Genetic Improvement of Pigeonpea (*Cajanus cajan* (L.) Millsp.) for *Fusarium* wilt resistance in Tanzania

By

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Thesis Abstract

Fusarium wilt, caused by a soil-borne fungus (*Fusarium udum* Butler), is an important disease contributing to low yields in pigeonpea (*Cajanus cajan* (L.) Millspaugh) in the semi-arid tropics. In Tanzania, the disease is widespread in most pigeonpea growing areas, causing yield losses up to 100% to susceptible genotypes. Controlling diseases through chemicals is difficult and not economical for most resource limited farmers. The development of resistant varieties is the cheapest and most environmentally friendly method to control the disease. The overall objective of this study was to contribute to increased pigeonpea productivity in Tanzania, through the development of improved, high yielding and *Fusarium* wilt resistant cultivars with farmers and market preferred traits. The specific objectives were to: (1) identify production constraints and farmers preferred traits of pigeonpea in eastern and northern Tanzania, (2) determine the genetic variation in Tanzanian pigeonpea germplasm for *Fusarium* wilt resistance, grain yield and yield components, (3) study the phenotypic diversity of Tanzanian pigeonpea germplasm based on agro-morphological traits, (4) assess genetic diversity of pigeonpea germplasm from Tanzania using SSR markers, and (5) study gene action controlling the inheritance of *Fusarium* wilt resistance, grain yield and yield components of pigeonpea.

A participatory rural appraisal (PRA) study was conducted in six villages of three districts, namely Kilosa, Karatu and Babati in eastern and northern Tanzania. Data were collected involving 240 farmers using a questionnaire and 108 farmers participated in focus group discussions. Results indicated that maize intercropping with pigeonpea is the most common production system across all three districts. The major production constraints perceived by farmers were insects and diseases. *Fusarium* wilt was the major disease across all three districts. Other constraints mentioned were drought, late maturing varieties, high input prices and limited access to improved varieties. Farmers use different methods in managing the disease in the field, such as the use of different varieties, avoiding fields infested with *Fusarium* wilt, crop rotation, uprooting and burning infected plants, adding farmyard manure and applying pesticides. Farmers showed strong preference for varieties with high yield, diseases resistance, drought tolerance, early maturity, short cooking time, prolific branching and large, white or cream seed.

Field screenings were conducted to evaluate 32 pigeonpea genotypes for their response to *Fusarium* wilt for two consecutive seasons between January 2014 to October 2015 at Hombolo

and Ilonga. In addition, a pot experiment was conducted in the 2014/2015 season to confirm the field results, using the seed infection technique. Genotypes ICEAP 00040, ICEAP 00932, ICEAP 00557, ICEAP 00554 and ICEAP 00053 were selected as potential donor parents for introgressing *Fusarium* wilt resistance into susceptible genotypes. Field results showed that all long duration genotypes out-performed medium and short duration genotypes in terms of grain yield and other important agronomic traits. The results indicated that there is sufficient genetic diversity in pigeonpea germplasm that could be used as a base for the improvement of the yield, *Fusarium* wilt resistance and other important attributes, through direct selection and hybridization.

Phenotypic characterization of 48 pigeonpea genotypes collected from northern, eastern and central zones of Tanzania, were evaluated for 15 qualitative and 16 quantitative agro-morphological traits. Genotypes were evaluated under field conditions at two sites in one season. The Sharon-Weaver diversity index (H') revealed low to high genetic diversity among zones of collection, while the overall mean indicated low diversity for qualitative traits. The principal component analysis (PCA) showed that the first four PCs explained 73.42% of total variation with days to 50% flowering, days to maturity, number of pods per plant, number of seed per plant, grain yield, leaf width and leaf area being the most important traits in the PC1. Cluster analysis grouped genotypes into three clusters. Genotypes from Northern Zone were allocated into all three groups, genotypes from Eastern Zone were grouped into clusters I and II, while genotypes from Central Zone were grouped into clusters II and III. Crosses between genotypes from different clusters could results into desirable segregants.

Genetic diversity and relationships of 48 pigeonpea genotypes collected from Tanzanian germplasm were analysed using 35 simple sequence repeats (SSR) markers. All 35 SSR markers used were polymorphic. The sizes of amplified polymorphic DNA fragments (bands) ranged from 117 to 280 bp. A total of 162 alleles were amplified among 48 pigeonpea genotypes and the numbers of alleles scored for the 35 loci ranged from two to eleven with an average of 4.63. The maximum number of alleles (11) was detected at the CcM0246 and CcM0443 locus. The polymorphic information content (PIC) value of the SSR markers, which is a measure of allele diversity at a locus, ranged from 0.032 to 0.806 with an average of 0.412. Gene diversity values were in the range of 0.0322 to 0.8277, with an average of 0.4466. The observed heterozygosity values ranged from 0.0 to 0.635 with an average of 0.279. A dendogram constructed, based on unweighted pair group method with arithmetic mean (UPGMA), grouped the 48 eight genotypes

into five clusters and nine sub-clusters. The genetic similarity index revealed high similarity between long traditional landraces and long duration improved genotypes.

Six lines and four testers were crossed using a line x tester mating design. The 24 F_1 generations and their parents were evaluated in a row-column design under field conditions in two replications for two seasons at Hombolo and Ilonga in the 2014/15 and 2015/16. The general combining ability of lines, testers and crosses were evaluated for *Fusarium* wilt resistance, grain yield and important agronomic traits. The F_2 populations were evaluated under field conditions for their reaction to *Fusarium* wilt. A pot experiment was conducted to evaluate F_2 populations for their reaction to *Fusarium* wilt, using the seed infection technique. The study revealed that non-additive gene action was important in controlling *Fusarium* wilt resistance, grain yield and important agronomic traits except for days to maturity and plant height. The inheritance pattern for *Fusarium* wilt resistance for the F_2 populations yielded segregating ratios of 3 Resistant:1 Susceptible, 9 Resistant:7 Susceptible, 13 Resistant:3 Susceptible, R15 Resistant:1 Susceptible and ratios of 1 Resistant:3 Susceptible and 7 Resistant:9 Susceptible suggesting that the inheritance of *Fusarium* wilt resistance is governed by a variable number of genes such as one dominant gene or recessive gene, two complementary genes, dominant epistasis gene or duplicate genes.

The study identified potential sources of resistance to *Fusarium* wilt disease and superior candidate progenies with high yields and resistance to *Fusarium* wilt disease encompassing farmers preferred attributes.

Declaration

I, Didas Rogasian Kimaro declare that

1. The research reported in this thesis, except where otherwise indicated, is my original research.

2. This thesis has not submitted for any degree or examination at any university.

3. This thesis does not contain other person data, pictures, graph or other information, unless specifically acknowledged as being sourced from other persons.

4. This thesis does not contain other persons writing, unless specifically acknowledged as being sourced from other researchers. Where other sources have been quoted, then:

(a) The words have been re-written but the general information attributed to them has been referenced.

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Signed……..……..Date…….

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Prof Hussein Shimelis (Co-supervisor)

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Dedication

This thesis is dedicated to the Almighty god, from whom all good things come, to my parents' late father Dr. R.T. Kimaro and my mother Mamelta Kimaro, my wife Juliana, to my sons Colin, Jesse and my late son Coleman.

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Introduction to thesis

Importance of pigeonpea

Pigeonpea (*Cajanus cajan* (L.) Millspaugh) is the most important grain legume and food security crop for people living in dry areas of the world. The crop has the ability to adapt to adverse environmental conditions and gives reliable yield (Foley *et al*., 2011). Worldwide, pigeonpea is cultivated on an area of approximately 5.2 million ha of land. India is the major producer followed by Myanmar, Malawi, Kenya, Tanzania and Mozambique (FAO, 2010; ICRISAT, 2012). It is a cash and food crop with a high potential to enhance productivity per unit area as a companion crop with cereals and other legume (Sakala *et al*., 2000; Myaka *et al*., 2006). It is mainly cultivated in low-, mid- and high-altitude areas (Mligo and Craufurd, 2005; Mponda *et al*., 2013).

Pigeonpea is consumed as a dry grain in most parts of Tanzania and dehulling is common in the southern parts (Silim *et al*., 2005). According to Lo Monaco (2006), only 10% of pigeonpea produced is consumed as a green vegetable. Pigeonpea provides a cheap source of feed for livestock in an integrated crop-livestock system (Upadhyaya *et al*., 2006; Franzluebbers, 2007; Ezeaku *et al*., 2016). It has a higher nitrogen fixation rate, more than any other grain legume, and is an excellent candidate for crop rotation (Chikowo *et al*., 2004; Mafongoya *et al*., 2006). It increases soil organic matter content and improves soil structure and quality, nutrient recycling and tolerates low fertility soils. The deep root system of pigeonpea helps to mine nutrients and moisture from deep layers and offers less competition with other intercropping cereals (Mafongoya *et al*., 2006).

Pigeonpea grains are highly nutritious and may contain 18-25% protein, 51-58% carbohydrate and important minerals and vitamins (Odeny, 2007). Pigeonpea provides medicinal values in treating a number of human diseases (Yuan *et al*., 2010). In Chinese traditional medicine it is used as sedative to relief pain (Ahsan and Islam, 2009). In Nigeria pigeonpea seed is used to treat malaria (Aiyeloja and Bello, 2006). Pigeonpea may also act as a windbreak, especially the tall varieties, and provides good forage for animal production systems, providing high biomass (production, nutritional quality, and enhanced palatability). Its woody stem is used as cooking fuel and roofing material (Mallikarjuna *et al*., 2011).

The significance of pigeonpea production in Tanzania

Agriculture is among the leading sectors in the economy of Tanzania, which contribute to 43% of GDP and provides employment to over 80% of the people (Salami *et al*., 2010). Apart from employment, it is also a source of cash income. In Tanzania, pigeonpea is a highly valued legume crop, produced mainly for export and the surplus for domestic consumption. Due to a limited domestic market, the export markets remain the most significant commercial outlets. Yearly, approximately 135,000 tons of grain legumes valued US $ 54 million are exported from Tanzania, of which pigeonpea accounts for 56% (Abate, 2011). Pigeonpea has a great potential in meeting household food and financial needs for over 70% poor and food insecure inhabitants of northern and central districts of Tanzania. According to ICRISAT (2012), farmers in Babati earn more than 50% of their cash money from selling pigeonpea.

Pigeonpea varieties from northern Tanzania are generally considered of high quality due to their white color and large seed size and are exported to Kenya, India and Europe (Shiferaw *et al*., 2007). In recent years, the export of pigeonpea from Tanzania to India has increased significantly. Farmers have adopted *Fusarium* wilt-resistant varieties and tripled their yields, thus creating a thriving export market (Shiferaw *et al*., 2007, Shiferaw *et al*., 2008). In recenty years, the production area of pigeonpea has increased from 87,000 ha in 2001 to 2493,000 ha in 2014 (Table 1).

Table 1. Pigeonpea production in Africa during 2001 and 2014

	Production (000ha)		
Country	**2001**	**2014**	**% Increase**
Tanzania	87.1	249.3	186
Mozambique	31.6	120.9	282
Malawi	105.8	301.0	184
Kenya	73.4	274.5	274
Uganda	80.0	93.6	17
Africa	380.6	1047.3	175

Source: FAO 2014

Constraints to pigeonpea production in Tanzania

Pigeonpea production is severely affected by several abiotic and biotic constraints, which cause low yields. The average global yield of pigeonpea is reported to be 0.7 t/ha which is far below potential of 2.5-3.0 t/ha (Saxena *et al*., 2006). Mligo and Craufurd, (2005) reported yields >0.5 t/ha in farmers field in Tanzania. The major biotic constraints in Tanzania include diseases such as *Fusarium* wilt (*Fusarium udum* Butler), pod borer (*Helicoverpa armigera)* and grain weevils. The abiotic constraints include drought, salinity and water logging conditions (Chauhan, 1987). Other constraints include poor production practices, such as low plant densities, low soil fertility, insufficient weeding and inappropriate use of fungicides and herbicides and lack of high yielding varieties.

Fusarium wilt is a soil-borne disease and is regarded as a threat to pigeonpea production (Kannaiyan *et al*., 1984; Gwata *et al*., 2006). Once the field is infested with the disease, the fungus can stay in the soil for a long period of time, making it very difficult for poor farmers to control it without extended crop rotation or expensive chemicals. Farmers in Tanzania grow traditional landraces that are late maturing and low yielding, because they possess many desirable preferred traits. Farm saved seed is common in Tanzania, in addition to other sources, particularly from development projects (Silim *et al*., 2005).

Pigeonpea breeding in Tanzania

Pigeonpea breeding started in the early 1960s in Tanzania. In 1962/63 the first varieties collected from Ukiriguru were screened for *Fusarium* wilt at Ilonga station. However, this work discontinued after the departure of scientists in crop research. Breeding work started again in 1974/75, when the National Grain Legume Research was launched. Sixty varieties from ICRISAT were evaluated and three pure lines were identified, NPP610, RK201 and TRT201. The collaborative research between ICRISAT and the National Agricultural Research Institute in Tanzania started in 1986 with the objective of breeding high yielding, *Fusarium* wilt resistant, early maturing and cream coloured pigeonpea varieties. An early maturing variety ICPL 87091 (Komboa) was released in 1999, but it was susceptible to *Fusarium* wilt. Later a late maturing *Fusarium* wilt resistant variety ICEAP 00040 (released as Mali in 2002) and a medium maturing variety ICEAP 00068 (released as Tumia in 2003) (Kimani, 2001; Shiferaw *et al*., 2005).

The collaboration work continued from 2003-2015 and has resulted in the release of four varieties in 2015 by Ilonga Agriculture Research. Two varieties were late maturing ICEAP 00932 (Kiboko) and ICEAP 00053 (Karatu-long but compact), and two varieties were intermediate, namely ICEAP 00554 (Ilonga 14-M1) and ICEAP 00557 (Ilonga 14-M2) (Kaoneka *et al*., 2016). The current survey conducted in Arumeru, Karatu, Babati and Kondoa districts reported that the adoption of improved varieties is now 46% with an 11-56% increase in yield as compared to local varieties (Dalton *et al*., 2016). However, despite these efforts, farmers continued to grow landraces that are low yielding, susceptible to pests and diseases, and late-maturing because of their large seed size, good taste and colour (Manyasa *et al*., 2009). Delayed maturity has a negative impact on pigeonpea farming. This is because intercropping pigeonpea with cereals is a common practice and famers will have no time to rest the soil after harvesting pigeonpea, as they need to prepare their fields for next season. In addition, the quality of produce is low because harvesting is likely to coincide with the rainy season.

Statement of the problem and justification

Fusarium wilt is the major disease of pigeonpea worldwide (Kiprop *et al*., 2002; Gwata *et al*., 2006). In Tanzania, wilt incidence ranging from 10-96% has been recorded in pigeonpea fields (Mbwaga, 1988). The pathogen is primarily soil-borne, hence, disease control through chemicals is difficult and not economical. There is no current data on incidence level and yield loss in Tanzania, although, surveys and interviews with farmers revealed *Fusarium* wilt as a major threat to pigeonpea production. Developing resistant varieties is the cheapest and most environmentally friendly method to control the disease. Although varieties developed by ICRISAT were resistant to *Fusarium* wilt, not all have the farmers preferred traits. Farmers prefer medium duration varieties with large, white grain seeds for both local and export market and a high number of branches for other uses. In order to ensure long term disease resistance and good yield, there is a need to breed new resistant genotypes that are high yielding and have traits preferred by farmers and the export market. Moreover, addressing farmers preferred traits in the pigeonpea breeding programme would be important, as it can contribute to the acceptance and adoption of the newly bred cultivars.

Research objectives

Overall objective

The main objective of this study is to contribute to increased pigeonpea productivity in Tanzania through development of improved, high yielding and *Fusarium* wilt resistant cultivars with farmers and market preferred traits.

Specific objectives

The specific objectives of the study are;

a) Production constraints and farmers preferred traits of pigeonpea in eastern and northern Tanzania.
b) Genetic variation in Tanzanian pigeonpea germplasm for *Fusarium* wilt resistance, grain yield and yield components.
c) Phenotypic diversity of Tanzanian pigeonpea germplasm based on agro-morphological traits.
d) Assessment of genetic diversity of pigeonpea germplasm from Tanzania using SSR markers.
e) Gene action controlling inheritance of *Fusarium* wilt resistance, grain yield and yield components of pigeonpea.

Thesis Outline

The specific objectives mentioned in the foregoing were achieved and addressed in the various chapters that constitute this thesis. Chapters 2-6 are written in the form of discrete research chapters, each following the format of stand-alone research paper. The referencing style used in the chapter of this thesis is based on the Journal of Crop Science referencing system with some modification.

Thesis Chapters

Introduction to thesis.

Chapter 1. Literature review.

Chapter 2. Production constraints and farmers preferred traits of pigeonpea varieties: implications for breeding in Tanzania.

Chapter 3. Genetic variations of Tanzanian pigeonpea germplasm for *Fusarium* wilt resistance, grain yield and yield components.

Chapter 4: Phenotypic diversity of Tanzanian pigeonpea germplasm based on agro-morphological traits.

Chapter 5: Assessment of genetic diversity of pigeonpea germplasm from Tanzania using SSR markers.

Chapter 6: Gene action controlling inheritance of *Fusarium* wilt resistance, grain yield and yield components of pigeonpea.

Chapter 7: Research overview, implications of the findings and way forward.

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Chapter 1 : LITERATURE REVIEW

1.1 Introduction

This review covers the origin and distribution of pigeonpea and *Fusarium* wilt of pigeonpea including symptoms, spread and distribution, biology and ecology. Studies of pathogenic variation of *Fusarium udum*, disease management, genetic studies of *Fusarium* wilt resistance, screening techniques and marker assisted breeding in pigeonpea will be reviewed. Finally, this review highlights the future use of marker assisted selection (MAS), hybrid technology, farmers preference on pigeonpea and participatory research, genetic diversity studies in pigeonpea and general combining ability studies in pigeonpea for *Fusarium* wilt resistant, grain yield and yield components.

1.2 Origin and distribution of pigeonpea

Pigeonpea belongs to the family *Leguminosae*. The origin of pigeonpea is still confusing and several authors have reported on the origin of pigeonpea. Vavilov (1951) was the first author to report that pigeonpea originated from India. According to Van der Maesen (1980), the crop was believed to exist in Africa 2000 B.C (Van der Maesen, 1980). Several authors have reported that pigeonpea originated form its closest wild relative *Cajanus cajanifolius* (Van der Maesen, 1980; Krishna and Reddy, 1982; Panigrahi *et al*., 2007). Nadimpalli *et al*. (1992) reported the evolution of pigeonpea was through interspecific hybridization of *Cajanus cajanifolia* and *Cajanus scarabaeoides*. It was also suggested that pigeonpea had originated through a single gene mutation of *Atylosia species* (Dey, 1974; Reddy, 1973). According to FAO (1988), pigeonpea originated in both Asia and Africa. Pigeonpea is believed to have been introduced into East African by Indian migrants in the 19th century (Hillocks *et al*., 2000). Later it spread to Egypt through the Nile valley and thereafter to West Africa and America (Odeny, 2007; Mallikarjuna *et al*., 2011). India was believed to be the centre of diversity of cultivated pigeonpea, while Africa and Australia were the secondary and tertiary centre of diversity due to the presence of several wild relative species (Smartt, 1990; Van der Maesen, 1990).

1.3 *Fusarium* wilt disease of pigeonpea

Fusarium wilt (*Fusarium udum* Butler) is caused by a soil-borne fungus and is the most devastating disease of pigeonpea globally. The disease was described in 1906 in Bihar state, India (Butler, 1906). The infection may start at an early growth stage, but the most critical stage is at flowering and podding. According to Kannaiyan and Nene (1981), the yield loss can

approach 100% when it occurs at flowering stage, and 67% and 30% when it occurs at post flowering and 30% at pre-harvest stage. The economic loss has been estimated at US $ 36 million in India alone and US $ 5 million in eastern Africa (Kannaiyan *et al*., 1984). In recent years, no data for economic loss due to *Fusarium* wilt of pigeonpea has been reported.

1.4 Symptoms of *Fusarium* wilt

Fusarium wilt infects pigeonpea plants at all growth stages. According to Singh (1973), *Fusarium* wilt disease is characterized by gradual, yellowing, withering and drying of leaves followed by drying the all plant. Wilting plants showed loss of leaf turgidity, interveinal chlorosis and finally death of entire plant. Reddy *et al*. (1993) reported wilting of plants at different stages, but mostly at flowering and podding stages. A typical symptoms of *Fusarium* wilt is that one side of plant is wilting. On peeling off the bark, black streaks are found to extend in the vascular tissue from the dead branches downward to one or more infected lateral roots (Kiprop, 2001). At the adult stage the main characteristics of wilt is a purple band extending upwards from the base of the main stem.

1.5 Causal organism of *Fusarium* wilt

Fusarium wilt of pigeonpea is caused by a soil-borne fungi called *Fusarium udum. Fusarium* species were reported for the first time in 1906 in India (Butler, 1906). In the, *Fusarium udum* was identified as a new species (Butler, 1910). According to Wollenweber and Reinking (1935), the official name of *Fusarium udum* was accepted in 1935. The name *Fusarium udum Butler* var cajani was proposed by Padwick (1940), after he studied cultural characters of *F. udum* and *F. vasinfectum* and found macroconidia of *F. udum* are distinguished by a prominent hook.

1.6 Spread and distribution of *Fusarium* wilt disease

Fusarium wilt disease was first reported by Butler (1906) in India. The distribution and spread of *Fusarium* wilt in India were reported from all major growing areas (Kannaiyan *et al*., 1984). Recently *Fusarium* wilt surveys in Southern and Central of India from 2013 to 2015 reported that wilt incidence ranges between 0-45.3% and 0-70.8% (Ravikumara, 2015). A *Fusarium* wilt survey conducted in farmers fields in Malawi, reported over 50% wilt incidence (Marley and Hillocks, 1996). According to Changaya *et al*. (1996), wilt disease was widely distributed in all districts in Malawi. In Tanzania, a survey conducted in 1988 in Kilosa districts reported incidence of 10-90% in farmers field (Mbwaga, 1988). At present, *Fusarium* wilt occurs in all major growing areas around Babati, Karatu, Kilosa, Mtwara and Lindi. Gwata *et al*. (2006) reported on wilt incidence in Kenya (87.5%), Malawi (92.0%) and Tanzania (90.9%) for the

susceptible genotype Komboa (ICEAP 00068). Hillocks and Songa (1993) conducted a *Fusarium* wilt survey in farmers field in eastern Kenya and observed a high incidence level of *Fusarium wilt*. The disease has also been reported in Mozambique and Southern Africa (Gwata *et. al*., 2006). Although *Fusarium* wilt has been observed in Mozambique, the distribution and incidence of the disease is not known.

1.7 Biology and ecology

1.7.1 Transmission and spread of Fusarium wilt disease

Fusarium wilt disease starts from an infected area and will expand further every year, especially when susceptible pigeonpea varieties are grown successively in the same field. The disease is spread through plant debris and during soil tillage operation. The pathogen can survive in soil for three years as a saprophyte, but nutrient level and soil type will determine its persistence (Nene, 1980). *Fusarium udum* can also spread through contaminated water and soil and farm implements (Upadhyay and Rai, 1983). Upadhyay and Rai (1992), reported the spread of the pathogen from plant to plant through root contact, rainwater, irrigation and termites. Dissemination of the pathogen is also possible through seed-borne infection. Haware and Kannaiyan (1992) confirmed transmission from seed to seedling.

1.7.2 Life cycle and survival of the pathogen

The pathogen that causes *Fusarium* wilt survives in a perfect state (*Gibberella indica*) and an imperfect state (*Fusarium udum*).The pathogen occurs intercellularly, intracellularly and ectotrophically on the collar region as well as on the roots of the infected plants. In order to complete the wilt disease cycle, both imperfect and perfect stages are important. However, the imperfect state is considered most important and more predominant in nature (Upadhyay and Rai, 1992). According to Upadhyay and Rai (1983), the survival of the perfect state is yet to be confirmed and needs further investigation. The pathogen is heterothallic and sexual stages of *F. udum* and *G. indica* have been observed on the roots and collars of wilted pigeonpea plants (Rai and Upadhay, 1982). To produce spores, the two opposite sexual stages need to come into contact and produce perithecia. Reddy *et al*. (1998) investigate *F. udum* under laboratory conditions and observed optimum temperature to produce perithecia at 25±2^{o}C. The ascus contains eight ascospores, which are borne as two or three celled. The ascospores germinate and produce macro and microconidia (Reddy et al., 1998).

The fungus *F. udum* survive in the soil through inactive resting structures called chlamydospores and remain viable in fallow soil for two or more years (Elmer and Lacy, 1987). The entry of the pathogen into the plant is through lateral branches of the roots, where it causes infection and grows into the xylem vessels. The fungus survives saprophytically in both imperfect and perfect state during wilting and after death of the host plant for several years (Nene *et al*., 1980; Upadhyay and Rai, 1983). As the mycelium ages it produces chlamydospores, which after a dormancy period, germinate and infect the roots of the plants. During the cropping season, the ectotrophic growth of the pathogen can occur on infected roots and the collar.

1.8 Factors influencing *Fusarium* wilt disease development

Temperature is one of the factors affecting infection and spread of *Fusarium* wilt (Agrios, 2005; Chand and Khirbat, 2009). According to Singh and Bhargava (1981), low soil temperature favours *Fusarium* wilt disease development. The highest fungal population of *F. udum* has been observed at soil temperatures between 20-30^{o}C (Singh and Bhargava, 1981). Cook and Baker (1983) reported that at 28^{o}C, the growth of *Fusarium udum* is optimal, while above 33^{o}C the growth is inhibited. The temperature below 17^{o}C didn't favour disease development (Cook and Baker, 1983).

Nutrient status has been cited among the factors influencing *Fusarium* wilt disease development (Nene *et al*., 1981). Organic nitrogen sources have been shown to promote the disease more than inorganic sources (Walker, 1971; Warren and Kommedahl, 1973). Woltz and Engelhard (1973) reported that a high nitrogen level in the soil favours *Fusarium* wilt diseases development. A similar result was observed in an earlier study by Walker (1971). Fertilization with NO_3-N retards *Fusarium* wilt disease development as compared with NH^{+}-N (Byther, 1965). Nitrate has also been shown to reduce a wilt disease caused by *F*. *oxysporum* (Loffler *et al*., 1986). Rai and Upadhyay (1983) observed urea to promote activity of *F*. *udum* in the soil and suggested should not be used in the infected areas.

Slightly acidic and alkaline soils favour *Fusarium* wilt disease development (Upadhyay and Rai, 1992; Hillocks *et al*., 2000). Heavy and black cotton soils (18%) have a lower *Fusarium* wilt incidence than sandy soils (94%) (Shukla, 1975). High soil pH reduces the disease incidences due to a decreased nutrient level. Nematodes have been reported to increase the development of wilt disease through injuries to the roots of the plants (Edward and Singh, 1979; Hillocks *et al*., 2000). Two species of nematode, namely cyst nematode (*Heterodera cajani*) and reniform

nematode (*Rotylenchulus reniforms*), have been reported to increase susceptibility of pigeonpea to *Fusarium* wilt in India (Sharma and Nene, 1990; Jain and Sharma, 1996).

1.9 Studies of pathogenic variation of *Fusarium udum*

1.9.1 Biochemical variation

Biochemical markers have been used in studies of pathogenic fungi (Tudzynski and Weltring, 1983). *Fusarium udum* produces a number of biological substances such as enzymes, toxins and polysaccharides (Thomas, 1949; Neema, 1992; Pandey *et al*., 1995). According to Sadasivan and Subramanian (1963), pectic enzymes are secreted by fungi only. Enzymes have been reported to play key role in breakdown of cell walls and maceration of plant tissues, thus allowing entry of pathogens into the plant (Gothoskar *et al*., 1955). Kumar *et al*. (2007) investigated the extent of variability among 11 isolates of *F. udum* on the basis of biochemical characteristics and the results revealed a high variation in total sugar, total protein and amino acids and the most aggressive isolate was found to be rich in total sugar content. They further reported that certain morphological, physiological and biochemical characteristics of the pathogen govern the aggressiveness of isolates. Similar results were reported in earlier findings by Prasad and Chaudhary (1974), Shit and Gupta (1980) and Paterson and Rutherford (1991).

1.9.2 Cultural or morphological variability

Variation of the *F. udum* pathogen based on morphological, nutritional, physiological and spore germination has been studied by several researchers. The first study on cultural variation was reported by Butler (1910). Sharma and Mathur (1971) reported variation of *F. udum* in morphological characteristics and identified a diversity in pathogenicity. Variations in morphological characteristics has also been reported by several other authors (Shit and Gupta, 1978; Gupta *et al*., 1988; Gaur and Sharma, 1989). Reddy and Chaudhary (1985) reported variation amongst six isolates of *F. udum* and categorized these into three groups based on radial growth and colony characteristics. Kiprop *et al*. (2002) analysed isolates collected from various districts of Kenya. The 56 isolates of *F. udum* showed a high level of variability in aerial growth, pigmentation and radial mycelia growth (colony diameter) on PDA (potato dextrose agar). Madhukeshwara and Seshadri (2011) studied variability of six isolates from six distinct places. The isolates showed variation in pigmentation colour from white to dusky red and in the size of macro and microconidia. Further classification grouped the six isolate into three clusters and confirmed the existence of difference races.

1.9.3 Genetic variability

DNA molecular markers are the most common technique used to study variability of *F. udum*. The study of genetic diversity of *Fusarium* species using molecular markers, offers advantages over other techniques as it gives more detailed information on genetic differences, without interference from environmental factors (Garcia *et al*., 2004; Saker *et al*., 2005). Several genetic variability studies of *Fusarium* species have been conducted at molecular levels (Beladid *et al*., 2004; Kiprop *et al*., 2005, Bogale *et al*., 2007; Wang *et al*., 2010). Using random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP), Sivaramkrishnan *et al*. (2002) studied the variability of 36 isolates. Cluster analysis grouped the 36 isolates into four groups by mycelial colour, three groups by aerial mycelium growth and three groups by substrate colour and suggesting the existence of specific races. Mesapogu *et al*. (2011) studied the genetic diversity using 13 RAPD markers and UPGMA dendrogram analysis separated 30 different *F. udum* isolates into three clusters. Datta and Lal (2013), used 24 RAPD, 12 SSR and ITS-RFLP markers to study variability of 14 isolates and reported isolates were grouped based on their cultural characteristics and pathogenticity. Cluster analysis based on ITS-RFLP grouped isolates into three clusters.

1.10 Management approaches of *Fusarium* wilt

1.10.1 Cultural practices

The cultural practices are a long-term and sustainable management practice against *Fusarium* wilt (Bhatnagar, 1995). Cultural practices are easy to apply, inexpensive and environmentally friendly. Several researchers have reported on the effect of different cropping systems, soil types, crop combinations on *Fusarium* wilt (Natarajan *et al*., 1985; Singh *et al*., 1990). Different pigeonpea cropping systems, such as crop rotation, intercropping and multiple cropping, have been regarded as the efficient methods in reducing the disease (Thurston, 1992). According to Ingole *et al*. (2005), a pigeonpea/ sorghum mixed cropping or a pigeonpea sorghum rotation reduces the *Fusarium* wilt infestation to below 20%. A crop rotation between pigeonpea and *Crotalaria juncea* as green manure reduces the incidence level by 30% (Upadhay and Rai, 1981). Solarization of fields could is effective in reducing the population of *Fusarium* inoculum (Reddy *et al*., 2012).

Pigeonpea is usually intercropped with cereals, such as maize, and legumes. Several authors have reported that intercropping pigeonpea with sorghum results in a reduction of the *Fusarium* wilt incidence (Dey, 1974; Naik *et al*., 1997; Sharma *et al*., 1987). Naik *et al*. (1997) reported a

39% incidence of *Fusarium* wilt in pigeonpea intercropped with sorghum, compared to 57% in a sole crop. A *Fusarium* wilt incidence reduction was also reported upon intercropping with castor (ICRISAT, 1994; Bhatnagar, 1995). Low level of *Fusarium* wilt incidence (<10%) was observed in pigeonpea intercropped with cotton (Bhatnagar, 1995).

1.10.2 Chemical control methods

Several fungicides have been used to control *Fusarium* wilt in pigeonpea under laboratory and field conditions (Ghosh and Sinha, 1981; Upadhyay and Rai, 1981). Ghosh and Sinha (1981) studied the effect of systemic fungicides Benlate and Campogram-M at 50 ppm *in vitro* and *in vivo* and found that spore germination of *F. udum* was completely inhibited. Seed treatment with carbendazim 500 g/kg and BAS 38601F were found highly effective in controlling the mycelial growth of *F. udum.* Other studies, by Haware and Kannaiyan (1992) and Kotasthane *et al*. (1987), reported the effective seed treatment with Benomyl and Thiram.

1.10.3 Integrated disease management

Integrated disease management (IDM) is a combination of cultural practice, chemical control, biological control and the use of resistant varieties, and an effective management of the pigeonpea *Fusarium* wilt (Pande *et al*., 2012). Several authors have reported on the successful use of integrated disease management in reducing *Fusarium* wilt incidences (Locke *et al*., 1985; Upadhyay and Rai, 1989). A significant reduction of wilt incidence, by 27.1%, was reported with the combined use of *Trichoderma viride* as a seed treatment with other recommended practices (Bidari and Gundappagol, 1997). A combination of carbendazim seed treatment @ 2 g/kg of seeds + soil application of *P. fluorescens, Trichoderma* viride each @ 2.5 kg/ha in farm yard manure applied at 50 kg/ha recorded the lowest *Fusarium* wilt incidence of 5.32%, as compared with pigeonpea intercrop with sorghum @ 1:1, which recorded *Fusarium* incidence level of 9.44% (Mahesh *et al*., 2010). Carbendanzim seed treatment @ 2 g/kg of seeds + T. viride soil application @ 2.5 kg/ha in farm yard manure @ 50 kg/ha recorded *Fusarium* wilt incidence level of 9.30% as compared to carbendazim seed treatment @ 2g/kg of seed + *P. fluorescens* in soil application, which recorded *Fusarium* wilt incidence of 19.09% (Mahesh *et al*., 2010).

1.10.4 Host plant resistance

Host plant resistance in pigeonpea is considered a reliable, economical and effective method of disease management (Sharma and Ghosh, 2016). Considerable efforts by the International

Crop Research Insttitute for the Sem-Arid Tropic (ICRISAT) have led to the identification of different sources of resistance, which can be utilized in national breeding programs in Asia and Africa (Park *et al*., 2008). Nene *et al*. (1989), in multilocational trials in India and east Africa, have identified several resistant lines such as ICP 4769, ICP 7118, ICP 7182, ICP 8863, ICP 9168, ICP 10958 and ICP 11299. According to Reddy *et al*. (1993), the following lines were moderately resistance to *fusarium* wilt: ICP 8863, ICP 9145, ICP 9174, ICP 12745, ICPL 333, ICPL 8363, ICPL 88047, BWR 370, DPPA 85-2, DPPA 85-3, DPPA 85-8, DPPA 85-13, DPPA 85-14, Bandapalera, ICP 4769, ICP 9168, ICP 10958, ICP 11299, C 11 (ICP 7118), BDN 1. Gwata et al. (2006) reported the resistance of ICEAP 00040 to *Fusarium* wilt after field evaluations in Kenya, Malawi and Tanzania. Changaya (2007) identified a traditional long duration AP10 as a high yielding genotype, *Fusarium* wilt resistant landrace in in Malawi. In India, Sharma and Pande (2011), reported several lines which were moderately to highly resistant, both in greenhouse and field, such as ICP 14976, ICP 15049, ICP 7903, ICP 12031, ICP 12059, ICP 12771 and ICP 12775. Sharma *et al*. (2012) also reported several lines that are resistant in greenhouse and field, namely ICP 6739, ICP 8860, ICP 11015, ICP 13304 and ICP 14819.

1.11 Inheritance of resistance to *Fusarium* wilt in pigeonpea

The genetics of *Fusarium* wilt resistance in pigeonpea is still not well understood. Several studies suggested that resistance was controlled by major genes, which are either dominant or recessive, acting singly or with dominant epistatic effects (Delhaize *et al*., 1993; Saxena *et al*., 2012) or by two independent dominant genes (Kochian *et al*., 2005). Pal (1934) reported a multiple genetic control. Silim *et al*. (2005) reported that the wilt disease was controlled by a single dominant gene and his findings were corroborated by Pandey *et al*. (1996) and Kotresh *et al*. (2006). Karimi *et al*. (2010) also confirmed that resistance to *Fusarium* wilt was controlled by a single dominant and a recessive gene. Saxena *et al*. (2012), on the other hand, reported that resistance to wilt was due to one dominant and one recessive gene with epistatic effects. Odeny *et al*. (2009), studying the genetics of resistance in two genotypes [African (ICEAP 00040) and an Indian (ICP 8863)], found that the gene for *Fusarium* wilt resistance in ICEAP 00040 was controlled by a single recessive gene, while in ICP 8863 two pairs of recessive genes governed the resistance. Changaya *et al*. (2012) reported that resistance to *Fusarium* wilt was controlled by a single or two independent/ complimentary dominant genes.

1.12 Screening techniques for *Fusarium* wilt resistance in pigeonpea

Several techniques have been widely used for screening pigeonpea for *Fusarium* wilt resistance. However, each technique has advantages and disadvantages. Root dipping in inoculum (Phipps and Stipes, 1973; Prasad *et al*., 2003), growing seed or seedlings in infected soils (Russell, 1978), soaking seed in inoculum (Sakar *et al*., 1982) and injecting inoculum into plants (Jindal *et al*., 1982) have all been used. Usually plant injury, especially to the roots, enhances infection (Henderson and Winstead, 1961).

Hubbeling (1980) reported that growing varieties in infected soil was the most effective method, because disease symptoms usually appeared at the early growth stages and the level of wilting is usually enough to differentiate between susceptible and resistance varieties. In cotton, Hillocks (1984) screened for resistance *Fusarium oxysporum f. vasinfectum,* however, the disadvantage of this method is that it is difficult to quantify the amount of inoculum in the soil.

The root dipping method is considered a reliable method, because even at low inoculum levels, the difference between susceptible and resistant genotypes are easy to differentiate (Phipps and Stipes, 1973). According to ICRISAT (1986), the root dipping method involved transferring a 7-10 day old seedlings from a germination medium, washing their roots, pruning it so as to create entry for the pathogen, dipping the roots in a conidial suspension and transplanting into a non-infested soil. Reddy and Raju (1993), found that for the dip inoculation method, at concentration of 1×10^6, colony forming was optimal. Screening for *Fusarium* wilt in pots was reported by Nene (1982), Mishra and Dhar (2005) and Prasanthi *et al*. (2009). Pandey et al. (1996), reported survival of the susceptible and resistant genotypes of 0-7% and 87-94%, respectively, using this technique.

The field screening method is considered the simplest and most common method. Butler (1908) screened a number of pigeonpea genotypes in a field infested with *F. udum* fungus. Later McRae and Shaw (1926) selected resistant versus susceptible genotypes in *Fusarium* wilt sick plots. According to Kimani (2001), the field sick plots were developed by incorporating chopped stems and roots of diseased plants. The susceptible genotypes were grown in those plots to check the presence of the pathogen and they confirmed the presence of *F. udum* with laboratory techniques by isolation from wilted plants. The same procedure has to be repeated for four consecutive seasons until a susceptible check reaches a mortality rate of over 60%. Field screening has its shortfall because the pathogen in the soil is not always evenly distributing leading to escapes. According to Burgess *et al*. (1994), the expression of disease symptoms depends on the moisture content, temperature and structure of the soil. To increase

the disease pressure, and make more even distribution of inoculum in the field, diseased debris were incorporated into the field.

1.13 Marker assisted breeding in pigeonpea

DNA molecular markers are a useful tool to increase the efficiency and precision in crop improvement. However, the progress in the development of genomic tools in cultivated pigeonpea has been very slow due to the low level of polymorphism (Kiprop *et al*., 2002; Odeny, 2007). The objective to integrate molecular markers and conventional breeding is to develop superior varieties with enhanced tolerance to abiotic and biotic stresses. A number of molecular markers such as restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs) and amplified fragment length polymorphisms (AFLPs) have been used in the past for genetic analysis (Varshney *et al*., 2007). Currently, simple sequence repeats (SSRs), single nucleotide polymorphisms (SNPs) and diversity array technology (DarT) markers have become the markers of choice for genetic analysis and breeding applications (Varshney *et al*., 2007). Compared to other markers, SSR markers have the advantages of being multi-allelic and co-dominant (Prasanthi *et al*., 2009). SNP markers offer high-throughput and cost-effective genotyping options and represent the most abundant class for polymorphism. Diversity array technology (DArT) markers are high-throughput and have been used in many crop species because no sequence information is needed for developing these markers (Minja and Shanower, 1999).

1.14 Use of marker assisted selection for *Fusarium* wilt resistance of pigeonpea

Marker assisted selecton (MAS) in pigeonpea is used to shorten the breeding cycle for candidate genotypes developed through conventional breeding methods, by selecting phenotypes that have the genes of the targeted traits (Xu *et al*., 2005; Collards *et al*., 2005). At present, several markers and maps for different traits are available and have been proven to be useful in pigeonpea breeding (Gupta and Varshney, 2000; Varshney *et al*., 2005). Molecular mapping of quantitative traits loci for important agronomic traits have been developed (Kumawat *et al*., 2012). A large number of SSRs and SNPs, for agronomic traits and diseases of economic importance, have been developed (Raju *et al*., 2010; Varshney *et al*., 2010; Bohra *et al*., 2011). Saxena *et al*. (2010) developed a mapping population for *Fusarium* wilt and sterility mosaic disease resistance using SSR markers.

Screening pigeonpea for resistance to *Fusarium* wilt, using molecular markers, has been made possible following the discovery of marker based technology, using the polymerase chain

reaction (PCR) (Bussel *et al*., 2005). Markers allows for a quick evaluation of progenies for the presence of a desired gene in an early generation. RAPD markers were developed in 1990s by Williams *et al*. (1990) and proven to be effective because of the simplicity of the technique, the low quantity of target DNA necessary for genetic analysis and the possibility of automization. RAPD markers in pigeonpea have been used to screen for *Fusarium* wilt resistance (Kotresh *et al*., 2006). Prasanthi *et al*. (2009) used 24 RAPD markers to screen pigeonpea breeding lines for resistance to *Fusarium* wilt. Due to the development of mapping populations in pigeonpea more markers are now available. Chaithanya *et al*. (2011) used the sequenced characterized amplified regions (SCAR) marker to trace the introduction of *Fusarium* wilt resistant genes in segregating populations. In a recent study, Ganesh *et al*. (2014) identified markers for *Fusarium* wilt resistance, using simple sequence repeats (SSRs) and bulk segregant analysis (BSA). The use of PCR based molecular markers and bulk segregant analysis (BSA) has proven to be a very powerful technology in identifying markers tightly linked to genes of interest (Nakamura *et al*., 2001; Shen *et al*., 2003). Singh *et al*. (2016) reported that associated SSRs ASSR-1, ASSR-23 and ASSR-148, are useful markers for introgressing *Fusarium* wilt resistance into adapted susceptible varieties.

1.15 Use of hybrid technology

Pigeonpea is a unique legume because of its partial out-crossing nature. Saxena *et al*. (1990) reported a large out-crossing range of 0-70% in India. In China, a single study observed a natural out-crossing range of 0-60% (Yang *et al*., 2003). However, the outcrossing nature of pigeonpea has been a problem in maintaining the purity of genotypes in farmers field and in seed production. Scientists at ICRISAT have made use of the outcrossing nature to exploit the hybrid vigour of pigeonpea by developing high-yielding commercial hybrids. The special features of hybrids are the greater adaptation, greater drought tolerance, faster growth rate, uniformity, high grain yield and greater disease resistance (Saxena and Nadarajan, 2010). Hybrids offers 20-40% yield advantage as compared to open-pollinated varieties (Shiferaw *et al*., 2008). Saxena *et al*. (2002) reported a yield advantage of 30-60% over pure lines.

1.16 Farmers variety preference and participatory research

According to Mergeai *et al*. (2001), the preference expressed by farmers in Kenya for pigeonpea varieties were large seed size, high yield, earliness, white/cream seed colour, insect pest resistance, resistance to *Fusarium* wilt and large pod size. A similar observation was reported by Changaya (2007) in Malawi. Farmers prefer landraces above improved varieties because of compatibility in the intercropping system, a high expansion rate of the seed after

cooking and big stems for firewood (Mergeai *et al*., 2001; Changaya, 2007). Shiferaw *et al*. (2005) conducted a survey in Tanzania and reported that farmers prefer pigeonpea genotypes which are high yielding, resistant to pests and diseases, early maturing with even/uniform maturity, white large grain colour, drought tolerance and medium plant height. Snapp and Silim (2002) reported that farmers in Kenya and Malawi desire improved varieties with a suitable for intercropping, deep rooted, indeterminate, and long duration which can be intercropped with short duration legumes. Ogbe and Bamidele (2007) reported that farmers in Nigeria prefer pigeonpea genotypes with a short cooking time, high yield, pest resistant, day neutral and suitable for home gardens.

Rao *et al*. (2012) conducted a participatory varietal selection (PVS) in farmers fields in Tanzania and Malawi and found that farmers select genotypes which are high yielding, large, cream coloured seed, resistant to *Fusarium* wilt disease, drought tolerance and vegetable types with green pods. Buyers prefers large and white coloured grains because they fetch higher prices in the export market. Moreover, the colour of grains should be deep and consistent throughout the pea. Buyers also prefers pigeonpea with short cooking and milling times. Lo Monaco (2006) reported that round shape grains are preferred above oval shape grains in industrial processing into *dhal*.

1.17 Genetic diversity in pigeonpea

Systematic determination of the genetic diversity and the genetic relationships within a germplasm collection is an important aspect of genetic resource conservation (Clark *et al*., 1997). The genetic variability of cultivated species and their wild relatives are a potential source for developing new and improved varieties with farmers desirable traits (Govindaraj *et al*., 2015). Knowledge of genetic diversity in a crop helps breeders to choose desirable parents for the breeding program and introgression distantly related germplasm. Genetic diversity in pigeonpea has been studied using agro-morphological traits (Upadhaya *et al*., 2005, Upadhaya *et al*., 2007: Manyasa *et al*., 2008; Hamid *et al*., 2011). However, DNA molecular markers are found to be effective in estimating genetic diversity in plants (Varshney *et al*., 2005). Since the development of DNA molecular markers, SSR markers have been the most commonly used in analyzing genetic diversity and genetic relationships in pigeonpea (Odeny et al., 2007; Sousa *et al*., 2011; Njung'e *et al*., 2016). A better understanding of genetic diversity in pigeonpea will facilitate further improvement of important traits.

1.18 Combining ability studies in pigeonpea for *Fusarium* wilt resistance, yield and yield components

The term combining ability was defined by Sprague and Tatum (1942). General combining ability (GCA) is the average performance of a line in hybrid combination, while specific combining ability (SCA) is a performance of parents in specific crosses. The most effective tool for the identification of appropriate parents for hybridization is through combining abilities studies. Combining ability plays an important role in breeding programmes as it characterize the nature and magnitude of genetic effects governing yield and yield components traits and it identifies the most promising parents to be used in development of suitable genotypes (Iqbal *et al*., 2012). GCA measures additive gene effects and SCA measures non-additive gene effects, including dominance and epistasis.

Combining ability studies in pigeonpea on line x tester mating designs have been widely reported by several authors (Sarode *et al*., 2009; Kumar *et al*., 2009; Shoba and Balan, 2010; Gupta *et al*., 2011; Kumar *et al*., 2012; Yerimani *et al*., 2013, Changaya, 2007). Using line x tester mating designs scientists were able to gather information on GCA and SCA performance of parents and their progenies (Ceyhan *et al*., 2008). Pandey *et al*. (2014) showed that SCAs were higher than GCA for all traits except plant height. Several studies have reported predominance of SCA effects over GCA for seed yield and important yield components (Jayamala and Rathinasamy, 2001; Jahagirdar, 2003; Kumar *et al*., 2003; Banu *et al*., 2006; Kumar *et al*., 2009; Vaghela *et al*., 2009; Shoba and Balan, 2010). Changaya *et al*. (2012) reported that SCA was predominant for *Fusarium* wilt with incidence ranged from 0 to 97.3%, days to 50% flowering and number of secondary branches, while GCA was predominant for yield and other secondary traits. For an evaluation of disease resistance, crosses with significant and high negative SCA are desirable, as they are considered resistant. Makelo (2011) reported a significant and high negative SCA for *Fusarium* wilt. Changaya *et al*. (2012) reported that both GCA and SCA are important for the expression of *Fusarium* wilt resistance.

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Chapter 2 : Production constraints and farmers preferred traits of pigeonpea varieties: implications for breeding in Tanzania

Abstract

Understanding production constraints and farmers preferred traits of crop varieties is crucial for the uptake and sustainable use of production technologies. The aim of this study was to identify major production constraints, farming systems, control strategies of *Fusarium* wilt disease and farmers preferred traits for pigeonpea in Tanzania. A participatory rural appraisal (PRA) and structured survey were conducted in three selected districts viz. Babati, Karatu and Kilosa with a total of 240 individuals for the semi-structured survey and 108 individuals for the focus group discussions. Results indicated that maize intercropping with pigeonpea is the most common production system across the three districts. Maize and beans are the main crops grown for household consumption, while pigeonpea is primarily grown for cash income. Other crops such as hyacinthbean, barley, wheat, tomatoes, simsim, sunflower, millet and horticultural crops are grown for both cash income and household consumption. The major production constraints perceived by farmers were insects and diseases. *Fusarium* wilt was the major disease in the three districts. Both field and storage pests were considered limiting factors. Other constraints are drought, lack of early maturing varieties, high input prices and limited access to improved cultivars. Farmers have demonstrated the awareness of disease symptoms and control options. Farmers showed a strong preference for high yield, disease resistance, drought tolerance, early maturity, short cooking time, determinate (bush) growth habit and large seed size and white/cream seed colour. Farmers expressed their needs towards improved production systems, and supply and distribution of high yielding varieties resistant to *Fusarium* wilt. Since these traits conditions farmers response to new varieties, breeders should strictly consider these traits as their selection criteria in future cultivar development

2.1 Introduction

Pigeonpea (*Cajanus cajan* (L.) Millsp) is a drought-tolerant grain legume and one of the most important crops adapted to diverse growing conditions in Tanzania. Despite the breeding effort, the average global productivity of pigeonpea is still very low (Choudhary *et al*., 2013). Pigeonpea plants have both physiological and morphological attributes that may reduce interspecific competition in mixed culture. Pigeonpea is grown mostly in Southern Asia and a few countries in Africa. The crop was probably domesticated in India. East Africa is considered a secondary centre of diversity of pigeonpea (Smartt, 1990; Van der Maesen, 1990).

Pigeonpea is an important legume crop in Tanzania. The crop accounts for about 5% of total output of pulses and 4% of total area under pulses, making it the third most important pulse after beans and cowpeas in the country (Simtowe *et al*., 2011). Pigeonpea grown in the northern zone districts of Tanzania is sold in several African and Asian countries (Van der Maesen, 2006). Given the limited domestic market, export markets are a key driver for commercialization in Africa.

Pigeonpea has been considered an important crop due to its adaptability to semi-arid environments, tolerance to low soil fertility and a capacity to recycle nutrients (Whiteman *et al*., 1985; Nene and Sheila, 1990; Mapfumo *et al*., 1998; Adjei-Nsiah *et al*., 2007; Mula and Saxena, 2010). It has been reported that intercropping pigeonpea with cereals resulted in an increase of 40-90 kg N/ha in the succeeding crop and additional 20-30 kg N/ha in the second season (Peoples *et al*., 2015). Pigeonpea grains contain an average of 22% crude protein and have a high nutritional value for both humans and livestock. It is also a feed for domestic livestock during dry seasons (Ezeaku *et al*., 2016).

A participatory rural appraisal (PRA) is a rapid and a cost effective method of identifying farmers preferred cultivars and it can reveal a number of important traits that would otherwise not have been considered by breeders in developing a new cultivars (Danial *et al*., 2007). It involves the researcher and farmers working together in the identification of farmers breeding priorities. Changaya (2007) reported that farmers in Malawi prefer local landraces over improved varieties due to the high expansion rate after cooking, short cooking time, big stems for firewood, early maturation, capacity to improve soil fertility, drought tolerance and pest tolerance. The objective of the PRA was to identify the crops intercropped with pigeonpea,

crops grown in rotation and to identify the major pigeonpea productions constraints and farmers preferred traits. An additional objective was to explore the levels of *Fusarium* wilt disease infestation, the effect on yield and the management methods used to control disease. For the structured survey a questionnaire was used to interview individual farmers. The aim of this study was to identify the major pigeonpea production constraints, the strategies used to control *Fusarium* wilt disease, the farmers preferred traits and the breeding priorities of pigeonpea in Tanzania.

2.2 Materials and methods

2.2.1 Study area description

The study was conducted in three selected districts namely Babati, Karatu and Kilosa. Per district two villages were selected. The studies were conducted in the 2014 growing season (at Babati and Karatu districts) and the 2015 off-season (Kilosa district). These districts were selected because they are high potential areas for pigeonpea production in Tanzania and farmers have been growing pigeonpea for many years. The descriptions of the study sites are summarized in Table 2.1.

Table 2.1 Description of study sites

District	Village	Latitude	Longitude	Altitude (meter above sea level)
Kilosa	Magubike	S 03º33.031'	E 037º09.526'	934
	Berega	S 06º11.559'	E 037º04.856'	950
Babati	Sigino	S 04º12.472'	E 035º 41.019'	1459
	Nakwa	S 03º 22.563'	E 036º39.898'	1387
Karatu	Bashay	S 03º21.401'	E 035º37.91'6	1536
	Rhotiakati	S 03º12.472'	E 035º 40.778'	1527

2.2.2 Sampling procedures

Both formal and informal approaches were employed to collect data for the study. Two hundred and forty farmers were sampled for a structured survey. The number of farmers who participated in the different regions is presented in Table 2.2. The survey involved individual interviews with all 240 farmers. This enabled individual farmers to express their own views without the influence of the community. The informal approach of the participatory rural

appraisal was used to generate information on farmers perception related to aspects of pigeonpea production. Prior to the survey the list of villages in each district was provided by District Agricultural, Irrigation and Cooperative Officer of each district. The participants were selected from each village with the help of village chairman, village agricultural extension officer and village executive officer by providing list of farmers. The criteria used to select participants were based on their experience in pigeonpea production and marketing, education levels, farm size i.e. small, medium and big farmers, gender, age balance and government retired officers. A total of 240 farmers participated (123 men and 117 women). During the structured survey the language used was ‘’***Swahili***’’. This is the widely used language in Tanzania. For the focus group discussions eighteen farmers were selected per village providing a total of 108 participants. Gender was considered in the selection of focus group discussion participants (Table 2.2).

Table 2.2 Number of participants during surveys and focus group discussions

Formal Survey		**Gender of respondent**		
District	**Village**	**Male**	**Female**	**Total**
Babati	Nakwa	20	20	40
	Sigino	20	20	40
Karatu	Bashay	21	19	40
	Rhotiakati	21	19	40
Kilosa	Magubike	21	19	40
	Berega	20	20	40
Total		123 **(51.2%)**	117 **(48.7%)**	240 **(100%)**
Focus Group Discussion				
District	**Village**	**Male**	**Female**	**Total**
Babati	Nakwa	9	9	18
	Sigino	9	9	18
Karatu	Bashay	10	8	18
	Rhotiakati	9	9	18
Kilosa	Magubike	11	7	18
	Berega	8	10	18
Total		**56 (51.8%)**	**52 (48.1%)**	**108 (100%)**

2.2.3 Data collection

Data for structured survey was collected using questionnaires. The focus group discussions (FGD) were undertaken to collect additional information using PRA tools such as pairwise ranking, matrix scoring, transect walk and direct observation. Only a few farms were visited in Karatu and Babati Districts due to logistic reasons i.e. hills and road accessibility in villages. A transect walk was conducted in a few fields after the focus group sessions to promote discussion amongst farmers about the pigeonpea production and the associated constraints (Figure 2.1). Participants were given a flip chart to list problems facing pigeonpea production, strategies used to control *Fusarium* wilt, and were asked to rank production problems according to their importance and score. *Fusarium* wilt disease is widely known as ***"mnyauko"*** *in Swahili language* meaning wilting and is easy to identify. As such, no pictures or cards were used to show *Fusarium* wilt disease symptoms to focus group discussion.

2.2.4 Data analysis

The data collected through the questionnaires was processed and analysed using Statistical Package for Social Science (SPSS) version 21.0 (SPSS, 2012). Analysis was done for descriptive statistics, frequencies and percentages.

2.3 Results

2.3.1 Crops and cropping systems

The results indicated that over 50% of the farmers own land of less than 1ha (Table 2.3). It was noted that only 20% of farmers have more than 2 ha of land (Table 2.3). It is also appeared that land owned by female farmers is smaller than land owned by males farmers (Table 2.3).

Table 2.3 Land of the study area and gender

	District					
	Babati		Karatu		Kilosa	
Land holding size (hectares)	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
0.1-0.5	3	15	5	10	5	8
0.6-1.0	10	20	11	12	11	19
1.1-2.0	14	5	14	12	12	6
Above 2.0	13	0	12	4	14	5
Total	**40**	**40**	**42**	**38**	**42**	**38**

None of the farmers interviewed practiced sole cropping. Intercropping is the most common farming practice (91.7%). Other cropping systems were mixed cropping (0.4%), multiple cropping (0.8%); intercropping and multiple cropping (7.1%) (Table 2.4).

Table 2.4 Pigeonpea farming system in Babati, Karatu and Kilosa districts

	District				
Farming system	**Babati**	**Karatu**	**Kilosa**	**Total**	**% Total**
Intercropping	62	79	79	220	91.7
Mixed cropping	0	1	0	1	0.4
Multiple cropping	1	0	1	2	0.8
Intercropping and Mixed cropping	17	0	0	17	7.1
Total	**80**	**80**	**80**	**240**	**100.00**

Maize was the most common crop for intercropping with pigeonpea (Table 2.5). Overall the maize and pigeonpea intercropping accounted for 70.8% of the cropping systems. Other crop combinations grown either in intercrop and/or mixed cropping were maize, beans and pigeonpea (19.6%); maize, pigeonpea and hyacinthbean (6.7%) and maize, pigeonpea and cowpea (2.9%). In Karatu and Babati districts hyacinthbean and beans were grown in a mixed culture with maize and pigeonpea (Table 2.5). In Kilosa district cowpea was grown in intercropped with maize and pigeonpea (Table 2.5). In the intercrop system of maize and pigeonpea, maize is harvested first.

Table 2.5 Crop combinations used in the cropping system at Babati, Karatu and Kilosa Districts of Tanzania

Crops used in the cropping system	District			Total	% Total
	Babati	Karatu	Kilosa		
Maize and pigeonpea	54	57	59	170	70.8%
Maize, beans and pigeonpea	17	16	14	47	19.6%
Maize, hyacinth bean and pigeonpea	9	7	0	16	6.7%
Maize, cowpea and pigeonpea	0	0	7	7	2.9%
Total	**80**	**80**	**80**	**240**	**100%**

The relative importance of other crops grown by farmers in addition to pigeonpea across the three districts was estimated based on the number of growers of each crop and is presented in Table 2.6. Tomatoes were grown by 2.9%, of farmers across the three districts, barley 5.8%, wheat 7.9%, millet 3.3% sunflower 9.2% and simsim 7.9%, hyacinth bean 12.1% and beans 47.1%. These crops were grown by farmers in order to enhance food and nutritional security and increase the level of household income (Table 2.6).

Table 2.6 Numbers of farmers growing different crops at Babati, Karatu and Kilosa Districts of Tanzania

	District				
Crops	**Babati**	**Karatu**	**Kilosa**	**Total**	**%Respondent**
Beans	63	33	17	**113**	**47.1**
Hyacinthbean	16	13	0	**29**	**12.1**
Sunflower	0	1	21	**22**	**9.2**
Simsim	0	0	19	**19**	**7.9**
Barley	0	14	0	**14**	**5.8**
Wheat	0	19	0	**19**	**7.9**
Tomatoes	1	0	6	**7**	**2.9**
Millet	0	0	9	**9**	**3.8**
Cowpea	0	0	8	**8**	**3.3**
Total	**80**	**80**	**80**	**240**	**100.0**

2.3.2 Constraints to pigeonpea production

Several pigeonpea production constraints were identified by farmers. Pests and diseases (72.1%) such as *Fusarium* wilt and pod borer were considered to be the most important constraints across the districts (Table 2.7). Other constraints were combinations of diseases and drought (20%); pests, diseases, drought and lack of improved seeds (2.5%); pests, diseases and lack of improved seeds (3.3%); lack of improved seeds (1.2%) and drought (0.8%) (Table 2.4). Of the three districts, Babati had the highest number of farmers who perceived pests and diseases as the main constraints (73), followed by Karatu (56) and Kilosa (44) (Table 2.7).

Table 2.7 Major constraints of pigeonpea production across the three districts

Constraints	Babati	Karatu	Kilosa	Total	% Total
Pests and diseases	73	56	44	173	72.1
Drought	0	1	1	2	0.8
Lack of improved seeds	2	0	1	3	1.2
Pests, diseases and drought	4	23	21	48	20.0
Pests, diseases drought and lack of improved seeds	0	0	6	6	2.5
Pests, diseases and lack of improved seeds	1	0	7	8	3.3
Total	**80**	**80**	**80**	**240**	**100**

The focus groups listed the most important constraints in their areas and ranked them in a direct pair-wise ranking (Table 2.8). Diseases ranked as the most important constraint in three districts. Insect pests ranked second, followed by drought. It was mentioned that pigeonpea yields are reduced during the years of drought, despite pigeonpea being known as a drought tolerant crop. Other constraints mentioned were lack of early maturing improved varieties and high input costs. Lack of early maturing varieties was considered a constraint in Babati and Karatu, because most of pigeonpea varieties grown by farmers are long duration genotypes which mature between 210-270 days. During the flowering stage, insecticide application to control pod borer is essential in order to realize good yields with high quality grains.

Table 2.8 Pair-wise ranking of the major constraints of pigeonpea at Babati, Karatu and Kilosa Districts of Tanzania

	Constraints	A	B	C	D	E	F	Score	Rank
Babati									
A	Diseases	-	A	A	A	A	A	6	1
B	Insect pests		-	B	C	B	B	4	3
C	Late maturity			-	C	C	C	5	2
D	Drought				-	D	D	3	4
E	Lack of improved varieties					-	E	2	5
F	High input price						-	1	6
Karatu									
A	Diseases	-	A	A	A	A	A	6	1
B	Insect pests		-	B	B	B	B	5	2
C	Late maturity			-	C	C	D	3	4
D	Drought				-	D	D	4	3
E	Lack of improved varieties					-	F	1	6
F	High input price						-	2	5

Kilosa									
A	Diseases	-	A	A	A	A	A	6	1
B	Insect pests		-	C	B	B	B	5	2
C	Late maturity			-	D	F	F	2	5
D	Drought				-	D	D	4	3
E	Lack of improved varieties						F	1	6
F	High input price							3	4

Key: Score: 1= lowest, 6= highest; Rank: 1 most important, 6=least important

In the survey, 96.2% of the farmers reported *Fusarium* wilt as the major disease (Table 2.9). Farmers fields infested with *Fusarium* wilt were observed during the transect walk. The majority of the farmers interviewed (80.8%) mentioned that they have knowledge of the *Fusarium* wilt disease symptoms (Table 2.9).

Table 2.9 Knowledge on *Fusarium* wilts disease problem and symptoms

Respondents (%)	Wilt problem	Respondents (%)	Wilt symptoms knowledge
96.2	Major problem	80.8	Yes
3.8	Not a problem	19.2	No

2.3.3 Incidence of *Fusarium* wilt, effect on yield and management options used to control *Fusarium* wilt

The incidences of *Fusarium* wilt on farmers field were as follows: 69.2% of farms had *Fusarium* wilt on a small part of the farm; 1.2% on half of the farm; 17.9% on one third of the farm; 7.5% on two third of the farm; 0.4% on the whole farm and 3.8% didn't specify incidence levels (Table 2.10). The results indicate that *Fusarium* wilt is a major problem.

Table 2.10 *Fusarium* incidence levels in farmers fields

	District				
Farm incidence levels	**Babati**	**Karatu**	**Kilosa**	**Total**	**%Total**
Some part of the farm	60	67	39	166	69.2
Half of the farm	3	0	0	3	1.2
One third of the farm	13	4	26	43	17.9
Two third of the farm	4	2	12	18	7.5
Whole farm	0	0	1	1	0.4
Not known	0	7	2	9	3.8
Total	**80**	**80**	**80**	**240**	**100.0**

Results from the structured surveys indicated that 50.0% of farmers interviewed reported low yield, 27.9% very low yield and 22.1% no yield at all (Table 2.11).

Table 2.11 Effect of *Fusarium* wilt on yield

	District				
Grain yield in the presence of wilt	**Babati**	**Karatu**	**Kilosa**	**Total**	**% Total**
Very low yield (50 kg- 125 kg per ha)	12	22	33	67	**27.9**
Low yield (250-500 kg per ha)	44	32	44	120	**50.0**
No yield (0 kg per ha)	24	26	3	53	**22.1**
Total	**80**	**80**	**80**	**120**	**100**

Different management options to control *Fusarium* wilt used by farmers in all three districts are presented in Table 2.12. The overall results of the focus group discussions indicated that farmers in all the districts have more or less similar control methods. Crop rotation, use of different seeds, uprooting and burning were the most adopted management practices for *Fusarium* wilt control in all districts (Table 2.12). Overall most farmers (47.1%) avoid fields infested with *Fusarium* wilt to prevent spread of disease to the other fields. The ranking by focus groups are presented in (Table 2.12).

Table 2.12 Different control methods used by farmers to control *Fusarium* wilt

Control methods	District			Total	% Total
	Babati	Karatu	Kilosa		
Use of different types of seeds	10	8	3	21	8.8
Avoiding field infested with *Fusarium* wilt	34	39	42	115	47.9
Crop rotation and applying pesticides	5	3	3	11	4.5
Crop rotation, uprooting and burning	1	4	9	14	5.8
Adding farmyard manure	3	1	2	6	2.5
Applying pesticides	6	4	7	17	7.1
Uprooting and burning	3	11	7	21	8.8
Crop rotation	18	10	7	35	14.6
Total	**80**	**80**	**80**	**240**	**100.0**

Table 2.13 Pair-wise ranking for different method of controlling *Fusarium* wilt disease

Control methods		A	B	C	D	E	F	Score	Rank
Babati									
A	Avoiding of infected field	-	A	A	C	A	A	5	1
B	Crop rotation		-	B	B	B	C	3	2
C	Uprooting and burning			-	C	C	B	2	3
D	Use of different seeds				-	A	A	2	3
E	Adding farmyard manure					-	B	1	5
F	Applying pesticides						-	1	5
Karatu									
A	Avoiding of infected field	-	B	B	A	A	A	3	2
B	Crop rotation		-	B	B	B	B	4	1
C	Uprooting and burning			-	C	B	A	1	4
D	Use of different seeds				-	D	D	2	3
E	Adding farmyard manure					-	E	1	4
F	Applying pesticides						-	1	4

Control methods		A	B	C	D	E	F	Score	Rank
Kilosa									
A	Avoiding of infected field	-	A	A	A	A	A	5	1
B	Crop rotation		-	B	B	B	B	4	2
C	Uprooting and burning			-	A	C	C	2	4
D	Use of different seeds				-	D	D	3	3
E	Adding farmyard manure					-	E	1	5
F	Applying pesticides						-	1	5

2.3.4 Source of seed and pigeonpea varieties preferred by farmers at Babati, Karatu and Kilosa districts of Tanzania

Different sources of seed used for planting were mentioned by farmers. Farm saved seed (70.8%) is the main source of seed in all surveyed areas (Table 2.14). Other sources are seed purchased from the market (11.7%); provided by NGOs (3.8%); purchased from market and farm saved seeds (12.9%) and farm saved seed and provided by NGOs (0.8%). Borrowing of seeds from neighbours or relatives with the agreement of paying back after harvest and exchange of pigeonpea seed with other crop varieties is also practiced by a few farmers before planting. The majority of farmers reported using recycled seeds for more than four years.

Table 2.14 Different source of seeds for pigeonpea varieties in three selected districts of Tanzania

	District				
Source of seed	**Babati**	**Karatu**	**Kilosa**	**Total**	**% Total**
Purchased from market	4	11	13	28	11.7
Farm saved seed	65	56	49	170	70.8
NGO's	0	0	9	9	3.8
Purchased from market and farm saved seed	11	13	7	31	12.9
Farm saved seed and NGO's	0	0	2	2	0.8
Total	**80**	**80**	**80**	**240**	**100**

The results showed that 50.4% of the farmers interviewed across districts used improved varieties, 43.3% use traditional varieties and 6.2% use both improved and traditional varieties (Table 2.15). All farmers interviewed in Babati district grow traditional varieties, while in the other districts farmers use both improved and traditional varieties (Table 2.15). The majority of

improved seeds used by farmers were obtained from research station and different development projects aimed at enhancing pigeonpea production.

Table 2.15 Types of pigeonpea varieties grown in three districts

	District				
Pigeonpea varieties grown	**Babati**	**Karatu**	**Kilosa**	**Total**	**%Total**
Improved	0	71	50	121	50.4
Traditional	80	1	23	104	43.3
Improved and traditional	0	8	7	15	6.2
Total	**80**	**80**	**80**	**240**	**100**

The surveys revealed that Mali (42.1%) was the most preferred variety in all districts (Table 2.16). The second most preferred variety was Bangili (30.0%), a well-known traditional long duration variety, followed by short variety (6.7%), Komboa (5.4%) and Tumia (5.0%). Some varieties grown by farmers were not identified by their names, instead farmers mention ‘’*short* or *long*’’ means they take short or long duration to reach maturity.

Table 2.16 The most preferred pigeonpea varieties across three districts

Variety	District			Total	% Total
	Babati	Karatu	Kilosa		
Bangili	72	0	0	72	30.0
Tumia	0	0	12	12	5.0
Komboa	0	0	13	13	5.4
Mali and Tumia	0	0	8	8	3.3
No. 40	1	0	0	1	0.4
Mali	0	76	25	101	42.1
Babati White	1	0	1	2	0.8
Short	1	0	15	16	6.7
Long	0	4	6	10	4.2
Bangili and short	3	0	0	3	1.2
Bangili and long	1	0	0	1	0.4
Bangili, short and Long	1	0	0	1	0.4
Total	**80**	**80**	**80**	**240**	**100**

2.3.5 Farmers ranking of the preferred traits

Farmers have demonstrated a good understanding of the preferred traits during the focus group discussions. The ranking of the traits considered important in pigeonpea production are represented in Table 2.17. There is a strong positive correlation for preferred pigeonpea traits ranked by farmers among villages in all districts ($r_{s=}$ 0.903, 0.89 and 0.975) (Table 2.17).

Table 2.17 Farmers ranking of preferred pigeonpea traits in three districts

	District						
	Babati		Karatu		Kilosa		
	Village						Overall
Pigeonpea Traits	Nakwa	Sigino	Bashay	Rhotiakati	Magubike	Berega	Rank
Pest/Disease resistance	1	1	2	1	3	3	**1**
Yield potential	2	2	1	2	2	2	**2**
Earliness to maturity	3	3	4	3	1	1	**3**
Drought resistance	5	6	3	4	4	4	**4**
Good colour	4	4	5	6	5	5	**5**
Taste	6	5	8	5	6	7	**6**
Good for firewood	9	7	6	7	7	6	**7**
Good for livestock	8	8	7	8	8	9	**8**
Short cooking time	7	1	10	9	9	8	**9**
Easy for shelling	10	9	9	10	10	10	**10**
Correlation	**0.903**		**0.89**		**0.975**		

Key: low score= most important, high score= least important

2.3.6 Farmers evaluation of pigeonpea varieties grown in study areas

The focus groups listed the most common pigeonpea varieties in each district and their preferred characteristics in a matrix score (Table 2.18). The most preferred characteristics for Bangili, No. 40 and Kiboko were white colour of grains, indeterminate growth habit, high yield potential and good resistance to insect, diseases and drought (Table 2.18). The same characteristics were important for Babati White, except for disease resistance. Babati white is known to be susceptible to *Fusarium* wilt. Other characteristics important for Babati White were the short cooking time and the good taste. For the varieties Tumia and Komboa, the important characteristics were earliness, a good colour of grains and a high yield potential (Table 2.18).

Table 2.18 Matrix score of preferred trait of the most common varieties

	District							
	Babati			Karatu		Kilosa		
Pigeonpea traits	**Bangili**	**N0. 40**	**Babati White**	**Mali**	**Kiboko**	**Mali**	**Tumia**	**Komboa**
Early maturing	4	4	4	4	4	2	1	1
Low input use	4	4	4	3	3	3	4	4
Determinate growth habit	1	1	2	1	1	2	3	4
Good disease resistance	2	3	4	1	1	1	4	4
Good insect resistance	2	2	3	3	3	2	3	3
Good colour	1	2	1	2	2	1	2	2
High yield potential	1	1	1	1	1	1	1	1
Drought tolerant	3	3	3	3	2	4	4	4
Compatibility in intercropping	4	4	2	4	3	4	4	4
Short cooking time	4	4	2	3	4	4	4	4
Good Taste	3	3	2	3	4	4	4	4

Key 1: most important: 4: least important

2.4 Discussion

2.4.1 Crops and cropping system

The findings from this study indicate that land allocated to pigeonpea production was relatively small. The main crops grown by farmers in all three districts are maize and pigeonpea. In addition, farmers plant dry beans, hyacinth bean, sunflower, simsim, cowpea, tomatoes, millet, barley and wheat. Maize and dry beans are primarily grown as subsistence food crops, but in some high-potential areas in the highlands, they are planted both as a cash and subsistence food crops. Pigeonpea is mainly grown as a cash crop and surplus is used for food.

Maize intercropped with pigeonpea (70.8%) is the most common cropping system practiced in the surveyed areas. These results are in agreement with Rao and Mathuva (2000), Snapp *et al*. (2002) and Ghosh *et al*. (2006). Farmers are aware of the positive contribution of pigeonpea

to soil fertility enhancement, fodder and firewood availability, medicinal use, the crop's ability to break hardpan, and its positive impact on moisture retention and weed control (Snapp, 1998; Myaka *et al*. 2006; Richard and Marietha 2007). Yield of maize intercropped with pigeonpea in semi-arid conditions is often similar to or less than that of sole-cropped maize (Rao and Mathuva, 2000; Snapp *et al*., 2002; Myaka *et al*., 2006), indicating yield decrease due to competition for soil nutrients. Dass and Sudhishiri (2010) reported that the addition of considerable organic matter to the soil, through nitrogen fixation, improves microbial biomass, enriched the soil and improves the soil properties.

It was observed that farmers like to intercrop medium and long duration genotypes with maize because it will result in less competition between crops. Sogbedji *et al*. (2006), reported a maize crop yield increase of 32%, when maize was planted in association with pigeonpea. According to Ali (1988), pigeonpea based intercropping with other legumes helps to suppress weed by 30-40%, compared with 22% by sorghum and pigeonpea. In all surveyed areas farmers prefer to grow indeterminate varieties (bush and spreading) type rather than the determinate (compact) varieties because of intercropping compatibility.

2.4.2 Pigeonpea production constraints

Diseases, especially *Fusarium* wilt caused by soil-borne fungus (*Fusarium udum* Butler), was considered a main threat to pigeonpea cultivation. These results are in agreement with the earlier reports by Soko (1992), Subrahmanyam *et al*. (1992), Changaya-Banda (1997), Hillocks *et al*. (2000), Gwata *et al*. (2006) and Changaya (2007). The increase in disease severity on most farmers fields is due to the continued use of genotypes that are susceptible to *Fusarium* wilt. However, according to the farmers, the implementation of *Fusarium* wilt the screening programme initiated by ICRISAT in collaboration with ARI-Selian in early 2000, has resulted in a reduced incidence compared in the late 1980s.

Farmers identified insects, especially pod borer (*Helicoverpa armigera* Hubner) and pod sucker (*Clavigralla. tomentosicollis*), as the second major challenge to pigeonpea production. Pod borer is a serious pest of pigeonpea because of its extensive host range and the severe damage it can cause. The two pests have an impact on the quality and marketability of the grain. Farmers expressed their needs for insect resistant varieties, which will enable them to reduce production. Storage pests were considered the minor constraints, as farmers don't store the pigeonpea for long periods, as they dispose of the grain shortly after harvest.

Other production constraints mentioned by farmers were drought and lack of improved seeds which contribute to low productivity. Farmers perceived drought as constraint in years where rainfall was not reliable, but it was considered a minor limiting factors. Most farmers rely on recycled seeds for their production— saving the best pigeonpea grains from the harvest to use as seed in the next planting period. A large gap exists between the production potential of improved pigeonpea varieties in research environment (1200-2500 kg/ha) and the yield obtained at farm level (500-1200 kg/ha). A study by Mergeai *et al*. (2001) in pigeonpea found that the low yields obtained were a result of the use of unimproved, long duration varieties, susceptible to diseases and insects and to the poor production practices.

2.4.3 Management options used to control *Fusarium* wilt and other constraints

Crop rotation, uprooting and burning diseased plants are the important control options for *Fusarium* wilt practiced by farmers. A large percentage of farmers take any control measures after fields became infested with *Fusarium* wilt. Farmers in Karatu and Kilosa districts perceived that use of resistant varieties such as Mali is a cheapest and affordable control measure. In Babati districts, farmers still use local landraces due to the unavailability of improved varieties.

For insects pod borer and pod sucker the main control method is through application of pesticides. Despite the high price of chemicals, poor supply of insecticides, lack of sprayers, and the ignorance of control measures, pesticides are applied by the majority of farmers. Farmers usually spray insecticides three to four times per season. The seed carried over to the next growing season, is treated with ashes, actellic dust, smoke and plant extract to control the bruchids. Minja *et al*. (1999) observed traditional methods in Malawi for the control of storage pests, such mixing of wood ashes and grains.

2.4.4 Pigeonpea varieties and source of seeds

The study revealed that both traditional varieties and improved varieties are grown by farmers. Farmers in Babati district only grow traditional varieties, while farmers in the other two districts grow both improved and traditional varieties. According to focus group discussions, the reasons why farmers are still growing traditional varieties are the lack of seed of improved varieties, lack of information about availability of improved varieties and preferred traits that are lacking in the improved varieties. According to Shiferaw *et al*. (2007), productivity of pigeonpea will increase with improved seed production systems, and supply, distribution and marketing channels.

Interviewed farmers showed a strong preference for early maturing pigeonpea varieties, which takes 3 to 5 months to reach maturity. Komboa is a short duration variety, which matures between 70-90 days, but is not preferred due to susceptibility to *Fusarium* wilt disease. In high altitude areas of northern Tanzania, Komboa was not popular, due to the long duration of the rainy season. Long duration genotypes are preferred in high altitude areas in northern Tanzania, while the medium and short duration genotypes are preferred in low altitude areas in eastern Tanzania.

The structured surveys revealed that Mali was the most preferred varieties in all three districts. The second most preferred variety was Bangili, followed by Komboa and Tumia. Shiferaw *et al*. (2007) reported the adoption of Mali in northern districts of Tanzania is due to its high yield potential and resistant to *Fusarium* wilt disease. Several new varieties have been introduced and tested by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in collaboration with the Agricultural Research Institute-Selian farmers conditions, but were not preferred by farmers because they failed to match with their preference.

A majority of farmers use farm saved seed (70.8%), while others purchase seed at the market (11.7%). These results are consistent with a study by Cromwell *et al*. (1992) who reported that farmers' primarily plant farm saved pigeonpea seed and occasionnally purchase grain from local market. The seed supply system for pigeonpea is not well developed in Tanzania. Tripp (2000) reported the absence of an organized commercial market where farmers can access improved pigeonpea varieties. In all six villages the focus groups mentioned the lack of improved varieties as a challenge to pigeonpea production.

Interviewed farmers perceived that the use of poor quality seed may contribute to low grain yield. The farm saved seed can easily be contaminated with fungal diseases and therefore compromises vigour and germination. Mine (2012) reported that in northern Tanzania, only 28% of sample fields were sown with improved varieties. These results are in agreement with Shiferaw *et al*. (2007) who reported that 25–34% of farmers in Tanzania were growing improved pigeonpea on 32% of the area. Therefore, government should encourage small scale enterprises to provide farmers with improved seeds.

2.4.5 Farmers preference for pigeonpea varieties

Farmers perceived earliness, high yield potential, disease resistance, insect resistance, drought resistance, large white/cream seed and indeterminate (bush) growth habit as the most important preferred traits. These preferences were identified during both the focus group

discussion and the structured survey. In all three districts farmers grow medium and long duration genotypes. According to interviewed farmers, improved varieties such as Komboa, are not preferred, as their seeds are small and have a poor cookability, which make them more difficult to market.

White large seed is considered an important trait in the international market standard. The indeterminate growth habit is preferred because it offers multiple benefits, such as high yield, big stems for firewood and extended period of harvesting. A study from Malawi reported that farmers prefer large, white and round seed is positively associated with high market prices (Changaya, 2007). Farmers showed a preference for varieties with a short cooking time, as they require less fuel. This is in agreement with other studies by Yeung *et al*. (2009).

Conclusions

The study identified pigeonpea production constraints and farmers preferred traits. The information obtained from this study will assist breeders to use farmers' preference as selection criteria in future cultivar development. The use of participatory rural appraisal and structured survey gave insights and understandings to explore the preferences of the end users for the pigeonpea improvement programmes in Tanzania.

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Appendices

Appendix 2.1 Semi-structure questionnaire used during the survey

Questionnaire serial number……..

1. Background information

(a) Name of farmer……(b) Name of the village…….

(c) Ward…….. (d) District…… (e) Region……. (f) Date……..

2. What is the total land area you own?

a) 0.5 - 1.5 acres ( )

b) 2.0 - 3.0 acres ( )

c) 3.5 - 5.0 acres ( )

d) Above 5.0 acres ( )

3. What type of crops did you grow in the last three consecutive seasons?

Season	crop	acreage
a) 2012/2013	----------	----------
b) 2011/2012	----------	----------
c) 2010/2011	----------	----------

4. Land use and cropping intensity

i. Gross cropped area : _____ ha
ii. Net Area sown : _____ ha
iii. Fallow lands : _____ ha
iv. Cultivable waste lands : _____ ha
v. Forest cover : _____ ha
vi. Barren lands : _____ ha
vii. Cropping intensity : _____ ha

5. Of the crops you are cultivating, which are for food and which are for sale?

a) Food crops? ----------

b) Cash crops? ----------

6. What is the most important crop in your area?

7. Do you grow pigeonpea? a) Yes b) No

8. What is the source of pigeonpea seeds used for planting?

(a) Purchase from shop

(b) Farmer own saved seeds

(c) Given by NGOs

(d) None of the above

9. What varieties of pigeonpea do you grow? a) ..... b)…… c)……..

10. Are these improved or landraces?

11. What varieties of pigeonpea do you prefer the most?

12. What is your varieties preferred traits?

13. What seed colour do you prefer?

14. What are the major problems that threaten pigeonpea cultivation?

a) Pests and Diseases

b) Poor soils

c) Drought

d) Unimproved seeds

e) None of the above

15. Is a *Fusarium* wilt disease a major problem in your area?

16. If yes, what is the extent of the problem or how widespread is it?

17. Is there any knowledge of the symptoms of *Fusarium* wilt disease?

18. If yes, what is the plant stage when *Fusarium* wilt symptoms start showing?

19. What do you think are the causes of *Fusarium* wilt disease? list

20. What management practices do you use to control *Fusarium* wilt disease?

21. Have there been any awareness campaigns on *Fusarium* disease?

22. How do you compare *Fusarium* wilt disease with other constraints?

23. How is the grain yield in the absence of *Fusarium* wilt disease?

24. How is the grain yield in presence of Fusarium wilt disease?

25. Which cropping system do you use?

(a) Intercropping ( )

(b) Mixed cropping ( )

(c) Monoculture ( )

(d) Crop rotation ( )

(e) Shifting cultivation ( )

(f) Others (specify) ( )

26. If answer is (a) which crop is intercropped with pigeonpea?

(a) Maize

(b) Sorghum

(c) Beans

(d) None of the above

27. What do you think are the advantages and disadvantages of the cropping system that you are using?

Advantages	Disadvantages
----------	----------
----------	----------
----------	----------

28. Where do you sell your product?

(a) At the local market

(b) Nearby villages

(c) Transport to big towns.

Thanks very much for your cooperation.

Chapter 3 : Genetic variation of Tanzanian pigeonpea germplasm for *Fusarium* wilt resistance, grain yield and yield components

Abstract

Fusarium wilt (*Fusarium udum* Butler) is the major disease of pigeonpea (*Cajanus cajan* (L.) Millsp.) in Tanzania. The aim of this study was to identify parental sources of resistance against *Fusarium* wilt under field and controlled conditions, and to study grain yield and yield components. Thirty two pigeonpea genotypes, obtained from various sources, were evaluated for two seasons, 2013/2014 and 2014/2015, at Hombolo and Ilonga sites. A pot experiment was conducted under controlled conditions at the Chinese Agricultural Demonstration Centre. All genotypes were grown in an 8 x 4 row-column design with two replications in the field and a completely randomized design with three replications under controlled conditions. Genotypes ICEAP 00040, ICEAP 00932, ICEAP 00557, ICEAP 00554 and ICEAP 00053 were selected as the best parents for *Fusarium* wilt resistance. The study revealed highly significant differences ($P<0.001$) for most of the traits studied. The long duration genotypes showed a better performance in terms of yield and yield components. The results indicated that there is sufficient genetic diversity in pigeonpea germplasm that could be used as a base for the improvement of yield, *Fusarium* wilt resistance and other important attributes of this crop through direct selection or hybridization.

3.1 Introduction

Pigeonpea (*Cajanus cajan* [L.] Millsp) is an important crop that is grown in a wide range of cropping systems and environments (Nene and Sheila, 1990). The crop provides better economic returns than beans and cowpea in Tanzania, although it ranks third in importance (Mligo and Myaka, 1994; Joshi *et al*., 2001). Pigeonpea is grown in the tropics and sub-tropics, but primarily in Southern Asia and Eastern Africa (Yadav *et al*., 1997). India accounts for about 70% of the world production and 74% of the total area planted with pigeonpea (Bohra *et al.* 2012).

Pigeonpea can be intercropped with maize without a negative effect on the yield of maize (Egbe and Adeyamo, 2006). The crop is important for low-resource households in drought prone areas (Choudhary and Nadarajan, 2011). Apart from human consumption, it offers multipurpose usages such as feed and fodder for livestock, fencing materials, firewood, and material for roofing and basket weaving. The seed protein is also of excellent quality, being high in lysine. The crop is an important complement to cereal and root and tuber crops based diets (Van Der Maesen, 1990).

Several authors have reported on the cause of low productivity in pigeonpea. Poor production practices, inappropriate use of fungicides and herbicides, and the use of traditional varieties, are among of the factors contributing to low yields (Jones *et al*., 2001). Other factors are field insect pests, lack of seed of improved genotypes and storage pests. *Fusarium* wilt, caused by *Fusarium udum* Butler, is a major disease of pigeonpea in Africa. The extent of the disease is supported by several surveys, which reported on the incidence in Kenya (15.9%), Malawi (36.3%) and Tanzania (20.4%) (Mbwaga, 1995). According to Gwata *et al*. (2006), the *Fusarium* wilt incidence levels are increasing over time. It is estimated that annual losses caused by *Fusarium* wilt in these countries is over US$ 5 million (Kannaiyan *et al*., 1984).

In contrast to cereals and other crops, pigeonpea in Tanzania is ranked low in research priority. There has been no concerted research effort to promote the crop, despite it being of importance in subsistence agriculture. The local evaluation of pigeonpea for resistance to *Fusarium* wilt and with other important agronomic trait, is an essential prerequisite in the hybridization-based crop improvement program. In the present study, the genetic variation for *Fusarium* wilt resistance and other important agronomic traits was studied with the following objectives: (i) to identify pigeonpea genotypes with exploitable levels of *Fusarium* wilt resistance (ii) to identify pigeonpea genotypes with superior key agronomic traits.

3.2 Materials and methods

3.2.1 Plant material

Plant material used in this study was collected from the National Plant Genetic Resources Centre (NPGRC-Arusha), ARI-Selian, ARI-Ilonga and farmers from Hombolo, Kiteto and Babati. Twenty five traditional pigeonpea genotypes and seven improved genotypes were included in the study. The details of the genotypes are summarized in Table 3.1.

Table 3.1 Pigeonpea germplasm

Genotypes	Wilt reaction	Source	Maturity Group	Growth habit
ICEAP 00040	Resistant	ARI-Selian	Long	Semi-indeterminate
ICEAP 00053	Resistant	ARI-Selian	Long	Determinate
TUMIA	Susceptible	ARI-Ilonga	Medium	Semi- indeterminate
KOMBOA	Susceptible	ARI-Ilonga	Short	Semi-inderminate
ICEAP 00932	Resistant	ARI-Selian	Long	Semi-inderminate
ICEAP 00557	Resistant	ARI-Selian	Medium	Semi-inderminate
ICEAP 00554	Resistant	ARI-Selian	Medium	Semi-inderminate
TZA 2466	Unknown	NPGRC	Medium	Semi-inderminate
TZA 5463	Unknown	NPGRC	Medium	Semi-inderminate
TZA 2514	Unknown	NPGRC	Medium	Semi-inderminate
TZA 5596	Unknown	NPGRC	Medium	Semi-inderminate
TZA 197	Unknown	NPGRC	Medium	Semi-inderminate
TZA 253	Unknown	NPGRC	Medium	Semi-inderminate
TZA 250	Unknown	NPGRC	Medium	Semi-inderminate
TZA 2692	Unknown	NPGRC	Medium	Semi-inderminate
TZA 5464	Unknown	NPGRC	Medium	Semi-inderminate
TZA 2496	Unknown	NPGRC	Medium	Semi-inderminate
TZA 2439	Unknown	NPGRC	Medium	Semi-inderminate
TZA 5555	Unknown	NPGRC	Medium	Semi-inderminate
TZA 2807	Unknown	NPGRC	Medium	Semi-inderminate
TZA 2456	Unknown	NPGRC	Medium	Semi-inderminate
TZA 5541	Unknown	NPGRC	Medium	Semi-inderminate
TZA 2785	Unknown	NPGRC	Medium	Semi-inderminate
TZA 2509	Unknown	NPGRC	Medium	Semi-inderminate
TZA 2464	Unknown	NPGRC	Medium	Semi-inderminate
TZA 5557	Unknown	NPGRC	Medium	Semi-inderminate
TZA 5582	Unknown	NPGRC	Medium	Semi-inderminate
No. 40	Unknown	Babati	Long	Semi-inderminate
Hombolo	Unknown	Hombolo	Medium	Semi-inderminate
Kiteto	Unknown	Kiteto	Medium	Semi-inderminate
Bangili	Unknown	Babati	Long	Semi-inderminate
Babati White	Susceptible	Babati	Long	Semi-inderminate

3.2.2 Description of study sites

Field screening was conducted in two growing seasons (2013/2014 and 2014/2015) at two sites, namely Hombolo and Ilonga stations. Ilonga station is located at 37º 2´ ´E 6º 42´ ´S with a temperature range between 20.0 and 36.7ºC at an altitude of 506 m above seas level (masl) and Hombolo station which is located at 35º 59´´E 05º 52´´S with a temperature range between 20 and 32ºC at an altitude less than 760 masl. The rainfall data for Hombolo and Ilonga station are presented in Figure 3.1. Pot experiment was done at the Chinese Demonstration Centre using the inoculation and transplanting method. The Centre is located at Dakawa at 37º 40´´ E 6º 5´ ´S with a temperature range between 20 and 35ºC at an altitude of 360 masl.

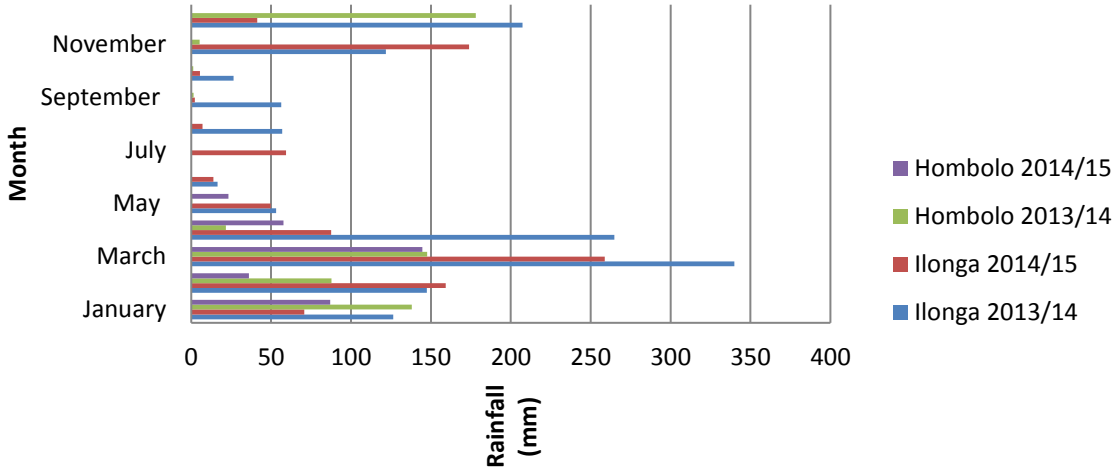

Figure 3.1 Rainfall data for Hombolo and Ilonga in the 2013/2014 and 2014/2015 seasons

3.2.3 Field experiment

The selected genotypes were planted during March at Ilonga and January at Hombolo. The 25 traditional pigeonpea genotypes and seven improved genotypes were planted in four row plots row plots, 3 m length, with an inter-row and intra-row spacing of 1 m and 0.5 m, respectively. The middle two rows were evaluated. The experimental design was an 8 x 4 row-column design with two replications. Field screening for *Fusarium* wilt at Ilonga station was done using a sick plot (see chapter 1.12), while in Hombolo the screening was done on a field previous planted with pigeonpea. The soil of Ilonga is characterised as loamy soils. The soil of Hombolo site is characterised as reddish-brown sandy clay soil, classified as Ustic Torriorthents (Budotela, 1995). A threshold level of *Fusarium udum* was maintained by incorporating chopped wilted pigeonpea plants annually at both sites in order to increase disease pressure. The crops were

raised following a recommended package of practices, and observations on the levels of the *Fusarium* wilt damage were recorded after harvest, following standard procedures. No fertilizer was applied to adopt farmers practice

3.2.4 Controlled experiment

The selected genotypes were planted in a pot experiment in June 2015. All the test genotypes were grown in 12 l plastic pots in a complete randomized design with three replications. A 1-9 disease scale was used, where 1= no visible symptoms and 9= very severely diseased or dead. In this experiment, no yield data was taken since this experiment was primarily conducted to verify *Fusarium* wilt results obtained from field.

3.2.5 Isolation procedure and inoculation of pathogen

The causal organism of pigeonpea wilt was isolated on potato dextrose agar (PDA) from wilted plants at the African Seed Health Centre laboratory, SUA-Morogoro. The infected tissues were surface sterilized using 3% hypochlorite, rinsed in sterile distilled water three times and subsequently placed on the potato dextrose agar (PDA). The tissues were incubated at 24^{o}C for 48 hours for sporulation. The fungal colony/conidia, which had a pink colour at the back of the petri dishes, were then sub-cultured on fresh PDA for purification left for seven days, after which the spore suspension (500 ml) was spread on the sterile sorghum grains in a flask and incubated for 14 days to obtain enough sporulation.

One week old seedlings were inoculated by pruning the tip of root systems for about 3 mm, after uprooting the seedlings from the sterilized soil and thorough washing of the root system with running tap water. The pruned seedlings were briefly dipped into the sorghum seeds containing *Fusarium udum* conidia and were subsequently transplanted, together with a few sorghum seeds, into plastic pots filled with sterilized soil. Each genotype was replicated three times with one pot containing three plants representing one replication.

3.2.6 Data collection for field trial and analysis

The *Fusarium* wilt scoring scale, described by Nene and Kannaiyan (1982) for the wilt incidence, was used where:

0 – 20% plant mortality= resistant,
21 – 40% plant mortality= moderately resistant,
41 – 60% plant mortality= susceptible,
61 – 80% plant mortality = moderately susceptible, and

81 – 100% plant mortality= highly susceptible,

Grain yield was measured on the basis of plot harvest adjusted to a 15.5% moisture content. Agronomic data recorded were number of pods per plant, days to flowering and maturity, recorded when 50% of plants in each plot had flowers and dry brown pods, respectively. Other data recorded included plant height, 100-grain weight (determined from 100 clean seeds on a plot basis), number of primary and secondary branches.

Table 3.2 Analysis of variance for genotypes tested over the years and locations

Source of variation	df	MS	EMS
Replication within location and year	ly(r-1)	M1	$\sigma^2_e + g\sigma^2_r$
Year	y-1	M2	$\sigma^2_e + r\sigma^2_{gly} + rg\sigma^2_{gy} + rl\sigma^2_{gly} + rlg\sigma^2_y$
Location	l-1	M3	$\sigma^2_e +$
Genotype	g-1	M4	$\sigma^2_e + r\sigma^2_{gly} + ry\sigma^2_{gl} + rl\sigma^2_{gy} + rly\sigma^2_g$
Year x location	(y-1) (l-1)	M5	$\sigma^2_e + r\sigma^2_{gly} + rg\sigma^2_{ly}$
Genotype x year	(g-1) (y-1)	M6	$\sigma^2_e + r\sigma^2_{gly} + rl\sigma^2_{gy}$
Genotype x location	(g-1) (l-1)	M7	$\sigma^2_e + r\sigma^2_{gly} + ry\sigma^2_{gl}$
Genotype x location x year	(g-1) (l-1) (y-1)	M8	$\sigma^2_e + r\sigma^2_{gly}$
Residual	ly(g-1) (r-1)	M9	σ^2_e

Key: df=degree of freedom; MS=mean square; EMS=error mean square; l=location; r=replication; y=year; g=genotype; σ^2_e=environmental variance; σ^2_g=genotypic variance; σ^2_{gly}=variance due to genotype x location x year; σ^2_{gl}=variance due to genotype x location; σ^2_{gy}=variance due to genotype x year

Data for individuals sites were also analysed separately to determine the significance of genotype effects and homogeneity of error variances (Steel and Torrie, 1980), using Genstat 14th edition (Payne *et al*., 2011). Combined analysis for field experiments were performed as suggested by Gomez and Gomez (1984). Least significant differences were used for the mean separation. Principal components analysis and cluster analysis were performed using Genstat 14th edition.

3.3 Results

3.3.1 Evaluation for reaction to *Fusarium* wilt under field and controlled conditions

At Hombolo there was not enough inoculum to develop enough wilt disease symptoms in both seasons, and the genotypes were therefore solely evaluated for yield and other agronomic traits. All genotypes at Hombolo were grown in a field which was previously planted with groundnut and sorghum. The analysis of variance showed that the genotypes were significantly different for *Fusarium* wilt incidence at Ilonga ($P<0.05$) in the 2014/2015 season (Table 3.3). The combined analysis over seasons revealed that the genotypes showed significant differences at Ilonga ($P<0.05$) (Table 3.3). However, the genotypes were not significantly different for *Fusarium* wilt in 2013/2014 season (Table 3.2). Among the 32 genotypes screened under field conditions, TZA 2785 had a very low incidence of *Fusarium* wilt followed by TZA 2439, Hombolo, TZA 5463, TZA 2456, TZA 5541, Tumia, TZA 2807 and TZA 250 (Table 3.3). All the above genotypes had wilt incidence below 20 %. Genotypes with a zero incidence score under field conditions in both seasons were ICEAP 00040, ICEAP 00932, Bangili, No. 40, TZA 5464, TZA 2692, TZA 253, TZA 5582, ICEAP 00053, ICEAP 00557 and ICEAP 00554.

Other genotypes such as TZA 197, TZA 2514, Babati white, TZA 2464, TZA 2496, TZA 5557, Komboa, Kiteto and TZA 2466 had moderate *Fusarium* wilt incidence ranging from 20% to 50% (Table 3.2). In the 2013/2014, the genotypes showed a high incidences include Kiteto, Komboa, TZA 197 and TZA 2807 (Table 3.3). In 2014/2015 the highest level of *Fusarium* wilt incidence was recorded on genotype TZA 2466 (75%) (Table 3.3). The known susceptible genotypes, Babati White and Komboa, showed disease symptoms in both seasons.

Under controlled conditions, the genotypes with zero incidence were the improved genotypes known to be resistant to *Fusarium* wilt, namely ICEAP 00040, ICEAP 00932, ICEAP 00053, ICEAP 00557 and ICEAP 00554 (Table 3.3).

Table 3.3 *Fusarium* wilt incidence recorded in pigeonpea genotypes under field and greenhouse conditions

	Field experiment			Pot experiment	
	2013/2014	2014/2015		2014/2015	
Genotypes	**Incidence (%)**	**Incidence (%)**	**Combined Analysis**	**Score**	**FWR**
ICEAP 00040	0.00	0.00	0.00	1	R
ICEAP 00932	0.00	0.00	0.00	1	R
TZA 5463	0.00	12.50	6.25	9	S
TZA 2514	8.00	50.00	29.00	9	S
TZA 2439	0.00	6.25	3.13	9	S
TZA 197	37.45	9.37	23.40	9	S
Komboa	41.60	50.00	45.80	9	S
Tumia	8.30	25.00	16.70	9	S
TZA 2456	0.00	25.00	12.50	9	S
Kiteto	54.20	43.75	48.90	9	S
Hombolo	8.30	12.50	10.40	9	S
Bangili	0.00	0.00	6.50	9	S
Babati White	25.00	34.37	28.80	9	S
No. 40	0.00	0.00	0.00	9	S
TZA 2466	25.00	75.00	50.00	9	S
TZA 2464	0.00	62.50	31.25	9	S
TZA 2807	33.30	0.00	16.70	9	S
TZA 2785	8.00	0.00	4.00	9	S
TZA 5464	0.00	0.00	0.00	9	S
TZA 5555	16.70	62.50	39.6	9	S
TZA 5557	25.00	62.50	43.80	9	S
TZA 5582	0.00	0.00	0.00	9	S
TZA 5596	25.00	12.50	18.80	9	S
TZA 2496	0.00	62.50	31.25	9	S
TZA 2692	0.00	0.00	0.00	9	S
TZA 253	0.00	0.00	0.00	9	S
TZA 250	33.30	0.00	16.70	9	S
TZA 2509	0.00	0.00	0.00	9	S
TZA 5541	16.70	12.50	14.70	9	S
ICEAP 00053	0.00	0.00	0.00	1	R
ICEAP 00554	0.00	0.00	0.00	1	R
ICEAP 00557	0.00	0.00	0.00	1	R
Mean	**26.90**	**35.50**	**31.80**		**-**
CV	**48.94**	**68.20**	**61.70**		
Lsd $_{(0.05)}$		**8.50**	**6.30**		
P value	**0.08 ns**	**0.05***	**0.0206***		

*, **, *** significant at 0.05, 0.01, 0.001; ns=non-significant

Pot experiment score: R=resistant; S=susceptible; 1=Resistant; 9=Susceptible; CV=coefficient of variation; LSD= least significance difference at 5%; Pr =probability level

3.3.2 Grain yield and yield components

Grain yield

The combined analysis of variance for grain yield revealed that there were highly significant differences between genotypes (P<0.001) (Table 3.4). Late duration genotypes namely ICEAP 00040, ICEAP 00932, ICEAP 00053 and Bangili produced mean grain yields above 2000 kg/ha (Table 3.4). Among the medium duration genotypes, Kiteto produced the lowest yields with a mean of 768.8 kg/ha, while ICEAP 00557 produced the highest yields with a mean of 1614 kg/ha. Komboa produced the lowest yields among all the tested genotypes with a mean of 738 kg/ha (Table 3.5).

Analysis of variance revealed that there were significant differences for grain yield at Hombolo (P<0.001) and Ilonga (P<0.01) in 2013/2014 (Appendix 3.1). In 2014/2015 the analysis of variance showed significant difference for grain yield at Hombolo (P<0.001) and Ilonga (P<0.05) (Appendix 3.1). In 2013/2014 yields in Hombolo site was adversely affected by a long periods of drought. In 2013/2014 at Ilonga the genotypes yielded above 2000 kg/ha include ICEAP 00040, ICEAP 00932, ICEAP 00053, Bangili, ICEAP 00557, ICEAP 00554 and TZA 253 (Appendix 3.1). In 2014/2015 the highest yielding genotypes at Ilonga were ICEAP 00040, ICEAP 00932, Bangili, ICEAP 00557, ICEAP 00053 and No. 40, all them yielded above 2000 kg/ha (Appendix 3.1).

Number of pods per plant

The combined analysis of variance for number of pods per revealed that there were highly significant differences for genotypes, sites, seasons and genotypes x season interaction (P<0.001) (Table 3.4). Genotypes x site interaction effects were significant differences (P<0.01) (Table 3.3). Bangili, ICEAP 00932, TZA 250, ICEAP 00040 produced the highest mean number of pods per plant, above 120 (Table 3.5).

The analysis of variance showed highly significant differences for the number of pods per plant at Hombolo (P<0.001) and Ilonga (P<0.01) both in 2013/2014 and 2014/2015 (Appendix 3.1).

Table 3.4 Combined analysis of variance for *Fusarium* wilt, grain yield and yield components

***Fusarium* wilt disease**			**Grain yield and yield components**				
Source of Variation	**DF**	***Fusarium***	**Source of variation**	**DF**	**Grain Yield**	**NPP**	**DT50%F**
Replication	1	11.32	Genotypes	31	1315056***	2611***	1959***
Genotypes	31	602.36*	Sites	1	19039895***	598516***	43142***
Season	1	724.52	Season	1	8004	21142***	11917***
Genotypes x Season	31	235.87	Genotypes x Site	31	333989***	1915**	371***
Residuals	121	305.845	Genotype x Season	31	53024	2093***	31***
			Site x Season	1	65728	450	9218***
			Site x Replicates	2	5822	72	5
			Genotypes x Site x Season	31	55503	2127***	33***
			Residuals	121	105130	898	45

Table 3.4 Continued

Source of Variation	**DF**	**DTM**	**PH**	**100-GW**	**NPB**	**NSB**
Genotypes	31	1996***	5931***	52.81***	130***	2529***
Site	1	25306***	123336***	1.06	14918***	292057***
Season	1	3475***	6400***	166.43***	414***	27960***
Genotype x Site	31	428***	974***	10.39***	47***	1729***
Genotype x Season	31	31***	759***	11.40***	35***	1560***
Site x Season	1	2231***	2751***	0.34	343***	25974***
Site x Replicates	2	3	2028***	3.32	29	1880***
Genotypes x Site x Season	31	29***	882***	3.016***	33**	1520***
Residuals	121	3	386	2.98	18	233

*, **, ***, significant at 0.05, 0.01, 0.001

Key**:** DF=degree of freedom; NPP-number of pods/plant; DT50%=days to 50% flowering; DTM=days to maturity; PH=plant height; 100-GW=100 grain weight; NPB-number of primary branches; NSB-number of secondary branches

Table 3.5 Combined means of 32 pigeonpea genotypes for grain yield and yield components

		Yield components						
Genotype	**Grain Yield**	**NPP**	**D50%F**	**DTM**	**PH**	**100-GW**	**NPB**	**NSB**
ICEAP 00040	2345.4	131.6	141.7	177	193.2	20.7	20.9	53.3
ICEAP 00932	2365.6	135	141.9	175.9	204.2	20	22.7	48.9
Bangili	2170.7	157.6	147.6	184.7	193.4	20.4	19.7	65
ICEAP 00053	2123.6	119.3	146	180.4	204.6	17.7	24.1	104.6
No. 40	1962.0	116.5	139	172.7	205	17.2	21.1	88
ICEAP 00557	1682.5	87.4	108.1	141.2	153.2	16	10.9	52
Babati White	1614.1	134	142.7	175.9	211.5	21.2	21.2	55.4
ICEAP 00554	1614.1	120.4	109.1	144.2	151.6	18.1	17.1	39.6
TZA 253	1572.4	71.7	106.6	140.4	150.1	16.1	12.6	32.6
TZA 5464	1489.3	98.5	102.7	135.5	160.4	14.7	14.5	43
TZA 2514	1455.4	73.6	106.7	139.7	138.8	13.2	11	64.1
TZA 2807	1453.6	67.8	103.7	136.5	144.2	16.2	11.7	40
TZA 2509	1374.1	71.7	107.6	140.9	122.1	14.8	14.6	51.4
TZA 2439	1370.6	96.9	103.6	136.2	116.8	17.5	14.7	35.4
TZA 2464	1343.9	82.1	104.2	137.4	126.2	13.2	12.4	22.2
TZA 5463	1309.9	99.1	107.9	141	142.1	15.1	11.9	38.9
TZA 2692	1307.6	92.4	100.5	137.7	146.7	13	10.4	24.6
TZA 5596	1302.5	106	105.9	138.7	155.4	14.2	14.6	45.5
TZA 5541	1284.9	92.1	103.7	136.5	140.1	13.5	10.7	37.7
TZA 5582	1249.4	98.7	103.1	136	142.1	15.2	13.7	42.5
TZA 250	1246.8	132.2	105	138	154	13.1	10.4	33.6
TZA 2785	1213.5	79.9	105.1	137.5	158.2	12.5	15.1	28.2
Tumia	1189.4	85.5	106.7	141.7	145.6	16.9	13.6	27.6
TZA 5557	1143.3	59.4	106.1	139.1	144.1	12.2	10.7	46.2
TZA 2496	1126.3	77.2	105	137.7	141.2	15.4	9.9	40
TZA 2456	1088.9	96.9	104.9	137.6	151.4	12.5	12.9	35.4
TZA 197	1055.4	79.2	105.7	141.1	127.9	16.2	16.1	43.6

	Yield components							
Genotype	**Grain Yield**	**NPP**	**D50%F**	**DTM**	**PH**	**100-GW**	**NPB**	**NSB**
Hombolo	1025.4	109	108.2	141.7	144.6	15.6	14.9	67.7
TZA 2466	1006.0	71.5	106.1	138.9	147.2	15	8	23
TZA 5555	944.6	110.2	103.9	136.7	133.5	12.2	13.6	38.8
Kiteto	768.8	98.7	107.4	140.7	153.2	14.6	15.4	47.8
Komboa	739.7	65.1	78.3	117.4	88.8	11.3	9.7	17.4
Mean	**1397.8**	**97.9**	**111.5**	**145.2**	**152.9**	**15.5**	**14.6**	**44.6**
CV	**25.7**	**67.8**	**17.0**	**10.0**	**22.7**	**16.8**	**43.5**	**50.2**
Lsd	**30.9**	**2.9**	**1.9**	**5.6**	**3.9**	**3.2**	**4.3**	**5.9**
P value	**<0.001*** **	**<0.001*** **	**<0.001*** **	**<0.001*** **	**<0.001*** **	**<0.001*** **	**<0.001*** **	**<0.001*** **

Key: *** significant at 0.001

NPP=number of pod per plant; DT50%F=days to 50% flowering; DTM=days to maturity; PH=plant height; 100-GW=100 grain weight; NPB=number of primary branches; NSB=number of secondary branches

Days to 50% flowering

The combined analysis of variance for days to 50% flowering revealed that genotypes, sites, seasons and interaction between genotype x season were all highly significantly different (P<0.001) (Table 3.4). Among the tested genotypes Komboa (78d) was the earliest and Bangili (147d), a traditional long duration genotype, the latest to flower (Table 3.5). It was noted that during 2014/2015 there was little rain.

The analysis of variance revealed that days to 50% flowering was highly significantly different at Hombolo (P<0.001) and Ilonga (P<0.001) in 2013/2014 (Appendix 3.2). In 2014/2015 days to flowering was highly significantly different at Hombolo (P<0.001) and Ilonga (P<0.001) (Appendix 3.2). Flowering was significantly earlier in short season genotypes and medium genotypes, compared with long duration genotypes (Appendix 3.2).

Days to maturity

The combined analysis of variance for days to maturity showed highly significantly different (P<0.001) for genotypes, sites, season, interaction between genotype x site, genotype x season and genotype x site x season (Table 3.4). Komboa reached maturity in 117 days and was significantly different from long duration genotypes that matured in 185 days and 180 days for Bangili and ICEAP 00053, respectively (Table 3.5).

The analysis of variance revealed that days to maturity was highly significantly different at Hombolo (P<0.001) and Ilonga (P<0.001) in 2013/2014 (Appendix 3.2). At Hombolo, the earliest genotypes to reach maturity were Komboa, TZA 5464, TZA 2439 and TZA 5582. The late maturing genotypes were Bangili, ICEAP 00040, No. 40 and ICEAP 00053. At Ilonga, the earliest maturing genotypes were Komboa, TZA 2785, TZA 5555, TZA 2464 and TZA 250. In 2014/2015, at Hombolo, Komboa was the earliest genotype to reach maturity, in 118 days, while Bangili and Babati white were the last to reach maturity in 153 and 152 days, respectively. At Ilonga, Komboa was the earliest genotype to reach maturity and all long duration varieties reached maturity after 180 days (Appendix 3.2).

Plant height

The combined analysis of variance for plant height showed highly significant differences (P<0.001) for genotypes, sites, seasons, interaction between genotype x site and genotype x site x season (Table 3.4).The interaction between genotype x season were significantly different

(P<0.01) (Table 3.4). Plant height ranged from 88.75 cm to 204.57 cm for genotypes Komboa and ICEAP 00053, respectively (Table 3.5).

The analysis of variance showed highly significant differences in plant height at Hombolo (P<0.001) and Ilonga (P<0.001) in 2013/2014 (Appendix 3.3). At Hombolo, plant height ranged from 81 cm to 208 cm for the genotypes Komboa and ICEAP 00040 respectively. At Ilonga, plant height varied from 79 cm (Komboa) to 228.5 (ICEAP 228.5 cm) (Appendix 3.3). In 2014/2015, analysis of variance showed highly significant differences in plant height at Hombolo (P<0.001) and Ilonga (P<0.001) (Appendix 3.3). At Hombolo, plant height ranged from 77.0 cm to 212.5 for the genotype Komboa and ICEAP 00040, respectively. At Ilonga, plant height varied from 98 cm to 223 cm for the genotype TZA 2439 and Babati White, respectively (Appendix 3.3).

100-grain weight (gm)

The combined analysis of variance for 100-grain weight showed highly significant differences (P<0.001) for genotype, season, interaction between genotype x season and genotype x site x season (Table 3.4). On an individual basis, Bangili (21.2 gm) had the highest 100-grain weight and Komboa (11.3 gm) had the lowest weight (Table 3.5). All long duration genotypes, both improved and traditional, had higher 100-grain weight than the short duration genotypes. It was observed that most of the long duration genotypes have large creamy seeds, while the short duration genotypes have small seeds.

The analysis of variance showed significant differences for 100-grain weight at Hombolo (P<0.01) and Ilonga (P<0.001) in 2013/2014 (Appendix 3.3). Hundred grain weight varied from 12.0 gm to 21.5 gm for the genotypes Komboa and ICEAP 00040, respectively. At Ilonga, the 100-grain weight varied from 10.0 gm to 22.5 for the genotypes TZA 5596 and Bangili, respectively. In the 2014/2015 season the analysis of variance showed highly significant differences for 100 grain weight at Hombolo (P<0.001) and Ilonga (P<0.001) (Appendix 3.3). At Hombolo, the 100-grain weight varied from 10 gm to 21 gm for the genotypes TZA 5541 and Bangili, respectively. At Ilonga 100-grain weight varied from 10 gm to 21 gm for genotypes TZA 5596 and Babati White, respectively (Appendix 3.3).

Number of primary branches

The combined analysis of variance for the number of primary branches showed significant differences (P<0.001) for genotypes, sites, seasons, and interaction between genotype x site (Table 3.4). Interaction between genotype x season and genotypes x site x season were

significantly different (P<0.01) (Table 3.4). In the overall performance over the two seasons, ICEAP 00053 recorded the highest number of primary branches (24) and TZA 2466 the lowest (8) (Table 3.5).

The analysis of variance showed significant differences for the number of primary branches at Hombolo (P<0.001) and Ilonga (P<0.01) in 2013/2014 (Appendix 3.4). The highest numbers of primary branches at Hombolo were recorded for Bangili (21) and ICEAP 00040 (18). Genotypes TZA 5555, TZA 5464, TZA 2496 and TZA 2466 all have four primary branches (Appendix 3.4). At Ilonga, the highest number of primary branches was recorded for genotypes ICEAP 00053 (32) and ICEAP 00040 (29). The lowest number of primary branches was recorded for genotypes Komboa (15) and TZA 2785 (16) (Appendix 3.4). In 2014/2015, the number of primary branches were significantly different at Hombolo (P<0.001) and Ilonga (P<0.001) (Appendix 3.4). The number of primary branches recorded at Hombolo ranged from 4 to 17 for the genotypes TZA 5596 and Bangili, respectively. At Ilonga the highest number of primary branches was recorded for Hombolo (64) and the lowest for TZA 5557 (9) (Appendix 3.4).

Number of secondary branches

The combined analysis of variance for the number of secondary branches showed highly significant differences (P<0.001) for genotype, site, season, interaction between genotype x site, genotype x season, site x season and genotype x site x season (Table 3.4). Overall performance in terms of the number of secondary branches over the two season revealed that the lowest and highest numbers were recorded for genotypes Komboa (17) and ICEAP 00053 (104), respectively (Table 3.5)

The analysis of variance showed significant differences for the number of secondary branches at Hombolo (P<0.001) and Ilonga (P<0.01) in 2013/2014 (Appendix 3.4). At Hombolo, the highest number of secondary branches was recorded for genotype Bangili (32) and the lowest for TZA 5541 (3). At Ilonga, the highest number of secondary branches was recorded for genotype ICEAP 00053 (117) and the lowest for Komboa (17). The analysis of variance also revealed that there were highly significant differences for the number of secondary branches at Hombolo (P<0.001) and Ilonga (P<0.001) in 2014/2015 (Appendix 3.4). At Hombolo, genotype Bangili (36) had the highest number of secondary branches, while four genotypes, namely TZA 5582, TZA 2807, TZA 2785 and TZA 250, had only four secondary branches (Appendix 3.4). At Ilonga, the number of secondary branches ranged from 32 to 255 for genotype TZA 2466 and No. 40, respectively (Appendix 3.4).

3.4 Discussion

3.4.1 Reaction to *Fusarium wilt* under field and controlled conditions

Fusarium wilt is an economicaly important disease of pigeonpea worldwide. In the present study the incidence of *Fusarium* wilt disease under field conditons ranged from <10 to 60%. First symptoms of wilting started to appear during the flowering stage with partial wilting of the leaves and finally the whole plant dries out completely. In the 2014/2015 season wilting symptoms were observed at the seedling stage in genotype TZA 2466. Choudhary (2010) and Prasad *et al*. (2003) also reported disease at the seedling stage and Okikor (2002) reported that resistance may be expressed at the seedling, adult or maturity stages. The most susceptible genotypes evaluated across seasons were TZA 2466, Kiteto, Komboa, TZA 5557 and Babati White (Table 3.3).

Artificial disease inoculation was done to confirm the resistance to *Fusarium* wilt found in field trials, using the seed inoculation technique (Changaya, 2007). Among the 32 genotypes screened in field conditions, all genotypes were susceptible under screenhouse conditions, except for ICEAP 00557, ICEAP 00554, ICEAP 00932, ICEAP 00040 and ICEAP 00053 (Table 3.3). These genotypes can be used as donor parents in breeding for pigeonpea resistance to *Fusarium* wilt in Tanzania. Similarly, Gwata *et al*. (2006), Changaya (2007) and Sharma *et al*. (2012) have also reported genotype ICEAP 00040 as a good source of resistance in pigeonpea.

The information presented in this study contributes to efforts to develop *Fusarium* wilt resistant pigeonpea. There are several reports where successful identification of resistance sources for wilt diseases have been documented, namely Gwata *et al*. 2006, Changaya, 2007, Choudhary (2010), Choudhary and Nadarajan (2011), Singh *et al*. (2011), Sharma *et al*. (2014), Jaggal *et al*. (2014) and Pawar *et al*. (2015).

3.4.2 Field evaluation for important agronomic traits

Field evaluation under high disease pressure for important agronomic traits was conducted in order to identify pigeonpea genotypes with superior key agronomic traits among the selected genotypes. There was large variation for grain yield ranging from 902 kg/ha to 2344 kg/ha in Hombolo and 380 kg/ha to 2579 kg/ha in Ilonga (Appendix 3.1). The results also demonstrated that the influence of genotype X site X season interactions resulted in variations in genotype performance. The mean grain yield ranged from 739 kg/ha to 2365 kg/ha (Table 3.5). The variation in grain yield is due to vaiations in maturing group, growth habit, susceptibility to wilt disease and environmental conditions. The findings from this study are similar to those reported

by Atta *et al*. (2008). Most of long duration genotypes, such as ICEAP 00040 and ICEAP 00932, are resistant to *Fusarium* wilt, had semi-indeterminate growth habit and outyielded medium and short duration genotypes at both sites across the two seasons. The above genotypes produce flowers for a long period of time and had more branches (both primary and secondary) as compared to the medium (Tumia) and short duration genotypes (Komboa). This result is in contrast to that of Dasbak *et al*. (2012) and Sharma *et al*. (1981), who found medium maturing genotypes producing a higher grain yield than early and late flowering genotypes.

The two sites had very different moisture conditions, as Hombolo is very dry, while Ilonga very wet (Figure 3.1). At Ilonga all genotypes had higher grain yield than in Hombolo, despite the greater presence of *Fusarium* wilt at Ilonga. The grain filling stage is critical in most plants and is the stage when adequate water is required. This observation was also reported by Nam *et al*. (2001) and Lopez *et al*. (1996) who recorded lower grain yields with reduced amount of water application from flowering to harvest.

There was variation in number of pods per plant over the two sites. All genotypes at Hombolo had less than 100 pods per plant in 2013/2014, while in 2014/2015 only four genotypes had more than 100 pods per plant. The low mean number of pods per plant at Hombolo could be attributed to low moisture supply and high temperature. This is similar to findings by Turnbull (1986) who reported that at high constant temperatures flower abortion increases, leading to low pod set. Genotypes at Ilonga had more pods per plant in 2014/2015 than it was in 2013/2014. From other studies, drought occuring during the growth of legumes may result in a reduction of the number of pods per plant by up to 65% (Mwanamwenge *et al*., 1999).

Most of the traditionally grown pigeonpea genotypes represented in the medium and long duration maturity groups that mature in 150 to 210 days. The earliest flowering genotype Komboa reached maturity in 95 days and 120 days in Hombolo and Kilosa, respectively. Other studies (Upadhaya *et al*., 2007) classified maturity duration of Kenyan genotypes that flowered <130 days as being early and those that flowered in >160 days as late flowering. Plant height varied significantly at Hombolo and Ilonga sites. It was observed that all long duration genotypes were generally tall.

The number of branches, both primary and secondary, varied across all sites for the two seasons. Comparing the two sites, most genotypes at Ilonga produced a higher number of primary and secondary branches as compared to Hombolo. An increase in number of primary branches, secondary branches, number of pods per plant and plant height would result in increased seed yield per plant (Rekha *et al*., 2013). Santosh and Madrap (2007) reported that pirmary branches

and 100-gram weight had a direct positive effect on seed yield. Hence, simultaneous selection based on these characters could lead to improved yield.

Conclusions

The study revealed that reistance sources for *Fusarium* wilt disease exist in Tanzania pigeonpea germplasm. Genotypes ICEAP 00040, ICEAP 00554, ICEAP 00932, ICEAP 00053 and ICEAP 00557 were identified as a useful sources of *Fusarium* wilt resistance with adaptation to Tanzania growing conditions. These sources can be used in breeding programmes for the development of pigeonpea *Fusarium* wilt resistance cultivars, which can effectively help to reduce the incidence and yield loss arising from *Fusarium* wilt disease.

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Appendices

Appendix 3.1 Mean values of grain yield (kg/ha) and number of pods per plant at Hombolo and Ilonga evaluated in 2013/14 and 2014/2015

	Grain yield				Number of pods per plant			
	Hombolo		Ilonga		Hombolo		Ilonga	
Genotype	**2013/14**	**2014/15**	**2013/14**	**2014/15**	**2013/14**	**2014/15**	**2013/14**	**2014/15**
ICEAP 00040	2322.5	2225.0	2445.0	2579.0	58.5	138.5	172.0	177.5
ICEAP 00932	2344.5	2245.0	2420.0	2453.0	56.0	128.5	219.5	136.0
Bangili	2210.0	2142.5	2085.0	2265.0	61.0	132.5	201.0	187.5
ICEAP 00053	2015.0	2122.5	2167.5	2135.0	47.0	67.5	158.0	168.5
No. 40	1931.0	1937.5	1950.0	2029.0	47.0	128.0	164.0	124.0
ICEAP 00557	1295.0	1200.0	2042.5	2192.5	29.0	39.5	177.0	104.0
Babati White	2169.0	2127.5	1140.0	1020.0	55.5	104.5	187.5	188.5
ICEAP 00554	1310.0	1255.0	2000.0	1891.5	23.5	67.5	193.0	197.5
TZA 253	1433.5	1210.0	2000.0	1720.0	26.5	45.5	115.0	128.0
TZA 5464	1259.5	1220.0	1602.5	1875.0	30.0	43.5	116.0	204.5
TZA 2514	1339.0	1280.0	1820.0	1382.5	28.0	43.5	121.5	101.5
TZA 2807	1382.5	1387.5	1255.0	1789.5	29.0	61.0	110.0	71.5
TZA 2509	1261.5	1257.5	1232.5	1745.0	27.5	92.0	80.5	87.0
TZA 2439	1087.5	930.0	1810.0	1655.0	26.0	34.0	211.0	116.5
TZA 2464	1187.5	1175.0	1778.0	1235.0	24.5	48.0	161.0	95.0
TZA 5463	994.5	937.5	1842.5	1465.0	31.0	37.0	113.0	215.5
TZA 2692	955.5	1210.0	1540.0	1525.0	26.5	54.5	114.0	174.5
TZA 5596	1395.0	1302.5	1235.0	1277.5	27.5	93.5	108.5	194.5
TZA 5541	1174.5	1115	1410.0	1440.0	28.5	74.0	107.5	158.5
TZA 5582	1045.0	922.5	1250.0	1780.0	26.0	66.5	130.0	172.5
TZA 250	902.0	940.0	1365.0	1780.0	24.5	46.0	265.0	193.5
TZA 2785	1178.0	907.5	1124.0	1644.5	28.5	30.5	81.5	179.0
Tumia	1227.5	1127.5	1310.0	1092.0	28.5	42.0	206.0	157.5
TZA 5557	1159.0	1180.0	1379.0	855.0	28.0	37.5	107.0	63.0
TZA 2496	1167.5	1137.5	1165.0	1035.0	27.0	43.5	130.5	108.0
TZA 2456	963.0	942.5	1250.0	1200.0	26.0	38.5	212.0	111.0
TZA 197	1184.0	1217.5	672.5	1147.5	28.0	38.0	124.0	127.0
Hombolo	889.0	880.0	922.5	1410.0	24.5	46.0	110.0	255.5
TZA 2466	1087.5	1057.5	1115.0	764.0	25.0	28.5	122.5	110.0
TZA 5555	1025.0	995.0	900.0	858.5	28.0	90.0	204.0	119.0
Kiteto	1065.0	1065.0	565.0	380.0	22.5	50.5	114.0	208.0
Komboa	934.0	920.0	550.0	460.0	27.0	39.5	113.0	105.0
Mean	**1315.5**	**1299.1**	**1478.9**	**1499.1**	**31.3**	**63.4**	**148.7**	**148.5**
CV	**5.9**	**6.1**	**27.7**	**32.3**	**8.9**	**28.7**	**28.1**	**15.5**
$Lsd_{(0.05)}$	**33.0**	**30.4**	**30.6**	**24.6**	**1.6**	**1.5**	**4.8**	**3.1**
P value	**<0.001*****	**<0.001*****	**0.0039****	**0.01751***	**<0.001*****	**<0.001*****	**0.00106****	**<0.001*****

Appendix 3.2 Mean values of days to 50% flowering and days to maturity at Hombolo and Ilonga evaluated in 2013/14 and 2014/15

	Days to 50% flowering				Days to Maturity			
	Hombolo		Ilonga		Hombolo		Ilonga	
Genotype	**2013/14**	**2014/15**	**2013/14**	**2014/15**	**2013/14**	**2014/15**	**2013/14**	**2014/15**
ICEAP 00040	125.0	111	169.0	175.5	158.0	151.5	200.0	210.0
ICEAP 00932	122.5	110.5	168.5	166.0	152.5	151.0	200.0	198.5
Bangili	125.0	112.0	171.0	171.0	162.0	153.0	210.5	202.0
ICEAP 00053	123.0	110.0	170.0	169.5	154.0	151.5	202.0	201.0
No. 40	124.0	95.0	168.5	168.5	155.0	136.5	199.0	200.5
ICEAP 00557	111.5	82.0	119.0	120.0	140.0	125.0	149.0	151.0
Babati White	123.0	112.0	172.0	164.0	153.0	152.5	203.0	195.0
ICEAP 00554	107.0	82.0	124.5	123.0	142.0	125.0	157.0	153.0
TZA 253	114.5	81.5	119.0	116.0	145.0	124.5	149.0	146.0
TZA 5464	101.0	80.5	115.5	114.0	129.0	124.0	145.5	143.5
TZA 2514	112.5	80.5	118.0	116.0	142.0	123.5	148.0	145.5
TZA 2807	106.0	81.5	112.5	115.0	134.0	124.5	143.0	144.5
TZA 2509	113.5	81.5	119.5	116.0	143.0	124.5	150.0	146.0
TZA 2439	101.0	81.5	117.0	115.0	129.0	124.5	147.0	144.5
TZA 2464	112.5	81.0	112.0	111.5	142.0	124.0	141.5	142.0
TZA 5463	113.5	82.0	118.0	118.0	143.0	125.0	148.0	148.0
TZA 2692	103.0	82.5	114.5	116.0	134.5	125.5	144.5	146.5
TZA 5596	111.0	80.5	116.0	116.0	140.0	123.5	146.0	145.5
TZA 5541	106.0	80.0	116.0	113.0	135.0	123.0	146.0	142.0
TZA 5582	101.0	81.5	115.0	115.0	129.5	124.5	145.0	145.0
TZA 250	114.5	80.5	112.0	113.0	145.0	123.5	141.5	142.0
TZA 2785	114.5	82.0	111.0	113.0	142.0	125.0	141.0	142.0
Tumia	101.0	82.0	122.5	121.5	137.0	125.0	153.0	152.0
TZA 5557	112.5	81.0	117.0	114.0	141.5	124.0	147.0	144.0
TZA 2496	106.0	81.0	116.0	117.0	134.0	124.0	146.0	147.0
TZA 2456	105.0	80.5	119.0	115.0	134.0	123.5	149.0	144.0
TZA 197	113.5	81.5	114.0	114.0	153.0	124.5	144.0	143.0
Hombolo	114.0	80.5	121.5	117.0	143.5	123.5	153.0	147.0
TZA 2466	114.0	82.0	117.5	111.0	143.0	125.0	147.5	140.0
TZA 5555	108.5	82.0	111.5	113.5	138.0	125.0	141.0	143.0
Kiteto	115.0	80.5	116.5	117.5	145.0	123.5	146.5	148.0
Komboa	87.0	61.5	83.0	84.0	120.0	118.0	112.0	117.0
Mean	**110.9**	**85.7**	**125.5**	**124.4**	**141.3**	**128.8**	**156.1**	**155.1**
CV	**1.6**	**4.4**	**0.4**	**2.9**	**0.3**	**2.6**	**0.4**	**0.2**
$Lsd_{(0.05)}$	**2.9****	**3.8**	**5.4**	**4.4**	**0.3**	**3.9**	**1.1**	**1.8**
P value	**0.00357****	**<0.001*****	**<0.001*****	**<0.001*****	**<0.001*****	**<0.001*****	**<0.001*****	**<0.001*****

Appendix 3.3 Mean values of plant height (cm) and 100-grain weight (gm) at Hombolo and Ilonga evaluated in 2013/14 and 2014/15

	Plant height				100-grain weight			
	Hombolo		Ilonga		Hombolo		Ilonga	
Genotype	**2013/14**	**2014/15**	**2013/14**	**2014/15**	**2013/14**	**2014/15**	**2013/14**	**2014/15**
ICEAP 00040	208.5	212.5	147.0	205.0	21.5	20.5	21.0	20.0
ICEAP 00932	201.0	208.5	193.5	214.0	20.0	20.0	20.5	19.5
Bangili	129.0	200.0	205.5	205.5	20.0	19.0	20.0	22.5
ICEAP 00053	188.0	177.5	228.5	216.0	15.0	17.0	20.0	17.5
No. 40	165.0	205.0	227.5	222.5	17.5	15.0	19.0	17.5
ICEAP 00557	117.0	121.5	179.5	195.0	15.0	15.0	19.0	15.0
Babati White	206.5	204.0	212.5	223.0	20.0	21.0	22.0	22.0
ICEAP 00554	115.5	134.5	167.5	189.0	20.0	20.0	17.5	15.0
TZA 253	164.5	100.5	178.5	164.0	20.0	15.0	14.0	15.0
TZA 5464	116.0	127.5	189.0	209.0	15.0	15.5	16.0	12.5
TZA 2514	102.0	103.0	150.5	199.5	15.0	10.0	13.0	15.0
TZA 2807	106.5	143.5	170.0	157.0	15.0	10.0	20.0	20.0
TZA 2509	105.0	104.0	162.5	117.0	15.0	18.5	16.0	10.0
TZA 2439	107.0	104.5	157.5	98.0	15.0	20.0	20.0	15.0
TZA 2464	122.5	96.5	147.5	138.5	15.5	10.0	17.5	10.0
TZA 5463	116.5	107.0	140.0	205.0	15.0	18.0	15.0	12.5
TZA 2692	118.5	130.5	157.0	181.0	15.0	14.0	13.0	10.0
TZA 5596	114.0	139.0	171.5	197.0	20.0	10.0	17.0	10.0
TZA 5541	115.5	115.5	136.0	193.5	14.5	14.5	10.0	15.0
TZA 5582	109.0	144.5	149.0	166.0	16.0	15.0	15.0	15.0
TZA 250	141.5	120.5	146.5	207.5	15.0	10.0	15.0	12.5
TZA 2785	136.5	124.5	162.0	210.0	15.0	10.0	15.0	10.0
Tumia	89.5	150.0	160.5	182.5	20.0	15.0	17.5	15.0
TZA 5557	129.0	113.0	165.0	169.5	17.5	10.0	11.5	10.0
TZA 2496	107.0	124.0	144.5	189.5	16.5	15.0	17.5	12.5
TZA 2456	117.0	114.0	164.0	210.5	15.0	10.0	12.5	12.5
TZA 197	81.0	93.5	158.5	178.5	15.0	20.0	15.0	15.0
Hombolo	130.5	112.0	131.0	205.0	15.0	15.0	15.0	17.5
TZA 2466	127.5	115.5	168.5	177.5	15.0	15.0	15.0	15.0
TZA 5555	102.5	124.0	162.5	145.0	13.0	10.0	13.5	12.5
Kiteto	136.0	106.5	186.5	184.0	13.5	15.0	15.0	15.0
Komboa	89.5	77.0	79.0	109.5	12.0	10.5	12.0	11.0
Mean	**127.7**	**132.9**	**167.3**	**183.6**	**16.3**	**14.8**	**16.2**	**14.6**
CV	**19.1**	**14.6**	**13.3**	**7.8**	**11.6**	**10.9**	**8.5**	**13.3**
$Lsd_{(0.05)}$	**4.0**	**3.7**	**3.8**	**2.4**	**3.7**	**4.4**	**3.1**	**3.9**
P value	**<0.001*** **	**<0.001*** **	**<0.001*** **	**<0.001*** **	**<0.001*** **	**<0.001*** **	**<0.001*** **	**<0.001*** **

Appendix 3.4 Mean values of number of primary and secondary branches at Hombolo and Ilonga evaluated in 2013/14 and 2014/15

	Number of Primary branches				Number of secondary branches			
	Hombolo		Ilonga		Hombolo		Ilonga	
Genotype	**2013/14**	**2014/15**	**2013/14**	**2014/15**	**2013/14**	**2014/15**	**2013/14**	**2014/15**
ICEAP 00040	18.0	16.5	29.0	24.0	31.5	34.0	76.0	83.0
ICEAP 00932	16.5	16.5	29.0	29.0	30.0	31.5	53.5	80.5
Bangili	21.0	17.0	22.5	19.0	32.0	35.5	53.5	122.5
ICEAP 00053	10.0	7.5	32.0	40.0	16.0	16.5	112.0	229.5
No. 40	12.0	12.5	21.5	38.5	21.5	24.0	21.5	255.0
ICEAP 00557	4.5	3.5	22.5	13.0	5.0	7.0	60.0	136.0
Babati White	14.5	16.0	20.5	34.0	28.5	33.0	68.5	91.5
ICEAP 00554	6.0	7.5	24.5	30.5	8.5	14.5	87.5	48.0
TZA 253	4.0	4.5	19.5	32.0	6.5	6.5	52.5	97.0
TZA 5464	3.5	6.0	21.5	27.0	4.0	8.0	93.5	66.5
TZA 2514	4.5	3.5	19.0	17.0	4.5	7.5	59.5	185.0
TZA 2807	7.5	5.0	16.5	18.0	10.5	3.5	66.5	79.5
TZA 2509	5.5	5.5	17.0	30.5	6.5	9.5	51.5	138.0
TZA 2439	4.0	6.0	18.5	30.5	2.5	6.0	44.5	88.5
TZA 2464	4.0	5.5	18.5	21.5	4.5	4.0	38.5	42.0
TZA 5463	4.0	5.5	17.5	20.5	7.5	10.5	43.5	94.0
TZA 2692	4.0	4.5	15.0	18.5	2.5	4.0	51.0	41.0
TZA 5596	4.0	3.5	22.0	29.0	3.5	4.0	64.0	110.5
TZA 5541	4.0	5.0	18.5	15.5	2.5	5.5	80.5	62.5
TZA 5582	5.0	5.5	18.5	26.0	5.5	3.5	71.5	89.5
TZA 250	5.0	5.5	12.0	19.0	7.5	3.5	27.0	96.5
TZA 2785	4.5	5.0	15.5	35.5	3.5	3.5	35.0	71.0
Tumia	5.0	5.5	16.0	28.0	11.5	11.5	35.5	52.0
TZA 5557	4.5	6.0	23.5	9.0	3.0	6.5	104.5	71.0
TZA 2496	3.5	5.5	17.5	13.0	7.0	8.5	32.0	112.5
TZA 2456	4.0	5.5	14.5	27.5	8.0	7.5	38.5	87.5
TZA 197	8.5	5.0	18.0	33.0	11.5	7.5	63.0	92.5
Hombolo	5.0	5.5	18.5	30.5	9.5	7.0	41.0	213.5
TZA 2466	3.5	4.0	15.0	9.5	6.5	8.5	45.0	32.0
TZA 5555	3.5	5.0	17.5	28.5	4.5	4.5	59.0	87.0
Kiteto	5.0	6.0	25.5	25.0	5.0	6.0	71.5	108.5
Komboa	4.0	5.0	15.0	17.5	7.5	8.5	17.0	36.5
Mean	**6.3**	**6.9**	**19.7**	**24.6**	**9.5**	**6.9**	**58.1**	**100.1**
CV	**34.8**	**27.8**	**23.7**	**25.9**	**47.7**	**27.8**	**27.9**	**27.1**
$Lsd_{(0.05)}$	**1.4**	**2.2**	**2.6**	**3.6**	**2.7**	**1.5**	**5.5**	**10.1**
P value	**<0.001*** **	**<0.001*** **	**0.0016** **	**<0.001*** **	**<0.001*** **	**<0.001*** **	**0.0021** **	**<0.001*** **

Chapter 4 : Phenotypic diversity of Tanzanian pigeonpea germplasm based on agro-morphological traits

Abstract

Phenotypic characterization of crop genetic resources generates important information for plant breeders. The objective of this study was to assess the genetic diversity of Tanzanian pigeonpea germplasm using qualitative and quantitative traits and to identify the best and complementary parents that could be used in a pigeonpea breeding programme. A total of 48 entries collected from the Northern, Eastern and Central Zones of Tanzania were evaluated for 15 qualitative and 16 quantitative agro-morphological traits. Genotypes were evaluated using a 8 x 6 row-column design with two replication at two sites in one season. Results from the combined analysis of variance showed that genotypes varied significantly for most of traits studied. The Sharon-Weaver diversity index (H') revealed a low to high genetic diversity among the zones of collection, while the overall mean indicated a low diversity for qualitative traits. High heritability was recorded for days to 50% flowering and days to maturity, while grain yield had a medium heritability. The correlation analysis identified the important traits for simultaneously selection for improvement. The principal component analysis (PCA) showed that the first four PCs explained 73.4% of total variation with days to 50% flowering, days to maturity, number of pods per plant, number of seed per plant, grain yield, leaf width and leaf area being the most important traits in the PC1. The cluster analysis grouped genotypes into three groups. The most desirable genotypes with distinct attributes useful in future pigeonpea breeding were Bangili, TZA 5463, Babati White, ICEAP 00040 and ICEAP 00932.

4.1 Introduction

Pigeonpea (*Cajanus cajan* (L.) Millsp.) is an important crop for millions of people living in dry regions of the world. It's a multipurpose crop that integrates crop and livestock production, thus contributing to food security. Africa, especially Eastern Africa, is considered as a secondary centre of diversity due to the presence of wild relatives. The crop has gained popularity among the farming community in many parts of Tanzania and area of production has been increased in recent years (Abate *et al*., 2012). In Tanzania, over 80% of pigeonpea dry seed is sold to external markets and the remaining is consumed locally mainly as a green vegetable (Lo Monaco, 2006).

Pigeonpea is the only drought tolerant crop in the *Leguminosae* family that can survive in drought affected areas, where other legume crops have failed. It can also survive in poor soils, various climates and altitudes (Silim *et al*., 2006). The crop fits in different cropping system and can be used in conservation agriculture (Lal *et al*., 1978). It reduces weed competition and the level of root-knot nematodes in the soil (Daniel and Ong, 1990; Venzon *et al*., 2006). The fallen leaves of pigeonpea provides an important source of nutrients to the soil and pigeonpea improves soil fertility through its ability to fix atmospheric nitrogen (Varshney *et al*., 2010). It provides materials for industrial and medicinal purposes. The crop is referred to as poor man's meat because of high protein content. Pigeonpea seed have a higher protein content than other legume crops (Saxena *et al*., 2010). It is also a source of protein to vegetarians (Singh *et al*. 1990; Saxena, 2009).

Traditional landraces are the donor parents for improved varieties, because they are rich in genetic variability and have a high level of stability under diverse environments (Kobayashi *et al*., 2006). The diversity in crop species usually depends on mutation, recombination, selection and genetic drift. The search for diversity in a germplasm collection is a way of identifying desirable genes for future utilization in breeding (Aggarwal *et al*., 2002; Brondani *et al*., 2006; Thomson *et al*., 2007). The cultivated pigeonpea has low polymorphism (Odeny, 2007).

Genetic diversity of crop species can be studied using different methods viz. morphological and/or phenotypic, biochemical and molecular markers (Mehmood *et al*., 2008). Morphological characterization is considered as a traditional method because it is simple and inexpensive, without requiring special facilities or procedures. Morphological characterization provides an understanding of the crop species, based on the phenotype, under field conditions, but highly affected by the environment. In the past years, morphological traits, both qualitative and quantitative, have been successfully used to study genetic diversity in pigeonpea (Upadhaya *et al*., 2007; Manyasa *et al*., 2008). Understanding the level of genetic diversity in pigeonpea

germplasm is useful to prevent genetic erosion and ensure sufficient genetic variation for selection of desirable parents for introgression. Therefore the objective of this study was to assess genetic diversity based on qualitative and quantitative traits and to identify the best parents for different traits that could be used in pigeonpea breeding programme.

4.2 Materials and methods

4.2.1 Plant material

The experimental materials consisted of 48 pigeonpea germplasm collected from Northern, Eastern and Central Zones of Tanzania. Table 4.1 summarizes the genotypes and place of collections.

Table 4.1 Genotypes used and their place of collection in Tanzania

Genotype	Place of Collection	Genotype	Place of Collection	Genotype	Place of Collection
ICEAP 00040	Eastern	Ilonga	Eastern	ICEAP 01147	Northern
ICEAP 00053	Northern	Hombolo	Central	ICEAP 01179/1	Northern
ICEAP 00932	Eastern	No. 40	Northern	ICEAP 00576-1	Northern
ICEAP 00936	Northern	TZA 2509	Northern	ICEAP 001154/15	Northern
ICEAP 00911	Northern	TZA 2466	Northern	TZA 253	Northern
ICEAP 00540	Northern	TZA 2464	Northern	TZA 2496	Northern
ICEAP 00557	Eastern	TZA 5582	Northern	TZA 2692	Northern
ICEAP 00554	Eastern	TZA 5555	Northern	TZA 5596	Northern
Komboa	Eastern	TZA 5557	Northern	TZA 2439	Northern
Arumeru	Northern	TZA 2514	Northern	TZA 2456	Northern
ICEAP 01179	Northern	TZA 2807	Northern	Kiteto	Central
ICEAP 01154/2	Northern	TZA 2785	Northern	Tumia	Eastern
TZA 250	Northern	TZA 5463	Northern	Kondoa	Central
Mthawanjuni	Northern	TZA 5464	Northern	ICEAP 00979/1	Northern
Babati white	Northern	TZA 197	Northern	Kondoa	Central
ICEAP 0673/1	Northern	TZA 5541	Northern	Bangili	Northern

4.2.2 Experimental details

The experiment was conducted at Hombolo and Ilonga (see Chapter 3.2.2 for detailed sites description).The experiment was laid out in a 8 x 6 row-column design, with two replications. The

48 genotypes were planted in four row plots row plots, 3 m length, with inter-row and intra-row spacing of 1 m and 0.5 m, respectively. Data were collected on the middle rows. The genotypes were evaluated for one rainy season at Ilonga during December-August 2016 and at Hombolo from January–July 2016. All standard field management procedures were followed and no fertilizer was applied to simulate farmer practice.

4.2.3 Data collection

Data collected of the15 qualitative and 16 quantitative traits are presented in Table 4.2. All data were recorded following the descriptors for pigeonpea (IBPGR and ICRISAT, 1993).

Table 4.2 Descriptors for qualitative and quantitative traits studied

Qualitative traits	
Traits	**Descriptors**
Growth habit	1-Erect and compact, 2-Semi-spreading, 3-Indeterminate
Stem colour	1-Green, 2-Sun- red, 3-Purple, 4-Dark purple
Flower base colour	Main colour of the petals 1– Ivory, 2-Light yellow, 3-Yellow, 4-Red
Pattern of streak	Pattern of streak= 1-Sparse, 2-Medium, 3-dense, 4-Uniform
Flowering pattern	1-Determinate, 2-Semi-determinate, 3- Indeterminate
Pod colour	1-Green, 2-Purple, 3-Mixed, 4-Dark purple
Seed eye colour	1-None, 2-Purple, 3-Light brown, 4-Reddish brown, 5-grey/dark, 6-Cream/white
Pod hairiness	1-Glabrous, 2-Pubescent
Pod form	1-Flat, 2-Cylindrical
Seed eye width	1-Narrow, 2-Medium, 3-Wide, 4-None
Base seed colour	1-White, 2-Cream, 3-Orange, 4-Ligt brown, 5-Reddish brown, 6-Light grey, 7-Grey, 8-Purple, 9-Dark purple, 10-Dark grey
Seed shape	1-Oval, 2-Round, 3- Square 4-Elongated
Seed colour pattern	1-Plain, 2-Mottled, 3-Speckled, 4-Motlled and speckled, 5-Ringed
Leaf shape	1-Ovate, 2-Triangullar, 3-Trullate
Leaf hairiness	1-Hairy, 2-Non-hairy
Days to 50% flowering	Number of days from sowing until 50% of plants flower

Qualitative traits	
Traits	**Descriptors**
Days to maturity	Days from sowing to the stage when 90% of pods have matured and turned brown
Plant height (cm)	From base to the top of plant, measured at maturity
Number of pods per plant	Number of pods per plant recorded at maturity
Number of seed per pod	Average number of seeds of 10 randomly selected pods from three randomly selected plants in a row
Number of seeds per plant	Average seed yield of three randomly selected plants
100-grain weight	Weight of air dried (10% moisture) seeds estimated from a random sample taken from total row yield
Primary branches	Branches born on main stem
Secondary branches	Branches born on primary branches
Grain yield	Grain yield was measured by weighing grains of selected 10 middle plants in a plot
Pod length (cm)	Maximum average length of 10 randomly selected mature pods Recorded at physiological maturity
Stem thickness	Measure of diameter of the stem
Leaf area	Leaf area was calculated using image scanner
Leaf length	Leaf length was measured using a meter ruler
Leaf width	Leaf width was measured using a meter ruler
Number of raceme	Mean of 5 plants at flowering

Source: IBPGR and ICRISAT, 1993

4.2.4 Data analysis

Analysis of variance was done for the 16 quantitative traits over two sites using the SAS 9.3 version (SAS Institute, 2010) after the homogeneity of variances tests (Gomez and Gomez, 1984). The principal component principal biplot and cluster analysis were calculated using Genstat 14th edition (Payne, *et al*., 2011). The Shannon-Weaver diversity index (H') was computed using the phenotypic frequencies to assess the phenotypic diversity for each character for all accessions as described by Perry and McIntosh (1991). Each H' value was divided by its maximum value

(logen) and normalized in order to keep the values between 0 and 1. Frequency of each category in each trait were also calculated.

Shannon-Weaver (1949) diversity index (H), given as:

$$H = -\sum_{i=1}^{s} pi \ln (pi)$$

Where:

s is the number of phenotypic classes for a character and *pi* is the relative proportion of the total number of entries (N) in the ***i***th class.

Partitioning of the total variance into components due to genotype (σ^2 g), environment (σ^2 e) and genotypes by location interaction (σ^2 gl) variances were performed from the analyses of variance by assuming various observed mean squares equal to their expected mean squares (Table 4.3) as suggested by Singh and Chaudhary (1985).

σ^2 g = [(σ^2 e + R σ^2 gl + RLσ^2 g) – (σ^2 e + Rσ^2 gl)]/RL= (MS3- MS4)/RL

σ^2 e = MS5

σ^2 gl = [(σ^2 e + Rσ^2 e) – (σ^2 e)]/R= (MS4-MS5)/R

Where σ^2 g = genotype variance, σ^2 e = environmental variance, σ^2 gl = genotype by location interaction variances, and G, L and R = number of genotypes, location and replication, respectively.

Broad-sense heritability (h_b^2) was calculated as:

$h_b^2 = \sigma^2$ g / [σ^2 g + σ^2 gl / L + σ^2 e)/ RL] X100

where the components of the equation are described above

Table 4.3 Combined analysis of variance over location

Source of variation	Degree of freedom	Mean Square (MS)	Expected Mean Square (EMS)
Locations	L-1	MS1	$\sigma^2 e + G\sigma^2 r + GR\sigma^2 l$
Replications/location	L(R-1)	MS2	$\sigma^2 e + G\sigma^2 r$
Genotypes	G-1	MS3	$\sigma^2 e + R\sigma^2 gl + RL\sigma^2 g$
Genotype x Location	(G-1) (L-1)	MS4	$\sigma^2 e + R\sigma^2 gl$
Error	L(G-1) (R-1)	MS5	$\sigma^2 e$

4.3 Results

4.3.1 Qualitative traits

Data for the frequency distribution for the 15 quantitative traits are presented on Table 4.4. All genotypes had hairiness on the pods and leafs (100%), an ovate leaflet shape (100%), an oval on seed shape (100%) and a flower streak pattern (100%). Semi-spreading was the dominant growth habit (97.9%) with only one erect/compact genotype and most pods were flat (97.9%), while one genotype had cylindrical pods (2.1%). There was variation in stem colour with green (66.7), purple (17.0%) and dark green (17.0%) stems. Cream was the dominant seed colour (89.6%), others had light grey (4.2%) and light brown (6.3%) seeds. The flowering patterns were semi-indeterminate (4.2%), determinate (89.6%) and indeterminate (8.3%). The highest variation was observed for flower colour with yellow (39.6%), light yellow (31.3%), ivory (12.5%) green (10.42%) and red (6.25%). The seed coat patterns were plain (64.6%), speckled (27.1 %) and few had mottled/speckled (8.33%). The eye on seed colour was highly variable from no colour (83.3%), to light brown (4.17%), purple (4.2%) and reddish brown (4.2%).

Table 4.4 Frequency distribution for qualitative traits studied of 48 pigeonpea genotypes

Traits and categories	Frequency	Traits and categories	Frequency
Stem colour		**Pattern of streak**	
Green	32 (66.7%)	Sparse streaks	48 (100%)
Purple	8 (17%)	Medium amount	
Dark green	8 (17%)	Dense streaks	
Base flower colour		Uniform coverage	
Ivory	6 (12.5%)	**Pod form**	
Yellow	19 (39.6%)	Flat	47 (97.9%)
Light yellow	15 (31.3%)	Cylindrical	1 (2.1%)
Green	5 (10.4%)	**Seed shape**	
Red	3 (6.3%)	Oval	48 (100%)
Flowering pattern		Globular	
Determinate	2 (4.2%)	Square	
Semi-determinate	43 (89.6%)	Elongate	
Indeterminate	3 (6.3)	**Base seed colour**	
Seed colour pattern		Cream	43 (89.6%)
Plain	31 (64.6%)	Dark purple	
Speckled	13 (27.1%)	Light grey	2 (4.2%)
Mottled/Speckled	4 (8.3%)	Light brown	3 (6.3%)
Mottled		**Seed eye width**	
Seed eye colour		Narrow	20 (41.7%)
None	40 (83.3%)	Medium	28 (58.3%)
Light brown	2 (4.2%)	None	
Purple	2 (4.2%)	Wide	
Reddish brown	2 (4.2%)	**Growth habit**	
Grey/dark		Erect and compact	1 (2.1%)
Cream	2 (4.2%)	Semi-spreading	47 (97.9%)
Pod hairiness			
Pubescent	48 (100%)		
Non pubescent			
Leaflet shape			
Ovate	48 (100%)		
Triangular			
Trullate			
Pod colour			
Purple	6 (12.5%)		
Green	30 (62.5%)		
Black	3 (6.3%)		
Mix	9 (18.8%)		
Leaf hairiness			
Hairiness	48 (100%)		
Non-hairy			

4.3.2 Diversity index for 15 qualitative traits

The results of the Shanon-Weaver index (H') for diversity index are shown on Table 4.5. There is remarkable variation among the genotypes collected from three zones of Tanzania. The diversity index vaues for genotypes collected from Northern Zone vary from 0.18 to 1.0 with a

mean of 0.35 ± 0.062. For the Central Zone the H' values varied from 0.17 to 0.55 with a mean of 0.26 ± 0.037. The H' values for the Eastern zone varied from 0.21 to 0.64 with a mean of 0.37 ± 0.045. Across the three zones the diversity index (H') ranged from 0.19 to 0.62 with a mean of 0.33 ±0.048. Collections from the Northern Zone were highly diverse. In particular for pod colour, stem colour and seed eye colour. Collections from the Central Zone were highly diverse for stem colour, seed colour pattern, seed eye colour and growth habit. Collections from the Eastern Zone had a high diversity for stem colour, seed colour patern and seed eye colour.

In this study 38 out of 48 genotypes were collected from Northern Zone and the majority are from medium duration and long duration types. The overall perfomance of collections from Central Zone had the lowest diversity. Only three genotypes from Central Zone were included in this study. Genotypes collected from Eastern Zone were all improved and are the most commonly grown in Tanzania. These genotypes are in all maturity group, vz. early, medium and late duration. In general, stem colur, seed colour, seed colour pattern, seed eye colour and pod colour were polymorphic. Seed eye width and growth habit were relatively polymorphic, while the rest of the traits showed monomorphic characters.

Table 4.5 Shanon-Weaver index (H') for different qualitative traits of 48 pigeonpea genotypes collected from three zones of Tanzania

	Diversity index (H') across zones				
Characters	**Northern**	**Central**	**Eastern**	**Mean**	**SE**
Stem colour	0.51	0.45	0.64	0.53	0.056
Base flower colour	0.23	0.21	0.23	0.22	0.007
Flowering pattern	0.18	0.17	0.21	0.19	0.012
Seed colour pattern	0.55	0.48	0.79	0.61	0.094
Seed eye colour	0.66	0.55	0.64	0.62	0.034
Pod hairness	0.18	0.17	0.28	0.21	0.035
Leaflet shape	0.18	0.17	0.28	0.21	0.035
Pod colour	1.00	0.23	0.34	0.52	0.024
Leaf hairness	0.18	0.17	0.23	0.19	0.019
Seed shape	0.29	0.23	0.34	0.29	0.032
Pattern of streak	0.18	0.17	0.28	0.21	0.035
Pod form	0.23	0.19	0.31	0.24	0.035
Seed eye width	0.32	0.28	0.37	0.32	0.026
Base seed colour	0.19	0.18	0.28	0.22	0.032
Growth habit	0.27	0.53	0.38	0.39	0.754
Mean	0.35	0.26	0.37	0.33	
SE	0.062	0.037	0.045	0.048	

4.3.3 Quantitative traits

The results from the combined analysis of variance are presented on Table 4.6. There were significant differences (P<0.05) among genotypes for the number of seed per pod, 100-grain weight, number of branches, pod length, stem diameter, leaf length, leaf width and leaf area. There were significant differences (P<0.01) among genotypes for the number of pod per plant, number of seed per plant and number of raceme. Highly significantly different (P<0.001) among genotypes were observed for the days to 50% flowering, days to maturity, plant height and grain yield. Across the zones, there were highly significantly different (P<0.001) for all traits except for the number of seeds per pod, number of seeds per plant, 100-grain weight, number of primary branches, leaf area, leaf width and length. The interaction effects among genotypes and location were highly significantly different (P<0.001) for the days to 50% flowering, days to maturity, plant height and grain yield.

Data for mean, minimum and maximum values for the 16 quantitative traits from three zones are displayed in Table 4.7. There was variation for the different quantitative traits for genotypes collected from three zone. Genotypes from northern zone showed variation for days to 50% flowering (103-149 days), days to maturity (149-200 days), plant height (143.2-239.2cm), grain yield (560.3-2020 kg/ha), number of pods per plant (103-561), number of seeds per pod (4-7), number of seeds per plant (571-2435), 100-grain weight (12.5-25.9 gm), number of primary branches (14-135) and number of secondary branches (39-231)

Genotypes from central zone showed a variation for days to 50% flowering (108-114 days), days to maturity (155-160 days), plant height (187.3-228.4 cm), grain yield (870-1262 kg/ha), number of pods per plant (156-243), number of seeds per pod (5-6), number of seeds per plant (954-1293), 100-grain weight (14.8-23.1 gm), number of primary branches (22-58) and number of secondary branhces (40-94).

Genotypes from eastern zone showed a varition for days to 50% flowering (81-131 days), days to maturity (126-187 days), plant height (90-209.8 cm) grain yield (1029-2030 kg/ha), number of pods per plant (184-398), number of seeds per pod (5-6), number of seeds per plant (910-2324) 100-grain weight (15.4-23.1), number of primary branches (28-52) and nmber of secondary branches (52-119).

Table 4.6 Combined analysis of variance for 16 quantitative traits of 48 pigeonpea genotypes and genetic parameters

Source	DF	DT50F	DTM	PH	NPP	NSOP	NSP	100-gw	NPB
Location (L)	1	49.667.89***	29957.40***	607484.91***	960037.91***	4.86**	19599301.48**	133.37**	3275.66*
Genoype (G)	47	494.89***	750.16***	1678.59***	32347.47**	1.95*	822118.86**	28.36*	1275.62*
G X E	47	144.73***	130.88***	1571.39	26085.88*	1.21**	580108.83	16.29	786.59
Error	71	26.35	45.33	733.88	16790.64	0.76	415836.5	4.05	672.94
Mean		115.05	162.09	200.95	247.96	5.76	1397.21	19.68	38.78
CV		4.4	4.15	13.48	52.26	13.27	46.15	20.57	72.31
Vg		87.52	154.82	26.8	1565.39	0.18	60502.51	3.02	122.26
Vp		59.19	42.75	418.76	4647.64	0.22	82135.92	6.12	56.83
Vgxe		123.19	187.54	419.65	8086.87	0.49	205529.7	7.09	318.91
H		0.71	0.83	0.06	0.19	0.38	0.29	0.43	0.19

Source	DF	NSB	GY	PL	SD	LFL	LW	LA	RCM
Location (L)	1	80512.33***	10252919.86***	22.54***	529.71***	0.59	0.03	1085.17**	11395.31***
Genotype (G)	47	4491.38*	519644.12***	2.42*	4.07*	2.81*	0.62*	394.27*	415.69**
G x E	47	3430.15	227358.82	1.86	2.26*	1.69	0.45	226.67	216.68
Error	71	2602.6	175918.66	1.61	2.15	1.67	0.4	210.47	215.71
Mean		85.07	1237.65	7.8	8.4	8.01	3.53	48	31.34
CV		59.97	33.89	17.4	24.01	16.12	17.96	30.22	46.97
Vg		265.31	73071.07	0.14	0.45	0.28	0.04	41.9	49.75
Vgxe		413.78	25720.08	1.86	0.05	0.01	0.03	7.98	0.48
Vp		1122.84	129911.03	0.61	1.02	0.70	0.16	98.57	103.92
H		0.24	0.56	0.23	0.44	0.39	0.27	0.43	0.48

*, **,*** significant at 0.05, 0.01, 0.001

Key: DF=degree of freedom; DT50F=days to 50% flowering; DTM=days to maturity; NPP=number of pods/plant; NSOP=number of seeds/pod; NSP=number of seeds/plant; 100-gw=100 grain weight; NPB=number of primary branches; NSB=number of secondary branches; GY=grain yield; PL=pod length; SD=stem diameter; LFL=leaflet length; LW=leaflet width; LA=leaf area; RCM=number of racemes; CV=coefficient of variation; Vg=variance due to genotype; Vgxe=variance due to interaction between genotype and environment; Vp= Phenotypic variance; H=heritability

Table 4.7 Mean, minimum and maximum of 16 quantitative traits of 48 pigeonpea collected from Northern, Eastern and Central Zones of Tanzania

Traits	Northern				Central				Eastern			
	Mean	Min	Max	CV	Mean	Min	Max	CV	Mean	Min	Max	CV
DT50F	116	103	149	12.6	110.6	108	114	2.86	112.3	81.07	130.8	16.2
DTM	163	149	200	10.6	157.3	155	160	1.78	161.1	126	186	13.7
PH	202.5	143.2	239.2	11.2	202.4	187.3	228.4	11.2	181.6	90	209.8	25.2
NPP	249.5	102.9	560.6	40.9	198.5	156.5	242.6	21.7	262.5	184	398.1	34
NSOP	6	4	7	11.4	6	5	6	11.2	6	5	6.	7.66
NSP	1370	570.9	2435	34.9	1159	953.8	1293	15.6	15.8	910	2324	32.9
100-GW	19.8	12.5	25.9	16.6	18.6	14.8	23.1	22.6	20.2	15.4	23.1	14.9
NPB	41.2	13.9	135.2	49	35.4	21.8	58	55.9	39.9	28.0	51.7	22.3
NSB	91.7	39	231	42.8	63.5	40	94	43.6	85.7	51.8	119.4	35.1
GY	1240	560.3	2020	33.1	1032	870	1262	19.8	1440	1029	2030	25.3
PL	7.9	6.4	9.92	10.3	8.3	7.5	9.8	15.4	7.00	5.6	7.9	12.4
SD	8.4	6.2	11.2	12.5	8.9	7.7	10.6	17.5	7.9	7.0	8.8	8.9
LFL	7.9	5.6	10.8	15.9	8.6	7.7	9.4	9.8	7.8	6.6	8.9	11.7
LW	3.5	2.6	5.3	21.9	3.9	3.2	4.9	22.2	3.3	2.9	4.0	11.7
LA	49.1	22.8	78.6	27.9	46.7	22.9	81.8	66.4	42.1	26.5	62.32	31.3
RCM	32.9	15.9	58.6	33.7	19.5	13.3	23.9	28.6	30.5	21.2	49.9	33.8

Key: DT50%F=days to 50% flowering; DTM=days to maturity; PH=plant height; NPP=number of pod per plant; NSOP=number of seed per pod; NSP=number of seed per plant; 100-GW=100 grain weight; NPB= number of primary branches; NSB=number of secondary branches; GY=grain yield; PL=pod length; SD= stem diameter; LFL=leaflet length; LW=leaf width; LA= leaf area; RCM=number of racemes

4.3.4 Heritability estimates

The heritability was estimated for all 16 quantitative traits (Table 4.6). The estimated heritability ranged from a low of 6% for the plant height to a high of 83% for the number of days to maturity (Table 4.6). In this study a heritability greater than 70% was considered high, 50-69% moderate, 20-49% low and 1-19% very low. The heritabilty was high for days to maturity (83%) and days to 50% flowering (71%). A moderate heritability was recorded for grain yield (56%). A low heritability was recorded for the number of racemes (48%), stem diameter (44%), leaf area (43%), 100-grain weight (43%), length of leaflet (39%),number of seeds per pods (38%), number of seeds per plant (29%), leaf width (27%), number of secondary branches (24%) and pod length (23%). A very low heritability was recorded for the number of pods per plant (19%), number of primary branches (19%) and plant height (6%) (Table 4.6).

4.3.5 Correlation

The results for the correlation analysis for the 16 quantitative traits are presented in Table 4.8. A strong positive correlation was recorded between days to 50% flowering and days to maturity (r = 0.99), grain yield and number of seeds per plant (r = 0.93), number of primary branches and number of secondary branches (r = 0.85), leaf legth and leaf width (r = 0.83), number of seeds

per plant and number of seeds per pod (r = 0.75) and number of pods per plant and grain yield (r = 0.72). This indicates that all yield components are important for grain yield. Positive correlation was recorded for number of racemes and number of pods per plant (r = 0.61), leaf area and days to 50% flowering (r = 0.060), grain yield and days to maturity (r = 0.58), leaflet length and stem diameter (r = 0.57), leaf width and leaf area (r = 0.56), leaf width and days to maturity (r= 0.56), number of racemes and grain yield (r = 0.56), grain yield and days to 50% flowering (r = 0.55), number of seeds per pod and days to 50% flowering (r = 0.54), leaf width and stem diameter (r = 0.54), number of seeds per plant and days to maturity (r = 0.53), plant height and days to 50% flowering (r = 0.52), leaf width and pod length (r = 0.52), leaflet length and pod length (r = 0.52), number of seeds per pod and days to maturity (r = 0.51), plant height and days to maturity (r = 0.51), number of primary branches and number of pods per plant (r = 0.51) and leaflet length and days to 50% flowering (r = 0.50). It was observed that all these characters in this category are important yield components.

A weak positive correlation was recorded for grain yield and stem diameter (r = 0.001) and grain yield and 100-grain weight (r = 0.01) (Table 4.8). A negative correlation was recorded for leaf width and 100-grain weight (r = -0.44), while a weak negative correlation was recorded for number of seed per plant and leaf width (r = -0.01), number of raceme and stem diameter (r = -0.03), number of seed per pod and number of primary branches (r = -0.11). The strong positive correlation between different traits allows for simultaneous selection.

Table 4.8 Correlation analysis for 16 quantitative traits of pigeonpea

	Traits	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	DT50F	-															
2	DTM	0.99***	-														
3	PH	0.52***	0.51***	-													
4	NPP	0.37*	0.39**	0.14	-												
5	NSOP	0.54***	0.51***	0.12	0.17	-											
6	NSP	0.48***	0.53***	0.25	0.75***	0.39*	-										
7	GW	-0.15	-0.12	0.16	0.06	-0.14	0.08*	-									
8	NPB	0.06	0.09	0.11	0.51***	-0.11	0.31*	0.14	-								
9	NSB	-0.12	-0.11	0.08	0.25	-0.12	0.17	0.15	0.85***	-							
10	GY	0.55***	0.58***	0.25	0.72***	0.35*	0.93***	0.01	0.32*	0.17	-						
11	PL	0.26*	0.22	0.18	0.11	0.17	-0.23**	-0.38*	-0.29*	-0.37*	-0.21	-					
12	SD	0.45*	0.40**	0.24	0.21	0.29*	0.13	-0.27	0.03	-0.11	0.19	0.46**	-			-	
13	LFL	0.50***	0.47***	0.12	0.04	0.26	0.02	-0.41*	-0.06	-0.21	0.00	0.52***	0.49***	-			
14	LW	0.60***	0.56***	0.24	0.07	0.30*	-0.01	-0.44*	-0.12	-0.30*	0.07	0.52***	0.57***	0.82***	-		
15	LA	0.54***	0.49***	0.17	0.33*	0.46***	0.25	-0.06	0.08	0.03	0.35	0.23	0.54***	0.31*	0.56***	-	
16	RCM	0.20	0.18	0.13	0.61***	0.15	0.51***	0.08	0.26	0.26	0.56***	-0.18	-0.03	-0.21	-0.13***	0.16	-
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

*, **,*** significant at 0.05, 0.01, 0.001

Key: DT50F=days to 50% flowering; DTM=days to maturity; NPP=number of pods/plant; NSOP=number of seeds/pod; NSP=number of seeds/plant; 100-gw=100 grain weight; NPB=number of primary branches; NSB=number of secondary branches; GY=grain yield; PL=pod length; SD=stem diameter; LFL=leaflet length; LW=leaflet width; LA=leaf area; RCM=number of racemes

4.3.6 Principal component analysis (PCA)

Data for principal component analysis for the first four components are given in Table 4.9. The first four principal components with eigenvalue greater than 1.0 together accounted for about 73.4 % of the total variation among collection (Table 4.9). The relative discriminating power of the principal axes as indicated by the eigenvalues was high (5. 3%) for axis 1 and low (1.2%) for axis 4. The first principal component (PC1) explained 32.8% of the variation and was associated mainly with days to 50% flowering, days to maturity, grain yield, leaf area and leaf width.

The PC2 was responsible for about 23.8% of the variation and was associated mainly with pod length, number of seondary branches, number of primary brannches, number of raceme, leaf width and number of pods per plant. The proportion of variance explained by PC3 was 9.3% and associated with number of primary branches and number of secondary branches. PC4 accounted for 7.6% of variation and was associated with plant height and 100-grain weight. Days to 50% flowering, days to maturity, number of seed per pod, number of seed per plant, grain yield, pod length, stem diameter, leaflet length, leaf width and leaf area were considered the most important for the characterization of pigeonpea germplasm, as they appeared in the four PCs three times. The remaining characters had lesser contribution to the variation in four PCs and therefore were of minor importance in the characterization of pigeonpea germplasm.

All long duration genotypes collected from the Northern and Eastern Zones tended to be scattered on the lower negative side of the PC1 and PC2 biplot (Figure 4.1). These genotypes were characterized by long days to flower and maturity, and a high number primary and secondary branches, number of racemes and grain yield. Genotypes from the Central Zone tended to be scattered on the positive upper side of PC2 suggesting that they had fewer branches and medium days to flower and mature. The medium duration genotypes collected from the northen and eastern zones tended to scatter on the positive upper side of PC1. The earliest genotype, Komboa, scattered on the positive end of PC1. The genotype is characterized by short days to flower and mature, short plant and 4-5 number of seed per pod. Genotypes collected from the central zone tended to scatter on the positive upper side of PC2. These genotypes were characterized by medium days to flower and maturity, a low number of primary and secondary branches and yielded less than the long duration genotypes.

Table 4.9 Principal component analysis (PCA) for 16 quantitative traits

	PC1	PC2	PC3	PC4
Eigenvalue	5.245	3.72	1.484	1.217
Proportion of variance (%)	32.78	23.75	9.28	7.61
Total variance (%)	32.78	56.53	65.81	73.42
Eigenvectors (loadings)	Eigen vectors			
Days to 50% flowering	**0.396**	0.044	0.103	-0.217
Days to maturity	**0.388**	0.018	0.120	0.230
Plant height	0.197	-0.033	0.035	**-0.687**
Number of pod per plant	0.254	**-0.308**	-0.062	0.224
Number of seed per pod	0.260	0.043	0.248	0.136
Number of seed per plant	0.279	-0.313	0.204	0.119
100-grain weight	-0.099	-0.230	0.187	**-0.464**
Number of primary branches	0.075	**-0.332**	**-0.569**	-0.088
Number of secondary branches	-0.014	**-0.331**	**-0.546**	-0.107
Grain yield	**0.300**	**-0.300**	0.176	0.143
Pod length	0.136	**0.351**	-0.099	0.005
Stem diameter	0.261	0.178	-0.233	0.160
Leaflet length	0.247	0.294	-0.237	-0.058
Leaf width	**0.285**	**0.310**	-0.184	0.052
Leaf area	**0.297**	0.049	-0.104	0.088
Number of racemes	0.145	**-0.315**	0.116	0.226

Values bolded made substantial contribution to total variation

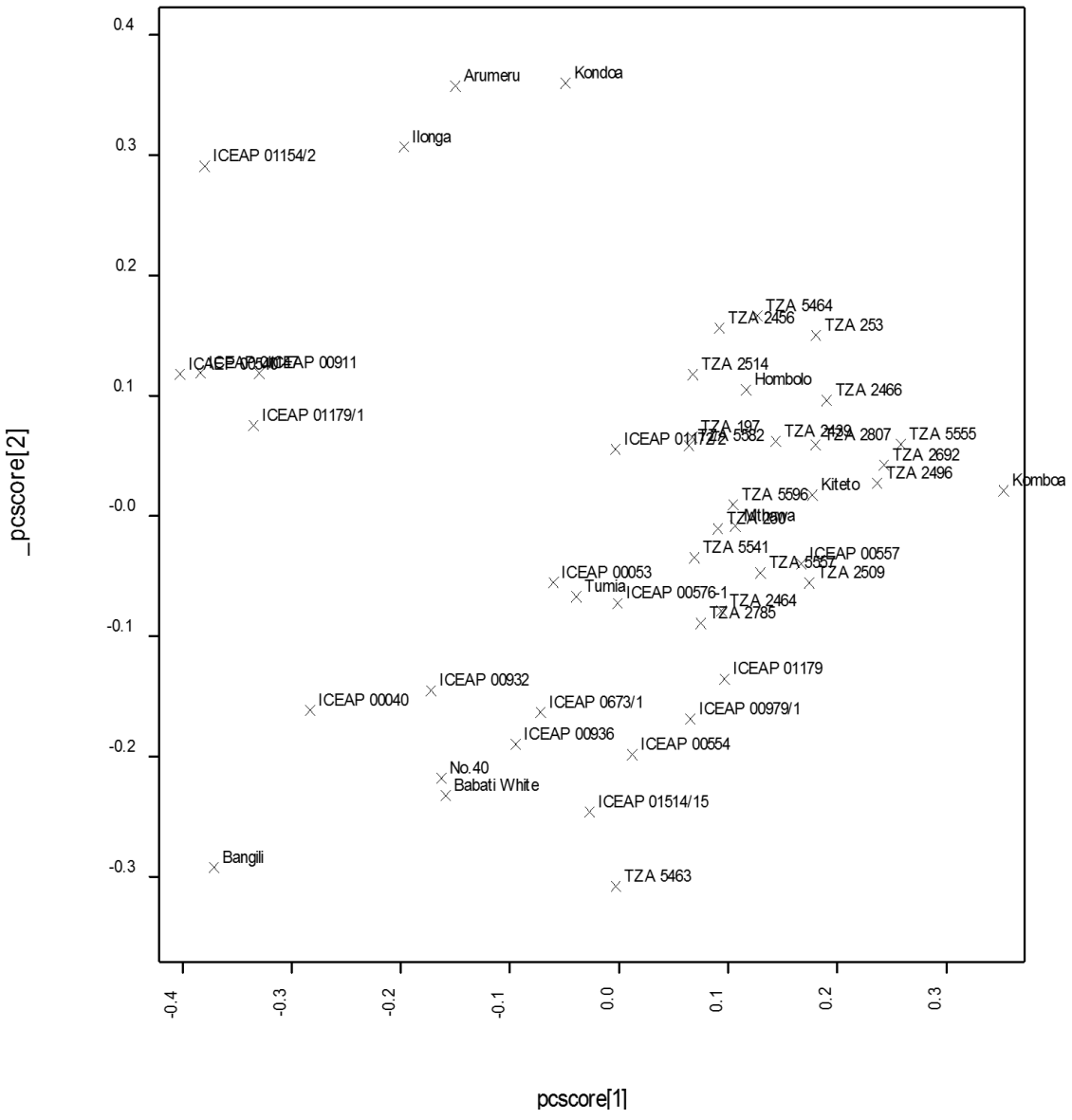

Figure 4.1 Scatter plot for the first two principal component analysis for quantitative traits of pigeonpea genotypes collected from three zones of Tanzania.

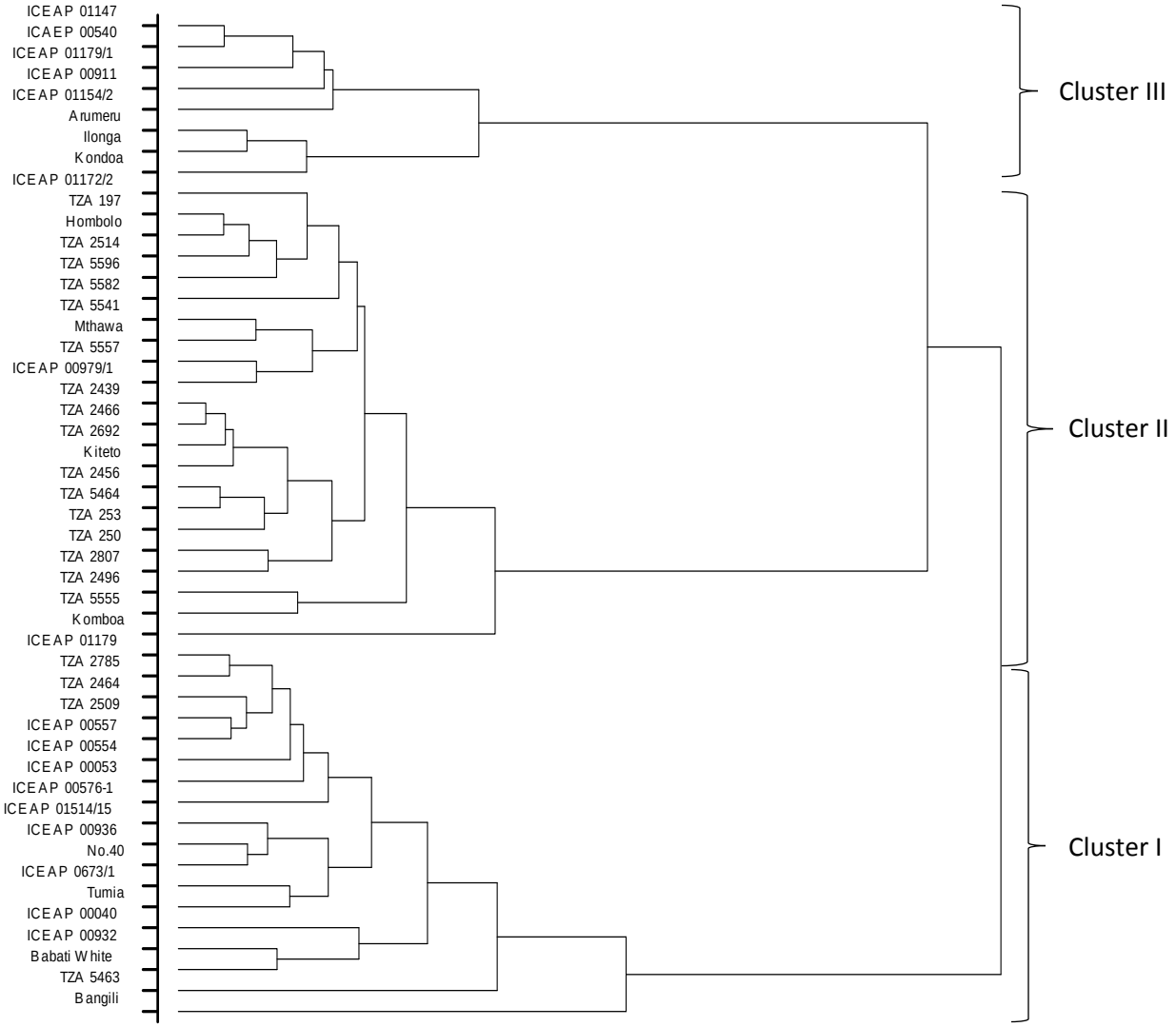

Figure 4.2 Dendrogram showing phenotypic cluster of 48 pigeonpea genotypes based on 16 quantitative traits

4.3.7 Cluster analysis

Table 4.8 showed distribution of 48 genotypes into 3 clusters. The results of multivariate analysis grouped the pigeonpea germplasm into 3 clusters (Figure 4.2). Cluster 1 contained mostly genotypes collected from the northern zone and eastern zone. Cluster 2 comprised of genotypes from all three zones. Cluster 3 contained genotypes from the northern and eastern zone (Figure 4.2). The genotypes in cluster 1 are characterised by late flowering and maturing, have a high

number of primary and secondary branches, number of seeds per plant, number of pods per plant and high grain yield

Table 4.10 Cluster distribution of 48 genotypes based on 16 quantitative traits

Cluster	No. of genotypes	Genotypes
I	17	Bangili, TZA 5463, Babati White, ICEAP 00932, ICEAP 00040, Tumia,ICEAP 0673/1, No. 40, ICEAP 00936, ICEAP 01154/15, ICEAP 00576-1, ICEAP 00053, ICEAP 00554, ICEAP 00557, TZA 2509, TZA 2464, TZA 2785
II	22	ICEAP 01179,Komboa, TZA 5555, TZA 2496, TZA 2807, TZA 250,TZA 253, TZA 5464,TZA 2456, Kiteto, TZA 2692, TZA 2466, TZA 2439, ICEAP 00979/1, TZA 5557, Mtwawanjuni, TZA 5541, TZA 5582, TZA 5596, TZA 2514, Hombolo, TZA 197,
III	9	ICEAP 00172/2, Kondoa, Ilonga, Arumeru, ICEAP 01154/2, ICEAP 00911, ICEAP 01179/1, ICEAP 00540, ICEAP 01147

4.4 Discussion

4.4.1 Qualitative traits

The most important traits responsible for variability observed in this study were stem colour, growth habit, flowering pattern, seed eye colour, base flower colour, pod from, base seed colour and seed colour pattern. Green was the dominant stem colour followed by purple and dark green. The dominance of the green stem colour in African pigeonpea genotypes has previously been reported by Upadhaya *et al*. (2005) and Manyasa *et al*. (2008). However, in an earlier study, Saxena and Sharma (1990) reported that purple was the dominant colour on African landraces. Semi-determinate was the dominant growth habit (97.9%). It has been reported that farmers in Africa prefer the semi-determinate types because of their compatibility in intercropping system. Upadhaya *et al*. (2007) reported that 91% of 1290 pigeonpea collection form Caribbean and Central American regions consisted of semi-determinate genotypes. Semi-determinate was also the dominant flowering pattern. Most of genotypes in this study had indeterminate flowering (89.6%), followed by semi-indeterminate (6.3%) and determinate (4.2%). Several authors

reported the dominance of indeterminate flowering pattern in pigeonpea (Remanandan *et al*., 1988; Upadhaya *et al*., 2005; Manyasa *et al*., 2008; Upadhaya *et al*., 2014).

Out of five phenotypic classes of base colour flowers used in this study, yellow and light yellow colour were predominant with 39.6% and 31.3%, respectively. Seed colour pattern had high variability on plain (64.6%), speckled (27.1%) and mottled/speckled (6.3%). The base seed colour was dominated by cream colour (89.6%). In Northern districts of Tanzania, where pigeonpea is commercialized, farmers prefer large plain, white/cream pigeonpea. These preferences were found important for the external market. The preference for large white/cream pigeonpea was previously reported by Silim *et al*. (2005) and Shiferaw *et al*. (2007). According to Lo Monaco (2006), large, white/cream colour and round grains were associated with high market prices. In this study all genotypes had an oval seed shape. For processing to *dhal* seed shape was the most important grain traits.

All genotypes had a flat pod shape, except TZA 2456. Pod shape is among the consumer preferred traits of vegetable pigeonpea. According to Saxena *et al*. (2010), immature pods that look good in appearance fetch a good price in the market. The consumers of pigeonpea prefer green colour pods because they believed green colour stays attractive for 3-5 days after harvest. Several researchers have reported the preference for green colour in pods (Saxena *et al*., 1983; Pandita and Dahiya, 1988; Patel *et al*., 1996).

The diversity was calculated using Shanon-Weaver diversity index (H') for all 15 qualitative traits and based on the index, the flowering pattern (0.19) and leaf hairiness (0.19) had low diversity index (H') showing little or no variation among genotypes. This results are similar to those report by Upadhaya *et al*. (2005). A high diversity index (H') was recorded for seed eye colour (0.62) and seed colour pattern (0.61). The mean diversity index was 0.33, showing a low general diversity. The low diversity for pigeonpea has also been reported by Manyasa *et al*. (2009).

4.4.2 Quantitative traits

The combined analysis of variance for mean square revealed significant variation for the different traits. According to Upadhaya *et al*. (2005), important quantitative traits to characterize pigeonpea germplasm include days to maturity, plant height, number of pods per plant, number of seeds per pod, number of seeds per pod, number of racemes, number of secondary branches and pod length. Genotypes collected from eastern zone are comprised with short, medium and long duration. The number of days to 50% flowering were ranged from 81-131 and days to maturing ranged of 126-186 days. Genotypes collected from the northern zone had the least days to 50%

flowering (103-148.9 d) and days to maturity (148.6-200 d). The early duration genotypes in this study are best suited for the dry areas in the central zone and some part of western zone.

Genotypes from the northern zone were taller than those from the eastern and central zones. All genotypes from the central zone were medium duration and the plant height ranged between 90.0 and 209.8 cm. Generally long duration genotypes take a long time to mature, they grow taller and produce higher yields than the medium and early duration genotypes.

A high number of seeds per pod has been important in areas where pigeonpea is mainly grown for domestic market and consumed as green vegetable. This is in agreement with observation by Omanga *et al*. (1995) and Shiferaw *et al*. (2007), who reported preferences of farmers for a high number of seeds per pod and seed weight (100-grain weight). A similar observation was also reported by Saxena *et al*. (2010), who reported strong consumer preference for genotypes with many seeds per pod. The number of seeds per pod across all three zones was similar with a mean of 7. A high number of seeds per pod recorded in this study confirms the earlier findings by Silim *et al*. (2005), Upadhaya *et al*. (2005) and Manyasa *et al*., (2008), who reported a higher number of seeds per pod in the African germplasm than in the Indian germplasm.

Grain yield was generally higher for genotypes from the eastern zone (1.4 t/ha) than the northern (1.2 t/ha) and central zones (1.0 t/ha). The number of pods per plant, number of seeds per plant, gram weight and number of primary and secondary branches, were higher for the genotypes collected from northern zone than the genotypes from the central and eastern zones. Genotypes collected from northern zone had high number of primary and secondary branches than genotypes collected from central and eastern zone because of their semi-spreading growth habit. According to Baldey (1998), the semi-spreading long duration genotypes possess higher number of branches than the medium and early maturing genotypes.

4.4.3 Heritability and correlation

The estimate for heritability in broad sense for the different traits ranged from very low to high. The highest heritability was recorded for days to maturity (83%) and days to 50% flowering (71%). According to Bello *et al*. (2010), traits that have a high heritability respond positively to a selection pressure. Grain yield (56%) exhibited a medium heritability. Other traits exhibited low heritability, for example number of racemes (48%), stem diameter (44%), 100-grain weight (43%), leaf area (43%), leaflet length (39%), number of seeds per pod (38%), number of seeds per plant (29%), leaf width (27%), number of secondary branches (24%) and pod length (23%). Based on findings from this study, traits that have medium to high heritability such as grain yield, days to 50%

flowering and days to maturity are the most important for characterization of pigeonpea germplasm.

The correlation coefficient between traits ranged from strong positive to strong negative. Strong positive correlation coefficient were found between days to 50% flowering and days to maturity, number of primary and secondary branches, leaf length and leaf width, number of seed per pod and number of seed per plant number of pod per plant and grain yield. Findings from this study were similar to those reported by Sreelakshmi *et al*. (2011). Upadhaya *et al.* (2014) reported high correlations between days to 50% flowering and days to 75% maturity and between number of seeds per plant and number of pods per plant. According to Singh *et al*. (1990), a strong correlation between yield and yield components would imply that it is possible to improve both traits simultaneously. The 100-grain weight and number of primary branches exhibited a weak positive correlation. This findings is in contrast with what was observed by Musaana and Nahdy, (1998), who reported a strong positive correlation.

A negative correlation was recorded between leaf width and 100-grain weight, while a weak negative correlation was observed between number of seeds per plant and leaf width, number of racemes and stem diameter, number of seeds per pod and number of primary branches. The negative correlation between number of seeds per pod and number of primary branches implies that the number of primary braches was not an important yield component. The same observation was reported by Musaana and Nahdy (1998). In this study the days to 50% flowering, days to maturity, number of seeds per pod, number of seeds per plant, number of pods per plant, grain yield, number of primary and secondary branches, leaf length and leaf width were identified as selection criteria for obtaining good parents in a pigeonpea breeding programme.

4.4.4 Principal component analysis (PCA) and cluster analysis

The main reason for germplasm collection is to obtain natural variability that can be useful for crop improvement. According to Johnson (1998), the PCA is the most useful statistical tool for screening multivariate data. Information obtained through a PCA may assist the plant breeders to identify a limited number of highly differentiated populations for use in a hybridization and selection programs. From the principal component analysis, PC1 (32.7%) accounted for most of the variation with positive loadings. The most important traits to distinguish pigeonpea genotypes were days to 50% flowering, days to maturity, grain yield, leaf width, leaf area, number of seeds per plant stem diameter number of seeds per pod and number of pods per plant. Using the PCA,

some of these traits have also been used extensively to characterize pigeonpea by Rao *et al*. (2010) and Rheka *et al*. (2013).

The hierarchical cluster analysis conducted on the 16 quantitative traits grouped the genotypes into three clusters, indicating sufficient variability to warrant selection. Manyasa *et al*. (2008) grouped 123 pigeonpea into six clusters. The grouping of pigeonpea into six clusters have been reported by Birhan *et al*. (2013) and Rupika *et al*. (2014) who observed 100 and 90 genotypes grouped into six clusters. Shunyu *et al*. (2013) observed 30 pigeonpea genotypes grouped into seven phenotypic clusters. In this study, it was observed that clustering of genotypes from different Zones into one cluster could be attributed to exchange of breeding materials. A greater diversity in the pigeonpea germplasm will offer a good scope for pigeonpea improvement programmes.

Conclusions

The results showed significant variations for a range of qualitative and quantitative traits in pigeonpea germplasm. The heritability estimates suggests that days to 50% flowering, days to maturity and grain yield are important traits for pigeonpea characterization. Positive correlation among different traits indicates that simultaneously selection of two traits can be done. The principal component analysis was performed to identify the most important traits for characterization. Days to 50% flowering, days to maturity, number of pods per plant, number of seeds per plant, number of primary branches, number of secondary branches, grain yield, stem diameter, leaf width and leaf area were identified as the most desirable traits. Furthermore, the cluster analysis grouped the genotypes into three clusters. The genotypes Bangili, TZA 5463, Babati White, ICEAP 00040 and ICEAP 00932 had desirable phenotypic attributes and were identified as good parents for a pigeonpea breeding programme in Tanzania.

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Chapter 5 : Assessment of genetic diversity of pigeonpea germplasm from Tanzania using SSR markers

Abstract

Understanding the genetic diversity and genetic relationships in plant species is crucial for a breeding programme and the efficient utilization of germplasm for genetic improvement. The aim of this study was to assess the genetic diversity of pigeonpea genotypes grown in Tanzania. The 48 pigeonpea genotypes were genotyped using 35 simple sequence repeat (SSR) markers that were polymorphic. All amplifications were in a range of 117 to 280 bp. The informative marker combinations revealed a total of 162 alleles at 35 loci, with an average of 4.63 alleles detected per marker. Variations in the gene diversity (H_e) were in a range of 0.03-0.83, with an average of 0.45. The heterozygosity (H_o) values ranged from 0.00-0.635, with an average of 0.279. The polymorphism information content (PIC) varied from 0.032 to 0.806, with an average of 0.412. The Tanzania pigeonpea germplasm was grouped into five main clusters and nine sub-clusters. The genetic similarity index revealed a high similarity between long duration landraces and long duration improved genotypes. The released pigeonpea varieties in Tanzania were grouped into the various clusters, which indicates that there is sufficient genetic diversity among the cultivated pigeonpea germplasm.

5.1 Introduction

Pigeonpea (*Cajanus cajan* (L.) Millsp) is an important legume crop grown by small-scale farmers in Africa and Asia in diverse environments (Mligo *et al*., 2001). The crop has the ability to produce economic yield under low moisture condition making it an important crop in dry areas (Goud *et al*., 2012). It is one of the under-researched crops in many countries. In Tanzania, the crop is grown in several regions as a food crop and cash crop (Technoserve-TA and ICRISAT/SARI, 1990; Shiferaw *et al*., 2005). Tanzania is one of the top six global exporters of pigeonpea to Asian market.

Pigeonpea is considered as a valuable crop of poor people and has diverse uses. It improves soil physical properties and yield of associated intercropped crops while simultaneously yielding marketable grain and thick stems are mainly used as fuel wood and roofing material (Odeny, 2007). It is mainly used as animal feed in Nigeria (Kabuo *et al*., 2015). It provides an important source of protein for low-resource farmers who cannot afford animal products (Saxena, 2010). In Australia, pigeonpea is used to reduce population of *Helicoverpa armigera* in Bt cotton production (Baker and Tann, 2013). Furthermore, as a legume, pigeonpea fix atmospheric N more than any other legume crops and saves money that could have been used to purchase N fertilizers (Peoples *et al*., 1995). Pigeonpea acts as windbreak and contour hegdes to control erosion (Tesema, 2007) and It also provides cash income for household (Mergeai *et al*., 2001).

Genetic diversity in pigeonpea has been studied using morphological and agronomic traits (Upadhaya *et al*., 2005; Upadhaya *et al*., 2007; Manyasa *et al*., 2008). In recent years, genetic diversity of pigeonpea has been analysed by using DNA molecular markers, as these are not influenced by environment (Saxena *et al*., 2009). However, the choice of markers to be used depends on the availability of genetic information about the genome sequence, cost for marker development, ease of documentation and polymorphism (Mittal and Dubey, 2009). Among the DNA molecular markers, the SSR markers have been found the most suitable for use in pigeonpea (Gupta and Varshney, 2000). A number of studies have been reported using SSR markers (Sousa *et al*., 2011; Njung'e *et al*., 2016). The SSR markers are chosen because of high polymorphism, detection of multi-allelic variation, co-dominance, reproducible, ease of detection by PCR, relatively abundance with a uniform coverage, require small amount of DNA and act as universal genetic marker for the genetic reagent mapping (Powel *et al*., 1996; Mittal and Dubey, 2009; Saxena *et al*., 2009). Cultivated pigeonpea is known to have low polymorphism, hence SSR markers are ideal for studying the genetic diversity.

In Tanzania, no study of molecular characterization of pigeonpea has been reported. Knowledge of genetic diversity and relationships among pigeonpea germplasm grown in Tanzania using simple sequence repeats (SSR) molecular markers will play a significant role in breeding programmes to improve production, quality traits, biotic and abiotic stresses that plant breeders can use as a parental selection tools. Thus, estimation and quantification of genetic diversity are pre-requisite for genetic improvement. Therefore, the aim of this study was to assess the genetic diversity of Tanzanian pigeonpea germplasm using SSR markers.

5.2 Materials and methods

5.2.1 Plant material

The study material consisted of 48 pigeonpea genotypes representing traditional landraces, farmers varieties and improved genotypes collected from northern, central and eastern zones of Tanzania. The detailed information about the material used in this study is given in Chapter 4.

5.2.2 DNA extraction

Prior to deoxyribonucleic acid (DNA) extraction, seeds of all 48 genotypes were planted at Mikocheni screenhouse in pots. The genomic DNA was extracted from fresh leaf material from 10-14 days old plants of each of the 48 pigeonpea entries and was ground to fine powder in liquid nitrogen, following the cetyl trimethyl ammonium bromide (CTAB) method as described by Mace *et al*. (2003), with some modification. For all samples, the DNA quality was determined by agarose gel electrophoresis (0.8% (w/v) stained with 5 µ/100 ml Gel® (Biotium Inc., USA), while the quantity was determined by spectrophotometry (Nanodrop© 100, Thermo Scientific, USA). The DNA samples were analysed at ICRISAT-laboratory, Centre of Excellence in Genomics, India.

5.2.3 PCR amplification

The study used 35 SSR markers (Table 5.1). The markers used for PCR amplification were all polymorphic and selected based on sequence information. The markers used and their characteristics are presented in Table 5.2. The PCR amplification was optimized and conducted in a reaction buffer of 12.5 µL containing 1 x PCR buffer; 1 Unit Taq DNA polymerase; 0.2 mM each of dATP, dGTP and dTTP; 3 mM of MgCl2, 0.1 µM of respective forward and reverse primer and 40ng of genomic DNA. The PCR amplification was carried out in a Bioer XP Thermal Cycler (Hangzhou Bioer Technologies, Hangzhou, China). The thermal cycling conditions were as

follows: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation (94°C) for 1 min, annealing (56-72°C) for 1 min, primer extension (72°C) for 1 min, followed by an extension at 72°C for 20 min. The amplification products were analysed by electrophoresis on a 2.8% agarose gel, stained with ethidium bromide and photographed under short wavelength UV light in a gel documentation system. A 100 bp DNA ladder (MBI Fermentas, Germany) was used as standard.

5.2.4 Data analysis

PowerMarker 3.25 (Liu and Muse 2005) was used to determine the polymorphic information content (PIC), gene diversity and heterozygosity values for each SSR marker used in the study. The expected heterozygosity (H_e) and observed heterozygosity (H_o) values were used to evaluate the genetic diversity within the set of genotypes. Expected heterozygosity, i.e. the probability that two alleles from the same locus would be different when chosen at random, was calculated for each SSR locus according to Nei (1973) as:

$$H_e = 1 - \sum (p_i)^2$$

Where $p_{i\ iS}$ the frequency of the ith allele in a locus for individual p.

Observed heterozygosity was calculated by dividing the number of heterozygous individuals by the number of individuals scored. Polymorphic information content for the SSR markers in the sample DNA was calculated as:

$$PIC = 1 - \sum p_i^2$$

For co-dominant markers such as SSRs and RFLPs, data can be scored as allele frequencies and as binary traits (1 = allele presence, 0 = allele absence) (Warburton and Crossa, 2002). The allele frequency data from PowerMarker 3.25 was used to export the data in binary format for analysis with NTSYS-PC 2.1 software (Exeter Software, Setauket, NY, USA) (Dice, 1945). NTSYS-PC 2.1 only accepts binary data coding. The 0/1 matrix was used to calculate genetic similarity based on the dice coefficient (Dice, 1945). A dendrogram was constructed using the unweighted paired group method using arithmetic averages (UPGMA) method as implemented in NTSYS-PC 2.1 to infer genetic relationships and phylogeny. The MXComp function was used to determine the R value for the dendogram (Rohlf, 1998).

5.3 Results

5.3.1 Genetic diversity

The marker pairs, allele number and frequency, gene diversity, heterozygosity and PIC are presented in Table 5.2. The sizes of amplified polymorphic DNA fragments (bands) ranged from 117 to 280bp (Table 5.1). A total of 162 alleles were amplified among the 48 pigeonpea and the numbers of alleles scored for 35 loci ranged from two to eleven with an average of 4.63 (Table 5.2). The maximum number of (11) alleles were detected at the CcM0246 and CcM0443 locus. The PIC value of the SSR markers, which is a measure of allele diversity at a locus, ranged from 0.032 to 0.806 with an average of 0.412. Ten SSR loci (CcM0195, CcM0246, CcM0381, CcM0443, CcM0492, CcM0721, CcM0956, CcM0974, CcM2704 and CcM2895) exhibited PIC values higher than 0.6. PIC is an indicator of how well a marker is able to distinguish the samples tested due to the diversity of alleles detected across the samples. The gene diversity values were in the range of 0.0322 to 0.8277, with an average of 0.4466. The recorded heterozygosity values ranged from 0.0 to 0.635 with an average of 0.279 (Table 5.2).

Table 5.1 Primer sequences of the 35 SSR markers used in this study

[a]Marker name	GenBank ID	[b]SSR motif	Forward primer (5'-3')	Reverse primer (5'-3')	Product size (bp)
CcM0121	FI191501	(TA)17	AGAAATTGGAGGCTTGGTCA	GGTATAAGGCTCAAACCCGA	273
CcM0443	FI200654	(TA)17n(AT)5	TGACAAAATAATGCGGTCAC A	CAAGCCAAAGTTTGTTTGAAC T	261
CcM2044	FI245729	(TAT)9	ATCACTCCAAGCACCCAAAC	TGCAAATGGAAGGGAATAGC	212
CcM0444	FI200657	(TA)7	TGTCATGAGTGGCTGATCCT	TCAACCAAAATCCAAACCAA	184
CcM0494	FI202253	(AT)21	ACGTGAAAAATCCGCAACTT	GCTTGTGTTTCAAAATCCAAC TT	117
CcM1251	FI224872	(CCA)9	CAAATGGCAGAACAGAGCAG	CGGAGATTGCATTGTTCCTT	228
CcM2097	FI246959	(CT)12	TGATAGGAATATTTCGGCGG	CCTTTGAAATTGAAGGCGAG	193
CcM2409	FI255641	(TTA)6	TGAAGGTTGATCCAAGGAGG	CGTGCAAAATAATTGTCCAA AA	186
CcM0974	FI216621	(AT)13	CGTCTTACAGACGATCTGCAT C	CAAAGAAACAGACATGATAA AGAGAGA	161
CcM2379	FI254391	(TC)10	CCGGAAAAATTGCCTATTGA	TTCGATGACAGAATTTAGGTG C	151
CcM2895	FI272645	(AT)24	AATGATAATTGGACACTTCTT TTTC	TGCGTTAATTAAACAAGCAA GC	268
CcM0246	FI195094	(AT)16	ATGGAGCCAAAGTGTCCAAG	ATGAAAAGCAACTACGCGCT	226
CcM0721	FI209310	(AT)19	ATCCAACCACGTGTTTCACA	TTTGAAATGGTATCGATGATT AAA	169
CcM0195	FI193462	(AT)11	CAACAATAAAGCATAAACCA CCA	TGACGTAGATTGGGTAGTTA GGA	223
CcM0361	FI198648	(TA)9	TCTTCCTGTCCTCATCCTCG	TGGAAACCAAAGTTGTGCAT	172
CcM0374	FI198903	(TA)11	GAACCGTCTTAAAATTTCTCA TTT	CAATGGCACATTGTCAAAAA	161
CcM0484	FI201979	(T)12n(ATT)5n(AT)5	TGGAAATTAAACACCATGAA ACA	TGCATGCTACCAAGGAATTG	248
CcM2049	FI245893	(TAT)9	GCGACCAGGTACTTTCAAGC	CGAAAAGCGATTTCAGAATT T	260
CcM0594	FI205393	(GA)9n(TC)9	GGCTTGGTTCTTTCTTGGTG	AAGTCCCTGACTTTCCCCAT	185
CcM0956	FI216271	(AT)16	AGCCCCAACTCAATTATCAA A	TTCCTTGCGGTTTGAGCTAT	224
CcM1357	FI227664	(AT)15n(ATA)5	TCTAGCATCTCCATTAAACCA TTT	ACACATATGACATTTAGCAA ATAAAAA	280
CcM2004	FI244896	(CT)7n(AG)12	AGGAATGCGACATTTTGGAG	TCCCCATCCCTTTCTTTCTT	209
CcM0492	FI202198	(AT)21	AAAATTTACGAGCACTAAAA TGAAAAA	TCAACAATAAATTGTCATATG TCTGG	271
CcM0698	FI208758	(AAT)17	CTCTTCTTGTTGTCCCTCGC	GCAGTTCTGGAATACCTCGC	188
CcM0785	FI210851	(AT)9	GCATGTGTTTTTACTTGAGTC GTC	TGGAGGCGATCTCTTTCTTG	277
CcM1982	FI244391	(TC)17	TATCAAACCTGGCGATCACA	ATTCCGCAAACACATCACAA	246
CcM2704	FI265930	(AT)10	AAAAATGTTCAATGTCGTAGT ATTTGA	TGCCATATATCATGCCCTCA	127
CcM0248	FI195265	(TA)8	CAAACTCAACCCTACCAATG C	CATTCCTTGTCATCAATGAAG TTT	280
CcM0673	FI208212	(AT)6(AG)9	TGACCACCAACCATTACCAA	CATGCACCAGACCAGAATCA	272
CcM1045	FI219229	(AT)6	AACCTTAGTTGGTGATAGATT TCAGA	ACCGTCAAGTCCCAAATCAC	262
CcM2394	FI255036	(TC)12	TGGAAACGATTTCCTACCACA	ACAAGGGGAAAAGGGAAAG A	260
CcM0381	FI199172	(TA)21	CGATCCCTGCTTGAAATCAT	GGTTCAAGCGATGCACTACA	267
CcM0834	FI212739	(AT)10	GTCCGGCTTGCCTATAAGGT	AAGGCAACCTCCCCAGTATT	262
CcM2505	FI259241	(GAA)8	CCTCGGAAGAGATTGCAGTT	TGATGAATTGGGAAGCAACA	201
CcM2697	FI265781	(CT)9n(T)14	AGAGTTCGGTGACGGTTACG	GATCTGTCGAGGTTGAGGCT	242

Source: Bohra *et al*. 2011

Table 5.2 Details of polymorphisms and genetic parameters of 35 SSR markers across 48 pigeonpea genotypes

Marker	Major Allele Frequency	No. of Obs.	Allele No.	Availability	Gene Diversity	Heterozygosity	PIC
CcM0121	0.7131	61	5	0.9683	0.4621	0.213	0.432
CcM0195	0.4206	63	4	1.0000	0.6721	0.413	0.607
CcM0246	0.2984	62	11	0.9841	0.8225	0.387	0.801
CcM0248	0.7258	62	4	0.9841	0.4437	0.371	0.412
CcM0361	0.5702	57	4	0.9048	0.6125	0.035	0.569
CcM0374	0.9127	63	3	1.0000	0.1616	0.079	0.153
CcM0381	0.3167	60	7	0.9524	0.7750	0.267	0.742
CcM0443	0.2698	63	11	1.0000	0.8277	0.508	0.806
CcM0444	0.9524	63	4	1.0000	0.0921	0.095	0.091
CcM0484	0.8934	61	2	0.9683	0.1904	0.180	0.172
CcM0492	0.2917	60	9	0.9524	0.8024	0.467	0.776
CcM0494	0.5794	63	6	1.0000	0.6167	0.302	0.583
CcM0594	0.9000	60	2	0.9524	0.1800	0.133	0.164
CcM0673	0.6066	61	2	0.9683	0.4773	0.328	0.363
CcM0698	0.9500	60	3	0.9524	0.0961	0.000	0.094
CcM0721	0.4754	61	6	0.9683	0.7052	0.508	0.671
CcM0785	0.8629	62	2	0.9841	0.2366	0.209	0.209
CcM0834	0.8571	63	4	1.0000	0.2523	0.191	0.233
CcM0956	0.5196	51	6	0.8095	0.6561	0.333	0.613
CcM0974	0.4435	62	8	0.9841	0.7375	0.468	0.709
CcM1045	0.9127	63	2	1.0000	0.1594	0.143	0.147
CcM1251	0.5769	52	2	0.8254	0.4882	0.346	0.369
CcM1357	0.6000	60	5	0.9524	0.5419	0.433	0.471
CcM1982	0.8607	61	4	0.9683	0.2517	0.197	0.240
CcM2004	0.9180	61	3	0.9683	0.1517	0.164	0.142
CcM2044	0.4127	63	4	1.0000	0.6565	0.635	0.587
CcM2049	0.8548	62	3	0.9841	0.2582	0.258	0.242
CcM2097	0.5574	61	5	0.9683	0.5752	0.426	0.503
CcM2379	0.9836	61	2	0.9683	0.0322	0.000	0.032
CcM2394	0.9435	62	3	0.9841	0.1073	0.081	0.103
CcM2409	0.7381	63	3	1.0000	0.3907	0.206	0.320
CcM2505	0.9590	61	2	0.9683	0.0786	0.082	0.076
CcM2697	0.5397	63	4	1.0000	0.5704	0.349	0.488
CcM2704	0.3934	61	10	0.9683	0.7647	0.508	0.735
CcM2895	0.3197	61	7	0.9683	0.7839	0.459	0.752
Mean	**0.6608**	**60.94**	**4.63**	**0.9673**	**0.4466**	**0.279**	**0.412**

Table 5.3 Genetic similarity among 48 pigeonpea genotypes generated using 35 SSR primer combinations based on Dice's similarity coefficient

Geno	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
Tumia	1.00																																															
No.40	0.59	1.00																																														
Hombolo	0.54	0.56	1.00																																														
I 001172	0.58	0.60	0.65	1.00																																												
T 5582	0.58	0.48	0.60	0.65	1.00																																											
T 5555	0.55	0.52	0.66	0.61	0.73	1.00																																										
T 2464	0.56	0.63	0.63	0.60	0.60	0.65	1.00																																									
T 5541	0.54	0.64	0.64	0.65	0.57	0.57	0.62	1.00																																								
I 00040	0.56	0.57	0.61	0.65	0.60	0.57	0.51	0.53	1.00																																							
I 0576-1	0.57	0.60	0.62	0.62	055	0.55	0.55	0.57	0.82	1.00																																						
I 001179	0.57	0.61	0.66	0.65	0.62	0.64	0.63	0.61	0.62	0.60	1.00																																					
T 2466	0.52	0.65	0.58	0.68	0.63	0.59	0.66	0.63	0.63	0.62	0.61	1.00																																				
I 0673/1	0.49	0.58	0.67	0.72	0.57	0.64	0.56	0.64	0.53	0.52	0.64	0.57	1.00																																			
Ilonga	0.60	0.66	0.64	0.69	0.61	0.65	0.67	0.72	0.58	0.53	0.65	0.69	0.66	1.00																																		
T 5464	0.48	0.58	0.55	0.65	0.57	0.52	0.59	0.57	0.52	0.43	0.49	0.53	0.49	0.63	1.00																																	
Komboa	0.42	0.48	0.48	0.55	**0.37**	0.51	0.48	0.48	0.41	0.44	0.43	0.45	0.58	0.46	0.42	1.00																																
T 197	0.54	0.72	0.55	0.63	0.61	0.62	0.54	0.71	0.56	0.57	0.57	0.65	0.54	0.67	0.53	0.42	1.00																															
I 1154/2	0.56	0.57	0.62	0.67	0.57	0.63	0.57	0.57	0.60	0.60	0.60	0.57	0.62	0.57	0.53	0.49	0.51	1.00																														
T 250	0.61	0.62	0.59	0.54	0.46	0.55	0.63	0.58	0.53	0.57	0.59	0.59	0.53	0.59	0.45	0.46	0.51	0.59	1.00																													
I 00936	0.53	0.57	0.59	0.63	0.60	0.57	0.49	0.51	**0.94**	0.79	0.59	0.60	0.51	0.56	0.52	0.38	0.56	0.59	0.53	1.00																												
I 01147	0.60	0.58	0.60	0.65	0.54	0.63	0.63	0.57	0.64	0.60	0.73	0.65	0.64	0.65	0.57	0.45	0.54	0.65	0.69	0.62	1.00																										
I 00911	0.56	0.63	0.61	0.62	0.58	0.57	0.56	0.59	0.58	0.59	0.64	0.60	0.55	0.58	0.51	0.52	0.66	0.59	0.56	0.63	0.59	1.00																									
Kiteto	0.59	0.66	0.68	0.69	0.56	0.69	0.59	0.67	0.72	0.68	0.67	0.65	0.64	0.71	0.58	0.48	0.67	0.60	0.63	0.69	0.69	0.57	1.00																								
I 00053	0.68	0.62	0.56	0.55	0.55	0.53	0.63	0.51	0.67	0.71	0.67	0.63	0.47	0.58	0.53	0.38	0.53	0.59	0.58	0.64	0.62	0.64	0.62	1.00																							
Kondoa	0.48	0.56	0.61	0.61	0.63	0.68	0.60	0.57	0.79	0.64	0.64	0.60	0.57	0.59	0.55	0.44	0.61	0.53	0.53	0.74	0.66	0.55	0.66	0.60	1.00																						
T 5463	0.64	0.72	0.70	0.74	0.61	0.62	0.67	0.67	0.64	0.63	0.71	0.67	0.66	0.69	0.69	0.51	0.62	0.65	0.64	0.62	0.67	0.68	0.69	0.67	0.61	1.00																					
T 5557	0.62	0.69	0.53	0.63	0.62	0.67	0.65	0.61	0.55	0.51	0.62	0.59	0.57	0.70	0.65	0.44	0.64	0.61	0.53	0.52	0.59	0.57	0.65	0.60	0.59	0.65	1.00																			
T 2509	0.59	0.65	0.66	0.70	0.59	0.63	0.65	0.60	0.64	0.61	0.62	0.73	0.59	0.67	0.53	0.45	0.67	0.65	0.53	0.62	0.61	0.71	0.64	0.64	0.54	0.69	0.65	1.00																	
I 00540	0.54	0.67	0.63	0.75	0.50	0.59	0.58	0.64	0.66	0.60	0.64	0.69	0.69	0.66	0.51	0.57	0.64	0.66	0.59	0.63	0.66	0.65	0.68	0.61	0.60	0.70	0.64	0.71	1.00																
T 253	0.42	0.47	0.47	0.45	**0.35**	0.49	0.49	0.48	0.46	0.41	0.46	0.43	0.42	0.42	**0.36**	0.39	0.43	0.46	0.53	0.46	0.51	0.51	0.41	0.48	0.53	0.49	0.42	0.47	0.47	1.00															
T 5596	0.53	0.65	0.58	0.59	0.51	0.61	0.67	0.57	0.62	0.58	0.60	0.63	0.51	0.61	0.52	0.40	0.62	0.58	0.58	0.59	0.68	0.57	0.62	0.52	0.57	0.63	0.62	0.72	0.59	0.59	1.00													
I 00557	0.62	0.65	0.68	0.67	0.58	0.61	0.64	0.66	0.64	0.62	0.72	0.61	0.64	0.66	0.58	0.47	0.62	0.71	0.58	0.61	0.63	0.64	0.62	0.66	0.59	0.71	0.76	0.70	0.74	0.46	0.66	1.00												
I 01147/1	0.51	0.55	0.59	0.63	0.51	0.56	0.59	0.49	0.55	0.55	0.60	0.65	0.58	0.59	0.46	0.53	0.53	0.59	0.48	0.52	0.61	0.55	0.60	0.60	0.55	0.54	0.63	0.64	0.68	0.40	0.53	0.72	1.00											
I 00554	0.59	0.64	0.59	0.62	0.49	0.59	0.58	0.58	0.56	0.64	0.61	0.57	0.62	0.60	0.47	0.50	0.59	0.65	0.56	0.53	0.61	0.61	0.63	0.63	0.53	0.66	0.64	0.65	0.69	0.51	0.64	0.70	0.59	1.00										
Mtawa	0.47	0.50	0.48	0.62	0.41	0.51	0.46	0.52	0.48	0.53	0.59	0.43	0.56	0.51	0.39	0.56	0.50	0.55	0.59	0.46	0.61	0.51	0.53	0.43	0.52	0.57	0.51	0.47	0.59	0.48	0.51	0.54	0.59	0.57	1.00									
T 2456	0.59	0.63	0.69	0.65	0.63	0.69	0.71	0.66	0.64	0.66	0.68	0.66	0.62	0.65	0.56	0.52	0.68	0.57	0.56	0.60	0.59	0.62	0.68	0.58	0.65	0.73	0.70	0.71	0.62	0.47	0.67	0.69	0.61	0.61	0.53	1.00									
I 1514/15	0.36	0.48	0.45	0.50	0.40	0.56	0.48	0.48	0.46	0.44	0.51	0.48	0.54	0.48	0.42	0.53	0.50	0.52	0.43	0.46	0.45	0.57	0.48	0.42	0.51	0.55	0.44	0.45	0.52	0.47	0.50	0.50	0.51	0.43	0.61	0.49	1.00								
T 2496	0.56	0.62	0.62	0.63	0.62	0.61	0.65	0.62	0.55	0.59	0.62	0.62	0.56	0.64	0.58	0.48	0.64	0.59	0.62	0.55	0.63	0.59	0.62	0.56	0.58	0.70	0.71	0.65	0.64	0.50	0.66	0.67	0.58	0.61	0.51	0.78	0.53	1.00							
T 2692	0.52	0.66	0.59	0.69	0.57	0.62	0.61	0.74	0.61	0.62	0.62	0.65	0.64	0.66	0.62	0.51	0.66	0.56	0.56	0.59	0.64	0.61	0.66	0.51	0.58	0.68	0.62	0.59	0.61	0.44	0.59	0.59	0.54	0.63	0.58	0.67	0.51	0.63	1.00						
Aru	0.53	0.59	0.51	0.55	0.48	0.47	0.48	0.51	0.84	0.74	0.54	0.60	0.47	0.54	0.42	0.37	0.48	0.52	0.49	0.84	0.55	0.55	0.59	0.67	0.63	0.59	0.49	0.62	0.61	0.48	0.59	0.61	0.49	0.58	0.45	0.60	0.42	0.48	0.54	1.00					
Bbt White	0.52	0.63	0.62	0.64	0.61	0.56	0.55	0.56	**0.89**	0.74	0.58	0.66	0.50	0.57	0.51	0.37	0.58	0.61	0.57	0.89	0.58	0.59	0.65	0.62	0.72	0.67	0.53	0.63	0.62	0.50	0.63	0.63	0.54	0.55	0.46	0.68	0.45	0.59	0.60	0.82	1.00				
I 1179/1	0.57	0.66	0.64	0.59	0.52	0.53	0.56	0.58	0.82	**0.94**	0.63	0.66	0.52	0.54	0.43	0.44	0.57	0.58	0.62	0.76	0.60	0.59	0.68	0.73	0.67	0.67	0.53	0.62	0.62	0.43	0.59	0.65	0.55	0.63	0.50	0.68	0.44	0.59	0.62	0.73	0.79	1.00			
I 00932	0.55	0.61	0.60	0.64	0.60	0.56	0.51	0.53	**0.93**	0.78	0.58	0.64	0.51	0.57	0.52	0.40	0.56	0.61	0.55	0.93	0.64	0.60	0.68	0.63	0.73	0.66	0.54	0.63	0.65	0.47	0.61	0.66	0.56	0.57	0.48	0.63	0.45	0.54	0.58	0.86	**0.93**	0.81	1.00		
T 2514	0.51	0.58	0.67	0.64	0.69	0.59	0.61	0.61	0.59	0.55	0.60	0.67	0.50	0.62	0.63	0.38	0.65	0.56	0.49	0.56	0.56	0.56	0.58	0.62	0.62	0.60	0.66	0.71	0.57	0.44	0.66	0.72	0.63	0.56	0.48	0.72	0.41	0.71	0.59	0.53	0.63	0.57	0.61	1.00		
T 2439	0.61	0.59	0.67	0.70	0.67	0.67	0.66	0.65	0.63	0.57	0.72	0.66	0.63	0.79	0.59	0.39	0.63	0.63	0.59	0.60	0.69	0.56	0.69	0.63	0.64	0.67	0.73	0.69	0.65	0.54	0.65	0.72	0.66	0.66	0.52	0.72	0.43	0.72	0.66	0.58	0.66	0.59	0.64	0.73	1.00		
T 2785	0.56	0.54	0.65	0.60	0.61	0.64	0.69	0.72	0.61	0.62	0.61	0.65	0.57	0.66	0.53	0.42	0.56	0.59	0.64	0.58	0.64	0.58	0.70	0.60	0.65	0.61	0.64	0.61	0.61	0.53	0.56	0.63	0.59	0.54	0.53	0.68	0.44	0.68	0.63	0.53	0.62	0.64	0.60	0.64	0.77	1.00	
Bangili	0.54	0.60	0.59	0.63	0.58	0.55	0.54	0.54	**0.93**	0.80	0.60	0.66	0.52	0.54	0.48	**0.38**	0.55	0.60	0.54	0.93	0.63	0.61	0.67	0.65	0.72	0.65	0.53	0.67	0.64	0.48	0.65	0.65	0.55	0.56	0.49	0.68	0.46	0.56	0.59	**0.91**	**0.90**	0.80	**0.94**	0.60	0.61	0.62	1.00	
T 2807	0.65	0.66	0.59	0.66	0.58	0.59	0.60	0.56	0.81	0.77	0.64	0.62	0.50	0.59	0.58	0.43	0.64	0.59	0.57	0.75	0.60	0.59	0.64	0.68	0.67	0.67	0.61	0.70	0.62	0.48	0.63	0.69	0.58	0.57	0.49	0.69	0.40	0.60	0.63	0.72	0.78	0.79	0.77	0.63	0.69	0.63	0.76	1.00

Key: Geno= Genotype, I= ICEAP, T= TZA, Mtawa= Mtawanjuni, Aru= Arumeru, Bbt White= Babati White

Figure 5.1 Computer Screen shot of GeneMapper ® peaks for marker CcM0381. Samples A, B, C and D shows different allele size (bp) for the same marker

5.3.2 Genetic similarity

The estimates of genetic similarities matrices based on SSR marker data for the 48 pigeonpea genotypes are presented on Table 5.3. The genetic similarity, which is used to compare genotypes were relatively high (r = 0.84). The estimates of similarity coefficients among the 48 pigeonpea ranged from 35% to 94% (Table 5.3). The lowest similarity index of 35% was obtained between TZA 5582 and TZA 253, followed by TZA 2464 and TZA 253 (36%) and Komboa and TZA 5582 (36%). The highest similarity index of 94% was obtained between ICEAP 0673/1 and ICEAP 00936, followed by ICEAP 0673/1 and Bangili (93%), ICEAP 0673/1 and ICEAP 00932 (93%) and ICEAP 00936 and ICEAP 00932 (93%) (Table 5.3).

Based on their molecular profiles, the 48 pigeonpea genotypes were grouped into five main clusters (Figure 5.2). Cluster 1, 2 and 3 are the smallest clusters. Cluster 3 is comprised of genotypes that are the most divergent. Cluster 1 contains one genotype (TZA 253) with the lowest genetic similarity of 35% (TZA 5582 and TZA 253), which is a traditional landraces collected from Northern Zone of Tanzania. Cluster 2 consists of three improved genotypes (Komboa, Mthawanjuni and ICEAP 001514/15). Two genotypes (Mthawanjuni and ICEAP 001514/15) were collected from Northern Zone. Mthawanjuni is a popular traditional genotype commonly grown in Malawi. Cluster 3 consists of one improved genotype (ICEAP 001147/1) and two landraces (TZA 5464 and TZA 250).

Cluster 4 is the largest group and contains 28 genotypes, which was divided into seven sub-clusters. Sub-cluster 4.1 has two traditional landraces (TZA 5582 and TZA 250). Sub- cluster 4.2 has two improved genotypes (ICEAP 001154/2 and ICEAP 00554). Sub-cluster 4.3 has three traditional landraces (TZA 2514, TZA 2496 and TZA 2456) and one improved genotype (ICEAP 00557). Sub-cluster 4.4 has four traditional landraces (TZA 5596, TZA 2509, TZA 2466 and TZA 2464). Sub-cluster 4.5 has six traditional landraces (Kiteto, TZA 2785, TZA 2439, Ilonga, TZA 2692 and TZA 5541). Sub-cluster 4.6 consists of five improved genotypes (ICEAP 01147, ICEAP 001179, ICEAP 0673/1, ICEAP 00540 and ICEAP 001172) and two traditional landraces (Hombolo and TZA 5463). Sub-cluster 4.7 consists of one improved genotype (ICEAP 00911) and two traditional landraces (TZA 197 and No.40).

Cluster 5 is a second largest group and has twelve genotypes which divided into two sub- clusters. Sub-cluster 5.1 has five improved genotypes (ICEAP 00040, ICEAP 00936, ICEAP 00932, ICEAP 00576-1 and ICEAP 001179/1) and five traditional landraces (Kondoa, Arumeru, TZA 2807, Bangili and Babati White). Sub-cluster 5.2 has two improved genotypes (Tumia and ICEAP

00053). All genotypes in Cluster 5 are late maturing genotypes except Tumia, Kondoa and TZA 2807

In the present investigation, it was observed that all late maturing genotypes were grouped into cluster 5 except genotype No. 40. The majority of the long duration genotypes grown in Northern Zone were improved genotypes developed by ICRISAT- Kenya (ICEAP 00936, ICEAP 00576-1 and ICEAP 001179/1). Genotype ICEAP 00040, popular known as Mali, and ICEAP 00932 released as Karatu in Tanzania, and ICEAP 00053, released as Kiboko are known for their resistance to the *Fusarium* wilt disease. These late duration genotypes have a high level of genetic similarity. Babati White and ICEAP 00040 have a genetic similarity of 89%, ICEAP 00932 and ICEAP 00936 have a genetic similarity of 93% and a traditional landrace Babati White and ICEAP 00936 have a genetic similarity of 89%. This could indicate that these ICRISAT genotypes have been developed from the local landraces.

Several genotypes collected from the Central and Eastern Zone were grouped in the same cluster. Genotypes collected from Northern Zone were grouped into clusters 1, 2, 3, 4 and 5. Genotypes collected from Eastern Zone were grouped into cluster 2, 4 and 5. Genotypes from Central Zone were grouped into cluster 4 and 5. Grouping of genotypes in the same clusters indicates that those genotypes shared many characteristics. Morphologically long duration landrace genotype No.40 resembled improved long duration genotype ICEAP 00040. Genotype No. 40 was resulted from the farmers' mixture between genotype ICEAP 00040 and several long duration landraces in late's 1990 when *Fusarium* wilt resistance genotype ICEAP 00040 was introduced to farmers' in Babati districts to reduce *Fusarium* wilt disease.

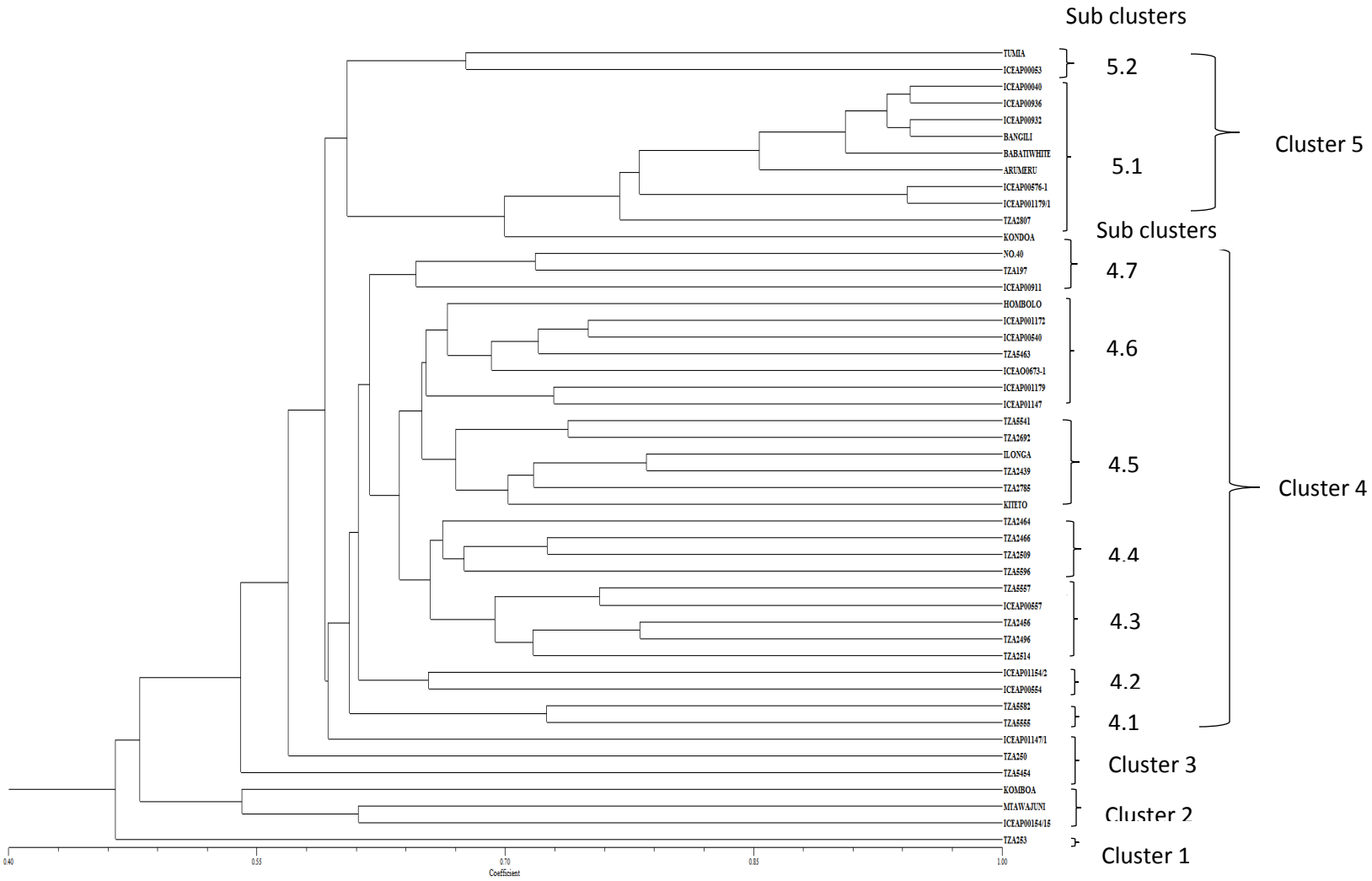

Figure 5.2 UPGMA dendrogram of 48 pigeonpea genotypes from Tanzania germplasm based on 35 SSR markers

5.4 Discussion

5.4.1 Genetic diversity of pigeonpea using SSR molecular markers

Genetic improvement of crops depends on the amount of genetic variation among the breeding material. In the present study the average number of alleles detected per marker is 4.63. Previous studies of genetic diversity in Brazilian pigeonpea genotypes by Sousa *et al*. (2011) and Malawian germplasm by Njung'e *et al*. (2016), using SSR markers reported a higher average number of alleles of 5.1 and 5.58, respectively. An earlier study of Kenyan pigeonpea and Indian accessions by Songok *et al*. (2010) using 88 genotypes and six SSR markers reported a high average number of alleles per locus. This shows that genotypes studied in Kenya, Malawi and Brazil has a higher diversity than that of Tanzania. The low average number of alleles observed in this study could be attributed to the fact that the genotypes were collected from a relative narrow geographical area.

The average PIC value observed was 0.462. Earlier studies using SSR markers reported a PIC value of 0.49 (Sousa *et al*., 2011). Njung'e *et al*. (2016) reported a low PIC value of 0.30 using 38 SSR markers. Singh *et al*. (2012) analysed 40 genotypes, including four wild relative and reported PIC value of 0.523. A study by Singh *et al*. (2013) reported a PIC value of 0.515. Low PIC values of 0.18 using SSR marker and high PIC value of 0.90 using RAPD markers was reported by Walunjkar *et al*. (2015). Rani *et al*. (2015) using 10 RAPD and 10 ISSR markers for 42 genotypes reported PIC values of 0.73 and 0.77, respectively. The low levels of genetic diversity for cultivated pigeonpea genotypes have been reported by several researchers (Smart, 1990; Souframaniem *et al*., 2003; Saxena, 2008).

The average gene diversity (H_e), which is a measure of genetic diversity observed, in the present study was 0.45 (Table 5.2). Songok *et al*. (2010) reported a higher genetic diversity in Indian genotypes than East African germplasm. Wasike *et al*. (2005) investigated the Asian and African accession using AFLP markers and revealed that Indian accession are more diverse than African accession. These findings and several other reports suggest that India is the centre of origin and domestication of pigeonpea and East Africa is the second genetic diversity. In the present study, the observed heterozygosity of the genotypes was low. Comparable results were obtained from a microsatellite-based study that involved 77 accessions from Brazil using 43 SSR markers (Sousa *et al*., 2011). The low level of observed heterozygosity is mostly likely attributable to the out-crossing nature of pigeonpea and farmers selection pressure that might have reduced polymorphism in the populations. Farmers traditionally sample, retain and use relatively small

seed sample from the previous harvest for planting the following cropping season (Cromwell *et al*., 1992).

The most informative SSR markers for detecting polymorphism in this study were CcM0246, CcM0381, CcM0443, CcM0492, CcM0974, CcM2704 and CcM2895.These markers have high values in alleles number >7, gene diversity >0.7 and PIC values >0.7. However, there is no report whether these markers were linked to important traits such as *Fusarium* wilt resistance, maturity and insect resistance.

5.4.2 Genetic relationships and cluster analysis

The results from the genetic similarity index using SSR markers identified 5 clusters. Genotypes ICEAP 00040, ICEAP 00932, ICEAP 00053, ICEAP 00557, ICEAP 00554 are known for their resistance to *Fusarium* wilt disease and were released in Tanzania. The first three genotypes are the late maturing and were grouped into cluster 5, while the last two genotypes are medium duration and grouped into cluster 4. In addition, Tumia and Komboa are improved and released varieties grouped into different clusters. Tumia was grouped into cluster 5 while Komboa was grouped into cluster 2.

Genetic similarity observed among 48 pigeonpea genotypes showed that most of the long duration genotypes have a high level degree of genetic similarity. Long duration landraces Bangili and Babati White have been found to have a high genetic similarity levels with most of long duration improved genotypes (ICEAP 00936, ICEAP 00576-1 and ICEAP 001179/1). Bangili and ICEAP 00936 showed the highest genetic similarity (93%). The higher levels of genetic similarity indicates that these genotypes are related and the degree of variation was low. In similar study by Odeny (2006) observed a high similarities (94%) between ICP 12058 and ICP 13092 genotypes collected from Tanzania and Kenya, respectively. High genetic similarity (85%) for six pairs of genotypes has been reported by Sing *et al*. (2013) using 24 SSR for 36 pigeonpea genotypes. Shende and Raut (2013) observed 98% genetic similarity between BDN-2029 and Vipula using 5 RAPD for 15 pigeonpea genotypes. In this study, most of the late maturing genotypes had a high level of genetic variation. In breeding programmes genotypes which are not closely related are desired.

Conclusions

Appropriate characterization of pigeonpea genotypes is required for detection of duplicates and reduce the cost for germplasm maintenance. Study of genetic diversity using SSR markers provides a foundation towards the genetic improvement of pigeonpea in Tanzania. In the present investigation the SSR markers revealed a low genetic diversity in the cultivated pigeonpea of Tanzania germplasm. This might be due to the limited number of germplasm used for the study. The most polymorphic SSR markers, based on alleles (>6), gene diversity (He) (>0.7) and PIC values (>0.7) were CcM0246, CcM0381, CcM0443, CcM0492, CcM0974, CcM2704 and CcM2895. The SSR identified five clusters and nine sub-clusters, which suggests that there is sufficient diversity. The distantly related genotypes TZA 253, Komboa, Mtawanjuni, ICEAP 001514/15, TZA 2464, TZA 5582, Tumia, ICEAP 00932 and ICEAP 00040 were selected based on genetic similarity index.

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Chapter 6 : Gene action controlling inheritance of *Fusarium* wilt resistance, grain yield and yield components of pigeonpea

Abstract

Pigeonpea (*Cajanus cajan* (L.) Millsp.) is one of the important legumes grown by smallholder's farmers in Tanzania for food, feed and income. Understanding genetic control of *Fusarium* wilt resistance is crucial in pigeonpea breeding programme. The aims of this study was to establish the gene action controlling *Fusarium* wilt resistance, grain yield and yield components and to study inheritance of *Fusarium* wilt resistance. Six lines were crossed with four testers in a line x tester mating design. The 24 F_1 generations were advanced to F_2 population. The 24 F_1 generations were evaluated under field conditions at two seasons in two sites using a row-column design. The F_2 populations were evaluated for *Fusarium* wilt reaction under field conditions. Both F_1 and F_2 generations were also evaluated in a pot experiment for the *Fusarium* wilt reaction. Results indicated that parents and crosses were highly significantly different ($P<0.001$) for yield and important agronomic traits. General combining ability effects of lines and general combining ability effects of testers were also significant. Specific combining ability were also significant for some crosses. Non-additive gene action was important than additive gene action in controlling of *Fusarium* wilt disease, grain yield and important yield components except for days to maturity and plant height. The inheritance pattern for *Fusarium* wilt resistance of F_2 populations expressed segregating ratios of 3R:1S, 9R:7S, 13R:3S, 15R:1S and ratios of 1R:3S and 7R:9S. Findings from this study confirm inheritance of *Fusarium* wilt resistance is governed by variable number of genes such as one dominant gene or recessive gene, two complementary genes, dominant epistasis gene or duplicate genes.

6.1 Introduction

Pigeonpea (*Cajanus cajan* (L.) Millsp.) is a major cash crop legume in the northern districts of Tanzania. The major growing regions are Arusha, Manyara, Dodoma and Mtwara, which accounts for over 90% of total production (Mponda *et al*., 2013). It is the main source of income for small scale farmers' in Babati district of the Manyara region (ICRISAT, 2012). The crop is popular in all parts of Tanzania due to an increased demand and attractive prices when compared to decreased price of cereal crops. There is opportunity for expansion of pigeonpea production in the southern districts of Tanzania, because of reduced cost of transportation to the seaport.

Fusarium wilt disease caused by *Fusarium udum* is the main cause of low grain yields in pigeonpea. The global average yield of the crop is 729 kg/ha (Abate *et al*., 2012). The search for sources of disease resistance has been the breeding priority (Saxena *et al*., 2010). Variety ICEAP 00040, popularly known as Mali in Tanzania, has been identified as a potential source of *Fusarium* wilt (FW) resistance to reduce *Fusarium* wilt disease (Gwata *et al*., 2006). Changaya (2007) identified long duration traditional genotype AP10 with resistance to *Fusarium* wilt and high yield. In order to ensure sustainability of pigeonpea production in the region, several lines that confer resistance to *Fusarium* wilt have been identified and released in eastern and southern Africa regions (ICEAP 00932, ICEAP 00053, ICEAP 00554 and ICEAP 00557) (Kaoneka *et al*., 2016). However, only two released varieties have been studied for gene action and inheritance of *Fusarium* wilt (FW) disease (ICEAP 00040 and ICEAP 00554) (Changaya *et al*., 2012).

General and specific combining ability for FW resistance in pigeonpea have been studied by many researchers (Makelo, 2011; Changaya *et al*., 2012; Tikle *et al*., 2016). Combining ability using line x tester mating design has been widely used in pigeonpea (Changaya *et al*., 2012; Kumar *et al*., 2009; Sarode *et al*., 2009; Shoba and Balan, 2010; Patil *et al*., 2014). The study is important because it offers an understanding of genetic information about parents (both male and female) and their hybrids for future use in breeding programmes (Pandey *et al*., 2014). The inheritance pattern for FW will also give detailed information on the number of genes governing the resistance. FW resistance conditioned by dominant single gene has been reported by several authors (Karimi *et al*., 2010; Changaya *et al*., 2012; Singh *et al*., 2016). Odeny *et al*. (2009) found a single recessive gene controlling inheritance of FW resistance. Control by two dominant, two complementary gene and recessive gene has been reported by Karimi *et al*. (2010) and Changaya *et al*. (2012). The inheritance pattern of FW resistance is still inconclusive. Therefore the objectives of this study were to study general combining ability and specific combining ability

effects for FW resistance, yield and important agronomic traits, and study the inheritance of resistance to FW.

6.2 Materials and methods

6.2.1 Development of breeding population

Four pigeonpea testers obtained from the Agricultural Research Institute-Ilonga were crossed to six lines to obtain F_1. The four testers were used for their *Fusarium* wilt resistance (Table 6.1). A line x tester mating design was adopted to combine *Fusarium* wilt resistance genes to the susceptible lines for combining ability tests and for segregation analysis. F_1 seeds were divided into two parts, some of F_1 seeds were retained for evaluation, and some part were advanced to F_2. After harvesting the F_2 seeds was stored for evaluation. Crossing was done by removing the keel with forceps, exposing the stamens and stigma. The stamens was then removed by firmly grasping of the filaments carefully to insure that all 10 stamens were removed, without rupture and all contact with stigma was avoided. After emasculation pollination was done by brushing mature pollen to a stigma of an opened flower. After pollen transfer the wings were returned to the closed position and secured with a 3 cm strip of adhesive tape to prevent desiccation and contamination by visiting insects. Crosses were done daily between 6.30-10.30 a.m. at the Chinese Agricultural Demonstration Centre-Dakawa-Tanzania.

Table 6.1 List of lines and testers used in this study

Lines	Wilt reaction	Testers	Wilt reaction
TZA 197	Susceptible	ICEAP 00040	Resistant
TZA 2439	Susceptible	ICEAP 00554	Resistant
TZA 5463	Susceptible	ICEAP 00053	Resistant
TZA 2514	Susceptible	ICEAP 00932	Resistant
Tumia	Susceptible		
Komboa	Susceptible		

6.2.2 Field experimental details for F_1 and F_2 generations

All 24 F_1 generations, representing crosses between resistant x susceptible (R x S) genotypes and parents (Line x tester), were evaluated in four environments in the 2014/2015 and 2015/2016 at Hombolo and Ilonga using a 17 x 2 row-column design with two replications. At Hombolo

planting was done in January 2015 (H14/15) and January 2016 (H15/16). At Ilonga planting was done in March 2015 (Ilg14/15) and January 2016 (Ilg15/16). Each plot consisted of five rows, 3 m long and spaced at 50 cm between rows and 50 cm within rows. The 24 F_2 populations were planted along with their parents in plots consisting of 70, 60 and 63 number of plants per plot in a space of 1m x 1m at Ilonga. Fields were ploughed and harrowed. Sowing of the experimental field was done after heavy rain to ensure even germination. Two seeds were sown per hole and, after germination, were thinned to one plant per hole. Field management followed standard agricultural practices recommended for the site.

6.2.3 Pot screening for F_1 and F_2 generations for *Fusarium* wilt resistance

All 24 F_1 generations were screened for resistance to *Fusarium* wilt along with their parents using seed infection technology as described in Chapter 3. Seeds of 24 F_1 generations along with their parents were planted in a 12 L capacity plastic pots. After two weeks, the seedlings of each genotype was removed and washed with running water and roots were bruised to allow entry for the pathogen. Five seedlings of each F_1 generation along with their parents were inoculated with sorghum seeds containing *Fusarium udum* conidia and transplanted in each pot and replicated three times. Number of plants showing typical symptoms of *Fusarium* wilt were recorded up to the podding stage.

The *Fusarium* wilt assessment scale, described by Nene and Kannaiyan (1982) for the *Fusarium* wilt incidence, was used where:

0 – 20% plant mortality= resistant,
21 – 40% plant mortality= moderately resistant,
41 – 60% plant mortality= susceptible,
61 – 80% plant mortality = moderately susceptible,
81 – 100% plant mortality= highly susceptible

Forty seeds from each of 24 F_2 populations along with their parents were planted in 12 L plastic pots. After two weeks, each seedling was inoculated with sorghum seeds containing *Fusarium udum* conidia and transplanted one plant per pot. A total of 40 plant per each of 24 F_2 populations was evaluated for *Fusarium* wilt disease. Data was collected by counting the number of plants showing typical *Fusarium* symptoms up to the podding stage.

Table 6.2 ANOVA Table for Line X Tester mating design

Source	df	MS	Expected mean squares	
			Model I	Model II
Replication	$r-1$			
Genotypes	g-1			
Parents	p-1			
Parents Vs Crosses	1			
Crosses	c-1			
Lines	$m-1$	M_1	$\sigma^2 + rf\frac{1}{m-1} + \sum_i g_i^2$	$\sigma^2 + v_{sca} + rf_{gca(m)}$
Testers	$f-1$	M_2	$\sigma^2 + rm(\frac{1}{f-1})\sum_j g_j^2$	$\sigma^2 + rv_{sca} + rm_{gca(f)}$
Line x tester	$(m-1)(f-1)$	M_3	$\sigma^2 + r\left[\frac{1}{(m-1)(f-1)}\right]\sum_i\sum_j s_{ij}$	$\sigma^2 + rv_{sca}$
Error	$(r-1)(mf-1)$	M_4	σ^2	σ^2

Key: df=degree of freedom; MS=mean square; r=number of replication; g=genotypes; p=parents; c=crosses; m=number of male parents (lines); f=number of female parents (testers); M_1=mean square of lines; M_2=mean square of tester; M_3=mean square of line x tester; M_4= error mean square, gca=general combining ability; sca=specific combining ability; V_{gca} = Covariance of half-sibs; V_{sca} = Cov. Full-sibs – 2 Cov. Half-sibs

The variances for general and specific combining ability were tested against their respective error variances, derived from the analysis of variance of the different traits as follows:

Covariance of half-sib of line

= Cov.H.S. (line) (1)

$=M_1-M_3 / rt$

Covariance of half-sib of tester

= Cov.H.S. (tester) (2)

$=M_2-M_3 / rl$

Covariance of full sib

= Cov.F.S. (3)

$= [(M_1-M_4) + (M_2- M_4) + (M_3-M_4)] / 3r + [6r (Cov.H.S) – r(l-t) Cov.H.S] /3r$

While Cov.H.S. (average) was calculated by the formula

Cov.H.S. average (4)

$= 1 / [r (2lt-l-t)] \times [((l-1) (M_1) + (t-1) (M_2) / (l+t-2) –M_4]$

6.2.4 Data collection and analysis for F_1 and F_2 generations in both field and controlled conditions

Data was collected for *Fusarium* wilt incidence (%) of F_1 generation grown under both field and controlled conditions using *Fusarium* wilt assessment scale as described in 6.2.3. Other data recorded were grain yield, days to 50% flowering, days to maturity, plant height, number of seeds per pod, 100-grain weight, number of primary branches and number of secondary branches. Data collection for the F_2 populations were scored either as susceptible or resistance by counting the number of dead or wilted and survived plants.

A combined analysis of variances for all F_1 generations recorded data under both field and controlled conditions was performed using SAS 9.3 version (SAS Institute, 2010) computer package and Genstat 14th edition (Payne *et al*., 2011). Crosses were considered fixed, while replications and environment was considered as random. A Line x Tester analysis was performed using the following model (Dabholkar, 1999):

$$Yijk = \mu + gi + gj + sij + rk + e_l + \varepsilon ijkl$$

Where Yijk = mean phenotypic value; μ = grand mean effect; gi = GCA effect of ith parent (lines); gj = GCA effect of jth parent (testers); sij = SCA effect of cross i x j; rk = replication effect; e_l = environment; εijkl = random error. Estimates of the combining ability were computed according to Kempthorne (1957).

Various genetic ratios were reported for the inheritance of *Fusarium* wilt resistance in pigeonpea (Odeny *et al*., 2009, Karimi *et al*., 2010, Changaya *et al*., 2012). It appears that there are no consensus on the number of genes controlling the inheritance of *Fusarium* wilt resistance in pigeonpea. Consequently, this study examined different genetic ratios including 3:1, 9:7, 13:3 and 15:1 to establish the number of genes conditioning the inheritance of resistance involving 24 cross combinations. The aim was to identify and pinpoint the best goodness of fit for different phenotypic ratios using the Chi-square (χ^2) analysis to propose the number of genes governing *Fusarium* wilt resistance (Snedecor and Cochran, 1967; Singh *et al*., 2016).

$$\chi^2 = \sum \frac{(\text{Observed value} - \text{Expected value})^2}{(\text{Expected value})}$$

6.3 Results

6.3.1 *Fusarium* wilt reaction, yield and yield components and combining ability

The combined analysis of variance revealed significant variation for all traits (Table 6.3). *Fusarium* wilt reaction were highly significantly different (P<0.001) among the genotypes under field conditions and significantly different (P<0.01) among the genotypes in the pot experiment. There were highly significantly different (P<0.001) among the genotypes for all traits across the sites except for the number of seeds per pod. The combined analysis of variance for yield trial revealed that genotypes were highly significantly different (P<0.001). Parents also showed highly significantly different for all traits (P<0.001) except for the number of primary and secondary branches. The parents versus crosses were highly significantly different (P<0.001) for days to 50% flowering and significantly different (P<0.01) for days to maturity and *Fusarium* wilt reaction under field conditions.

The crosses were highly significantly different (P<0.001) for days to maturity, 100-grain weight and grain yield. Highly significant variation (P<0.01) was recorded among the crosses for number of seeds per pod and number of primary branches, and significant differences (P<0.05) were found between crosses for days to maturity, plant height, number of pods per plant, number of secondary branches and *Fusarium* wilt reaction in the pot experiment. The general combining ability (GCA (fm)) for lines was significant for all traits, except for *Fusarium* wilt in a pot experiment. The general combining ability (GCA (m)) for testers was significant for the days to maturity, and 100-grain weight and *Fusarium* disease in field and pot experiments. The specific combining ability (SCA) was significant for days to maturity, number of seeds per pod, 100-grain weight, grain yield and *Fusarium* wilt reaction in a pot experiment (Table 6.3).

Table 6.3 Combined analysis of variance with significant tests for combining ability evaluation of 34 genotypes (10 parents and 24 F_1 generation) of pigeonpea for yield and yield components, and *Fusarium* wilt resistance in four testing sites under field and pot experiments

		Yield and yield components								*Fusarium* wilt disease			
										Field		Pot	
Source	Df	DT50%F	DTM	PH	NSOP	100-GW	NPB	NSB	GY	df	FWF	df	FWP
Site	3	20661.94***	28078.88***	30736.23***	0.37	305.29***	40364.42***	175172.91***	2358807.40***	1	2059.21**		
Rep(site)	4	22.18	204.48	3549.66	0.36	10.69	659.64	1041.21	116495.24	2	306.6	2	136.61***
Genotype	33	1343.09***	209.11***	12124.74***	3.35***	96.11***	2058.09***	6624.46***	799861.96***	33	527.72***	33	1365.47**
Parents (P)	9	4144.06***	6374.18***	25006.44***	4.17***	56.33***	3263.14*	9565.54*	1092515.57***	9	356.63***	9	1642.96**
P Vs C	1	3017.91***	4823.06**	53.68	2.21	1.25	2026.59	6618.53	17344418.31	1	4211.16**	1	1790.38
Crosses (C)	23	172.846***	295.29*	6943.88**	2.98**	116.19***	1508.61**	5086.2*	592616.72***	23	434.51	23	1238.41*
GCA(fm)	5	379.09***	522.39*	11381.07**	3.85**	223.12***	210.13*	8434.71*	1227299.50***	5	231.43*	5	410
GCA(m)	3	161.58***	117.35	8462.74*	1.87	199.16***	1209.19	4006.79	295992.34	3	340.67*	3	1561.11*
SCA	15	106.35***	255.18	5161.04*	2.91***	63.95***	1371.31	4185.91	440380.67**	15	520.97	15	1450.11*
Error	132	12.35	153.08	3130.18	0.79	5.58	791.99	2964.48	176493.04	66	204.58	66	444.35
Mean		118.14	158.49	173.35	6.11	17.21	48.31	98.22	1204.15		17.37		34.12
CV (%)		3.97	7.81	32.27	14.58	13.73	58.25	55.43	34.89		82.17		61.78
%GCA(fm) to SS		47.68	38.46	35.63	28.18	41.75	30.27	36.05	45.02		11.58		7.20
%GCA (m) to SS		12.19	5.18	15.90	8.18	22.36	10.45	10.28	6.51		10.23		16.44
% SCA to SS		40.13	56.36	48.47	63.64	35.89	59.28	53.67	48.47		78.19		76.36

, **, ** significant at 0.05; 0.01 and 0.001 Key: df=degree of freedom; DT50%F=days to 50%flowering; DTM=days to maturity; PH=plant height; NSOP=number of seed per pod; 100-GW=100-grain weight; NPB=number of primary branches; NSB=number of secondary branches; GY=grain yield per plot; FWF=*Fusarium* wilt in field; FWP=*Fusarium* wilt at pot experiment; CV=coefficient of variation; GCA=general combining ability; SCA=specific combining ability; fm=female; m=male; SS=sum of squares

6.3.2 Evaluation of F_1 generations of various crosses for *Fusarium* wilt resistance, yield and agronomic traits

The performance of the F_1 generations of various crosses of pigeonpea genotypes in both field and pot experiment are presented in Table 6.4. *Fusarium* wilt disease had a mean incidence of 37.5% in the pot experiment with a minimum of 6.7% and maximum of 73.3% *Fusarium* wilt incidence. Under field conditions *Fusarium* wilt had a mean incidence of 21.0%, with a minimum of 5.2% and maximum of 41.7%. The highest *Fusarium* wilt disease incidence in a pot experiment was observed in cross TZA 5463 X ICEAP 00554 (73.3%) followed by crosses TZA 197 X ICEAP 00932 (66.7%), Tumia X ICEAP 00040 (66.7%) and TZA 2514 X ICEAP 00557 (60.0%). The cross TZA 2439 X ICEAP 000932 had the least *Fusarium* wilt disease incidence (6.7%). Under field conditions, the cross TZA 2439 and ICEAP 00557 had the lowest *Fusarium* wilt disease incidence (5.2%).

Grain yield and important agronomic traits are presented in Table 6.4 and Table 6.5. Days to 50% flowering had a mean of 116, minimum of 105.5 and maximum of 123.6 days. The cross between Tumia X ICEAP 00557 had the shortest number of days to flowering (105.5 d) and days to maturity (146.1 d). The cross TZA 2439 X ICEAP 00554 had the least days to flowering (165 d) and maturity (165 d). The mean yield for the F_1 generation was 1153 kg/ha, with a minimum of 725.2 kg/ha and a maximum of 1719 kg/ha. The cross TZA 2514 X ICEAP 00554 had the lowest yield of 725.3 kg/ha, while the highest yield of 1719 kg/ha was recorded for cross TZA 197 X ICEAP 00932. The cross TZA Tumia and ICEAP 00040 had the tallest plants (272.3 cm) followed by the cross TZA 197 X ICEAP 00932 while the cross TZA 197 X ICEAP 00557 had the shortest plants (127.9 cm). The number of seeds per pod was highest for the cross Tumia X ICEAP 00554 (7). The lowest number of seeds per pod were observed for the cross Tumia X ICEAP 00932 (4.6). The cross Komboa X ICEAP 00040 had the smallest seed (13.1 gm per 100-gw) and the cross TZA 2439 X ICEAP 00557 the largest (26.1 gm per 100-gw). The highest number of primary branches was recorded for the cross TZA 2439 X ICEAP 00932 (74.3) and the lowest number for the cross TZA 5463 X ICEAP 00932 (26.5). The highest number of secondary branches was recorded for the cross Tumia X ICEAP 00040 (137) and the lowest for the cross TZA 5463 X ICEAP 00932 (58).

Table 6.4 *Fusarium* wilt incidence in field and pot experiments and grain yield for 24 F_1 generations of various crosses of pigeonpea genotypes

	***Fusarium* wilt disease**		**Yield**
Crosses	**Pot- Wilt %**	**Field-Wilt %**	**Grain yield**
TZA 2439 X ICEAP 00557	53.3	5.2	1035.0
TZA 2439 X ICEAP 00554	46.7	12.5	931.3
TZA 2439 X ICEAP 00932	6.7	37.5	1493.8
TZA 2439 X ICEAP 00040	40.0	20.8	1056.5
TZA 2514 X ICEAP 00557	60.0	7.3	1041.6
TZA 2514 X ICEAP 00554	33.3	15.6	725.3
TZA 2514 X ICEAP 00932	53.3	12.5	1137.5
TZA 2514 X ICEAP 00040	13.3	24.0	1021.4
TZA 197 X ICEAP 00557	26.7	41.7	1115.8
TZA 197 X ICEAP 00554	53.3	17.7	1068.1
TZA 197 X ICEAP 00932	66.7	12.5	1718.8
TZA 197 X ICEAP 00040	20.0	20.8	1444.4
TZA 5463 X ICEAP 00557	10.0	40.6	840.6
TZA 5463 X ICEAP 00554	73.3	18.8	1250.0
TZA 5463 X ICEAP 00932	13.3	14.6	833.1
TZA 5463 X ICEAP 00040	30.0	30.2	1125.4
Tumia x ICEAP 00557	20.0	14.6	1363.4
Tumia X ICEAP 00554	53.3	31.3	1641.5
Tumia X ICEAP 00932	20.0	16.7	1348.8
Tumia X ICEAP 00040	66.7	24.0	1439.1
Komboa X ICEAP 00557	26.7	31.3	1363.8
Komboa X ICEAP 00554	46.7	19.8	774.1
Komboa X ICEAP 00932	20.0	5.2	983.0
Komboa X ICEAP 00040	26.7	28.1	910.6
Mean	37.5	21.0	1153.0
Min	6.7	5.2	725.2
Max	73.3	41.7	1719.0
CV (%)	54.2	49.7	23.6

Key: CV=coefficient of variation

Table 6.5 Mean values of yield components for 24 F_1 generations of various crosses of pigeonpea under field evaluations

Crosses	DT50%F	DTM	PH	NSP	100-GW	NPB	NSB
TZA 2439 X ICEAP 00557	117.5	156.1	173.6	6.0	26.1	60.5	108.1
TZA 2439 X ICEAP 00554	123.1	165.0	179.4	7.3	22.4	41.0	82.1
TZA 2439 X ICEAP 00932	118.6	153.3	151.1	6.8	20.1	74.6	134.8
TZA 2439 X ICEAP 00040	123.6	162.0	194.0	6.3	16.1	31.8	75.3
TZA 2514 X ICEAP 00557	116.4	153.3	162.4	5.8	18.4	58.4	112.6
TZA 2514 X ICEAP 00554	120.3	161.3	150.8	4.9	20.3	39.8	78.5
TZA 2514 X ICEAP 00932	111.0	151.9	160.0	6.3	19.1	36.4	72.8
TZA 2514 X ICEAP 00040	119.9	157.5	151.9	6.3	18.4	33.0	66.3
TZA 197 X ICEAP 00557	113.9	154.0	127.9	5.9	19.6	60.3	103.5
TZA 197 X ICEAP 00554	112.0	155.1	175.4	6.6	18.9	52.5	99.1
TZA 197 X ICEAP 00932	114.8	155.0	224.0	6.6	14.1	65.0	132.6
TZA 197 X ICEAP 00040	109.0	152.3	176.6	6.4	13.5	35.8	73.6
TZA 5463 X ICEAP 00557	118.3	159.9	150.0	5.5	19.5	41.9	87.5
TZA 5463 X ICEAP 00554	120.4	162.0	144.9	5.8	13.9	30.3	64.1
TZA 5463 X ICEAP 00932	115.4	156.5	169.8	5.9	14.8	26.5	57.5
TZA 5463 X ICEAP 00040	121.9	164.8	177.9	6.4	14.3	30.0	64.8
Tumia x ICEAP 00557	105.5	146.1	198.6	6.4	13.4	34.3	86.1
Tumia X ICEAP 00554	116.1	157.3	175.1	7.0	19.5	60.4	123.4
Tumia X ICEAP 00932	112.3	154.1	186.4	4.6	14.1	42.3	99.9
Tumia X ICEAP 00040	116.3	160.5	272.3	6.1	17.3	63.1	136.5
Komboa X ICEAP 00557	120.3	163.6	173.1	5.5	14.3	57.3	130.3
Komboa X ICEAP 00554	115.1	155.9	152.6	5.9	17.8	35.1	68.9
Komboa X ICEAP 00932	110.5	163.4	147.8	5.5	15.4	51.8	107.8
Komboa X ICEAP 00040	111.9	150.6	178.1	5.9	13.1	55.5	115.0
Mean	116.0	157.1	173.1	6.1	17.3	46.6	95.0
Min	105.5	146.1	127.9	4.6	10.1	26.5	57.5
Max	123.6	165.0	272.2	7.3	26.1	74.6	136.5
CV (%)	4.0	3.1	17.0	10.1	22.1	29.5	26.5

Key: DT50%F=days to 50% flowering; DTM=days to maturity; PH=plant height (cm); NSP=number of seed per pod; 100-GW=100-grain weight (gm); NPB=number of primary branches; NSB=number of secondary branches; CV=coefficient of variation

6.3.3 Contribution of general combining ability and specific combining ability to the total sum of squares

The proportional contribution due to GCA (fm), GCA (m) and SCA to the total sum of square are presented in Table 6.3. The GCA for lines contributed more to the total sum of square for the days to 50% flowering (47.6%) and 100-grain weight (41.7%). The GCA for testers contributed less to the total sum of square for all traits. The SCA contributed more to the total sum of square for the days to maturity (56.3%), plant height (48.4%), number of seeds per pod (63.6%), number of primary branches (59.2%), number of secondary branches (53.6%), grain yield (48.4%), *Fusarium* wilt incidence in the field (78.2%) and *Fusarium* wilt incidence in a pot trial (76.3%).

6.3.4 Estimates of genetic components for the nine agronomic traits in pigeonpea

Data for the estimates of genetic parameters are presented in Table 6.6. The variance components estimated due to SCA were larger than those due to GCA except for days to maturity and plant height. Dominance genetic variance was larger than additive genetic variance, except for days to maturity and plant height. This were supported by the ratio of variance of general to specific combining ability (δ^2_g / δ^2_s) which was smaller than a unity (<1), except for days to maturity and plant height. The higher magnitude of SCA than the GCA variances, except for days to maturity and plant height, signifies the presence of non-additive gene action for *Fusarium* wilt, grain yield, days to maturity, number of seeds per pod, 100-grain weight, number of primary and secondary branches. Based on the findings on genetic parameters, days to maturity and plant height were controlled by additive gene effects.

Table 6.6 Estimates of genetic parameters for nine agronomic traits of pigeonpea

Traits	δ^2_g	δ^2_s	δ^2_A	δ^2_D	δ^2_g / δ^2_s
FWF	3.27	75.53	6.54	75.53	0.04
FWP	5.34	224.9	10.68	224.9	0.02
GY	32,126.52	67,690.76	64,253.04	67,690.76	0.47
DT50%F	6.47	38.2	12.94	38.2	0.17
DTM	16.39	14.25	32.78	14.25	1.15
PH	76.08	63.29	152.16	63.29	1.20
NSP	0.03	1.05	0.05	1.05	0.03
100-GW	1.97	67.74	3.94	67.74	0.03
NPB	5.19	354.3	11.8	354.3	0.05
NSB	34.06	1105.54	68.12	1105.54	0.03

Key: δ^2_g=Variance due to general combining ability; δ^2_s=variance due to specific combining ability; δ^2_A=additive variance; δ^2_D=dominance variance; δ^2_g / δ^2_s=ratio of variances due to combining ability and specific combing ability; FWF=*Fusarium* wilt in field experiment; FWP=*Fusarium* wilt in pot experiment; GY=grain yield; DT50%F=days to 50% flowering; DTM=days to maturity; PH=plant height; NSP=number of seeds per pod; 100-GW=100-grain weight; NPB=number of primary branches; NSB=number of secondary branches

6.3.5 General combing ability effects for lines and testers

The general combining ability effects for lines and testers are presented in Table 6.7. The desirable significant and negative GCA for *Fusarium* wilt was exhibited by line TZA 2514 and tester ICEAP 00932 in the field trial. Under the controlled conditions significant and negative GCA were exhibited by line TZA 5463 and tester ICEAP 00932. A significant and negative GCA for the days to 50% flowering was exhibited by line TZA 197 and tester ICEAP 00554. A significant and negative GCA for days to maturity was exhibited by the line TZA 2439. The highly significantly and positive GCA for grain yield was exhibited by the lines TZA 197 and Tumia and the tester ICEAP 00932. The negative GCA were exhibited by lines TZA 2439, TZA 2514, TZA 5463 and Komboa and testers ICEAP 00557 and ICEAP 00554.

A highly significant and positive GCA for plant height was exhibited by line Tumia and tester ICEAP 00040. For number of seeds per pod positive GCA were exhibited by lines TZA 2439 and TZA 197 and testers ICEAP 00554 and ICEAP 00040. Significant and positive GCAs for 100-grain weight were exhibited by lines TZA 2439 and TZA 2514 and tester ICEAP 00554. Line TZA

197 had significant and positive GCA for number of primary branches. Lines Tumia and Komboa and testers ICEAP 00557 and ICEAP 00932 have significant and positive GCA for number of secondary branches.

Table 6.7 General combining ability effects for nine agronomic traits in pigeonpea

	***Fusarium* disease**		**Yield and yield components**							
Lines	**FWF**	**FWP**	**GY**	**DT50%F**	**DTM**	**PH**	**NSP**	**100-GW**	**NPB**	**NSB**
TZA 2439	-0.9	-0.42	-15.17	2.26	3.30	0.37	0.26	1.95**	1.40	2.41
TZA 2514	-3.06*	1.25	-88.92	0.36	-0.66	-8.76	-0.13	1.34*	-2.05	-6.36
TZA 197	1.10	2.08	88.83**	-1.83*	-2.65*	1.09	0.16	-0.84	3.63*	3.49
TZA 5463	1.0	-2.92*	-73.54	1.41	2.07	-6.57	-0.09	0.82	-6.98	-13.39
Tumia	0.33	3.75	141.21**	-1.31	-0.41	16.91**	-0.01	-0.57	2.02	8.10**
Komboa	0.06	-3.75	-52.42	-0.86	-1.64	-3.04	-0.18	-1.05	1.98	5.75*
se(gl)	1.44	1.70	7.10	0.65	1.27	2.61	0.34	0.49	1.82	2.58
se(gi-gj)	2.88	3.40	14.19	1.30	2.53	5.23	0.68	0.98	3.63	5.15
Testers										
ICEAP 00557	1.24	-1.53	-2.92	-0.08	0.41	-3.33	-0.11	0.60	2.11	5.14*
ICEAP 00554	-0.85	6.81	-47.17	-0.82*	-0.80	-5.90	0.09	1.11*	-1.47	-4.61
ICEAP 00932	-2.24*	-3.75*	46.67**	1.21	2.01	-0.30	-0.06	-0.81	1.65	2.81*
ICEAP 00040	1.85	-1.53	3.42	0.46	0.81	9.53**	0.08	0.90	-2.29	-3.34
se(gt)	1.18	1.39	5.79	0.53	1.03	2.13	0.28	0.40	1.48	2.10
se(gi-gj)	2.35	2.78	11.59	1.06	2.07	4.27	0.56	0.79	2.96	4.21

* , ** Significant at 0.05; 0.01

Key: FWF=*Fusarium* wilt in field; FWP=*Fusarium* wilt in a pot trial; GY=grain yield; DT50%=days to 50% flowering; DTM=days to maturity; PH=plant height (cm); NSP=number of seeds per pod; 100-GW=100-grain weight (gm); NPB=number of primary branches; NSB=number of secondary branches

6.3.6 Specific combining ability effects of crosses

Data for specific combining ability effects (SCA) are presented in Table 6.8. Specific combining ability were considered to be the best criteria for selection of superior hybrids. Under field conditions significant and negative SCA for *Fusarium* wilt were recorded in crosses TZA 2439 X ICEAP 00557, TZA 2439 X ICEAP 00554, TZA 2514 X ICEAP 00557, TZA 197 X ICEAP 00932, TZA 197 X ICEAP 00040, TZA 5463 X ICEAP 00554, TZA 5463 X ICEAP 00932, Tumia X ICEAP 0055 and Komboa X ICEAP 00932. In a pot experiment significant and negative SCA for *Fusarium*

wilt were recorded in crosses TZA 2439 X ICEAP 00554, TZA 2514 X ICEAP 00554, TZA 2514 XICEAP 00040, TZA 197 X ICEAP 00557, TZA 197 X ICEAP 00554, TZA 197 X ICEAP 00040, TZA 5463 X ICEAP 00557, TZA 5463 X ICEAP 00932, Tumia x ICEAP 00557, Tumia x ICEAP 00554, Tumia x ICEAP 00932 and Komboa x ICEAP 00932. The negative SCA are desirable because it contributes to disease resistance while positive SCA contributes to susceptibility of disease.

Significant and negative SCA for days to flowering were recorded in crosses TZA 2439 X ICEAP 00557, TZA 2514 X ICEAP 00932, TZA 197 X ICEAP 00554, Komboa X ICEAP 00932 and Komboa X ICEAP 00040. Significant and negative SCA for days to maturity were recorded in crosses TZA 2439 X ICEAP 00932, TZA 2514 X ICEAP 00554, TZA 2514 X ICEAP 00932, TZA 197 X ICEAP 00554, TZA 197 X ICEAP 00040 and Tumia X ICEAP 00557. Crosses with negative SCA for days to flowering and maturity were desired while with positive SCA were not desired.

Significant and positive SCA for grain yield were exhibited by crosses TZA 2439 X ICEAP 00932, TZA 2514 X ICEAP 00557, TZA 2514 X ICEAP 00932, TZA 2514 X ICEAP 00040, TZA 197 X ICEAP 00932, TZA 197 X ICEAP 00040, TZA 5463 X ICEAP 00554, Tumia X ICEAP 00554 and Komboa X ICEAP 00557. Crosses with positive SCA were desired for grain yield.

Table 6.8 Specific combining ability effects of 24 F1 generations of various crosses of pigeonpea genotypes for grain yield and yield components and *Fusarium* wilt disease reaction under field and pot experiments

	***Fusarium* disease**		**Yield and yield components**							
Crosses	**FWF**	**FWP**	**GY**	**DTF**	**DTM**	**PH**	**NSOP**	**SW**	**NPB**	**NSB**
TZA 2439 X ICEAP 00557	-16.28**	19.72	-88.17	-3.04**	5.13	5.73*	-0.33	12.44**	-4.89	-2.20
TZA 2439 X ICEAP 00554	-4.28*	-3.61**	-103.67	0.76	-0.05	16.67**	0.51	7.92**	-4.96	-8.71
TZA 2439 X ICEAP 00932	22.96	-22.5	271.66**	0.32	-4.53**	-22.82	0.30	9.53**	22.4**	29.06**
TZA 2439 X ICEAP 00040	-1.88	6.39	-79.84	1.97	-0.57	0.41	-0.47	5.68**	-12.57	-18.12
TZA 2514 X ICEAP 00557	-10.02**	23.06	66.33**	-0.34	-3.05*	12.79**	0.20	0.97	12.64**	19.82**
TZA 2514 X ICEAP 00554	2.46	-20.28**	-162.17	1.76	6.17	0.04	-1.08	6.33**	0.7	5.2*
TZA 2514 X ICEAP 00932	2.12	20.83	63.16**	-3.48**	-5.11**	4.34	0.58	6.33**	-8.93	-15.41
TZA 2514 X ICEAP 00040	5.43	-23.61**	32.66**	-2.07	1.95	-17.13	0.31	5.05**	-4.41	-9.59
TZA 197 X ICEAP 00557	16.01	-11.94**	-215.17	1.93	1.03	-41.42	-0.27	4.47**	2.64	-8.99
TZA 197 X ICEAP 00554	-3.78	-1.95*	-174.67	-2.17**	-3.75*	11.23**	0.07	3.24**	2.07	6.14*
TZA 197 X ICEAP 00932	-6.2**	32.5	288.66**	4.69	6.57	48.63**	0.36	-3.43	8.31**	24.77**
TZA 197 X ICEAP 00040	-6.05**	-18.61**	101.16**	-4.46	-3.87**	-18.44	-0.17	0.11	-13.04	-21.9
TZA 5463 X ICEAP 00557	12.12	-28.6**	-165.42	-0.54	-1.92	-3.99	-0.14	2.42	5.49**	8.76**
TZA 5463 X ICEAP 00554	-5.6*	28.06	332.08**	-0.24	-0.5	-3.94	-0.30	-4.22	1.04	4.89
TZA 5463 X ICEAP 00932	-7.0**	-10.83**	-272.59	-1.18	0.12	9.76**	0.11	0.5	-8.97	-16.6
TZA 5463 X ICEAP 00040	0.48	11.4	105.91	1.97	2.28	-1.81	0.37	0.16	2.43	2.97
Tumia x ICEAP 00557	-9.52**	-1.94*	-98.92	13.13	-6.85**	-4.34	0.55	-6.15	-19.51	-35.66
Tumia X ICEAP 00554	11.32	-5.28**	294.58**	0.93	-0.03	-20.69	0.80*	-1.12	13.17**	21.15**
Tumia X ICEAP 00932	-0.48	-17.5**	-186.09	1.19	3.89	-20.69	-1.29	-2.65	-11.21	-17.2
Tumia X ICEAP 00040	-1.34	24.73	-9.59	1.84	2.95	45.64**	-0.05	0.64	17.56**	31.74
Komboa X ICEAP 00557	7.69	-0.27	501.33**	5.99	5.61	31.26**	0.03	-2.14	3.6	18.31**
Komboa X ICEAP 00554	0.4	3.06	-186.17	-1.01	-1.87	-3.26	0.02	0.31	-12.0	-28.63
Komboa X ICEAP 00932	-11.42**	-2.5*	-164.84	-1.55*	-0.95	-19.29	-0.07	1.78	-1.63	-4.62
Komboa X ICEAP 00040	3.31	-0.27	-150.34	-3.4**	-2.79	8.66	0.04	-0.31	-10.02**	14.65**
se (sij)	0.74	3.12	8.20	0.74	2.01	2.71	0.28	0.57	2.35	1.89
Se (sij-skl)	4.10	2.78	10.23	1.85	3.23	6.03	0.58	0.74	4.56	6.23

* , ** Significant at 0.05, 0.01; Key: FWF=*Fusarium* wilt in field; FWP=*Fusarium* wilt in pot; GY=grain yield; DT50%=days to 50% flowering; DTM=days to maturity; PH=plant height (cm); NSP=number of seeds per pod; 100-GW=100-grain weight (gm); NPB=number of primary branches; NSB=number of secondary branches

6.3.7 Segregation ratios of F_2 populations for *Fusarium* wilt resistance

The inheritance study for *Fusarium* wilt resistance was conducted in both field and pot experiments. Uneven distribution of inoculum, slow disease development and disease escape were observed in the field evaluation. However, the pot experiment, using the seed infection technique, was found to be the most appropriate. The analysis of chi-square for segregation ratios for F_2 populations for *Fusarium* wilt resistance under both controlled and field conditions are presented in Table 6.9 and Table 6.10. The P-values for all crosses showed a non-significant difference for the genetic ratios of 3:1, 9:7, 13:3 and 15:1 (Tables 6.9 and 6.10). This implies that the expected phenotypic ratio did not deviate from the observed ratio. The observed phenotypic ratios in field conditions were 3R:1S recorded for the following crosses: TZA 2439 x ICEAP 00557, TZA 2514 X ICEAP 00557, TAZA 197 X ICEAP 00554, TZA 5463 X ICEAP 00040, Tumia X ICEAP 00554, Tumia X ICEAP 00040, Komboa X ICEAP 00557 and Komboa X ICEAP 00040. The phenotypic ratio of 13R:3S was recorded for crosses TZA 2439 X ICEAP 00554, TZA 2439 X ICEAP 00932, TZA 2439 X ICEAP 00040, TZA 5463 X ICEAP 00554, TZA 5463 X ICEAP 00932, Tumia X ICEAP 00557, Tumia X ICEAP 00932, Komboa X ICEAP 00554. The ratio of 15R:1S was recorded for crosses TZA 2514 X ICEAP 00040, TZA 197 X ICEAP 00557, TZA 197 X ICEAP 00932, TZA 197 X ICEAP 00040, TZA 5463 X ICEAP 00557 and Komboa X ICEAP 00932.The phenotypic ratio of 9R:7S was recorded for the cross TZA 2514 X ICEAP 00554.

In the pot experiment different phenotypic ratios were recorded (Table 6.10). The F_2 phenotypic ratio for *Fusarium* wilt resistance of 3R:1S was recorded for the crosses TZA 2514 X ICEAP 00554, TZA 2514 X ICEAP 00932, TZA 197 X ICEAP 00557, TZA 197 X ICEAP 00554, TZA 5463 X ICEAP 00557, TZA 5463 X ICEAP 00932, TZA 5463 X ICEAP 00040, Tumia X ICEAP 00554, Tumia X ICEAP 00040, Komboa X ICEAP 00557 and Komboa X ICEAP 00040. The ratio of 9R:7S was recorded for crosses TZA 2514 X ICEAP 00557, TZA 2514 X ICEAP 00040, TZA 197 X ICEAP 00040, TZA 5463 X ICEAP 00554, Tumia X ICEAP 00557, Tumia X ICEAP 00040, Komboa X ICEAP 00557 and Komboa X ICEAP 00040. Crosses TZA 197 X ICEAP 00932 and Komboa X ICEAP 00932 showed a ratio of 15R:1S. Two crosses showed ratios of 1R:1S and 13R:3S such as TZA 2439 X ICEAP 00040 and Tumia X ICEAP 00932, in that order. The ratios of 1R:3S and 7R:9S were recorded for crosses TZA 2514 X ICEAP 00932, TZA 2514 X ICEAP 00557 and TZA 2514 X ICEAP 00554. The phenotypic ratios of 3R:1S, 1R:3S, 9R:7S, 7R:9S, 13R:3S, and 15R:1S recorded implies that inheritance of *Fusarium* is controlled by one dominant

gene, one recessive gene, two complementary genes, duplicate genes or a dominant epistasis gene.

Table 6.9 Segregation ratio of 24 F_2 populations of pigeonpea derived from crosses between four resistant and six susceptible parents for *Fusarium* wilt under field evaluation

Cross	No. of F_2 Plants	Observed count		Expected count		Expected ratio		χ^2	P value
		R	S	R	S	R	S		
TZA 2439 X ICEAP 00557	63	48	15	47	16	3	1	0.048	0.827
TZA 2439 X ICEAP 00554	63	55	8	51	12	13	3	1.514	0.218
TZA 2439 X ICEAP 00932	63	55	8	51	12	13	3	1.514	0.218
TZA 2439 X ICEAP 00040	63	53	10	51	12	13	3	0.342	0.559
TZA 2514 X ICEAP 00557	63	52	11	47	16	3	1	1.910	0.167
TZA 2514 X ICEAP 00554	63	40	23	35	28	9	7	1.343	0.247
TZA 2514 X ICEAP 00932	63	47	16	47	16	3	1	0.005	0.942
TZA 2514 X ICEAP 00040	63	57	6	59	4	15	1	1.152	0.283
TZA 197 X ICEAP 00557	63	58	5	59	4	15	1	0.306	0.580
TZA 197 X ICEAP 00554	63	44	19	47	16	3	1	0.894	0.344
TZA 197 X ICEAP 00932	63	59	4	59	4	15	1	0.001	0.974
TZA 197 X ICEAP 00040	63	58	5	59	4	15	1	0.306	0.580
TZA 5463 X ICEAP 00557	63	56	7	59	4	15	1	2.541	0.111
TZA 5463 X ICEAP 00554	70	58	12	51	12	13	3	0.910	0.340
TZA 5463 X ICEAP 00932	60	51	9	51	12	13	3	2.894	0.089
TZA 5463 X ICEAP 00040	60	50	20	47	16	3	1	1.307	0.253
Tumia x ICEAP 00557	70	57	13	51	12	13	3	0.779	0.377
Tumia X ICEAP 00554	70	51	19	47	16	3	1	0.968	0.325
Tumia X ICEAP 00932	60	60	3	51	12	13	3	0.238	0.626
Tumia X ICEAP 00040	60	47	13	47	16	3	1	0.481	0.488
Komboa X ICEAP 00557	70	49	21	47	16	3	1	1.815	0.178
Komboa X ICEAP 00554	70	67	3	51	12	13	3	1.290	0.256
Komboa X ICEAP 00932	70	66	4	59	4	15	1	1.625	0.366
Komboa X ICEAP 00040	60	43	17	47	16	3	1	0.481	0.488

Key: R= Resistant S=Susceptible

Table 6.10 Segregation ratio of 24 F_2 populations of pigeonpea derived from crosses between four resistant and six susceptible parents for *Fusarium* wilt under controlled experiment

		Observed count		Expected count		Expected ratio			
Cross	No. of F_2 Plants	R	S	R	S	R	S	χ^2	P value
TZA 2439 X ICEAP 00557	40	17	23	18	22	7	9	0.025	0.873
TZA 2439 X ICEAP 00554	40	17	23	17	22	7	9	0.025	0.873
TZA 2439 X ICEAP 00932	40	12	28	10	30	1	3	0.533	0.465
TZA 2439 X ICEAP 00040	40	21	19	20	20	1	1	0.100	0.752
TZA 2514 X ICEAP 00557	40	22	18	22	18	9	7	0.025	0.873
TZA 2514 X ICEAP 00554	40	28	12	30	10	3	1	0.533	0.465
TZA 2514 X ICEAP 00932	40	29	11	30	10	3	1	0.133	0.715
TZA 2514 X ICEAP 00040	40	24	16	22	18	9	7	0.229	0.633
TZA 197 X ICEAP 00557	40	31	9	30	10	3	1	0.133	0.715
TZA 197 X ICEAP 00554	40	32	8	30	10	3	1	0.533	0.465
TZA 197 X ICEAP 00932	40	35	5	38	2	15	1	2.667	0.102
TZA 197 X ICEAP 00040	40	26	14	22	18	9	7	1.244	0.265
TZA 5463 X ICEAP 00557	40	31	9	30	10	3	1	0.133	0.715
TZA 5463 X ICEAP 00554	40	22	18	22	18	9	7	0.025	0.873
TZA 5463 X ICEAP 00932	40	7	33	30	10	3	1	1.200	0.273
TZA 5463 X ICEAP 00040	40	27	13	30	10	3	1	1.200	0.273
Tumia x ICEAP 00557	40	21	19	22	18	9	7	0.229	0.633
Tumia X ICEAP 00554	40	27	13	30	10	3	1	1.200	0.273
Tumia X ICEAP 00932	40	34	6	33	7	13	3	0.369	0.543
Tumia X ICEAP 00040	40	26	14	22	18	9	7	1.244	0.265
Komboa X ICEAP 00557	40	24	16	22	18	9	7	0.299	0.633
Komboa X ICEAP 00554	40	28	12	30	10	3	1	0.533	0.465
Komboa X ICEAP 00932	40	35	5	38	2	15	1	2.667	0.102
Komboa X ICEAP 00040	40	25	15	22	18	9	7	0.635	0.426

Key: R= Resistant S=Susceptible

6.4 Discussion

6.4.1 General combining ability (GCA) and specific combining ability (SCA) effects for *Fusarium* wilt, grain yield and yield components

The findings from this study showed that non-additive gene effects were more important than additive gene effects for the seven traits, except for days to maturity and plant height. The higher magnitude of SCA than the GCA variances for *Fusarium* wilt, suggests that non-additive gene action was important in controlling *Fusarium* wilt disease. Similar findings were reported by (Tikle *et al*., 2016), who observed non-additive gene effects in controlling *Fusarium* wilt resistance. The

additive gene action for plant height recorded in this study was reported by Pandey *et al*. (2014). Changaya *et al*. (2012) reported the importance of both additive and non-additive gene action in the controlling of *Fusarium* wilt resistance, yield and yield components.

Estimated GCA effects for *Fusarium* wilt under field conditions revealed that line TZA 2514 and tester ICEAP 00932 showed significant negative GCA. These parents were the best combiner responsible for increased disease resistance. In the pot experiment the significant and negative GCA for *Fusarium* wilt incidence were recorded for line Komboa and tester ICEAP 00932. These parents were also considered the best due to their negative GCA responsible for increased *Fusarium* wilt disease resistance.

High significant and positive GCA for grain yield were recorded for lines TZA 197, TZA 5463 and Tumia and tester ICEAP 00932. The importance of additive gene for grain yield and yield components have been reported by several workers (Kumar *et al*., 2003; Shoba and Balan, 2010; Changaya *et al*., 2012; Pandey *et al*., 2013; Patil *et al*., 2014).. Tester ICEAP 00557 and line TZA 197 had a significant and negative GCA. Line TZA 197 and tester ICEAP 00554 had a significant and negative GCA for days to maturity. These parents were the best in terms of days to maturity and therefore could be utilized in breeding for earliness. The importance of additive gene for earliness have been reported by several workers (Shoba and Balan, 2010; Changaya *et al*., 2012; Pandey *et al*., 2013).

Tester ICEAP 00040 and the line Tumia had significant positive GCA for plant height. Plant height is a desirable character in pigeonpea for achieving high yield. Similar findings were reported by several authors (Chandirakala *et al*., 2010; Vaghela *et al*., 2011; Pandey *et al*., 2013). However, Reddy (1990) and Changaya *et al*. (2012) reported tall plants are always late mature and are not desirable. Although farmers prefer early to medium maturity, late maturing plants offers some benefits. According to Odeny (2007), tall varieties are preferred in African villages for roofing material, firewood and basket weaving.

The high SCA effects in a crosses where the parents had a high and a low GCA indicated an additive x non-additive gene effects. This observation has been reported by several authors (Vanniarajan *et al*., 1999; Devi *et al*., 2011; Kumar *et al*., 2012; Patil *et al*., 2014). Some crosses had parents with average GCA effects, for grain yield but produced crosses with a significant SCA, for example as in TZA 2514 X ICEAP 00557.Similar findings were reported in earlier studies (Devi *et al*., 2011; Patil *et al*., 2014). Some crosses have both parents with high GCA effects for

grain yield and produced significant and positive SCA. This was recorded for cross TZA 197 and ICEAP 00932. Similar observations were reported in previous studies (Pandey and Singh, 2002; Pandey *et al*., 2014). This suggest involvement of additive gene effects in controlling grain yield.

The findings from this study also revealed that some crosses combinations have significant SCA effect for most of yield components. The same findings were reported in earlier studies (Shoba and Balan, 2010; Pandey *et al*., 2014). It is also noted that some of the parents showing significant and positive GCA for grain yield also exhibited significant and positive GCA for the yield components such as plant height, primary and secondary branches. Similar results were reported by Kumar *et al*. (2012) and Pandey *et al*. (2014). The dominance gene actions for *Fusarium* wilt resistance, grain yield and some yield components recorded in this study suggests that the development of hybrids is a feasible option is advocated by ICRISAT (Makelo, 2011; Saxena *et al*., 2013).

6.4.2 Genetics of *Fusarium* wilt resistance

The inheritance of *Fusarium* wilt resistance was analysed using chi-square test. Under field conditions the F_2 crosses segregated into different ratios, namely of 3R:1S, 9R:7S, 13R:3S and 15R:1S. Out of 24 crosses, nine crosses gave 3R:1S ratio, one cross gave 9R:7S, eight cross gave 13R:3S and six cross gave 15R:1S. In a pot experiment F_2 populations segregated into ratios of 1R:1S, 3R:1S, 9R:7S, 13R:3S and 15R:1S. In addition, ratio of 1R:3S and 7R:9S was recorded in three crosses. Nine crosses segregated into 3R:1S, one cross gave 1R:1S, one cross gave 13R:3S, two crosses gave 15R:1S, eight cross gave 9R:7S, one cross gave ratio of 1R:3S and two crosses gave 7R:9S ratios. Under field conditions and pot experiment predominance of 3R:1S ratio was recorded.

A segregating ratio of 3R:1S ratio recorded in this study was previously reported in several studies (Joshi, 1957; Pandey *et al*., 1996; Changaya *et al*., 2012; Saxena *et al*., 2012: Singh *et al*., 2016). This monogenic model of inheritance will allow for a simple introgression of *Fusarium* wilt into susceptible genotypes. The ratio of 9R:7S recorded in this study confirmed earlier reports that two complementary dominant genes control resistance of *Fusarium* wilt (Okikor, 2002; Singh, 2005; Changaya *et al*., 2012; Singh *et al*., 2016). The ratio of 13R:3S suggests that dominant epistasis genes control the inheritance of *Fusarium* wilt resistance (Changaya *et al*., 2012). The 15R:1S ratio suggests that duplicate genes with equal effects confer inheritance of *Fusarium* wilt resistance. A similar observation was also reported by several researchers (Singh, 2005;

Changaya *et al*., 2012; Singh *et al*., 2016). The recorded ratio of 1R:3S and 7R:9S was reported by several authors (Jain and Reddy, 1995; Odeny *et al*., 2009; Changaya *et al*., 2012). This implies that the resistance was inherited as a recessive trait. The involvement of one or more recessive genes for control of *Fusarium wilt* resistance has been reported by Karimi *et al*. (2010). The reversed ratio of 1R:3S and 7R:9S indicates the presence of recessive resistance genes result from the recombination of two genotypes.

Conclusions

The present study suggests that SCA were more important than GCA in controlling *Fusarium* wilt disease resistance, grain yield and some yield components except for days to maturity and plant height. The most promising parents for breeding for *Fusarium* wilt disease resistant were lines TZA 2439 and TZA 197 and tester ICEAP 00932, due to their significant and negative GCA effects for *Fusarium* wilt incidence. In addition to that, parents TZA 197, TZA 5463 and Tumia and ICEAP 00932 were identified as the best parents for increased grain yield. Furthermore, crosses TZA 2439 X ICEAP 00932, TZA 2514 X ICEAP 00557 were identified as the best for disease resistance and high grain yield due to their significance SCA effects for grain yield and negative SCA effects for *Fusarium* wilt incidence. The inheritance pattern for *Fusarium* wilt across selected populations suggested phenotypic segregation ratios of 3R:1S, 9R:7S, 13R:3S, 15R:1S and ratio of 1R:3S and 7R:9S. This implies that in the tested populations the inheritance of *Fusarium* wilt resistance is governed by a variable number of genes such as one dominant gene or recessive gene, two complementary genes, dominant epistasis gene or duplicate genes.

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Chapter 7 : Research overview

7.1 Introduction

Pigeonpea (*Cajanus cajan* (L.) Millsp.) is an important legume crop widely grown by smallholder's farmers in Asia and Africa. Pigeonpea has a higher protein content than other legume crops and serves as a supplementary protein for people who cannot afford animal products. The crop has the ability to fix atmospheric nitrogen and improve the soil fertility. Pigeonpea production and productivity is affected by a number of pests and diseases. *Fusarium* wilt is one of the devastating diseases of the crop causing yield losses reaching up to 100% to a susceptible varieties. Breeding for *Fusarium* wilt resistance is an economic and important component of integrated disease management of pigeonpea.

7.2 Summary of the major findings of the study

Production constraints and farmers-preferred traits of pigeonpea varieties: implications for breeding in Tanzania

A participatory rural appraisal (PRA) study was conducted in Kilosa, Babati, Karatu Districts in Eastern and Northern Tanzania. The core findings of the study were:-

- Pigeonpea intercropping with maize is the main cropping system in all three districts.
- Farmers grow long duration traditional pigeonpea varieties.
- *Fusarium* wilt disease and pod borer were the main farmers perceived production constraints causing low grain yields of the crop.
- Farm save seed is the main source of seed used by farmers for pigeonpea cultivation.
- Production constraints, in order of importance were diseases, field pests, storage pests, drought, high input prices, late maturing varieties and limited access to improved varieties.
- Farmers preferred pigeonpea traits were high yield, disease resistance, drought tolerance, early maturity, short cooking time, determinate (bush) growth habit and large, white or cream seed.

Genetic variation of Tanzanian pigeonpea germplasm for *Fusarium* wilt resistance, grain yield and yield components

Thirty two pigeonpea genotypes including traditional and improved genotypes, were evaluated under field conditions for *Fusarium* wilt resistance, grain yield and important agronomic traits at Hombolo and Ilonga. A pot experiment was conducted to verify field trial results for *Fusarium* wilt disease resistance. The main outcomes were:

- Under field conditions most of the genotypes did not develop symptoms of *Fusarium* wilt disease.
- Pot trials confirmed the genotypes ICEAP 00040, ICEAP 00932, ICEAP 00053, ICEAP 00557 and ICEAP 00554 as being the most resistant to *Fusarium* wilt disease.
- All long duration genotypes outperformed medium and short duration types in terms of yield and yield components.

Phenotypic diversity of Tanzanian pigeonpea germplasm based on agro-morphological traits

Forty eight pigeonpea genotypes were evaluated under field conditions to assess the genetic diversity, using qualitative and quantitative traits and to identify best, and complementary parents that could be used in pigeonpea breeding programme. The core findings were:

- The Shanon-Weaver diversity index revealed a low diversity in Tanzania pigeonpea germplasm.
- The principal component and cluster analyses grouped the 48 pigeonpea genotypes into three clusters.

Assessment of genetic diversity of pigeonpea germplasm from Tanzania using SSR markers

The 48 pigeonpea genotypes used in the phenotypic diversity study were assessed for genetic diversity, using 35 simple sequence repeats (SSR) markers. The main results of this study were:

- The marker combinations revealed a total of 162 alleles at 35 loci with an average of 4.63 alleles detected per marker. Variation in gene diversity (H_e) were in a range of 0.03-0.83 with an average of 0.45. The heterozygosity (H_o) values ranged from 0.00-0.635 with an average of 0.279. The polymorphism information content (PIC) varied from 0.032 to 0.806 with an average of 0.412.

- The genetic relationships similarity index revealed a high similarity between long duration traditional genotypes and long duration improved genotypes.
- The cluster analysis grouped the 48 pigeonpea genotypes into five clusters and seven sub-clusters.
- The genotypes TZA 253, Komboa, Mtawanjuni, ICEAP 001514/15, TZA 2464, TZA 5582, ICEAP 00932 and ICEAP 00040 were selected for breeding.

Gene action controlling inheritance of *Fusarium* wilt resistance, grain yield and yield components of pigeonpea

Six lines susceptible to *Fusarium* wilt disease and four testers known to be resistant to *Fusarium* wilt were crossed using a line x tester mating design. The F_1 and F_2 generations were evaluated under field conditions for *Fusarium* wilt resistance, grain yield and yield components. These genotypes were also evaluated using a pot experiment for reaction to *Fusarium* wilt resistance. The main findings were:

- Non-additive gene action was more important than additive gene action in controlling *Fusarium* wilt resistance, grain yield and yield components except for days to maturity and plant height.
- The best general combiner parents for resistance to *Fusarium* wilt resistance were TZA 2439, TZA 197, among the tested lines and ICEAP 00932 among the testers. These parents were selected because of their negative and significant general combining ability (GCA) effects for *Fusarium* wilt disease resistance.
- The most promising crosses were TZA 2439 X ICEAP 00557, TZA 2439 X ICEAP 00554, TZA 2514 X ICEAP 00557, TZA 197 X ICEAP 00932, TZA 197 X ICEAP 00040, TZA 5463 XICEAP 00554, TZA 5463 X ICEAP 00932, Tumia X ICEAP 00557 and Komboa X ICEAP 00932 for *Fusarium* wilt resistance under field conditions. In the pot experiment the most promising crosses were TZA 2439 X ICEAP 00932, TZA 2514 X ICEAP 00554, TZA 2514 X ICEAP 00040, TZA 197 X ICEAP 00557, TZA 197 X ICEAP 00554, TZA 197 X ICEAP 00040, TZA 5463 X ICEAP 00557, Tumia X ICEAP 00554, Tumia X ICEAP 00932. These families were selected based on negative and significant specific combining ability (SCA) effect for *Fusarium* wilt disease resistance.
- The most promising progenies for grain yield were TZA 2439 X ICEAP 00932, TZA 2514 X ICEAP 00557, TZA 2514 X 00932, TZA 2514 X ICEAP 00040, TZA 197 X ICEAP 00932, TZA 197 X ICEAP 00040, TZA 5463 X ICEAP 00554, Tumia X ICEAP 00554, and Komboa

X ICEAP 00557. These were selected due to their positive and significant SCA values for grain yield.

- The inheritance studies for *Fusarium* wilt resistance provided segregation ratios of 3R:1S, 9R:7S, 13R:3S, 15R:1S and ratios of 1R:3S and 7R:9S implying that the inheritance pattern for *Fusarium* wilt resistance is governed by one, two dominant, two complementary or recessive genes in that order.

7.3 Implications of the research findings to breeding pigeonpea for higher yield and resistance to *Fusarium* wilt resistant and way forward

The following implications were noted:-

- The participatory rural appraisal (PRA) identified breeding priorities encompassing farmers preferences. This is important for the acceptance and adoption of new breeding materials. Farmers preference should be considered in future pigeonpea breeding.
- Screening for resistant to *Fusarium* wilt identified ICEAP 00040, ICEAP 00932, ICEAP 00053, ICEAP 00557 and ICEAP 00554 as the potential donors for genetic improvement of pigeonpea for *Fusarium* wilt resistance in Tanzania
- There is considerable variation for pigeonpea genotypes grown by farmers as revealed by the phenotypic characterization based on qualitative and quantitative traits. This variation could be utilized in the improvement of pigeonpea for important traits.
- The molecular characterization and genetic relationships, using SSR markers, identified the most diverse and the closely related genotypes, to be used for *Fusarium* wilt resistance breeding. Crossing of parents with wider genetic distance enables the selection of superior candidate progenies.
- Non-additive genetic effects were predominantly noted in controlling of *Fusarium* wilt resistant, enhanced grain yield and yield components except for days to maturity and plant height. The dominance gene action suggests that hybrid breeding method could be a desirable method for genetic improvement.
- The inheritance of *Fusarium* wilt resistance among the F_2 populations showed phenotypic ratios of 3:1, 9:7, 3:3, 15:1 and ratios of 1:3 and 7:9. This suggests that the inheritance of *Fusarium* wilt resistance is governed by a variable number of genes across the tested populations. The variable number of genes found in these populations could be incorporated to susceptible varieties using a well-designed breeding method. To speed up the breeding process and to reduce the breeding cycle marker assisted selection (MAS)

should be integrated with the conventional breeding approach, as this will accelerate development and improvement of varieties.

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