CHARACTERIZATION AND INCORPORATION OF FUSARIUM WILT DISEASE RESISTANCE IN AFRICAN AND ASIAN PIGEONPEAS [*CAJANUS CAJAN* (L.) MILLSP.] GERMPLASMS

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ABSTRACT

Fusarium wilt is a plant disease caused by *Fusarium udum* (Butler). It is a soil and seed borne fungus which causes significant yield losses in susceptible cultivars throughout the pigeonpea growing areas. Numerous control measures have been suggested to alleviate the problem but their success has still remained low due to prohibitive costs. Development of resistant pigeonpea varieties is sought as an alternative for control of the disease. However, the task of developing resistant pigeonpea varieties has been complicated by variability in the pathogen. To undertake the study, the whole task was divided into three sub studies: Variability and aggressiveness of *Fusarium udum*, genetic diversity, inheritances and SSR marker segregation for the disease resistance, combining ability in yield and yield components. The Variability and aggressiveness of *Fusarium udum* isolates against 21 pigeonpeas were examined using pathogenicity and morphological characterizations, high level of resistance was noted in ICEAP 00040, ICEAP 00540 and ICEAP 00557 genotypes hence could be used as new source of resistance thus further studies using molecular markers were recommended. Sixty genotypes and 16 primers were used in diversity analysis. Close relationship was observed between Indian and East African collections. This could be diversified through selection, recombination and introduction of new source of variability from genetically diverse pigeonpeas. The study of genetic inheritance revealed a 3:1 ratio in F_2, thus proved a single dominant gene which could be used to donate genes for disease resistance into genotypes where Fusarium wilt is of an economic problem. The F_2 populations with resistant and susceptible parents were characterized using six SSR markers. Only CZ681922, CZ681962 and CZ681928 showed high correlation to phenotypic expression, hence application of these markers was recommended for sorting resistant genotypes. Path analysis showed that number of pods per plant, seeds per pod and 100 seeds weight had good contribution to yield and could be used as selection criteria in breeding programs. With

regard to combining ability parents, P2, P_5 and P_6 with crosses $P_1 \times P_2$, $P_1 \times P_4$, $P_1 \times P_5$, $P_2 \times P_3$, $P_2 \times P_4$, $P_3 \times P_6$ and $P_5 \times P_6$ were good general and specific combiners hence could be used by breeders as source of composite and hybrid materials.

DECLARATION

I, Maryanna Maryange Mayomba, do hereby declare to the Senate of Sokoine University of Agriculture, that this thesis is my original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

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DEDICATION

I dedicate this work to the ALMIGHTY GOD for keeping me healthier and strong enough to accomplish this study. Secondly I dedicate this work to my beloved family for continuous prayers and encouragement during the course of this work.

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LIST OF ABBREVIATIONS

$(HI/D)^{1/2}$	Average dominance variance
BC	Backcross
dH_2O	distilled water
DNA	Deoxy-ribo Nucleic Acid
dNTP	DeoxyriboNucleotide TriPhosphate
EDTA	Ethylene-Diamine-Tetra-Acetic acid
ESA	Eastern and Southern Africa
F_1,F_2	First and second feliar generation
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Statistics
GCA	General Combining Abilities
H_2O	Water
HBP	Heterosis Better Parent
HSD	Honestly Significant Difference
ICRISAT	International Crop Research Institude for Semi- Arid Tropics
MAS	Marker Assisted Selection
PCA	Principal Component Analysis
PCR	Polymerize Chain Reaction
PDA	Potato Deoxy Agar
pH	Hydrogen ions concentration
QTL	Quantitative Trait Loci
Rr	Heterozygous
SCA	Specific Combining Abilities

SMD	Sterility mosaic disease
SSR	Simple Sequence Repeats
SUA	Sokoine University of Agriculture
TBE	Tris Borate EDTA
v/v	volume volume ratio
Wr	Covariance of offspring and non recurrent parents
vr	Variance of offspring

CHAPTER ONE

1.0 INTRODUCTION

1.1 Pigeonpeas

Pigeonpeas *Cajanus cajan (*L.) Millspaugh are normally grown as an annual shrub or more usually short-term perennial shrub that may reach 4-5m in height, but usually 1-2m only, woody at the base, with a variable habit, but usually erect, with deep and quick growing tap root. Angular stem resulting from three ribs starting from the base of each petiole. The crop has trifoliate leaves, alternate set in a spiral along the stem. Leaflets are oblong, lanceolate, 5-10 cm long x 2-4 cm wide, pubescent likewise the stem. Lateral petioles, 2-3 mm the terminal one reaching 10-20 mm. Stipules are linear 2-3 mm long, stipulets filiform 1-2 mm long. Flowers usually yellow but they may also be striated with purple streaks or plain red, corolla20-25 mm, with the flag 18-20 mm wide. Calyx 10-12 mm long, with 5 linear teeth. Inflorescence composed of racemes having 5-10 flowers on top of an axillary, little divided peduncle. Pods flat, with an acuminate tip, pubescent and of variable colour, 5-9 cm long x 12-13 mm wide, containing 2-9 seeds in shades of brown, red or black (Osman *et al*., 2012).

Pigeonpea may continue to grow up to 5 years and develop into small trees (Phatak *et al*., 1993). It gives additional yield after the first harvest if sufficient moisture is available, and it has great flexibility in a wide range of cropping systems. The crop has a wide range of maturity (80 to 250 days) and time to maturity is greatly affected by temperature and photoperiod. The crop is used for intercropping with mainly *Zea mays* (maize), or *Sorghum bicolor* (sorghum) or *Manihot esculenta* (cassava) (Gwata *et al*., 2005). In intercropping, the crop performs well with 2

rows of cereals (e.g. sorghum, millets, maize), cotton or groundnut (Marer *et al*., 2007; Mwangi *et al*., 2010). After harvest of the intercrop, long-duration pigeonpea continues to grow and protects the soil.

Pigeonpea is regarded as a good plant for restoration of fertility and is used in a rotation with crops such as maize-groundnut-tobacco-pigeonpea for three to four years in Uganda. In Uganda, it is usually sown in alternate rows with sesame or African finger millet (*Eleusine coracana*), and in Malawi with maize. In Tanzania, the main intercrop is cassava. In Kenya, sorghum and maize are the most common intercrops with pigeonpea. In these cropping systems farmers grow traditional pigeonpeas varieties or landraces with long maturity duration of 9–10 months (Silim *et al*., 2005; Gwata *et al.,* 2005; Mahesh *et al.,* 2010). However, due to its high demand, there is a tendency to move away from traditional intercropping to monocropping. In Ukambani and Coastal strip, Kenya, the crop is grown commercially in large plots. In these cropping systems farmers grow traditional, long duration (9–10 months) landraces that yield approximately 0.4 ton/Ha of grain (Gwata *et al.,*2005). Although, short-duration types have been developed in Australia and India that mature in less than 100 days with a yield potential of over 5,000 kg/ha and can be grown as sole crop in multiple cropping systems (Agyare *et al.,*2002). Pigeonpeas are commonly grown as an annual crop, a hardy, widely adapted and drought tolerant crop with a large variable maturity ranging from 90-300 days. Therefore, its widely adaptation and variability of maturity time allows its production in a range of environments and cropping systems (Saxena, 2008).

Pigeonpea is belongs to the genus *Cajanus*, sub-tribe Cajaninae of the economically important leguminous tribe Phaseoleae which contains soybean (*Glycine max* L.), common bean (*Phaseolus vulgaris* L.), dolichos (*Dolichos lablab*) and mungbean (*Vigna radiata* L. Wilczek) (Young *et al*., 2003). The crop is a perennial shrub with a diploid genome comprising 11 pairs of chromosomes (2n=2x=22) and a genome size of 858Mbp. The genus *Cajanus* comprises of 32 species where by India sub-continent, Australia, and Africa accounting for 17, 13 and 2 wild species, respectively (Odeny, 2007; Bohra *et al*., 2011a).

It is an important grain legume grown in tropics and subtropics with its primary centre of diversity being in India and secondary centre of diversity being in the regions along Eastern and Southern Africa (ESA) extending from Uganda, Kenya, Malawi, and Tanzania to Mozambique where it has been grown for at least 4,000 years (Villiers *et al*., 2008). A wide diversity of cultivated pigeonpea is also available in developing country for production and crop improvement against biotic and abiotic stresses.

1.2 Fusarium Wilt Disease

Fusarium wilt disease (FWD) is a plant disease caused by *Fusarium udum* (Butler). *Fusarium udum* (Butler) is a soil and seed borne fungus which causes significant yield losses in susceptible cultivars throughout the pigeonpea growing areas and resulting in up to 100% yield loss (Gwata *et al*., 2005; Odeny *et al.,* 2009; Prasanthi*et al.,* 2009; Karimi *et al*., 2010; Pande *et al*., 2013; Ajay *et al.,* 2013). It is capable of surviving in the soil for many years (Pinto *et al.,* 2010). The pathogen lives in the soil, between crops it survives in residual plant debris as mycelium and

in all its spore forms. The germ tube of the mycelium or spore penetrates seedlings through root tips, wounds or point of formation of lateral roots. The mycelium advances through the xylem causing infection at flowering and pod-filling stages (Gwata *et al*., 2005). It causes significant yield losses in susceptible cultivars throughout the pigeonpeas growing areas (Karimi *et al.,* 2010). Surveys carried out estimated wilt incidence to be 60% in Kenya, 36.3% in Malawi and 20% in Tanzania (Pande *et al*., 2013).

In some of the fields wilt incidence was as high as 90% with annual losses of US$5 million in each of these countries (Mesapogu *et al*., 2012). Although numerous control measures have been suggested to alleviate the problem of FWD and increase productivity of pigeonpeas, their success still remains low due to prohibitive cost of practices and labourconstraints among smallholder producers. Development of well-adapted resistant pigeonpea varieties is sought as an alternative for control of FWD. However, the task of developing resistant pigeonpea varieties has been complicated by the reported variability in the pathogen (Mahesh *et al*., 2010). The presence of strains of Fusarium wilt pathogens of varying degrees of aggressiveness on pigeonpeas has been observed (Harlapur *et al*., 2007).

The reactions of pigeonpea to Fusarium wilt pathogen vary considerably between and within African and Asian continents. Songa *et al*. (1995) found that pigeon pea line ICP 9145, which was wilt resistant at Ilonga (Tanzania), Katumani (Kenya), International Crop Research Institude for Semi- Arid Tropics (ICRISAT) Asia Centre (India), and Malawi was highly susceptible (71% wilt) at Kiboko (Kenya). In addition, line ICP 2376 that was susceptible in India, had an average wilt of 47%

in Kenya compared with 100% usually reported at ICRISAT in India. Other pigeonpea lines that were reported as resistant to wilt in other locations failed to give uniform performance at ICRISAT in India (Reddy and *et al*., 1998).

The heritability of resistance trait is quantitative and not clearly understood suggesting a need for identification of candidate quantitative trait loci (QTL) responsible for resistance to FWD. Despite extensive pathological and molecular studies, the nature and extent of pathogenic variability in *F. udum* has not been clearly established. This situation complicates the process to be used in the selection of genotypes that have multiple resistances for wider range of pathogenic races. Therefore, the information on characterization of *F. udum* is needed to help identify race differentials. Furthermore, genetic diversity and relatedness of Asian and African pigeonpea genotypes with respect to wilt is not clearly understood. Understanding diversity and relatedness is an important component of crop improvement, while characterization of genetic diversity allows for better selection of diversified parents in order to combine desirable traits for crop improvement.

1.3 Geographical Distribution of Fusarium Wilt Disease

Fusarium wilt was first recorded by Butler (1906) in India. Although the disease is more prevalent in India, East Africa and Malawi where field losses of over 50% are common, it also occurs in Bangladesh, Grenada, Indonesia, Mauritius, Mynmar, Nepal Nevis, Venezuela, Trinidad, and Tobago (Karimi *et al*., 2012). Recently, this pathogen was reported to be spreading in Southern Africa reaching areas in Mozambique (Southern Zambezi province) (Gwata *et al*., 2006).

Although the incidence and distribution information is not available, the disease has also been reported in Zambia (Reddy *et al*., 1993). Ghana is also included in the distribution list but the presence of the disease in the country has not been confirmed (Reddy *et al*., 1993). In Kenya, the disease was first reported in 1983 when the first released variety (Munaa) broken down with F.W.D and was withdrawn from the production (Kimani, 1991). The disease was found in all pigeonpea growing areas but incidences are high in the eastern areas (Kannaiyan *et al.* 1984; Hillock and Songa, 1993). In Tanzania the distribution occurs around Babati in the north, in the southern zone around Mtwara and along the coast near Dar es Salaam. Although the FWD has been observed in Uganda, the present distribution and incidence of the disease is not known (Karimi *et al*., 2012).

The existence of races of *F. udum* has been reported in Zambia and Ghana, but information on the incidence and distribution is not available (Reddy *et al.,* 1993). There is variability in the pathogen races and ecotypes of *F. udum* in different locations (Patel *et al*., 2011), where the presence of strains of fusarium wilts pathogens of varying degrees of virulence on pigeonpea has been observed. Lines that were reported as resistant to FWD in other locations failed to give uniform performance at ICRISAT in India (Nene *et al*., 1979).

1.4 Problem Statement and Justification

Among the biotic factors affecting pigeonpeas productivity is fusarium wilt, a soil-borne disease caused by fungus *Fusarim udum,* capable of surviving in the soil for at least eight years (Nene *et al*., 1981). It causes significant yield losses in susceptible cultivars throughout the pigeonpeas growing areas. Pigeonpea yields

have been declining due to serious and recurring occurrence of this disease. In India, the annual loss due to this disease is estimated at US $71 million (Reddy *et al*., 1993). Surveys carried out estimated wilt incidence to be 15.9% in Kenya, 36.3% in Malawi and 20% in Tanzania. In some of the fields wilt incidence was as high as 90% with annual losses of US$5 million in each of these countries (Reddy *et al*., 1998). Although numerous control measures have been suggested to alleviate the problem of Fusarium wilt and increase productivity of pigeonpeas, their success still remains low due to prohibitive cost of practices and labour-constrains among smallholder producers.

Development of well adapted resistant pigeonpea varieties is sought as an alternative for control of Fusarium wilt. However, the task of developing resistant pigeonpea varieties has been complicated by the reported variability in the pathogen (Saxena, 1990). The presence of strains of Fusarium wilt pathogens of varying degrees of virulence on pigeonpeas has been observed. The reactions of pigeonpea to Fusarium wilt vary considerably between and within African and Asian continents. Songa *et al*. (1995) found that pigeonpea line ICP 9145, which was wilt resistant at Ilonga (Tanzania), Katumani (Kenya), ICRISAT Asia Centre (India), and Malawi was highly susceptible (71% wilt) at Kiboko (Kenya). In addition, line ICP 2376 that was susceptible in India, had an average wilt of 47% in Kenya compared with 100% usually reported at ICRISAT in India. Other pigeonpea lines that were reported as resistant to wilt in other locations failed to give uniform performance at ICRISAT in India (Allen and Lenne, 1998)

The heritability of fusarium wilt resistance trait is quantitative and not clearly understood suggesting a need for identification of candidate quantitative trait loci (QTL) responsible for resistance to fusarium wilt. Also extent of variability of *F. udum* strainsi*s* not clearly understood thus complicating the process to be used in the selection of genotypes that have multiple resistances for wider range of pathogenic races.

Furthermore, genetic diversity and relatedness of Asian and African pigeon pea accessions with respect to wilt disease is not clearly understood. Understanding diversity and relatedness is an important component of crop improvement, while characterization of genetic diversity allows for better selection of diversified parents in order to combine desirable traits for crop improvement

1.5 Overall Objective

To improve Fusarium wilt disease resistance in pigeonpea [(*Cajanus cajan* (L.) Millsp] genotypes preferred by consumers in Tanzania.

1.5.1 Specific objectives

i. To identify and determine *Fusarium udum* strains and extent of variability among isolates affecting pigeonpea genotypes.
ii. To determine genetic diversity and relatedness of selected Asian and African pigeonpea genotypes.
iii. To develop FWD resistant pigeonpeas lines for improved yield and quality characteristics.
iv. To identify morphological and molecular markers associated with resistance to FWD in pigeonpeas.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin of Pigeonpeas

Pigeonpea, *Cajanus cajan* (L.) Millspaugh is one of the major grain legume crops grown in about 50 countries in the tropics and subtropics (Wasike *et al*., 2005; Akande, 2007; Vange and Moses, 2009; Kamlesh and Dubey, 2012). Pigeonpea name was first derived from plants used in Barbados as an important pigeon feed. Based on the range of genetic diversity of the crop, India was considered as centre of origin of pigeonpeas (Kassa *et al*., 2012). Although several authors also considered Eastern Africa to be the centre of origin of pigeonpea, as it occurs there in the wild form. According to Mula and Saxena (2010), Songok *et al.* (2010) review the pigeonpea origin has been agreed in favour of India. Vange and Moses (2009), Malviga and Yadav (2010) concluded that India was the primary centre of origin and Africa was the secondary centre of origin of pigeonpea, therefore both are centres for origin and diversity. It is clear that the species has been under cultivation for a long period. Several countries in Africa (in the central, western, and southern regions), North America, Central America, and South America have been identified as potential areas for pigeonpea production (Kassa *et al*., 2012).

2.2 Ecology of Pigeonpeas

The crop reported to have wide adaptability in different climatic and soil condition (Odeny, 2007). It grows well on a broad range of well-drained soils, from sands to clays over sedimentary, igneous and metamorphic parent materials. It is remarkably tolerant to pH of ranges from 4.5 to 8.4 and some varieties tolerate 6 to 12 mmhos/cm of salinity. Pigeonpeas grow in areas that receive rainfall between 600-

1000 mm. In contrast to other legumes, which rapidly close their stomata, pigeonpeas allow for stomatal adjustment in response to water stress, allowing for osmotic adjustment until a critical internal water status occurs. In addition, solutes and other compounds in pigeonpea help to maintain integrity of the cells, preventing protein denaturation (Odeny, 2007). The ability of pigeonpea to withstand severe drought is attributed by its morphology and vessel diameter(deep root) which allows extraction of moisture from deep layers of the soil and thus makes it a crop that produces biomass including protein-rich grain while utilizing residual moisture (Porter and Didlack, 2011). Its unique polycarpic flowering habit further enables the crop to shed reproductive structures in response to stress. However, the pigeonpea does not tolerate water logging and frostbut producing seed profusely under dry zone conditions, as the crop matures early and the incidence of pest damage is low (Odeny, 2007).

The crop is more or less photoperiod-sensitive; short days decrease time to flowering. Under humid conditions pigeonpea tends to produce luxuriant vegetative growth and rain during the time of flowering causes defective fertilization and permits attack by pod-caterpillars. The plant is remarkably hardy to both low temperatures (as low as 5° to 10°C) and high temperatures (up to 40°C) and, thus, is an ideal crop to fit into cropping systems in many parts of the world. Pigeonpea is intolerant of shade and tolerates only moderate competition. It does best in full sun on bare ground but can grow with side shade or broken shade from trees and a low cover of grass and forbs. Growth is moderately slow during the first 2 months to 3 months of life during which time seedlings are not competitive with grass and

weeds; afterwards pigeonpea competes well with vegetation equal or lower in height (Van der Maesen, 1990).

2.3 Importance and Uses of Pigeonpeas

Pigeonpea is an important food in developing tropical and subtropical countries (Vange and Moses, 2009). Its major benefits include food for human consumption (proteins, carbohydrates, and minerals) (Table 2.1), income generation (Shiferaw *et al*., 2007; kunjeku and Gwata, 2011; Infonet-biovision, 2013). The protein content of commonly grown pigeonpea cultivars ranges between 17.9 and 24.3 g/100g for whole grain samples, and between 21.1 and 28.1 g/100 g for split seed (Sheahan, 2012). Wild species of pigeonpea have been found to be a very promising source of high-protein and several genotypes were developed with a protein content as high as 32.5% (Singh *et al*., 1990). The high-protein genotypes also contain significantly higher (about 25%) sulphur-containing amino acids, namely methionine and lysine which assist in break down of fats and thereby prevents the build-up of fat in the arteries, as well as assisting with the digestive system (Oluwaseun, 2013). Pigeonpea seeds contain about 57.3 to 58.7% carbohydrate, 1.2 to 8.1% crude fibre, and 0.6 to 3.8% lipids (Hassan *et al*., 2013). It is also a good source of soluble vitamins thiamine, riboflavin, niacin, and choline (Valenzuela, 2007). Since pigeonpeas contain high protein value they supplement the diets for millions of people, especially traditional cereal-, banana- or tuber-based diets of resource-poor farmers that are generally protein-deficient. The perennial nature of pigeonpea allows farmers to take multiple harvests with surpluses traded in both local and international markets (Odeny, 2006).

Pigeonpeas also is used in various areas where it is consumed in various ways, for example, the green seeds (and pods) serve as vegetable while ripe seeds are a source of flour, used split (dhal) in soups or eaten with rice also the ripe seeds may be germinated and eaten as sprout. Tender leaves are rarely used as a potherb. The crop has also widely been used as a traditional folk medicine. The leaves have been reported to arrest blood flow, relive pain and kill worms. They can be used to cure measles, dysentery, jaundice, diarrhea, cough, sore, bronchitis, bladder-stones, diabetes and many other illnesses (Saxena *et al*., 2010).

Pigeonpea is an important component in the integrated crop and livestock systems of the semi-arid tropics as it's by-products of split and shrivelled seed are used as livestock feed (Troedson *et al.,* 1990). The present high cost of animal sources of protein feeds, such as fish and bone meal, makes pigeonpea ideal to be used as a good plant protein substitute as it is less expensive. Due to its role as excellent forage/ feed for livestock there is a great scope for selecting cultivars with not only higher grain yields but also higher forage yields and crude protein (Morton, 1976). Pigeonpea produces more nitrogen from plant biomass per unit area of land than many other legumes although it usually produces fewer nodules than legumes. The crop can fix about 70 kg N/ha per season by symbiosis until the mid-pod-fill stage. This is around 88% of the total nitrogen content of the plant at that stage of growth. The residual effect on a following cereal crop can be as much as 40 kg N/ha (Phatak *et al.,* 1993).

For optimum production the crop requires a supply of mineral nutrients, the most important of which is nitrogen. The exhausted soils are often low in nitrogen, meaning that farmers are normally applying inorganic fertilizers. However, as fertilizer costs increase, farmers struggle to obtain good yields. This problem can be addressed by incorporating pigeonpeas into the cropping system. Leguminous plants have a special relationship with nitrogen-fixing bacteria (*Rhizobium*). By biologically fixing nitrogen levels in the soil, legumes provide a relatively low-cost method of replacing nitrogen in the soil, improving soil fertility and boosting subsequent crop yields (Saxena *et al*., 2010).

Table 2.1: Nutritive Value per 100 g of edible portion of pigeonpea

Raw or Cooked Pigeonpea	Food Energy (Calories / %Daily Value*)	Carbohydrates (g / %DV)	Fat (g / %DV)	Protein (g / %DV)	Calcium (g / %DV)	Phosphorus (mg / %DV)	Iron (mg / %DV)	Potassium (mg / %DV)	Vitamin A (I.U)	Vitamin C (I.U)	Vitamin B 6 (I.U)	Vitamin B 12 (I.U)	Thiamine (mg / %DV)	Riboflavin (mg / %DV)	Ash (g / %DV)
Pigeonpeas (Red Gram) cooked	121 / 6%	23.2 / 8%	0.4 / 1%	6.8 / 14%	43.0 / 4%	119.0 / 12%	1.1 / 6%	384 / 11%	3.0 IU / 0%	0.0 / 0%	0.1 / 3%	0.0 / 0%	0.1 / 10%	0.0 / 0%	1.1
Pigeonpeas (Red Gram) raw	343 / 17%	62.8 / 21%	1.5 / 2%	21.7 / 43%	130 / 13%	367 / 37%	5.2 / 29%	1392 / 40%	28.0 IU / 1%	0.0 / 0%	0.3 / 14%	0.0 / 0%	0.6 / 43%		

* Percent Daily Values (DV) are are based on a 2000 calorie diet. Your daily values may be higher or lower depending on your calories needs.

Source: infonet-biovision (2013)

Pigeonpea gains a high popularity level and it is proven by the fact that it is cultivated in more than 25 countries of the world. As compared to the other pulses produced in the world, pigeonpea holds the sixth rank in the context of production. The world production of this crop stand at around 3.25 million tons annually valued at around US$ 1,600 million (Odeny, 2007). Dominant producers of this crop are the countries in the Indian sub-continent, Africa and Central America as the climate conditions suit the development of the crop (Odeny, 2006). The leading producer is India producing about 85 % of the world's total produce. Pigeonpea is consumed throughout the world as a staple food. Though India is the largest producer of the crop but it is not into the exports of the crop at all, as the domestic consumption demand in the country is quite high but Myanmar, the neighbouring country to India leads the crop exporting countries' list. The major pigeonpea exporting countries are Myanmar, Republic of Tanzania, Kenya, Malawi, Uganda and Mozambique. The pigeonpea importing list is topped by the India and the European Union.

2.4 Growth and Management of Pigeonpea

Pigeonpea is normally sown directly into prepared ground. Seeding rates for pure stands are 12 to 25 kg of seed/ha. Seeding depths of 2.5 to 5.0cm are recommended (Center for New Crops and Plants Products, 2002). Optimal moisture and temperature (29°C-36°C) is required for germination to occur. The crop is a slow-growing crop and mostly cultivated during the rainy season. Mostly pigeonpea suffers from early weed infestation as it fails to compete with weeds at the early stage of development. Therefore, it is necessary to keep the crop weed-free during the early growth period (4-6 weeks) (Reddy, 1990).

Pigeonpea is largely grown as a rain-fed crop; however, during the flower initiation and pod setting stages are the most crucial to drought stress. Therefore, irrigation at these stages usually helps the crop from drought development symptom which includes leaves pointing towards the sun at noon (Chauhan *et al*., 1988). However, excessive moisture is detrimental to pigeonpea as it promotes vegetative growth and enhances the incidence of *Phytophthora* and *Alternaria* blights. Therefore, irrigation should be given only when the crop experiences drought stress after flowering and at pod filling stage.

2.5 Pigeonpea Production in Tanzania

Pigeonpea *(Cajanus cajan*) is an important grain legume in the semi-arid regions of Tanzania. It ranks third among pulses (after beans and cowpeas) in total production. The crop is grown in several parts of the country. The major growing areas include Lindi, Mtwara, Kilimanjaro, Arusha, Manyara and Shinyanga regions. The crop is also grown along the coast, Dar es Salaam, Tanga and in Morogoro regions in the eastern zone where it is used mainly as a vegetable (green peas). About 14 districts are primary producers mainly located in the southern and northern zones of the country. In these districts, pigeonpea is mainly harvested and consumed or sold as dry grain, while it is mainly harvested at the green stage and consumed as a vegetable (green peas) in the secondary production areas. In the northern zonedistricts including Babati, pigeonpea is mainly grown as a cash crop. Traditionally, the farmers in the northern zone prefer to consume other legumes such as beans and cowpeas while their counterparts in the southern zone districts lack these alternative food sources and therefore use a larger share of their pigeonpea produce for home consumption. The quality of pigeonpea from the

northern zone districts is also considered to be superior and hence more suited for the export market, especially the large and white colored grains grown in Babati (Shiferaw *et al*., 2005).

The crop is grown under both sole and intercropping systems. Normally there are four distinct durations for pigeonpea varieties - extra short (mature in <100 days), short (100-120 days), medium (140-180) days and long duration (>200 days). Each group is suited to a particular agro-ecosystem, which is defined as a function of altitude, temperature, latitude and day length (van den Beldt, 1988).

Mostly the early -maturity (100-120 days) genotypes are grown as a sole crop. These genotypes have a higher harvest index with an average of 34% compared to medium-maturing genotypes at 24% (Faujdar and Oswalt, 1992). Therefore, from the cropping system point of view, early-maturity genotypes are able to provide an increased opportunity for a second crop. Post rainy season pigeonpeas are also cultivated as a sole crop. This avoids the wet conditions associated with the rainy season, gives fewer incidences of insect pests and diseases, and makes better use of residual soil moisture. Moreover, post rainy season sole crops were found more efficient than the rainy season sole crops also intercropped with maize, sorghum, beans, and cowpea.

The survey results indicated that most farmers in Tanzania use tall long-duration landraces that are susceptible to abiotic (drought) and biotic (insect pests and diseases) stresses (Mligo, 1994). According to FAO statistics, pigeonpea accounted for about 11% of the total annual production of pulses in Tanzania between 1992

and 2000 (Shiferaw *et al*., 2005). Thus the government now places great emphasis on increasing the production of low volume, low-value, and developing the non-traditional export sector (Table2.2).

Table 2.2: Mean annual production, cultivated areas, and shared pulses in Tanzania (1992- 2000)

Crops	Production (1000t)	Share %	Cultivated areas 1000ha	Share %
Beans	241.1	60.5	350.6	47.4
Cowpeas	44.1	11.1	146	19.7
Pigeonpea	44	11	62.9	8.5
Other pulses	69.2	17.4	180.7	24.4
Total pulses	398.4	100	740.1	100

Source Shiferaw *et al*., 2005

2.6 Constraints in Pigeonpea Production

Although pigeonpea is the most important crop in Tanzania and Eastern African region in general little concerted research effort has been directed at either crop improvement or technology transfer situation which causes slow growth of pigeonpea as compared to other pulses like common beans and cowpeas (Lyimo and Myaka, 2001, Varshney *et al*., 2012). The production of pigeonpea has remained static over the last several years. From this observation many farmers were abandoning pigeonpea cultivation (Odeny, 2006). Recent surveys indicate that biotic factor like Fusarium wilt caused by *Fusarium udum*, sterility mosaic disease (SMD), leaf spot (*Mycovellosiella cajani*), *Macrophomina* stem canker, rust and to a lesser extent powdery mildew (*Leveillula taurica*) are diseases of economic importance of pigeonpea (Prasanthi *et al*., 2009; Dialoke*et al*., 2010; Kamlesh and Choure, 2012). In surveys conducted in Kilosa district during the 1988 cropping season, it was observed that FWD was a major constraint in pigeonpea production, with wilt incidence ranging from 0% to 100% on farmers' fields depending on stage

of infection (Kiprop *et al.,* 2005). Screening of large number of lines needs creating sick plots, artificial inoculation and time taking as the disease occurs at any stage of crop and rapid screening may not be possible. As diferent needs and opportunities surface, pigeonpea breeders need to incorporate new genetic sources using various breeding methods aided with modern tools such as biotechnology.

Other factors like traditional landraces were also responsible for the decline of crop yield on farmer's field in Tanzania. Year after year use of these traditional landraces, which frequently suffer from different biotic and abiotic stresses due to lack of quality seeds results into decrease in productivity of pigeonpea. Poor production practices such as low plant densities, low soil fertility, insufficient weeding and inappropriate use of fungicides and herbicides also limit the production. Pigeonpea is mostly underutilized as compared with other legumes like common beans and cowpeas, a situation which lead in low production rate of the crop (Akande, 2007). Not only this also abiotic factor such as drought, soil with poor water holding capacity and socio-economic factor like infrastructure, marketing and exploitation by middlemen) affecting pigeonpea productivity in major crop growing regions of Tanzania.

2.6.1 Fusarium wilt disease in pigeonpeas

Pigeonpea is attacked by more than 100 pathogens including fungi, bacteria, and viruses, nematodes and mycoplasma-like organisms, but *Fusarium udum* is considered the most important soil borne pathogen of pigeonpea (Parde *et al*., 2012; Sharma, 2013; Chhetry and Devi, 2014). Fusarium wilt disease caused by fungal pathogen *Fusarium udum* Butler, is the vascular disease that trasmit through seed

and soil (Pande *et al.,* 2007) which is difficult to handle through chemical, biological and other cultural practice like crop rotation, and field sanitation.

It is the most devastating seed and soil borne disease of pigeonpea affecting plants at all stage of growth eventually causing significant yield losses in susceptible cultivars throughout the pigeonpea growing areas (Kiprop *et al.,* 2005; Karimi *et al*., 2010, Datta and Lal 2012). In India, the annual loss due to this disease is estimated at US $71 million (Reddy *et al*., 1993). Fusarium wilt causes economic loss in pigeonpea of about 470, 000 t of grains in India and 30,000 t of grains in Africa. Karimi *et al*. (2012) reported wilt incidence (and range) in Kenya, Malawi and Tanzania of 15.9% (0-90%), 36.6 (0-90) and 20.4% (0-60%) respectively with annual loss estimated at US $ 5 million in each of the countries. According to Mbwaga (1995) in Tanzania, an incidence of fusarium wilt disease is as high as 96% been observed.

The disease can occur at any stage of the crop with the entry of fungus into the hosts' vascular system at root tip leading to progressive chlorosis of leaves, branches and finally wilting of the whole plant. After infestation in the soil of some areas it can rapidly spread to new areas (Prasanthi *et al*., 2009).

2.6.2 Description and symptoms of Fusarium wilt disease on pigeonpeas

Patches of dead plants in the field, usually when the crop is at flowering stage or pod set, are the first indications of wilt. Isolated wilted plants are noted about a month after sowing. The most characterized wilt symptom in the adult is purple colour band extending upwards from the base of the main stem (Plate.2.1). This

band is more easily seen in pigeonpeas with green stems than in lines with coloured stems. Partial wilting of the plant is a definite indication of wilt and distinguishes this disease from termite damage, drought and *Phytophthora* blight which also kill the plants. Partial wilting is associated with lateral root infection while total wilt is a result of tap root infection (Reddy *et al.,* 1998).

Other symptoms of wilt are browning of the stem below the purple band and browning or blackening of the xylem which is visible when the main stem or primary branches are split open. Intensity of browning or blackening decreases from the base to the tip of the plant. Sometimes, branches especially lower ones, are affected even when there is no band on the main stem. These branches show dieback symptoms with purple band extending from the tip downwards, and intensive blackening of internal xylem. When young plants (1-2 months old) die from wilt; they usually do not show external banding but have obvious internal browning or blackening. Plant infected by *F. udum* also exhibit loss of turgidity in leaves, and interveinal clearing followed by slight chlorosis and yellowing of the leaves before wilt. Withered leaves are retained on wilted plants before death (Reddy *et al*., 1998).

Plate 2.1: Partial wilting in pigeonpea caused by infection of *Fusarium udum* pathogen

2.6.3 Epidemiology of *Fusarium udum*

The disease is both soil borne and seed borne (Kannaiyann, 1992). Untreated seeds showed levels of internal infection with *F. udum* of 13- 19%. Thus effective seeds treatment eradicates the pathogen. Infected seeds may be the primary means of spread of *F. udum* over long distances and new areas. The pathogen can survive on infected plant debris in the soil for more than three years. Disease incidence is more severe on vertisols than on alfisols and rationing predisposes the plant to wilt. Early sowing, weed management and vigorous crop growth favour wilt development. Long and medium duration pigeonpea varieties suffer more than short and extra short duration types. Sole system of cropping favours more on wilt infection than intercropping system of crop production.

Though infection may occur in the seedling stage, maximum expression of the disease is at flowering and podding (Reddy *et al.,* 1998). This seems to be due to the extended time needed by the fungus to colonize the plant. The recent work in ICRISAT has shown that infected plants wilt only after the basal half of the main stem is colonized by the fungus, which takes approximately 3- 4 months (Reddy *et al.,* 1998). This explains why there are low levels of wilt infestation in short duration types compared to long-duration and ratooned pigeonpeas, as the former morphotypes are escaping wilt. Any practice which leads to increased plant biomass in pigeonpeas was found to increase susceptibility to wilt (Reddy *et al*., 1994). Higher biomass is produced when the crop is sown early, under weed-free and well-drained conditions in fertile fields at low plant density and when rains are well distributed.

2.6.4 Biology of *Fusarium udum*

Fusarium udum is described as parasitic within the roots of the plant, or saprophytic. The hyphae are hyaline slender, much branched, usually with aerial growth. Micro conidia are produced successively on the ends of short simple or clustered, vertically branched conidiosphores. They are usually aseptate, elliptical, hyaline singly but salmon – pink in mass, occasionally develop from the surface of spherical stromata, and are 6 -11 x 2- 3 μmin diameter. In culture, the microconidial stage is usually white to salmon-pink, occasionally orange-red but never green or purple. Macroconidia are formed on short conidiophores and detach soon after abjunction. They are hyaline, three to five septate, 15-50 x3-5μm in size, falcate with a distinct foot cell and an atypical cell of decreasing diameter towards the tip which may be curved or hooked. The chlamydospores are round or oval, rather

thick-walled, hyaline, intercalary in the mycelium, sometimes in short chains and 5-10 µm in diameter (Reddy *et al.,* 1998).

Limited information on the perfect state considered to be associated with *F. udum* is available. Perithecia of *Gibberella indica* (a perfect stage of *F. udum*) were formed in the fields but infrequently on wilted pigeonpea. If formed on exposed roots or in the collar region of the plant, they are superficial, commonly aggregated, globose to subglobose, sessile and smooth walled. The ascus contains eight ascospores which are 2-3 celled. Ascospores germinate to produce micro and macroconidia as illustrated (Fig 2.1) below. The association of the perfect state with *F.udum* and its role in pathogenesis, however, needs further investigation (Reddy *et al.,* 1998).

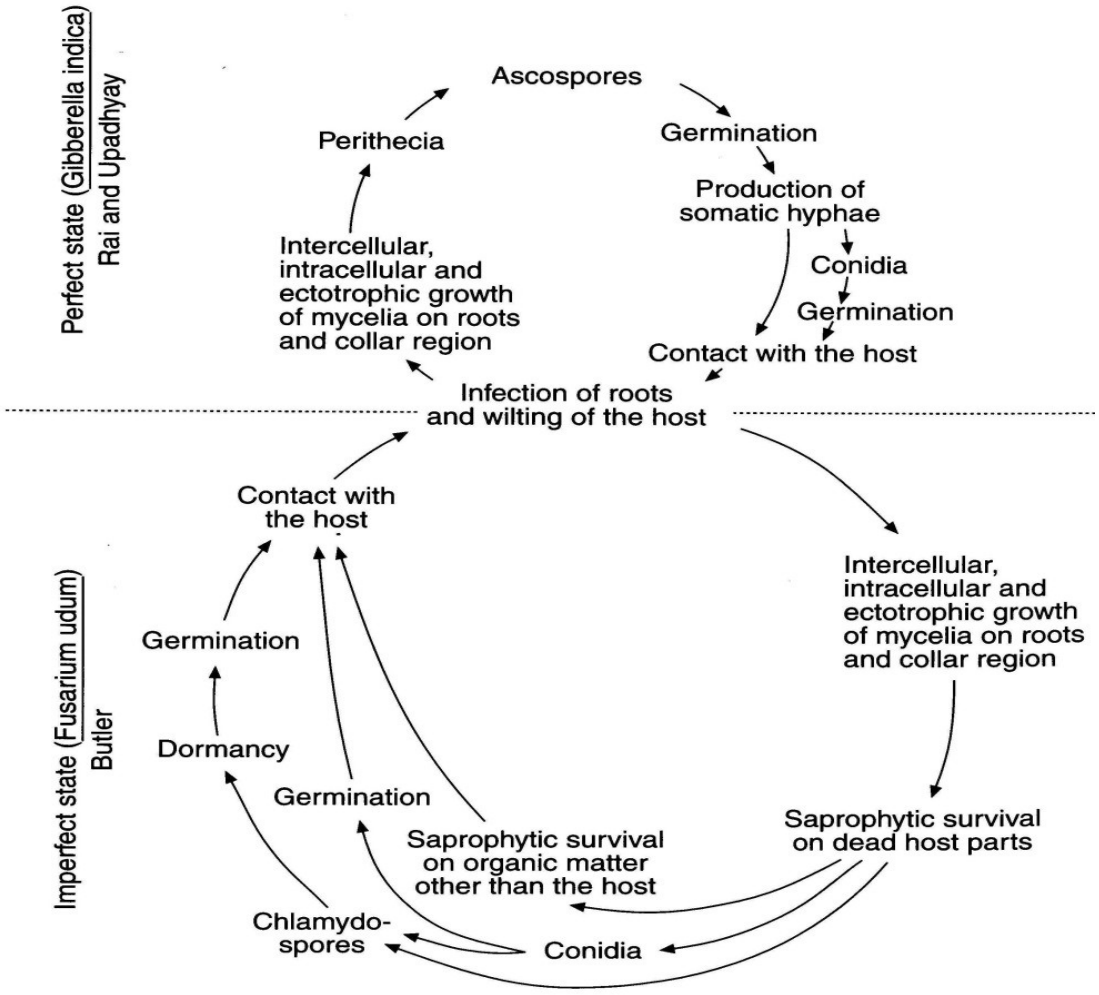

Figure 2.1: Life cycle of *F. udum* causal agent of wilt of pigeonpea. (Reddy *et al*., 1998)

2.6.5 Factors affecting infection and spread of the *Fusarium udum* pathogen

2.6.5.1 Temperature

Temperature has been cited as one of the weather related factor for the development of the wilt disease (Karimi *et al*., 2010). The optimum growth of *F. oxysporum* f.sp. *Ciceris* was at 24.5–28.5°C and chickpea cultivar was moderately resistant to *F. oxysporium* f.sp. *Ciceris* when grown in temperatures ranging from21–24°C, but highly susceptible at a temperature regime of 25–27°C (Josh *et al*., 2009; Karimi *et al*., 2012).

The physiological study with *F. oxysporum* f.sp. *cubense* isolates revealed that the optimum temperature was 25°Cfor all the isolates, no growth was observed at temperatures of 5 and 40°C, while very little growth was observed at 10 and 35°C (Karimi *et al.,* 2012) through modelling reported positive correlation between wilt severity and soil temperature.

2.6.5.2 Nutrient status of the soil

Decreases or quiescence of *Fusarium* population in soil depends on the stage of growth of the crop, the ecological balance and nutrient availability. Nene (1981) reported that the persistence of this pathogen is influenced by soil type and nutrient status. High levels of nitrogen fertilization in agricultural soils generally lead to an increase in Fusarium wilt disease development. Studies have also shown that the form of nitrogen in the soil is important in the development of FWD. The *F. oxysporum* cultured on ammonium nitrogen was more virulent than the same fresh weight of organism cultured on nitrate nitrogen (Wolts and Jones, 1973). Effects on nitrate and ammonium sources on disease were apparently related to soil pH effects. Nitrate causes an elevation in soil pH while ammonium causes a reduction. The nitrate form of nitrogen becomes increasingly unfavourable for the disease with increasing rate of application, while the ammonium form becomes more favourable as the nitrogen application rate is increased. Relatively low levels of calcium appear more conducive to disease than normal levels (Karimi *et al*., 2012).

2.6.5.3 Soil and soil pH

The amount of wilt incidence is influenced by water retentive nature of the soil and the disease is favoured by slightly acidic and alkaline soils with sand content of

more than 50% as the crop prefers soil pH of 5- 7, but can tolerate pH of 4.5- 8.4 (Upadhyay and Rai, 1992). Hillocks *et al*., (2000) observed more Fusarium wilt inoculum in sand (94%) than in heavy black cotton soil (18%). However, some soils are suppressive to the pathogen due to their physico-chemical characteristics (Upadhyay and Rai, (1992) found that a higher soil pH reduced the disease incidences.

2.6.5.4 Soil antagonists

The susceptibility of pigeonpea cultivars to FWD is increased by the presence of certain nematodes in the soil (Hillocks *et al.,* 2000). The association between Fusarium wilt and root-knot nematodes is well established (Hillocks and Songa, 1993; Marley and Hillocks, 1994; Marley and Hillocks, 1996). In India, reports have shown that the cyst nematode, *Heterodera cajani* (Hasan, 1984; Sharma and Nene, 1989) and reniform nematode, *Rotylenchulus reniformis* (Sharma and Nene, 1990; Jain and Sharma, 1996) increase susceptibility to the disease. Its growth is also influenced by soil antagonists especially the bacterium *Bacillus subtilis* that produces the antibiotic bulbiformin (Pursey, 1989). The microflora has also been reported to affect the pathogenicity of the fungus (Upadhyay and Rai, 1987).

2.6.5.5 Pigeonpea genotypes

The disease begins in the field in a small patch, which enlarges with each successive year that a susceptible cultivar is grown. Since FWD is seedborne disease may be carried to the field and another generation (Upadhyay and Rai, 1983). This may explain why wilt is common in areas where the crop is grown year after year making it more devastating in small-scale farmers who retain their seeds.

Also the continous use of rationing and knife which acts as a carrier of inoculum from plant to plant could be the major means of transfering Fusarium wilt disease.

2.6.5.6 Host plant resistance

Several resistant pigeonpea lines have been developed at ICRISAT in India, Kenya and Malawi (Reddy *et al.,* 1990). However, inconsistence in reaction to FWD is common and reports have shown that resistance is environmentally unstable and pathogenecity is cultivar specific (Patel and Patel, 2012). Several cultivars that were earlier claimed to possess resistance genes against the FWD failed to give uniform performance at ICRISAT (Nene *et al*., 1979). It has also been shown that due to segregation of varieties for resistance to FWD, some varieties succumb to the disease in subsequent generations (Hillocks*et al*., 2000).

Testing of pigeonpea against FWD has shown that reaction can vary considerably between countries and even sites within the same country (Songa *et al*., 1995; Hillocks*et al*., 2000). For instance, pigeonpea line ICP 2376 was highly susceptible to F.W.D (100%) in India (17^{o}N, 78^{o}E), while in Kenya an average wilt incidence of 47% was observed (Songa *et al*., 1995). Also it was found that pigeonpea line ICP 9145, was resistant to FWD isolate at Katumani-Kenya, India and Malawi but highly susceptible (71% wilt) to FWD isolate at Kiboko-Kenya. The differences among genotypes reaction to Fusarium wilt under field conditions indicate possibilities for genetic variations among varieties of pigeonpeas as well as variability pathogen virulence over locations.

Despite success through sustained breeding efforts over the last decade as evidenced from commercially accepted resistant pigeonpea varieties, breakdown of Fusarium wilt disease resistance has been observed (Hillocks*et al*., 2000). This is because there are different virulence levels that are environment specific and also high chance of break-off of resistance. There is therefore, a need to characterize specific virulent ecotypes of FWD pathogens and identify alternative sources of resistance as well and widen the genetic base of resistance and pyramiding the genes in one genotype background.

Development and use of resistant cultivars is effective, economical, and environmentally sound strategy for disease control, although variable responses with cultivation conditions had been a matter of concern in the use of resistant varieties in diseases control (Saxena *et al.,* 2012). This strategy is gaining importance due to difficulty of widespread on application of available cultural, biological, and chemical control measures for FWD, especially for resource poor farmers, therefore considerable emphasis has been placed on the development of resistant cultivars (Reddy*et al.,* 1998). Thus, process of developing improved pigeonpea varieties focuses on evaluation of local germplasm, collections and introductions or making crosses among cultivars that possess traits of economic importance, among others FWD resistance.

The pathogen produces both macro- and microconidia and the formed sporodochial and pinnotes are salmon to raised buff coloured (Reddy *et al*., 1998). *Fusarium udum* is a facultative parasite that survives in the soil even in the absence of living host for a period of 3 years (Upadhyay and Rai, 1992, Reddy*et al*., 1993). Because

it is a soil-borne pathogen that normally infects the host through the roots (Mehrotra, 2007), infection occurs in the early seedling stage although symptoms are not visible until later in crop developmental stages (Hillocks *et al*., 2000). According to Okiror (1986), wilting suddenly appears in ratooned crops probably because of the continued use of ratooning knife which acts as a carrier of inoculum from plant to plant.

2.6.6 Control strategies for Fisarium wilt disease

The pathogen is primarily a soil inhabitant hence controlling the disease is very difficult since infected plant debris, seeds and soil are the main sources of infection and the principal means of dissemination of the pathogen. Due to this it is necessary the control measure of this disease to lies upon factors such as: cultural, chemical, biological and genetic resistance. However, *F. udum* shows great variation on cultural and morphological characteristics due to environmental conditions, the age of the isolates, sub culturing, method of storage and culturing condition (Kiprop *et al*., 2005).

2.6.6.1 Cultural methods

Several cultural methods are recommended for restricting the severity of the FWD of pigeonpea. Because Fusarium persists several years in soil, a long crop rotation (4 to 6 years) has been found to free the field completely of the Fusarium wilt pathogens (Verma and Rai, 2008).The Crop rotation with sorghum [*Sorghum bicolor* (L.) Moench], tobacco (*Nicotiana tabacum* L.), or castor (*Ricinus communis* L.) every three years has been found to free pathogen completely from the field (Verma and Rai, 2008). Rotations of pigeonpea with tobacco, intercropping and/or

mixed cropping of pigeonpea with sorghum reduce wilt significantly (Reddy *et al*., 1993). Pigeonpea intercropped with sorghum had only incidence of 24% wilt against 85% in sole crop treatment whileone-year break with either sorghum or fallow reduced wilt to below 20% (Verma and Rai, 2008), and by avoid using any solanaceous crop (potato, tomato, pepper, eggplant) or other host plants in the rotation. In case of acidic soil, lime or farmyard manure can be applied and avoid use of different materials which contribute in raising acid in the soil like chicken manure. It is important to keep the moisture constant through irrigation, and keeping field free of weeds. It has been reported that a one-year break between pigeonpea crop by fallowing, with growing sorghum or tobacco reduces the wilt to 22%, 20% and 44%, respectively (ICRISAT, 1987).

Application of nitrogen in form of farmyard manure and green manuring with *Crotalaria juncea* and mixed cropping with *Crotalaria medicaginea* reduce the incidence of FWD considerably (Nageswara *et al*., 2014). Under intensive agriculture, solarization, by covering the soil with transparent polythene sheet for 6-8 weeks during summer months effectively controls FWD in pigeonpeas. This method reduced FWD in a susceptible pigeonpea genotype (LRG 30) and enhanced growth and yield in a wilt-resistant ICPV 1 (Shukla and Dwivedi, 2011). However, this is not a practical option for majority of farmers in the developing countries.

2.6.6.2 Biological control

Biological control of *F. udum* has attracted attention throughout the world and currently, the idea of controlling soil-borne plant pathogens, including *F. udum*, with chemical pesticides or fungicides is being challenged by its effect in the

environment and agriculture (Devi and Chhery, 2012). Several microorganisms such as bacteria have been evaluated and reported as biocontrol agents against *F. udum* by several workers. Bacterial antagonists in a dominant population in soil along with other bacteria like *Serratia, Azotobacter, Clostridium, Bacillus, Arthrobacter, Alcaligens, Agrobacterium, and Bradyrhizobium* are potential biocontrol agents. Collectively these bacteria have been termed ‘‘Rhizobacteria’’. Certain fluorescent pseudomonads increase yield or control one or more soilborne plant pathogens when applied as seed or seed piece inoculants in agricultural crops (Anjaiah *et al*., 2003; Maisuria*et al*., 2008).

In a bio-control experiment, Anjaiah *et al*. (2003) reported that inoculation of pigeonpea and chickpea seeds with *Pseudomonads aeruginosa* (PNA1) significantly reduced the incidence of FWD in naturally infested soil. Certain soil antagonists are known to suppress the development of wilt through induction of resistance (Upadhyay and Rai, 1981; ICRISAT, 1987; Upadhyay and Rai, 1992). Studies on antagonism found that *Aspergillus niger, Aspergillus flavus, Micromonospora globosa* and *Aspergillus terreus* highly suppressed the population of *F. udum* (Upadhyay and Rai, 1981). In a different study on tomatoes (*Lycopersicon esculentum*), Khan and Khan (2002) observed that root-dip application of *Bacillus subtilis, Pseudomonas fluorescens, Aspergillus awamori*, *Aspergillus niger* and *Penicillium digitatum* resulted in significant decline of *Fusarium oxysporum* f.sp *lycopersici* population in the rhizosphere. Biological control by *Bacillus* spp. (Siddiqui *et al.,* 2005) had also been reported. Soil amendment with *Trichoderma harzianum* at all pathogen levels had been reported to give a disease control of 22-61.5% (Prasad *et al*., 2002). However, biocontrol microbes often are not thought of

as acceptable alternatives due to lack of broad spectrum activity, inconsistent performance, and slower (and sometimes lesscomplete) action by the biocontrol. Therefore, the inconsistent field performance caused by a large number of biotic and abiotic factors (Susan *et al.,* 2002) often restricts commercial development of biocontrol agents.

2.6.6.3 Chemical control

Seed treatment with systematic chemical like mixture of benomyl and thiram at equivalent rates completely eradicate the *Fusarium udum* (Reddy *et al*., 1993). Also seeds treatment with 4g *Trichoderma viridae* formulation + 3 g thiram kg^{-1} seed or seed treatment with carbooxin thiram (1: 2) at the rate of 3g/kg of seed weight is recommended. Although application of carbendazim in controlling the disease is effective and convenient, but their use and abuse are causing serious ecological, economic and social problems which limits their wider adoption (Devi and Chhetery, 2012). Also the frequent application of fungicides to the soil has caused environmental hazards causing water and soil pollution in addition to killing the non-target beneficial microorganisms in soil which adversely affect fertility, as well as pollute the environment. Alternative approaches are needed to substitute the chemicals with resistant cultivars because of the potential threat for development of chemical resistance, especially systemic fungicides by fungal pathogens and non-target side effects on other plant pathogens and beneficial microorganisms. Sustainable fertility of the soil, blending of the chemical fertilizers with chemical-adaptive strains is one approach that may derive synergistic benefits (Vargas *et al*., 2000; Kamlesh and Choure, 2012).

2.7 Genetic Variability in Fusarium Wilt Pathogen

Recent study on cultural, morphological characterization and rate of reaction of *Fusarium udum* pathogen suggested the existence of different virulence groups (Harlapur *et al*., 2007,; Mahesh *et al*., 2010,; Karimi *et al*., 2010). According to Muhhamad*et al*. (2011) there appears to be no apparent reason as to why these already tested wilt resistant materials showed such a variable wilt reaction and which creates a doubt about the possibility of existence of physiological forms of the pathogen. However, the effectiveness of host resistances is curtailed by the occurrence of pathogenic races in *F. oxysporium* f.sp. *ciceris*.Therefore, integrated management strategies are the only solution to maintain plant health. These strategies should includes minimum and efficient use of chemicals for checking the pathogen population, encouragement of beneficial biological agents to reduce pathogen inoculum, modification of cultural practices and use of resistant varieties (Karimi *et al*., 2012).

To develop high yielding and Fusarium wilt resistant varieties of pigeonpea, it is essential not only to identify sources of resistance, but also to understand the genetics of inheritance. This study was carried out to determine different races of *F. udum* found in pigeonpeas growing areas of Tanzania as in any study the knowledge of genetic variation of a pathogen is a pre-requisite for any resistance breeding programme. Pathogen variability is one of the main causes of failure of the resistant variety or fungicidal control of plant diseases. In nature, new races may arise through mutation, hybridization and different cytoplasmic inheritance (heterokaryosisand parasexuality). Hence, the resistant varieties of today may become susceptible due to the development of new races of the pathogen in the

future, so in any breeding programme, the detailed knowledge about the existing races of pathogen and their possible occurrence in future is quite essential. Assessment of extent and distribution of genetic variation in a pathogen and its relatives is essential in understanding pattern of diversity and evolutionary relationships (Karimi *et al*., 2010).

The disease is prevalent in all pigeonpeas growing areas, but its severity differs from place to place. In some areas, the disease is more severe killing almost 90% of plants in the farmer's field. Limited studies on variability in the wilt fungus *Fusarium udum* have indicated that the fungus exhibit physiological specialization. However, information on the variation of the pathogen in the isolates collected from different agro –ecological zones is lacking (Mbwaga, 1995). Such information will help in developing disease resistant pigeonpea varieties. It has been observed variability of FWD reactions between countries (Hillocks*et al*., 2000) and even sites within the same country (Mahesh *et al*., 2010) as a result of existence of different virulent isolate.

2.7.1 Genetic diversity and relatedness of pigeonpeas

Compared to other grain legumes such as beans and cowpeas cultivated in the region, pigeonpeas has received relatively little research attention in crop improvement, and the same traditional varieties are still being cultivated by the farmers with limited improved varieties in use (Varshney *et al*., 2010). It is therefore, important to determine the genetic diversity of the crop as a prerequisite to crop improvement (Vange and Moses, 2009). Also it is necessary to know the potentials for use of any available collections for efficient utilization (Wasike *et al*.,

2005, Akande, 2007). The ICRISAT currently has a collection of more than 13,000 pigeonpea germplasm accessions in the genebank. This germplasm has been morphologically characterized and found to contain variation among accessions (Odeny, 2006). Morphological studies alone do not provide sufficient information to understand genetic diversity within the species as well as its relatedness to other species (Malviya and Yadav, 2010). Molecular analysis using SSRs can provide additional information on genetic diversity that would be useful for breeding programs through selection of diverse parents (Odeny, 2006).

The current interest in the genetic potential of wild relatives could be further enhanced through the use of molecular markers in identification of the most closely related parents for inter-specific crossing. The on-going breeding emphasis on development of hybrid pigeonpea will also require a quick and efficient way of predicting and identifying inbred lines that can produce highly heterotic hybrids precisely.

2.7.2 Genetic variability of fusarium wilt disease resistance in pigeonpeas

Conflicting reports have been made by other researchers on the inheritance of resistance to FWD in pigeonpeas. Saxena (2012) reported one dominant and one recessive gene with dominant suppressive epistatic effects were found responsible for controlling resistance to Fusarium wilt. Swarna *et al.,* (2012), Karimi *et al*., (2012) observed that resistance to be controlled by a single dominant gene. Sharma (1986) discussed the probable causes of variability in a proportion of resistant and susceptible plants in different generations of different pigeonpea populations to be conditioned by confirmed the dominance of resistance over susceptibility. This

suggested that the resistance to FWD in pigeonpeas is conferred by quantitative trait loci (QTLs) with major genes.

Other reports have shown that resistance to wilt in pigeonpea could be governed either by a single dominant (Singh *et al*., 1998) or a recessive gene (Jain and Reddy, 1995) in different crosses. However, inconsistent findings have been detected from different studies. Parmita *et al*., (2005) detected dominant epistatic gene ($,^2_{DI}$) interaction and a single dominant gene. Ajay *et al*. (2013) detected that the resistance to Fusarium wilt disease is governed by a dominant, a recessive gene and complementary action of two dominant genes. Odeny (2001) detected digenic and quantitative genes that are resistant to Fusarium wilt and reported that quantitative inheritance is often influenced by environment. However, the resistance depends on the source of the gene. In a cross between two resistant and one susceptiblepigeon peas, Okiror (2002) through qualitative genetic analysis, indicated that resistance was dominant over susceptibility and was controlled by two genes. Effects of duplicate genes and multiple factors on Fusarium wilt in pigeonpeas have also been observed. Kotresh *et al*. (2006) reported monogenic inheritance for FWD in pigeonpea.

2.7.3 Relationship between various characters in pigeonpeas

Shah-Al-Emran*et al*., (2014) pointed out the importance of assessing relationship between components of yield. The knowledge of such interrelation can indicate the ease with which simultaneous improvement of the components can be affected. Compensation between components at different stages in the development of the plant may lead to negative correlations between them, resulting in limitations to

increase yields by component selection approach. These adverse relationships can be attributed to genetic and environmental factors. Thus, there is a need for detailed investigation and analysis at the genetical, physiological and developmental levels of yield components and their interrelationships. In plant breeding the knowledge of combining ability is of economic importance in study and compares performance of lines in hybrid combination. Diallel analysis proposed by Hayman (1954) was designed to allow detection of dominance and other effects of importance in crossing and breeding programme in regressing the parent-offspring covariance on the offspring variance on n parents in n x n diallel crosses (Ojo, 2003).

2.7.4 General and specific combining abilities in pigeonpeas

The information on combining ability studies is more reliable as they provide useful information on additive and non-additive gene action controlling the inheritance of several traits in the respective crop. This information assists to determine general combiner to be used as donor parent for improvement of specific trait and in this case Fusarium wilt resistance. It also involved elucidating the nature and magnitude of various types of gene actions involved in the expression of quantitative trait (John *et al.,* 2011). Combining ability such as general combining ability (GCA) and specific combining ability (SCA) studies are useful in classifying parental lines in terms of their hybrid performance. In self-pollinated crops like pigeonpea, these studies are useful in assessing the combining ability of the parents which, when crossed, would give more desirable segregates (Hassan *et al*., 2010).

2.7.5 Fusarium wilt disease marker-assisted selection

The genetic variation of *F .udum* in pigeonpeas causes screening of large number of lines, needs creating infected plots, artificial inoculation and time taking as the disease occurs at any stage of crop and rapid screening may not be possible. Also most of the important characters are controlled by several (quantitative traits) genes (Collard *et al*., 2005). The genetic factors responsible for a part of the observed phenotypic variation for a quantitative trait are called quantitative trait loci (QTL). This indicates a region on the genome and could be comprised of one or more functional genes (Falconer and Mackay, 1996).

Traditionally, this problem has been dealt with through several replicated trials, which allow identification of genotypic differences through statistical analysis (Odeny, 2006). This process can be laborious and especially slow for a late-maturing crop like pigeonpea. Therefore, availability of adequate SSRs in pigeonpea would enable breeders to know the location of specific genes and QTLs making it possible to improve the efficiency of breeding through MAS. Also application of molecular marker (primers) in assessment of susceptibility or resistance (FWD) at early crop stage will be possible with the closely linked molecular marker, and it will eliminate the need for maintaining virulent isolates of the wilt pathogen and development of sick plots for artificial screening techniques (Magadum *et al.,* 2013).

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CHAPTER THREE

3.0 Variability and Aggressiveness of Tanzanian *Fusarium udum* Isolates Against Selected Pigeonpea Varieties from Africa and Asia

ABSTRACT

Fusarium wilt disease (FWD) is a plant disease caused by *Fusarium udum* (Butler), a soil and seed borne fungus which causes remarkable yield losses in susceptible cultivars throughout the pigeonpea growing areas. It has been recorded to cause up to 100% yield loss. Although numerous control measures have been suggested to alleviate the problem of Fusarium wilt disease and increase productivity of pigeonpeas, their success still remain low due to limited studies on variability of *F. udum*, thus existence of genetic variability of *Fusarium udum* pathogens lead into difficult in control of disease. The study was conducted to assess variability and aggressiveness of *Fusarium udum* isolates. Both pathogenicity and morphological characterizations were analysed to test *F. udum* variability. Twenty one pigeonpea genotypes were screened against nine *F. udum* isolates collected from the major pigeonpeas growing areas of Tanzania. The great variability was observed, this variability in disease reaction could be due to difference in pathogen gene and environmental factor surrounding it. Also the variability in disease reaction could be due to poor stability in pigeonpea gene resistance. Varieties ICEAP 00040, ICEAP 00540 and ICEAP 00557 showed remarkable resistance hence could be used as new source of resistance to Fusarium wilt, therefore further studies using molecular markers were recommended to validate the results of this study.

Key words: *Fusarium udum*, genetic variability, isolates, pigeonpeas.

3.1 INTRODUCTION

Tanzania is the world's sixth largest pigeonpea producer after India, Myanmar, Kenya, Malawi and Uganda with over 68,000 ha cultivated annually (Odeny *et al.,* 2009; Vange and Moses, 2009). Pigeonpeas in Tanzania are the third most important food legumes after common beans and cowpeas in terms of acreage and production. However, the production of pigeonpea is hindered by many biotic and abiotic factors. Among these factors Fusarium wilt disease, caused by *Fusarium udum* Butler, is the most important constraint of crop production (Kiprop *et al.,* 2005; Lamontagne- Godwin *et al*., 2012; Mahesh *et al.,* 2010, Mula and Saxena 2010). Fusarium is a soil and seed borne fungus which causes significant yield losses in susceptible cultivars throughout the pigeonpea growing areas and resulting in up to 100% yield loss (Gwata *et al*., 2006; Odeny *et al*., 2009; Prasanthi *et al*., 2009; Karimi *et al.,* 2010).

The fungus is capable of surviving in the soil for at least eight years (Nene *et al*., 1981) where it survives between crops in residual plant debris as mycelium and in different spore forms. The germ tube of the mycelium or spore penetrates seedlings through root tips, wounds or point of formation of lateral roots. The mycelium advances through the xylem causing the disease during flowering and pod-filling stages (Gwata *et al.,* 2005). Previous surveys estimated wilt incidence to be 15.9% in Kenya, 36.3% in Malawi and 20% in Tanzania. In some of the fields wilt incidence was as high as 90% with annual losses of US$ 5 million in each of these countries (Karimi *et al*., 2010; Mesapogu *et al*., 2012).

Although numerous control measures have been suggested to alleviate the problem of Fusarium wilt and increase productivity of pigeonpeas, their success still remain low due to limited studies on variability of *F. udum*, thus existence of genetic variability of *Fusarium udum* pathogens lead into difficult in control of disease. In eastern Africa in particular, research on pigeonpea Fusarium wilt started in 1980's and focused mainly on identification and testing of newly bred and imported sources of resistance. Two sources of resistance on pigeonpea have been identified and conformation of the imported cultivars has been done (Odeny*et al*., 2009). Recent molecular characterization of Eastern African isolates suggested the existence of different virulence groups (Kiprop *et al*., 2002). It is essential not only to identify source of resistance, but also to understand on the genetic variability of *Fusarium udum*. Presently, the information on the genetic variability of *F.udum* strains in the world in general and more particularly in Tanzania is lacking. This study was therefore carried out to determine the genetic variability and aggressiveness of *F. udum* isolates found in Tanzania as information on variability of the pathogen is prerequisite for any disease resistance breeding programme.

3.2 MATERIALS AND METHODS

3.2.1 Collection of diseased samples

Dry dead stems of plants infected with Fusarium pathogen as well as seeds of local pigeonpeas varieties were collected from major crop growing zones of Tanzania viz. Eastern zone, (Morogoro) Central zone, (Dodoma) and Northern zone (Manyara). From each region collection was done in selected district in which three samples from three farms were collected. Plant samples were obtained from

pigeonpea stems showing typical symptoms of the disease. Samples were packed in separate paper bags sealed and labelled to indicate location, altitude and date of collection where by location of each site was determined by using GPS. Along with the collection of diseased samples, seeds of local pigeonpea varieties were collected from the same locations. The data recorded during collection of the samples included, name of variety, growth habit, seed size, seed colour and name of the location from which was collected (Table 3.1, Fig 3.1).

Table 3.1: Sources and names of *Fusarium udum* diseased sample used in the study

Zone	Region	District	Ward/Village	Name of the isolate	GPS Location and Altitude
			Kondoa	CDKK1	4°53' 30.44"S, 35°46' 0.464"E, 1404masl
Central	Dodoma	Kondoa	Kondoa	CDKK2	4°53' 57.97"S, 35°45' 47.71"E, 1403.91masl
			Kondoa	CDKK3	4°54' 07.11"S, 35°46' 24.73"E, 1396.29masl
Eastern	Morogoro	Kilosa	Msowero	EMKM2	6°32' 19.62"S , 37°12' 23.53"E, 435.25masl
			Chanzuru	EMKIL1	6°42' 0.00"S , 37°2' 0.00"E, 506masl
		Mvomero	Mikese	EMMM1	6°44' 12.32"S , 37°55' 21.86"E, 383.44masl
		Morogoro Municipal	Kihonda	EMMK1	6°46' 10.25"S , 37°38' 10.93"E, 495masl
	Manyara	Babati	Mamire	NMBM1	4°11' 36.56"S , 35°45' 11.63"E, 1313.99masl
Northern			Galapo	NMBG1	4°14' 24.98"S , 35°53' 25.38"E, 1252.73masl

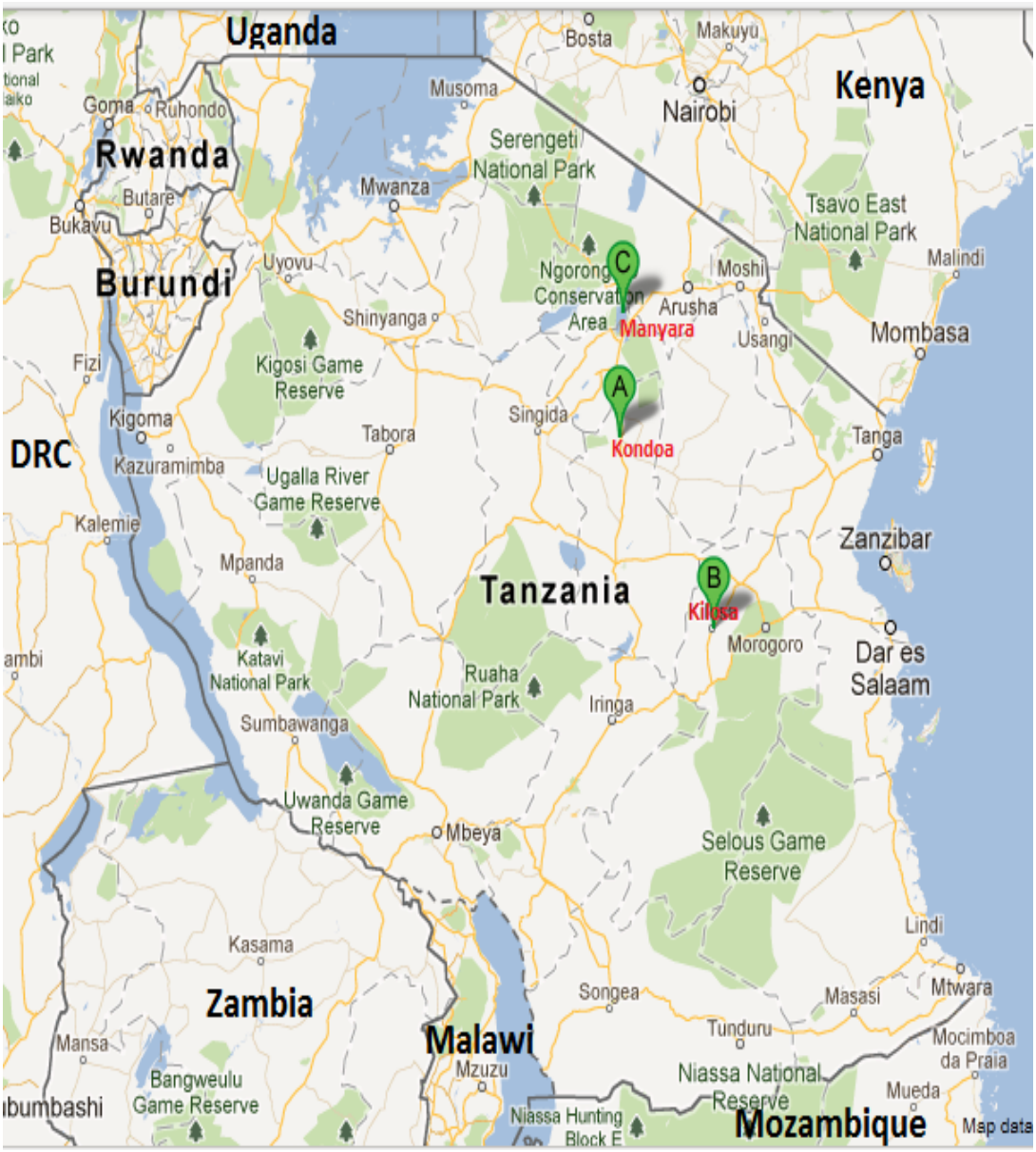


Figure 3.1: Map of Tanzania showing different locations where fusarium wilt disease samples and local varieties were collected (source Google map, 2012)

3.2.2 Isolation of fusarium pathogen in the laboratory

Stems from diseased pigeonpea plants were cut into small pieces of 1 cm using a sterilized scalpel and surface sterilized in 1% sodium hypochlorite (NaOCl) for one minute then rinsed three times using sterile distilled water. The pieces were blotted into paper for seven days to allow growth. After seven days the observations were

made to detect the growth of the specific fungus. The tissue mycelia were then transferred into V8 agar. The plates were incubated at 25^{o}C in a 12 hour light/dark cycles for 72 hours in a growth chamber.

3.2.3 Identification of *Fusarium udum* isolates

Identification of *F. Udum* was done after ten days by mounting spore on a slide with a drop of water and a cover slip and then observing it under camera-mounted compound microscope 40X. A photograph of the conidia was made for their confirmation (Plate 3.1).

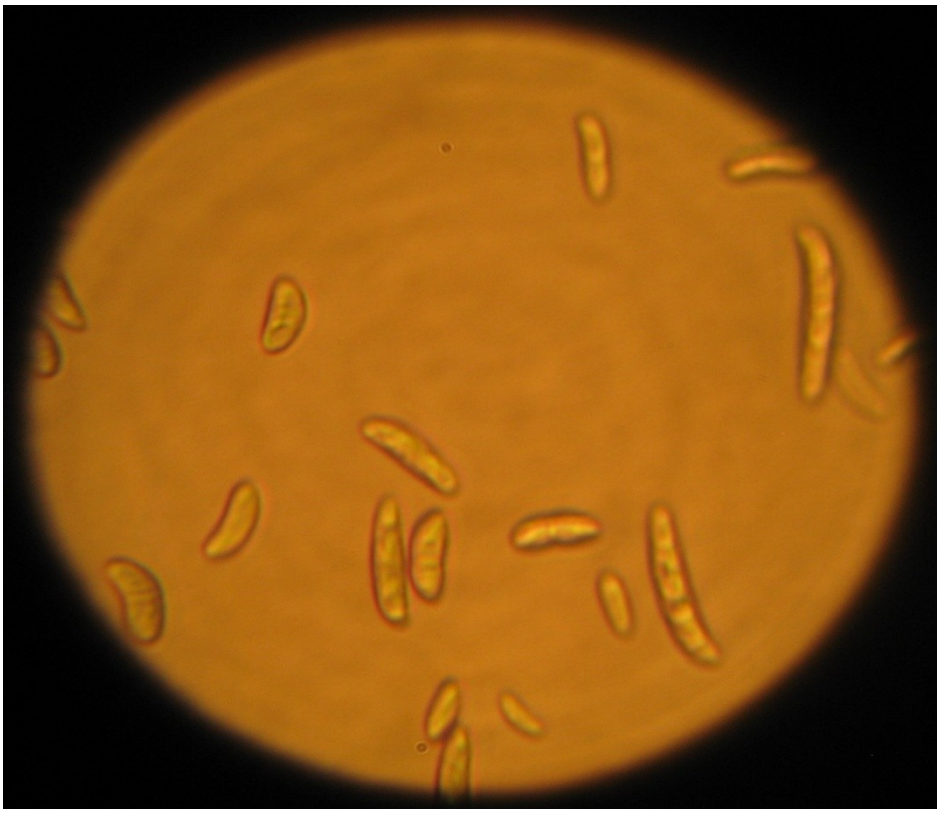


Plate 3.1: Conidia of *Fusarium udum* taken from EMKM2 isolate collected from Msowero village of Morogoro region visualized using compound microscope at a magnification of 40X.

3.2.4 Inoculum preparation

Fusarium wilt isolates were then multiplied in Petri dishes with fresh prepared Tape Water Agar (TWA). The inocula from the culture isolates were prepared by rinsing the surfaces of 14-day old cultures in TWA with sterile water. The conidial suspension was then filtered through cheese cloth to remove the mycelia, and the inoculum concentration was adjusted to 1.0×10^6 conidia ml^{-1} based on the counts made with a haemocytometer.

3.2.5 Screening of pigeonpea genotypes against *F. udum* isolates

Twenty one genotypes were screened for resistance against nine *F. udum* isolates under screen house conditions at Sokoine University of Agriculture, Morogoro. The trial also assessed the variability in aggressiveness of the nine fungal isolates. A Complete Randomized Design (CRD) experiment with three replications was used. The pigeon pea genotypes were planted in polythene bags containing sterilized sand, after seven days of germination the seedlings were gently pulled out from the sand and sand on the roots removed by shaking followed by washing in sterile distilled water. By using sterile scissors, 1 cm of the distal end of the roots system was cut. The cut plants were then dipped in the inoculum prepared from the nine (9) *F. udum* isolates (Table 3.1) at a concentration of 1.0×10^6 conidia ml^{-1} for 10 minutes to allow the conidia to enter the wounds created in the root systems. Genotypes ICPL 211 and ICPL 161 were used as resistant and susceptible checks respectively. The seedlings were then transplanted in plastic pots with the diameter of 15.24 cm filled with a mixture of sterile soil and sand (3: 1 v/v) with extra handling with continous watering at an interval of two days.

3.2.6 Cultural and morphological characterization of *Fusarium udum* isolates

The variation in cultural and morphological characters among the nine isolatesof *F. udum* collected in Tanzania was studied on V8 medium. The cultural characters likecolonydiameters (cm) colony growth, spore germination (%) and sporulation were recorded. Colony diameter was recorded by measuring the radial growth of the myceliumin cm after seven days of incubation at 25^{o}C.

3.2.7 Data collection and analysis

Disease severity was assessed using a scale described by Nene *et al*. (1981) with some modification, where by; 0-30% mortality was considered as highly resistant, 31-40% mortality as moderately resistant, 41-60% mortality as moderately susceptible and 61% -100% mortality as highly susceptible. The number of wilted seedlings (mean infection rates) was collected ten days after inoculation, when the susceptible checks started wilting. The final scores were taken two months later from inoculation date when all the susceptible checks had died. Percentage plant mortality was determined as the proportion of wilted plants divided by the total number of plants x 100%. Data collected were analyzed using Genstat software and mean performance was done by using Honestly Significant Difference (HSD).

3.3 RESULTS

3.3.1 Screening of pigeonpea genotypes against *F. udum* isolates

Significant ($p \leq 0.001$) effects due to pigeonpea genotypes for disease severity were observed also effects due to isolates were significant ($p \leq 0.001$) while there was no significant difference due to G × I interaction for disease severity (Table 3.2).

Table 3.2: Fusarium wilt disease incidence on 21 pigeonpea genotypes

Source of variation	Df	Mean Square
Isolates	8	1872.8299***
Pigeonpea genotypes (G)	20	11346.1062***
Pigeonpea genotypes (G) × Isolate (I)	160	225.4232
Error	360	202.6531
CV		12.08%

*** = significant ($p \leq 0.001$).

The mean infection rate of all *Fusarium udum* isolates indicated a susceptible reaction in all 21 pigeonpea genotypes. Among these EMKM2 showed the highest mean infection rate (52.183) followed by EMMK1, EMMM1 CDKK2, EMKIL1, CDKK3, CDKK1, NMBG and NMBM1 (Table 3.3, Plate 3.2).

Table 3.3: Infection rate of 9 Fusarium wilt isolates evaluated on pigeonpea genotypes

Isolate	Mean infection rate of the isolate
EMKIL1	50.496
EMKM2	52.183
EMMM1	43.254
EMMK1	41.27
CDKK1	38.79
CDKK2	42.758
CDKK3	39.782
NMBM1	36.31
NMBG1	38.194
Mean	42.56
L.S.D (at 5%)	7.9156
C.V	12.08%

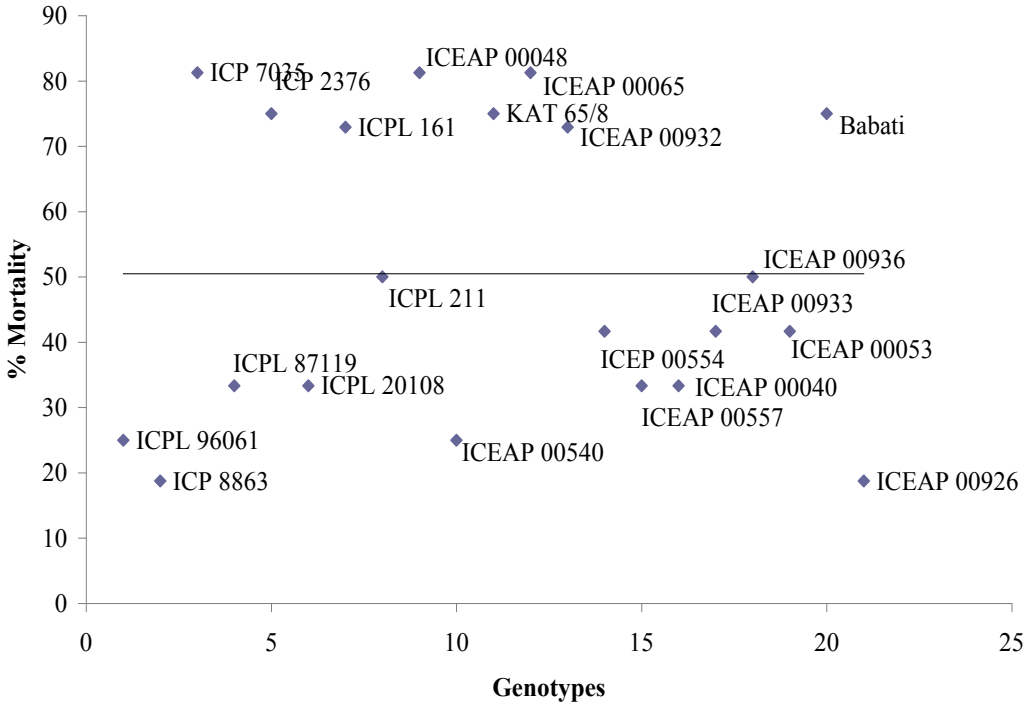
Plate 3.2: Pigeonpea genotypes ICPL 161 and ICPL211 thirty days after inoculating with EMMK1 isolate under screen house condition at SUA

The reaction of the African and Asian pigeonpea genotypes to FWD isolates differed with percent wilt incidence for EMKIL1, EMKM2, EMMM1 and EMMK1 ranging from 18.75% - 81.25%, 16.18% - 87.70%, 18.75% - 81.25% and 12.5% – 75.0% respectively (Table 3.4). *Fusarium udum* isolates CDKK1, CDKK2 and CDKK3 caused disease severity ranging from 18.75% - 75%, 12.5% - 66.67% and 12.7% - 87.5% (Table 3.4), while NMBM1 and NMBG1 ranged from, 6.2% - 66.67% and 12.5%- 66.67% respectively (Table 3.4). All isolates were agressiveto the susceptible genotypes Babati, ICEAP 00065 and ICP 7035 and less virulent to resistant genotypes ICP 8863, ICP 20108, ICEAP 00540, ICPL 211 and ICEAP 00557. However, isolates showed variation in aggressiveness on genotypes ICPL 96061, ICP 7035, ICP 2376, ICEAP 00936 and ICEAP 00053. The comparison of nine *Fusarium udum* isolates is presented in Fig 3.4 to Fig 3.12)

3.3.2 Aggressiveness of nine *Fusarium udum* isolates against twenty one pigeonpea genotypes

3.3.2.1 *Fusarium udum* isolate EMKIL1

Genotypes ICP 8866, ICEAP 00540, ICEAP 00926 and ICPL 96061 showed high level of resistance to *Fusarium udum* isolate EMKIL1. Pigeonpea genotypes ICPL 87119, ICPL 20108, ICEAP 00557 and ICEAP 00040 were moderately resistant while ICP 7035, ICPL 161, ICEAP 00048, KAT 65/8, ICEAP 00065, ICEAP 00932 were highly susceptible to *Fusarium udum* isolate EMKIL1 collected from Ilonga. The rest of pigeonpea genotypes gave moderately susceptible disease reaction level (Table 3.4, Fig. 3.2)

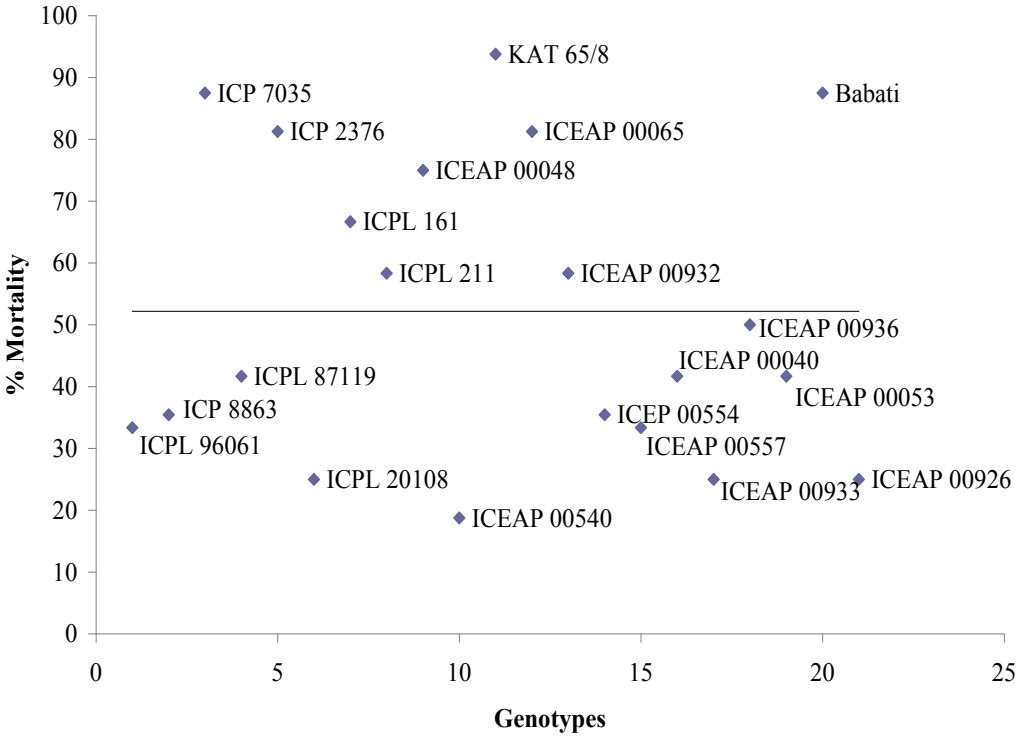


Mean =50.04, CV= 26.43%, L.S.D (0.05)= 41.60%

Figure 3.2: Disease severities of 21 pigeonpea genotypes inoculated with *Fusarium udum* isolate EMKIL1

3. 3.2.2 *Fusarium udum* isolate EMKM2

Interaction between *F. udum* isolate EMKM2 collected from local landrace genotype from Msowero village in Morogoro region withthe 21 pigeon pea genotypes when scored eight weeks after inoculation exhibited high level of susceptibility to ICPL 161, ICP 7035, ICP 2376, ICEAP 00048, KAT 65/8, ICEAP 00065 and Babati. However, ICPL 20108, ICEAP 00540, ICEAP 00933 and ICEAP 00926 were highly resistant to *F. udum* isolate EMKM2 while ICPL 96061, ICP 8863, ICEP 00554 and ICEAP 00557 showed moderate resistance to the same isolate. The rest of the pigeonpea genotypes were moderately susceptible (Table 3.4, Fig. 3.3).

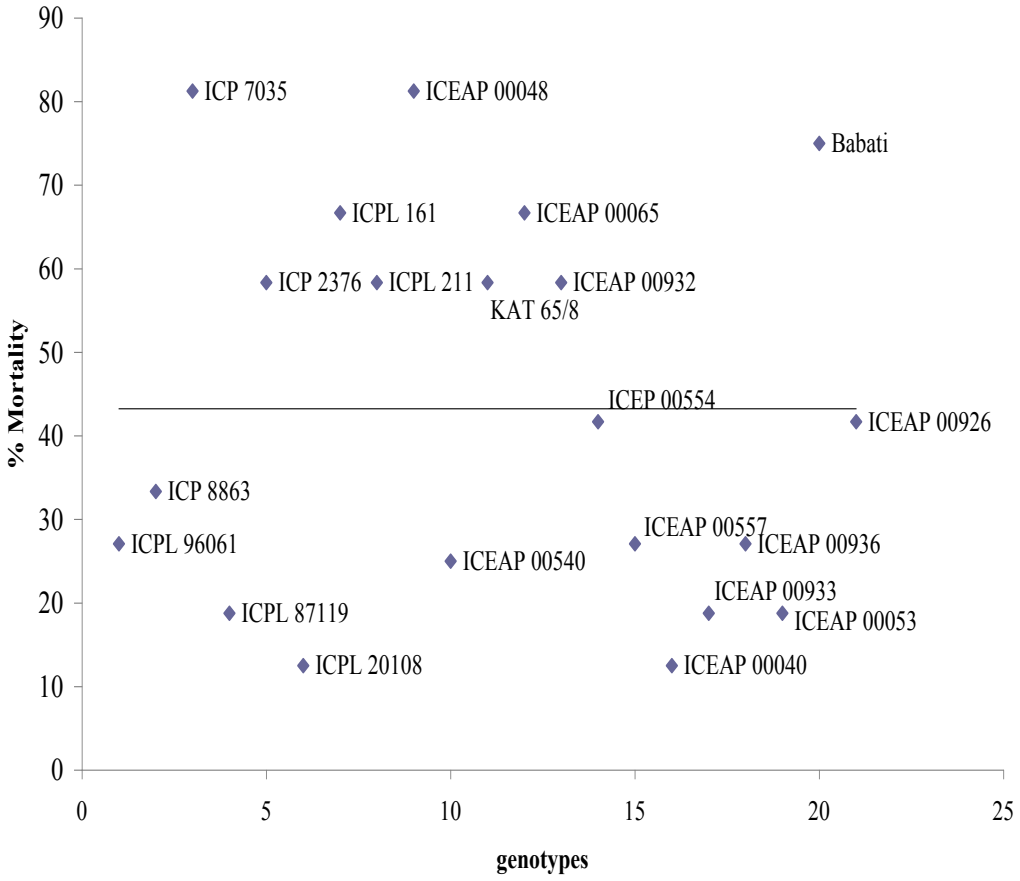


Mean =52.18, CV= 27.41%, L.S.D(0.05) = 44.59%

Figure 3.3: Disease severities for 21 pigeonpea genotypes against *Fusarium udum* isolate EMKM2

3. 3.2.3 *Fusarium udum* isolate EMMM1

Four pigeonpea genotypes, ICPL 161, ICEAP 00048, ICP 7035 and ICEAP 00065 were highly susceptible to *F. udum* isolate EMMM1 with a percentage score between 66.67 and 81.25. However, genotypes ICPL 96061,ICPL 87119**,** ICPL 20108**,** ICEAP 00540, ICEAP 00933, ICEAP 00936 and ICEAP 00053 were highly resistant to this isolate with percentage scores between 12.05 and 27.08 and the remaining genotypes exhibited moderate susceptibility to the disease reaction (Table 3.4, Fig. 3.4).

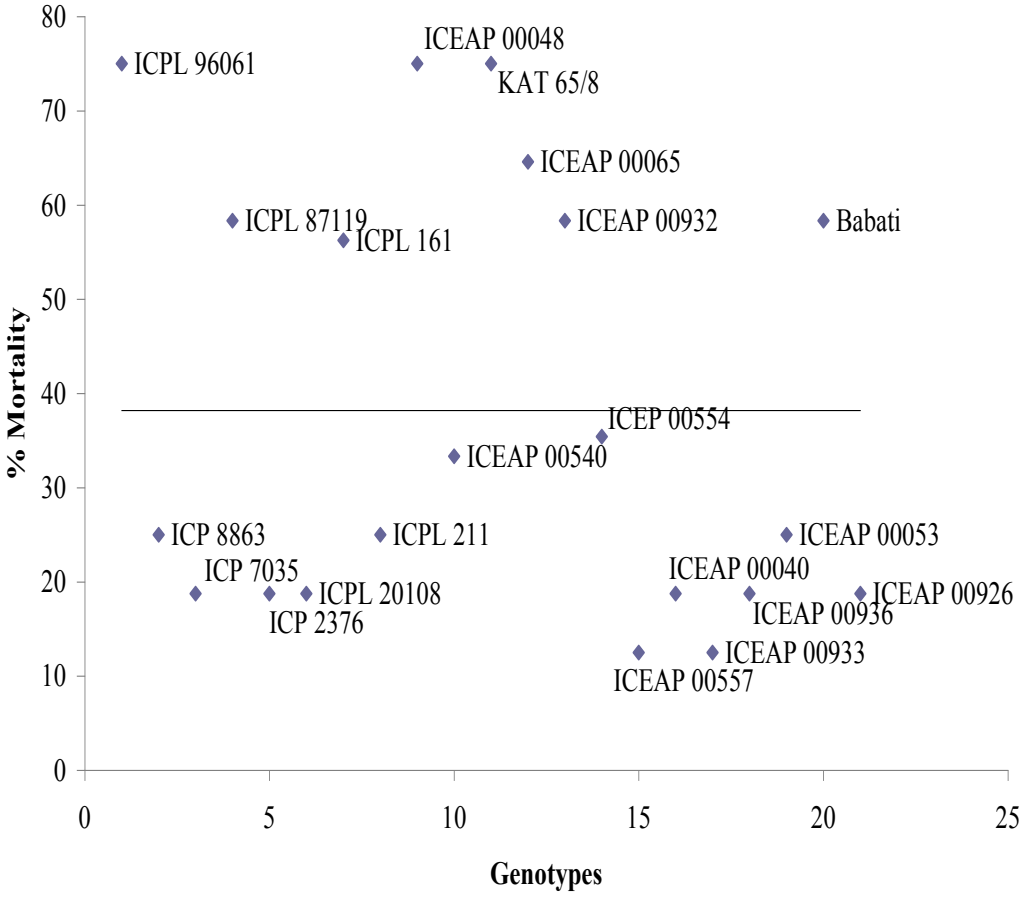


Mean= 43.25, CV=33.79%, L.S.D (0.05) =45.57%

Figure 3.4: Disease severities for 21 pigeonpea genotypes against *Fusarium udum* isolate EMMM1

3.3.2.4 *Fusarium udum* isolate EMMK1

When the 21 pigeonpea genotypes were tested against *F. udum* isolate EMMK1, eleven of the genotypes showed resistancereaction with scores of between 12.05 and 25.0 percentages. These were ICP 8863, ICP 2376, ICPL 20108, ICP 211, ICEAP 00557, ICEAP 00040, ICEAP 00933, ICEAP 00936, ICEAP 00053 and ICEAP 00926. However, genotype KAT 65/8 and ICEAP 00065 were highly susceptible to EMMK1 isolate. The rest of the genotypes scored moderately resistant and moderately susceptible percentages (Table 3.4, Fig. 3.5).

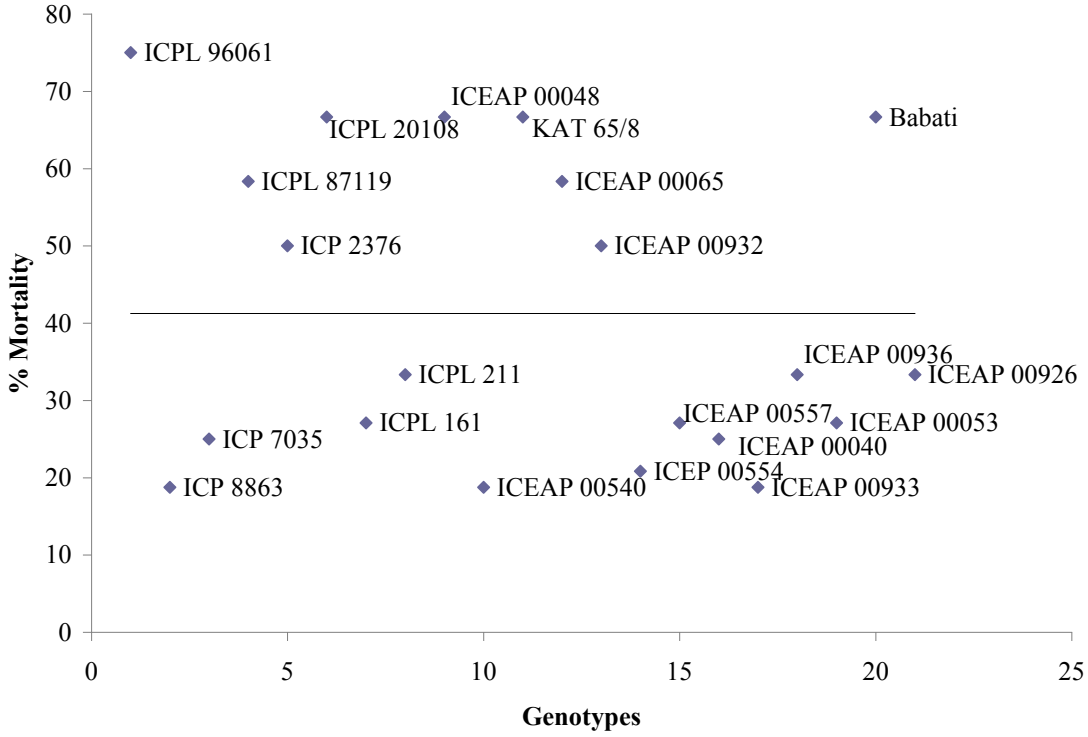


Mean = 38.19, CV= 37.18%, L.S.D (0.05) = 44.27%

Figure 3.5: Disease severities for 21 pigeonpea genotypes against *Fusarium udum* isolate EMMK1

3.3. 2.5 *Fusarium udum* isolate CDKK1

The reaction of 21 pigeonpea genotypes screened against *F. udum* isolate CDKK1 showed genotypes ICP 8863, ICP 7035, ICPL 161, ICEAP 00540, ICEP 00554, ICEAP 00557, ICEAP 00040, ICEAP 00933 and ICEAP 00053 to be resistant to this isolate. Where as, genotypes ICPL 211, ICEAP 00936 and ICEAP 00926 were revealed to be moderately resistant. The genotypes ICPL 87119, ICP 2376, ICEAP 00065 and ICEAP 00932 were moderately susceptible while ICPL 96061, ICPL 20108, ICEAP 00048, KAT 65/8 and Babati were revealed to be highly susceptible (Table 3.4, Fig. 3.6).

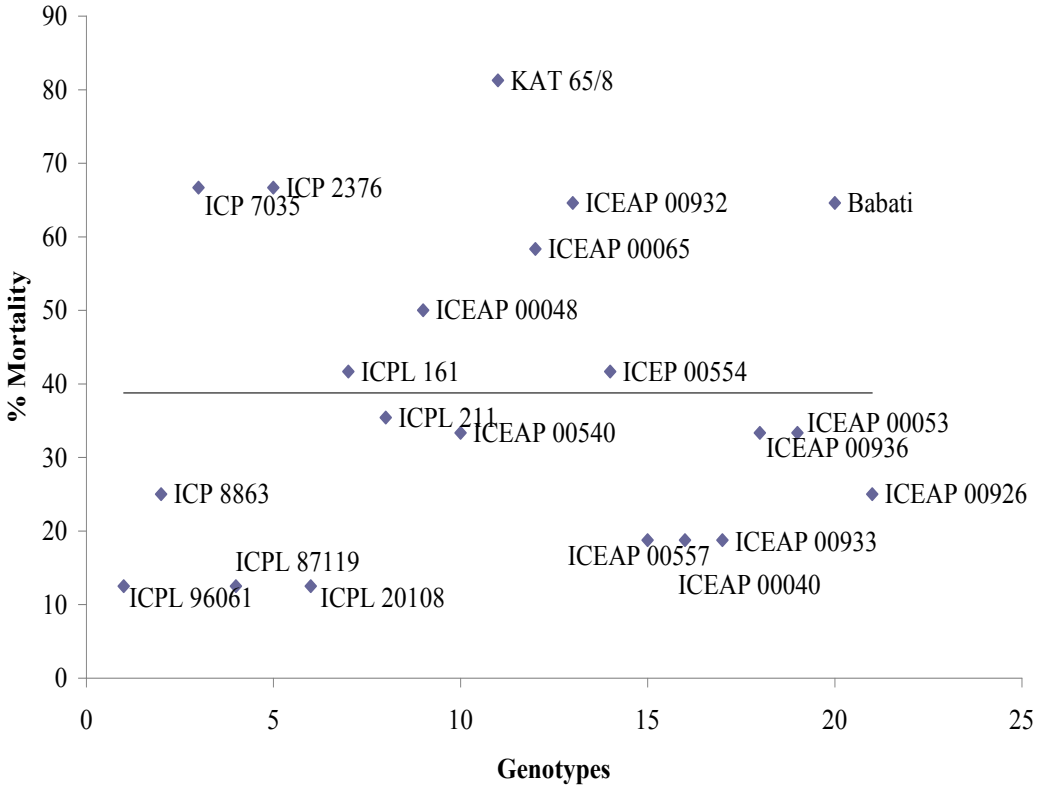


Mean = 41.26, CV= 34.81%, L.S.D (0.05) = 44.79%

Figure 3.6: Disease severities for 21 pigeonpea genotypes against *Fusarium udum* isolate CDKK1

3.3.2.6 *Fusarium udum* isolate CDKK2

When the 21 pigeonpea genotypes were screened against the *F. udum* isolate CDKK2, eight genotypes ICPL 96061, ICP 8863, ICPL 87119, ICPL 20108, ICEAP 00557, ICEAP 00040, ICEAP 00933 and ICEAP 00926 were found to be resistant. Four genotypes, ICPL 211, ICEAP 00540, ICEAP 00936 and ICEAP 00053 were found to be moderately resistant. Genotypes ICPL 161, ICEAP 00048, ICEAP 00065 and ICEAP 00554 were found to be moderately susceptible while ICP 7035, ICP 2376, ICEAP 00932, Babati and KAT 65/8 were found to be highly susceptible (Table 3.4, Fig. 3.7).

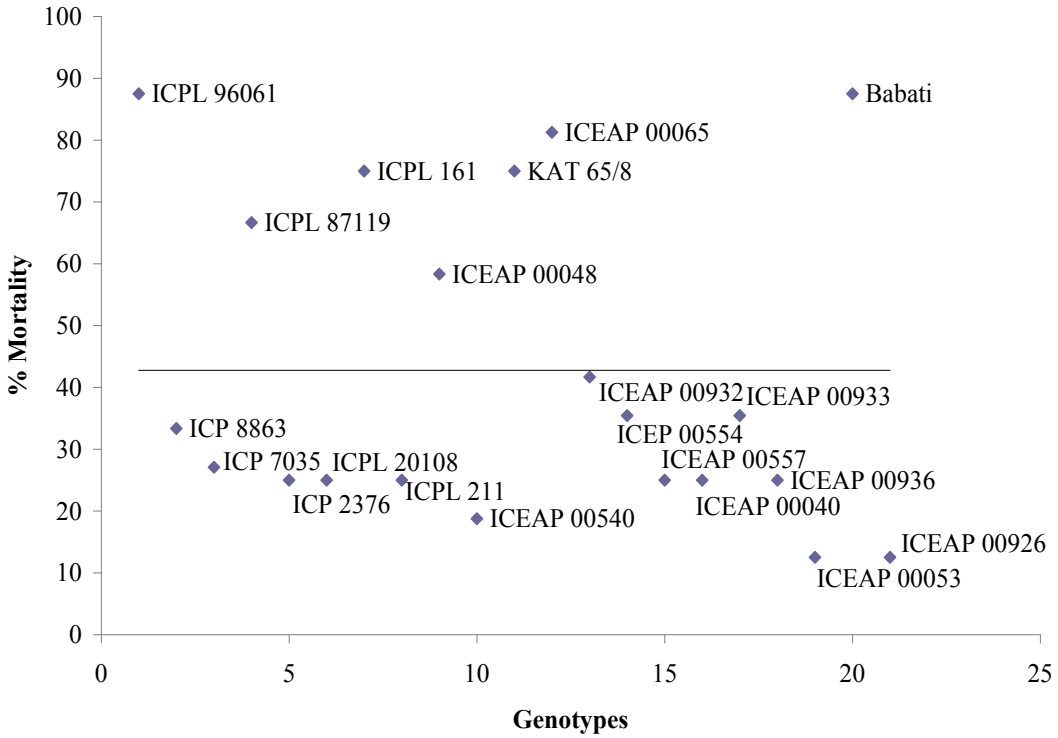


Mean= 38.78, CV= 39.62%, L.S.D (0.05) = 47.92%

Figure 3.7: Disease severities for 21 pigeonpea genotypes against *Fusarium udum* isolate CDKK2

3.3.2.7 *Fusarium udum* isolate CDKK3

Ten of the twenty one screened genotypes appeared to show resistance to this isolate. The resistant genotypes were ICP 7035, ICP 2376, ICPL 20108, ICPL 211, ICEAP 00540, ICEAP 00557, ICEAP 00040, ICEAP 00936, ICEAP 00053 and ICEAP 00926. Three genotypes were moderately resistant; these were ICP 8863, ICEP 00554 and ICEAP 00933. Two genotypes, ICEAP 00048 and ICEAP 00932, showed moderate susceptibility, while the remained six, ICPL 96061, ICPL 87119, KAT 65/8, ICEAP 00065 and Babati were susceptible (Table 3.4, Fig. 3.8).

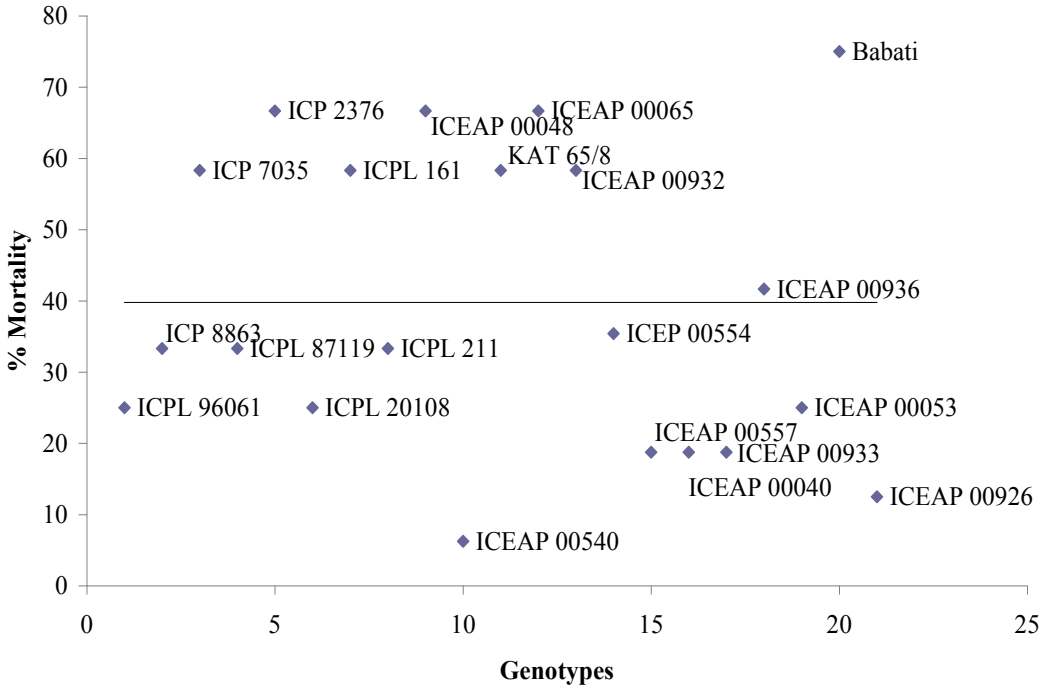


Mean = 42.75, CV = 31.59%, L.S.D (0.05) = 42.00%

Figure 3.8: Disease severities for 21 pigeonpea genotypes against *Fusarium udum* isolate CDKK3

3.3.2.8 *Fusarium udum* isolate NMBM1

Pigeonpea genotypes ICP 2376, ICEAP 00048, ICEAP 00065 and Babati showed high level of susceptibility to *F. udum* isolate NMBM1 collected from Mamire village in Manyara region. However, genotypes ICPL 96061, ICPL 20108, ICEAP 00540, ICEAP 00557, ICEAP 00040, ICEAP 00933 ICEAP 00053 and ICEAP 00926 were highly resistant to the same isolate. Genotype ICP 8863, ICPL 87119, ICPL 211 and ICEAP 00554 showed moderately resistant to NMBM1.While ICP 7035, KAT 65/8, ICEAP 00932 and ICEAP 00936 were moderately susceptible to isolate (Table 3.4, Fig. 3.9).

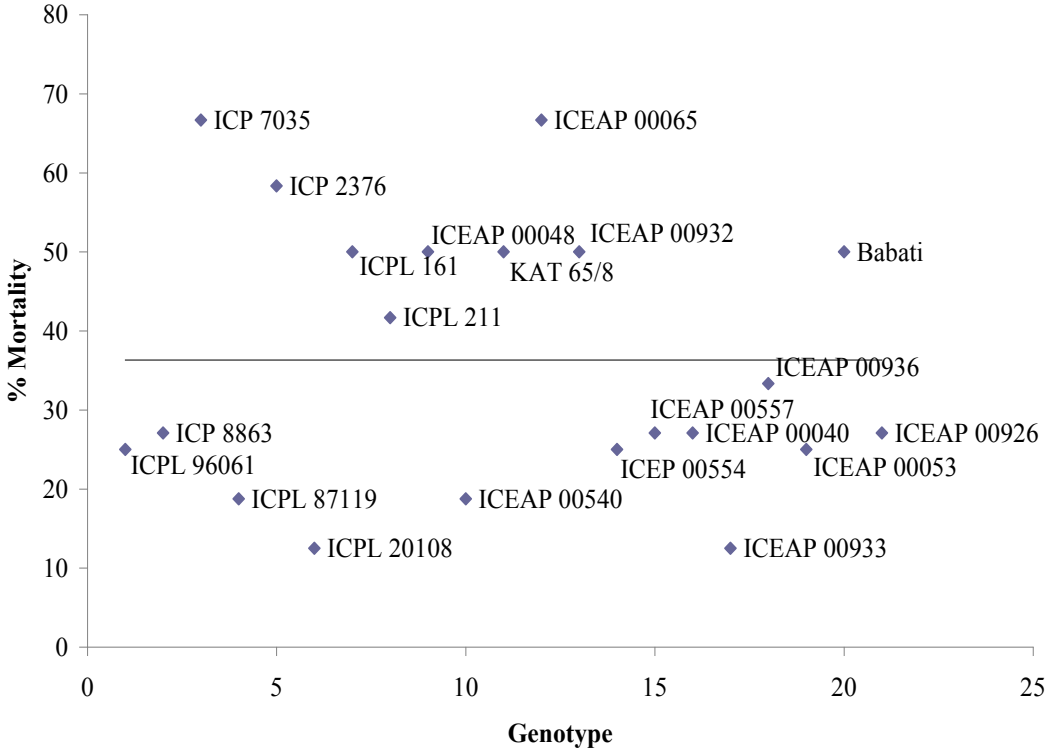


Mean= 39.78 CV = 35.53%, L.S.D (0.05) = 44.06%

Figure 3.9: Disease severities for 21 pigeonpea genotypes against *Fusarium udum* isolate NMBM1

3.3.2.9 *Fusarium udum* Isolate NMBG1

The *F. udum* isolate NMBG1 collected from Galapo village in Babati district in Manyara region, was less virulent to 10 genotypes namely ICPL 96061, ICP 8863, ICPL 87119, ICPL 20108, ICEAP 00540, ICEP 00554, ICEAP 00557, ICEAP 00040, ICEAP 00933, ICEAP 00053 and ICEAP 00926. However, only two genotypes namely ICP 7035, ICEAP 00065 showed more susceptibility to the isolate.Genotype ICEAP 00936 was moderately resistant with 33.3% while remaining genotypes were moderately susceptible with percentage reactions of between 41.67 and 58.33% (Table 3.4, Fig. 3.10).



Mean= 36.30, CV= 38.99%, L.S.D (0.05) = 44.14%

Figure 3.10: Disease severities for 21 pigeonpea genotypes against *Fusarium udum* isolate NMBG1

3.3.3 Reaction of twenty one pigeonpea genotypes against nine *Fusarium udum* isolates

3.3.3.1 Pigeonpea genotype ICPL 96061

The genotype ICPL 96061 showed wide variation against *F. udum* isolates. The genotype was highly resistant to the isolates CDKK2, NMBM1, NMBG1, EMKIL1, and EMMM1 with percentage mortalities of 12.50, 25.00, 25.00, 25.00 and 27.08% respectively. It was also found to be highly susceptible to the isolates CDKK3, EMMK1 and CDKK1, with percentage mortalities of 87.5, 7500 and 75.00% respectively. It was moderately resistant to EMKM2 with percentage mortality of 33.33% (Table 3.4).

3.3.3.2 Pigeonpea genotype ICP 8863

The genotype ICP 8863 was found to be highly resistant to EMKIL1, CDKK1, EMMK1, CDKK2 and NMBG1 with percentage mortalities of 18.75, 18.75, 25.00, 25.00 and 27.08% respectively. With the rest of the isolates (EMMM1, CDKK3, NMBM1 and EMKM2) this genotype expressed moderately resistance with percentage mortalities of 33.33, 33.33, 33.33 and 35.42% respectively (Table 3.4)

3.3.3.3 Pigeonpea genotype ICP 7035

The genotype ICP 7035 was found to be highly resistant to the three isolates EMMK1, CDKK1 and CDKK3 with percentage mortalities of 18.75, 25.00 and 27.08% respectively. It was found to be moderately susceptible to NMBM1 with percentage mortality of 58.33% and highly susceptible to the isolates CDKK2, NMBG1, EMKIL1, EMMM1 and EMKM2 with their respective percentage mortalitesof 66.67, 66.67, 81.25, 81.25 and 87.50% (Table 3.4).

3.3.3.4 Pigeonpea genotype ICPL 87119

This genotype expressed a wide variation of reaction to the nine isolates studied. The genotype expressed high resistance to the isolates CDKK2, EMMM1 and NMBG1 with as low percentage mortalities as 12.50, 18.75 and 18.75. Moderate resistance was observed against two isolates EMKIL1 and NMBM1 both with percentage mortality of 33.33. Moderate susceptibility reaction was observed in the isolates EMKM2, EMMK1 and CDKK1 with their percentage mortalities of 41.67, 58.33 and 58.33 respectively. The genotype expressed it's highly susceptibility to the isolate CDKK3 with percentage mortality of 66.67 (Table 3.4).

3.3.3.5 Pigeonpea genotypes ICP 2376

The genotypes also expressed a varying reaction to the nine *F. udum* isolates studied. It expressed highly resistance to the two isolates EMMK1 and CDKK3 with percentage mortalities of 18.75 and 25.00. This genotype expressed moderate susceptibility to the isolates CDKK1, EMMM1 and NMBG1 with percentage mortalities of 50.00, 58.33 and 58.33 respectively. Highly susceptibility reactions were expressed with the isolates CDKK2, NMBM1, EMKIL1 and EMKM2 with their percentage mortalities of 66.67, 66.67, 75.00 and 81.25 respectively (Table 3.4).

3.3.3.6 Pigeonpea genotype ICPL 20108

This genotype expressed varying reactions against *F. udum* isolates studied with high resistance to majority of them while only one was highly susceptible. The genotype was highly susceptible to the isolates EMMM1, CDKK2, NMBG1, EMMK1, EMKM2, CDKK3 and NMBM1 with their respective percentage

mortalities of 12.50, 12.50, 12.50, 18.75, 25.00, 25.00 and 25.00. This genotype expressed moderate resistance to the isolate EMKIL1 with percentage mortality of 33.33 and highly susceptibility of percentage mortality of 66.67 to the isolate CDKK1 (Table 3.4).

3.3.3.7 Pigeonpea genotype ICPL 161

The genotype ICPL 161 expressed high resistance against isolate CDKK1 only with percentage mortality of 27.08. It expressed moderate susceptible to the isolates CDKK2, NMBG1, EMMK1 and NMBM1 with percentage mortalities of 41.67, 50.00, 56.25 and 58.33 respectively. This genotype also expressed high susceptibility to the isolates EMKM2, EMMM1, EMKIL1 and CDKK3 with their percentage mortalities of 66.67, 66.67, 72.92 and 75.00 respectively (Table 3.4).

3.3.3.8 Pigeonpea genotype ICPL 211

This genotype showed varying response of reaction to the isolates studied. It expressed high resistance reaction to the isolates EMMK1 and CDKK3 with percentage mortalities of 25.00 and 25.00. It also expressed moderate resistance to the isolates CDKK1, NMBM1 and CDKK2 with the percentage mortalities of 33.33, 33.33 and 35.42 respectively. The genotype expressed moderate susceptibility to the isolates NMBG1, EMKIL1, EMKM2 and EMMM1 with percentages mortalities of 41.67, 50.00, 58.33 and 58.33 respectively (Table 3.4).

3.3.3.9 Pigeonpea genotype ICEAP 00048

The genotype showed moderate to high susceptibility to the isolates studied. It expressed moderate susceptibility to the isolates CDKK2, NMBG1 and CDKK3

with their percentages mortalities of 50.00, 50.00, and 58.33 respectively. The genotype also expressed high susceptibility to the isolates CDKK1, NMBM1, EMKM2, EMMK1, EMKIL1 and EMMM1 with their percentage mortalities of 66.67, 66.67, 75.00, 75.00, 81.25 and 81.25 respectively (Table 3.4).

3.3.3.10 Pigeonpea genotype ICEAP 00540

This genotype expressed impressive reaction against all the isolates studied. It showed resistance to all the nine isolates studied. The pattern was such that resistance reaction was observed to the isolates NMBM1, EMKM2, CDKK1, CDKK3, NMBG1, EMKIL1 and EMMM1 with their percentage mortality values 6.25, 18.75, 18.75, 18.75, 18.75, 25.00 and 25.00 respectively. The genotype also expressed moderate resistance to the two isolates, EMMK1 and CDKK2 with percentage mortality of 33.33 (Table 3.4).

3.3.3.11 Pigeonpea genotype KAT 65/8

The genotype KAT 65/8 expressed overall susceptibility to the isolates studied. It expressed moderate susceptibility to NMBG1, EMMM1 and NMBM1 with percentage mortality rates of 50.00, 58.33 and 58.33 respectively. The genotype also showed high susceptibility to the isolates CDKK1, EMKIL1, EMMK1, CDKK3, CDKK2 and EMKM2 with percentage mortality rates of 66.67, 75.00, 75.00, 75.00, 81.25 and 93.75 respectively (Table 3.4).

3.3.3.12 Pigeonpea genotype ICEAP 00065

The genotype ICEAP 00065 expressed overall susceptibility to the isolates studied. This genotype expressed moderate susceptibility to the isolates CDKK1 and

CDKK2 with percentage mortality rates of 58.33 for both. The genotype also showed high susceptibility to the isolates CDKK2, EMMK1, EMMM1, NMBM1, NMBG1, EMKIL1, EMKM2 and CDKK3 with percentage mortality rates of 58.33, 64.58, 66.67, 66.67, 66.67, 81.25, 81.25 and 81.25 respectively (Table 3.4).

3.3.3.13 Pigeonpea genotype ICEAP 00932

The genotype ICEAP 00932 also expressed susceptibility to all the isolates selected for this study. It expressed moderate susceptibility to the isolates CDKK3, CDKK1, NMBG1, EMKM2, EMMM1, EMMK1 and NMBM1 having percentage mortality rates of 41.67, 50.00, 50.00 and 58.33 for the rest four genotypes. The genotype also expressed higly susceptibility to the isolates CDKK2 and EMKIL1 with the percentage mortalities of 64.58 and 72.92 (Table 3.4).

3.3.3.14 Pigeonpea genotype ICEP 00554

The genotype ICEP 00554 expressed a wide variation in reaction to the isolates studied from highly resistance to moderate susceptibility. The genotype expressed high resistance to the isolates CDKK1 and NMBG1 with percentage mortalities of 20.83 and 25.00 respectively. The genotype showed moderate resistance to the isolates EMKM2, EMMK1, CDKK3 and NMBM1 with percentage mortality of 35.42 for all the four and moderate susceptibility to the three isolates EMKIL1, EMMM1 and CDKK2 with a percentage mortality of 41.67 for all the three isolates (Table 3.4).

3.3.3.15 Pigeonpea genotype ICEAP 00557

This genotype ICEAP 00557 expressed remarkable reaction against all the isolates studied. It showed high resistance to most of the isolates. The pattern was such that highly resistance reaction was observed to the isolates EMMK1, CDKK2, NMBM1, CDKK3, EMMM1, CDKK1 and NMBG1 with percentage mortalities of 18.75, 18.75, 25.00, 27.08, 27.08 and 27.08 respectively. The genotype also expressed moderate resistance to the two isolates EMKIL1 and EMKM2 each with percentage mortality of 33.33 (Table 3.4).

3.3.3.16 Pigeonpea genotype ICEAP 00040

This genotype expressed remarkable reaction against most of the isolates studied. It showed high resistance to almost all the isolates studied but one. The pattern was such that highly resistance reaction was noted in the isolates EMMM1, EMMK1, CDKK2, NMBM1, CDKK1, CDKK3 and NMBG1 with percentage mortalities of 12.50, 18.75, 18.75, 18.75, 25.00, 25.00 and 27.08 respectively. The genotype also expressed moderate resistance to the isolate EMKIL1 with percentage mortality of 33.33 and moderate susceptible in the isolate EMKM2 with percentage mortality of 41.67 (Table 3.4).

3.3.3.17 Pigeonpea genotype ICEAP 00933

This genotype expressed a remarkable reaction against most of the isolates studied. It showed high resistance to almost all the isolates studied but one. The pattern was such that highly resistance reaction was noted in the isolates EMMK1, NMBG1, EMMM1, CDKK1, CDKK2, NMBM1 and EMKM2 with percentage mortalities of 12.50, 12.5.0, 18.75, 18.75, 18.75, 18.75 and 25.00 respectively. The genotype also

expressed moderate resistance to the isolate CDKK3 with percentage mortality of 35.42 and moderate susceptible in the isolate EMKIL1 with percentage mortality of 41.67 (Table 3.4).

3.3.3.18 Pigeonpea genotype ICEAP 00936

The genotype ICEAP 00936 expressed a remarkably varying reaction to the nine *F. udum* isolates studied. It expressed high resistance to three isolates EMMK1, CDKK3 and EMMM1 with percentage mortalities 18.75, 25.00 and 27.08 respectively. This genotype also expressed moderateresistance to the isolates CDKK1, CDKK2 and NMBG1 with percentage mortality of 33.33 for each. Moderate susceptibility was expressed to the three remained isolates NMBM1, EMKIL1 and EMKM2 with percentage mortalities of 41.67, 50.00 and 50.00 respectively (Table 3.4).

3.3.3.19 Pigeonpea genotype ICEAP 00053

This genotype showed a varying response of reaction to the isolates studied. It expressed high resistance reaction to the isolates CDKK3, EMMM1, EMMK1, NMBM1, NMBG1 and CDKK1 with percentage mortalities of 12.50, 18.75, 25.00, 25.00, 25.00 and 27.08 respectively. The genotype also expressed moderately resistance to CDKK2 with percentage mortality of 33.33 and moderate susceptible to isolates EMKIL1 and EMKM2 with percentages mortality of 41.67 each (Table 3.4).

3.3.3.20 Pigeonpea genotype Babati

Babati showed susceptible reaction against all the isolates studied. The genotypes expressed moderate susceptibility to the isolates NMBG1 and EMMK1 with percentage mortalities of 50.00 and 58.33 respectively. It expressed high susceptibility to the rest of the isolates in the order CDKK2, CDKK1, EMKIL1, EMMM1, NMBM1, EMKM2 and CDKK3 with percentage mortalities of 64.58, 66.67, 75.00, 75.00, 75.00, 87.5 and 87.5 respectively (Table 3.4).

3.3.3.21 Pigeonpea genotype ICEAP 00926

This genotype showed a well varying response of reaction to the isolates studied. It expressed highly resistance reaction to the isolates CDKK3, NMBM1, EMKIL1, EMMK1, EMKM2, CDKK2, and NMBG1 with percentage mortalities of 12.50, 12.50, 18.75, 18.75, 25.00, 25.00 and 27.08 respectively. It also expressed moderate resistance to the isolate CDKK1 with a percentage mortality of 33.33 and moderate susceptibility to the isolate EMMM1 with a percentage mortality of 41.67 (Table 3.4).

Table 3.4: Disease severity/Reaction of twenty-one pigeonpea genotypes against nine *Fusarium udum* isolates at 60 days after inoculation

		Plant mortality (%) across the nine *Fusarium udum isolates*												
s/n	**Genotypes**	**EMKIL1**	**EMKM2**	**EMMM1**	**EMMK1**	**CDKK1**	**CDKK2**	**CDKK3**	**NMBM1**	**NMBG1**	**Mean**	**LSD**	**SE±**	**CV %**
1	ICPL 96061	25	33.33	27.08	75	75	12.5	87.5	25	25	42.82333	4.603	2.341	12.08
2	ICP 8863	18.75	35.42	33.33	25	18.75	25	33.33	33.33	27.08	27.77667			
3	ICP 7035	81.25	87.5	81.25	18.75	25	66.67	27.08	58.33	66.67	56.94444			
4	ICPL 87119	33.33	41.67	18.75	58.33	58.33	12.5	66.67	33.33	18.75	37.96222			
5	ICP 2376	75	81.25	58.33	18.75	50	66.67	25	66.67	58.33	55.55556			
6	ICPL 20108	33.33	25	12.5	18.75	66.67	12.5	25	25	12.5	25.69444			
7	ICPL 161	72.92	66.67	66.67	56.25	27.08	41.67	75	58.33	50	57.17667			
8	ICPL 211	50	58.33	58.33	25	33.33	35.42	25	33.33	41.67	40.04556			
9	ICEAP 00048	81.25	75	81.25	75	66.67	50	58.33	66.67	50	67.13			
10	ICEAP 00540	25	18.75	25	33.33	18.75	33.33	18.75	6.25	18.75	21.99			
11	KAT 65/8	75	93.75	58.33	75	66.67	81.25	75	58.33	50	70.37			
12	ICEAP 00065	81.25	81.25	66.67	64.58	58.33	58.33	81.25	66.67	66.67	69.44444			
13	ICEAP 00932	72.92	58.33	58.33	58.33	50	64.58	41.67	58.33	50	56.94333			
14	ICEP 00554	41.67	35.42	41.67	35.42	20.83	41.67	35.42	35.42	25	34.72444			
15	ICEAP 00557	33.33	33.33	27.08	12.5	27.08	18.75	25	18.75	27.08	24.76667			
16	ICEAP 00040	33.33	41.67	12.5	18.75	25	18.75	25	18.75	27.08	24.53667			
17	ICEAP 00933	41.67	25	18.75	12.5	18.75	18.75	35.42	18.75	12.5	22.45444			
18	ICEAP 00936	50	50	27.08	18.75	33.33	33.33	25	41.67	33.33	34.72111			
19	ICEAP 00053	41.67	41.67	18.75	25	27.08	33.33	12.5	25	25	27.77778			
20	Babati	75	87.5	75	58.33	66.67	64.58	87.5	75	50	71.06444			
21	ICEAP 00926	18.75	25	41.67	18.75	33.33	25	12.5	12.5	27.08	23.84222			
	MEAN	50.49619	52.182857	43.253333	38.19381	41.269048	38.789524	42.758095	39.781429	36.309048	42.559259			
	LSD 0.05	3.013												
	SE±	1.533												
	CV%	12.08												

1= Disease rating scale was based on Nene *et al*. (1981) with modifications: where 0-30% mortality was considered as highly resistant, 31-40% mortality =moderately resistant, 41-60% mortality = moderately susceptible and 61% -100% mortality = highly susceptible.2= Means within columns followed by common letters are not significantly different ($p \leq 0.05$); mean separation was done using Honestly Significant Difference (HSD). Values are means of 3 replications.

3.3.4 Cultural and morphological characterization of *Fusarium udum* isolates

A great difference in morphological characters was noted among the nine *F. udum* isolates (Table 3.5). Based on colony character, isolates were categorized into two major groups: i) flattened at the centre with isolates NMBG1 and NMBM1 and ii) colony character raised at the centre which accommodated isolates CDKK1, CDKK2, CDK3, EMKK1, EMKM2, EMMK1 and EMMM1 (Plate. 3.3). Based on spore formation there were three groups classified as (+++) good with 15 to 20 conidia per microscopic field, this consist of EMKM2 and CDK2, the second group hadfair number of conidia per microscopic field (++) consisted of EMMM1, NMBG1, EMK1, CDKM1and CDK1 and the third group had poor number of conidia per microscopic field (+) consisted of CDKK2, and CDKK3. Based on colony growth isolates were categorized into two groups of maximum and minimum.Maximum radial growth was observed in the isolates CDKK1, EMKM2, EMMM1, NMBG1, NMBM1 and CDKK3 with (4.8 cm, 4.75 cm, 4.7 cm, 4.5 cm, 4.4 cm and 4.0 cm) respectively while EMMK1, EMKIL1 and CDKK2 scored minimum radial growth of 3.3 cm, 3.4 cm and 3.7 cm respectively. Maximum spore germination of 90% was recorded in EMMK1 followed by EMKM2 85% and EMMM 75%. Minimum spore germination was observed in CDK1 43% (Table 3.5).

Table 3.5: Cultural and morphological characteristics of *Fusarium udum* isolates when grown on V8 Agar

Isolates	Colony Characters	Colony growth diameter (cm)	Spore Germination (%)	Sporulation
CDKK1	flattened at the centre	4.8	90	+++
EMKM2	raised at the center,	4.5	85	+++
EMMM1	raised at the centre	4.4	75	++
NMBG1	flattenedd at the centre	4.75	62	++
NMBM1	flattened d at the centre	4.7	65	++
CDKK3	raised at the center	4.0	67	++
EMMK1	raised at the center	3.3	43	+
EMKIL1	raised at the center	3.4	45	+
CDKK2	raised at the center	3.7	50	+

Key: conidia per microscopic field, +++ -Good -15-20 conidia per microscopic field, ++-Fair -10-15 conidia per microscopic field and + -poor < 10 conidia per microscopic field. 4.8 -4.0 maximum and <4 min. 60-100 max and <50 min

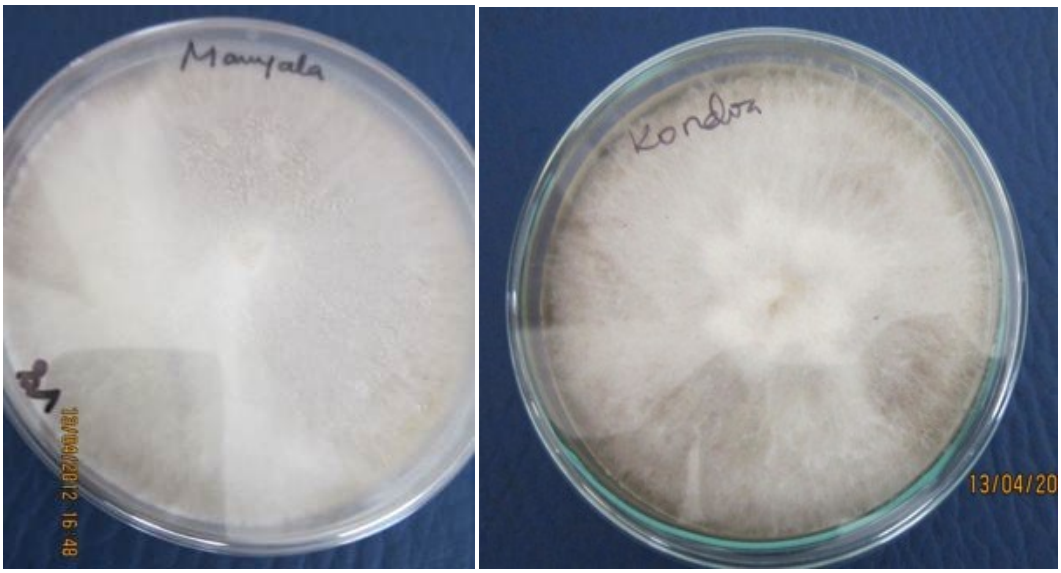


Plate 3.3: Growth pattern of *Fusarium udum* mycelia NMBM1 to the Left and CDKK2 to the right

3. 4 DISCUSSION

Results obtained from the screening of twenty one pigeonpea genotypes against nine *Fusarium udum* isolates in this study demonstrated the existence of variability in *Fusarium udum* pathogen. Similarly, Karimi *et al.* (2010) and Pandey (2013

reported that development of vascular wilt resistance caused by *F.udum* may become extensive because of the high level of genetic variability in the pathogenic population. The differential reaction of genotypes against the *F.udum* isolates observed in this study implies that races may exist within the population. Gwata *et al*., (2005) observed that there were differential host responses to *F. udum* observed for genotype ICEAP 00053 during the evaluation of Fusarium wilt disease which was done in Kiboko (Kenya), Ngabu (Malawi) and Ilonga (Tanzania). These isolates also showed high level of variability in their morphological features like sporulation, spore germination and radial mycelia growth (colony diameter) and colony character on V8 agar (tomato juice). Results are in agreement with observations of Kiprop *et al*., (2005); and Mahesh *et al.* (2010) that *F. udum* isolates from the same site or different geographical locations have been shown to exhibit high variability on morphological and pathogenicity of this pathogen.

The observed variability in pathogenicity of *Fusarium udum* isolates and morphological features could probably be due to the genetics of the pathogen as well as environmental factors surrounding it. This was concurred by Karimi *et al*.(2012), that among some pigeon pea resistant varieties segregation to Fusarium wilt disease does occur in subsequent generations due to genetic variation in isolates. Similarily, Kiprop *et al.* (2002); Kiprop *et al.* (2005); Mahesh *et al*. (2010); Karimi *et al*. (2010); Pandye *et al.,* (2010); and Mesapogu *et al*. (2012) reported prevalence or development of new strains of *F. udum* in different geographical locations.The disease incidence of up to 10 percent in some locationswas common to the cultivars reported as wilt resistant varieties and therefore released for use by

farmers. According to Gwata *et al*. (2005) the variation of *Fusarium udum* isolates could be influenced by different environmental factors.

The saprophytic ability of *F.udum* pathogen to survive in the soil for a long period of time could be one of the factors for its pathogenic variability. The pathogen can survive for at least eight years during which it may have to go through different environmental stresses and biological competition. This tends to modify the expression of some genes of that pathogen as a result it lead into different disease causing abilities (Nene *et al*., 1981; Mahesh *et al*., 2010; Muhhamad *et al*., 2011).

In this study, pigeonpea genotypes also expressed their differences in resistance against *F. udum* isolates. Babati, the local susceptible check expressed moderate to high susceptibility to all the isolates used in this study. Also while each genotype was highly resistant to 7 out of 9 isolates used, ICEAP 00962, ICEAP 00933, ICEAP 00040 and ICEAP 00557 also expressed different resistance patterns from each other by differing in the isolates to which they were resistant.Genotype ICPL 00926 was highly resistant to isolate EMMK1 while CDKK3 andICEAP 00933 expressed moderate resistance to moderate susceptibility to these isolates. The differences in disease reaction among pigeonpea genotypes against *F. udum* isolates could clearly demonstrate differences in their genetic makeup, which could be due to different sources of gene and its stability against different environments. Other authors such as Kumar *et al*. (2009); and Karimi *et al.* (2010) reported that sources and genetic backgrounds of the resistant materials used in the study could have contributed to the differences.

3.4.1 Conclusion and Recommendation

The genetic variability and differences in aggressiveness were observed on nine *Fusarium udum* strains collected from different crop growing areas of Tanzania. Among the nine isolates characterized, EMKM2 isolate was the most aggressive. It may therefore be useful for screening pigeonpea genotypes in breeding work. As *Fusarium udum* is dynamic, it is important to keep on continuous evaluating the pathogen before growing pigeonpea genotypes. There was wide genetic variability among pigeon pea genotypes evaluated against nine *Fusarium udum* isolates. Out of these genotypes ICEAP 00040, ICEAP 00540, ICEAP 00557 and ICEAP 00933expressed resistances against all the isolates studied. Therefore ICEAP 00040, ICEAP 00540 and ICEAP 00557 could be used as new source of Fusarium wilt resistance hence further studies are using molecular markers is recommended to confirm the finding.

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CHAPTER FOUR

4.0 Genetic Diversity, Inheritances and SSR Marker Correlated with Fusarium Wilt Disease Resistance in Pigeonpea (*Cajanus cajan* (L.) Millsp.) Among Asian and African Genotypes

ABSTRACT

Fusarium Wilt Disease (FWD) caused by *F. udum* is the most serious disease of pigeonpeas [*Cajanus cajan* (L.) Millsp.]. Significant yield losses which could results up to 100% were reported in susceptible cultivars throughout the pigeonpeas growing areas. The reported yield loss is results of low information on genetic diversity and gene responsible with fusarium wilt disease resistance and its mode of inheritance. A total of 60 pigeonpea genotypes were characterized using simple sequence repeats (SSR). Diversity analysis results showed close relationship between the East African and Indian populations, implying that Asian and African genotypes originated from the same gene pool hence could be improved through mutation, more selection and recombination. Studies on genetic inheritance of Fusarium wilt resistance, showed the F_2 progenies ratio 3:1 that agreed with Mendelian ratio, thus concluded that the resistance to Fusarium wilt disease is under the control of a single dominant gene. The observed single dominant gene could be used to donate gene for Fusarium wilt disease resistance into genotypes where Fusarium wilt disease is of an economic problem. Identification of SSR markers correlated to fusarium wilt disease revealed that, the markers CZ681922, CZ681962 and CZ681928 were highly correlated to phenotypic observation. Therefore its application is recommended for sorting resistant genotypes rather than use of phenotypic method which is tedious and time consuming.

Key words: Pigeonpeas, SSR, virulence, MAS,

4.1 INTRODUCTION

Pigeonpea [*Cajanus cajan* (L.) Millspaugh] is one of the major grain legumes (pulse) crops which are devastated by *Fusarium udum* the major causal agent of soil and seed borne disease on pigeonpea (Odeny *et al*., 2009). Significant yield losses of up to 100% were reported in susceptible cultivars throughout the vaious pigeonpeas growing areas (Gwata *et al*., 2005; Odeny *et al.,* 2009; Prasanthi *et al.,* 2009; Karimi *et al*., 2010). Fusarium wilt disease was known since the 1930s, especially in India, yet the genetics of resistance to this disease remains to be understood. Some of the reports are conflicting and inconclusive regarding the genetics of this destructive disease. Reports indicated that resistance to FWD in pigeonpea was controlled by multiple factors (Odeny *et al*., 2009) while Shaw (1936) observed two complementary genes. Later studies by Pathak (1970) confirmed the presence of two complementary genes while Pawar and Mayee (1986) reported the control of this trait by a single dominant gene.

The information on gene responsible for Fusarium wilt disease resistance and genetic diversity of pigeonpeas is still inadequate. In order to breed for fusarium wilt resistance on pigeonpea it is important to understand genetic variation of genes within populations and mode of inheritance of FWD.Therefore, the objectives of this study was to determine the genetic diversity and gene responsible for FWD and its mode of genetic inheritance in pigeonpeas in order to control significant yield losses caused by *F.udum.*

4.2 MATERIALS AND METHODS

4.2.1 Diversity analysis of pigeonpeas

Sixty (60) pigeonpea genotypes (Table 4.1) collected from East Africa and Asia were analysed using 16 primers (Table 4.2). These genotypes were planted in the screen house at Sokoine University of Agriculture. Two weeks after germination leaf samples were harvested into a sterile eppendorf 1.5 ml tubes and kept into ice box for DNA extraction. The genomic DNA was extracted by using plant mini kit DNA extraction method. The total volume of 20 µl was used for PCR mixture containing 4 µl of 10 × Taq buffer, 0.25 µl of 5 units/µl Taq polymerase, 1.2 µl of 25 mM of MgCl, 0.4 µl of 10 mM dNTPs, 0.8 µl of forward and reverse primers, 11.55 µl of 50 ml PCR H_2O and 1 µl (1.25 ng/µl) of 25 ng DNA template.

Table 4.1: Pigeonpea populations from India and East Africa

S/N	Genotypes	Country of origion	Pedigree
1	ICEAP 00053	Kenya	KibWB□-KibWB
2	ICP 7169	Uganda	Unknown
3	ICP 6927	Uganda	Unknown
4	ICP 40	Uganda	Unknown
5	ICP 7271	Uganda	Unknown
6	ICP 2812	Uganda	Unknown
7	ICP 12062	Uganda	Unknown
8	ICP 7173	Uganda	Unknown
9	ICP 13410	Uganda	Unknown
10	ICP 7171	Uganda	Unknown
11	SINGIDA	Tanzania	unknown
	ICEAP 00053	Kenya	KibWB□-KibWB
12	ICP 6927	uganda	
13	ICEAP 00936	Kenya	ICEAP 00040-6
14	ICEAP 00040	Uganda	KibWB□-KibWB□
15	ICEAP 00932	Kenya	ICEAP 00040-1
16	ICP 87051	Kenya	unknown
17	ICEAP 00048	Kenya	KibB-13
18	ICEAP 00554	Kenya	NA-6-KibB16-KIbB3-KibB12
19	ICEAP 00926	Kenya	ICEAP 00020-24
20	KAT 60/8	Kenya	TAz 9-KIbB01-KibB10-KibB6
21	ICEAP 00068	Kenya	TAz 9-KIbB01-KibB10-KibB6
22	ICEAP 00850	Kenya	14)-1-1
23	ICEAP 00557	Kenya	NA-7-KibB21-KibB8
24	ICEAP 00540	Kenya	LINDI-4-KIbB-KibB
25	ICP7035	India	DISR 55
26	ICPB 2101	Kenya	unknown
27	ICPL 96058	Kenya	unknown
28	ICPR 2433	Kenya	unknown
29	ICP 87051	Kenya	unknown
30	ICPL 96053	Kenya	Unknown
31	ICPL 20186	Kenya	unknown
32	ICP 132092	Tanzania	Unknown
33	ICP 13092	Tanzania	unknown
34	ICPL 96061	India	ICPL 87051 X ICPL83057
35	ICP 8863	India	ICWR -6
36	ICP 7035	India	DISR 55
37	ICPL 87119	India	C11 X ICP-1-6 W(x)-W1(x)
38	ICP 2376	India	P 3888
39	ICPL 20108	India	IPH 487 inbred line (mb 3783 X ICPL 87119
40	ICPL 161	India	ICP-6 X PANT-A-2
41	ICPL 211	India	pusa
42	ICEAP 00125	Tanzania	uknown
43	MALI	Tanzania	
44	ICEAP 00932	Tanzania	ICEAP 00040-1
45	ICEAP 00554	Tanzania	NA-6-KibB16-KIbB3-KibB12
46	ICEAP 00933	Tanzania	ICEAP 00040-2
47	KOMBOA	Tanzania	unknown
48	ICEAP 0053	Tanzania	KibWB□-KibWB
49	ICEAP 01130	Tanzania	unkown
50	TUMIA	Tanzania	unknown
51	ICEAP 01117/9	Tanzania	unknown
52	ICEAP 01139	TanzaniaI	unknown
53	ICEAP 00936	Tanzania	ICEAP 00040-6
54	ICEAP 00932	Tanzania	ICEAP 00040-1
55	00040 (MALI)	Tanzania	unknown
56	TUMIA	Tanzania	unknown
57	ICEAP 00554	Tanzania	NA-6-KibB16-KIbB3-KibB12
58	00053	Tanzania	KibWB□-KibWB
59	KOMBOA	Tanzania	unknown
60	ICEAP 00557	Tanzania	NA-7-KibB21-KibB8

Table 4.2: List of primers used in diversity analysis of pigeonpeas

Primer	Forward sequence	Reverse sequence	Tm	References	bp
CZ681994	gggaaacaaaatatcccctaatc	taatcacacacatcacacctagca	57.1, 59.3	Odeny *et al*., 2007	23,24
CZ681983	tgggcatggtagaggaagtt	tcagaagtcgatggcaagtg	57.3,57.3	Odeny *et al.,* 2007	20,20
CZ681951	acatgtgtggcgtagtgtga	gcaaaaccgttccataaaaa	57.3,51.2	Odeny *et al*., 2007	20,20
CZ681964	gatagcacacacacacacaaca	taccttagggtcaccaacga	58.4,57.3	Odeny *et al*., 2007	22,20
CZ681914	Atcggcttttgtcttgatga	aagctacaagggatacacatgc	53.2,58.4	Odeny *et al.,* 2007	20,22
CZ681993	atcatcagattcttcagccgta	ggttagaccaatccaatcaagc	56.5,58.4	Odeny *et al*., 2007	22,22
CZ681926	gtagaggaggttccaaatgacata	atctgtctggtgttttagtgtgct	59.3,59.3	Odeny *et al.,* 2007	24,24
CZ681967	aggtgcaaaggaagcactaat	cagctccactgtcttcaacg	55.9,59.4	Odeny *et al*., 2007	21,20
CZ681922	acaccaccatgctaaagaacaag	ccaagcaagacacgagtaatcata	58.9,59.3	Odeny *et al.,* 2007	23,24
CZ681990	caggtctgctactgccatca	agcccacttctgcatcactc	59.4,59,4	Odeny *et al.,* 2007	20,20
CZ681998	catcataatcatacatgtcaatgcta	ggttttatctttgtctccaattctg	56.9,58.1	Odeny *et al.,* 2007	26,25
CZ681966	agtcgatgtggaacatgagga	tgttgtaagccgtgggtagg	57.9,59.4	Odeny *et al*., 2007	21,20
CZ681963	gttcttcttgttgttgttgttg	aattcgtggagttcattgg	54.7,52.4	Odeny *et al*., 2007	22,19
CZ681917	tgaaatgaacaaacctcaatgg	tgtattgcacattgacttggcta	54.7,57.1	Odeny *et al*., 2007	22,23
CZ681923	catcgcctacaatcatacaaaga	tcttgtccttttcagtcatcgt	57.1,57.1	Odeny *et al.,* 2007	23,23
CZ682019	aacacgcacctcaattcca	gaatgaggaatgaagggacaaa	54.7,56.5	Odeny *et al.,* 2007	19,22

PCR conditions consisted of 5 minutes initial denaturation of template DNA at 94°C followed by 30 cycles of 94°C for 30s, 60°C for 30s and 72°C for 60 s followed by a 5 minute final extension at 72°C. The annealing temperatures were changed depending on the marker used which ranged from 51.2 -62.1°C. PCR products were loaded on 2% agarose gels in 1×TBE (Tris Borate EDTA) buffer following electrophoretic separation at 120V for 2 hours. The DNA fragments from different loci size for each primer were scored in agarose gel or electropherograms and were entered in a computer file as binary matrices after staining it with 0.5 µg/ml ethidium bromide, where 0 and 1 represented absence and presence of a band, respectively.

4.2.1.1 Data collection and analysis

Each band or fragment was treated as a separate putative locus and scored using a binary system of present (1) and absent (0). The binary matrix was then subjected to phenetic analysis and tested further for a population structure. Clustering was done using NTSY-PC software and from them a dendogram was constructed depicting similarities of the genotypes used.

4.2.2 Genetic inheritance of Fusarium wilt disease in pigeonpeas.

All the populations [F_1, F_2, (BC_1F_1), (BC_2F_1), ICEAP 00048 and ICPL 96061] susceptible and resistant parents were established in screen house at Sokoine University of Agriculture. Seven days after germination these populations were screened using EMKM2 isolate. Using root tip inoculation techniques after seven days of germination, the individual seedlings from F_1, F_2, BC_1F_1, BC_2F_1 and parents were uprooted from the sand and shaked to remove the excess sand and washed in sterile dH_2O. The plants were immersed in sterile distilled water to remove sand from the roots and by using sterile scissors, one cm of the distal end of the root system was cut and dipped in the inocula at a concentration of 1.0×10^6 conidia ml^- and suspended for 10 minutes to allow the conidia to enter the wounds created on the root systems. The plants were then transplanted into pots containing mixture of sterile soil and sand (3:1 v/v) (Plate 4.1). Watering was done after transplanting in an interval of two days.

Plate 4.1 First days after inoculation of *F. udum* isolate EMKM2 on transplanted pigeonpea seedlings in the green house

4.2.2.1 Data collection and analysis

Numbers of wilted and health plants were recorded and Chi-square test was performed to test the goodness of fit between the theoretical expected and observed ratios of resistant and susceptible plants. It was hypothesized that the R: S ratio fits 3:1. Chi-square test was computed using the following formula:-

$$\chi^2 = \sum \frac{[Observed - Expected]^2}{Expected}$$

Where,

χ^2 = calculated chi- square value

Observed = frequency observed

Expected = frequency expected

4.2.3 SSR markers correlated with fusarium wilt disease resistance

The F_2 populations with resistant and susceptible parents were established in the screen house at Sokoine University of Agriculture. Two weeks after germination DNA was extracted from the population, DNA extraction was performed using DNeasy plant mini kit (50). Cat. NO69104, Lot NO139309262 DNA extraction methods. The extracted DNA from each group was subjected to the general SSR markers (Table 4.3). The total volume of 20 µl was used for PCR mixture containing 10× Taq buffer, 5units/µl Taq polymerase, 1.2 µl of 25 ml of MgCl, 0.4 µl, 10 mM dNTPs, 0.8 µl of forward and reverse primers, 11.55 µl of 50 ml PCR H_2O and (1.25 ng/µl) of DNA template. The PCR conditions consisted of 5 minutes initial denaturation of template DNA at 94°C followed by 30 cycles of 94°C for 30 s, (55°-60°C) for 30 s and 72°C for 60 s followed by a 5 minute final extension at 72°C. The annealing temperatures were adjusted depending on the

marker used ranging from 51.2 -62.1°C. PCR products were loaded on 2% agarose gels in 1×TBE (Tris Borate EDTA) buffer following electrophoretic separation at 120V for 2 hours. The DNA fragments from different loci size for each primer were scored in 2% agarose gel and were entered in a computer file as binary matrices after staining it with 0.5 µg/ml ethidium bromide, where 0 and 1 represented absence and presence of a band, respectively.

4.2.3.1 Data collection and analysis

Only the shared presence of a band was counted, and not the shared absence of band, the phenotypic scores was reported as 1 and 0 for resistant and susceptible respectively.These scores were analysed for their correlation using excels.

Table 4.3: Primers used for the identification of best markers identification

Primer	Forward sequence	Reverse sequence	Tm	Reference	bp
CZ681922	acaccaccatgctaaagaacaag	ccaagcaagacacgagtaatcata	58.9, 59.3		23,24
CZ681962	gggaaactcacctatattaccaa	cactaccgtctacagccatctc	57.6, 57.6	Odeny *et al*., 2007	23,22
CZ681928	tcttagcatgtcctctattttcgt	agtacatttcaaatccacacatcc	57.1, 62.1		24,24
CZ681998	catcataatcatacatgtcaatgcta	ggttttatctttgtctccaattctg	56.9,58.1	Odeny *et al.,* 2007	26,25
CZ681990	caggtctgctactgccatca	agcccacttctgcatcactc	59.4,59,4	Odeny *et al.,* 2007	20,20
CZ681914	Atcggcttttgtcttgatga	aagctacaagggatacacatgc	53.2,58.4	Odeny *et al.,* 2007	20,22

4.3 RESULTS

4.3.1 Diversity and relatedness of African and Asian pigeonpeas

The 51 and 9 pigeonpea populations from East Africa and India respectively were grouped into three clusters A, B, and C. These clusters were divided into subclusters (Table 4.4, Fig 4.1). From the cluster analysis it was observed that Indian and East African collections (Kenya and Uganda) were grouped together in clusters A and B while rest of the genotypes collected from Tanzania were grouped in cluster C.

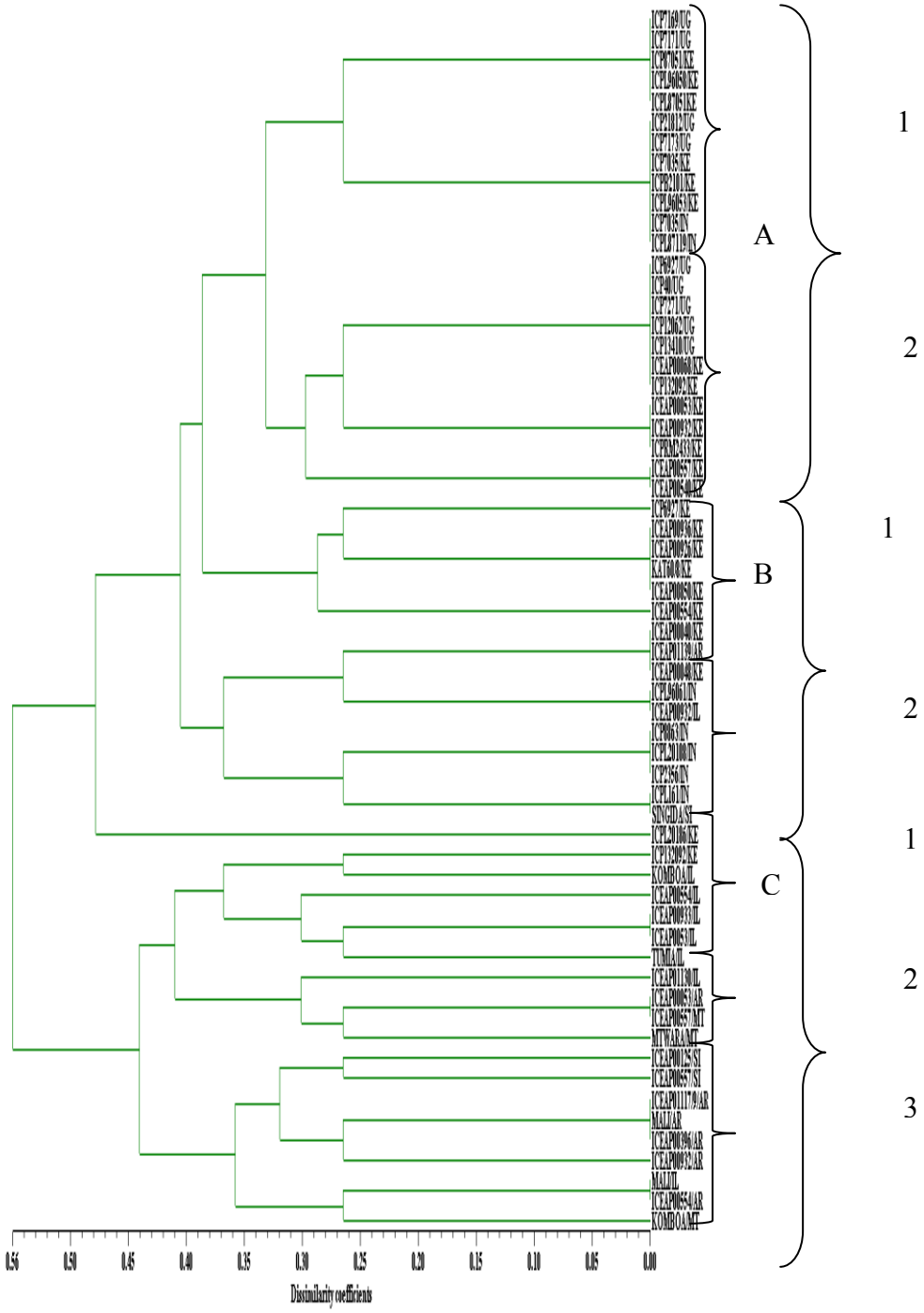


Figure 4.1: Genetic dissimilarity coefficients of pigeonpea accessions collected from India and parts of East Africa

Table 4.4: Genotypes clustering order of similarity

A		B		C		
A1	A2	B1	B2	C1	C2	C3
ICP 7169 UG	ICP6927UG	ICP6927KE	ICEAP 00040KE	ICP 132092KE	ICEAP 01130 IL	ICEAP 00125
ICP 7171 UG	ICP40UG	ICEAP 00936KE	ICEAP 01139AR	KOMBOA	ICEAP 00053 AR	ICEAP 00557AR
IC87051KE	ICP7271UG	ICEAP 00926KE	ICEAP 00048KE	ICEAP 00554 IL	ICEAP 00557 MT	ICEAP 01179 AR
ICPL 96050 KE	ICP12062UG	KAT 60/8KE	ICPL 96061IN	ICEAP 00933IL	MTWARA	MALI
ICPL 87051KE	ICP 13410UG	ICEAP 00850KE	ICEAP 00932IL	ICEAP 0053IL		ICEAP 00396AR
ICP 21812UG	ICEAP 00068KE	ICEAP 00554 KE	ICP 8863IN	TUMIA		ICEAP 00932
ICP7173UG	ICP 132092KE		ICPL 20108IN	ICEAP 01130 IL		ICEAP 00554
ICP 7035KE	ICEAP 00053KE		ICPL 161IN			KOMBOA
ICPB 2101KE	ICEAP 00932KE		SINGIDA			
ICPL 7035IN	ICP2433KE					
ICPL87119IN	ICEAP 00540KE					
ICPL96053KE						

4.3.2 Genetic inheritances of fusarium wilt disease resistance in pigeonpea

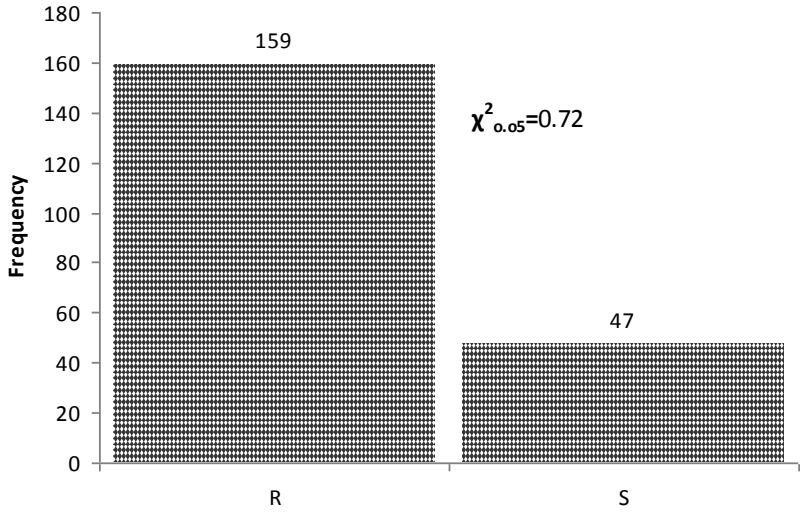
The resistant parents used in this study showed no reaction to EMKM2 while susceptible parents showed high level of virulence as 60 plants were all susceptible to the same inoculum (rr) used in resistant genotype .The F_1 progenies developed from the crosses of resistant × susceptible and their reciprocals were all resistant to FWD. The number of resistant and susceptible progenies obtained in F_2 populations derived from crosses between resistant parent ICPL 96061 and susceptible ICEAP00048 ($\chi^2_{(0.05)}$ = 0.72, P≥0.639), demonstrated a ratio of 3:1 (resistant:susceptible). All the BC_1F_1 (backcrosses to the resistant parents) showed a resistant reaction while their respective BC_2F_1 segregated into a ratio of 1:1 (Table 4.5, Fig 4.2).

Table 4.5: Genetic analysis of resistance to FWD in pigeonpea (*Cajanus cajan*) populations derived from ICEAP 00048 × ICPL 96061

Pedigree	Generations	Total plants	Observed frequencies R	S	Ratio R: S	χ^2	χ^2 Tabulated
Crosses using ICEAP 00048 and ICPL 96061 as parents							
ICEAP 00048	P_1	60		60			
ICPL 96061	P_2	60	60				
ICEAP 00048× ICPL 96061	F_1	80	80				
ICPL 96061× ICEAP 00048	F_1	80	80				
ICEAP 00048× ICPL 96061	F_2	206	159	47	3. 38:1	0.72	3.84
ICPL 96061× ICEAP 00048	F_2	206	157	49	3.20:1	0.35	3.84
ICEAP 00048× ICPL 96061*[2]	BC_1F_1	60	60				
ICPL9606×ICEAP00048 *[2]	BC_1F_1	54	49	5	9.8:1		
ICEAP00048×ICPL 96061*[1]	BC_2F_1	53	33	20	1.65:1	3.56	3.84
ICPL 96061× ICEAP 00048*[1]	BC_2F_1	54	33	21	1.57:1	3.20	3.84

Key: S= susceptible and R= resistant

(A)



(B)

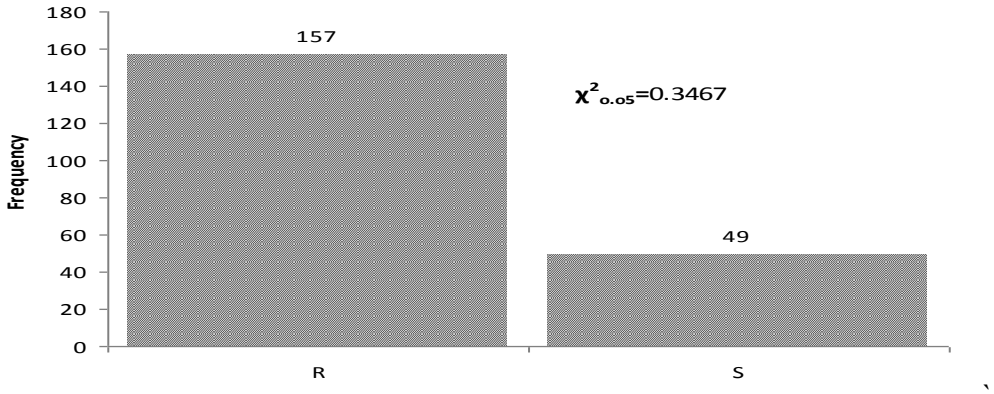


Figure 4.2: Segregation of F_2 pigeonpea (*Cajanus cajan*) populations derived from (A) ICEAP 0048 × ICPL 96061 (B) ICPL 96061 × ICEAP00048 against EMKM2 isolate

4.3.3 Identification of SSR correlated to Fusarium wilt disease

Twenty SSR primers were analysed for Fusarium wilt disease resistance for 38 pigeonpea individuals but only six primers amplified 4.3. Out of the six primers only three showed high, positive and significant correlations with phenotypic Fusarium wilt screening (Table 4.6). The primersCZ681922, CZ681962 and CZ681928 revealed correlation of 77%, 70% and 77% respectively (Table 4.7). The

markers CZ681922, CZ681962 and CZ681928 showed that out of 38 individuals there were 9, 10, and 10 bands scores respectively. When correlated with the phenotypic scores the individual genotypes challenged with Fusarium wilt. Genotypes with no bands were highly susceptible to the Fusarium wilt by showing more mortality.

Table 4.6: Markers correlated to Fusarium wilt disease resistance in pigeonpea F_2 of ICPL 96061 and ICEAP 00048

	PRIMERS								
GENOTYPES	**CZ681998**	**CZ681990**	**CZ681922**	**CZ681962**	**CZ681928**	**CZ681914**	**FWD Reaction**	**Resistant**	**Susceptible**
R1	0	1	1	1	1	1	R	5	
R2	1	1	1	1	1	1	R	6	
R3	0	1	0	0	0	1	S		4
R4	0	1	0	0	0	1	R	2	
R5	0	1	0	0	0	1	R	2	
R6	0	1	0	0	0	1	S		4
R7	0	1	0	0	0	1	R	2	
R8	0	0	0	0	0	1	S		5
R9	0	1	0	0	0	1	S		4
R10	0	1	0	0	0	1	S		4
R11	0	1	0	0	0	1	S		4
R12	1	1	0	0	0	1	S		3
R13	1	1	1	1	1	1	R	6	
R14	1	1	0	0	0	1	R	3	
R15	1	1	1	1	1	1	R	6	
R16	0	0	1	0	1	0	R	2	
R17	0	1	1	1	1	1	R	5	
R18	1	1	1	1	1	1	R	6	
R19	1	1	0	0	0	1	S		3
R20	1	1	0	0	0	1	S		3
R21	1	1	0	1	1	1	R	5	
R22	1	1	0	0	0	1	S		3
R23	1	0	0	0	0	0	S		5
R24	0	0	0	0	0	0	S		6
R25	0	0	0	0	0	1	S		5
R26	0	1	0	0	0	1	S		4
R27	0	1	0	0	0	1	S		4
R28	0	1	0	0	0	1	S		4
R29	0	1	0	0	0	1	S		5
R30	0	1	0	0	0	1	S		4
R31	0	1	0	1	0	0	S		4
R32	0	1	0	0	0	0	S		5
R33	0	1	0	0	0	1	S		4
R34	0	1	0	0	0	1	S		4
R35	0	0	0	0	0	0	S		6
R36	1	1	1	1	1	1	R	6	
1CPL 96061	1	1	1	1	1	1	R	6	
ICEAP00048	0	0	0	0	0	0	S		6
TOTAL	13	31	9	10	10	31	0	62	103

Table 4.7: Correlation of Fusarium wilt resistance in pigeonpea F_2 of ICEAP 0048 × ICPL 96061

Primer	Correlation coefficient	Mean	Std Dev	Probability
CZ681998	0.40	0.36	0.48	0.01
CZ681990	0.18	0.83	0.37	0.29
CZ681922	0.77	0.25	0.43	0.00
CZ681962	0.70	0.27	0.45	0.00
CZ681928	0.77	0.25	0.43	0.00
CZ681914	0.18	0.83	0.37	0.29

4.4. DISCUSSION

4.4.1 Diversity and relatedness of African and Asian pigeonpeas

The study on genetic relationship between East African and Indian pigeonpea genotypes as studied based on SSR revealed close relationship between these two populations. The close relationship between the accessions or collections could be due to the same origin of pigeonpeas (India) from which it was distributed to other places in the world through different means like human migration as they carried seed for cultivation. Similar observation was reported by Moyib *et al.* (2007) in cassava that the medium relationship observed might be a result of a common source of collection (IITA) from which Nigerian farmers choose their common desirable traits of cassava, such as high yielding and resistance to pests and diseases. According to Wasike *et al*. (2005) based on the range of genetic diversity of the crop, India was considered as centre of origin of pigeonpeas. Smartt (1990) supported the previous hypotheses that India is the primary centre of diversity, with East Africa being a secondary centre of diversity. Pigeonpea is believed to have been moved by ancient explorers from India to Malaysia then to East Africa (Vange and Moses, 2009; Songok *et al.,* 2010).

The small sample size of Indian population (9 accessions) used in this study to determine genetic relationship probably could account for inefficient of genetic variability. This observation is in agreement with the results of Mulualem *et al*. (2012). In addition, self pollinated mode of reproduction of pigeonpeas could lead into formation of hybrids and introgression of l genes between these populations of pigeonpea.

4.4.2 Genetic inheritances of fusarium wilt disease resistance

The results obtained shows that no reaction to EMKM2 isolates in all resistant progenies while all susceptible genotypes were highly infected with the EMKM2 isolate. The observation showed a 3:1 segregation that agreed with Mendelian ratio, thus the resistance to Fusarium wilt disease is under control of asingle dominant gene. This observation is in agreement with reports of Karimi *et al*. (2010) that resistance to FWD is controlled by single dominant and recessive gene. Also in field beans for example, resistance to FWD (*Fusarium oxysporium* f.sp. *phaseoli*) was reported to be controlled by major genes among germplasm of races Durango (Karimi *et al*., 2012). However, Odeny *et al.* (2001); Kumar *et al*. (2009) reported different observations that resistance to FWD (Kiboko isolate) in pigeonpea is controlled by recessive genes; a single recessive gene in cultivar ICEAP00040 of East African origin and duplicate recessive genes in the Indian resistant source, ICP8863, while polygenes controlled resistance in the Mesoamerican types (Odeny *et al.,* 2001).

4.4.3 Identification of SSR markers correlated to fusarium wilt disease

In this study phenotypic and genotypic characterization were used together to analyse Fusarium wilt markers correlated to FWD resistance in pigeonpea. The high, positive and significant correlation was revealed by markers CZ681922, CZ681962 and CZ681928. Correlated markers to wilt diseases will apply more for selection of resistant genotypes rather than phenotypic screening which is expensive in terms of time and resources. Young (1999) noted that to plant breeders it is more attractive to select desirable lines based on genotypes rather than analyzing phenotypes.

4.4.4 Conclusion and Recommendation

The close genetic relationship observed between two populations, could be diversified through mutation breeding, selection, recombination and introduction of new source of variability from genetically diverse pigeonpea populations. The study of genetic inheritance in pigeon pea revealed a ratio of 3:1 hence confirming a single dominant gene control. This single dominant genecan be used to donate genes for disease resistance into genotypes where Fusarium wilt disease is be of an economic problem.The markers CZ681922, CZ681962 and CZ681928 were highly correlated to phenotypic observation hence correlated to Fusarium wilt resistance. The application of these is recommended for sorting resistant genotypes rather than phenotypic method which is tedious and time consuming.

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CHAPTER FIVE

5.0 Combining Ability in Yield and Yield Components in Pigeonpea (*Cajanus cajan* (L.) Millspaugh)

ABSTRACT

Pigeonpea, (*Cajanus cajan* (L.) Millspaugh) is one of the important grain legume crops grown in many countries in the tropics and subtropics. The crop is reported to have wide adaptability in different climatic and soil conditions. The perennial nature of pigeonpea allows farmers to take multiple harvests with surpluses traded in both local and international markets. However, it is not benefited from intensive scientific research, the situation which results into a large gap between potential yield and yield obtained on farmers fields. A study was conducted on combining ability in yield and yield components on pigeonpea during the dry season in 2011. A diallel cross was evaluated for days to 50% flowering, days to 85% maturity, number of branches, plant height, pods per plant, seeds per pod, 100 seed weight and yield. With regard to performance of parents and their crosses, Wr/Vr graphical analysis revealed that days to 50% flowering, days to 85% maturity and pods per plant were controlled by dominant genes while seeds per pod were controlled by partial dominance genes. Estimate of general and specific combining ability revealed high significant effect in all yield components except number of branches and seeds per pod. This implies that, both additive and non-additive gene action in these characters were expressed. Phenotypic correlation was explained by the path analysis on days to 50% flowering and days to 85% maturity, showing negative correlation to yield. It was also observed that negative or positive indirect effect had

significant effect to direct and total correlation. These results revealed positive and significant in number of branches per plant, pods per plant and number of seeds per plant. Hence number of branches per plant, pods per plant and number of seeds per plant has high contribution to yield thus could be useful in the selection for yield improvement.

Key words: Combining ability, pigeonpea, gene action

5.1 INTRODUCTION

Pigeonpea, (*Cajanus cajan* (L.) Millspaugh) is a leguminous crop which plays a significant role in human nutrition, especially in tropical and subtropical countries where their consumption is high (Saeed, *et al*., 2007). Pigeonpea is intercropped with other crops like maize without any effects on yield and yield components (Vange and Moses, 2009). It is prepared into various meals and served as substitute for cowpea. It also provides fuel wood and fodder for the small - scale farmers (Vanisree, *et al*., 2013). However, pigeonpea is one of a range of orphan (neglected) crops that have not benefited from intensive scientific research, the situation which results into a large gap between potential yield (2,500kg/ha) and yield obtained on farmers fields (866.2kg/ha) in Asia and (736.2kg/ha) in Africa despite their importance for regional food security in the worlds poorest regions (Varshney *et al*., 2010). Thus plant breeders are interested in estimating the gene effects in order to apply the most effective breeding procedure for improvement of the attributes in question. Moreover, the choice of the most efficient breeding methodology mainly depends upon the type of gene action controlling the genetic variation.

Therefore, unambiguous tests of the genetic components help the breeder to make correct decisions about the most effective breeding method to be applied (Bayoumi and El- Bramawy, 2007). The study of combining ability, heritability and heterosis are more reliable as they provide useful information on selection of parents in terms of performance of their hybrids and elucidate the nature and magnitude of various types of gene action involved in the expression of the quantitative trait. In self pollinated crops like pigeonpea these studies are useful in assessing the combining ability of the parent which when crossed would give more desirable segregates (Kalapchieva, 2010). The limited studies on combining ability and heterosis contribute to limited selection of the best genotypes for specific traits in pigeonpea. The objective of this study was to determine combining abilityof yield and FWD resistance in East African and Indian pigeonpea populations.

5.2 MATERIALS AND METHODS

5.2.1 Development of F_1 population

During the 2010 dry season, the seeds of six parents KAT 60/8, ICP 7035, ICEAP 00557, ICPL 96061, ICPL 20108 and ICEAP 00540 were planted in 5 m rows plot of 100cm × 50cm spacing. During the crossing process diallel mating design was done using Griffins mating design I, model I (Griffins, 1956) to generate F_1 populations (Table 5.1 and Plate. 5.1). At the flowering stage the tightly closed buds of the female parents were emasculated by removing anthers from the staminal column with fine forceps one day before they were due to open. About 2 – 10 buds were emasculated per branch and all smaller buds removed to prevent competition within the inflorescence. Pollination was done one day after emasculation using unopened buds of the maleparent for which the anthers would dehisce on the same

day. Both emasculation and pollination were done in the morning before 10.00 a.m to avoid heat, which would otherwise rapture the stigma of the emasculated flower. At maturity, the pods were harvested and F_1 seeds divided into three lots. The first lot was planted and allowed to self into F_2. The second lot was planted and backcrossed to both the resistant and susceptible parents (BC_1F_1). The remaining seeds were kept in store for an emergency.

Table 5.1: Mating scheme of the six pigeonpea (*Cajanus cajan*) genotypes used to develop F_1 populations for genetic analysis

	KAT 60/8	ICP 7035	ICEAP 00557	ICPL96061	ICPL20108	ICEAP00540
KAT 60/8		×	×	×	×	×
ICP 7035	×		×	×	×	×
ICEAP 00557	×	×		×	×	×
ICPL 96061	×	×	×		×	×
ICPL 20108	×	×	×	×		×
ICEAP00540	×	×	×	×	×	

KAT 60/8 = Katumani 60/8, ICEAP= ICRISAT East African Pigeonpea

Plate 5.1: ICEAP 00557 used in this study as resistant genotype at flowering stage

5.2.2 Combining ability, fusarium wilt and genetic variances

The seeds of six parents (Table 5.2) and their F_1 were planted in the field at Sokoine University of Agriculture for evaluation of combining ability, genetic variances and phenotypic correlation. The experiment was laid down in randomized complete block design (RCBD) in three replications. Ten randomly selected competitive plants from each genotype were used in recording observation on the characters such as days to 50% flowering, days to 85% maturity, plant height, number of branches per plant, seeds per pod, 100 seed weight and yield per ha in kg.

Table 5.2: Names and pedigree of the parental genotypes of the pigeonpea

Code No.	Name	Pedigree
P1	KAT 60/8	TAz 9-KIbB01-KibB10-KibB6
P2	ICP 7035	DISR 55
P3	ICEAP 00057	NA-7-KibB21-KibB8
P4	ICPL 96061	ICPL 87051 X ICPL83057
P5	ICPL 20108	IPH 487 inbred line (mb 3783 X ICRISAT ICPL 87119
P6	ICEAP 00540	LINDI-4-KIbB-KibB

5.2.3 Fusarium wilt disease screening

The same pigeonpea genotypes and their F_1 were planted in polythene bags containing sterilized sand, after seven days of germination the seedlings were removed from the sand by shaking and washed in sterile distilled water. The plants were immersed in sterile distilled water to remove sand then using sterile scissors, cm of the distal end of the roots system were cut. The cut plants were then dipeding the inoculum at a concentration of 1.0×10^6 conidial ml^{-1} for 10 minutes to allow the conidia to enter the wounds created in the root systems of Resistant and susceptible genotypes were used as control to evaluate the severity of the disease. The plants were then transplanted in plastic pots with the diameter of 15.24 cm filled with a mixture of sterile soil and sand (3:1 v/v) with extra handling. The pots

were arranged in a Complete Randomized Design (CRD) with three replications. Immediately after transplanting, watering was done at an interval of two days. After 2 months the re-isolation of the *Fusarium udum* pathogen from diseased plants which developed typical wilting was carried out to assure the existence of the pathogen (Plate 5.2). The reaction of these genotypes with wilt disease was evaluated using the scale indicated in (Table 5.3).

Table 5.3: The scale of evaluation used through the study

Percent infection	Reaction
0- 30 mortality	Highly resistant
31-40 mortality	moderately resistant
41-60 mortality	moderately susceptible
61- 100 mortality	highly susceptible

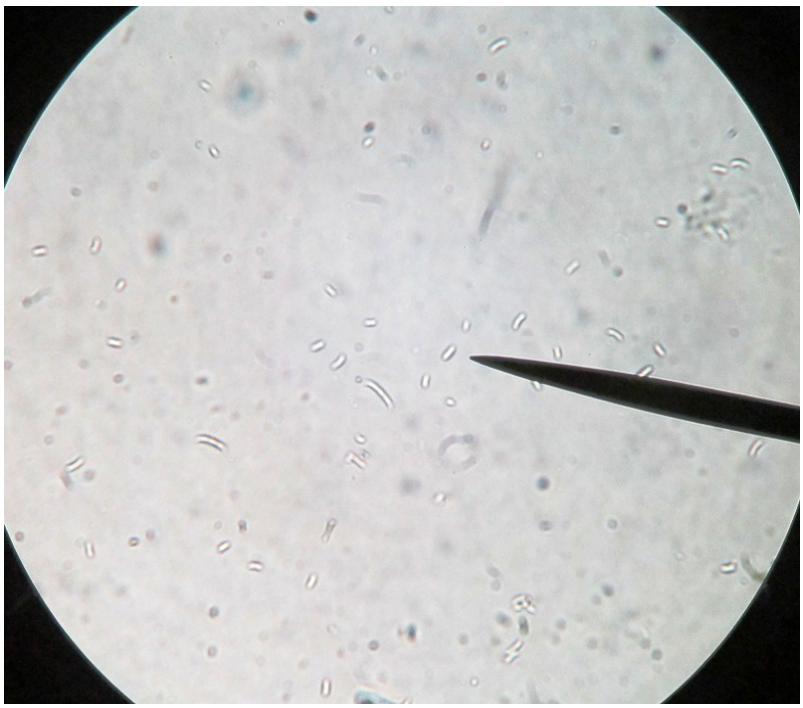

Plate 5.2: Conidial spores of *Fusarium udum* re-isolated from F_1 population derived from cross between ICP 7035x ICEAP00540 at 40X.

5.2.4 Data collection and analysis

Data collected were days to 50% flowering, days to 85% maturity, number of branches per plant, number of pods per plant, seeds per pod, 100 seed weight yield and percentage plant death.The data collected were statistically analysed using Genstat. The data were further subjected to the diallel analysis outlined by Griffing (1956) to determine the combinining ability and Hayman (1954) to separate out the components of genetic variance and their ratio.

5.3 RESULTS

5.3.1 Analysis of variance for parents

Yield and yield components including FWD incidence in the six parents were significanly different (p>0.001) (Table 5.4).

Table 5.4: Analysis **of variance for six parents**

		Mean Squares							
Source of variation	DF	Number of days to 50% flowering	Number of days to 85% maturity	Plant height (cm)	Number ofPods per plant	Number ofSeeds per pod	100 Seed weight	Yield kg/ha	FWD Incidence (%)
Genotype	5	489.56***	544.32***	3979.30***	1197.39***	0.72***	4.00***	15732.35***	2479.60***
Rep	2	10.16	201.72*	43.16	27.72	1.39***	0.22	40.22	21.71
Error	10	8.63	48.38	41.36	17.52	0.05	0.35	17.02	22.59
Total	17								

Significant (p>0.001)

Days to 50% Flowering

Days to 50% flowering followed the order P1, P4, P6, P2, P5 and P3, with 75, 97, 101, 102, 107 and 112 days respectively. P1 was observed to be the earliest in flowering while P3 was the latest variety (Table 5.5).

Table 5.5: Means of parents for the different variables

arent	Number of days to 50% flowering		Number of days to 85% maturity		Plant height (cm)		Number of Pods per plant		Number of Seeds per pod		Yield kg/ha		FWD Incidence (%)	
P1	75	c	109	d	175.33	c	74	d	4	ab	596.21	d	92.33	a
P2	102	b	139	b	257.04	ab	76	d	4	a	768.11	a	87.5	b
P3	112	a	144	a	205.33	abc	120	a	4	ab	599.11	d	32.22	c
P4	97	b	137	bc	180.89	bc	113	b	3	b	658.07	b	33.33	c
P5	107.	a	144	a	271	a	108	b	4	a	609.64	cd	24.33	d
P6	101	b	134	c	246.78	abc	97	c	3	ab	626.99	c	18.75	e
Ẋ	99		134		222.73		98		3.89		643.02		48.08	
SE±	1.39		1.12		23.27		1.82		0.322		5.48		0.8908	
C.V (%)	1.71		1.02		12.8		2.27		10.14		1.04		2.27	

Means within columns followed by common letters are not significantly different ($p \leq 0.05$), according to Tukey test. Values are means of 3 replications.

The Wr/Vr graphical analysis showed that the regression line cuts the Wr axis below the origin. Position of arrays indicates that P3, P4 and P6 were close to the origin while P1 was far from the origin. In relation to regression coefficient the b revealed a positive value and not significantly deviates from the unit slope (Fig. 5.1).

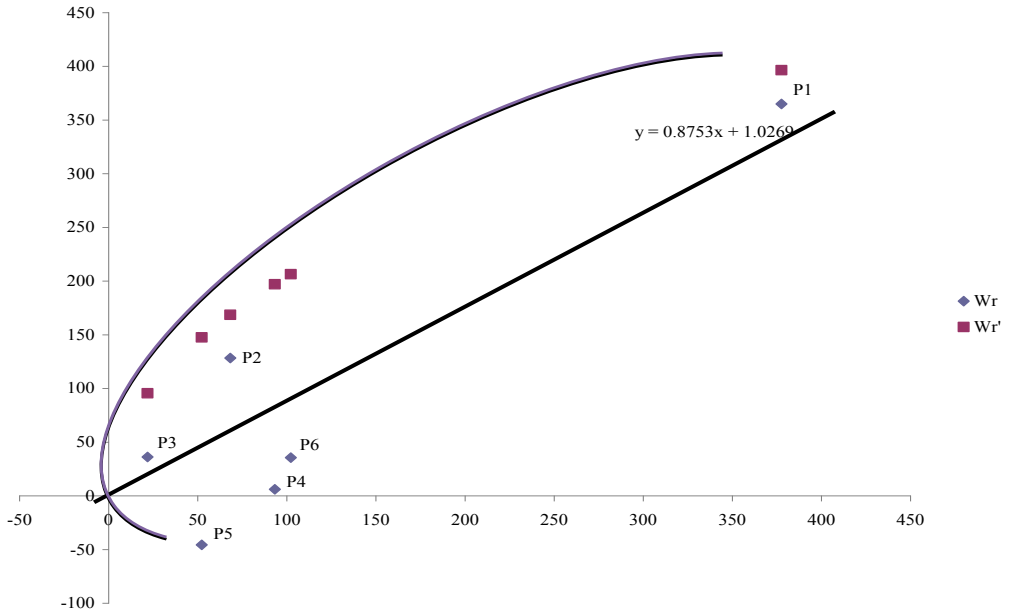

Figure 5.1: Covariance/variance graph for days to 50% flowering

On days to 85% maturity P1 showed the lowest number of days to maturity 109 days followed by P2 while P5 was the one with the highest number of days to maturity (144) days (Table 5.6). The Wr/Vr graph for days to 85% maturity showed that the regression line cuts the Wr axis below the origin. The position of arrays allocated P3 and P4close to the origin while P1 was far from the origin and the regression coefficient value was positive (Fig. 5.2).

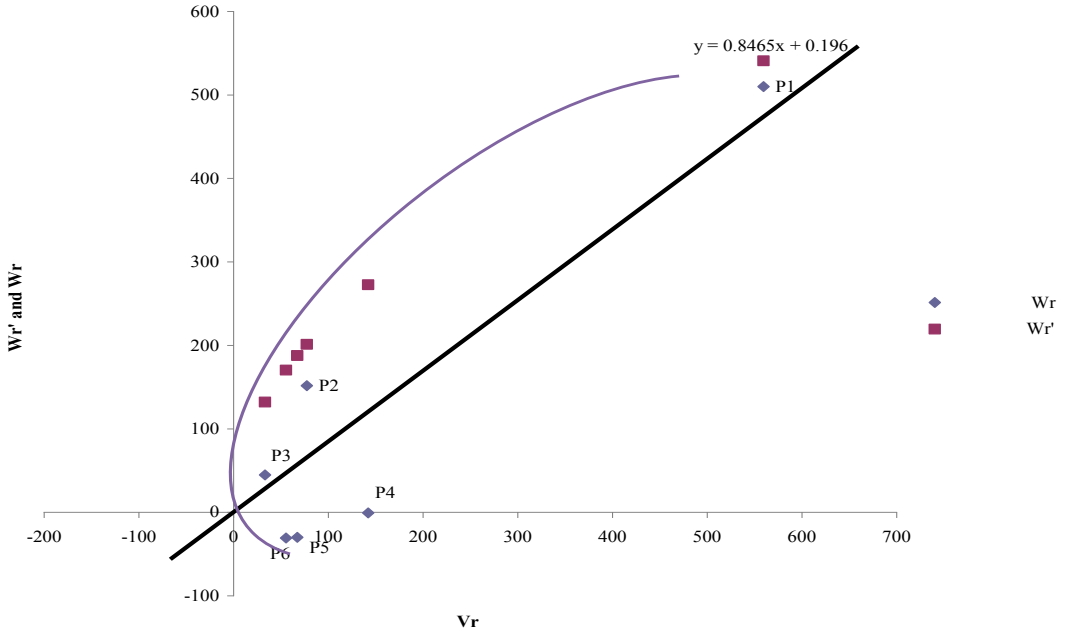

Figure 5.2: Covariance/variance graph for days to 85% maturity

Plant height showed the lowest height for P1 (175.30 cm) while P5 scored the highest height (271 cm) (Table 5.6).

Number of pods per plant: Fewest numbers of pods per plant was revealedby P1 and most pods were observed in P3. Analysis of pods per plant using graph showed that regression line cuts the wr axis through the origin. The position of arrays shows that P2 and P4 were more close to the origin while P3 is far from the origin and regression coefficient was positive (Fig. 5.3).

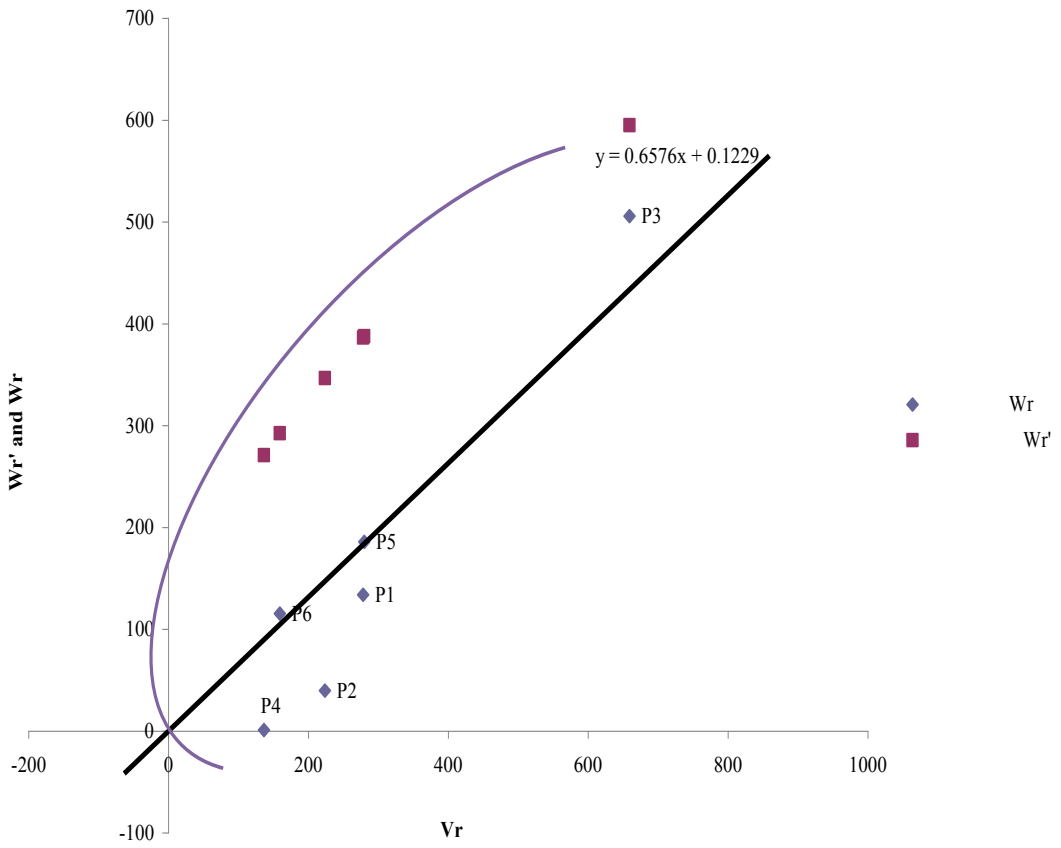

Figure 5.3: Covariance/variance graph for number of pods per plant

On number of seeds per pod P4 and P6 showed the lowest seeds per pod (3) while the rest genotypes had the highest number of seeds per pod (4) (Table 5.6). The graphical analysis of number of seeds per pod showed that the regression line cuts the Wr axis above the origin. The position of arrays shows that P1 and P4 were slightly close to origin while P6 was far from the origin and the regression coefficient is negative (Fig. 5.4).

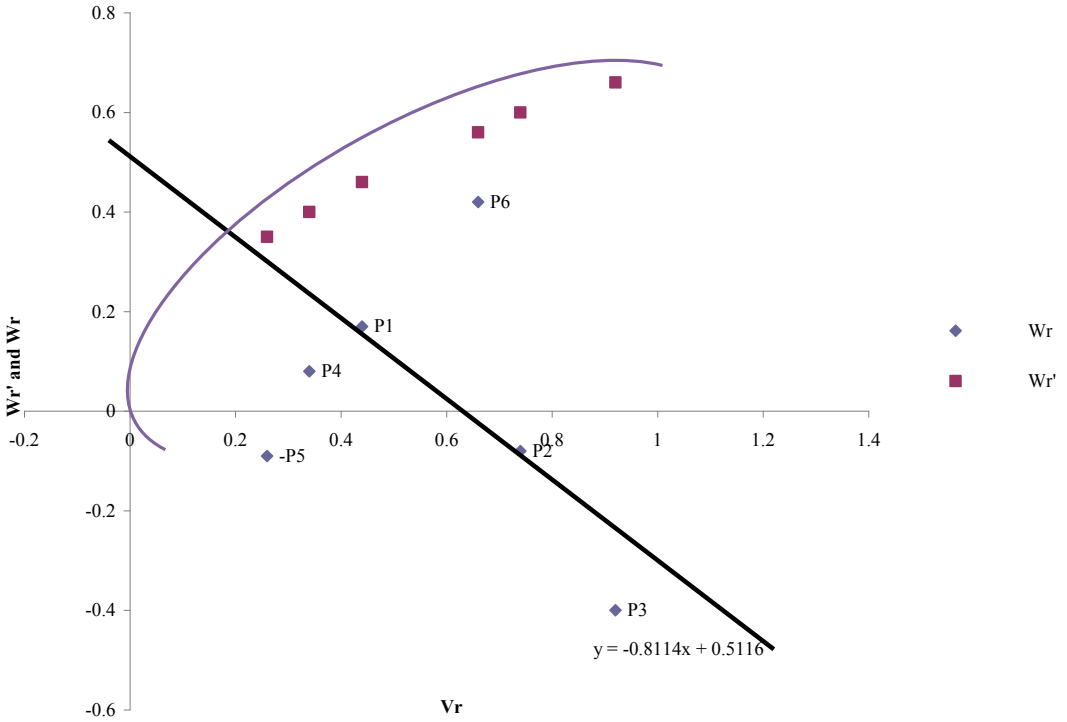

Figure 5.4: Covariance/variance graph for number of seeds per pod

In pigeonpea yield evaluation P1 was the least 596.21kg/ha while P2 was the most 768.11.kg/ha (Table 5.6).

Fusarium wilt disease showed more reaction to P1 and P2 while P5 and P6 were highly resistant to Fusarium wilt. The graphical analysis of fusarium wilt resistance showed that the regression line cuts the Wr axis below the origin. The position of arrays shows that P2, P3 and P6 were close to the origin while P1 was far from the origin (Fig. 5.6).

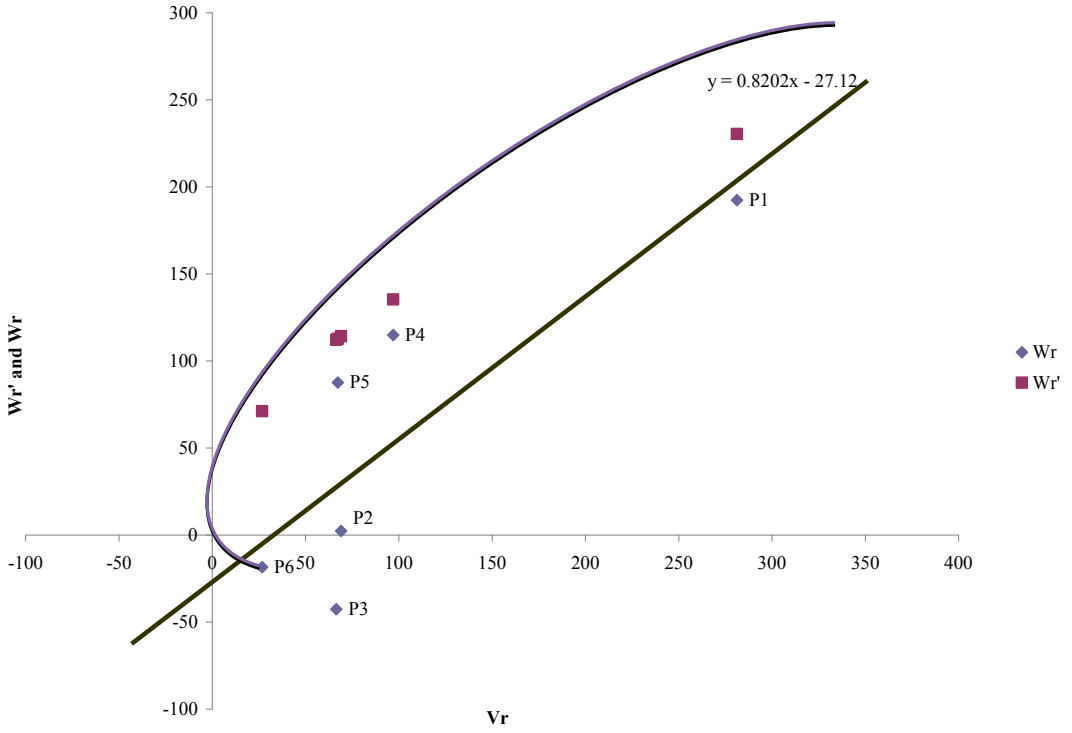

Figure 5.5: Covariance/variance graph for Fusarium wilt disease

Fifteen crosses showed significant differences on days to 50% flowering and days to 85% maturity ($p>0.05$), plant height and 100 seed weight were significant ($p>0.01$) while pods per plant and FWD showed significant difference ($p>0.001$) (Table 5.6).

Table 5.6: Analysis **of variances for crosses**

		Mean Squares								
Source of variation	DF	Number of days to 50% flowering	Number of days to 85% maturity	Plant height (cm)	Number of pods per plant	Number of seeds per pod	100 Seed weight	Yield kg/ha	FWD Incidence (%)	Number of branches/Plant
Crosses	14	114.93*	137.63*	2202.90**	1234.95***	1.55	3.57**	21874.59	430.60***	1.92
Rep	2	52.42	46.06	226.06	32.08	0.42	4.06*	3391.58	14.60	0.68
Error	28	43.96	54.85	623.11	100.25	0.75	0.93	10761.78	19.12	0.97
Total	44									

Significant :(p>0.05), (p>0.001) and (p>0.01),

The P_2 ×P_5 followed by P_1×P_4 flowered earliest; they took 95, and 97 days respectively (Table 5.7). The P_2×P_3 followed by P1×P3, and P4×P5 flowered latest. The rest of the genotypes were intermediate inflowering. Days to 85% maturity showed that P2×P_5, P_2×P_4 and P_4×P_6 were earliest to maturity on other hand P_2×P_3, P_1×P_3 and P_4×P_5 scored the highest number of days to maturity The cross P_5×P_6 followed by P3×P4 revealed minimum plant height while P2×P4, P1×P5 and P2×P6 showed maximum plant height. On number of pods per plant crosses P_1×P_2 and P5×P6 scored fewest numbers of pods, with P_3×P_4 and P_3×P_5 scored many pods. The crosses P_3×P_5 and P_1×P_3 had the lowest seed weight and the highest seeds weight was revealed by P_1×P_2. The lowest Fusarium wilt reaction (resistance) was observed on the P_1×P_2, P_4×P_6 and P_4×P_6 while the cross P_3×P_6 showed highest disease reaction.

Table 5.7: Means of F_1 crosses for the various variables

Cross	FW Incidence (%)		Number of days to 50% flowering		Number of days to 85% maturity		Plant height (cm)		Number of Pods per plant		100 Seeds weight		Yield kg/ha	
P1×s3														
P2	20.67	g	109	ab	138	ab	255	abc	74	g	16.91	a	753.58	a
P1×P3	41.67	cdef	114	ab	145	ab	245	abc	121	abcde	12.84	b	584.84	j
P1×P4	41.67	cdef	99	b	133	ab	261	ab	111	bcdef	14.73	ab	608	ghij
P1×P5	44.33	cde	107	ab	142	ab	271	ab	105	cdef	13.66	b	593.99	ij
P1×P6	53.33	bc	109	ab	133	ab	247	abc	95	defg	13.74	b	581.88	j
P2×P3	49	bcd	118	a	153	a	241	abc	131	abc	15.11	ab	753.1	a
P2×P4	49.33	bcd	99	ab	130	b	308	a	99	defg	14.95	ab	658.5	cd
P2×P5	48.33	bcd	95	b	129	b	254	abc	94	defg	14.49	ab	638.4	def
P2×5r														
P6	54.67	bc	101	ab	132	ab	263	ab	87	fg	14.85	ab	666.53	c
P3×P4	37.33	def	99	ab	138	ab	221	bc	144	a	13.99	b	629.9	efgh
P3×P5	58.33	ab	105	ab	140	ab	245	abc	140	ab	12.83	b	619.16	fghi
P3×P6	68.67	a	106	ab	141	ab	229	bc	124	abcd	13.6	b	709.12	b
P4×P5	31.33	efg	111	ab	144	ab	238	abc	120	abcde	13.21	b	582.46	j
P4×P6	31	fg	98	ab	130	b	249	abc	106	cdef	14.24	ab	605.96	hij
P5×P6	42.33	cdef	105	ab	138	ab	182	c	91	efg	12.93	b	766.97	a
Mean	44.8		104		137		247.33		109		14.14		647.98	
SE±	3.57		5.41		6.05		20.38		8.18		0.77			
CV (%)	9.76		6.37		5.37		10.09		9.13		6.81		1.3	

Means within the same columns followed by common letters are not significantly different ($p \leq 0.05$), according to Tukey test. Values are means of 3 replications.

5.3.2 Analysis of variance for combining ability

Mean squares due to general combining ability (GCA) and specific combining ability (SCA) were highly significant ($p \leq 0.001$) in all yield components and Fusarium wilt resistance except seeds per pod (Table5.8). The analysis of variance indicated that GCA variances were higher than their corresponding SCA for days to 50% flowering, days to 85% maturity, number of pods per plant, 100 seeds weight, yield and Fusarium wilt. General combining ability was greater than SCA hence the ratio of GCA mean square to SCA mean square was more than unit value for all variables except plant height and seeds per pod.

Table 5.8: Analysis of variance for combining ability for 6 x 6 F_1 diallel in pigeonpeas populations

		Mean Squares							
Source of variation	df	Number of days to 50% flowering	Number of days to 85% maturity	Plant height (cm)	Number ofPods per plant	Number ofSeeds per pod	100 Seed weight (g)	Yield kg/ha	FWD Incidence (%)
GCA	5	586.17***	1,017.19***	8,024.27***	5,778.60***	2.68	11.96***	15.21***	332.61***
SCA	**15**	**584.29***	**520.79***	**13,944.93***	**761.09***	**3.72***	**8.13***	**6.18***	**147.22***
error	20	45.57		524.84	92.87***		0.75		4.95
GCA: SCA		1	1.95	0.57	7.59	0.72	1.47	2.46	2.25

Significant ($p \leq 0.001$)

5.3.3 Estimates of general combining ability effects for fusarium wilt disease

The GCA effect of six parents studied for days to 50% flowering showed negative GCA and significantly different ($p \leq 0.001$) for P1, P2, P5 and P6 (Table 5.19a). Among these parents, P5 showed the highest negative value (-27.6339) (Fig. 5.6).

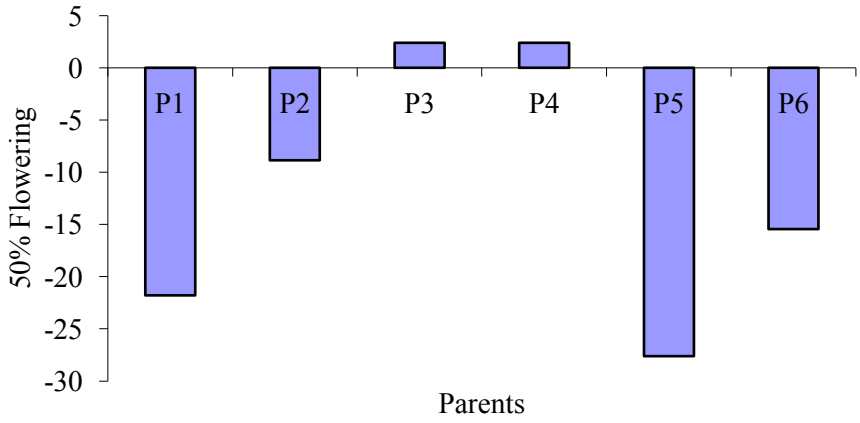

Figure 5.6: Relative GCA effects for days to 50% flowering

All parents used in this study showed negative and significant effects ($p \leq 0.001$) on days to 85% flowering (Table 5.9a). Among all parents used P4 (-21.9431) showed the highest negative value followed by P1 (-18.6887) and P5 (-16.3649) (Fig. 5.7)

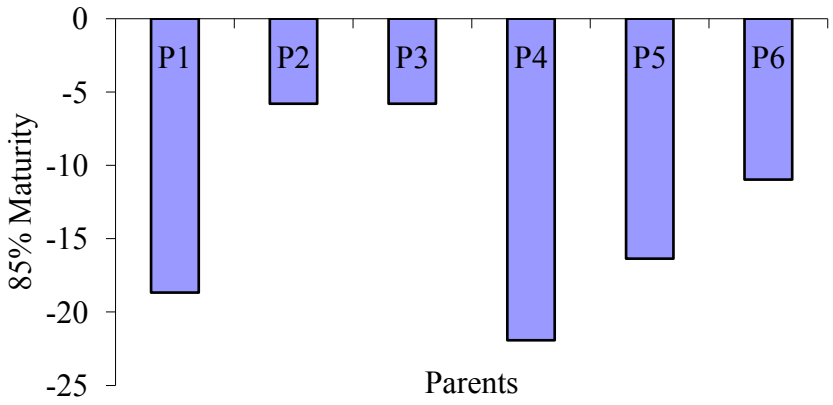

Figure 5.7 Relative GCA effects for days to 85% maturity

General combining ability showed significant difference (p≤ 0.001) on plant height for all six parents used in this study (Table 5.9a). Out of these six parents P2 showed highest and significant plant height compared with other parents (Fig. 5.8**)**

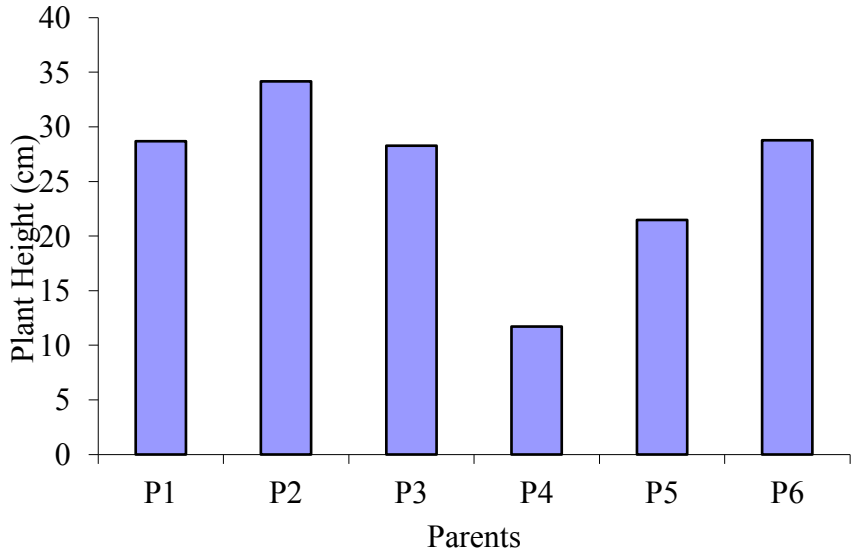

Figure 5.8: Relative GCA effects for plant height (cm)

Number of branches per plant was significant in GCA for all parents (p≤ 0.05), (p≤ 0.01), (p≤ 0.001) except for P4 while among these six parents P6 showed many number of branches per plant , positive and significant (Table 5.9a and Fig.5.9).

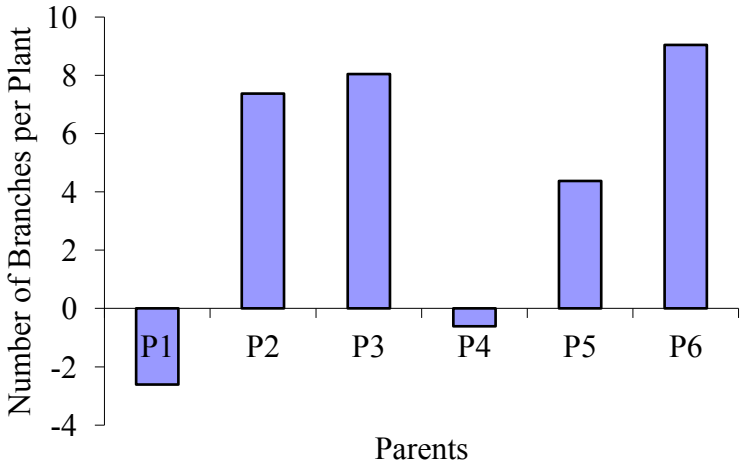

Figure 5.9: Relative GCA effects for number of branches per plant

All parents except P5 showed significant effects on number of on number of pods per plant (Table 5.9b). Among all six parents assessed in this study P6 scored the highest, positive and significant number of pods per plant followed by P4 while P3, P2 and P1 revealed negative GCA (Fig.5.10).

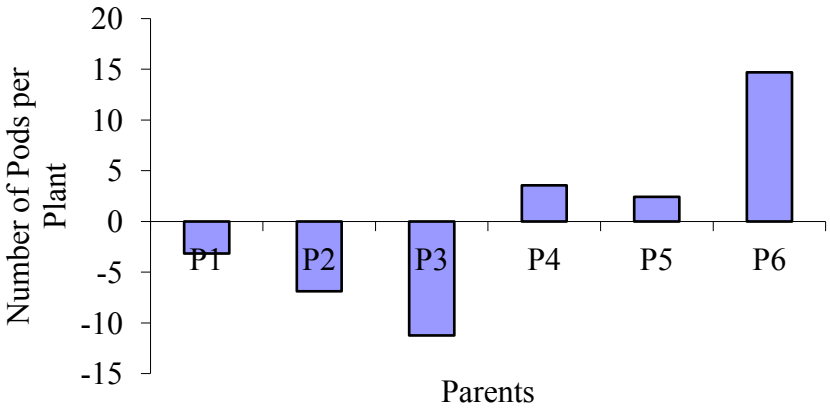

Figure 5.10: Relative GCA effects for number of pods per plant

Significant effect was observed for all parents except P5 in number of seeds per pod. P2 scored many and significant number of seeds per pod (Fig. 5.11).

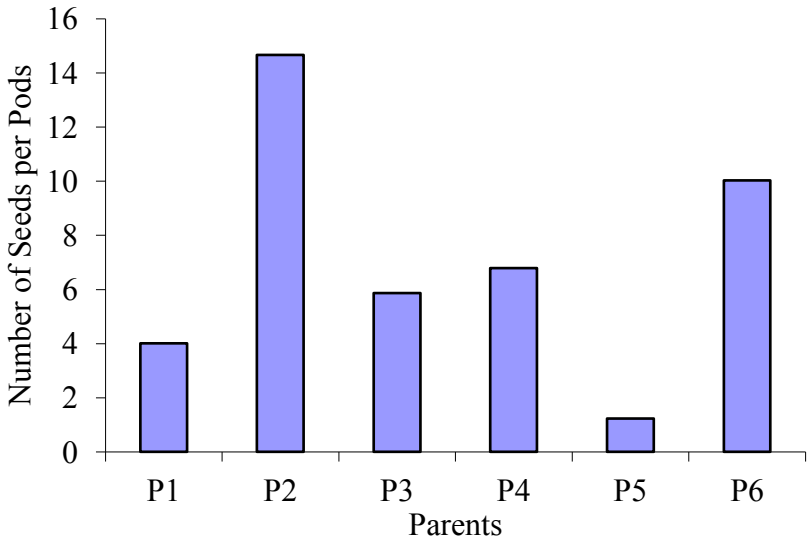

Figure 5.11: Relative GCA effects for number seeds per pods

All parents used in this study showed significant effect ($p \leq 0.01$) on 100 seeds weigt (Table 5.9b). Among these parents P6 revealed positive and highest seeds weight while P4 and P1 scored negative GCA (Fig. 5.12).

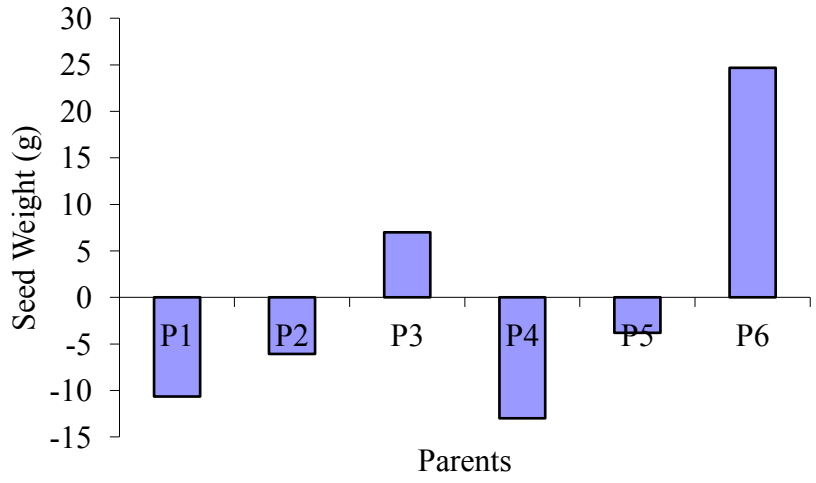

Figure 5.12: Relative GCA effects for 100 seeds weight (Kg)

Significant effects on yield for GCA were observed in pigeonpea yield for all parents ($p \leq 0.001$) except P1 and P5 (Table 5.9b and Fig. 5.13).

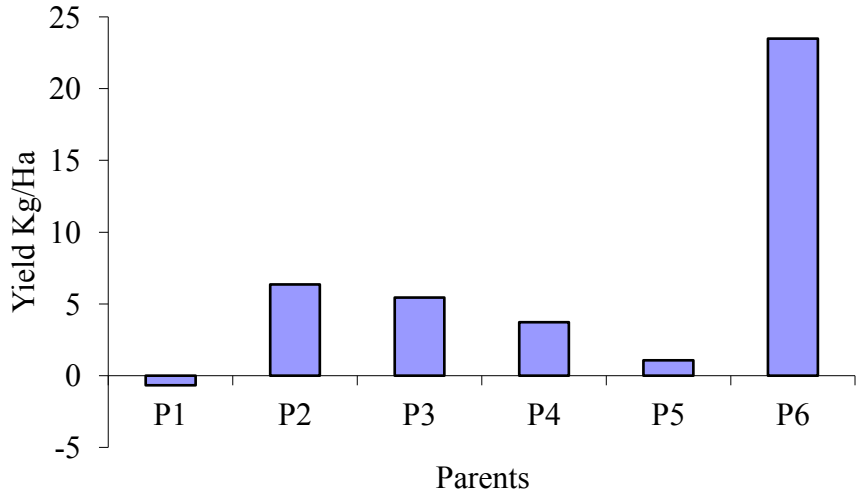

Figure 5.13: Relative GCA effects for yield (kg)

P3, P4, and P6 revealed significant effects in GCA in Fusarium wilt reaction, among these P6 showed the lowest disease reaction and significant (Table 5.9b and Fig. 5.14).

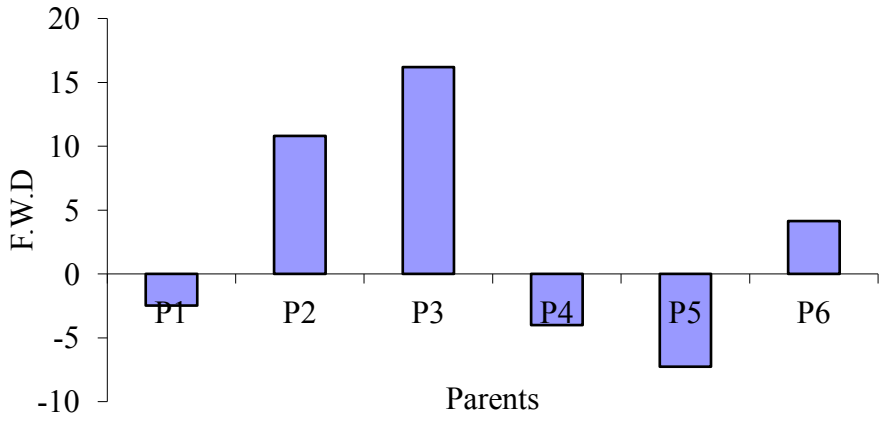

Figure 5.14: Relative GCA effects for FWD resistance

Table 5.9a: General combining ability effects in pigeonpeas yield

Parents	Number of days to **50 % Flowering**	Number of days to 85% maturity	Plant height (cm)	Number of branches/plant
P1	-21.7916***	-18.6887***	28.67814***	-2.60479*
P2	-8.86271***	-5.81088***	34.14458***	7.371257***
P3	2.389484	-5.81088***	28.26391***	8.041916***
P4	2.389484	-21.9431***	11.72146***	-0.61078
P5	-27.6339***	-16.3649***	21.45898***	4.377246**
P6	-15.4625***	-10.9715***	28.77395***	9.035928****

Table 5.9b: General combining ability effects in pigeonpeas yield

Parents	Number of pods per plant	Number of seeds per pod	Seed weight (g)	Yield (kg/ha)	FWD incidence (%)
P1	-3.15825*	4.008333**	-10.6364***	-0.6643	-2.47222
P2	-6.87176****	14.65833***	-6.09091***	6.357788***	10.80556***
P3	-11.2299***	5.866667***	7***	5.44063***	16.19444***
P4	3.549795**	6.791667***	-12.9924***	3.722815**	-4.00000
P5	2.412688	1.233333	-3.81818**	1.064706	-7.25000
P6	14.691***	10.03333***	24.67424***	23.49466***	4.13889**

** Significant ($p \leq 0.01$), *Significant ($p \leq 0.05$), *** Significant ($p \leq 0.001$)
Where by P1= KAT60/8, P2= ICP7035, P3= ICEAP00557, P4 = ICPL96061, P5 ICPL20108 = and P6= ICEAP00540.

5.3.4 Estimates of specific combining ability effects

The specific combining ability (SCA) effect on days to 50% flowering showed significant effects ($p \leq 0.01$) in P1×P4, P1×P5, P4 × P5 and P5 × P6 crosses where by P5 ×P6 showed highest positive effects (10.037) while P3 ×P6 and P3 × P4 showed negative score on days to 50% flowering -2.546 and -1.991 (Table 5.10 and Fig.5.15).

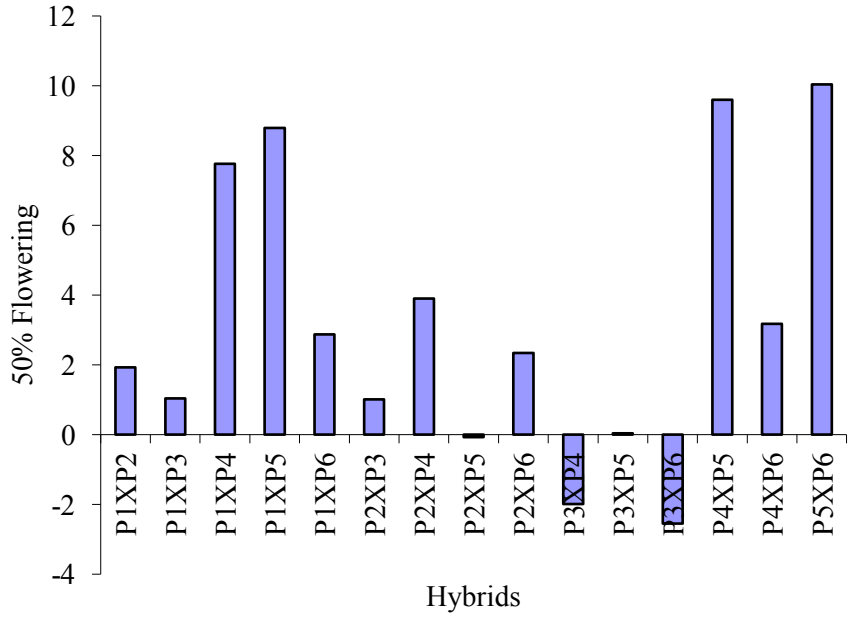

Figure 5.15: Relative SCA effects for days to 50% flowering

The SCA effects revealed significant effects on days to 85% maturity for the crosses P1× P4, P4 × P5 ($p \leq 0.01$), P1 × P5 ($p \leq 0.05$). Out of these P1 × P5 showed little number of days to 85% maturity (Table 5.10 and Fig. 5.16).

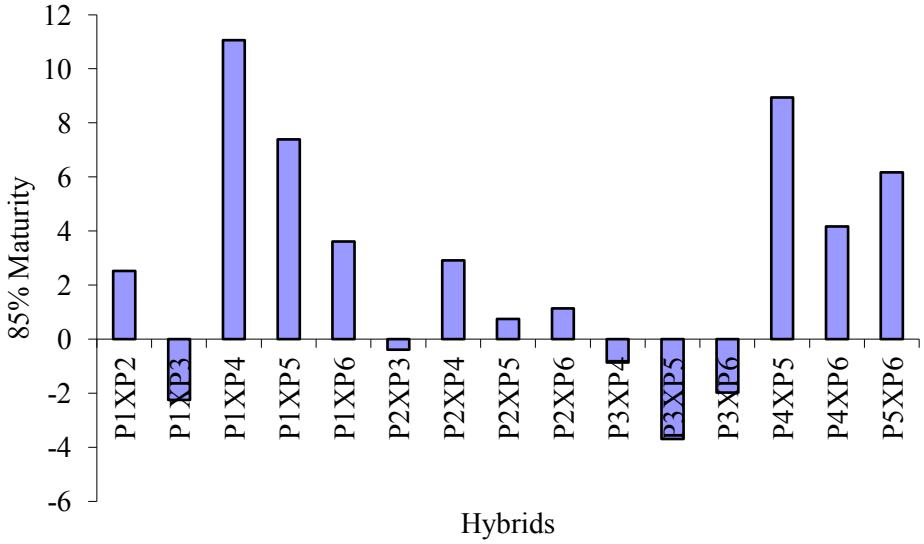

Figure 5.16: Relative SCA effects for days to 85% maturity.

The hybrids P1× P2, P1× P4, P1×P6 revealed significant effects ($p \leq 0.01$), P2 × P3, P2× P6 ($p \leq 0.001$) while crosses P2 × P5, P3× P5 and P5× P6 showed significant effects ($p \leq 0.05$) on plant height. On the other hand highest negative significant SCA effects were observed in P3×P5 (-3.694). The significant and lowest negative was revealed by P5× P6 (Table 5.10 and Fig 5.17).

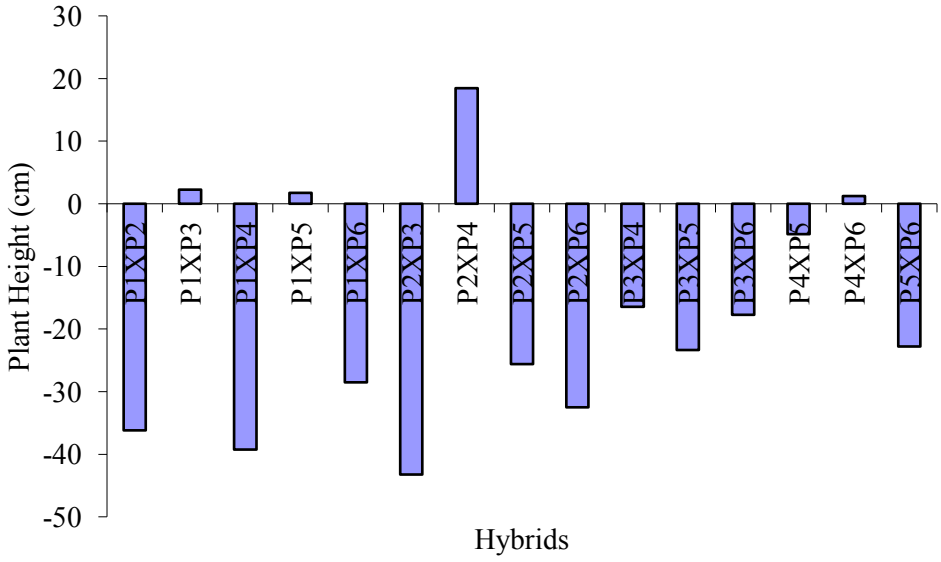

Figure 5.17 Relative SCA effects for plant height

Statistically the following crosses revealed significant effects P1× P4, P3 × P5 and P4 ×P5 ($p \leq 0.05$), P2× P3 ($p \leq 0.01$) on their number of branches. The hybrids P4×P5 showed the lowest, negative and significant on their number of branches (Table 5.10 and Fig. 5.18).

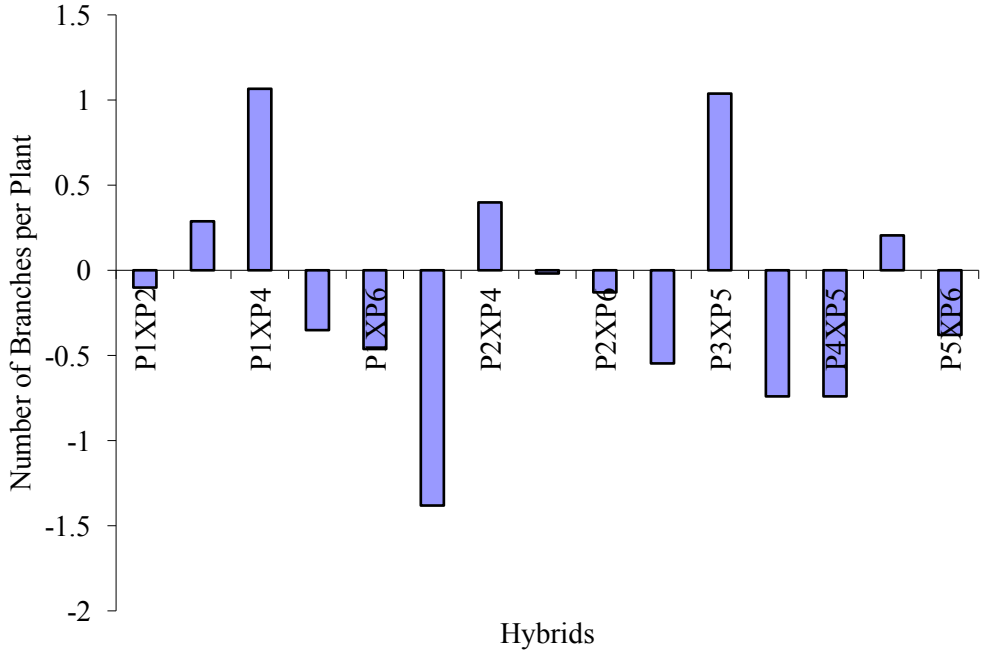

Figure 5.18: Relative SCA effects for number of branches per plant

Cross P1 × P2 showed significant ($p \leq 0.01$), P2 × P5 ($p \leq 0.001$), P2 × P3, P2 × P4 and P2×P6, revealed significant effect ($p \leq 0.01$) on number of pods per plant while cross between P2×P3 showed the highest positive SCA effect (25.231) (Table 5.10 and Fig. 5.19).

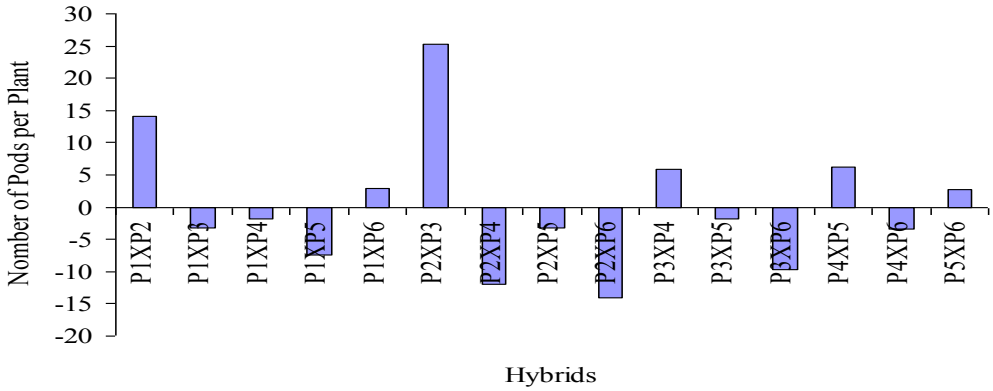

Figure 5.19: Relative SCA effects for number of pods per plant

The SCA effects showed significant effects at different levels P1×P6 and P2× P4, revealed significant effect ($p \leq 0.05$), where by P2 × P3 and P2 × P6 ($p \leq 0.01$). Out of all crosses hybrids P2×P4 showed positive and highest number of seeds per pod the rest showed positive but low while most of the crosses were responded negatively (Table 5.10 and Fig. 5.20).

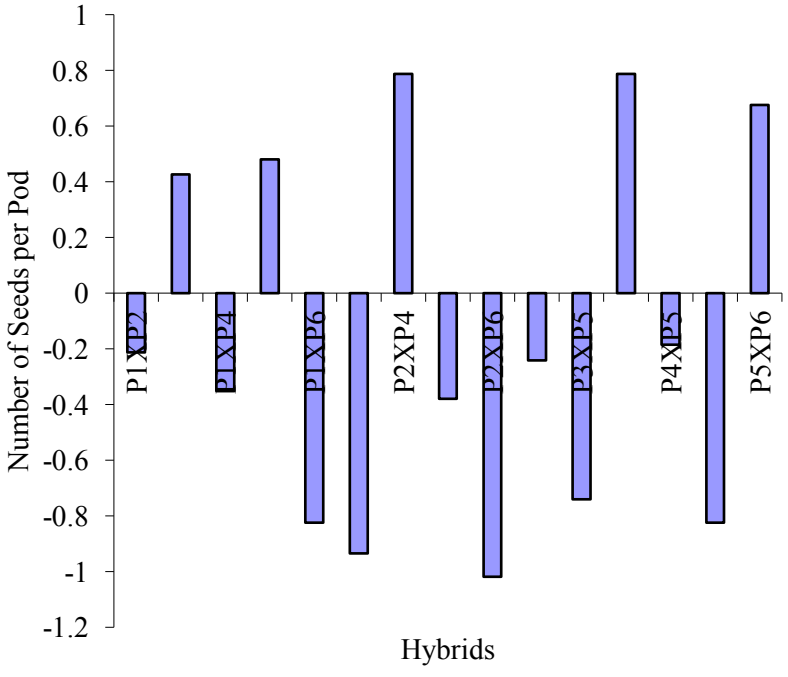

Figure 5.20: Relative SCA effects for seeds per pod

The SCA effects were statistically significant for 100 seed weight for the hybrid lines developed from the crosses of P1× P2, P1×P6, P2×P6 ($p \leq 0.01$), P4 × P6 ($p \leq 0.05$) . The hybrid P1× P2 was the one indicated the highest, positive and significant 100 seed weight (Table 5. 10 and Fig. 21)

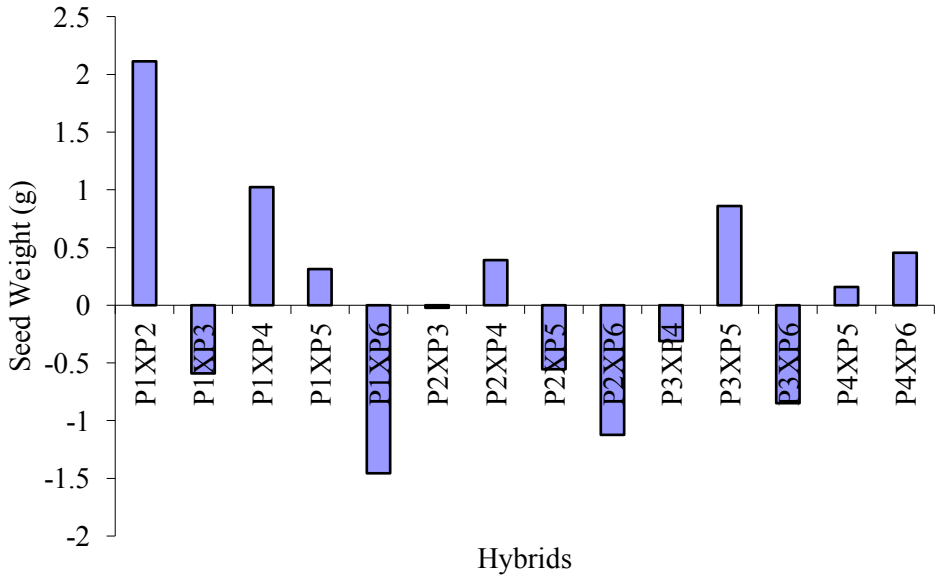

Figure 5.21: Relative SCA effects for seeed weight (g)

The SCA effects of these crosses P1 × P2, P1 × P3, P1× P6, P2 ×P4,P2×P5 ($p \leq 0.001$) P3×P4 ($p \leq 0.05$) and the cross P3 × P5, showed significant effect ($p \leq 0.01$) on pigeonpea yield per ha (Table 5.10 and Fig . 5.22).

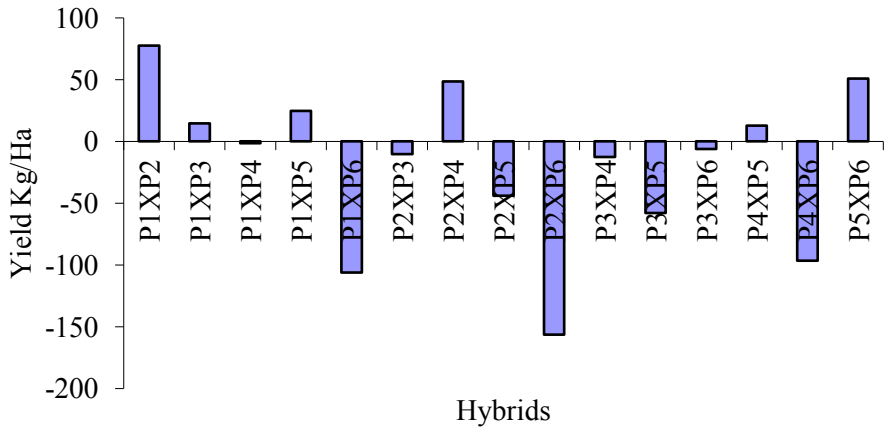

Figure 5.22: Relative SCA effects for yield in kg /ha

The estimate of SCA effects for Fusarium wilts disease resistance showed significant difference ($p \leq 0.001$) for crosses P1 × P2, P1 × P4, P1×P5, P1 × P6, P2 × P4, P2 × P5 while cross P3 ×P4 revealed the lowest and significant value for fusarium wilt ($p \leq 0.05$) (Table 5.10 and Fig. 5.23).

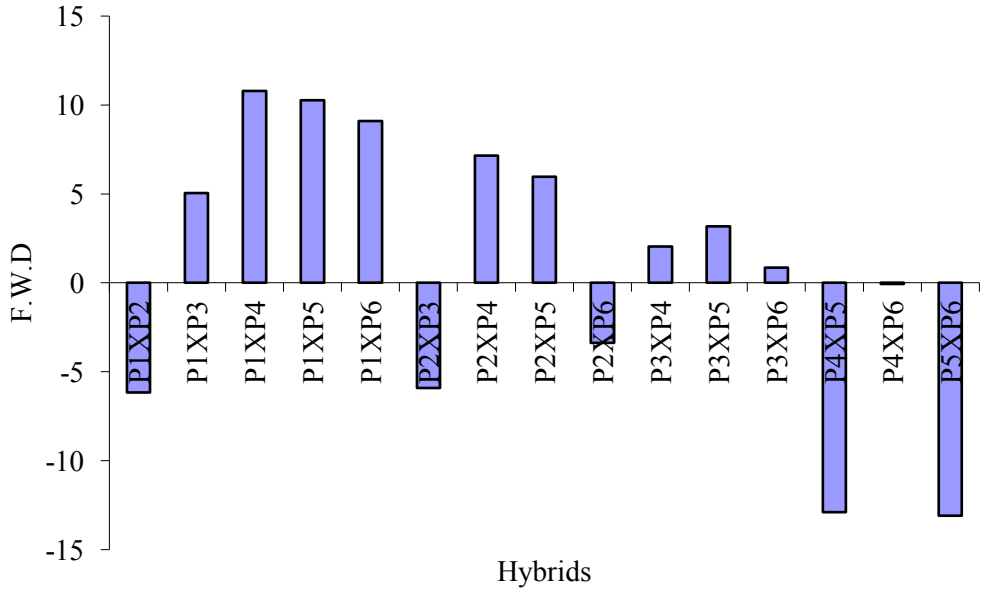

Figure 5.23: Relative SCA effects for FWD resistance

Table 5.10: Estimate of specific combining ability effects in eight characters and FWD in 6x6 F_1 diallel crosses

Hybrids	Number of days to 50% flowering	Number of days to 85% maturity	Plant height	Number of branches	Number of pods per plant	Number of seeds per pod	Seed weight (g)	Yield (kg/ha)	FWD Incidence (%)
P1×P2	1.926	2.528	-36.167**	-0.102	14.148**	-0.213	2.113***	77.477**	-6.17
P1×P3	1.037	-2.25	2.25	0.287	-3.213	0.426	-0.59	14.582	5.04***
P1×P4	7.759**	11.056**	-39.25**	1.065*	-1.88	-0.352	1.024**	-1.493	10.79***
P1×P5	8.787**	7.389*	1.722	-0.352	-7.38	0.481	0.313	24.566	10.26***
P1×P6	2.87	3.611	-28.528**	-0.463	2.954	-0.824*	-1.456**	-106.232**	9.10***
P2×P3	1.009	-0.389	-43.222***	-1.38**	25.231***	-0.935**	-0.023	-10.41	-5.92
P2×P4	3.898	2.917	18.444	0.398	-11.935**	0.787*	0.391	48.402	7.15***
P2×P5	-0.074	0.75	-25.583*	-0.019	-3.269	-0.38	-0.554	-44.19	5.96***
P2×P6	2.343	1.139	-32.5**	-0.13	-14.102**	-1.019**	-1.123**	-156.47**	-3.37
P3×P4	-1.991	-0.861	-16.472	-0.546	5.87	-0.241	-0.312	-12.81	2.04*
P3×P5	0.037	-3.694	-23.333*	1.037*	-1.796	-0.741*	0.86*	-58.08	3.18**
P3×P6	-2.546	-1.972	-17.75	-0.741	-9.63	0.787*	-0.859*	-6.182	0.85
P4×P5	9.593**	8.944**	-4.833	-1.019*	6.204	-0.185	0.157	12.691	-12.89
P4×P6	3.176	4.167	1.25	0.204	-3.463	-0.824*	0.455	-96.673*	-0.06
P5×P6	10.037**	6.167	-22.778*	-0.38	2.704	0.676*	-0.273	50.752	-13.09

** Significant (p≤0.1), *Significant (p ≤0.5), *** Significant (p≤0.01),PH- plant height, PP- Pods per plant, SW- 100 seed weight, SP- seeds per pod, F- Days to 50% flowering , G- Good trait, P- Poor trait and A- average. Where by P1 = KAT 60/8, P2 = ICP 7035, P3= ICEAP 00057, P4= ICPL 96061, P5= ICPL 20108 and P6= ICEAP00540.

5.3.5 Direct and indirect effects of different variables on pigeonpea yield

5.3.5.1 Yield versus days to 50% flowering

The correlation between yield and days to 50% flowering was positive but weak (r = 0.004). The direct effect of days to 50% flowering was (0.052) and indirect effects via days to 85% maturity, plant height, number of branches, pods per plant, seeds per pod and 100 seeds weight were (-0.03371, -0.00429, -0.00252,-0.00361, -0.04281, and 0.03876) respectively (Table 5.11, 5.18 and 5.19).

5.3.5.2 Yield versus days to 85% maturity

The days to 85% maturity correlated positively but not significant (r = 0.033) while the direct effect was negative and less than residual value (-0.038). The indirect influences of days to 85% maturity via days to 50% flowering, plant height, branches, pods per plant, seeds per pod and 100 seeds weight were (0.046124, -0.0034, -0.00097, 0.025886, -0.02003 0.02346) respectively (Table 5.12, 5.18 and 5.19)

5.3.5.3 Yield versus plant height

The correlation between yield and plant height was significant and positive (r = 0.23*). The direct influence of plant height on yield was positive (0.011) although too low. The indirect effects of plant height on yield via days to 50% flowering, days to 85% maturity, plant height, number of branches per pant, pods per plant and seeds per pod and 100 seed weight were (0.011742,0.003417, -0.02028,-0.03251, 0.216288 and 0.04182) respectively. They were also low with reference to residual value (Table 5.13, and 5.18 and 5.19).

5.3.5.4 Number of branches per plant versus yield

The phenotypic coefficient correlated positively and significantly on pigeonpea yield (r = 0.244**). The direct effect of number of branches was positive but low(0.017) while the indirect effects via days to 50% flowering, days to 85% maturity, plant height, per pant, pods per plant, seeds per pod and 100 seed weight were also low (0.03401, 0.003492, 0.002166, -0.0077, 0.1502 and 0.04522) respectively (Table 5.14, 5.18 and 5.19).

5.3.5.5 Yield versus pods per plant

The correlation between pods per plant and yield was high, positive and significant (r = 0.556***). The direct effect was very high thus significantly contributedto yield (0.602). The indirect effect via days to 50%, days to 85% maturity, plant height, number of branches, plant seeds per pod and 100 seeds weight were as follow (0.000986, -0.02629, -0.00059, -0.00163, 0.00031, and -0.01802) respectively (Table 5.15, 5.18 and 5.19).

5.3.5.6 Yield versus seeds per pod

The correlation between seed per pod and yield was very high, positive and significantly contributed to the yield (r = 0.7245***) the direct effect also was positive and very high (0.712). Its indirect effects via days to 50% flowering, to 85% maturity, plant height and number of branches, pods per plant and 100 seed weight were (-0.02306, -0.02107, -0.00204, 0.0034, 0.003168 and - 0.001026) respectively were not significant reference to the residual value (0.0621) in Fig. 5.24 and Table 5.16, 5.18 and 5.19).

5.3.5.7 Yield versus 100 seeds weight

The association of 100 seed weight and yield was not significant (r = 0.31), the direct effect showed significance (0.34) while its indirect effects via days 50% flowering, days to 85% maturity, plant height, number of branches per plant and pods per plant and seeds per pod were (-0.00451,-0.03191, 0.002261, 0.001353, -0.00262, and -0.02949) respectively (Table 5.17, 5.18 and 5.19).

5.3.5.8 Path analysis model

$r_{18} = P_{18} + r_{12}P_{28} + r_{13}P_{38} + r_{14}P_{48} + r_{15}P_{58} + r_{16}P_{68} + r_{17}P_{78}$

$r_{28} = r_{12}P_{18} + P_{28} + r_{23}P_{38} + r_{24}P_{48} + r_{25}P_{58} + r_{26}P_{68} + r_{27}P_{78}$

$r_{38} = r_{13}P_{18} + r_{23}P_{28} + P_{38} + r_{34}P_{48} + r_{35}P_{58} + r_{36}P_{68} + r_{37}P_{78}$

$r_{48} = r_{14}P_{18} + r_{24}P_{28} + r_{34}P_{38} + P_{48} + r_{45}P_{58} + r_{46}P_{68} + r_{47}P_{78}$

$r_{58} = r_{15}P_{18} + r_{25}P_{28} + r_{35}P_{38} + r_{45}P_{48} + P_{58} + r_{56}P_{68} + r_{57}P_{78}$

$r_{68} = r_{16}P_{18} + r_{26}P_{28} + r_{36}P_{38} + r_{46}P_{48} + r_{56}P_{58} + P_{68} + r_{67}P_{78}$

$r_{78} = r_{17}P_{18} + r_{27}P_{28} + r_{37}P_{38} + r_{47}P_{48} + r_{57}P_{58} + r_{67}P_{68} + P_{78}$

The direct effects appear in the diagonal as P's

Table 5.11: Days to 50% flowering with yield, r18= 0.004

	r	p	rp
Indirect effect via days to 85% maturity, r12p28	0.887	-0.038	-0.03371
Indirect effect via plant height, r13p38	-0.39	0.011	-0.00429
Indirect effect via number of branches, r14p48	-0.148	0.017	-0.00252
Indirect effect via pods per plant, r15p58	-0.006	0.602	-0.00361
Indirect effect via seeds per pod, r16p68	-0.057	0.751	-0.04281
Indirect effect via 100 seeds weight, r17p78	0.114	0.34	0.03876
Direct effect, P18			0.052
Total			0.003829

Table 5.12: Days to 85% maturity with yield, r28= 0.033

	r	p	rp
Iindirect effects via days to 85% maturity, r12p18	0.887	0.052	0.046124
Indirect effect via plant height, r23p38	-0.309	0.011	-0.0034
Indirect effect via number of branches, r24p48	-0.057	0.017	-0.00097
Indirect effect via pods per plant, r25p58	0.043	0.602	0.025886
Indirect effect via seeds per pod, r26p68	-0.027	0.751	-0.02003
Indirect effect via 100 seeds weght, r27p78	0.069	0.34	0.02346
Direct effect, P28			-0.038
Total			0.033074

Table 5.13: Plant height with yield, r38 = 0.231*

	r	p	rp
Indirect effect via days to 85% maturity, r23p28	-0.309	-0.038	0.011742
Indirect effect via number of branches, r34p48	0.201	0.017	0.003417
Indirect effect via days to 50% flowering, r13p18	-0.39	0.052	-0.02028
Indirect effect via pods per plant, r35p58	-0.054	0.602	-0.03251
Indirect effect via seeds per pod, r36p68	0.288	0.751	0.216288
Indirect effect via 100 seed weight, r37p78	0.123	0.34	0.04182
Direct effect , P38			0.011
Total			0.231479

Table 5.14: Number of branches per plant with yield, r48= 0.244**

	r	p	rp
Indirect effect via plant height, r34p38	0.201	0.011	0.03401
Indirect effects via pods per plant, r45 P58	0.058	0.0602	0.003492
Indirect effect via days to 85% Maturity, r24p28	-0.057	-0.038	0.002166
Indirect effect days to 50% flowering, r14p18	-0.148	0.052	-0.0077
Indirect effect via seeds per pod, r46p68	0.2	0.751	0.1502
Indirect efffects via 100 seeds weight, r47p78	0.133	0.34	0.04522
Direct effect , P48			0.017
Total			0.244392

Table 5.15: Number of pods per plant with yield, r58= 0.556*

	r	p	rp
Indirect effect via number of branches, r45p58	0.058	0.017	0.000986
Indirect effect via seeds per pod, r56p68	-0.035	0.751	-0.02629
Indirect effect via plant height, r35p38	-0.054	0.011	-0.00059
Indirect effect via days to 85% maturity, r25p28	0.043	-0.038	-0.00163
Indirect effect via days to 50% flowering, r15p18	-0.006	0.052	-0.00031
Indirect effect via 100 seeds weight, r57p78	-0.053	0.34	-0.01802
Direct effect, P58			0.602
Total	0.556		0.556141

Table 5.16: Number of seeds per pod with yield, r68= 0.7132*

	r	p	rp
Indirect effect via days to 50% flowering, r16p18	-0.057	0.052	-0.02306
Indirect effect via pods per plant, r56p58	-0.035	0.602	-0.02107
Indirect effect via 100 seeds weight, r67p78	-0.006	0.34	-0.00204
indirect effect via number of branches per plant,r46p48	0.2	0.017	0.0034
Indirect effect via plant height, r36p38	0.288	0.011	0.003168
Indirect effect via days to 85% maturity, r26p28	-0.027	-0.038	0.001026
Direct effect, p68			0.751
Total			0.71242

Table 5.17:100 seeds weight with yield, r78= 0.31

	r	p	rp
Indirect effect via number of seeds per pod, r67p68	-0.006	0.751	-0.00451
Indirect effect via number of pods per plant, r57p58	-0.053	0.602	-0.03191
Indirect effect via number of branches per plant,r47p48	0.133	0.017	0.002261
Indirect effect via plant height,r37p38	0.123	0.011	0.001353
Indirect effect via days to 85% maturity, r27p28	0.069	-0.038	-0.00262
Indirect effect via days to 50% flowering, r17p18	0.114	0.052	0.005928
Direct effect p78			0.34
Total			0.310508

Table 5.18: Phenotypic coefficient correlation analysis of yield components

	Number of Days to 50% flowering	Number of Days to 85% Maturity	Plant Height (cm)	Number of Branches/plant	Number of Pods/Plant	Number of Seeds/Pod	100 Seed Weight (g)	Yield (kg/ha)
Days to 50% flowering	1							
Days to 85% Maturity	0.887***	1						
Plant Height (cm)	-0.39	-0.309	1					
Number of Branches/Plant	-0.148	-0.057	0.201**	1				
Number of Pods/Plant	-0.006	0.043	-0.054	0.058**	1			
Number of Seeds/Pod	-0.057	-0.027	0.288**	0.2	-0.035	1		
100 Seed Weight (g)	0.114*	0.069	0.123	0.133	-0.053	-0.006	1	
Yield (kg/ha)	0.004	0.033	0.231*	0.244**	0.556***	0.712***	0.31**	1

Table 5.19: Direct and indirect effect of yield components on total correlation

Total correlation	Number of Days to 50% flowering	Number of Days to 85% Maturity	Plant Height (cm)	Number of Branches/ plant	Number of Pods/Plant	Number of Seeds/Pod	100 Seed Weight (g)
50% flowering r = 0.004	**0.052**	-0.0337	-0.0043	-0.0025	-0.0036	-0.0428	0.0388
days to 85% maturity r = 0.033	0.0461	**-0.038**	-0.0034	-0.001	0.0259	-0.0203	0.0235
Plant height r = 0.231*	-0.0203	0.0117	**0.011**	0.0034	-0.0325	0.2163	0.0418
Number of branches r = 0.244**	-0.0077	0.0022	0.0022	**0.017**	0.0349	0.1502	0.0452
pods/ plant r = 0.556**	-0.0003	-0.0016	-0.0006	0.001	**0.602**	-0.0263	-0.018
seds/ pod r = 0.712**	-0.003	0.001	0.0032	0.0034	-0.0211	**0.751**	-0.002
100 seeds weight r = 0.31**	0.0059	-0.0026	0.0014	0.0023	-0.0319	-0.0496	**0.34**

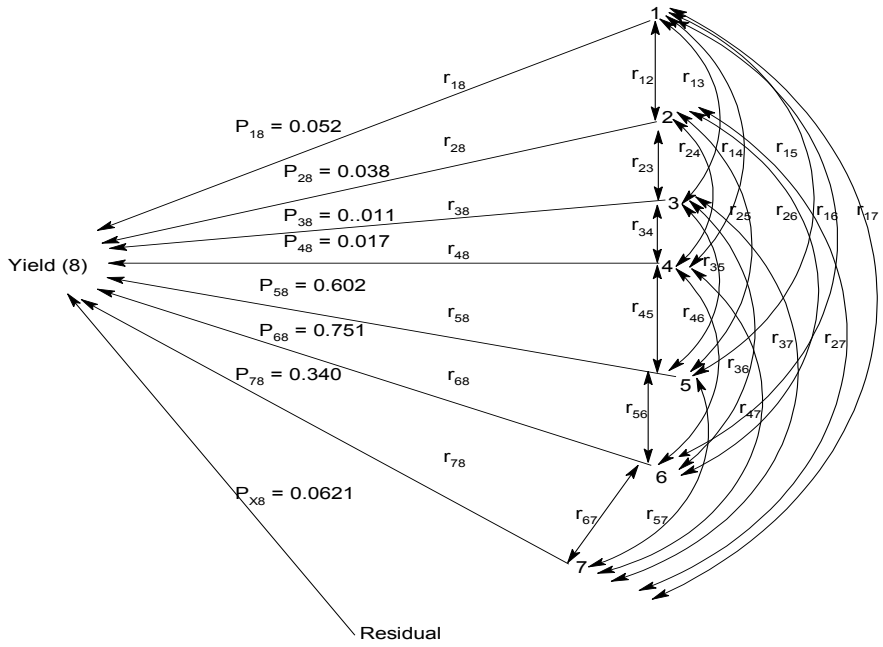

Figure 5.24: Path diagram showing relationship between yield and yield components

Where by: 1= days to 50% flowering

2= days to 85% maturity

3= plant height

4= number of branches per plant

5= pods per plant

6= seeds per pod

7= 100 seeds weight

8 = yield in kg / ha

P = direct effect

r = correlation coefficient

Pxy = residual value

5.3.6 Genetic components of variances

The study of genetic variance showed that both additive (D) and dominance gene effect (H1 and H2) were significant for days to 50% flowering, days to 85% maturity, pods per plant except seeds per pod. Also Fusarium wilt showed significance for dominance gene (H1 and H2) ($p \leq 0.01$) and for the additive gene (D) ($p \leq 0.001$). The mean degree of dominance (H1/D) 1/2 was greater than one in all traits include FWD. Signnificant environmental variance (E) was also observed for days to 50% flowering, days to 85% maturity and pods per plant ($p \leq 0.05$), ($p \leq 0.01$), and ($p \leq 0.001$) respectively. The value of (H2/4H1) was less than one quarter (0.25) for all traits including Fusarium wilt. The ratio (KD) dominance and (KR) recessive gene was more than one for all traits including FWD resistance (Table 5.20).

Table 5.20: Additive gene (D), dominant gene (H1 and H2), genetic variance and their derived parameters for the studied characters

	Number of Days to 50% flowering	Number of Days to 85% Maturity	Number of Pods/Plant	Number of Seeds/Pod	FWD Incidence (%)
E	37.17*	63.67**	78.35***	0.3	4.9691
D	379.04***	459.34***	458.97***	0.18	183.642***
H1	443.86***	546.93***	831.43***	1.86	356.6943**
H2	288.44**	361.08*	632.65***	1.56	284.5152**
(H1/D)1/2	1.08	1.09	1.35	3.23	1.393677
(H2/4H1)	0.16	0.17	0.19	0.21	0.199411
(KD/KR)	3.23	3.26	1.69	2.48	1.802714

5.4 DISCUSSION

5.4.1 Wr / Vr graphical analysis

Parental genetic variances showed significant variation on days to 50% flowering, days to 85% maturity, number of pods per plant, number of seeds per pod and fusarium wilt resistance. The Wr/ Vr graphical analysis showed that the regression line cuts the Wr axis through the origin on days to 50% flowering, days to 85% maturity and number of pods per plant.This implies that these variables are under influence of full dominance with additive gene effect. Position of arrays for days to 50% flowering indicate that P1 possess most recessive allels and P3 posses most dominant alleles. The remaining parents are in order of increasing recessiveness starting from the origin. The estimated regression line did not deviate significantly from unit slope indicating the absence of interaction. The results are in agreement with observation of Yao *et al*., (2011).

In days to 85% maturity P3 and P4 were allocated close to the origin point hence these genotypes posses more dominant alleles while P1 posses recessive alleles.

The number of seeds per pod could be controlled by partial dominance, using position of arrays P3 was controlled by recessive alleles and P5 is controlled by

dominant allele. The regression line deviated significantly from unit slope indicating the interaction of genes hence more backcrosses is required before selecting genotype based on number of seeds per pod. Hassan (2012) reported the similar results when studied inheritance of earliness, dry matter and shelling in pea. For FWD the regression line cuts Wr axis below the origin (negative position) indicated presence of over dominance gene effect while position of the arrays showed P6 and P2 were closed to the origin hence posse's dominant gene and P3 was far from the origin thus posse's recessive gene. The estimated regression line did not deviate significantly from unit slope indicating the absence of epistasis; hence direct selection of the genotype based on fusarium wilt could be done.

5.4.2 Estimate of general and specific combining ability

The significant effects due to GCA and SCA were observed in this study. The obtained results could be due to influence of additive and non-additive type of gene action (Hassan *et al.,* 2010; Sharma *et al*., 2013). Greater magnitude of GCA for character like days to 50% flowering, days to 85% maturity, pods per plant, 100 seed weight and yield in kg per ha in thiscould probably be due to additive genetic control on these characters while number of branches per plant and seed per pod were influenced by non additive genetic control. Similarly, Borah (2009); Hassan *et al.* (2010) reported dominance of both additive and non additive gene action of pea (*Pisum sativum* L). The ratio of GCA/SCA obtained was more than one for plant height, number of branches per plant and seed per pod which meant greater role of additive gene action in the inheritance of these traits. (Ercan and Mehmet 2005; Bayoumi and El-Bramawy 2007; Vaghela *et al*. 2009; Hassan *et al*. 2010).

GCA and SCA showed the highest negative value in P5 and P1×P4 for day's to50%. Similar results were also reported by Sharma *et al.* (2013) in pigeon pea. Negative GCA effect is preferable for days to flowering because it indicates the capacity of early parent to transmit its character to progenies in cross combination with other parents. Thus P5 and the cross P1×P4 could be the best general and specific for early flowering in pigeonpea.

The GCA and SCA for days to 85% maturity showed negative and significant for P4 and cross P1×P5. Positive GCA and SCA effects for days to 85% maturity would reflect combining ability for late harvest where by negative effects mean good combining ability for early harvest. Therefore P4 and P1×P5 was good combiner for both GCA and SCA (Hassan *et al*., 2010).

Positive, significant and the highest GCA as well as its SCA effects are preferable for plant height, number of branches per plant, number of pods per plant, number of seeds per pod, seeds weight and yield in general. On other hand GCA and SCA performance for parents and crosses scored the lowest disease reaction would be worth to be considered in future breeding programs. From the individual analysis of parents the P2, P5 and P6 with crosses P1×P2, P1×P4, P1×P5, P2×P3, P2×P4, P5×P6 were the good general and specific combiners for yield and yield components including fusarium wilt (Ojo, 2003; Bayoumi and El- Bramawy, 2007).

5.4.3 Phenotypic correlation

The yield of any crop depends on effects of different variables directly or indirectly (via other traits) hence makes that variable to be of predictive value towards the yield. As contribution of both direct and indirect effect is considered to the yield of pigeonpeas it create complex situation before plant breeders making selection (Vange and Moses, 2009; Sodavadiya *et al.,* 2009; Manggoel, 2012,). The path coefficient analysis provides more reality on interrelationship between yield and its yield components as both direct and indirect effects were considered. The path coefficient analysis in the present study revealed that plant height, number of branches per plant, pods per plant and seeds per pod had high and positive direct effect on yield. The result is in agreement with findings of Thanki and Sawargaonkar (2010); Chandirakala and Subbaraman (2010) and (Udensi *et al*. (2012).

The residual effect was low in this study indicating that most of the contributing characters were included in the path analysis. Therefore from the combine study of path analysis and correlation number of pods per plant, number of seeds per pod and 100 seeds weight observed to be most important.

5.4.4 Genetic components of variances

The study on genetic components of variances showed that both additive and dominant gene effect are important for yield components like days to 50% flowring, days to 85% maturity, number of pods per plant and fusarium wilt incideance. Although both are important in expression of these traits the dominant gene effect

(H1 and H2) has greater magnitude than additive gene effect (D). Similarly, Hayman (1954); Bayoumi and El- Bramawy (2007) reported the importance of additive and dominant gene effect in expression of plant height, number of fruiting branches per plant, number of capsule per plant and Fusarium wilt resistance in sesame. The mean degree of dominance [(H1/D) ½)] was greater than unity, for all the characters studied, indicating involvement of an over dominance in the expression of these traits (Sharma *et al*., 2013). The average frequency of negative versus positive alleles in the parent (H2/4H1) were less than one quarter for all traits studied including Fusarium wilt resistance, this indicating that positive and negative are not equally distributed. Similar results were reported by Bayoumi and Bramawy (2007). The study of dominance and recesive (KD/KR) ratio indicated excess of dominance alleles than recesive alleles as their ratio was more than one for all traits including Fusarium wilt resistance (Bayoumi and Bramawy 2007).

Significant environmental factor was observed on days to 50% flowering, days to 85% maturity and pods per plant except number of seeds per pod, implying that the environmental factor played a great role in their contribution to the total phenotypic variance (Bakheit *et al.,* 2000; Hoballah *et al*., 2001; Ammar *et al*., 2004).

5.4.5 Conclusion and Recommendation

The results of this study worth concluding that, number of pods per plant, seeds per pod and 100 seeds weight has good contribution to yield and recommended as selection criteria for good genotypes in pigeonpea breeding programs. Since other variables like days to 50% flowering, days to 85% maturity, plant height and

number of branches per plant had minimal contribution to yield; further selection is required to allow favourable gene recombination in a later generation before a final selection.

With regards to combining ability it was concluded that, P2, P5 and P6 with crosses P1×P2, P1×P4, P1×P5, P2×P3, P2×P4, P5×P6 were the good general and specific combiners for yield and yield components including fusarium wilt. Hence could be used by breeders as source of composite and hybrid materials.

It can also be concluded that, environmental factors played a great role in their contribution to the total phenotypic perfomance. Therefore for breeders it can be recommended that, there is a need to consider more locations and years for stabilization of the environmental variance with genotypic variance

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CHAPTER SIX

6.1 GENERAL CONCLUSIONS AND RECOMMENDATIONS

Studies on the Variability and aggressiveness of Tanzanian *Fusarium udum* isolates in selected pigeonpea varieties from Africa and Asia concludes that, among the nine isolates characterized EMKM2 isolate was the most virulent and was therefore useful to screen pigeonpea genotypes in breeding work. Both genetic and enviromental factors were reported as the major reason of variation of *Fusarium udum* pathogen, therefore it is recommended to keep on continous evaluating the pathogen before growing pigeonpea genotypes. It was also concluded that, there were wide genetic variability among pigeonpea genotypes evaluated against nine Fusarium wilt isolates. Out of these genotypes ICEAP 00040, ICEAP 00540 and ICEAP 00557 expressed highly resistance against all the isolates studied. Thus further analysis using molecular markers is recommended.

The study on Genetic diversity, inheritances and SSR marker segregation for Fusarium wilt resistance in pigeonpea concluded existence of close genetic relationship between two populations that could be diversified through mutation breeding, selection, recombination and introduction of new source of variability from genetically diverse pigeonpea populations. Also it is recommended to increase more number of Indian poulation for further analysis of diversity for future breeding program. The study also revealed 3: 1 in F_2 generation hence concluded the existence of single dominant gene which can be used to donate gene for disease resistance into genotypes where Fusarium wilt is of an economic problem. The SSR markers CZ681922, CZ681962 and CZ681928 were concluded highly correlated to

phenotypic observation hence linked to Fusarium wilt resistance. The application of these markers was recommended for sorting resistant genotypes rather than phenotypic method which is tedious and time consuming.

The study on Combining ability in yield and yield components on pigeonpea arrived at concluding that, number of pods per plant, seeds per pod and 100 seeds weight had good contribution to yield and recommended them as selection criteria for good genotypes in pigeonpea breeding programs. Since other variables like days to 50% flowering, days to 85% maturity, plant height and number of branches per plant had minimal contribution to yield, it was recommended for further selection to allow favourable gene recombination in subsequent generations before a final selection. With regards to combining ability it was concluded that, P2, P5 and P6 with crosses P1×P2, P1×P4, P1×P5, P2×P3, P2×P4, P5×P6 were the good general and specific combiners for yield and yield components including fusarium wilt. Hence could be used by breeders as source of composite and hybrid materials.

It was also being concluded that, environmental factors played a great role in their contribution to the total phenotypic perfomance. Therefore for breeders was recommended that, they needed to consider more locations and years for stabilization of the environmental variance with genotypic variance.

APPENDICES

Appendix 1: Collection of pigeonpea seed samples from Asia and their wilt record

Genotypes	Pedigree	Source	W	W	W
ICEAP 00040	KibB-KibB-KibW18□-KibWB□-KibWB□	ICRISAT	18	10	12
ICEAP 00053	KibB-KibB-KibW18□-KibWB□-KibWB	ICRISAT	20	11	22
ICEAP 00926	ICEAP 00020-24	ICRISAT	11	17	15
ICEAP 00932	ICEAP 00040-1	ICRISAT	32	16	24
ICEAP 00933	ICEAP 00040-2	ICRISAT	9	8	31
ICEAP 00936	ICEAP 00040-6	ICRISAT	30	12	30
ICP 9145	JM 2397	ICRISAT	50	26	74
ICEAP 00540	LINDI-4-KIbB-KibB	ICRISAT	19	24	30
ICEAP 00554	NA-6-KibB16-KIbB3-KibB12	ICRISAT	23	1	37
ICEAP 00557	NA-7-KibB21-KibB8	ICRISAT	28	8	43
ICEAP 00850	ICEAP 00073 (TANZANIA 14)-1-1	ICRISAT	69	39	85
ICEAP 00048	FA28-KibB-KibB2-KibB-7-KibB-13	ICRISAT	65	72	69
ICP 6927	CODE NO.15	ICRISAT	63	13	87
ICEAP 00158	KAT 60/8	ICRISAT	92	92	90
ICEAP 00068	TAz 9-KIbB01-KibB10-KibB6	ICRISAT	87	50	75

W –wilt

Appendix 2: Collection of pigeonpea seed samples from Africa and their wilt record

Lines	Pedigree	Source	Disease	Disease	Disease
`			W	W	W
ICPL 87119	C11 X ICP-1-6 W(x)-W1(x)	ICRISAT	9	8	0
ICPL 96061	ICPL 87051 X ICPL83057	ICRISAT	9	0	9
ICPL 20108	IPH 487 inbred line (mb 3783 X ICPL 87119	ICRISAT	0	11	0
ICP 8863	ICWR -6	INDIA (AP)	0	0	0
ICP 7035	DISR 55	INDIA (MP)	40	0	16
ICP 2376	P 3888	INDIA (AP)	100	94	100
ICPL 161	ICP-6 X PANT-A-2	ICRISAT	100	100	93
ICPL 211	pusa	ICRISAT	0	0	25

Appendix 3: Analysis of Variance of days to 50% flowering

Source	df	SS	MS	F	PROB
TOT	107	26903.63	251.4358	2.2347	
TRT	35	18875.63	539.3037	4.7931	
REP	2	151.9074	75.9537	0.6751	0
ERR	70	7876.093	112.5156	1	

Appendix 4: Analysis of Variance of days to 85% maturity

Source	df	SS	MS	F	PROB
TOT	107	36300.99	339.2616	1.7474	
TRT	35	22548.32	644.2378	3.3183	
REP	2	162.2407	81.12037	0.4178	0
ERR	70	13590.43	194.1489	1	

Appendix 5: Analysis of Variance for number of branches per plant

Source	df	SS	MS	F	PROB
TOT	107	210.9167	1.971184	1.3714	
TRT	35	107.5833	3.07381	2.1386	
REP	2	2.722222	1.361111	0.947	0.0035
ERR	70	100.6111	1.437302	1	

Appendix 6: Analysis of Variance for plant height

Source	df	SS	MS	F	PROB
TOT	107	387008.9	3616.905	2.3771	
TRT	35	272012.9	7771.796	5.1078	
REP	2	8486.741	4243.37	2.7888	0
ERR	70	106509.3	1521.561	1	

Appendix 7: Analysis of Variance for number of pods per plant

Source	df	SS	MS	F	PROB
TOT	107	70076.3	654.9187	2.7443	
TRT	35	53152.3	1518.637	6.3635	
REP	2	218.6852	109.3426	0.4582	0
ERR	70	16705.31	238.6474	1	

Appendix 8: Analysis of Variance for seeds per pod

Source	df	SS	MS	F	PROB
TOT	107	137.0741	1.281066	1.452	
TRT	35	72.40741	2.068783	2.3448	
REP	2	2.907407	1.453704	1.6477	0.0012
ERR	70	61.75926	0.882275	1	

Appendix 9: Analysis of Variance for 100 seed weight

Source	df	SS	MS	F	PROB
TOT	107	473.5957	4.426128	1.0492	
TRT	35	171.9956	4.91416	1.1649	
REP	2	6.31278	3.15639	0.7482	0.2892
ERR	70	295.2874	4.218391	1	

Appendix 10: Analysis of Variance for yield in kg per ha

Source	df	SS	MS	F	PROB
TOT	107	2042553	19089.28	1.4182	
TRT	35	1095323	31294.95	2.325	
REP	2	5007.987	2503.994	0.186	0.0014
ERR	70	942221.4	13460.31	1	

Appendix 11: Heterogeneity test for days to 50% flowering

Array	**Wr-Vr**					**t**	**t-tab**			**t**	**t-tab**
a1	-12.57	19.12	365.60	(a1-a2)	-72.66	-3.11	2.015				
a2	60.09	91.78	8424.15	(a1-a3)	-26.78	-1.15	2.015	(a2-a3)	45.88	1.97	2.015
a3	14.21	45.90	2106.90	(a1-a4)	74.60	3.20	2.015	(a2-a4)	147.26	6.31	2.015
a4	-87.17	-55.48	3077.98	(a1-a5)	85.42	3.66	2.015	(a2-a5)	158.08	6.78	2.015
a5	-97.99	-66.30	4395.23	(a1-a6)	54.15	2.32	2.015	(a2-a6)	126.81	5.44	2.015
a6	-66.72	-35.03	1227.02								
	-31.69	**VAR**	3266.15	**SD**	57.15	**SE**	23.331				

Appendix 12: Heterogeneity test for 50% flowering

		t	**t-tab**			**t**	**t-tab**				**t-tab**
(a3-a4)	101.38	4.35	2.015								
(a3-a5)	112.20	4.81	2.015	(a4-a5)	10.82	0.46	2.015				
(a3-a6)	80.93	3.47	2.015	(a4-a6)	-20.45	-0.88	2.015	(a5-a6)	-31.27	-1.34	2.015

Appendix 13: Heterogeneity test for days to 85% maturity

Array	**Wr-Vr**					**t**	**t-tab**			**t**	**t-tab**
a1	-49.52	-1.10	1.20	(a1-a2)	-123.64	-4.19	2.015				
a2	74.12	122.55	15018.20	(a1-a3)	-61.02	-2.07	2.015	(a2-a3)	62.63	2.12	2.015
a3	11.50	59.92	3590.79	(a1-a4)	93.34	3.16	2.015	(a2-a4)	216.98	7.35	2.015
a4	-142.86	-94.43	8917.79	(a1-a5)	47.94	1.62	2.015	(a2-a5)	171.58	5.81	2.015
a5	-97.46	-49.03	2404.37	(a1-a6)	36.81	1.25	2.015	(a2-a6)	160.46	5.44	2.015
a6	-86.33	-37.91	1437.03								
	-48.43	**VAR**	5228.23	**SD**	72.31	**SE**	29.519				

Appendix 14: Heterogeneity test for days to 85% maturity

		t	**t-tab**			**t**	**t-tab**				**t-tab**
(a3-a4)	154.36	5.23	2.015								
(a3-a5)	108.96	3.69	2.015	(a4-a5)	-45.40	-1.54	2.015				
(a3-a6)	97.83	3.31	2.015	(a4-a6)	-56.53	-1.91	2.015	(a5-a6)	-11.13	-0.38	2.015

Appendix 15: Heterogeneity test for pods per plant

Array	**Wr-Vr**					**t**	**t-tab**			**t**	**t-tab**
a1	-144.19	-18.43	339.56	(a1-a2)	39.64	2.14	2.015				
a2	-183.83	-58.07	3371.76	(a1-a3)	9.11	0.49	2.015	(a2-a3)	-30.53	-1.65	2.015
a3	-153.30	-27.54	758.38	(a1-a4)	-8.58	-0.46	2.015	(a2-a4)	-48.22	-2.61	2.015
a4	-135.61	-9.85	96.95	(a1-a5)	-50.35	-2.72	2.015	(a2-a5)	-89.99	-4.87	2.015
a5	-93.84	31.93	1019.37	(a1-a6)	-100.38	-5.43	2.015	(a2-a6)	-140.02	-7.57	2.015
a6	-43.81	81.95	6716.05								
	-125.76	**VAR**	2050.34	**SD**	45.28	**SE**	18.486				

Appendix 16: Heterogeneity test for pods per plant

	t	t-tab			t	t-tab				t-tab
(a3-a4)	-17.69	-0.96	2.015							
(a3-a5)	-59.47	-3.22	2.015	(a4-a5)	-41.77	-2.26	2.015			
(a3-a6)	-109.49	-5.92	2.015	(a4-a6)	-91.80	-4.97	2.015	(a5-a6)	-50.02	-2.71	2.015

Appendix 17: Heterogeneity test for number of seeds per pod

Seeds/Pods Array	Wr-Vr				t	t-tab			t	t-tab
a1	-0.27	0.28	0.08	(a1-a2)	0.55	3.39				
a2	-0.82	-0.28	0.08	(a1-a3)	1.05	6.43	2.015	(a2-a3)	0.50	3.04	2.015
a3	-1.32	-0.77	0.60	(a1-a4)	-0.01	-0.04	2.015	(a2-a4)	-0.56	-3.43	2.015
a4	-0.26	0.28	0.08	(a1-a5)	0.09	0.52	2.015	(a2-a5)	-0.47	-2.86	2.015
a5	-0.35	0.19	0.04	(a1-a6)	-0.03	-0.18	2.015	(a2-a6)	-0.58	-3.56	2.015
a6	-0.24	0.30	0.09							
	-0.54	**VAR**	0.16	**SD**	0.40	**SE**	0.163			

Appendix 18: Heterogeneity test for number of seeds per pod

	t	t-tab			t	t-tab				t-tab
(a3-a4)	-1.06	-6.47	2.015							
(a3-a5)	-0.96	-5.90	2.015	(a4-a5)	0.09	0.56	2.015			
(a3-a6)	-1.08	-6.60	2.015	(a4-a6)	-0.02	-0.14	2.015	(a5-a6)	-0.11	-0.70	2.015

Appendix 19: Analysis of variance summary for the different variables

Source	df	50% Flowering	85% Maturity	Branches per plant	Plant height	Pods per plant	Seeds	Seeds Weight	Yield
TOT	107	251.44	339.26	1.97	3616.91	654.92	1.28	4.43	19089.28
TRT	35	539.30***	644.24***	3.07***	7771.80***	1518.64***	2.07***	4.91***	31294.95***
REP	2	75.95***	81.12***	1.36***	4243.37***	109.34***	1.45***	3.16***	2503.99***
ERR	70	112.52	194.15	1.44	1521.56	238.65	0.88	4.22	13460.31

Appendix 20: Array variances and covariances

	FWD			50% Flowering		
	Wr'	Wr	Vr	Wr'	Wr	Vr
P1	230.32	192.36	281.25	396.44	365.04	377.61
P2	114.14	2.36	69.08	168.57	128.37	68.27
P3	112.05	-42.68	66.56	95.50	36.12	21.91
P4	135.28	114.89	97.03	197.02	6.09	93.26
P5	112.67	87.54	67.31	147.52	-45.70	52.28
P6	71.05	-18.49	26.77	206.35	35.59	102.31

Appendix 21: Array variances and covariances

	85% Maturity			Pods per branch			Seeds per Pod		
	Wr'	Wr	Vr	Wr'	Wr	Vr	Wr'	Wr	Vr
P1	540.94	509.96	559.48	386.56	133.92	278.11	0.46	0.17	0.44
P2	201.26	151.57	77.45	346.55	39.69	223.52	0.6	-0.08	0.74
P3	132.07	44.85	33.35	595.09	505.76	659.06	0.66	-0.4	0.92
P4	272.61	-0.76	142.1	270.86	0.92	136.53	0.4	0.08	0.34
P5	187.66	-30.12	67.34	387.69	185.89	279.73	0.35	-0.09	0.26
P6	170.25	-30.92	55.42	292.52	115.43	159.24	0.56	0.42	0.66