local landrace, emerged as the highest yielder. This landrace is large seeded (20.5 g 100 seeds^{-1}) and has brown mottled seed. Many farmers prefer this landrace because it is early maturing compared to most of the other local landraces and ICRISAT cultivars. The late duration genotypes yielded poorly due to the drought. Pigeonpea yields well with a range of 600 - 1400 mm of rainfall annually, and drought is one of the factors that affects its production (Chauhan, 1990). The distribution of the rainfall in the first year was poor towards the end of the season. In the second season, the distribution of rains was better than in the first year favouring late duration genotypes because they require more water to support their maturation. However, many insect pests, despite pesticide spraying, affected the trial. The pests affected medium maturing more than late maturing genotypes because they flowered earlier than long maturing genotypes. Reed and Lateef (1990) reported similar results. Pest infestation calls for pigeonpea genotypes with resistance/tolerance to pests. In the third year, the rains were heavy and this affected yield. The results showed that most local landraces were low yielding because they yielded less than 1 t ha^{-1} on average. This might be attributed to low genetic potential and poor agronomic practices.

The combined yield analysis showed that there is great potential for high yields in the local landraces that needs to be exploited. AP10 emerged as the highest yielder and could be utilized well in areas with low rainfall. The results also showed that medium duration genotypes are good for Malawi because they yield well, even with less rain.

ICEAP00068 and KAT60/8 were high yielding genotypes that could be recommended (upon release) for Fusarium wilt disease-free areas. ICEAP00068 was released in Tanzania as Tumia for high yields, medium duration, and adaptation to diverse agro-ecological zones and cropping systems (Gwata et al., 2007). The medium duration genotypes which were resistant to wilt should be tested further for yield and secondary traits, and released for farmers' cultivation, if they are acceptable to farmers because of desirable attributes such as taste and cooking time. In addition, three medium duration genotypes (ICEAP00540, ICEAP00554 and ICEAP00557) were high and stable yielding and resistant to Fusarium wilt. These genotypes have shown potential in Kenya and Tanzania ([2]Dr Said Silim, personal communication, 2008) and should be evaluated for their potential in Malawi or used in the breeding programme.

Researchers (cited by Reddy, 1990) have established that the number of branches plant^{-1} is correlated to yield. The genotypes studied varied significantly in the number of both primary and secondary branches. The genotypes that produced more primary and secondary branches were likely to give high yields if they retained more pods with a high number of seeds. Because of this, breeders should select for a high number of primary and secondary branches during the breeding process.

Most of the local landraces in the present study were large seeded (>15 g 100 seeds^{-1}). Seed size is a very important trait, both at household and industry levels. Farmers preferred large-seeded genotypes as food. During processing into dhal (split cotyledons), the small seeds tend to escape processing, which adds extra expense in the form of reprocessing or manual regrading. Medium and large seeds are easy to process, and they have potential in the canning industry for canned green peas (van der Maesen, 1990). Most of the local Malawian landraces have this potential for the processing and canning industry. Breeders should thus select for large seeds during selection.

Pigeonpea cultivars are divided into maturity groups based on days to 50% flowering (Reddy, 1990). The results show that most of the local landraces, with exception of a few (AP8 and AP10), were late maturing types, taking 9 to 10 mo to mature. Most of the local landraces mature at the same time, if not later, than the two varieties (ICP9145 and

[2] Dr Said N. Silim, ICRISAT, Nairobi, Kenya

ICEAP00040) released in the country. Some of the landraces (AP9) were very late maturing; 1 – 2 mo later than the released varieties. Late maturing varieties are likely to give yield in southern Malawi where there is winter rainfall (Chiperoni), however, improved varieties need to be slightly earlier than the present landraces. For central and northern Malawi, a balance needs to be achieved, realizing that the best varieties should be medium duration but which can be intercropped with maize or groundnuts, and flower when temperatures are getting cooler so as to escape field pests.

The variations in plant height (m) both at Kandiya and Bvumbwe Research stations showed the potential for selection among the various genotypes for the cropping systems. Singh et al. (1996) and Pandey et al. (1998) reported similar results. Genotypes with tall plants are normally late maturing (Reddy, 1990). There were no short genotypes among the entries. Tall plants are also good for firewood. However, the differences between Kandiya and Bvumbwe in the height of plants grown there reflect the effects of genotype-by-environment interactions. Most likely, it is the effect of temperature because the cooler the area the shorter the plants (Lawn and Troedson, 1990).

Stem diameter signifies plant vigour and it shows potential for selection among the genotypes. A bigger stem shows the ability of a plant to support a large number of branches and pods, as some of assimilates are diverted to the stem to support pod set (Lawson and Troedson, 1990). The difference in stem diameters among the pigeonpea genotypes shows that some genotypes have the potential for supplying more fuel wood and other construction materials than others. Breeders should select for bigger stems during the breeding process for plant vigour.

The results showed that most of the local landraces, with the exception of three, AP3, AP4 and AP10, were susceptible to Fusarium wilt. However, farmers selected all these local landraces due to certain desirable attributes. The resistant landraces should therefore be evaluated further for wilt and other traits, and can be released directly if they prove to be suitable; or they could serve as sources of resistance in the breeding programme. The results showed that there were sources of Fusarium wilt resistance among the landraces which should be exploited. Despite the resistance in ICRISAT cultivars, it is important to assess their suitability for consumption and marketability.

ICEAP00068 and KAT60/8 were reported to be susceptible to wilt in Kenya. However, the results showed that ICEAP00068 is less susceptible than KAT60/8 in Malawi, which made it an unsuitable indicator for wilt susceptibility in this trial. There were several local landraces which were highly susceptible such as AP29, AP9, and AP6, that can still be used as susceptible checks in wilt trials.

The genotypes have exhibited significant variations ($P < 0.001$) for various yield and yield components, including descriptors such as days to 50% flowering, plant structure (height), seeds pod^{-1}, seed weight, stem diameter, and reactions to Fusarium wilt disease. These variations form a basis for morphological and genetic improvements. There were no correlations between Fusarium wilt incidence and phenotypic characteristics which showed that wilt is not genetically or phenotypically influenced by the plant descriptors. The pattern of disease incidence between the wilt susceptible and resistant genotypes implies that resistance to wilt in pigeonpea should remain high from the seedling stage.

The local landraces showed considerable weaknesses: susceptibility to Fusarium wilt, late maturity, tall plants, longer days to 50% flowering, and low yields. From the evaluation results, these landraces need to be improved through breeding. The recommendation is, therefore, to breed for Fusarium wilt resistance, early maturation, high yield, more seed pod^{-1}, large seeds, and medium plant in the selected local landraces while maintaining the farmer- and consumer-preferred traits. Hybridization with improved genotypes which possess the missing traits can achieve this through pedigree breeding.

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Appendices

Appendix 3.1: Weather information for the 2004/05 and 2005/06 seasons

Month	Rainfall distribution (mm)		
	2004/05	2005/05	2006/07
October	41	0	0
November	153	46	151.3
December	318	170	196.5
January	240	199	401.5
February	98	121	189.5
March	61	214	106
April	0	47	31.5
May	13	0	0
Total	924	797	1076

Appendix 3.2: Yield performance of pigeonpea genotypes at Kandiya and Bvumbwe Research, Malawi

Genotype	Kandiya		Combined for (1) & (2)	Kandiya	Bvumbwe	Combined for (3) & (4)
	2004/05 (1)	2005/06 (2)		2006/07 (3)	2004/05 (4)	
AP1	6.16[a] (550) [b]	7.75[a](2518)[b]	6.96 [a] (1534) [b]	4.00 [a] (83) [b]	5.839 [a] (370)[b]	4.92 [a] (226) [b]
AP2	6.52 (900)	7.37 (1852)	6.94 1376)	6.13 (524)	6.786 (895)	6.46 (710)
AP3	3.36 (75)	7.33 (1521)	5.34 (798)	2.91 (17)	6.781 (912)	4.846 (464)
AP4	3.41 (90)	7.96 (2883)	5.69 (1486)	3.20 (45)	7.319 (1515)	5.257 (780)
AP5	2.30 (0)	5.29 (250)	3.79 (125)	2.68 (7)	6.486 (683)	4.583 (345)
AP6	2.30 (0)	5.63 (968)	3.96 (484)	2.30 (0)	6.652 (772)	4.477 (386)
AP7	5.55 (265)	7.18 (1544)	6.36 (904)	3.75 (52)	7.031 (1221)	5.389 (636)
AP8	6.95 (1104)	6.63 (806)	6.79 (955)	4.97 (332)	6.996 (1155)	5.985 (743)
AP9	2.30 (0)	5.52 (831)	3.91 (416)	2.30 (0)	6.866 (1004)	4.584 (502)
AP10	8.56 (5389)	7.19 (1456)	7.87 (3422)	6.62 (771)	7.105 (1258)	6.863 (1015)
ACC2253	5.08 (454)	7.81 (2590)	6.45 (1522)	5.70 (318)	5.869 (404)	7.787 (361)
ACC2291	5.77 (326)	7.71 (2285)	6.74 (1305)	2.80 (12)	7.012 (1119)	4.907(565)
ACC2298	7.61 (2510)	7.32 (1605)	7.47 (2058)	5.08 (248)	5.666 (624)	5.371 (436)
ICP9145	3.69 (67)	7.80 (2488)	5.74 (1278)	4.54 (125)	6.744 (861)	5.642 (493)
ICEAP00040	4.09 (122)	6.89 (1335)	5.49 (729)	3.56 (143)	7.364 (1678)	5.464 (911)
ICEAP00068	8.26 (4058)	7.53 (1846)	7.89 (2952)	5.62 (390)	6.894 (1070)	6.258 (730)
KAT60/8	8.11 (3378)	7.26 (1525)	7.68 (2451)	6.66 (934)	7.135 (1254)	6.899 (1094)
ICEAP00020	3.83 (60)	6.96 (1260)	5.40 (660)	3.98 (84)	7.288 (1536)	5.635 (810)
AP19	2.30 (0)	7.05 (1541)	4.67 (770)	2.73 (9)	2.303 (0)	2.517 (4)
ICEAP00554	8.02 (3053)	6.81 (1337)	7.42 (2195)	5.52 (287)	7.116 (1248)	6.316 (768)
ICEAP00540	7.86 (3092)	7.59 (2036)	7.72 (2564)	6.54 (690)	7.016 (1106)	6.779 (898)
ICPL87051	6.74 (851)	6.94 (1152)	6.84 (1001)	4.10 (65)	7.191 (1437)	5.647 (751)
AP23	2.30 (0)	5.63 (975)	3.96 (488)	3.45 (32)	5.232 (552)	4.342 (292)
ICEAP00557	7.64 (2773)	7.45 (1765)	7.55 (2269)	5.99 (508)	6.924 (1014)	6.459 (761)
ICEAP00053	2.30 (0)	3.33 (70)	2.82 (35)	2.30 (0)	6.249 (556)	4.276 (278)
ICEAP00932	3.83 (88)	5.01 (737)	4.42 (412)	2.30 (0)	7.394 (1647)	4.848 (823)
ICEAP00933	2.30 (0)	6.67 (929)	4.49 (465)	3.33 (24)	7.042 (1243)	5.185 (634)
ICEAP00936	4.03 (596)	7.54 (2187)	5.79 (1391)	2.74 (9)	7.495 (1841)	5.119 (925)
AP29	3.31 (65)	5.63 (270)	4.47 (167)	2.80 (12)	5.939 (732)	4.371 (372)
Mean	**4.98 (1030)**	**6.79 (1468)**	**5.88 (1249)**	**4.09 (197)**	**6.611 (1024)**	**5.351 (611)**
LSD	**1.869****	**1.90****	**1.676****	**1.652****	**1.1961****	**1.3643****
CV (%)	**22.9**	**17.1**	**24.9**	**24.7**	**11.1**	**22.3**

[a]Data transformed using Log_e; [b]Untransformed data in brackets; ** Significant at P < 0.001;

Appendix 3.3: Variations in number of primary and secondary branches for pigeonpea genotypes

Genotype	Number of branches at Kandiya				
	Primary branches			Secondary branches	
	2004/05	2006/07	Combined analysis	2004/05	2006/07
AP1	17.7	14.33	16	3.080[a] (12.3)[b]	2.850 [a] (7.33) [b]
AP2	13.7	11.33	12.5	2.781 (6.3)	2.502 (2.33)
AP3	15.7	14	14.83	3.125 (13)	2.957 (9.33)
AP4	19.0	13.67	16.33	3.104 (14.7)	2.848 (7.33)
AP5	17.3	15	16.17	2.996 (10.3)	2.724 (5.33)
AP6	13.3	12.33	12.83	2.740 (6.7)	2.761 (6)
AP7	15.7	11.67	13.67	3.256 (17.3)	2.781 (6.33)
AP8	19.3	13.67	16.50	3.144 (13.3)	2.693 (5.33)
AP9	15.7	15	15.33	2.813 (6.7)	2.902 (8.33)
AP10	16.0	15	15.50	2.781 (6.3)	2.730 (5.33)
ACC2253	14.3	13	13.67	2.632 (4)	2.642 (4.33)
ACC2291	17.0	13	15	3.073 (11.7)	2.946 (9.67)
ACC2298	17.7	10	13.83	3.201 (15)	2.665 (6.67)
ICP9145	23.0	16.33	19.67	3.072 (12.3)	2.811 (6.67)
ICEAP00040	18.3	14	16.17	3.090 (12.3)	2.794 (6.67)
ICEAP00068	16.7	12.67	14.67	2.821 (7.7)	2.637 (4)
KAT60/8	16.7	12.67	14.67	2.964 (10)	2.746 (6)
ICEAP00020	20.7	15	17.83	2.808 (6.7)	2.727 (5.33)
AP19	7.7	15	11.33	2.466 (2.1)	3.005 (10.33)
ICEAP00554	17.7	13	15.33	2.788 (6.3)	2.590 (3.33)
ICEAP00540	15.3	16	15.67	2.679 (4.7)	2.860 (7.67)
ICPL87051	20.7	16	18.33	3.164 (15)	2.932 (9)
AP23	12.0	15	13.50	2.538 (2.3)	3.099 (12.33)
ICEAP00557	14.3	13.33	13.83	2.744 (5.7)	2.716 (5.33)
ICEAP00053	18.3	8	13.17	3.399 (20.3)	2.578 (3.33)
ICEAP00932	21.0	5.33	13.17	3.173 (14.3)	2.476 (2)
ICEAP00933	23.0	15.33	19.17	3.397 (21.7)	2.712 (5.33)
ICEAP00936	21.0	8.67	14.83	2.848 (7.3)	2.634 (4)
AP29	17.3	14	15.67	3.071 (11.7)	2.998 (10.33)
Mean	**17.10**	**13.18**	**15.14**	**2.957 (10.28)**	**2.770 (6.32)**
LSD	**4.72****	**4.884***	**4.072***	**0.4301***	**0.2948***
CV (%)	**16.9**	**22.6**	**23.6**	**8.9**	**6.5**

*Significant at P < 0.01; ** Significant at P < 0.001;

[a]Data transformed for secondary branches only using Log_e (x+c, where c = 10);

[b]Untransformed data in brackets for secondary branches

Appendix 3.4: Variations in seed pod^{-1} and seed weight for pigeonpea genotypes

Genotypes	Seed pod^{-1}			Seed weight (100 seed weight g)		
	Kandiya		Combined analysis	Kandiya		
	2004/05	2006/07		2004/05	2005/06	2006/07
AP1	2.685[a] (4.7)[b]	2.728[a] (5.33)[b]	2.707[a] (5.0)[b]	18.07	18.1	3.292[a] (10.47)[b]
AP2	2.573 (3.3)	2.793 (6.33)	2.683 (4.83)	19.50	19.5	3.437 (18.93)
AP3	2.438 (1.7)	2.708 (5)	2.573 (3.33)	17.47	17.5	2.613 (4.60)
AP4	2.390 (1)	2.550 (3)	2.470 (2)	16.27	16.3	3.312 (5.30)
AP5	2.303 (0)	2.527 (2.67)	2.415 (1.33)	17.96	17.7	2.303 (5)
AP6	2.303 (0)	2.303 (0)	2.303 (0)	17.96	19.0	2.641 (0)
AP7	2.614 (3.7)	2.550 (3)	2.582 (3.33)	15.87	15.9	3.220 (9)
AP8	2.639 (4)	2.708 (5)	2.674 (4.50)	20.70	20.7	3.492 (19.87)
AP9	2.303 (0)	2.303 (0)	2.303 (0)	23.43	23.4	2.713 (0)
AP10	2.707 (5)	2.751 (5.67)	2.729 (5.33)	20.53	20.5	3.405 (20.40)
ACC2253	2.637 (4)	2.751 (5.67)	2.694 (4.83)	19.93	19.9	3.270 (16.93)
ACC2291	2.613 (3.7)	2.527 (2.67)	2.570 (3.17)	17.63	17.6	3.360 (4.50)
ACC2298	2.639 (4)	2.685 (4.67)	2.662 (4.33)	14.17	14.2	2.969 (15.40)
ICP9145	2.415 (1.3)	2.707 (5)	2.561 (3.17)	17.00	17	2.636 (16.73)
ICEAP00040	2.415 (1.3)	2.415 (1.33)	2.415 (1.33)	21.30	21.3	2.993 (6.70)
ICEAP00068	2.707 (5)	2.708 (5)	2.707 (5)	16.13	16.1	3.252 (16.73)
KAT60/8	2.708 (5)	2.730 (5.33)	2.719 (5.17)	15.33	15.3	3.274 (15.30)
ICEAP00020	2.527 (2.7)	2.708 (5)	2.618 (3.83)	17.93	17.9	2.680 (11.67)
AP19	2.303 (0)	2.415 (1.33)	2.359 (0.67)	17.96	19.3	2.620 (5.27)
ICEAP00554	2.730 (5.3)	2.708 (5)	2.719 (5.17)	16.20	16.2	3.217 (16.37)
ICEAP00540	2.662 (4.3)	2.751 (5.67)	2.707 (5)	17.20	17.2	3.249 (15.80)
ICPL87051	2.639 (4)	2.685 (4.67)	2.662 (4.33)	14.30	14.3	2.596 (13.65)
AP23	2.303 (0)	2.525 (2.67)	2.414 (1.33)	17.96	17.1	3.024 (12.40)
ICEAP00557	2.751 (5.7)	2.751 (5.67)	2.751 (5.67)	16.53	16.5	3.024 (16.70)
ICEAP00053	2.303 (0)	2.303 (0)	2.303 (0)	17.96	15.4	2.303 (0)
ICEAP00932	2.415 (1.3)	2.303 (0)	2.359 (0.67)	20.73	20.7	3.048 (0)
ICEAP00933	2.303 (0)	2.573 (3.33)	2.438 (1.67)	17.93	14.1	2.303 (9.37)
ICEAP00936	2.573 (3.3)	2.303 (0)	2.438 (1.67)	19.80	19.8	2.653 (6.20)
AP29	2.303 (0)	2.685 (4.67)	2.494 (2.33)	17.10	17.1	2.303 (5.40)
Mean	**2.514 (2.56)**	**2.591 (3.57)**	**2.553 (3.07)**	**17.96**	**17.78**	**2.938 (10.28)**
LSD	**0.1790****	**0.1854****	**0.14374****	**1.754****	**1.63****	**0.6544****
CV (%)	**4.4**	**4.4**	**4.9**	**5.9**	**5.6**	**13.6**

** Significant at P < 0.001;

([a]) Data transformed using Log_e (x+c, where c = 10);

[b]Untransformed data in brackets

Untransformed data for seed weight 2004/05 and 2005/06

Appendix 3.5: Days to 50% flowering for various pigeonpea genotypes at Kandiya Research Station, Lilongwe, Malawi

Genotypes	Days to 50% flowering			Combined analysis
	2004/05	**2005/06**	**2006/07**	
AP1	145.3	120	133.3	132.89
AP2	147	118	150	138.33
AP3	158.3	127	160	148.44
AP4	191	127.3	186.3	168.22
AP5	175.3	176.7	155	169
AP6	217	117.6	220	206.43
AP7	173.7	131.3	171.3	158.78
AP8	128.7	106.7	112.7	116
AP9	226	117.6	246	223.93
AP10	130	95.3	124	116.44
ACC2253	137.7	114	130.7	127.44
ACC2291	163	125	143.7	143.89
ACC2298	132	104.3	127	121.11
ICP9145	176.7	121.3	177.3	158.44
ICEAP00040	178.3	124.7	186.7	163.22
ICEAP00068	125	79	125.7	109.89
KAT60/8	113.7	83	105	100.56
ICEAP00020	164.7	119.7	180	154.78
AP19	205.7	117.6	175.7	178.6
ICEAP00554	128.7	91.3	110.3	110.11
ICEAP00540	123.7	91.3	119	111.33
ICPL87051	138	99	115.7	117.56
AP23	203	117.6	214.3	196
ICEAP00557	115.3	90.3	117.7	107.78
ICEAP00053	202	149.3	210	187.11
ICEAP00932	183	123.3	176.7	161
ICEAP00933	174	132.3	179.3	161.89
ICEAP00936	181.3	124.3	185.7	163.78
AP29	217	164.3	222.3	204.94
Mean	**163.97**	**117.56**	**160.7**	**150.29**
LSD	**8.98****	**14.78****	**29.04****	**13.4****
CV (%)	**3.3**	**7.7**	**11.0**	**9.6**

** Significant at P < 0.001

Appendix 3.6: Variations in plant height (m) and stem diameter (mm) for pigeonpea genotypes

Genotypes	Plant height (m)				Stem Diameter (mm)		
	Kandiya		Bvumbwe	Combined analysis	Kandiya		Bvumbwe
	2004/05	2006/07	2004/05		2004/05	2006/07	2004/05
AP1	2.117	2.393	1.46	1.990	22.1	23.3	12.6
AP2	2.19	2.813	1.547	2.183	22.1	20.0	13.4
AP3	2.31	2.75	1.603	2.221	22.1	21.7	14.1
AP4	2.3	2.267	1.487	2.018	23.4	18.0	12.8
AP5	2.257	2.46	1.467	2.061	22.1	20.0	12.8
AP6	2.203	2.483	1.517	2.068	20.9	31.7	14.1
AP7	2.113	2.303	1.607	2.008	20.9	23.3	14.7
AP8	1.893	2.23	1.31	1.811	23.4	16.7	12.9
AP9	1.963	2.743	1.597	2.101	20.9	21.7	14.6
AP10	2.467	2.78	1.453	2.233	23.4	26.7	12.9
ACC2253	2.14	2.403	1.45	1.998	20.9	21.7	11.2
ACC2291	1.86	2.47	1.52	1.950	22.1	23.3	12.8
ACC2298	2.097	2.02	1.343	1.820	22.1	21.7	12.2
ICP9145	3.31	2.56	1.407	2.092	22.1	21.7	11.4
ICEAP00040	2.467	2.437	1.713	2.206	23.4	26.7	14.4
ICEAP00068	1.447	2.207	1.18	1.611	19.7	23.3	11.2
KAT60/8	1.73	2.173	1.207	1.702	20.9	16.7	11.9
ICEAP00020	2.243	2.487	1.673	2.134	22.1	20.0	14.2
AP19	1.577	2.493	1.04	1.703	7.4	267	11.3
ICEAP00554	1.52	2.083	1.3	1.634	20.9	20.0	12.2
ICEAP00540	1.73	2.193	1.33	1.751	20.9	21.7	12.4
ICPL87051	2.097	2.06	1.45	1.869	22.1	18.3	12.9
AP23	1.803	2.507	1.4.3	1.904	16.0	30.0	11.9
ICEAP00557	1.977	2.043	1.293	1.778	22.1	25.0	11.7
ICEAP00053	2.187	1.997	1.37	1.851	22.1	20.0	12.5
ICEAP00932	2.467	1.41	1.69	1.856	23.4	20.0	14.9
ICEAP00933	2.597	2.543	1.523	2.221	24.6	26.7	12.4
ICEAP00936	2.447	1.773	1.703	1.974	23.4	23.3	15.4
AP29	2.153	2.79	1.66	2.201	23.4	28.3	14.4
Mean	**2.092**	**2.34**	**1.459**	**1.964**	**21.4**	**22.7**	**12.99**
LSD	**0.365****	**0.416****	**0.179****	**0.254****	**0.354****	**0.6355****	**0.209****
CV (%)	**10.7**	**10.9**	**7.5**	**13.9**	**10.1**	**17.1**	**9.8**

** Significant at P < 0.001

Combined analysis for plant height excludes Bvumbwe

Appendix 3.7: Reaction of genotypes to Fusarium wilt disease at Bunda College and Bvumbwe sick plots

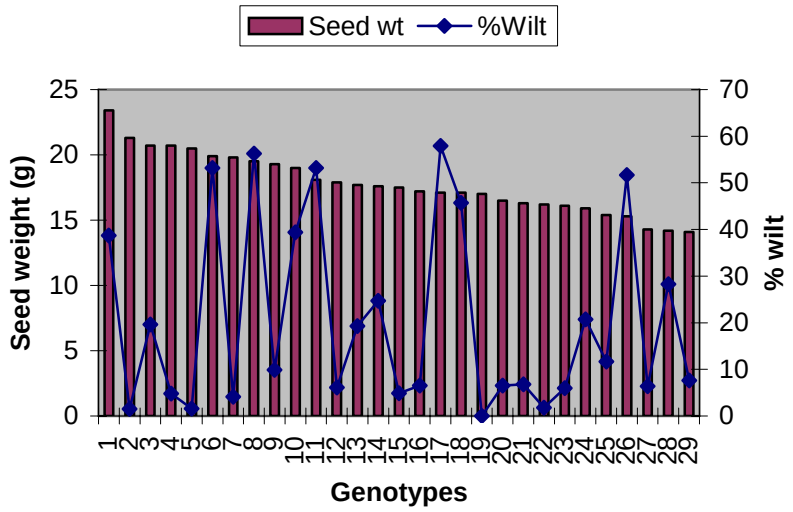
Genotypes	Incidence of Fusarium wilt (%)				
	Bunda College sick plot		Bvumbwe Research sick plot		Combined analysis
	2004/05	2005/06	2004/05	2005/06	Seasons/sites (***)
AP1	4.122[a] (53.2)[b]	3.917[a](42.7)[b]	0	3.977 [a] (50.8) [b]	4.005 [a] (48.9) [b]
AP2	4.156 (56.3)	4.277 (63.3)	4.5	4.499 (80.6)	4.311 (66.7)
AP3	2.664 (4.9)	3.049 (14.3)	0	3.914 (40.2)	3.209 (19.8)
AP4	2.742 (6.8)	3.432 (21.4)	13.6	3.713 (33.1)	3.296 (20.4)
AP5	3.262 (19.3)	3.646 (29.1)	1.5	4.307 (68.2)	3.738 (38.9)
AP6	3.899 (39.4)	4.455 (76.4)	0	4.567 (86.6)	4.307 (67.4)
AP7	3.415 (20.7)	4.338 (67.6)	3.0	4.161 (56)	3.972 (48.1)
AP8	3.285 (19.6)	3.449 (23)	4.6	3.701 (31.6)	3.478 (24.7)
AP9	3.884 (38.7)	4.373 (69.3)	0	4.579 (87.9)	4.279 (65.3)
AP10	2.433 (1.6)	2.953 (11.1)	0	3.602 (28.1)	2.996 (13.6)
ACC2253	4.141 (53.2)	4.153 (54.4)	1.5	4.424 (75.6)	4.239 (61.1)
ACC2291	3.542 (24.7)	3.091 (12.1)	5.3	3.864 (38.7)	3.499 (25.2)
ACC2298	3.644 (28.3)	3.532 (31.3)	3.3	4.291 (63.1)	3.822 (40.9)
ICP9145	2.303 (0)	2.873 (9.4)	3.3	3.430 (20.9)	2.868 (10.1)
ICEAP00040	2.426 (1.5)	2.518 (3)	1.5	2.780 (6.2)	2.575 (3.6)
ICEAP00068	2.713 (6.0)	3.730 (32.6)	4.5	3.544 (24.6)	3.329 (21.2)
KAT60/8	4.119 (51.7)	4.258 (61.2)	1.5	4.087 (51.4)	4.155 (54.7)
ICEAP00020	2.720 (6.1)	2.988 (10.6)	0	3.595 (27.9)	3.101 (14.9)
AP19	2.976 (9.9)	4.047 (47.4)	17.6	4.477 (78.3)	3.833 (45.2)
ICEAP00554	2.444 (1.8)	3.073 (13.1)	1.5	2.426 (1.5)	2.648 (5.5)
ICEAP00540	2.740 (6.5)	2.721 (5.2)	5	3.415 (24.6)	2.959 (12.1)
ICPL87051	2.749 (6.4)	2.588 (3.6)	10.8	3.272 (17.8)	2.870 (9.3)
AP23	4.012 (45.7)	4.390 (70.7)	0	4.314 (65.4)	4.239 (60.6)
ICEAP00557	2.732 (6.5)	2.788 (6.4)	1.5	3.005 (12.7)	2.842 (8.5)
ICEAP00053	2.900 (11.7)	3.136 (13.8)	7.8	3.064 (12.6)	3.033 (12.7)
ICEAP00932	2.599 (4.8)	3.246 (16.2)	1.8	2.981 (10.4)	2.942 (10.4)
ICEAP00933	2.698 (7.6)	2.749 (6.4)	0	3.119 (13)	2.855 (9)
ICEAP00936	2.622 (4.1)	2.740 (6.5)	3	2.551 (3.7)	2.638 (4.8)
AP29	4.127 (57.9)	4.360 (69.1)	0	4.597 (89.4)	4.361 (72.1)
Mean	**3.175 (20.5)**	**3.478 (30.7)**	**3.36**	**3.733 (41.4)**	**3.462 (30.89)**
LSD	**0.6175****	**0.5732****	**12.399Ns**	**0.5414****	**0.3601****
CV (%)	**11.9**	**10.1**	**225.4**	**8.9**	**11.2**

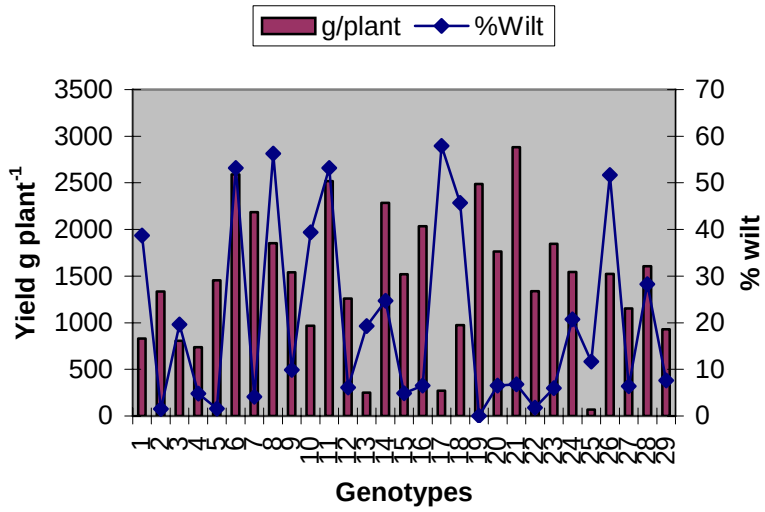
** Significant at P < 0.001; ns = Not significant

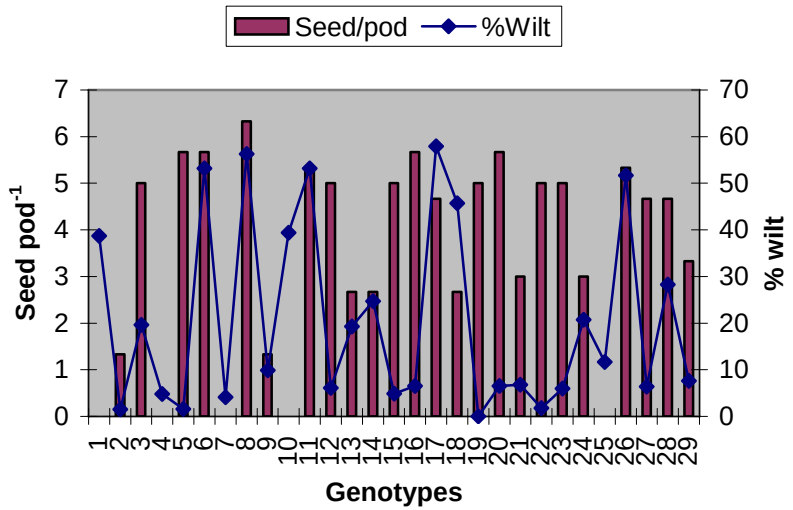
*** Combined analysis excluded Bvumbwe Research sick plot 2004/05

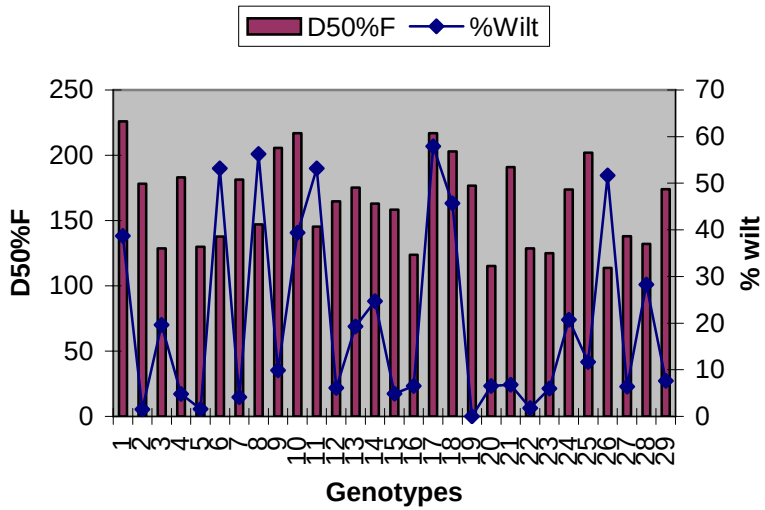
([a]) Data transformed using Log_e (x+c; where c = 10);

[b]Untransformed data in brackets except for Bvumbwe 2004/05


Seed wt
%Wilt
Seed weight (g)
% wilt
Genotypes


g/plant
%Wilt
Yield g plant-1
% wilt
Genotypes


Seed/pod
%Wilt
Seed pod-1
% wilt
Genotypes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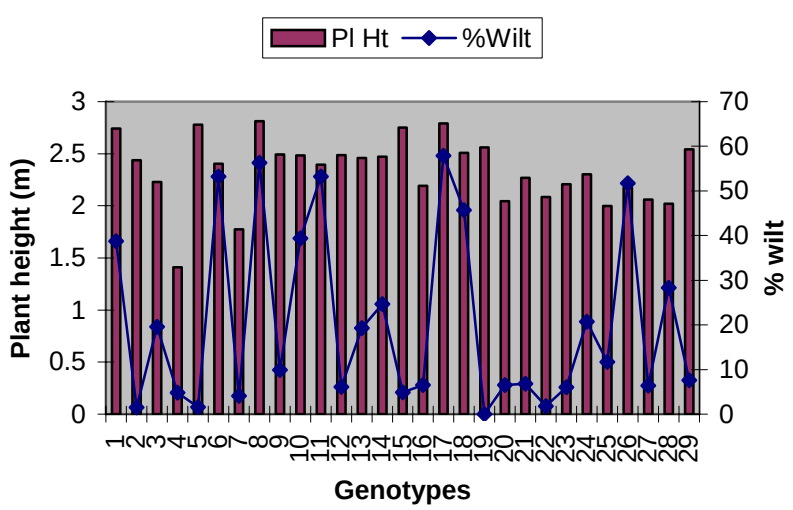
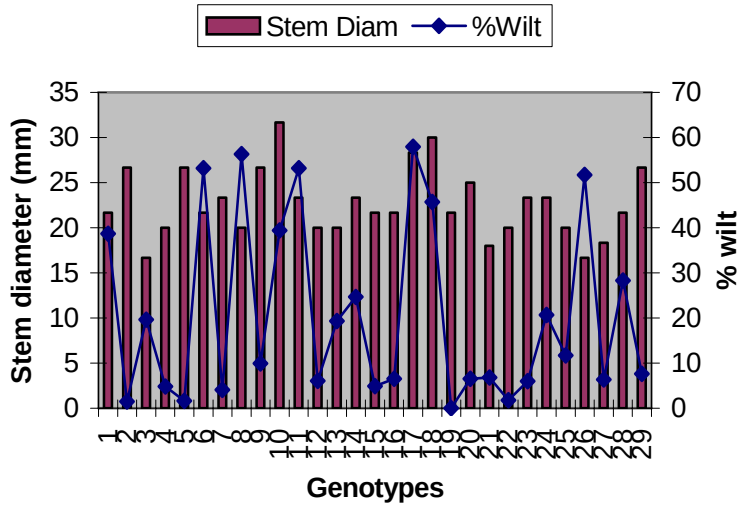
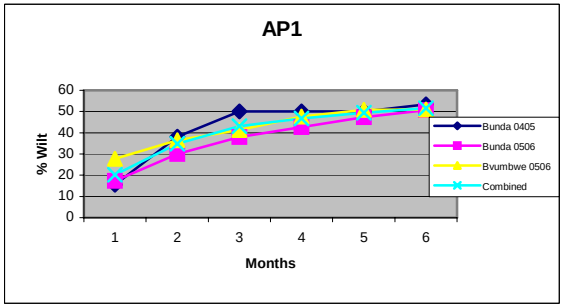
%Wilt
D50%F
% wilt
Genotypes

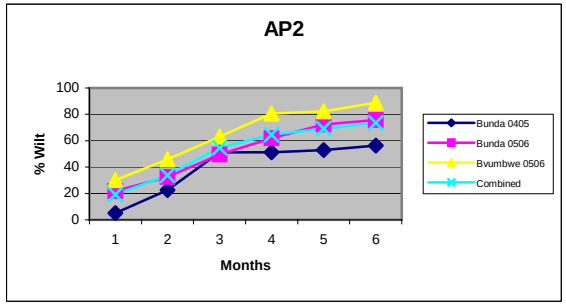


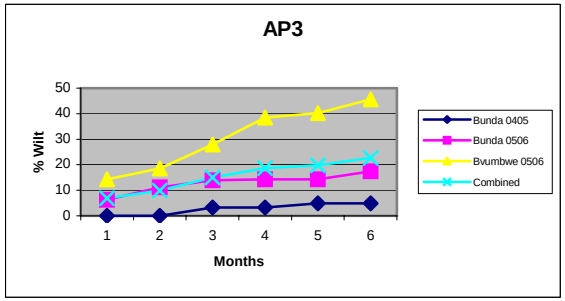


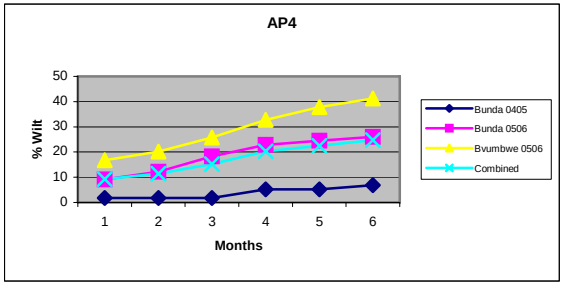
Appendix 3.8: Correlations between Fusarium wilt and plant descriptors among pigeonpea genotypes

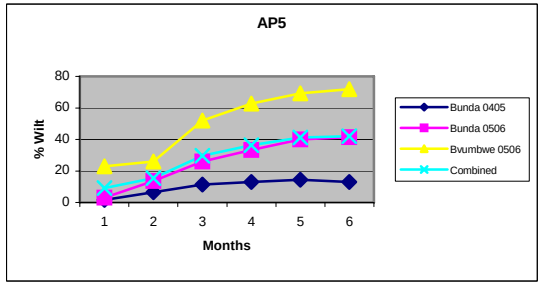
Legend: ***1****=AP1,* ***2****=AP2,* ***3****=AP3,* ***4****=AP4,* ***5****=AP5,* ***6****=AP6,* ***7****=AP7,* ***8****=AP8,* ***9****=AP9,* ***10****=AP10,* ***11****=ACC2253,* ***12****=ACC2291,* ***13****=ACC2298,* ***14****=ICP9145,* ***15****=ICEAP00040,* ***16****=ICEAP00068,* ***17****=KAT60/8, 18=ICEAP00020,* ***19****=AP19,* ***20****=ICEAP00554,* ***21****=ICEAP00540,* ***22****=ICPL87051,* ***23****=AP23,* ***24****=ICEAP00557,* ***25****=ICEAP00053,* ***26****=ICEAP00932,* ***27****=ICEAP00933,* ***28****=ICEAP00936,* ***29****=AP29.*

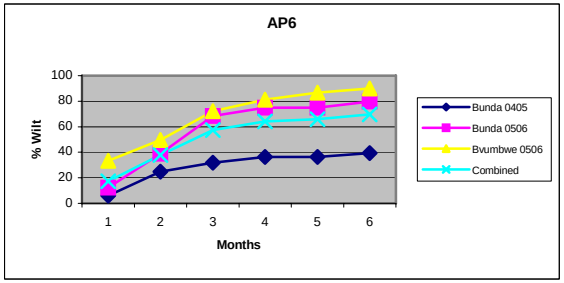







AP4
% Wilt
Months
Bunda 0405
Bunda 0506
Bvumbwe 0506
Combined


AP5
% Wilt
Months
Bunda 0405
Bunda 0506
Bvumbwe 0506
Combined


AP6
% Wilt
Months
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Bunda 0506
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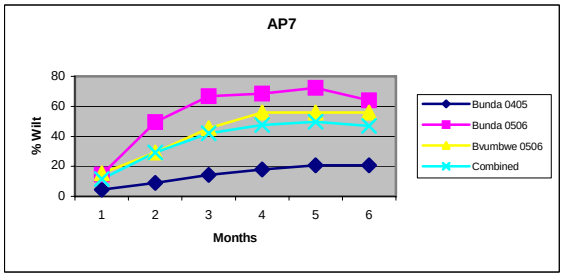
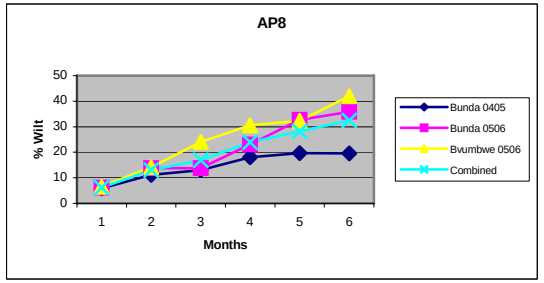
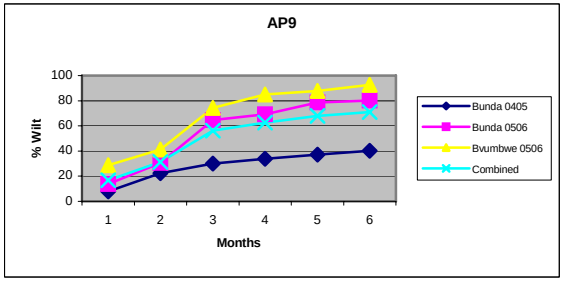
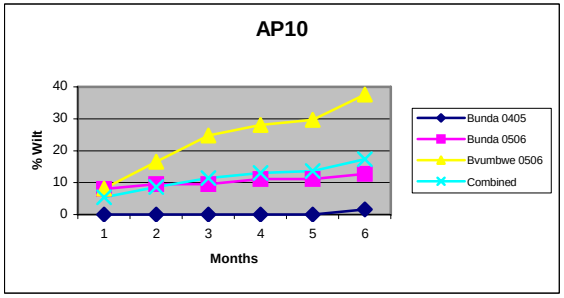
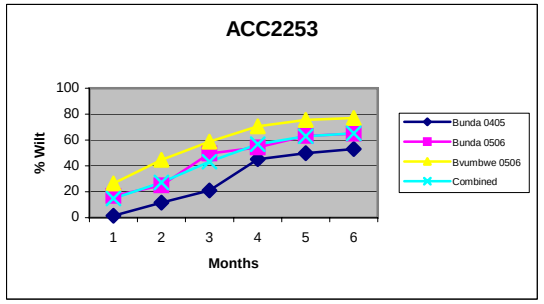
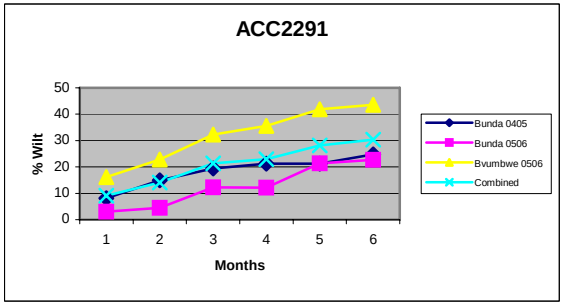

AP7
% Wilt
Months
Bunda 0405
Bunda 0506
Bvumbwe 0506
Combined

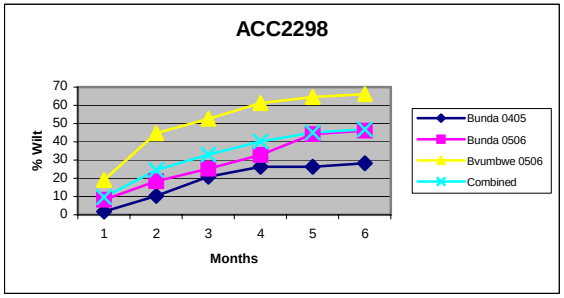

AP8
% Wilt
Months
Bunda 0405
Bunda 0506
Bvumbwe 0506
Combined

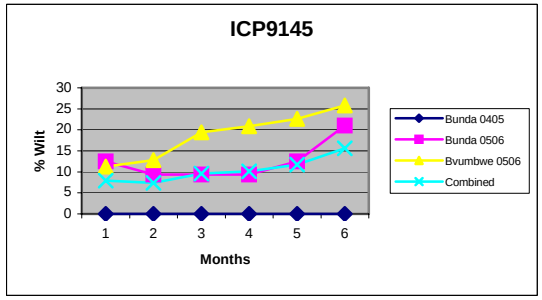

AP9
% Wilt
Months
Bunda 0405
Bunda 0506
Bvumbwe 0506
Combined

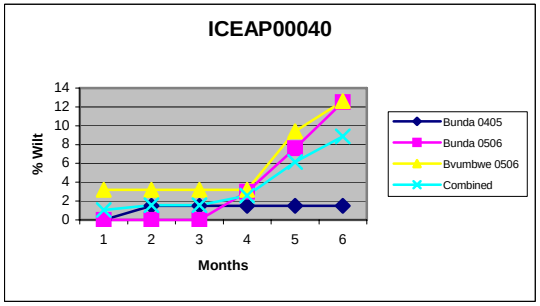

AP10
% Wilt
Months
Bunda 0405
Bunda 0506
Bvumbwe 0506
Combined

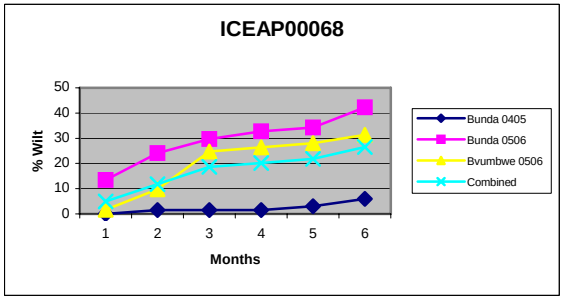

ACC2253
% Wilt
Months
Bunda 0405
Bunda 0506
Bvumbwe 0506
Combined


ACC2291
% Wilt
Months
Bunda 0405
Bunda 0506
Bvumbwe 0506
Combined


ACC2298
% Wilt
Months
Bunda 0405
Bunda 0506
Bvumbwe 0506
Combined


ICP9145
% Wilt
Months
Bunda 0405
Bunda 0506
Bvumbwe 0506
Combined


ICEAP00040
% Wilt
Months
Bunda 0405
Bunda 0506
Bvumbwe 0506
Combined


ICEAP00068
% Wilt
Months
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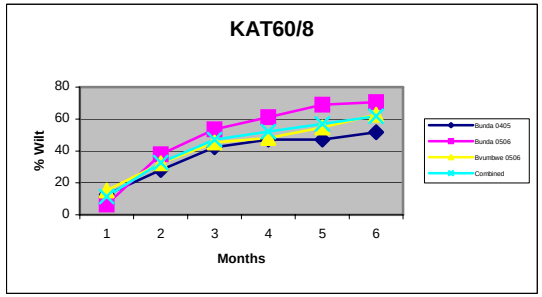
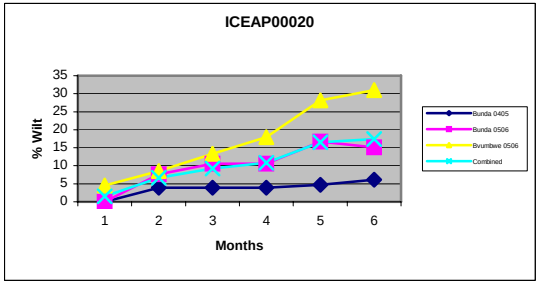
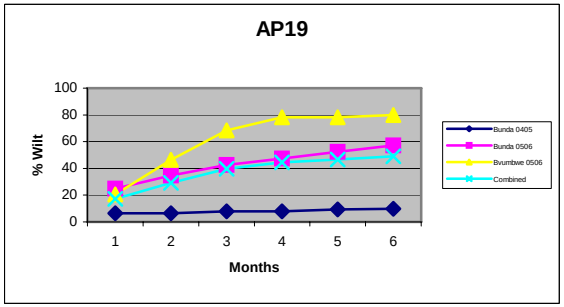
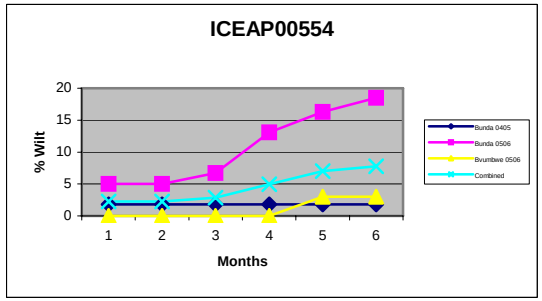
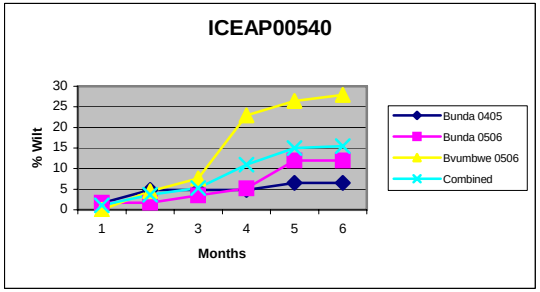
Bunda 0506
Bvumbwe 0506
Combined

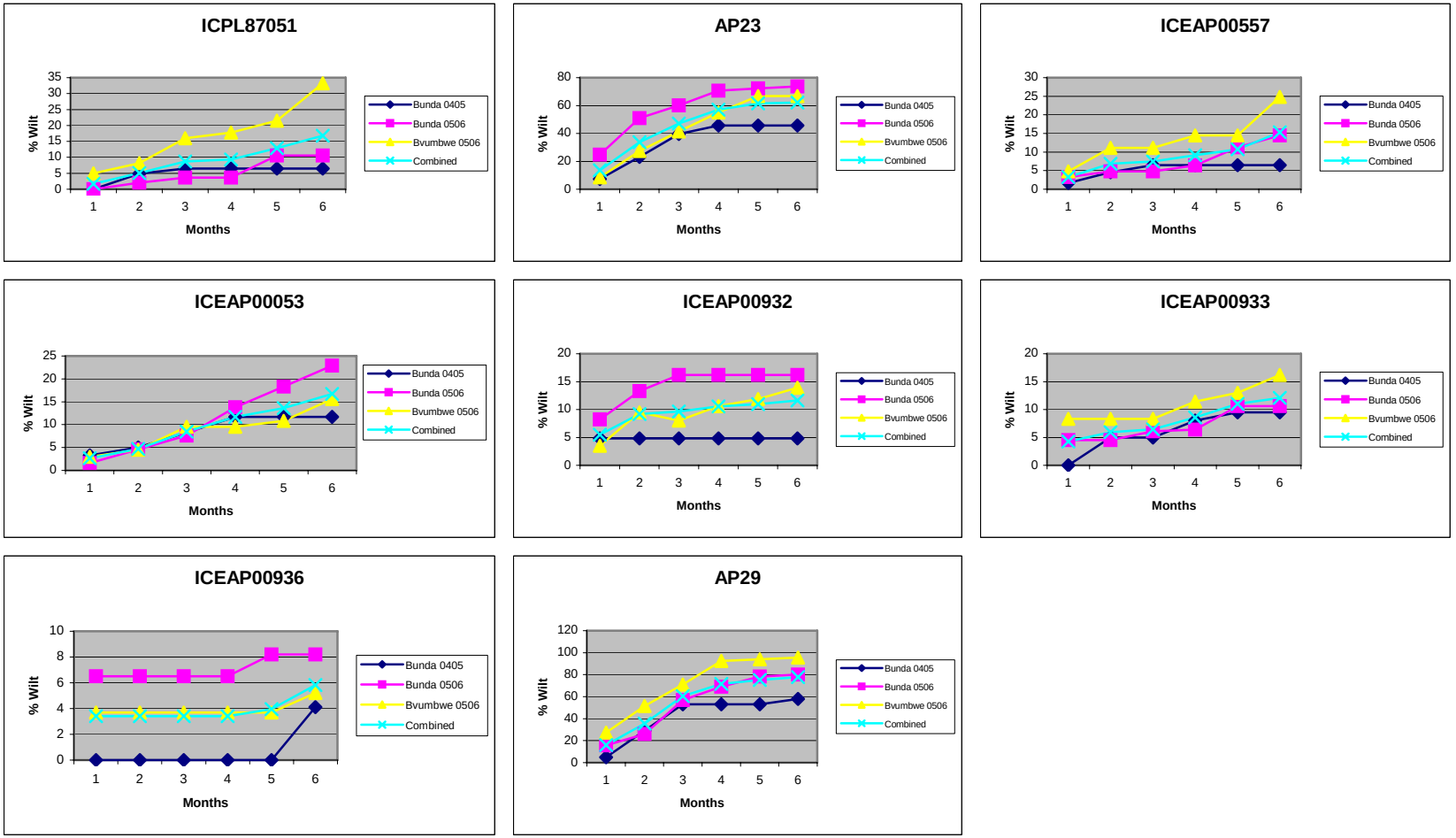

KAT60/8
% Wilt
Months
Bunda 0405
Bunda 0506
Bvumbwe 0506
Combined


ICEAP00020
% Wilt
Months
Bunda 0405
Bunda 0506
Bvumbwe 0506
Combined


AP19
% Wilt
Months
Bunda 0405
Bunda 0506
Bvumbwe 0506
Combined


ICEAP00554
% Wilt
Months
Bunda 0405
Bunda 0506
Bvumbwe 0506
Combined


ICEAP00540
% Wilt
Months
Bunda 0405
Bunda 0506
Bvumbwe 0506
Combined



Appendix 3.9: Variations in the incidence of Fusarium wilt among pigeonpea genotypes during the months of January to June.

Chapter 4: Development of a new screening technique for Fusarium wilt in pigeonpea

Abstract

Several methods to screen pigeonpea germplasm for resistance to Fusarium wilt exist, but each of the methods has both strengths and shortfalls. It is important to develop more efficient and user-friendly techniques to screen pigeonpea for resistance to Fusarium wilt disease in the breeding programme, and to explore new methods of preserving the isolates and multiplying the inoculum for use in the artificial inoculation. The objectives of this research, therefore, were to investigate effective and user-friendly methods of preserving Fusarium udum *isolates; to develop cheap but effective techniques of multiplying the pathogen; and to develop a new technique of screening pigeonpea germplasm for its resistance to Fusarium wilt disease.* Fusarium udum *was isolated from infected pigeonpea plants, purified and multiplied in three sterilized substrates, namely, finger millet, sorghum and wheat seeds. Concurrently, the isolates were preserved using three methods, namely, double sterilised distilled water at room temperature, potato dextrose agar slants, and silica gel in viral bottles under refrigeration. The isolates were first tested for pathogenicity, and subsequently used in the infested-seed inoculation technique using eight pigeonpea cultivars with a known reaction to Fusarium wilt infection. The results showed that* F. udum *can be stored for more than two and half years in double sterile distilled water, PDA and silica gel techniques. The double sterilized distilled water technique was the most economical and user-friendly of the tested methods. However, isolate virulence should be tested at intervals. Inoculum was successfully multiplied in the three substrates, but easily handled, large wheat seed medium was recommended during inoculation. The results also showed that the infested-seed inoculation technique used, the first of its kind for pigeonpea, is viable and effective, and should be used in the screening of pigeonpea germplasm for resistance to wilt. The recommendation is that any inoculum level can be used to establish the resistance status of the germplasm; however, high inocula levels should be used in the breeding programme to advance resistant lines in the filial generations.*

4.1 Introduction

4.1.1 Screening pigeonpea for Fusarium wilt resistance

Pigeonpea (*Cajanus cajan* (L.) Millspaugh) germplasm can be screened for its resistance to Fusarium wilt, caused by *Fusarium udum* (Butler), under natural or artificial conditions. The natural screening process relies on planting various pigeonpea germplasm accessions in Fusarium wilt infested soils (sick plots or sick soils) (Butler, 1908; Deshpande et al., 1963; Nene and Kannaiyan, 1982; Hillocks, 1984; Reddy et al., 1995; Infantino et al., 2006). However, the results of these natural screening methods have often been inconsistent (Nene et al., 1981) due to poor distribution of the pathogen and varying concentrations of inoculum in the soil. In addition, the expression of the disease varies across season, depending on the environmental conditions in the soil, such as moisture content, soil temperature, and soil structure (Burgess et al., 1994). Malawi has monomodal type of rainfall, hence screening germplasm or advancement of the filial generations can only be done during rainy season. It is imperative therefore to test other techniques for greenhouse use to avoid the problems associated with the ineffectiveness of the field screening method and to advance the filial generations during dry season.

There are a number of actual and possible artificial screening techniques for Fusarium wilt on pigeonpea. A root dip and transplanting technique involves dipping roots of healthy plants/seedlings into an inoculum and transplanting them into sterile soils (Roberts and Kraft, 1971; Phipps and Stipes, 1973; Sakar et al., 1982; Hillocks, 1984; ICRISAT, 1986; Gupta et al., 1988; DeVay et al., 1997; Marley and Hillocks, 2002; Wang and Roberts, 2006). The success of this technique depends on inoculum concentration, spore germination, the age of the plants, and the time period for which the roots are dipped into the inoculum. Several researchers have reported the use of various inocula concentrations ranging from 3×10^2 to 3×10^6 colony forming units (cfu) ml^{-1} (Bugbee and Sappenfield, 1972; Sakar et al., 1982; Gupta et al., 1988; Reddy and Raju, 1993; DeVay et al., 1997; Marley and Hillocks, 2002). Seven-day-old seedlings are bruised in order to stress them and then dipped in a conidial suspension for different time periods (Sakar et al., 1982; ICRISAT, 1986; Reddy and Raju 1993). Okiror (1998) reported that wilting started late and progressed slowly when using the root dip and transplanting method, showing that spore germination was slow. A further limitation of this technique

is that sterile soil may be unsuitable because the exclusion of microflora drastically changes the most important component of the soil environment (Tuite, 1969). Microflora are often responsible for creating avenues for the entry of pathogens (Agrios, 2005). The root dip and transplanting method also tends to be tedious when large numbers of plants are involved because of the need to carefully uproot and transplant each one (Hillocks, 1984).

Nene and Haware (1980), Nene and Kannaiyan (1982) and Kraft et al. (1994) used a water culture technique. Seven-day-old chickpea seedlings were planted in a 2.5% inoculum concentration of spores and mycelia, in test tubes, in the laboratory, to screen for resistance to Fusarium wilt. The plants were routinely supplied with sterilised distilled water. A similar technique called the incubator-shaker method has also been reported (Roberts and Kraft, 1971). Pea seedlings were dipped in 1×10^4 cfu in wide mouth jars (250 ml) and these jars were then put on an incubator shaker (100-120 cycles min^{-1}) (Roberts and Kraft, 1971). Wilt symptoms were observed within 10 d after inoculation.

Many scientists have reported the use of infested soils (Tuite, 1969; Kiraly et al., 1970; Russell, 1978; Nene and Kannaiyan, 1982; Sakar et al., 1982; Hillock, 1984; Burgess et al., 1994; Kraft et al., 1994; Katsantonis et al., 2003). The inoculum was mixed with soil before planting seeds. The seeds or seedlings were planted in pots containing infested soils with a known concentration of inocula. This is similar to the natural techniques of using a sick plot, but the concentrations of inoculum are controlled, in contrast to the sick plot where it is difficult to quantify the inoculum in the soil under field conditions (Okiror, 1998). The disadvantage of using infested soils is that many plants escape infection due to differential distribution and inoculum dormancy, and there is no way of knowing when infection occurred on each plant (Hillocks, 1984).

Sakar et al. (1982) and Kraft et al. (1994) reported immersing pea seed in a conidial suspension as inoculum and then planting the seed. This seed inoculation is contamination, not infection (Kiraly et al., 1970); the plants become infected as they germinate. However, the success of the seed inoculation technique depends on the spore concentration and/or period of soaking (Okiror, 1998) and the prevailing soil environmental conditions at planting.

Another screening technique involves injecting inoculum into plants, and is known as stem injection or stem puncture (Jindal et al., 1981; Hillocks, 1984; Katsantonis et al., 2003). The stem injection method has been widely used in the USA and Tanzania, in cotton, against Fusarium wilt, and the results have been comparable to field screening (Hillocks, 1984). In Tanzania the method was used to make single plant selections in cotton when breeding against Fusarium wilt (Hillocks, 1984). Sewing machine needles (or a syringe needle) are used to puncture the stem. The effectiveness of this technique depends on the age of the plant and spore concentration. Although it prevents disease escape, it works better for chemical rather than mechanical resistance. Sharma et al. (1977) used stem puncture to inoculate plants 45, 75 and 127 d after planting, by means of a longitudinal slit cut in the stem 10-15 cm above soil level, with inoculum from agar cultures.

Mak et al. (2004) reported the use of the double-tray technique as a rapid method for early screening of bananas at the seedling stage against Fusarium wilt disease. The technique requires a double-tray construction; a perforated inner tray contains sterilised river sand in which to grow hardened tissue cultured – plantlets (TC), and a larger outer container tray collects surplus Hoagland nutrient solution and pathogen washout. This method could also be applied to pigeonpea.

The effectiveness of all these techniques depends on such factors as spore concentration, plant age at inoculation, and environmental conditions such as temperature and humidity (Ribeiro and Hagedorn, 1979). Exerting stress on the plants through root bruising, reduced moisture availability and extremes of temperature enhances infection (Henderson and Winstead, 1961; Burgess et al., 1994). The inoculum source can also greatly influence the kind and amount of infection obtained (Tuite, 1969). It is difficult to generate inoculum at exactly the same time and physiological state for each trial.

4.1.2 Isolation of *Fusarium udum* from plants

Fusarium udum is normally isolated from infected pigeonpea plants before it is used in artificial inoculations, using the technique described by Tuite (1969), Kiraly et al. (1970), Burgess et al. (1994) and Agrios (2005). The isolates are also purified using the single-spore technique as described by Tuite (1969), Burgess et al. (1994) and Agrios (2005).

However, isolation of the pathogen from the diseased plant part is affected by the nature of the diseased tissue, the method of surface sterilisation, the planting procedures, the medium and the incubation conditions (Burgess et al., 1994).

4.1.3 Preservation of *Fusarium* spp isolates

Several techniques exist to preserve inoculum or isolates and these vary according to the type of inoculum and duration of preservation. For *Fusarium* spp, a number of methods exist and some, depending on the available equipment, have proven successful.

The first and most common method is that of periodic transfer of the culture. It generally consists of transferring cultures on agar slants twice a year (Tuite, 1969). Although it provides ready access to the organism, it is laborious, especially when a large number of cultures are involved. Repeated subculturing often results in undesirable mutations by most organisms, and contamination by extraneous microbes.

A second method is lyophilisation which consists of drying spores or cells from the frozen state and storing them in a vacuum. The method uses three processes, namely, pre-freezing, freeze-drying centrifugation and vacuum drying (Tuite, 1969; Kiraly et al., 1970; Burgess et al., 1994).

A third method, a silica gel technique, is an attractive method because it does not require expensive apparatus and is easy to use. The inoculum is stored in silica gel granules in viral bottles and stored under refrigeration. Cultures can be taken repeatedly from a single storage tube, although contamination must be avoided. The major disadvantage is a gradual decline in viability, but this can be overcome by replacement with fresh materials. The survival of *Fusarium* spp preserved by this method depends upon the abundant production of conidia. The procedure was described by Tuite (1969) and Burgess et al. (1994).

The last method to be discussed is water preservation. It consists of placing small pieces of mycelium, spores, and agar into small capped test tubes containing double sterilised-distilled water. The test tubes are then refrigerated at a temperature of 10^0C (Tuite, 1969). The longer a fungus is cultivated under artificial conditions, the greater is the

possibility of small, or even large, changes occurring, not only in genetic and physiological characteristics, but also in the morphological ones. By means of parasexuality and mutation, new characteristics can arise or some of the original characteristics can disappear during cultivation. With extended cultivation of the fungus, pathogenicity may be lost, or at least, virulence decreased (Kiraly et al., 1970; Burgess et al., 1994).

Regardless of the preservation technique used, the pathogen needs to be tested for virulence before it can be used. A pathogenicity test, therefore, should be done using a technique that enables the reproduction of typical symptoms of the disease, over the relevant time-scale, in the commercial glasshouse or field situation. The guidelines are outlined by Burgess et al. (1994). Fresh cultures should be used for the preparation of the inoculum. The nature and amount of inoculum used in pathogenicity tests should conform to the level of inoculum causing the disease under field conditions. The cultivars used in the pathogenicity test should be identical to those observed in the field. The pathogenicity tests for suspected soil borne pathogens is a more difficult design because the nature and amount of inoculum, and the characteristics of the soil, have a significant effect on infection and disease development. The soil selected for a pathogenicity test should be similar to that associated with the disease under investigation, physically and chemically (Burgess et al., 1994).

4.1.4 Multiplication of inoculum

Several methods have been used to produce or multiply the Fusarium wilt pathogen. One method is to multiply single spore isolates in 250 ml conical flasks containing 100 ml of potato dextrose broth (PDB). The flasks are placed on a rotary shaker for 10 d at room temperature (25 – 30^0C) (Roberts and Kraft, 1971; Ribeiro and Hagedorn, 1979; Nene and Kannaiyan, 1982; Infantino et al., 2006). Bugbee and Sappenfield (1972) reported the use of Czapek's broth on a rotary shaker for 3 - 4 d to multiply the inoculum. Another method involves growing the isolate on 20 ml PDA medium. After 1 wk, the spores and mycelia are scraped off and sieved or filtered through cheesecloth. The spores are diluted accordingly and used for inoculation (Sakar et al., 1982; DeVay et al., 1997; Wang and Roberts, 2006; Xing and Westphal, 2006).

Lindell et al. (1986) reported the use of wheat, oats, barley, and corn chaff as substrates for *F. graminearum*. The chaff was spread evenly into soil and then the infested soil was put in pots. The crop was planted into the inoculated soil. This method could also be applied to pigeonpea and other crops. Trimboli and Burgess (1983) reported the use of chaff grain medium (CGM), consisting of oat chaff and crushed oat grain, for multiplying *F. moniliformae*. For a month, sterile sand-cornmeal (1 l of sand, 56 ml of food-grade cornmeal, and 325 ml water) was used to multiply *F. solani* f. sp. *glycines* (Xing and Westphal, 2006).

Nene and Haware (1980) and Nene and Kannaiyan (1982) described the multiplication of single-conidium culture of *F. udum* on 100 g of 9:1 sand using pigeonpea meal medium for 15 d at 28 - 30^{0}C. Two hundred grams of this inoculum was mixed well with 2 kg of autoclaved soil in pots; pigeonpea seedlings were planted in the pots.

All the techniques used in inoculating *F. udum* in pigeonpea in the greenhouse as well as preserving and multiplying isolates have shortfalls. It was imperative, therefore, to develop suitable methods that are repeatable, require simple equipment and can be used in breeding programmes in Malawi. The methods should be effective in preserving and multiplying inoculum; and there was also the need to try new inoculation techniques. Therefore the objectives of this study were to:

1. Develop a suitable, user-friendly method of preserving isolates of *F. udum;*
2. Develop a cheap but effective technique of multiplying the pathogen; and
3. Develop a new technique to screen pigeonpea germplasm for its resistance to Fusarium wilt disease.

4.2 Materials and methods

4.2.1 Isolation, purification, and identification of the pathogen

The Fusarium wilt pathogen was isolated from infected pigeonpea plants from Bunda College and Bvumbwe Research Station sick plots in Malawi, as described by Tuite (1969), Blanchard and Tattar (1981) and Agrios (2005).

Purification of the isolates involved transferring the clean growing mycelium onto fresh Potato Dextrose Agar (PDA) medium (Laing, 2004; personal communication). The isolates were transferred from the first Petri dish onto a fresh PDA Petri dish; the second transfer was from this second Petri dish onto the third one. This method produced pure cultures similar to the single spore technique described by Toussoun and Nelson (1976).

The *F. udum* isolate was sent for identification to the Biosystematics Division: Mycology Unit Services of the Agricultural Research Council, Plant Protection Research Institute, Private Bag X134, 0121, Queenswood, Pretoria, South Africa. The sample was given reference number VERW NO. 06187 for identification.

4.2.2 Preservation of the isolates

A simple observational trial was set up in the laboratory to compare the three methods of storing Fusarium isolates. The three methods were: PDA slants (Tuite, 1969); silica gel (Tuite, 1969; Burgess et al., 1994); and double sterilised distilled water at room temperature (Tuite, 1969). From March 2005, the viability of the isolates was tested monthly for 2.5 years.

4.2.3 Multiplication of inoculum

The *F. udum* isolate was grown on Petri dishes containing PDA media for 10 d. Five ml of sterile, distilled water were poured onto the Petri dish, and the cultures scraped off the Petri dishes with a sterile scalpel; then the culture was poured onto the sterilized cereal grains and mixed thoroughly.

Three different cereal grains were used as substrates on which to multiply the inoculum. The substrates were finger millet, sorghum and wheat grains. The inoculum was multiplied on the sterilised cereal grains. This is similar to mushroom spawn multiplication (Fritsche, 1978; Elliot, 1987; Przybylowicz and Donoghue, 1988; Quimio et al., 1990). The method is a modification of Fritsche (1978) and Elliot (1987). The cereal grains were first boiled in water and the excess water drained off through a sieve. The 250 ml conical flasks were half filled with the cereal grains and autoclaved (steam-sterilised) for 2 hr at 121^0C at 15 pressure per square inch (p.s.i). The conical flasks were then inoculated with the *F. udum* isolate. The flasks containing infested cereal grains were incubated at 25^0C for 10 d until the fungus colonised the whole flask. The

flasks were shaken every 3 and 6 d during incubation, to ensure rapid growth, colonisation (Elliot, 1987; Przybylowicz and Donoghue, 1988), and mycelium revitalization (Adey, 1995).

4.2.4 Pathogenicity test for *Fusarium udum* pathogen

Pathogenicity tests were done to verify the virulence of the *F. udum* isolate grown on three different media. Both isolates from Bunda College and Bvumbwe Research sick plots were tested for their virulence. Ten-litre pots were filled with ordinary soil from a field where pigeonpea wilt had previously not been reported. The pots were watered 24h before transplanting the inoculated seedlings. Pigeonpea seeds were planted in composted pine bark media in black plastic tray cells (Figure 4.1). KAT60/8, a susceptible genotype from ICRISAT, was used in the pathogenicity test.

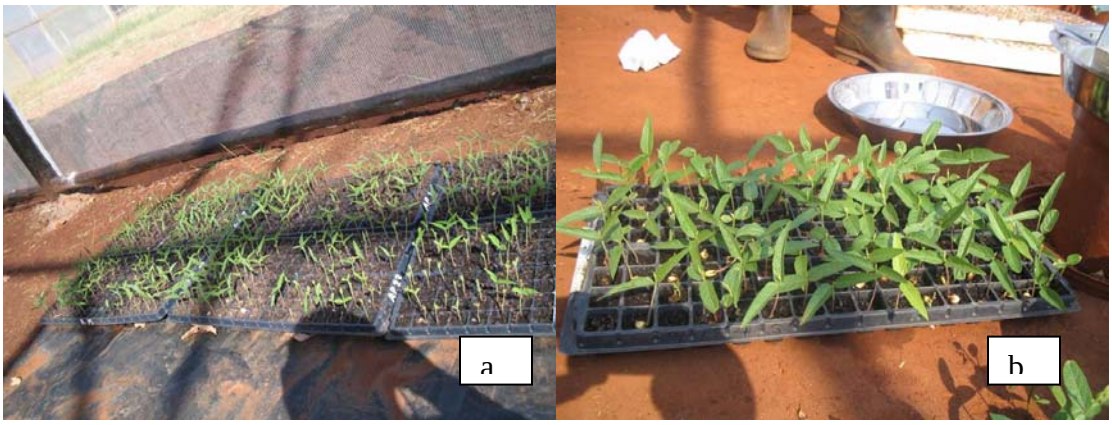


Figure 4.1: Raising of pigeonpea seedlings in plastic trays: a) young pigeonpea seedlings; b) seedlings ready for inoculation

During the pathogenicity test, five inoculum levels were used, that is, from one to five *F. udum* infested seeds plant^{-1}. The roots of the ten-day-old pigeonpea seedlings were bruised before inoculation (Nene and Kannaiyan, 1982; Gupta et al., 1988). The actual inoculation involved placing infested media seed/s permanently on the bruised part of the roots simultaneously and covering them with media (Figure 4.2). The inoculated seedlings were then transplanted into the pots (Figure 4.3a). The controls were bruised and transplanted into the pots. Water was withheld for 24 h after inoculation to stress the seedlings, because pathogen infection is enhanced through stress exerted through root bruising, reduced moisture availability, and temperature extremes (Henderson and Winstead, 1961; Burgess et al., 1994). Each pot contained five plants, each of the five

treatments (inoculum levels) was replicated five times, and laid out in a randomised complete block design (RCBD) in the screenhouse (Figure 4.3b). Symptoms were observed starting from 10 d after inoculation. The number of infected plants per each inoculum level was recorded.

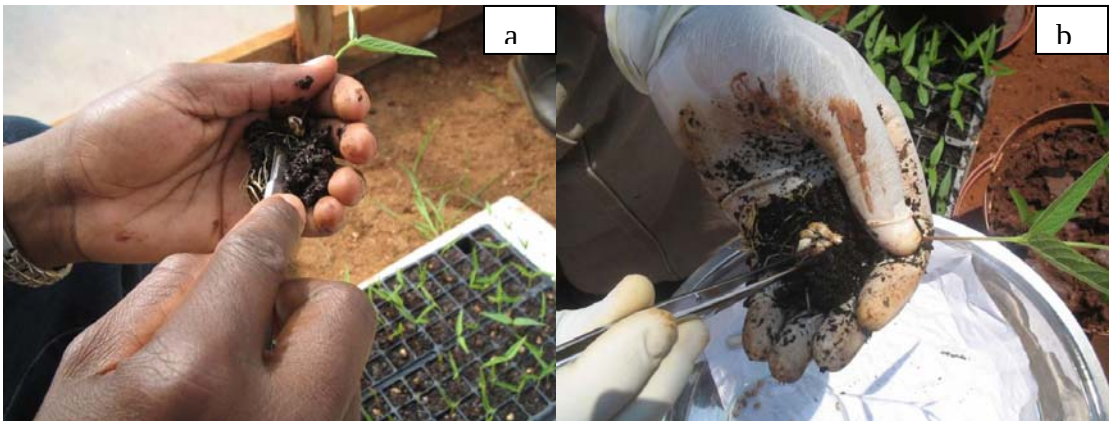


Figure 4.2: (a) Bruising of the roots with a scalpel; and (b) placing the inoculum on the bruised roots

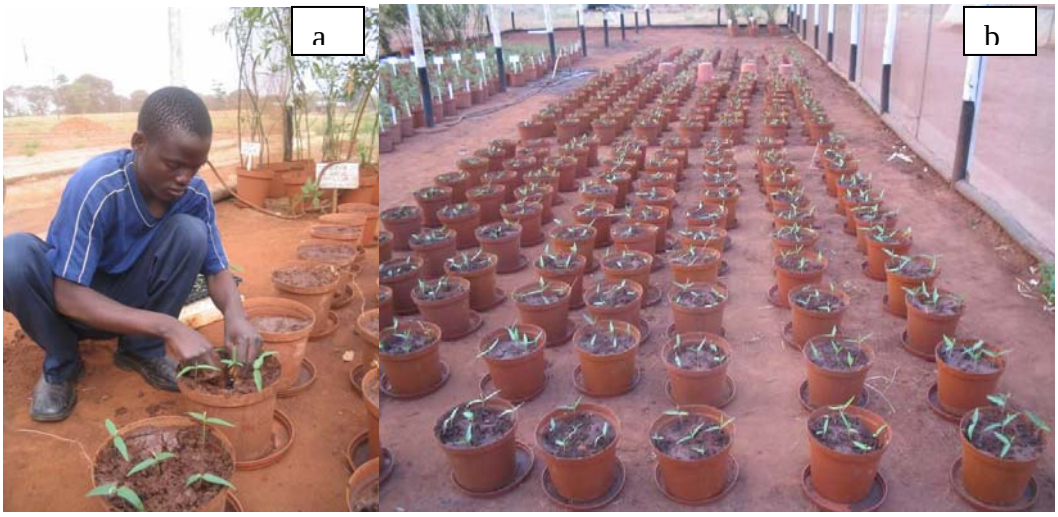


Figure 4.3: (a) Transplanting inoculated seedlings into pots and (b) trial layout in the screenhouse

4.2.5 Selection of the genotypes for the screening technique

Eight genotypes were used in the actual inoculation technique and these were carefully selected based on their resistance or susceptibility to Fusarium wilt disease and duration to maturity (Table 4.1). All the cultivars except one, AP29, were from ICRISAT. The seeds were provided by ICRISAT, Kenya. AP29 is a local landrace and was included in the experiment because of its high susceptibility to the disease, as evidenced from the sick plots evaluation trial.

Table 4.1: Characteristics of the pigeonpea genotypes used in the screening technique

Name of genotype	Type of resistance	Maturity duration
ICP9145	Resistant	Late maturing
ICEAP00040	Resistant	Late maturing
ICPL87051	Resistant	Medium maturing
ICEAP00554	Resistant	Medium maturing
ICEAP00020	Intermediate resistance	Late maturing
ICEAP00933	Intermediate resistance	Late maturing
KAT60/8	Susceptible	Early to medium maturing
AP29	Susceptible	Late maturing

Source: ICRISAT, Kenya and Malawi (2004)

4.2.6 Development of the infested seed inoculation technique

Ten inoculum levels, that is, one to ten *F. udum* infested wheat seeds, were used in the trial to inoculate each ten-day-old pigeonpea plant. The aim was to determine the optimum inoculum concentration for screening the pigeonpea germplasm for resistance to F. wilt.

One hundred and fifty pigeonpea seedlings genotype^{-1} were raised. Five seedlings pot^{-1} with a single inoculum level were inoculated; this treatment was replicated three times. Therefore, 15 pigeonpea seedlings were raised treatment^{-1}. Because there were 10 inoculum levels, requiring 15 seedlings each, a minimum of 150 seedlings were raised genotype^{-1}.

The ten-day-old pigeonpea seedlings were inoculated with *F. udum* infested wheat seed/s as described under the pathogenicity test (Section 4.2.4). Eight genotypes were therefore inoculated with ten inocula levels. The pots were laid in an RCBD in the screenhouse. The pots were watered with ordinary tap water as the need arose, beginning 24 hr after inoculation.

4.2.7 Disease assessment

Fusarium wilt symptoms were observed from 10 d after inoculation, continuing to 45 d. The numbers of dead and diseased seedlings were recorded weekly. The observations included: no apparent symptoms or disease (0 – 10 d); chlorosis and early wilting of seedlings (10 – 15 d); chlorosis, stunting, defoliation of lower leaves and late wilting (15 – 30 d); and chlorosis, defoliation, stunting, but no wilting (>30 d). Based on these

reactions, the cultivars were rated using a scale of 1 to 5 with modifications (Nene and Kannaiyan, 1982; Reddy and Raju, 1993) for the wilt incidence, where

1 = 0 – 20% (resistant),
2 = 21 – 40% (moderately resistant),
3 = 41 – 60% (susceptible),
4 = 61 – 80% (moderately susceptible)
5 = 81 – 100% (highly susceptible)

4.2.8 Data analysis

Data on dead and infected plants for each inoculum level during the pathogenicity test, and inoculation technique genotype^{-1} and inoculum level, were calculated and analyzed. Analysis of variance (ANOVA) of the Genstat statistical package was used and the analyzed data has been presented in the form of tables and graphs. Data on pathogenicity was transformed using $\log_{10}$ (x + c, where c = 10), while data on the inoculation technique was transformed using logit (c + c, where c = 100)

4.3 Results

4.3.1 Isolation, purification and identification of the pathogen

Isolation of *F. udum* from diseased pigeonpea plants was successful because precautions were followed (Burgess et al., 1994) in the isolation of the pathogen from the fresh samples. The pathogen was isolated several times from different samples with the same success. The characteristics of the isolates were typical of the *F. udum* (Figure 4.4) as described by Booth (1971, 1978) and Butler (1910). Making two transfers onto fresh PDA Petri dishes purified the cultures of the pathogens. The method is comparable to the single spore technique.

The isolate was identified as the true *F. udum* pathogen by Adriaana Jacobs, Project Leader of Biosystematics Division, Culture Collections, Plant Protection Institute, Agricultural Research Council, Pretoria, Republic of South Africa. This confirmed our identification of the pathogen, based on the description and experience of working with this pathogen.

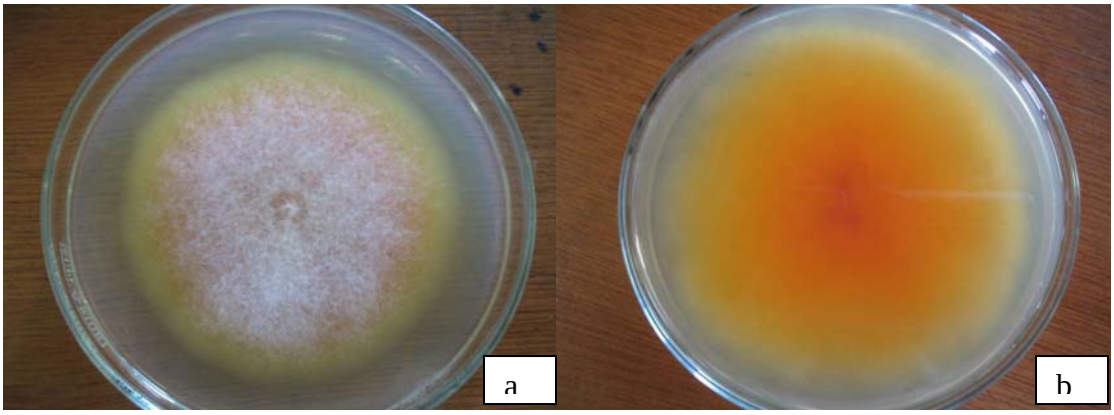


Figure 4.4: Cultures of *Fusarium udum* (Front (a) and back (b) of the Petri dishes)

4.3.2 Preservation of the isolates

The three methods of preserving the isolates (PDA slants, silica gel and double sterile distilled water) worked well. Since March 2005, all the isolates have been viable (Figure 4.5). The best choice for the Malawi location would be double sterilised distilled water since it is cheap, and the facilities readily available.

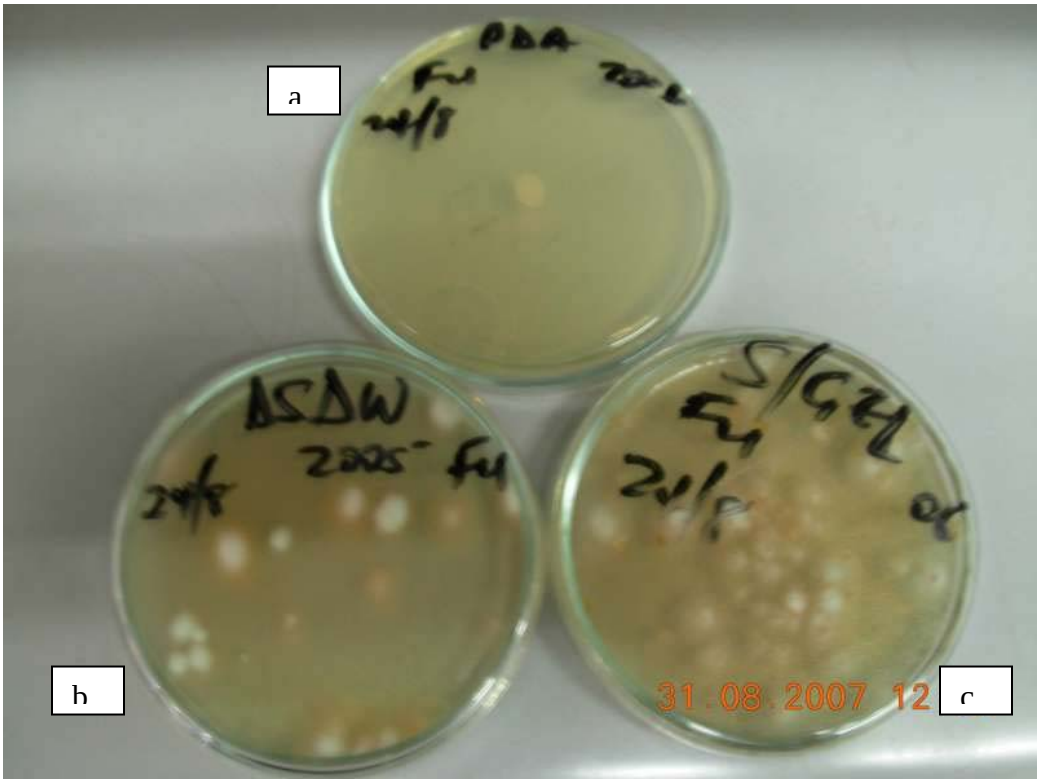


Figure 4.5: Viability test of the *Fusarium udum* isolates preserved in PDA slants (a); double sterilized distilled water (b); and silica gel (c).

4.3.3 Multiplication of inoculum

All the media (finger millet, sorghum and wheat) showed to be good substrates to multiply the *F. udum* pathogen (Figures 4.6 and 4.7). The fungus colonised the seeds in the jars or conical flasks within 7 to 10 d. Colonisation was faster in finger millet than in wheat and sorghum. All three substrates were used in the pathogenicity test.

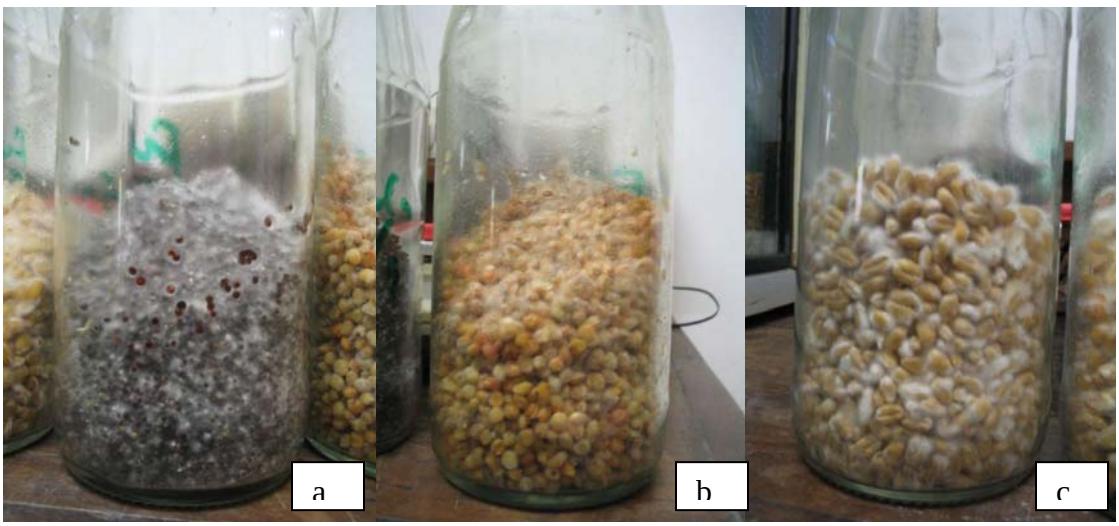


Figure 4.6: *Fusarium udum* multiplied on sterilized finger millet (a); sorghum (b); and wheat (c) seeds

Figure 4.7: *Fusarium udum* inocula on the three substrates ready for use in the inoculation trial

4.3.4 Pathogenicity test for *Fusarium udum* pathogens

Isolates of *F. udum* from Bunda College and Bvumbwe Research Station sick plots were similar in their cultural (colony margin, growth, density of the mycelia) and morphological characteristics. Both isolates were virulent, they caused Fusarium wilt in ten-day-old pigeonpea seedlings and the symptoms were typical of the disease. The isolates grown in the three different media: finger millet, sorghum and wheat were equally virulent. All the five inocula levels used in the pathogenicity test showed to be adequate to incite the disease in pigeonpea seedlings, although the incidence of disease resulting from one seed plant^{-1} of finger millet was less than the same treatments in sorghum and wheat substrates (Table 4.2). The Bunda isolate was chosen to be used in the inoculation trial.

Table 4.2: Fusarium wilt disease incidence at different inocula levels

Inoculum level (No. of infested seeds)	**Disease incidence (% wilt)**	**Disease incidence (% wilt) Transformed data (Log_{10})**
1	34.52	1.50
2	41.87	1.59
3	45.60	1.68
4	41.13	1.60
5	40.67	1.62
Mean		1.614
LSD (5%)		0.178
CV %		14.38

4.3.5 Inoculation technique for *Fusarium udum* pathogen

There was significant variation ($P<0.001$) in the reactions of the genotypes to artificial inoculations in the greenhouse (Figure 4.8 and Appendix 4.1). Two genotypes, KAT60/8 and AP29, showed susceptibility to the disease, while ICP9145, ICPL87051, and ICEAP00020, showed moderate resistance to Fusarium wilt.

There were no significant differences ($P>0.05$) in the genotype reaction when the inoculum levels were increased from one to ten seeds plant^{-1} (Figure 4.9 and Appendix 4.2). The incidence of the disease did not increase with the increase in inoculum levels. A single *F. udum* infested seed could initiate the disease in pigeonpea seedlings as effectively as ten infested seeds.

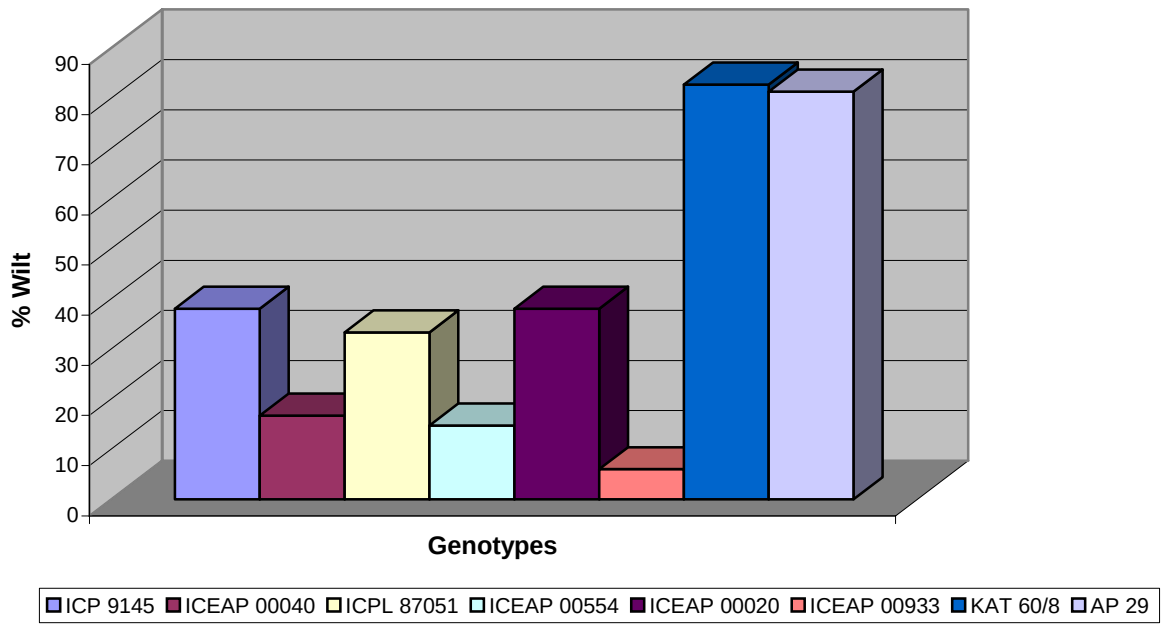


Figure 4.8: Reaction of pigeonpea genotypes to artificial inoculation with *Fusarium udum*

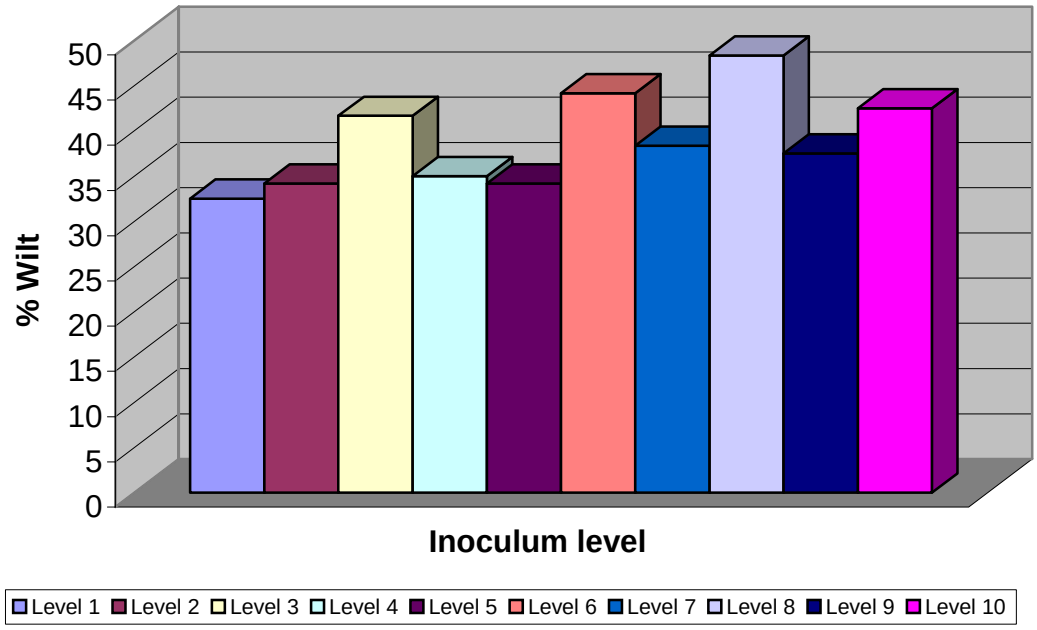


Figure 4.9: Fusarium wilt disease incidence in pigeonpea seedlings with increased inoculum level

4.4 Discussion and conclusion

Several artificial Fusarium wilt screening methods have been developed for pigeonpea, and their performance depends on many factors. The development of a new infested seed inoculation technique will assist in screening pigeonpea germplasm and filial generations in the greenhouse and enhance the breeding of pigeonpea for wilt resistance. The research showed that, with simple resources, isolates of the pathogen can be preserved, multiplied and inoculated.

Finger millet, sorghum and wheat have proven to be good substrates for multiplying *F. udum*. Cereal grains were used because they are high in nutrients that support fungal growth. Hence, the fungus colonizes them within a short time. Przybylowicz and Donoghue (1988) reported similar results. The applications of these substrates can extend towards the development of the sick soils. The infested substrates can easily be mixed with soils either in pots (greenhouse), or in sick plots, to develop sick soils or to increase the inoculum levels in the soils (Phipps and Stipes, 1973).

The pathogenicity results also showed that the three substrates do not affect the virulence of the pathogen. This may be attributed to the shorter storage period because the fungus loses virulence with long storage. Despite the three substrates giving similar results on inoculum multiplication and infection, the choice of the best substrate was wheat, due to its large seed size enabling easy handling and quantification.

The variations in the reaction of the genotype to *F. udum* showed differential genetic resistance. With reference to the background information, the results showed that six genotypes were resistant to Fusarium wilt. Two susceptible genotypes, KAT60/8 and AP29, showed that the technique worked and could be used to screen pigeonpea genotypes against Fusarium wilt disease.

The level of inoculum did not have any significant effect on the incidence of the disease because one infested seed was as infectious as ten or more seeds. A single seed carries enough inoculum to cause infection in the plant. However, this may be attributed to the presence of the mycelia in the inoculum and the accuracy of the placement of the inoculum. The mycelia continue growing from the substrate into the host tissue. The

combination of the mycelium and conidia assured an inoculum level that could cause the disease reliably.

The amount of inoculum has direct implications on the screening of pigeonpea germplasm. If the objective of screening germplasm is only to check resistance or susceptibility to the disease, then, any inoculum level can be used. However, if the aim is to screen the filial generations for resistance, it is imperative to use higher inoculum levels. The plants withstanding the disease are resistant and can be advanced to the next filial generation.

The infested seed inoculation technique has several advantages over the other inoculation techniques. Multiplication of the inoculum in wheat substrates does not require expensive equipment. It is easier to quantify the inoculum by just counting the number of seeds; a haemacytometer is not required to count spores. The inoculation is simple, requiring placement of the infested seeds on the bruised roots. In the methods where only spores are used in the inoculation process, dormancy of the spores before germination affects the results. There is no time variation in this technique compared to the root-dip and transplanting methods. Because of the presence of both mycelia and spores, there is an assurance of infection.

However, there is a need for further work on the methodology. It is important to determine how long the substrates can support the pathogen before it loses its virulence. It is also imperative to quantify inoculum, both mycelia and spores.

In conclusion, the infested seed inoculation technique is a more effective way of screening pigeonpea germplasm, and filial generations in the breeding programme, without any disease escaping. The technique uses readily available materials; the pathogen is multiplied in commonly available substrates such as wheat, sorghum and finger millet; the inoculation assures that infection takes place, and the results are a true reflection of the genetic make-up of the plant in terms of resistance or susceptibility of the germplasm to the disease. The applicability of the technique to other pathogens and crops is of interest to plant breeders.

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Appendices

Appendix 4.1: Reaction of pigeonpea genotypes to artificial inoculation with *Fusarium udum*

Genotypes	Disease incidence (% Wilt)	
	% Wilt (Untransformed data)	% Wilt (Transformed data - Logit)
ICP 9145	38.0	-80.40
ICEAP 00040	16.7	-84.84
ICPL 87051	33.3	-78.92
ICEAP 00554	14.7	-84.40
ICEAP 00020	38.0	-80.40
ICEAP 00933	06.0	-85.74
KAT 60/8	82.7	-63.44
AP 29	81.3	-65.06
Mean	38.84	-77.44
LSD (<0.001)		0.292***
CV (%)		13.2

*** Significant at P < 0.001

Appendix 4.2: Fusarium wilt incidence in pigeonpea seedlings with increased inoculum level

Inoculum level (No. of seeds)	Disease incidence (% wilt)	
	% wilt (Untransformed data)	% wilt (Transformed data - Logit)
1	32.50	-82.58
2	34.16	-78.44
3	41.66	-76.16
4	35.00	-79.26
5	34.16	-78.44
6	44.16	-72.14
7	38.34	-77.86
8	48.34	-75.70
9	37.50	-76.82
10	42.50	-75.24
Mean	38.84	-77.44
LSD (<0.05)		0.292 ns
CV (%)		13.2

Chapter 5: Inheritance of resistance to Fusarium wilt and yield traits in pigeonpea

Abstract

Fusarium wilt is the main constraint on pigeonpea production in Malawi; hence, deployment of disease resistant varieties would be valuable. The purpose of this study was to understand the nature and mechanism of inheritance of Fusarium wilt resistance, yield and secondary traits in pigeonpea. Forty-eight crosses were generated in a 12 lines x 4 testers mating scheme. Some F_1 plants were selfed in a screen house, while others were evaluated for wilt resistance, yield, and secondary traits in a randomised complete block design with three replications at three locations. Segregation analyses were conducted on the F_2 populations to determine the phenotypic ratios of resistance to susceptibility. There were significant variations among F_1 plants for wilt, days to 50% flowering, seed pod^{-1}, stem thickness, and number of secondary branches. Specific combining ability (SCA) effects were predominant for wilt, days to 50% flowering, and number of secondary branches. GCA effects, mainly due to maternal genotypes, were preponderant for yield and other secondary traits. The significance of GCA and SCA effects suggested that variations were due to additive gene action in both the testers and parental lines arising from the interactions of the testers, and the dominance effects, arising from the interactions of the parental lines. Testers and parental lines with good combining ability effects for wilt resistance, days to 50% flowering, seed pod^{-1}, plant height, stem diameter, number of primary and secondary branches were identified. ICEAP00554 was a good general combiner for wilt resistance and days to 50% flowering. Significant SCA effects were observed for crosses AP5 x ICP9145, AP6 x ICEAP00554, and AP10 x ICEAP00040 for wilt resistance. Crosses AP9 x ICEAP00040 and AP23 x ICP9145 displayed significant SCA effects for early maturity while AP 4 x ICEAP00040 displayed significant SCA for number of secondary branches. The Chi-square analysis suggested that resistance to wilt was dominant over susceptibility in most F_2 populations. The segregation ratios of 3:1, 15:1, and 9:7 suggested that either one dominant gene or two independent dominant genes, or two complementary genes, respectively, were conferring wilt resistance in these crosses. Involvement of a few genes governing wilt resistance suggested few complications, if any, should be faced when breeding for this trait in pigeonpea. Pedigree breeding method would be recommended for incorporating the various traits in pigeonpea.

5.1 Introduction

Pigeonpea is widely grown by small-scale farmers in Malawi, particularly in the southern region. It has a yield potential of more than 2 t ha^{-1} (Chauhan, 1990). However, the yields have stagnated below 800 kg ha^{-1}, despite the increase from 1995 to 2007 of 97891 ha to 158129 ha in area under production (Ministry of Agriculture and Food Security, 2007). Fusarium wilt is the main constraint reducing pigeonpea productivity in Malawi. The disease is aggravated by growing local landraces susceptible to the disease and practising very little, if any, rotation. In susceptible genotypes, yield losses were as high as 100% (Soko, 1992). Use of resistant cultivars, as an integral part of the integrated disease management strategy, has been reported as the most viable and economic option for controlling the disease, particularly for small scale farmers who are unable to afford chemical control measures or fallow, due to small land size (Reddy et al., 1990; Reddy et al., 1998; Ruckenbauer et al., 2001). Use of resistant cultivars can only be achieved through breeding for Fusarium wilt resistance.

Fusarium wilt resistant varieties, ICP9145 (Sauma) and ICEAP00040 (Kachangu), were released in the country in 1987 (Reddy et al., 1995) and in 2000 (Silim et al., 2005), respectively. ICP9145 is susceptible to wilt in some parts of Malawi. The adoption of these Fusarium wilt resistant cultivars has been declining since their release, due to several reasons. ICP9145 did not spread because of small seed size, difficulty in dehulling (reported by millers), lack of aroma and taking long to cook. In addition, there was no seed system to support the adoption of the variety. ICEAP00040 was bred specifically for resistance to wilt, ease of dehulling, excellent aroma and fast cooking. It is a recent release and has not had seed system support. Both varieties are introduction from Kenya and their attributes are not comparable to the local landraces in such aspects as flavour, taste, and short cooking time. Therefore, it is imperative to breed for Fusarium wilt resistance in farmer-preferred cultivars. A sound knowledge of the genetics of resistance to Fusarium wilt in pigeonpea will be useful in initiating an effective breeding programme aimed at developing Fusarium wilt resistant varieties (Kamboj et al., 1990; Snijders, 1990; Zhang et al., 2007).

A number of reports have been published on pigeonpea heritability estimates of various quantitative traits such as grain yield, pods $plant^{-1}$, seed size, primary and secondary

branches, protein content, plant height, days to 50% flowering, and days to maturity. Generally, characters such as yield, pod plant^{-1}, seed pod^{-1}, protein content, primary and secondary branches have low heritability (<50%), while days to flowering, plant height, and seed size have high heritability (>75%) (Saxena and Sharma, 1990). Manyasa et al. (2007) reported heritability estimates in Tanzanian pigeonpea for various traits such as days to 50% flowering (0.75), plant height (0.67), grain yield (0.28), seeds pod^{-1} (0.40), seed weight (0.63), and number of primary branches (0.58). The variations in the heritability estimates depend on the population and environments in which they are obtained (Saxena and Sharma, 1990). The high heritability estimates suggest that the character concerned can easily be selected for the test environment. However, not much work has been done on the heritability of Fusarium wilt resistance. Therefore, it was important to establish the narrow sense heritability (h^2_n) of Fusarium wilt resistance in local landraces.

Genetic studies, through analysis of segregation ratios, have been used to determine the number and nature of genes governing certain traits in pigeonpea and other crops (Gunduz et al., 2001; Ashry et al., 2002; Bahadur et al., 2002; Aher et al., 2003). Work done by many scientists, as cited by Saxena and Sharma (1990), indicate conflicting information on the number and nature of genes governing Fusarium wilt resistance in pigeonpea. The reports vary from multiple factors, to two complimentary genes, to a single dominant gene. It was also reported that resistance to wilt was dominant over susceptibility (Saxena and Sharma, 1990; Okiror, 2002). The variations depend on the populations and the methods used. Therefore, Malawian landraces might display a different number of genes and mode of inheritance.

Combining ability is the relative ability of a biotype to transmit desirable performance to its crosses (Dabholkar, 1999). It describes the breeding value of parental lines to produce hybrids (Ahuja and Dhayal, 2007). The general combining ability (GCA) is the average performance of a plant in a cross with different tester lines (Acquaah, 2007; Ahuja and Dhayal, 2007). Specific combining ability (SCA) is used to designate those cases in which certain combinations do relatively better or worse than would be expected on the basis of average performance of the lines involved (Dabholkar, 1999). In an 8 x 8 diallel, Ghodke et al. (1993) identified parents which were good combiners for yield, pods plant^{-1}, 100-grain weight, and number of primary and secondary branches in

pigeonpea. They also identified specific crosses which had significant SCA effects for yield and days to maturity. A line x tester mating design was used to reveal the predominance of non-additive gene action for seed yield plant^{-1} (Srinivas et al., 2000). Baskaran and Muthiah (2007) found significant SCA variances for yield. Therefore, it is imperative to determine the GCA and SCA of pigeonpea testers and lines to choose appropriate parents and crosses to combine effectively for Fusarium wilt resistance.

The purpose of this study was, therefore, to understand the nature and mechanism of the inheritance of Fusarium wilt resistance, yield, and secondary traits in pigeonpea crosses between local landraces and ICRISAT resistant testers. The intention was to use the findings to plan a proper breeding programme to develop high yielding and Fusarium wilt resistant pigeonpea cultivars with consumer and farmer-preferred traits. Therefore, the specific objectives of the study were to

a. Determine the type of gene action involved in Fusarium wilt resistance and certain quantitative traits;
b. Estimate the general and specific combining ability effects of the parental lines and testers for resistance and quantitative traits;
c. Estimate segregation of F_2 populations into resistant and susceptible crosses to estimate the number of genes governing the various traits.

5.2 Materials and methods

5.2.1 Pigeonpea genotypes used in the breeding programme

Farmers, through participatory variety selection (PVS), selected the local pigeonpea landraces used in the study (see Chapter 2). Twelve pigeonpea landraces with farmer preferred traits were selected, and four testers as sources of Fusarium wilt resistance, were obtained from ICRISAT[1] (Kenya). Two of the testers had already been released in Malawi as late maturing, Fusarium wilt resistant varieties (ICP9145 and ICEAP00040). The other two, ICPL87051 and ICEAP00554, which are medium maturing, had not yet been released in Malawi. The genotypes had different levels of resistance to Fusarium wilt, were medium to late maturing and had different flower colours (Table 5.1).

[1] ICRISAT = International Crops Research Institute for the Semi-Arid Tropics

Table 5.1: Origin and phenotypic characteristics of the 16 pigeonpea genotypes

Genotype	Source	Wilt reaction	Maturity period	Flower colour
AP1	Local landrace	Susceptible	Late maturing	Light green, medium purple streaks
AP2	Local landrace	Susceptible	Late maturing	Yellow, dense red streaks
AP3	Local landrace	Resistant	Late maturing	Ivory
AP4	Local landrace	Resistant	Late maturing	Yellow, sparse streaks
AP5	Local landrace	Susceptible	Late maturing	Yellow, medium purple streaks
AP6	Local landrace	Susceptible	Late maturing	Ivory
AP7	Local landrace	Susceptible	Late maturing	Yellow, medium purple streaks
AP8	Local landrace	Resistant	Medium maturing	Deep yellow, union coverage
AP9	Local landrace	Susceptible	Very late maturing	Yellow, sparse streaks
AP10	Local landrace	Resistant	Medium maturing	Deep yellow, union coverage
AP23	Local landrace	Susceptible	Late maturing	Yellow, medium red streaks
AP29	Local landrace	Susceptible	Late maturing	Deep yellow, dense red streaks
ICP9145	ICRISAT Kenya	Resistant	Late maturing	Ivory
ICEAP00040	ICRISAT Kenya	Resistant	Late maturing	Ivory
ICPL87051	ICRISAT Kenya	Resistant	Medium maturing	Yellow
ICEAP00554	ICRISAT Kenya	Resistant	Medium maturing	Ivory

5.2.2 Line x tester mating scheme

A total of 16 genotypes were used as parents in a 12 lines x 4 testers mating design. Each of the twelve local landraces was crossed to each of the four testers, in a screenhouse, at Kandiya Research Station in Lilongwe (Figure 5.1 and 5.2). The testers were used as males, while local landraces were used as females in crosses.

Figure 5.1: Screenhouse structure at Kandiya Research Station, Malawi

Figure 5.2: Arrangement of pots per genotype in the screenhouse

Twenty-four plants genotype^{-1} were raised in 15 L pots, two plants pot^{-1} (Figure 5.2). In planting, the testers were staggered to synchronise the flowering time with the local landraces. The first set was planted with the rest of the local landraces, while the second set was planted two weeks later. During the flowering stage, each landrace (line) was crossed to each tester. Pollination was done by hand (Figure 5.3) from 7.30 am to about 11.00 am to avoid heat affecting fertilization (Sharma and Green, 1980). Pigeonpea flowers are cleistogamous; self-pollination is done before the flower opens, and so the pollination team was attentive to the age of the flowers. The flowers were emasculated just before pollination. Each flower was tagged with a label indicating the parents used in the cross, the date of crossing, and the person who made the cross. Proper hygiene was followed to avoid pollen contamination; the forceps were dipped in 70% alcohol before using them on further flowers. The target in crossing was to produce a minimum of 60 seeds cross^{-1}; as many flowers as possible (>75) were therefore pollinated because the expected rate of successful crosses in pigeonpea is about 20% (Sharma and Green, 1980). Pod set on successful crosses was monitored on a daily basis, because the aim was to produce at least 20 pods with 3 – 4 seeds each. The success on the crosses was monitored by looking at development of the pods on tagged flowers, and in some cases selfed flowers were removed. The 48 F_1 crosses were advanced to F_2 by self-pollination

in the screenhouse at Kandiya Research Station. Six F_1 plants cross^{-1} were raised in three 15 L pots, two plants pot^{-1}. The target was to produce at least 150 F_2 seeds for segregation analysis.

Figure 5.3: Hand pollination of pigeonpea flowers in the screen house

5.2.3 Evaluation of pigeonpea F_1 crosses for Fusarium wilt resistance

Screenhouse trial: In the screenhouse, F_1 plants were evaluated for resistance to Fusarium wilt through artificial inoculation, using the infested seed inoculation technique (see Chapter 4). Each plant was inoculated with five *F. udum* infested wheat seeds after bruising, and transplanted into the pot. Two crosses were unsuccessful. Forty-six F_1 crosses were evaluated in the trial, and three seedlings of each F_1 cross pot^{-1} were inoculated. The trial was laid out as a randomized complete block design (RCBD) with three replications. The number of plants wilting or showing typical symptoms of Fusarium wilt was recorded weekly, starting from 10 days after inoculation, up to the podding stage. The Fusarium wilt assessment scale described by Nene and Kannaiyan (1982) was used to rate plants for resistance as follows:

0 - 20% infection – resistant;

21 - 40% infection – moderately resistant / tolerant;

41 - 60% infection - susceptible;

61 - 80% infection – moderately susceptible;

81 - 100% highly susceptible

Field trial: Five plants plot^{-1}, replicated three times, were planted at 0.5 m intervals in rows, 0.9 m apart in an RCBD. The trial was planted after the first rains at Kandiya Research Station (devoid of Fusarium wilt), and was kept weed free at all times. There was no yield trial at the Bunda sick plot, due to high disease pressure. Fertilizers were not applied, to simulate farmer practice. The season registered 1076 mm of rainfall. Data on yield (g plant^{-1}), plant height, stem thickness, days to 50% flowering, seed pod^{-1}, primary branches, secondary branches, and seed weight (g of 100 seed weight) were collected plot^{-1} at the end of the trial.

5.2.4 Screening pigeonpea F_2 lines for Fusarium wilt resistance

Field trial: The trial was planted at three sites, Bvumbwe Research Station, Bunda College and Kandiya Research Station. Some F_1 crosses were unsuccessful in giving 150 seeds in selfing. Nineteen and 23 F_2 populations were planted at Bvumbwe Research Station and Bunda College sick plots respectively. Fifty seeds were planted for each F_2 population, at 300 mm intervals, in rows 0.9 m apart. These F_2 populations were subjected to Fusarium wilt through natural inoculation. AP29, a susceptible check, was planted in a single row between plots. This was aimed at checking the distribution of the pathogen in the trial. The trials were planted with the first rains and kept weed free at all times. Fertilizers and chemicals were not applied to simulate farmer practice. Data was collected on the number of pigeonpea plants wilting, dying, and showing typical symptoms of Fusarium wilt; this was done at weekly intervals, starting one week after seedling emergence up to the podding stage.

Screenhouse trial: Thirty-two plants (four pot^{-1}) of 31 F_2 lines were artificially inoculated with *F. udum,* using an infested seed inoculation technique (ISIT) (see Chapter 4). Seventeen F_1 crosses failed to give enough seed for a trial. The number of available pots limited the number of plants to be screened per F_2 population. The inoculated seedlings were planted in 15 L pots in the screenhouse at Kandiya Research Station. The number of plants wilting, dying, and showing typical symptoms of Fusarium wilt was recorded at weekly intervals, starting from 10 days after inoculation to the podding stage.

5.2.5 Genetic analysis of Fusarium wilt, yield and secondary traits

The analyses of variances for percentage wilt, yield, and secondary traits were performed on the GenStat Computer Package. Data on percentage wilt and secondary

branches were transformed using logit (x + c, where c = 100), while yield data were transformed using log base 10 (x + c, where c = 10). Crosses were fixed while replications were considered as random. A North Carolina Design II (NCII) analysis was performed using the following model (Dabholkar, 1999):

$$Y_{ijk} = \mu + g_i + g_j + s_{ij} + r_k + e_{ijk}$$

Where Y_{ijk} = mean phenotypic value;
μ = grand mean effect;
g_i = GCA effect of i^{th} parent (lines);
g_j = GCA effect of j^{th} parent (testers);
s_{ij} = SCA effect of cross i x j;
r_k = replication effect;
e_{ijk} = random error.

The GCA effects of testers and parental lines were estimated using the following formula (Dabholkar, 1999):

$$\hat{g}_j \text{ (tester)} = (Y_{.j.} / rm) - (Y\ldots / rmf) \quad \text{and} \quad \hat{g}_i \text{ (lines)} = (Y_{i.} / rf) - (Y\ldots / rmf)$$

Where $\hat{g}_i$ and $\hat{g}_j$ = estimates of GCA effects of the ith line and jth tester, respectively;
$Y_{.j.}$ = sum of all crosses involving j^{th} tester;
$Y_{i.}$ = sum of all crosses involving i^{th} line;
$Y\ldots$ = grand total of all observations over all genotypes;
r = number of replicates;
m = number of male parents (testers);
f = number of females (lines).

The SCAs for all the significant traits were calculated using the following formula from Dabholkar (1999):

$$\hat{S}_{ij} = (Y_{ij.}/r) - (Y_{i..}/rf) - (Y_{.j.}/rm) + (Y\ldots/rmf)$$

Where $Y_{ij.}$ = sum of all crosses over all replications

$Y_{i..}$ = sum of all crosses involving ith line as one parent over all the testers and replications

$Y_{.j.}$ = sum of all crosses involving jth tester

Y… = grand total of all observations over all genotypes

r = number of replicates

m = number of male parents

f = number of females

The segregation ratios of the F_2 on wilt incidences were subjected to Chi-square analysis to test the goodness of fit between the observed, and the expected, means (Kamboj et al., 1990; Hill et al., 2006).

5.3 Results

5.3.1 Gene action controlling wilt resistance, yield and secondary traits

The Restricted Maximum Likelihood (REML) analysis of the F_1 crosses data (Table 5.2) were used to compare the gene action controlling the various traits in pigeonpea. Such traits included wilt resistance, yield plant^{-1}, days to 50% flowering, seed pod^{-1}, yield plant^{-1}, plant height, primary branches, secondary branches and stem thickness. The analyses show that, for some traits, both general combining ability for testers (GCA_t), general combining ability for parental lines (GCA_p), and the specific combining ability (SCA) effects were important in controlling the traits. While in other analyses, only the GCA_p effects due to parental lines were responsible for controlling the traits.

Table 5.2: REML analyses of variances of various traits of pigeonpea F_1 crosses

Fixed term	d.f.	MS	P value	Fixed term	d.f.	MS	P value
% Wilt				**Plant Height**			
Tester (GCAt)	3	17.02	<0.001	Tester (GCAt)	3	0.99	0.397
Parental lines (GCAp)	11	6.15	<0.001	Parental lines (GCAp)	11	2.42	0.005
Tester x Parental line (SCA)	31	2.66	<0.001	Tester x Parental line (SCA)	29	0.74	0.843
Yield (g plant^{-1}) (Transformed data)				**Stem Diameter**			
Tester (GCAt)	3	1.14	0.330	Tester (GCAt)	3	1.10	0.349
Parental lines (GCAp)	11	1.60	0.092	Parental lines (GCAp)	11	3.29	<0.001
Tester x Parental line (SCA)	21	1.22	0.223	Tester x Parental line (SCA)	29	1.06	0.380
D50%F				**Primary Branches**			
Tester (GCAt)	3	8.91	<0.001	Tester (GCAt)	3	0.44	0.727
Parental lines (GCAp)	11	15.83	<0.001	Parental lines (GCAp)	11	3.13	<0.001
Tester x Parental line (SCA)	29	3.14	<0.001	Tester x Parental line (SCA)	29	0.61	0.951
Seed pod^{-1}				**Secondary Branches**			
Tester (GCAt)	3	1.22	0.300	Tester (GCAt)	3	4.79	0.002
Parental lines (GCAp)	11	3.58	<0.001	Parental lines (GCAp)	11	4.64	<0.001
Tester x Parental line (SCA)	28	1.04	0.401	Tester x Parental line (SCA)	29	1.68	0.012

There were significant (P<0.001) variations among F_1 crosses in their reaction to Fusarium wilt. Wilt incidences ranged from 0% to 97.3%, with a mean of 31.7%. Twenty-one out of 46 F_1 crosses were highly resistant to wilt, eight were moderately resistant, while the rest were susceptible. About 63% of the F_1 crosses were resistant to Fusarium wilt at the end of the trial (Table 5.3).

Table 5.3: Evaluation of F_1 crosses for Fusarium wilt (% wilt) in screenhouse

Cross	% Wilt		Cross	% Wilt	
	Transformed	**Untransformed**		**Transformed**	**Untransformed**
AP1 X ICP9145	3.275	22.0	AP6 X ICEAP00554	2.789	11.0
AP1 X ICEAP00040	3.782	55.7	AP7 X ICP9145	2.789	11.0
AP1 X ICPL87051	3.955	44.3	AP7 X ICEAP00040	3.588	44.3
AP1 X ICEAP00554	3.469	33.3	AP7 X ICPL87051	4.150	55.7
AP2 X ICP9145	2.789	11.0	AP8 X ICP9145	3.955	44.3
AP2 X ICEAP00040	3.557	34.3	AP8 X ICEAP00040	3.081	18.5
AP2 X ICPL87051	3.955	44.3	AP8 X ICPL87051	3.872	38.7
AP2 X ICEAP00554	2.303	00.0	AP8 X ICEAP00554	2.789	11.0
AP3 X ICP9145	2.303	00.0	AP9 X ICP9145	3.081	18.5
AP3 X ICEAP00040	3.076	17.2	AP9 X ICEAP00040	3.761	33.0
AP3 X ICPL87051	2.789	11.0	AP9 X ICPL87051	4.268	66.7
AP3 X ICEAP00554	2.303	00.0	AP9 X ICEAP00554	2.303	00.0
AP4 X ICP9145	2.303	00.0	AP10 X ICP9145	4.463	78.0
AP4 X ICEAP00040	2.303	00.0	AP10 X ICEAP00040	2.789	11.0
AP4 X ICPL87051	2.789	11.0	AP10 X ICPL87051	4.150	55.7
AP4 X ICEAP00554	2.303	00.0	AP10 X ICEAP00554	2.789	11.0
AP5 X ICP9145	2.303	00.0	AP23 X ICP9145	3.469	33.3
AP5 X ICEAP00040	4.582	89.0	AP23 X ICPL87051	3.469	33.3
AP5 X ICPL87051	4.607	97.3	AP23 X ICEAP00554	3.275	22.0
AP5 X ICEAP00554	2.789	11.0	AP29 X ICP9145	2.789	11.0
AP6 X ICP9145	4.344	67.0	AP29 X ICEAP00040	3.955	44.3
AP6 X ICEAP00040	4.463	78.0	AP29 ICPL87051	3.955	44.3
AP6 X ICPL87051	4.582	89.0	AP29 X ICEAP00554	3.663	44.7
			Minimum	**2.303**	**00.0**
			Mean	**3.350**	**31.7**
			Maximum	**4.607**	**97.3**
			P value	**<0.001**	
			LSD (<0.05)	**1.074**	
			CV (%)	**19.8**	

The F_1 crosses (in Table 5.3) reacted differently to the disease infection over the evaluation period of four months (Figure 5.4). The resistant crosses had disease incidences below 20%, while the disease incidences for the tolerant crosses ranged from 21% to 40%. Seventeen crosses were susceptible, with a range of 41% to 100%. Most of the pigeonpea seedlings which wilted and died due to the disease did so within two months after inoculation. Two unsuccessful crosses, AP7 x ICEAP00554 and AP23 x ICEAP00040, were excluded from the graphs.

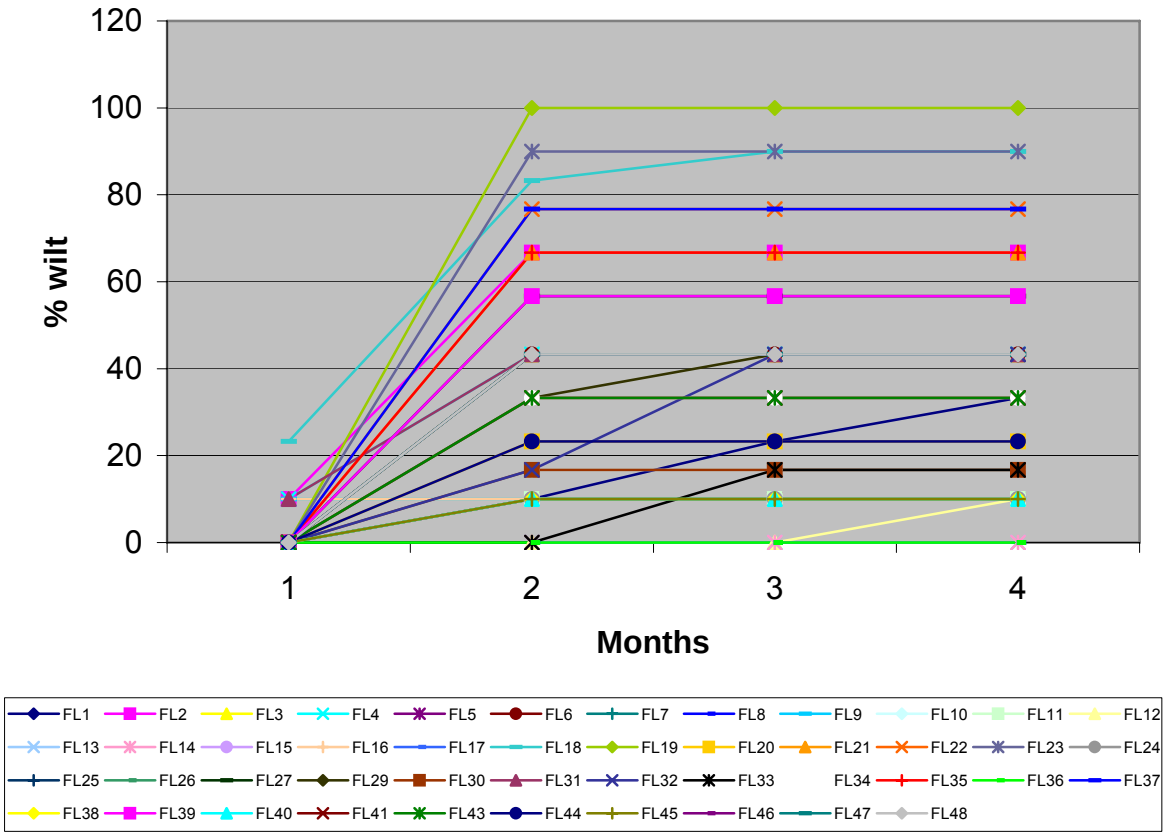

Figure 5.4: Cumulative Fusarium wilt disease incidence in F_1 crosses over four months in the screenhouse at Kandiya Research Station. Key: FL = F_1 line

The analysis of variance (ANOVA) for the transformed data showed that yield differed significantly (P = 0.05) among the F_1 crosses, with a range of 0.2 to 59.7 g plant^{-1} (Table 5.4). F_1 crosses also differed significantly (P<0.001) in the days to 50% flowering. The F_1 crosses ranged from 91 - 214 d to 50% flowering, with a mean of 148.5 d. Most of the F_1 populations were medium maturing. The results also showed that the F_1 crosses differed significantly (P<0.001) in the number of seeds pod^{-1}, ranging from 2.1 to 5.3. Most of the F_1s had high number of seeds pod^{-1}. The seed mass varied among the F_1 crosses, ranging from 11.9 - 20.7 g 100 seeds^{-1}, with a mean of 16.9 g 100 seeds^{-1} showing high seed mass. Most of them were large-seeded, with more than 15 g 100 seeds^{-1}.

Table 5.4: Evaluation of F_1 pigeonpea lines for yield, days to 50% flowering (D50%F) and number of seeds pod^{-1}

Cross	Yield g $plot^{-1}$		D50%F	Seed pod^{-1}	Seed mass (g)
	Transformed	Untransformed			
AP1 X ICP9145	4.06	49.2	125.7	3.3	16.9
AP1 X ICEAP00040	2.66	03.5	126.3	3.3	19.0
AP1 X ICPL87051	3.05	12.3	123.3	3.0	16.4
AP1 X ICEAP00554	3.25	22.6	121.7	4.0	14.6
AP2 X ICP9145	3.26	16.5	130.0	4.7	18.6
AP2 X ICEAP00040	3.48	24.1	133.7	5.3	18.3
AP2 X ICPL87051	3.80	34.6	128.0	5.3	16.9
AP2 X ICEAP00554	3.84	39.8	116.7	5.7	18.4
AP3 X ICP9145	2.39	00.2	156.7	4.0	-
AP3 X ICEAP00040	2.75	07.6	155.0	4.0	17.5
AP3 X ICPL87051	3.14	15.0	171.7	3.5	15.9
AP3 X ICEAP00554	3.16	16.5	147.0	4.3	13.6
AP4 X ICP9145	3.17	19.1	156.7	3.7	-
AP4 X ICEAP00040	2.79	08.3	145.0	5.3	15.8
AP4 X ICPL87051	2.47	00.5	168.0	5.0	16.0
AP4 X ICEAP00554	3.20	16.1	143.3	4.7	12.9
AP5 X ICP9145	4.19	56.9	150.0	4.3	19.2
AP5 X ICEAP00040	3.17	19.1	140.0	4.4	-
AP5 X ICPL87051	2.48	04.0	136.7	4.4	14.4
AP5 X ICEAP00554	3.15	16.4	121.7	5.0	17.8
AP6 X ICP9145	3.17	19.1	211.7	4.1	-
AP6 X ICEAP00040	3.17	19.1	214.0	4.4	-
AP6 X ICPL87051	2.50	04.2	180.0	4.0	18.8
AP6 X ICEAP00554	3.61	33.9	173.3	4.7	14.1
AP7 X ICEAP00040	3.31	21.2	140.0	5.0	15.6
AP7 X ICPL87051	2.75	06.3	153.3	3.7	16.4
AP8 X ICP9145	3.80	36.1	142.5	4.8	20.7
AP8 X ICEAP00040	3.42	26.9	138.7	4.3	14.7
AP8 X ICPL87051	3.49	29.9	098.3	4.3	16.6
AP8 X ICEAP00554	3.28	18.3	102.7	4.7	17.3
AP9 X ICP9145	3.17	19.1	091.0	4.5	-
AP9 X ICEAP00040	2.84	08.1	211.7	4.7	14.7
AP9 X ICPL87051	2.87	08.9	187.3	3.7	18.3
AP10 X ICP9145	3.58	27.6	142.7	5.3	20.5
AP10 X ICEAP00040	3.76	59.7	126.0	4.7	19.3
AP10 X ICPL87051	3.04	13.7	095.3	4.7	19.4
AP10 X ICEAP00554	3.03	10.9	100.0	4.7	18.6
AP23 X ICP9145	3.17	19.1	202.3	3.8	-
AP23 X ICPL87051	3.00	09.1	173.3	5.1	11.9
AP23 X ICEAP00554	2.61	04.0	124.7	3.7	18.0
AP29 X ICP9145	3.07	13.4	199.0	5.8	15.8
AP29 X ICEAP00040	3.17	19.1	203.7	4.0	-
AP29 ICPL87051	3.17	19.1	156.7	2.1	-
AP29 X ICEAP00554	3.04	12.7	169.0	3.0	17.8
Minimum	**2.39**	**00.2**	**91.0**	**2.1**	**11.9**
Mean	**3.17**	**19.1**	**148.5**	**4.4**	**16.9**
Maximum	**3.76**	**59.7**	**214**	**5.8**	**20.7**
P value	**0.05**		**<0.001**	**<0.001**	**<0.001**
LSD (<0.05)	**0.997**		**35.6**	**1.44**	**SD 2.14**
CV (%)	**18.9**		**14.8**	**20.3**	**12.7**

Plant height of the F_1 crosses varied significantly (P<0.005) using REML analysis. The plants ranged from 1.71 to 2.57 m in height (Table 5.5). Significant differences (P<0.001) in stem diameter among the F_1 crosses were also observed. The stem thickness ranged from 11.4 to 33.7 mm, with a mean of 20.5 mm. The results showed that F_1 crosses varied significantly (P<0.001) in the number of primary branches. The plants ranged from 10.5 to 22.33 branches plant^{-1}. The number of secondary branches differed significantly (P<0.05) among the F_1 crosses. The number of secondary branches ranged from 5 to 30, with a mean of 14 branches (Table 5.5).

Table 5.5: Evaluation of F_1 crosses for plant height, stem diameter, primary branches, and secondary branches

Cross	Plant Height (cm)	Stem Diameter (mm)	Primary Branches	Secondary Branches	
				Transformed	Untransformed
AP1 X ICP9145	232.7	2.14	15.87	2.842	07.33
AP1 X ICEAP00040	220.3	1.61	11.27	2.906	08.33
AP1 X ICPL87051	208.7	1.66	14.20	3.169	14.00
AP1 X ICEAP00554	230.7	1.80	12.90	3.061	11.67
AP2 X ICP9145	213.7	2.17	15.47	3.171	14.00
AP2 X ICEAP00040	252.7	2.40	14.10	2.687	05.00
AP2 X ICPL87051	217.0	2.23	14.77	2.961	09.67
AP2 X ICEAP00554	197.3	2.07	14.57	2.830	07.33
AP3 X ICP9145	215.3	1.87	15.83	3.156	13.67
AP3 X ICEAP00040	217.7	1.93	14.00	2.790	06.67
AP3 X ICPL87051	212.3	2.13	17.07	3.550	25.00
AP3 X ICEAP00554	217.7	2.03	16.47	3.012	11.33
AP4 X ICP9145	211.7	1.58	17.10	3.157	13.67
AP4 X ICEAP00040	215.3	2.50	18.10	3.519	24.00
AP4 X ICPL87051	196.7	1.73	14.53	3.163	13.67
AP4 X ICEAP00554	197.3	1.97	14.93	3.186	14.33
AP5 X ICP9145	242.0	2.37	17.93	3.091	12.00
AP5 X ICEAP00040	233.0	2.90	16.67	3.269	16.33
AP5 X ICPL87051	214.0	1.87	15.33	3.290	18.00
AP5 X ICEAP00554	213.3	2.13	16.33	3.271	16.33
AP6 X ICP9145	242.7	2.37	17.00	3.513	23.67
AP6 X ICEAP00040	222.3	2.03	14.27	3.348	18.67
AP6 X ICPL87051	239.7	2.17	15.33	3.256	17.33
AP6 X ICEAP00554	256.7	3.37	19.93	3.259	16.33
AP7 X ICEAP00040	220.0	2.17	16.23	3.130	13.00
AP7 X ICPL87051	223.7	2.00	16.67	3.113	12.67
AP8 X ICP9145	201.7	1.14	12.94	3.172	14.65
AP8 X ICEAP00040	198.7	1.93	11.57	3.224	15.33
AP8 X ICPL87051	198.3	1.90	13.17	3.304	17.33
AP8 X ICEAP00554	215.0	1.53	10.80	3.119	14.33
AP9 X ICP9145	240.7	2.47	18.00	3.077	12.33
AP9 X ICEAP00040	246.3	2.80	20.27	3.434	21.67
AP9 X ICPL87051	238.0	2.60	22.33	3.637	30.00
AP10 X ICP9145	227.7	2.27	14.83	2.945	10.00

Cross	Plant Height (cm)	Stem Diameter (mm)	Primary Branches	Secondary Branches	
				Transformed	Untransformed
AP10 X ICEAP00040	225.7	1.93	14.47	3.107	12.67
AP10 X ICPL87051	204.0	1.93	13.77	2.977	10.33
AP10 X ICEAP00554	204.7	1.93	12.43	2.802	06.67
AP23 X ICP9145	171.0	1.47	10.50	3.320	17.67
AP23 X ICPL87051	216.0	2.13	14.23	3.364	19.00
AP23 X ICEAP00554	193.3	1.90	16.60	2.938	10.00
AP29 X ICP9145	176.3	1.70	16.33	3.024	11.33
AP29 X ICEAP00040	222.0	1.93	11.83	2.844	08.00
AP29 ICPL87051	198.0	1.60	12.67	3.255	16.33
AP29 X ICEAP00554	222.3	1.83	12.60	2.957	10.33
Minimum	**171**	**1.14**	**10.50**	**2.687**	**5.00**
Mean	**217.4**	**2.05**	**15.14**	**3.14**	**14.14**
Maximum	**256.7**	**3.37**	**22.33**	**3.637**	**30.00**
P value	**0.005**	**0.020**	**0.001**	**<0.001**	
LSD (P<0.05)	**47.89**	**0.87**	**6.32**	**0.37**	
CV (%)	**13.60**	**26.00**	**25.70**	**7.30**	

5.3.2 Relative importance of the GCA and SCA effects

Table 5.6 shows the percentages of the sum of the squares attributable to GCA and SCA effects, for wilt and other traits. The GCA_p sums of squares were larger than GCA_t for all the traits. However, SCA effects contributed largely to wilt resistance and yield plant^{-1}. Overall, SCA effects were preponderant for yield, while GCA effects were preponderant for wilt resistance and secondary traits (Table 5.6).

Table 5.6: Percentages of cross sum of squares attributable to GCA and SCA effects for % wilt, yield and secondary traits

Traits	Percentage of cross sum of squares			
	GCA_p (Females)	GCA_t (males)	Total GCA	SCA
Percent Fusarium wilt	34*	25*	59	41*
Yield plant^{-1}	31*	6	37	63
Days 50% flowering	60*	9*	69	31*
Seed pod^{-1}	55*	5	60	40
Plant height	52*	6	58	42
Stem diameter	52*	5	57	43
Primary branches	68*	2	70	30
Secondary branches	45*	13*	58	42*

* Significant at P < 0.05

5.3.3 General combining ability in pigeonpea landraces and testers

Table 5.7 shows the GCA effects for local landraces. Lines with negative GCA for wilt resistance were desirable because they were contributing to disease resistance, while

those with positive GCA effects were undesirable as they were contributing to disease susceptibility. The lines AP2, AP3, and AP4 displayed highly significant negative GCA effects, while AP5 and AP6 showed significant positive GCA effects. The other lines did not show significant GCA effects. In the breeding programme, early maturing germplasm is required. Therefore, lines with negative GCA effects were desired for days to 50% flowering, while positive GCA effects were not desired. Lines AP1, AP2, AP5, AP8, and AP10 had significant negative GCA effects for days to 50% flowering; lines AP3, AP6, AP9, AP23, and AP29 had positive GCA effects. The rest did not show significant GCA effects. Positive GCA effects were desired for the number of seeds pod^{-1}, while negative GCA effects were not desired. Lines AP2, AP4, AP5, and AP10 had significant positive GCA effects; AP1, AP3, and AP29 had significant negative GCA effects for number of seeds pod^{-1}. Negative GCA effects were desired for plant height. Lines AP8, AP23 and AP29 had significant negative GCA effects, while AP6 and AP9 had significant positive GCA. Lines AP2, AP5, AP6, and AP9 had significant positive GCA effects for the stem diameter desired for plant vigour; while lines AP1, AP8, and AP23, had undesired negative GCA effects. Positive GCA effects were desired for the number of primary branches, while negative GCA effects were not desired. Lines AP5, AP6, and AP9 had significant positive GCA effects; lines AP1, AP8, AP10, AP23, and AP29 had significant negative GCA effects. Likewise, lines AP4, AP6, and AP9 had the significant positive GCA effects desired for an increased number of secondary branches; lines AP1, AP2, AP10, and AP29 had significant negative GCA effects for the number of secondary branches.

Table 5.7: General combining ability effects of parental lines for Fusarium wilt resistance and other traits

Genotypes	**GCA effects**						
	% Wilt	**D 50% F**	**Seed pod^{-1}**	**Plant Ht**	**Stem Diam**	**Pr Branches**	**Sec Branches**
AP1	7.11	-24.9**	-0.9**	5.5	-0.3**	-1.7*	-3.9**
AP2	-9.30*	-22.0**	0.9**	2.6	0.2*	-0.5	-5.3**
AP3	-24.75**	8.5*	-0.4*	-1.8	-0.1	0.6	-0.1
AP4	-28.97**	4.1	0.3*	-12.3	-0.1	0.9	2.2*
AP5	17.91**	-12.0*	0.3*	8.0	0.3**	1.3*	1.4
AP6	29.53**	45.6**	-0.1	22.8**	0.4**	1.4*	4.7**
AP7	5.28	-2.5	0.0	4.3	0	1.2	-1.4
AP8	-3.82	-28.6**	0.2	-14.1*	-0.4**	-3.2**	1.2
AP9	-2.41	14.2**	-0.1	24.1**	0.6**	4.9**	7.1**
AP10	7.20	-33.1**	0.5*	-2.0	0	-1.4*	-4.3**
AP23	-2.16	17.6**	-0.1	-24.1 **	-0.2*	-1.5*	1.3
AP29	4.36	33.0**	-0.6*	-12.9*	-.03	-1.9**	-2.8*
SE +	**3.65**	**3.62**	**0.15**	**4.73**	**0.089**	**0.61**	**0.94**

- Significant at P <0.05; ** Significant at P < 0.01
- D50%F = number of days to 50% flowering; Ht = Height; Diam = Diameter; Pr = Primary; Sec = Secondary

The males (testers) also differed significantly in their GCA variances for wilt and days to 50% flowering, but not seed pod^{-1}, plant height, stem diameter, and number of primary and secondary branches. ICEAP00554 had significant negative GCA effects for Fusarium wilt, and days to 50% flowering, while the rest were insignificant (Table 5.8).

Table 5.8: General combining ability effects of the testers for wilt and other traits

Genotype	GCA effects						
	%Wilt	D50%F	Seed pod^{-1}	Plant Ht	Stem Diam	Pr Branches	Sec Branches
ICP9145	-6.80	7.1	0.1	-1.4	-0.1	0.5	-0.3
ICEAP00040	7.15	9.5	0.2	7.5	0.1	-0.3	-0.4
ICPL87051	17.97	-0.5	-0.3	-3.5	-0.1	0.2	2.9
ICEAP00554	-18.32*	-16.1*	0.1	-2.6	0.0	-0.4	-2.2
SE +	**6.32**	**6.27**	**0.25ns**	**8.19ns**	**0.15ns**	**1.06ns**	**1.62ns**

- Significant at P <0.05
- D50%F = number of days to 50% flowering; Ht = Height; Diam = Diameter; Pr = Primary; Sec = Secondary

5.3.4 Specific combining ability effects for pigeonpea crosses

Table 5.9 presents data on the SCA effects for percentage wilt, days to 50% flowering, and number of secondary branches. Negative SCA effects were desired for % wilt and days to 50% flowering. The following crosses had highly significant negative SCA effects for Fusarium wilt reaction: AP5 x ICP9145, AP6 x ICEAP00554, and AP10 x ICEAP00040. Crosses AP5 x ICEAP00040, AP5 x ICPL87051, and AP29 x ICEAP00554 had significant positive SCA effects. Crosses AP9 x ICP9145 and AP23 x ICEAP00554 had significant negative SCA effects for days to 50% flowering, in the desired direction; AP9 x ICEAP00040 and AP23 x ICP9145 had significant positive SCA effects. Positive SCA effects were desired for the number of secondary branches, and the crosses, AP3 x ICPL87051 and AP4 x ICEAP00040, had significant positive SCA effects in the desired direction; AP3 x ICEAP00040 and AP9 x ICP9145 had significant negative SCA effects for the number of secondary branches.

Table 5.9: Specific combining ability effects for % wilt and days to 50% flowering and number of secondary branches

Cross	SCA effects		
	% Wilt	Days to 50%Flowering	Secondary Branches
AP1 x ICP9145	-9.79	-5.3	-2.7
AP1 x ICEAP00040	9.93	-7.1	-1.6
AP1 x ICPL87051	-12.23	-0.1	0.7
AP1 x ICEAP00554	13.06	13.9	3.5
AP2 x ICP9145	-4.37	-3.9	5.3
AP2 x ICEAP00040	5.01	-2.5	-3.6
AP2 x ICPL87051	4.19	1.8	-2.2
AP2 x ICEAP00554	-3.86	6.1	0.5
AP3 x ICP9145	0.08	-7.7	-0.2
AP3 x ICEAP00040	3.0	-11.7	-7.1*
AP3 x ICPL87051	-13.7	15.0	7.9*
AP3 x ICEAP00554	11.6	5.9	-0.7
AP4 x ICP9145	4.30	-3.3	-2.4
AP4 x ICEAP00040	-9.7	-17.4	8.0*
AP4 x ICPL87051	-9.5	15.6	-5.7
AP4 x ICEAP00554	15.8	6.5	0.1
AP5 x ICP9145	-42.59**	6.1	-3.3
AP5 x ICEAP00040	32.5*	-6.2	1.1
AP5 x ICPL87051	31.2*	0.5	-0.6
AP5 x ICEAP00554	-20.1	1.1	2.8
AP6 x ICP9145	12.80	10.2	5.0
AP6 x ICEAP00040	9.8	10.1	0.1
AP6 x ICPL87051	10.0	-13.9	-4.6
AP6 x ICEAP00554	-31.7*	-5.0	-0.5
AP7 x ICEAP00040	-18.95	-	0.6
AP7 x ICPL87051	0.4	-15.8	-3.1
AP7 x ICEAP00554	0.9	7.5	-
AP8 x ICP9145	23.48	15.2	-0.3
AP8 x ICEAP00040	-17.2	9.0	0.3
AP8 x ICPL87051	-7.0	-21.4	-1.0
AP8 x ICEAP00554	1.7	-1.4	1.0
AP9 x ICP9145	-4.69	-79.1	-8.7*
AP9 x ICEAP00040	-3.2	39.2	0.8
AP9 x ICPL87051	19.6	24.8	5.7
AP9 x ICEAP00554	-10.8	-	-
AP10 x ICP9145	46.13**	19.9	0.4
AP10 x ICEAP00040	-34.8*	0.9	3.2
AP10 x ICPL87051	-1.0	-19.8	-2.5
AP10 x ICEAP00554	-9.4	0.5	-1.1
AP23 x ICP9145	10.82	28.7	2.5
AP23 x ICPL87051	-14.0	-2.6	0.5
AP23 x ICEAP00554	11.0	-25.6	-3.4
AP29 x ICP9145	-18.04	10.1	0.2
AP29 x ICEAP00040	1.3	12.5	-3.1
AP29 x ICPL87051	-9.5	-24.5	1.9
AP29 x ICEAP00554	27.1*	3.4	1.0
SE +	**12.63**	**12.53**	**3.25**

5.3.5 Segregation analysis of F_2 population for resistance to Fusarium wilt

The F_2 lines segregated in their reaction to wilt. Table 5.10 shows the number of plants resistant, and susceptible, to Fusarium wilt. The Chi-square analysis of the lines showed that phenotypic ratios did not deviate significantly from the expected segregation ratios of 3:1, 15:1, and 9:7. A few lines did not fall into any of these ratios, and they showed a ratio of 1:1. Two crosses showed reverse ratios of 1:3 and 7: 9. From the segregation ratios, it is clear that resistance for Fusarium wilt in pigeonpea was dominant over susceptibility in most populations. The ratios show that one to two dominant genes confer wilt resistance in pigeonpea.

Table 5.10: Phenotypic ratios of resistant (R) to susceptible (S) segregating F_2 populations

Cross	Plants per plot	No F_2 Plants		Expected Ratio		X^2 value	P value
		R	S	R	S		
AP1 X ICPL87051	120	61	59	1	1	0.03	0.855
AP1 X ICEAP00554	32	17	15	1	1	0.12	0.724
AP2 X ICP9145	106	73	33	3	1	2.14	0.145
AP2 X ICEAP00040	101	51	50	1	1	0.01	0.921
AP2 X ICPL87051	113	85	28	3	1	0.00	0.957
AP2 X ICEAP00554	127	81	46	9	7	2.93	0.087
AP3 X ICP9145	104	97	7	15	1	0.04	0.839
AP3 X ICEAP00040	67	62	5	15	1	0.17	0.682
AP3 X ICPL87051	94	73	21	3	1	0.35	0.552
AP3 X ICEAP00554	112	89	23	3	1	1.19	0.275
AP4 X ICP9145	32	27	5	3	1	1.50	0.221
AP4 X ICEAP00040	32	32	0	15	1	2.13	0.144
AP4 X ICPL87051	64	66	2	15	1	1.27	0.260
AP4 X ICEAP00554	123	114	9	15	1	0.24	0.625
AP5 X ICP9145	63	59	4	15	1	0.00	0.974
AP5 X ICEAP00554	21	12	9	9	7	0.01	0.934
AP7 X ICPL87051	124	87	37	3	1	1.55	0.213
AP7 X ICEAP00554	129	105	24	15	1	2.81	0.930
AP8 X ICPL87051	116	66	50	9	7	0.02	0.888
AP8 X ICEAP00554	71	43	28	9	7	0.54	0.464
AP9 X ICPL87051	128	74	54	9	7	0.13	0.722
AP9 X ICEAP00554	32	19	13	9	7	0.13	0.722
AP10 X ICP9145	61	56	5	15	1	0.39	0.530
AP10 X ICEAP00040	121	100	21	13	3	0.15	0.694
AP10 X ICPL87051	113	108	5	15	1	0.64	0.423
AP10 X ICEAP00554	111	69	42	9	7	1.58	0.209
AP23 X ICPL87051	125	79	46	9	7	2.45	0.117
AP23 X ICEAP00554	32	22	10	3	1	0.67	0.414
AP29 X ICP9145	16	12	4	3	1	0.00	1.000
AP29 ICPL87051	29	21	8	3	1	0.10	0.748
AP29 X ICEAP00554	123	79	44	9	7	3.18	0.075

5.4 Discussion and conclusion

The genetic studies in this research were carried out to investigate the nature and mechanism of the inheritance of Fusarium wilt resistance in pigeonpea, to assist in developing an efficient resistance-breeding programme. Results pertain to the lines in the study because a fixed model was used.

The analysis of F_1 crosses data revealed the gene action controlling the various traits in pigeonpea. Both GCA_t and GCA_p effects were highly significant ($P \leq 0.001$) for wilt incidence, indicating the importance of additive gene effects. The SCA effects, represented by the interaction of the testers (father) and parental lines (mothers) were also highly significant ($P \leq 0.001$), indicating that non-additive gene effects were important in controlling Fusarium wilt resistance. Therefore, both additive and non-additive gene effects controlled the expression of wilt resistance in pigeonpea.

Most of the pigeonpea seedlings died due to the disease within the first two months after inoculation. The trend of the cumulative Fusarium wilt incidence in F_1 crosses showed that the level of resistance was higher in resistant and tolerant crosses than in susceptible crosses. This observation is consistent with additive gene action for resistance. However, there were no crosses that were immune to Fusarium wilt disease.

Yield $plant^{-1}$ also varied significantly among the crosses at $P = 0.05$. However, neither additive nor non-additive gene effects could be attributable to the trait at $P \leq 0.05$. The parental lines accounted for the variation at $P = 0.092$ (Table 5.2). The highly significant GCA and SCA effects for days to 50% flowering in pigeonpea indicate that both additive and dominance gene effects were important in controlling the trait. Only GCA_p effects due to parental lines were significant for the number of seeds pod^{-1}. The additive gene effects from the parental lines controlled the trait, showing that the trait was greatly influenced by the maternal genotype. However, maternal effects can only be confirmed with the analysis of reciprocal crosses. Seed mass was not analyzed using ANOVA, and the GCA or SCA effects cannot be assumed for this trait. GCA_p effects due to parental lines were significant for controlling plant height, stem diameter, and the number of primary branches. Both GCA and SCA effects were significant for the number of

secondary branches, indicating that both additive and non-additive gene effects were controlling the number of secondary branches in pigeonpea.

The results showed the relative importance of the GCA effects and the interaction effects. All the traits studied in this research were contributed largely by the parental lines used as females, reflecting the importance of the maternal effects. The female genotypes were predominant over the males for all the traits, except for Fusarium wilt resistance and yield, where the SCA effects contributed more than both parents. However, the number of testers and lines were not balanced; hence, the maternal effects could not be reliably estimated. In a balanced situation, where the number of testers = number of lines, Kearsey and Pooni (1996) showed that the ratio of female to male mean squares estimates the role of maternal effects. In wilt resistance, the non-additive gene effects were responsible for controlling the trait. The results suggest the need to exploit dominance gene action by crossing many parents.

The GCAs for Fusarium wilt incidence varied among parental lines and testers. The negative GCAs for Fusarium wilt incidence mean that the parental lines contributed towards introgressing Fusarium wilt resistance genes into their progenies and these parental lines showed low means of wilt incidence. Lines with positive GCA variances increased their susceptibility to Fusarium wilt in their progenies. The tester, ICEAP00554, with a negative GCA value, contributed resistance, while ICPL 87051 contributed increased disease susceptibility. It is the additive gene effects which are responsible for conferring resistance to Fusarium wilt in the parental lines and testers with negative GCA effects (Dabholkar, 1999; Singh, 2005; Acquaah, 2007).

In the pigeonpea breeding programme, early maturing germplasm is required because the food becomes available early to consumers. Earliness is loosely used to mean medium maturity, because very early maturing dwarf cultivars are no longer grown by farmers. Therefore, lines with negative GCA effects were desired for days to 50% flowering, while positive GCA effects were not desired. The additive gene effects in the lines with negative GCA effects contributed to early maturity. Eventually, the new cultivars mature earlier than the parents. Lines with positive GCA effects were responsible for late maturity, not desired in pigeonpea in Malawi. Positive GCA effects were desired for the number of seeds pod^{-1}, because the additive gene effects were

responsible for the high number of seeds pod^{-1}. High number of seed is positively correlated to high seed mass in contributing to high yields in pigeonpea. Negative GCA effects contributed to a reduced number of seeds pod^{-1}. In pigeonpea, tall plants are always late maturing (Reddy, 1990) and are not desirable in Malawi. Therefore, negative GCA effects were desired for plant height. Lines with significant negative GCA effects contributed to medium plants, while lines with significant positive GCA effects were responsible for tall plants. Positive GCA effects for stem diameter, which represent plant vigour, and for great secondary growth (Reddy, 1990) were desired, while lines with negative GCA effects were not desired. A vigorous plant survives better in a competitive environment, especially in utilizing limited resources. However, genotypes with thick stems are late maturing. Therefore, there is need to have a good selection criterion before advancing the generation. Positive GCA effects were desired for the number of primary branches because the number of primary branches is correlated to yield (Reddy, 1990), while negative GCA effects were undesirable. This explanation also holds true for the number of secondary branches. Positive GCA effects suggested that the additive genes contributed to an increased number of primary branches.

The SCA variances were significant for Fusarium wilt disease reaction, days to 50% flowering, and the number of secondary branches. Negative SCA effects were desired for percentage wilt because they contributed to reduced wilt incidence. Therefore, the following crosses are recommended for wilt resistance, because of highly significant negative SCA effects for their Fusarium wilt reaction: AP5 x ICP9145, AP6 x ICEAP00554, and AP10 x ICEAP00040. Crosses with significant positive SCA effects were not desired because they were highly susceptible to Fusarium wilt. Negative SCA effects were, however, also desired for days to 50% flowering, because they contributed to early maturity. Crosses AP9 x ICP9145 and AP23 x ICEAP00554 produced early maturing F_1 crosses. The crosses had significant SCA effects for days to 50% flowering, in the desired direction. The additive gene effects in crosses, with significant positive SCA effects, were not desired because they contributed to late maturity in pigeonpea. Positive SCA effects were desired for the number of secondary branches and the following cross, AP4 x ICEAP00040, was recommended for an increased number of secondary branches. More branching may mean more pods, therefore, high yield. However, in intercropping highly branched crops compete with cereals, and breeders must take caution. On the other hand, the additive gene action was responsible for the

reduced number of secondary branches in crosses AP3 x ICEAP 00040 and AP9 x ICP9145. While SCA effects are important, GCA would be more useful in pigeonpea, because the desired product would not be F_1 hybrids, but pure lines.

The Chi-square analysis of the F_2 progenies showed various phenotypic ratios of Fusarium wilt resistance. The results show that resistance to Fusarium wilt in pigeonpea is dominant over susceptibility in most populations. Okiror (2002) also reported similar results. The dominant nature of inheritance should make transferring Fusarium wilt resistance from resistant to susceptible cultivars relatively easy, with any selection method (Pastor-Corrales et al., 1994). One third of the crosses gave a 3:1 resistant (R): susceptible (S) ratio, which shows that one major dominant gene was contributing to Fusarium wilt resistance (Young and Kelly, 1996; Pathania et al., 2006). Resistance based on single dominant genes is generally considered to be vulnerable to genetic changes in pathogen virulence (Bjarko and Line, 1988). Significant deviations from the expected 3:1 (R:S) ratio were observed among the F_2 crosses. A third of the F_2 population gave 15:1 (R:S) ratios, while another third produced 9:7 (R:S) ratios, suggesting the effects of two genes. The 15:1 (R:S) ratio suggested that two independent dominant genes with equal effects confer resistance to Fusarium wilt (Singh, 2005). Kamboj et al. (1990), Pastor-Corrales et al. (1994) and Pathania et al. (2006) reported similar results in lentils and beans. The 9:7 (R:S) ratio signifies two complementary gene actions, indicating that one of the two, or both, genes influence resistance to Fusarium wilt in pigeonpea in a recessive state (Singh, 2005). Working on pigeonpea, Okiror (2002) reported that two genes controlled resistance to Fusarium wilt. Some crosses gave unexpected 1:1 ratios at F_2, not fitting into any of the known ratios.

In conclusion, line x tester mating design has been very useful in determining the variance components responsible for Fusarium wilt resistance, days to 50% flowering, seed pod^{-1}, plant height, stem diameter and number of branches. The GCA and SCA effects were significant for wilt resistance, days to 50% flowering, number of secondary branches in both parents, and in the interaction of the parents. It was showed that additive gene effect was significant in both parents, while dominance gene effects were significant in the interaction. However, maternal genotypes had greater influence than tester (male) genotypes in the seeds pod^{-1}, plant height, stem diameter, and number of primary branches. Through significant GCA and SCA, it is possible to select useful

parents that can be used in the hybridization programme to breed pigeonpea lines, are resistant to Fusarium wilt, early maturing, or that contain consumer preferred traits. It has been shown that pigeonpea landraces are also good sources of not only wilt resistance, but also days to 50% flowering, and other traits. The possible maternal effects in the seed pod^{-1}, plant height, stem diameter and number of primary branches provide insight for the breeder as to how parents can be selected for these traits before the hybridization programme. The low number of genes governing Fusarium wilt resistance suggests the ease of incorporating resistance in pigeonpea cultivars during the breeding process. Pedigree breeding would be recommended for conferring Fusarium wilt resistance, yield and secondary traits in pigeonpea.

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Research overview: Implications of research findings for pigeonpea breeding

Pigeonpea (*Cajanus cajan* (L.) Millspaugh) is one of the most important grain legume crops in Malawi. It provides food, cash, and firewood for subsistence farmers. Small-scale farmers dominate the production of pigeonpea, especially in the southern region of the country. Pigeonpea is intercropped with maize, cassava, groundnuts, sorghum, pearl millet, and beans. The crop has a yield potential of more than 2 t ha^{-1} but average yields have stagnated around 600-800 kg ha^{-1}. Pigeonpea production is hampered by, among other factors, Fusarium wilt disease caused by *Fusarium udum* (Butler). The disease can cause up to 100% yield losses in susceptible cultivars.

The long-term goal of the research was to breed for high yielding pigeonpea varieties that are resistant to Fusarium wilt with desirable characters to the consumer. The objectives of this research were, therefore, to identify the new sources of Fusarium wilt resistance in pigeonpea and to study the nature of inheritance of the resistance. It was important to get more information on farmers' preferences for local landraces, how farmers and consumers can be involved in developing new varieties, wilt disease, and the genetics of inheritance of resistance.

To accomplish these objectives, several methods and materials were used. A participatory rural appraisal was used in southern region of Malawi to identify pigeonpea production and marketing constraints. Participatory variety selection was used to identify landraces with desirable traits that could be used in the breeding programme. The selected pigeonpea local landraces and ICRISAT genotypes were evaluated for wilt resistance, yield, and secondary traits. Laboratory and screenhouse studies were concurrently performed to develop a new Fusarium wilt screening technique. Finger millet, sorghum, and wheat grains were tested for multiplying *F. udum* isolates. Pathogenicity tests were done, and the isolates were then used in an infested-seed inoculation technique against eight differential cultivars. The selected landraces were later crossed with wilt resistant testers in a 12 line x 4 tester mating scheme and 48 F_1 crosses were generated. These F_1 crosses were evaluated for wilt resistance, yield, and

secondary traits. Segregation analyses were conducted on F_2 populations to determine the resistance to susceptibility phenotypic ratios.

Significant findings were made in the participatory rural appraisal and the participatory variety selection studies with small-scale farmers and buyers, the evaluation of pigeonpea genotypes, the development of a new wilt screening technique and the inheritance studies on Fusarium wilt resistance. The findings include the following:

a) Farmers perceived Fusarium wilt as the most prevalent and devastative disease of pigeonpea and yet very little was being done to manage it.
b) Pigeonpea production is constrained by many factors such as poor soil fertility, flower abortion, poor weather conditions, lack of certified seed for improved cultivars, insect pests, and diseases.
c) Most local pigeonpea landraces were susceptible to Fusarium wilt and yet they accounted for more than 80% of the total pigeonpea production in the country.
d) Local landraces were preferred by farmers, despite the release of Fusarium wilt resistant varieties, because of taste (flavour) and fast cooking time which are lacking in the released varieties.
e) The local landraces were, however, mostly late maturing, low yielding with few seeds per pod.
f) Farmers and buyers were able to select ten promising pigeonpea landraces with desirable traits through participatory variety selection. These lines were used as parents in the breeding programme to develop Fusarium wilt resistant lines with farmer- and consumer preferred traits.
g) During genotype evaluation, some local landraces were identified with inherent resistance to wilt, high yielding ability, and early maturity. AP10, a local landrace, gave an outstanding performance. It is high yielding, Fusarium wilt resistant, large-seeded, and early maturing. It is highly preferred by farmers and consumers because of these traits.
h) The infested seed inoculation technique development is effective in screening pigeonpea germplasm for resistance to wilt. Wheat grains showed to be the best medium for multiplying *F. udum* and for easy inoculation compared to

sorghum and finger millet. The pathogen can be preserved in double sterile distilled water, PDA slants, or silica gel for more than 2.5 years.

i) Significant negative GCA effects for wilt resistance, days to 50% flowering and plant height were identified in the local landraces as were positive GCA effects for seed per pod, and number of secondary branches.

j) Significant negative SCA effects were also identified for wilt resistance and days to 50% flowering.

k) Inheritance studies showed that resistance to wilt was dominant over susceptibility in most F_1 crosses. Most F_1 crosses were resistant to Fusarium wilt which was due to both additive and non-additive gene effects.

l) The Chi-square analyses gave 3:1, 13:3, 15:1 and 9:7 (resistant: susceptible) segregation ratios, except for a few crosses which could not be fitted into any known segregation ratio at F_2 generation.

The implications of these findings, with regard to the management of Fusarium wilt and breeding for yield and yield components in pigeonpea, are far-reaching. Firstly, it would be easy to convince farmers to deploy new approaches to managing Fusarium wilt in their fields because they are aware of the disease and its damaging effects. Such approaches would include the use of resistant varieties, the use of certified seed, rotation, physically removing diseased plants and intercropping pigeonpea with crops non-host to *F. udum,* for example, sorghum. This would be aimed at reducing inoculum in the soil to below threshold levels.

The many pigeonpea production problems call for a multidisciplinary approach towards helping farmers to improve pigeonpea production. Scientists from different disciplines should work together and address farmers' problems.

There is a need to breed for Fusarium wilt resistant varieties with farmer- and consumer-preferred traits. The fact that local landraces account for a high percentage of pigeonpea production is a positive indicator that any variety with farmer-preferred traits would have a high adoption rate. Therefore, the continued involvement of farmers in the whole crop improvement programme is very important.

The fact that farmers and traders prefer local landraces is a “wake up call” for pigeonpea breeders not to ignore consumers’ demands in the breeding programme. Some traits that may not be related to yield, such as taste/flavour and cooking time, play a vital role in the whole varietal adoption process. Breeders should appreciate the importance of involving farmers in the whole breeding programme from variety selection to selection for desirable traits in the filial generations for additional reasons. The involvement of farmers or consumers in the variety selection process empowers them and gives them a sense of recognition, responsibility, and ownership for the varieties to be developed. Farmers look forward to the upcoming varieties because they feel they are part of the process.

Farmers also prefer high yielding and early maturing varieties. The breeding process should concentrate on selecting for such factors as early flowering, optimum branches per plant, more seeds per pod, and large-seed size in pigeonpea lines.

The results of the evaluation of local landraces for wilt, yield and other traits showed the existing potential in the local germplasm for the selection of potential landraces with desirable traits for genetic improvement. The farmers’ selection of promising landraces is an assurance of a high adoption rate of the resultant cultivars after incorporating the missing traits.

The answers to farmers’ problems mostly lie within reach, only that farmers do not know how to find solutions. This is evidenced by the outstanding performance of AP10. It is a local landrace with many desired attributes that farmers prefer, such as early maturity, large-seeds, high yield and Fusarium wilt resistance. This is a lesson to breeders as well, that they should first exploit the available resources before introducing foreign materials or make crosses between landraces with imported germplasm with desirable traits such as Fusarium wilt resistance.

Screening of pigeonpea and filial generations can be enhanced through the use of a newly developed screening technique which uses locally available materials. Preservation, multiplication and inoculation of the isolates can easily be done even in resource-strapped laboratories. The isolate can be preserved at room temperature in double sterilised distilled water. Wheat, sorghum and finger millet are crops that are grown in most African countries and their availability is guaranteed. The quantification of

the inocula levels during the inoculation process can be done through counting the infested seed. The new screening technique will speed up the breeding process, thereby making the much-needed pigeonpea cultivars available to farmers and consumers sooner.

The identification of significant GCAs for wilt resistance, days to 50% flowering, seeds per pod, plant height, stem diameter and number of branches showed the potential of additive gene effects in the local landraces of combining for these particular traits through hybridization. The dominance effects were also identified in specific crosses for wilt resistance, days to 50% flowering and number of secondary branches. Local landraces, therefore, are potential sources of traits that farmers and consumers prefer which can be incorporated through breeding.

The inheritance studies showed that breeding for Fusarium wilt resistance is possible through the pedigree method because resistance is dominant over susceptibility. The additive and dominant gene effects are both important for controlling wilt resistance in parents. The involvement of few genes governing Fusarium wilt resistance in pigeonpea indicates that few complications, if any, would be expected in breeding for Fusarium wilt resistance in pigeonpea. Pedigree breeding methods would be recommended for incorporating wilt resistance and other traits in pigeonpea.