EVALUATION OF PIGEONPEA (*Cajanus cajan* (L.) Millsp) GENOTYPES FOR YIELD PERFORMANCE, GENETIC DIVERSITY AND RESISTANCE TO INSECT PEST COMPLEX IN NORTH RIFT VALLEY- KENYA

BY

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2015

DECLARATION

DECLARATION BY THE STUDENT

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DEDICATION

To my beloved husband;- Solomon, my lovely children; Xavier, Genevieve and Hedwig. Finally, to my sister in-law, Prisca for their collective moral support.

ABSTRACT

Pigeonpea (*Cajanus cajan* (L.) is largely considered as an orphan crop and remains one of the most drought-tolerant legumes. However, insect pest complex pod borer (*Helicoverpa armigera)*, Sucking bug (*Clavigralla tomentosicollis*) and pod fly (*Melanogromyza cholcosoma*) is the major limiting factor to its production by causing tremendous loss ranging between 16-18% for pod borer, 69%-73% for sucking bug and 9-15% for pod fly in Kiboko and Kabete but no reported statistics in North Rift valley Kenya. The objective of this study was to evaluate yield performance, genetic diversity and level of resistance to insect pest complex among sixteen selected Pigeonpea genotypes. The study was carried out in three varied sites at (KALRO- Marigat, Fluorspar and ATC Koibatek) during the long rain season of April-November 2014. The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications in 75x25 cm inter and intra spacing. The results revealed six genotypes (ICEAPs 01147, 1147-1, 01159, 00911, 0979-1, and 1154-2) with a yield of more than 1.0 t/ha across the three sites. Grain yield was positively significantly correlated with height at maturity, secondary branches, pods /plant, seeds /pod and 100 seed weight. However days to 50% flowering was negatively correlated to yield. The seed damage by pod borer was 1.9%, 3.6% and 37.2% in Koibatek, Fluorspar and Marigat respectively. However Pod fly caused a damage of 5.9% in Marigat, 5.9% in Koibatek and 6.8% in Fluorspar while Seed damage by sucking bug was 39.3% in Marigat, 8.4% in Koibatek and 2.9% in Fluorspar. Genotypes ICEAPs 00902, 01541 and 1154-2 were tolerant to the insect pest complex at both podding and seed level. Grain yield associated negatively ($P \leq 0.05$) with pod borer eggs, pod borer pod damage, seed damage by sucking bug and pod borer. The molecular cluster analysis based on neighbor joining grouped the genotypes into three distinct clusters based on resistant/ susceptible levels. The markers generated 78 alleles in total with a mean of 3.67 alleles per locus. However, Polymorphism information content ranged from 0.13 to 0.70 with Marker CcM1348 being highly polymorphic and informative by providing 70% genetic diversity information. Therefore, there is need for further testing of the high yielding varieties identified in this study for other important agronomic traits in two seasons. Also mechanisms of resistance to this insect pest complex need to be identified and molecular markers to be developed using Single Nucleotide Polymorphism (SNP) markers through Genome Wide Association Studies (GWAS).

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ABBREVIATIONS AND ACRONYMS

AFLP	Amplified Fragment Length Polymorphism
ASALs	Arid and Semi -Arid Lands
CTAB	Cetyl Trimethyl Ammonium Bromide
DArT	Diversity Array Technology
DNA	Deoxyribonucleic Acid
dNTPs	deoxy Nucleotide Triphosphates
EDTA	Ethylene Diamine Tetra Acetic Acid
FAOSTAT	Food and Agriculture Organization Statistical databases
ICRISAT	International Research Institute for Semi-Arid Tropics
ng	Nanogram
PCR	Polymerase Chain Reaction
PIC	Polymorphic Information Content
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
RFU	Relative Fluorescence Unit
RNA	Ribonucleic acid
RNAse	Ribonuclease
SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeats
Taq	*Thermus aquaticus*
TBE	Tris/Borate/EDTA
TE	Tris EDTA

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CHAPTER ONE

INTRODUCTION

1.1 Background information

Pigeonpea, (*Cajanus Cajan* (L) Mill) is the third most important grain legume worldwide though largely considered as an orphan crop. It has a diploid genome consisting of 11 pairs of chromosomes (2n = 2x = 22) with an approximate genome size of 858 Mbp (Young *et al*., 2003). Globally, area under pigeonpea cultivation has increased by 56% since 1976 and currently stands at 4.92 million hectares with an annual production of 3.5 million metric tons and productivity of 885 kg ha^{-1} (ICRISAT, 2008, FAOSTAT, 2008). Globally, Kenya is fifth largest pigeonpea producer (2.1%) after India (62.7% of total production) and Tanzania (4.9% of total production). In Kenya, the crop is second only to field beans (*Phaseolus vulgaris*) as pulse and as a food legume in both acreage and production (FAOSTAT, 2013). Other dryland legumes of economic importance are groundnuts, cowpeas, chickpeas and green grams. In terms of legume breeding programs, pigeonpea lags further behind field beans (*Phaseolus vulgaris* (L.) and soybean (*Glycine max* (L.). The latter legumes are among the most researched crops worldwide even though, unlike pigeonpea, they are not as drought tolerant (Postel, 2000).

Pigeonpea average yield of 718 $Kgha^{-1}$ and maximum yield of 1087 Kg ha^{-1} has been recorded over the last 16 years under farmer conditions in tropics (FAOSTAT, 2007) and are far lower than its potential yield under research conditions (1500–2500 $Kgha^{-1}$) as reported by Mergeai *et al*., 2001. The major growing areas in Kenya are Eastern provinces (mainly in Machakos, Makueni, Kitui, Embu), Western (Alupe) and parts of

Coastal regions. Pigeonpea remains one of the most drought-tolerant legumes (Valenzuela and Smith, 2002) and is often the only legume crop that gives some grain yield during dry spells when other legumes such as field beans will have wilted and dried up (Okiror, 1986). It's a short lived perennial shrub traditionally cultivated as an annual crop in developing countries. Pigeonpea in Africa is primarily a subsistence crop though some countries have been reported to export significant amounts (Shanower *et al.,* 1999). Apart from being drought tolerant, pigeonpea is an important legume among the smallholder poor farmers in Kenya due to its high nutritive value (proteins, vitamins and minerals) for supplementation of low protein cereals. This has been considered as one of the best solutions to protein-calorie malnutrition in the developing world (Chitra *et al*., 1996).

Insect pests are major constraint to pigeonpea production in East Africa (Minja, 1999) and the tropics (Lateef and Reed, 1990; Shanower *et al.*, 1999). These pests include pod borer (*Helicoverpa armigera),* pod sucking bugs (*Clavigralla tomentosicollis)* and pod fly (*Melanagromyza chalcosoma)*. The pests affect the crop during its developmental and reproductive stage by feeding on flower buds, flowers, pods and seeds causing tremendous loss. Knowledge of the impact, dynamics, and ecology of the pests and natural enemies is essential (Minja, 1999), yet there has been relatively little research investment into the biology, ecology, and management of pigeonpea pests and their natural enemies. Research on losses and damage from insect pests are limited in East Africa particularly, Kenya. In Central and Eastern Kenya, losses of 18% due to *Helicoverpa armigera* have been reported in KALRO Kiboko and 16% in Kabete (Minja *et al*., 1999). However, little attention has been given to pests pod suckers and pod fly.

Several strategies have been recommended to control insect pests which include use of insecticides, crop rotation and use of tolerant genotypes among others. Chemical pesticides remain the primary means of pest management among farmers however 'pesticide treadmill' remains a challenge. The pesticide treadmill is a term indicating a situation in which it becomes necessary for a farmer to continue using pesticides regularly because they have become an indispensable part of an agricultural cycle (Klemm, 2007). Cultural control measure could have been easier on-farm options as compared to others but it rarely achieves desired results due to insect pest complexity. Use of host plant resistance to control insect pests is the best option as it is environmental friendly and cost effective under subsistence conditions as compared to other options. There is need therefore to develop improved genotypes that combine high yield and insect-resistance preferred agronomic characters (Minja, 1999). These are immense opportunities for enhancing the production and productivity of pigeonpea through a rational deployment of crop varieties with tolerance to these pests. Despite its importance there is little information on resistant varieties that are widely adapted in the current pigeon pea growing areas in rift valley Kenya. There is also limited information on incidence, distribution and reaction of commercial and improved varieties against the insect pest complex.

Pigeonpea improvement and conservation depend on the presence of genetic variability and the accurate characterization of the variability (Songok *et al.*, 2010). Understanding the distribution of genetic diversity among individuals is very crucial in plant breeding since it provides a selection base for parentage. Pigeonpea cultivated accessions have been reported to show little diversity using Diversity Array Technology analysis (Yang *et*

al., 2006). Therefore, Knowledge of the genetic basis of yield, resistance to pests are important factors for deciding the breeding strategies for genetic improvement of pigeonpea.

Several DNA-based molecular marker technology have been reported in Pigeonpea diversity study, they include; Random Amplified Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), Diversity Arrays Technology (DArT), Simple Sequence Repeats (SSR) and Single Nucleotide Polymorphisms. Most technologies have several demerits of high cost, low polymorphism, dominant, not PCR based, not transferable and not reproducible. SSR markers can overcome these limitations by having the advantage of high polymorphism information content, co-dominant, high abundance and random distribution in the genome.

In this study, 16 selected yield elite lines were evaluated in the drier parts of North Rift Valley with the aim of identifying resistant lines to insect pest complex (pod borer, pod fly and sucking bug); assess pigeonpea genetic diversity and yield performance among the genotypes. This was to identify promising high yielding, adaptable improved genotypes and resistance to the three pests which can possibly be used in breeding programs in Kenya.

1.2 Statement of the Problem

Pigeonpea though among the few drought-tolerant legumes that yield grains significantly during dry spells when other legumes such as field beans have wilted, it has remained static in production over the past decade due to prevalence of various abiotic and biotic factors (Choudhary, 2011). Insect pest complex (pod borer, pod fly and sucking bug) is

one of the biotic factors contributing to low yields. They cause damage to the crop from flowering to maturity stage by causing flower abortion, feeding on the pods and seeds hence not good for consumption and will not germinate when planted (Sharma, 2001). Globally, insect pest complex accounts to a yield loss of more than 1000 million dollars every year (Minja *et al*., 1999). Research on losses and damage from insect pests are limited in east Africa particularly Kenya. In Central and Eastern Kenya, losses due to *Helicoverpa armigera* (18%) have been reported in Kiboko and 16% in Kabete (Minja *et al*., 1999). However, little attention has been given to other pests like pod suckers and pod fly. In addition, total seed damage by sucking bug-73%; pod borer -18% and pod fly 9% in Kiboko while damage by sucking bug-69%,; pod borer-16% and pod fly 15% in Kabete have been reported while no statistics have been reported from dry parts of North Rift Valley (Minja *et al*., 1999). Knowledge of the genetic basis of yield, resistance to pests are important factors for deciding the breeding strategies for genetic improvement of pigeonpea (Yang *et al*., 2006). Genetic diversity encompasses several methods such as biochemical, morphological and molecular makers. Biochemical and morphological markers are time consuming and influenced by environment. However, molecular markers overcome these challenges by recognizing the presence or absence of a particular gene independent of plant part or age (Saxena, 2008). An understanding of the distribution of genetic diversity is essential for both utilization, production, improvement, promotion and conservation strategies (Songok *et al.*, 2010).

1.3 . Justification of the Study

The increasing concern of the effect of global climate change and its likely impact on agriculture has stimulated research for crops that can withstand extreme environmental

conditions. Among legumes, pigeonpea (*Cajanus cajan* (L.) has attracted attention as being both drought-tolerant and highly nutritious. The identification of pigeonpea genotypes which are resistant to insect pests would be of particular importance to most farmers in Kenya who are unable to access inputs like conventional pesticides. Though insect pest complex incidence can be controlled by application of chemicals, a variety possessing inbuilt resistance to the pest will be preferred for its manifold advantage like low input cost, through avoidance of pesticide cost besides eliminating residue problem and environmental pollution.

Conventional breeding has helped in identifying varieties but selection process are difficult, time consuming and most times pest symptoms are elusive because of the complexity of the pest and its occurrence. Classical breeding enhance efficient development of superior genotypes by complementing with more sophisticated technologies. Molecular marker technology (Wenzel, 2006) promises to facilitate pigeonpea breeding by providing information on genetic diversity (Sharma *et al.*, 2003), predicting and identifying promising genotypes for cultivar development, improving the efficiency of breeding through marker assisted selection (MAS).

These markers when used in combination with the available and proven breeding methods may be helpful for precision breeding as well as enhancing the process of breeding. Realizing the importance of such an investigation, the present study was carried out with the following objectives.

1.4. Objectives

1.4.1. Main Objective

To evaluate productivity, genetic diversity and resistance to insect pest complex among selected medium duration pigeonpea genotypes in North Rift Valley, Kenya to enhance food security.

1.4.2. Specific objectives

The specific objectives of the study are;

1. To evaluate yield and yield related traits of 16 selected pigeonpea genotypes
2. To assess resistance/tolerance level to insect pest complex among the selected pigeonpea genotypes
3. To characterize selected pigeonpea genotypes using SSR markers.

1.4 .3. Hypotheses (Ho)

1. There is no difference in yield performance and adaptability among pigeonpea genotypes when evaluated in 3 varied agro-ecological zones.
2. There is no difference in resistance to insect pest complex among pigeonpea genotypes during incidence and severity evaluation.
3. There is no genetic relatedness among 16 pigeonpea genotypes based on simple sequence repeats (SSR) markers

CHAPTER TWO

LITERATURE REVIEW

2.1. Botany and morphology of Pigeonpea

Pigeonpea belong to the family of fabaceae (pea family), is largely a self-pollinated crop but some are cross pollinated (20-30%) that occurs through insect pollination. The flower type, the abundance of insect pollinators and weather conditions during flowering can influence the degree of cross-pollination (Bramel *et al*., 2004). It has a diploid number of 2n=2x=22 and genome size of 858mbp. Their seedlings have cotyledon leaves that are oblong, 30-40mm long and 10-15 mm wide, with an indented central vein. The first true leaves and all subsequent leaves are trifoliate and the central leaflet longer than the side leaflets. Flowers are produced along slender stalks 20 -70 mm long that emerge from the leaf axils, with 6-12 flowers per stalk. Flowers are a typical pea-shape, bright yellow, with reddish-brown markings on the back of the petals. Pods are 45 -100 mm long, 8 –15 mm wide and contain 4-7 seeds. Seeds are ovoid, 4 - 7 mm in length and reddish-brown.

2.2: Economic importance of Pigeonpea

Pigeonpea remains one of the most drought-tolerant legume abundant in protein, making it an ideal supplement to traditional cereal-, banana- or tuber-based diets of most Africans which are generally protein-deficient (Odeny, 2007). It is also one of the indigenous crops being promoted for potential medicinal use (Mander *et al.*, 1996).

Pigeonpea production trends seem to be increasing since the turn of the century, perhaps with the decreasing quantity of rainfall in the region (Odeny, 2007). Pigeonpea is a multipurpose leguminous crop that is extensively used for food, fuel, thatching of huts and fodder for livestock (Rao *et al.,* 2002). It is also a source of green manure for soil fertility amelioration in local cropping systems (Osman *et al.,* 2012). It is the second food legume only to field beans (*Phaseolus vulgaris)* in Kenya in acreage and production (FAOSTAT, 2007). Although drought is the most important environmental constraints to plant survival and productivity (and hence food security) in the tropics pigeonpea is rarely affected (Speranza *et al.,* 2007). In addition, pigeonpea is the most preferred legume in the dry lands where it is intercropped with cereals or short duration annuals (Joshi *et al*., 2001).

The ability of the pigeonpea to withstand severe drought better than many legumes is attributed to its deep roots (Flower and Ludlow, 1987) and osmotic adjustment (OA) in the leaves (Subbarao *et al.,* 2000). The legume also maintains photosynthetic function during drought stress better compared to other drought-tolerant legumes such as cowpea. Furthermore, its unique polycarpic flowering habit also enables the crop to shed reproductive structures in response to stress. Its roots also help in releasing soil-bound P making it available for plant growth (Saxena, 2008). The total nitrogen produced per unit area from plant biomass is more than in other legumes. Moreover it also enriches the soil through addition of other valuable organic matter and micronutrients (ICRISAT, 2003). Egbe and Adeyemo (2006) reported that pigeonpea

can be intercropped with other crops like maize without negative effects on the yield and yield components of maize.

2.3. Agronomy and production of pigeonpea

Pigeonpea is reported to have a wide adaptability to different climates and soil (Traedson *et al*., 1990), but each specific group has its specific area of adaptation. The crop is purely grown under rain fed conditions with varying temperature, latitude and altitude. Its phenology is affected by temperature (altitude) and day length (latitude) by affecting floral development and maturity period (Silim *et al*., 2000). Photoperiod and temperature influence the phonological responses, hence playing a crucial role in the evolution of crop production systems. Photoperiod reaction is linked to days to flowering and amount of biomass accumulated by the plant (Wallis *et al*., 1981). The performance of genotypes depends on soil moisture, temperature, genetic potential of the cultivar. Pigeonpea is grown mostly in semi -arid areas with unreliable rainfall, where crop failures are frequent. It performs well with rainfall of 600-1000 mm. It grows in a wide range of soil types but gives optimum results in deep loam soils with a pH ranging from 5-7.Late planting, poor land preparation, low plant population and inadequate weeding contribute to low yields (Silim *et al*., 2000). Grain yields in farmers' fields in Eastern Africa have been reported to range between 800-1500t/ha compared to 2.6-4.3 reported from research trials in Kenya (Onim, 1984).Actual and yield potential gaps is contributed by a number of biotic(pests and diseases) and abiotic (drought, salinity, waterlogging) constraints. Pigeonpea is a quantitative short day plant. Pigeonpea is able to fix 40kg/ha of nitrogen per season in the soil due to presence of rhizobia bacteria in the nodules (Saxena, 2008). Nitrogen

fixing nodules are produced in pigeonpea by a number of rhizobia strains belonging to the ‘cowpea miscellomy’ group (Allen *et al.,* 1998).

The development and formation of nodules is affected by various biotic and abiotic factors such as moisture, soil type, day length, crop duration, salinity, insect damage, nutritional factors, and temperature. Most nodules are formed on the secondary roots and the majority of these are located in the top 30 cm of the soil profile. Small nodules are frequent in 120–150 cm soil zone and may occur at even greater depths (Kumar, 1990).

2.3.1 Types of pigeonpea

There are four types of pigeon pea based on maturity period; extra short, short, medium and long duration (table 1). There are now 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 suited to a particular agro-ecosystem (Odeny, 2007). Short duration line frequently is determinate, although determinate and indeterminate growth habits occur across maturity crops. Short duration, respond to drought stress by escaping and susceptible to pest infestation. The short-duration (SD) have a potential yield range from 1.2 to 2.0 tones with 8- 12 g of 100 seed mass while medium duration (MD) yield rang 1.8 to 3.0 tones with 15-18 hundred seed mass (g). On the other hand, long-duration (LD) has a potential yield of 1.8-3.5 tones with 18-25 hundred seed mass (g).

Table 1: Summary traits for pigeon pea types

Trait	Short duration	Medium duration	Long duration
Duration(days)	100-150	150-200	200 or more
Growth habit	Determinate/indeterminate	Indeterminate	Indeterminate
Drought response	Escape	Tolerance & resilience	Tolerance
Cook ability	Poor	Good	Very easy
Pest infestation	Susceptibility	Tolerant	Tolerant
Suitability	Sole & Ratoon	Intercrop & Ratoon	Maize intercrop
Yield potential (t)	1.2 – 2.0	1.8 – 3.0	1.8 - 3.5
100 Seed mass (g)	8-12	15-18	18-25
Fuel wood		More	More

Source (Odeny, 2007)

2.4 Genetic diversity and Molecular markers

Conventional plant breeding have had limited success in enhancing genetic resistance against pests due to lack of genetic information and complexity of genome. Genetic diversity is carried out for several purposes including germplasm characterization, genetic mapping, variety identification and breeding. Molecular markers offer great scope for improving the efficiency of conventional plant breeding. Varietal identification is important for the documentation of genetic resources (Gupta *et al*., 1999). Traditional techniques like morphometric traits observation and biochemical

techniques based on protein and isozyme polymorphism have been used before the advent of molecular markers. Morphological studies alone do not provide sufficient information to understand genetic diversity within the species as well as its relatedness to other species. Therefore, molecular markers such as Amplified fragment length polymorphic, Restriction fragment length polymorphism, Random Amplified Polymorphic DNAs, Simple Sequence Repeats and Single Nucleotide Polymorphism cannot be influenced by environmental factors and can be scored at any stage in plant growth (Gupta *et al.*, 1999).

2.4.1 Amplified fragment length polymorphic (AFLP) Marker

Amplified fragment length polymorphic marker is one of the important techniques that have been used for genetic characterization of plant pathogens (Vos *et al.,* 1995). They are based on the selective amplification of restriction fragments. AFLP techniques were more efficient in detecting polymorphism among closely related cultivars that could not be detected by other marker systems. AFLP markers have been proved as more reliable and reproducible as compared to RAPD markers and less cumbersome and time consuming than the RFLPs (Okori *et al*., 2003 and Panguluri *et al*., 2005). AFLP technique initially developed for fingerprinting plant genomes (Vos *et al.,* 1995) has emerged as an important technique for assessment of genetic diversity (Mackill 1995), phylogenetic analysis of closely related plant species (Sharma *et al*., 1996).

2.4.2 Restriction fragment length polymorphism (RFLPs)

RFLPs were the first molecular markers to be developed and have been used in pigeonpea, to overcome the problems associated with phylogenic grouping such as inconsistencies in taxonomic relationships based on data from morphology, cytology and cross ability. RFLP analysis has revealed that accessions of cultivated species *Cajanus cajan* share more DNA fragments with *Cajanus scarabaeoides* than with *C. cajanifolius* (Nadimpalli *et al.,* 1992). RFLP markers are Co-dominant, reproducible, difficult to automate labour intensive and have been used in diversity studies in pigeonpea (Miller, 1990).

2.4.3 Random Amplified Polymorphic DNAs (RAPDs)

Is one of the polymerase chain reaction (PCR) based molecular markers and involve use of arbitrary primers to amplify DNA at discreet random sequence (Williams *et al.,* 1990). These markers have been used for investigating quantitative trait loci (QTLs) in two strains of pigeonpea and in the F1and F2 progeny (Tyagi, 1997).They have also been used for somaclonal variation (Prasannalatha *et al*., 1999).

2.4.4 Simple Sequence Repeats (SSR) Markers

Microsatellite or simple sequence repeat markers are short tandem repetitive DNA sequences with a repeat length of a few (1-5) base pairs (Litt and Luty, 1989). Microsatellite markers have been increasingly used to assess the genetic diversity and population structure among plants (Li *et al*., 2000, Pillen *et al.,* 2000). There is an increase is number of polymorphic SSRs available due to recent initiative in pigeonpea genomics (Vashney *et al*., 2009). About 330 of these polymorphic SSRs

were used to construct a consensus pigeonpea genetic map showing the exact location in the chromosome (Bhora *et al*., 2012). Odeny *et al*. (2009) used 113 pigeonpea genomic SSRs, 73 of which amplified interpretable bands. Thirty-five of the primers revealed polymorphism among 24 pigeonpea breeding lines. The number of alleles detected ranged from 2 to 6 with a total of 110 alleles and an average of 3.1 alleles per locus. GT/CA and GAA class of repeats were the most abundant dinucleotide and tri-nucleotide repeats respectively. Additionally, 220 soybean primers were tested in pigeonpea, 39 of which amplified interpretable bands. Molecular analysis using SSRs can provide additional information on genetic diversity that would be useful for breeding programs through selection of diverse parents (Charcosset and Moreau 2004). They are abundant across the genome, highly polymorphic, codominant, transferable, multi allelic, highly reproducible, evenly distributed and very informative and PCR based (Edwards *et al*., 1996).

2.5 Major pests of prime importance of pigeonpea

Insect pests are a major biotic constraint limiting Pigeonpea production in southern and eastern Africa (Minja, 1997). More than 90 insect species attack pigeonpea in the region (Le Pelley, 1959; Lateef and Reed, 1990; Minja, 1997). Surveys in Kenya, Malawi, Tanzania and Uganda have shown that the most important pests of pigeonpea pods and seeds in the region are: the pod boring Lepidoptera (*Helicoverpa armigera*, *Maruca vitrata* (*testulalis)* Geyer, *Etiella zinkenella* Treitschke), pod sucking bugs (mainly *Clavigralla tomentosicollis* Stål) and pod fly (*Melanagromyza chalcosoma* (Minja *et al*., 1999). At Kiboko, sucking bugs accounted for 73% of the total seed damage, borers 18% and pod fly 9%. Seed damage at Kabete was similar to

that at Kiboko with pod sucking bugs accounting for 69%, pod borers 16% and pod fly 15%.

2.5.1 Sucking bugs

Pod sucking bugs are primary pests of pigeonpea. The most common pod-sucking bugs are: giant corbugs (Anoplocnemis spp) and spiny brown bugs (Clavigralla spp).These bugs suck developing seeds through the pod wall. The seeds become shriveled with dark patches. Attacked seeds do not germinate and are not acceptable as food. Fungal spores are sometimes transmitted with the mouthparts during feeding, resulting in rotting of the seeds. The spiny brown bug *Clavigralla tomentosicollis* is one of the most important pests of pigeonpea in Eastern Africa. Sucking bug adults are difficult to control since they are very mobile and can invade crops from neighboring sites (Faujdar and Oswalt 1992).

2.5.2 Pod borers

The African bollworm (*Helicoverpa armigera*), is the major pest of pigeonpea in East Africa. They feed on leaves, flowers and pods, destroying them. The caterpillars are 1.5 to 4cm long. They bore holes on pods and feed on the seeds. Usually develop in the pods and feed partly mature seeds. The legume pod borer (*Maruca vitrata*), the adult of the legume pod borer is a moth with a wing span of 15-30 mm. It has light brown forewings with white markings and pearly white hind wings. Though mainly nocturnal, the moth may also be seen during the day. They lay eggs in the flowers or buds, or on the pods. Caterpillars are whitish with black head and rows of conspicuous brown to black spots on the dorsal, lateral, and ventral surfaces of each

body segment. Fully-grown caterpillars measure about 15mm in length. They pupate in the soil. Caterpillars web leaves, flower buds and pods and feed inside the web. Flowers usually show little sign of damage until they wilt and drop. They typically attack pods at the point of contact between two pods, or between a pod and a leaf or stem (Faujdar and Oswalt 1992). The seasonal cycle of this pest varies in different parts of the country and also with cropping pattern. The lifecycle starts when eggs hatch in less than 3 days at optimum temperature of 27-28^{0}C. On hatching, the larvae feed for a short time on the tender leaflets by scrapping green tissue and then shift to flower buds and tender shoots. Larval and pupal periods last for 17-23 and 9-11 days, respectively in normal conditions as demonstrated in (Figure 1).

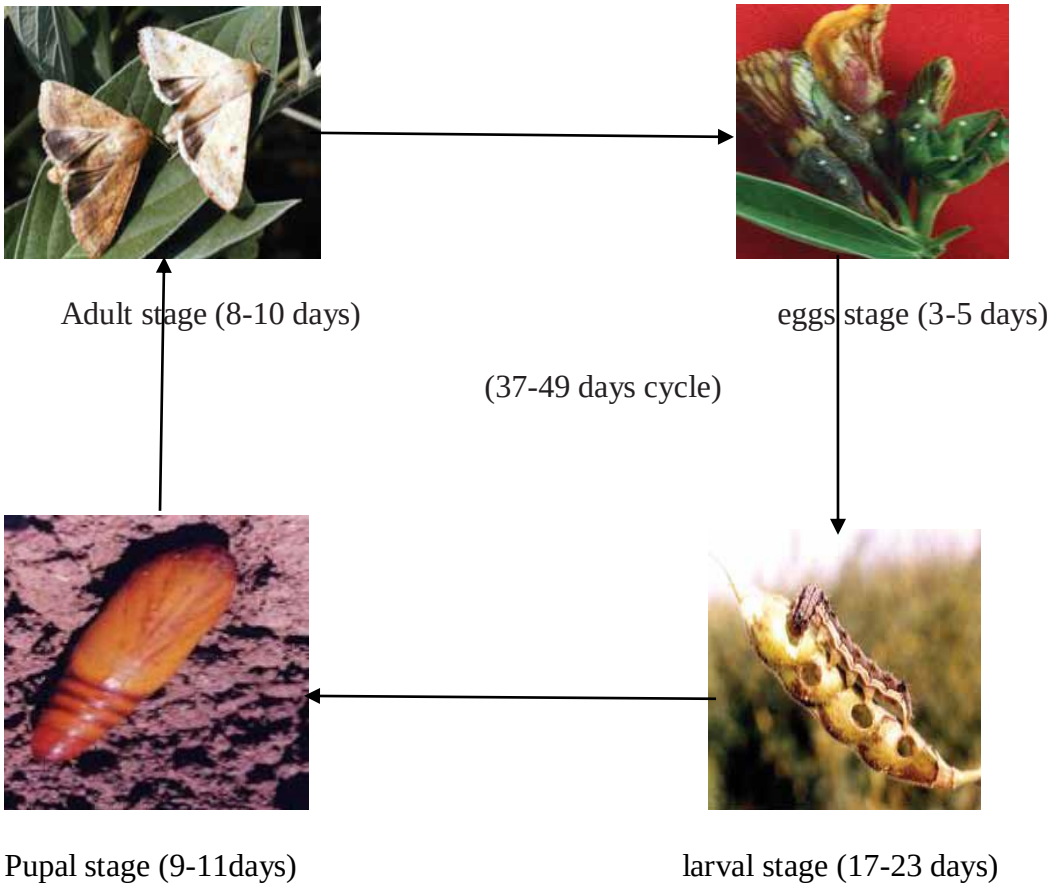


Figure 1: Pod borer lifecycle (37 to 49 days) as adapted from Sharma (2010).

2.5.3 The pod fly (Melanagromyza chalcosoma)

It is a small black fly that lays eggs through the walls of developing pods. The maggots (white in color and about 3 mm long) feed inside the green seed. The brown barrel shaped pupa is formed inside the pod but outside the seed. There are no obvious external symptoms of attack till the fully-grown maggot chews holes in the pod walls leaving a window through which the flies emerge after pupation in the pod. Damaged seeds are of no value. The pod fly causes most damage on pigeonpea maturing during cool weather and pigeonpea planted at altitudes higher than 500 m above sea level (Faujdar and Oswalt 1992). The lifecycle takes about 22-27 days as summarized in figure 2. Single fly is reported to lay 80-100 eggs which, incubate for 3 days and hatching maggot bores into the pod and feeds on developing seed. The pupal period may last for 7-15 days. All the immature stages remain within the developing pod and it is very difficult to monitor the pest without damaging the pod.

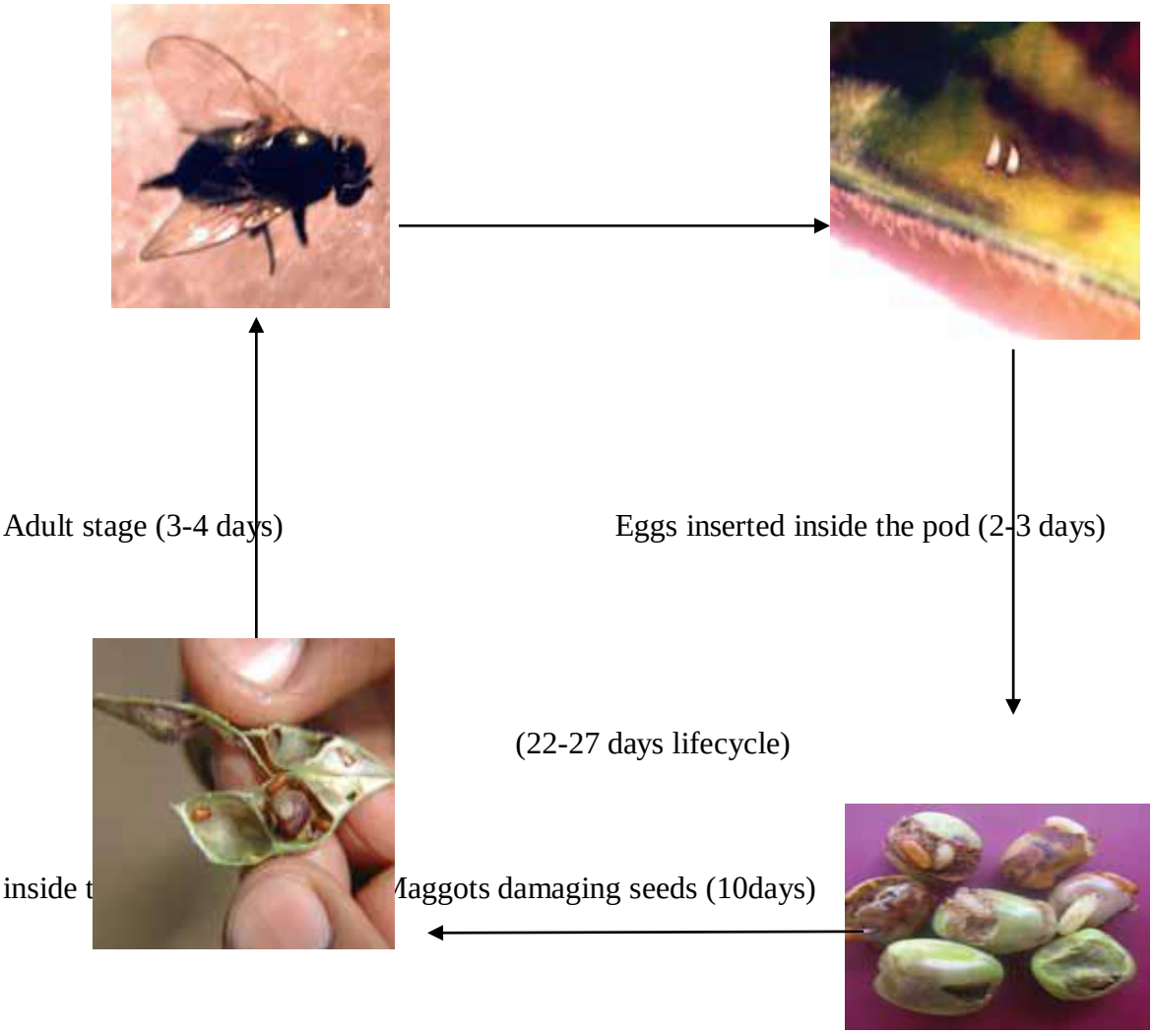


Figure 2: Pod fly lifecycle (Sharma, 2010)

2.6 Mechanism of resistance in pigeonpea against insect pests

Multiple types of resistance (Antixenosis, antibiosis, tolerance and escape) have been reported against insect pests in pigeonpea. Antixenosis are host plant characters responsible for non-preference of the insects for shelter, oviposition and feeding. Antixenosis denotes presence of morphological or chemical factor which alter insect behaviour resulting in poor establishment of the insect. Oviposition non preference is one of the major components of resistance to these pests in pigeonpea (Kumari *et al*., 2006; Sharma *et al*., 2001). Antibiosis are adverse effect of the host plant on the biology

(survival, development and reproduction) of the insects and their progeny due to the biochemical and biophysical factors present in it. It is manifested by larval death and abnormal larval growth.

Tolerance is the ability of plant to grow and yield despite pest attack. It is generally attributable to plant vigor, regrowth of damaged tissue, to produce additional branches, compensation by growth of neighboring plants. Finally escape is absence of infestation or injury to host plant due to transitory process like incomplete infestation. This pertains to few individuals of host. Acid exudates (PH 1.3) with a high concentration of malic acid secreted from the glandular hair on the leaves, stems and pods have been suggested as a marker for resistance (Rambold, 1981). All four known mechanisms of resistance are reported in pigeonpea against H. armigera. Antibiosis effects are expressed in terms of weight and size of insects into diapause. An experiment conducted at ICRISAT, Patencheru showed that H. armigera females did not lay eggs on the wild relatives *Cajanus playcarpus, C. scarabaeoides and C. sericaus* whereas egg laying was seen in cultivated pigeonpea. Pigeonpea pods produce chemicals from glandular trichomes that act as a phagostimulant to Helicoverpa larvae. Pods of C. *scarabaeoides* have a dense shoot and non- glandular trichomes that act as physical barriers to feeding by the young larvae (Green et al., 2002).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Description of the experimental sites

The experiments were carried out in three varied sites; (KALRO Perkerra-Marigat, ATC Koibatek in Baringo County and Fluorspar in Elgeyo Marakwet County (Appendix V) during the 2014 April- October long rain cropping season.

Kenya Agricultural and Livestock Research Organization (KALRO) Perkerra– Marigat is situated at 0°28'0" N and 36°1'0" E. It has an altitude of 1067m above sea level (A.S.L) with an average annual rainfall mean of 654mm. It falls in agro ecological zone 5 (LM 5) with an average temperature of the area range from 16-34^{0}C. Their soils are fluvisols of sandy/silt clay loam texture, slightly acid to slightly alkaline, highly fertile with adequate, P, K, Ca, Mg but low N and C (Jaetzold and Schmidt, 1983). National Youth Service (Chepsirei), Fluorspar falls in the agro-ecological zone 6 (LM6) and situated at 0°13'0" N and 35 °35'S with sandy loam soils, temperature range from 16 -30^{0}C, altitude 900-1500 m above sea level and receiving rainfall ranging from 400 to 800 mm per annum but the rainfall is usually erratic and unreliable (Jaetzold and Schmidt, 1983). ATC Koibatek is located 1°35'S, 36 °66'E at an elevation of 1890 meters A.S.L in agro-ecological zone UM4, with low agricultural potential. Average annual rainfall is767mm and mean temperature ranges between 18.2-24.3°C. Mean minimum and maximum temperatures are 10.9 °C and 28.8°C respectively. Soils are Vitric andosols with moderate to high soil fertility, well drained deep to sandy loam soils (Jaetzold and Schmidt, 1983).

3.2 Plant germplasm

Sixteen selected medium duration Pigeonpea genotypes sourced from ICRISAT were evaluated in this study during the long rains of April- October 2014 cropping season. The genotypes are yield elite lines as listed below (Table 2). Two among the 16 genotypes resistant ICEAP 00850 and susceptible KAT 60/8 are commercial varieties and were used as checks.

Table 2: List of 16 medium duration pigeonpea genotypes used in the study during long rains of April- October 2014 cropping season indicating their source and status (released or not)

Germplasm name	Source	Status
ICEAP 01147	ICRISAT	Pre release
ICEAP 01179	ICRISAT	Pre release
ICEAP 1147-1	ICRISAT	Pre release
ICEAP 01159	ICRISAT	Pre release
ICEAP 00554	ICRISAT	Pre release
ICEAP 01541	ICRISAT	Pre release
ICEAP 00540	ICRISAT	Pre release
ICEAP 00911	ICRISAT	Pre release
ICEAP 00902	ICRISAT	Released in Kenya (Egerton University)
ICEAP 01150	ICRISAT	Pre release
ICEAP 00068	ICRISAT	Released (Tanzania)
ICEAP 00557	ICRISAT	Released(Tanzania, Mozambique and Malawi)
ICEAP00850-R	ICRISAT	Released in Kenya (Leldet seeds)
ICEAP 0079-1	ICRISAT	Pre release
ICEAP 1154-2	ICRISAT	Pre release
KAT 60/8- S	ICRISAT	Released in Kenya (KALRO) and Uganda)

3.3 Description of experiments

The study involved three experiments: evaluation of 16 pigeonpea genotypes in three sites for yield performance and adaptability (Expt. I), evaluation of 16 genotypes for insect pest resistance /tolerance in three sites as experiment (Expt. II) and Expt. III which involved assessment of genetic diversity at molecular laboratory ICRISAT Nairobi .

3.3.1 Field layout and experimental designs

The field study involved evaluation of 16 genotypes of pigeon pea genotypes for one season in each site. Planting was done at the onset of the rains. The test entries were evaluated in a randomized complete block design (RCBD), in three replicates. Each plot consisted of 5 rows measuring 5 m in length, spaced 75 cm between the rows (inter-row) and 25 cm between the plants (intra-row). The evaluation was done under open field using natural pest population and rain fed conditions for yield and resistant/tolerance determination (EXP I &II).

3.4 Cultural practices

3.4.1 Land preparation

Land preparation was done in February and March 2014 in the three sites using a tractor for the first and second ploughing and then harrowing was done by hand.

3.4.2 Weeding

Weeding was done mechanically by hand and no fertilizers were applied in any site. To give the genotypes equal conditions, this practice was carried out the same day in all the

plots per site. The first weeding was 3-4 weeks after sowing, prior to flowering and podding at each site.

3.4.3 Pest management

Observations were monitored and recorded each day and the fields were guarded against wild animals especially in Marigat. Incidences of pests were monitored at different developmental stages (flowering and podding) as shown in (Plate 1).

Plate 1: Inspection of pests in the field

Source: Author (2014). Marigat field experimental site

3.4.4 Harvesting

The crop was harvested twice when the pods were dry; this is because medium duration genotypes have indeterminate growth habit. Fifty pods per plot were harvested randomly from the three middle rows for seed damage and number of seeds per plant assessment.

Pods were harvested from plants in the three middle rows (net plot) for grain yield evaluation. Finally, bulk harvesting was done for the remaining rows per plot.

3.5 Model

General linear model for three locations.

$$Y_{ijk} = \mu + b_i + e_j + g_k + eg_{jk} + \varepsilon_{ijk}$$

Where;

Y_{ijk} – plot observation

μ- Grand mean (effect for the whole experiment)

b_i – Added effect due to i^{th} blocking

e_j – Added effect due to j^{th} Environment

g_k – Added effect due to k^{th} Genotype

eg_{jk} – Added effect due to j^{th} *k^{th} Environment x Genotype interaction

ε_{ijk} – Error term

3.6 Data collection

The data was collected based on the parameters measured for each experiment as described below;

3.6.1 Experiment II: Evaluation of pigeonpea genotypes for yield performance and adaptation

Fifteen plants from three middle rows of each plot were tagged randomly for evaluation. The following data on yield and yield components was collected.

Days to 50% flowering: Days from date of emergence until 50% of the plants in a plot have at least one open flower.

Days to 75% maturity (days to physiological maturity): Days from date of emergence until 75% of the pods are physiologically mature (changing colour to yellow)

Plant height: Height of plants (cm) is recorded at maturity from the base of the plant to the top of five randomly selected plants in each plot and the average is recorded.

100 seed weight: Manual counting of 100 seeds from the bulk harvest of each plot then weighing (g) on an electronic balance.

Number of pods per plant: An actual count of number of pods on five randomly selected plants in each plot and the average is recorded.

Number of seeds per pod: Actual counts of number of seeds in 10 randomly selected pods from each plot and the average is recorded.

Number of secondary branches: Actual count of number of branches on five randomly selected plants borne on primary branches in each plot and the average was recorded.

Net plot yield (t/ha): Grain yield recorded from the net plot (2.25 M^2) that was cleaned dried and weighed (g) at 7-8% moisture content then converted to tons per hectare.

3.6.2 Experiment II: Screening for resistance to Insect pest complex by determining incidence and severity levels.

a) Larval and egg counts of pod borer (*Helicoverpa armigera)*

Larval population and eggs were counted on five random plants in each plot at 50% and 75% pod maturity. Pod borer eggs are spherical, white and later becoming greenish in color and usually laid singly on buds, leaves and flowers. Sucking bug eggs are smooth, shiny and brown in colour and laid normally in clusters form while for the pod fly, they are brown and laid singly on the pods and on leaves as indicated in (Plate 2).

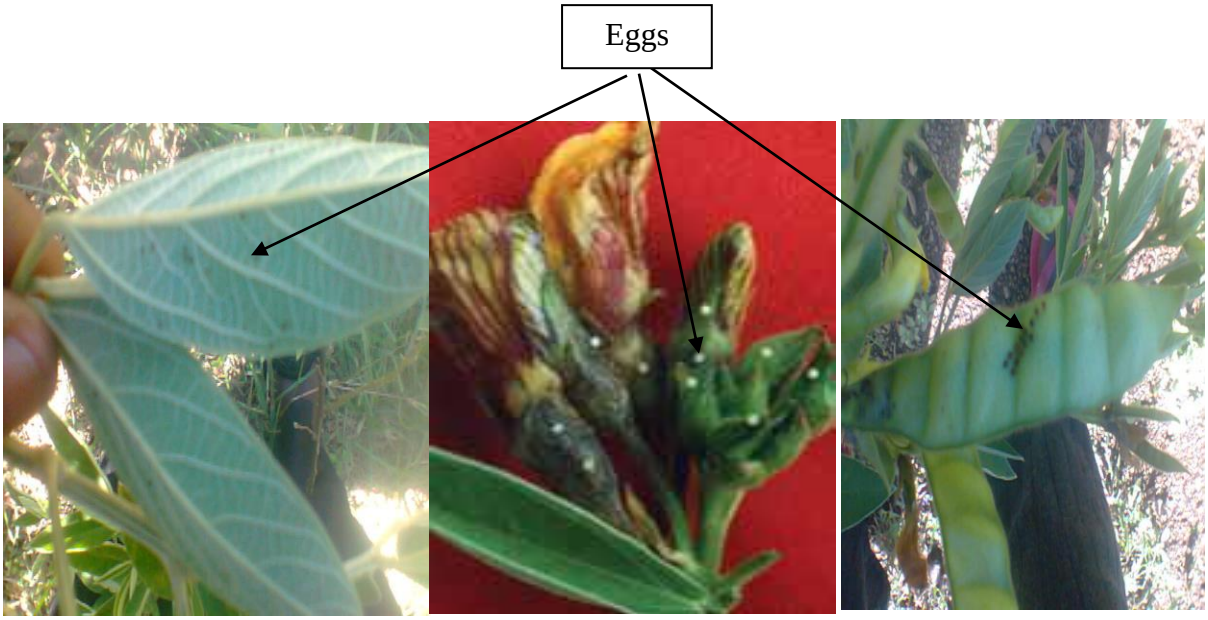


Plate 2: Eggs of Pod fly, pod borer and sucking bug from left to right

Source: Author (2014). Field experiment

Pod borer larvae (caterpillar) colouring is greenish and yellow to red-brown and the head is yellow with several spots though may camouflage depending on the habitat (colour of the flower, stem and leaf) as shown in (Plate 3).

Plate 3: Pod borer larvae attacking flower bud

Source: Author (2014). Field experiment

b) Pod damage by pod borer (*H, armigera*) and pod fly (*Melanagromyza chalcosoma*)

Pod- damage assessment was carried out at physiological maturity when the pods have turned colour from green to brown but not dry. Pods from 5 randomly tagged plants in the three middle rows of each plot were examined. Pod damage by pod borer was identified by the presence of round, large bored holes in pods and a pin shaped hole and immature drying for pod fly as presented in (Plate 4). Severity of damage and incidences of the insect based on symptoms and number of pods affected was established by rating them using 1-9 scale

Plate 4: Pods damaged by pod fly (A) and pod borer (B)

Source: Author (2014). Field experiment

The percentage pod damage was calculated based on Ojwang, (2010) formula demonstrated below.

$$\text{Pod damage \%} = \frac{\text{Number of pods damaged}}{\text{Total number of pods examined}} \times 100$$

$$\text{Seed damage \%} = \frac{\text{Number of seeds damaged}}{\text{Total number of seeds examined}} \times 100$$

c) Seed damage by sucking bug, pod fly and pod borer

Ten pods from each 5 plants from the three inner rows were harvested randomly, threshed and carefully cleaned. Seeds damaged by insect pests and wholesome seeds were separated, counted, recorded and percentage seed damage was calculated per plot. Seeds damaged by sucking bug become shriveled and develop dark patches, the injury being similar to that of drought stress. Seeds damaged by pod fly show presence of white maggots and a brown

puparium formed between the remnant of the seed and the pod wall and those damaged by pod borer can be identified by presence of round large holes on the seed as indicated in plate 5.

Source: Author (2014)

Plate 5: Seed damaged by pod fly (A), sucking bug (B) and pod borer (C)

Source: Author (2014). Sampled seeds from field experiments

Pod and seed damage was assessed using a nine point scale to rate the severity of the insect pest complex on genotypes based on Lateef and Reed (1990) formula as shown below,

$$\text{Pest resistance rating (\%)} = \frac{\text{(P.D. rating of check – P.D rating of test entry) x 100}}{\text{P.D rating of check}}$$

P.D = Mean of pod/seed damage (%).

The pest resistance percentage was converted to 1 to 9 scale as described (Table 3).

Table 3: A quantitative 1-9 point scale for resistance on genotypes

Pest resistance (%)	Resistance/susceptibility rating		
100	1	↑	Increasing resistance
75 to 99	2		
50 to 75	3		
25 to 50	4		
10 to 25	5		
-10 to 10	6		Equal to check
-25 to -10	7		
-50 to -25	8		
-50 to less	9	↓	Increasing susceptibility

3.6.3 Experiment II1. Assessment of genetic diversity of 16 pigeonpea genotypes using 20 SSR markers.

The assessment was carried out in ICRISAT Nairobi. Two seeds from individual 16 genotypes were planted in root trainers measuring 5 x 6 x 19cm filled with soil sourced from Karura forest in Nairobi. After two weeks of planting (13 days), one leaf from each plant was sampled and taken to the laboratory for DNA extraction using CTAB protocol (Mace *et al*., 2003).

3.6.3.1 Sampling

Sampling was done two weeks after germination from two individual seedlings of each genotype to ensure statistically sound representation from each accession as displayed in (Plate 6). One leaf weighing between 100-150g was sampled from each plant using a pair of scissor and was cut into small pieces to ease maceration of the samples and increase the surface for detergent activity then put into 2ml Eppendorf® tubes containing two stainless balls using a forcep. This was done on ice flakes to avoid degradation of the DNA

.

Plate 6: Sampling pigeon pea leaves for genomic DNA extraction from 13-day old Seedlings

Source: Author (2015). ICRISAT- Nairobi Glass house

3.6.3.2 Grinding

A volume of 450µ1 of pre-heated (65^0C) extraction buffer containing 3% (w/v) CTAB, 1.4M NaC1, 0.2% (v/v) β -Mercaptoethanol and 20mM EDTA was added to the leaf samples. Grinding was carried out using Geno-grinder (tissue lyser II) for 12 minutes. The macerated samples were then incubated for 30 minutes at 65^0C in a water bath with occasional mixing after 10 minutes.

3.6.3.3 Extraction

Solvent extraction was done by adding 450µ1 of Chloroform Isoamyl alcohol (CIAA) in a ratio of 24:1 respectively to each sample followed by thorough mixing by inverting the tubes two to four times. The tubes were centrifuged at 14000 rpm for 10 minutes at 4ºC using Eppendorf centrifuge 5417R machine. Approximately 400µ1 of the upper aqueous layer was transferred into fresh clean tubes. 280 µ1 of cold isopropanol (0.7 volumes) was added and gently mixed to precipitate the DNA. The samples were kept at -80 ºC for 1 hour. The tubes were centrifuged at 14000rpm for 20 minutes at 4^0C using the Eppendorf centrifuge 5417R machine. The supernatant was decanted and the DNA pellet washed with 70% ethanol and air-dried for 30 minutes in a laminar flow- hood.

3.6.3.4 RNA treatment

To each pellet, 250µl of low salt TE buffer (1mM Tris and 0.1mM EDTA pH 8.0) with 5µl of RNase was added and incubated at 37 ºC for 30 minutes in a water bath to digest RNA.

3.6.3.5 Solvent extraction

Two hundred and fifty (250) µl of Chloroform Isoamyl alcohol (CIAA) in a ratio of 24:1 was added to each sample and inverted twice to mix. The tubes were then centrifuged at 14000 rpm 4^0C using the Eppendorf centrifuge 5417R machine for 5 minutes to separate the DNA and the supernatant discarded. The aqueous layer (about 200µl) was transferred into clean tubes. 400µl of absolute Ethanol and 1/10 volume of 3M sodium acetate solution (pH 5.2) was added to each sample followed by incubation at -20ºC for overnight to allow precipitation. The tubes were then centrifuged at 14000 rpm using the

Eppendorf centrifuge 5417R machine for 25 minutes and the supernatant discarded. The DNA pellet was then washed with 200µl of 70% ethanol and the DNA pellet was air-dried for one hour and then re-suspended in 100 µl of low salt TE (10mM Tris, Im MEDTA pH 8.0) buffer.

3.6.3.6 DNA Quality check and quantification

The purity and quality of the DNA was verified by electrophoresis on a 0.8% (w/v) agarose gel stained with 5ul/100ml Gel Red $^{(R)}$ (Biotium inc. USA) for 30 minutes at 80volts. 20ng/ µ1 and 50 µ1 Lambda (ĥ) phage DNA was used as the standard. The concentration of the samples was determined by comparing band sizes and intensities of the test DNA with those of standard ĥ DNA. The good quality DNA shows a clear band while a poor quality shows a smear. A mixture of 1µl of DNA, 1µl of type1 water and 1µl of loading dye (25mg bromo phenol blue (0.25%), 25mg xylene xyanol (0.25%), 4g sucrose (40%), making a total volume of 3µl was electrophoresed in a 0.5 x TBE buffer (0.1M Tris base, 0.1M boric acid and 0.02M EDTA; pH 8.0). The fragments were visualized under UV light and photographed using a Scion camera (Scion Corporation, USA).The DNA quantity was determined by Qubit$^{(R)}$ 2000 (Appendix II). The DNA samples were then diluted to 20ng/µl based on the Qubit records and DNA stock was prepared and stored at 20^0c until use. The diluted genomic DNA was used for PCR.

3.6.3.7 Primer selection

40 SSR primers were selected from a pool of 288 that were well distributed across the 11 chromosomes of pigeonpea selected from consensus map constructed in 2009. The SSR markers were chosen based on genome position, repeat size (ranging from

dinucleotide to hexanucleotides repeats), polymorphism and the number of previously reported alleles ranging from two to six (Bohra et al., 2012).

3.6.3.8 Primer optimization

A pair of 40 SSR primers was optimized to avoid non-amplification. Eight primer set amplified at an annealing temperature of 59^0C. Thirty two primer set was then optimized using touch down PCR amplification procedure (60-52^0C) where twenty four primers amplified. Twenty primer pairs producing good and polymorphic amplification products were selected for final analysis (Appendix IV).

3.6.3.9 PCR Amplification

PCR amplification was optimized and conducted in a reaction of 5X My Taq reaction buffer (5Mm dNTPs, 15Mm $MgCl_2$, stabilizers and enhancers), 2µM labeled M13 fluorescent dye, 2µM M13-forward primer, 2µM reverse primer, 5U My Taq polymerase (Sib Enzyme Ltd, Russia) and 20ng template DNA. The volume for each PCR was topped to 10µl with sterile water. The concentrations and volumes for components in each PCR are shown in (Table 4).

Table 4: Concentrations and volumes for each PCR reagent in a single PCR reaction

PCR Component	Stock Concentration	Final Concentration	Volume for one PCR reaction in µl
My Taq reaction buffer	5x	2x	2
M13 label	2µM	0.9 µM	0.9
Forward primer	2µM	0.6 µM	0.6
Reverse primer	2µM	1.5µM	1.5
MyTaq DNA Polymerase	5U	0.04U	0.04
Sterile Water			2.96
Template DNA			2
Total volume			10

Reactions were performed on a thermo cycler (GeneAmp PCR system 9700®, Applied Biosystems, USA) in a 384-plate with initial denaturation of 95^{o}C for 5 minutes, followed by 35 cycles of 94^{o}C for 30 seconds, 59^{o}C for 1 minute and 72^{o}C for 2 minutes followed by final elongation at 72^{o}C for 20 minutes. PCR conditions were optimized by changing the annealing temperatures based on touch down PCR procedure as shown in Table 5 to ensure that all SSR makers were amplified. All the forward primers contained an M13 tag (CACGACGTTGTAAAACGAC to allow incorporation of a fluoro chrome during the PCR process (Shuelke, 2000). Labelled fluorescent reverse primer was also incorporated in each reaction, consisting only of the M13 sequence, which subsequently generated labeled PCR amplification products for capillary electrophoresis that also has the M13 sequence incorporated at the 5' end in the final PCR product (Shuelke, 2000). The fluorescent labels used

were 6-Carboxyfluorescein (6-FAM), NED®, VIC® and PET® (Life Technologies Corporation, Carlsbad, USA). After the PCR, a few samples were randomly selected and their PCR products run on a 2.0% agarose gel at 100 voltages for 45 minutes using 0.5X TBE buffer to check for amplification. The PCR products bands were scored using 100 bp molecular size ladder.

Table 5: PCR reaction set up

Step	**Temperature**	**Time**	**Cycles**
Initial denaturation	95^0c	5 min	1
Denaturation	95^0c	15 sec	8 (60-52^0c)
Annealing	60^0c	30 sec	
Extension	72^0c	20 sec	
Denaturation	95^0c	15 sec	28
Annealing	56^0c	45 sec	
Extension	72^0c	30 sec	
Final extension	72^0c	12 min	
Holding	15^0c	infinity	

.6.3.10 Capillary electrophoresis

PCR products were co-loaded post-PCR based on amplification intensity and fluorescent label on a 384 well to reduce the unit cost. During co-loading, 2 µl of

PCR products labeled with NED or PET and 1 µl of those labeled with VIC or FAM were loaded on the wells. 48 µl of LIZ and 3200 µl of highly deionized form amide (Hi Di) were added. The Hi Di help to keep the single stranded DNA in its shape while LIZ is a dye used by fragment analysis machine to recognize samples. DNA fragments were denatured at a 95^0C for 5 minutes. During capillary electrophoresis the amplification products passed through a detection window and a light excited the fluorescent dye.

3.7 Data analysis

Data from field evaluation was subjected to analysis of variance using SAS version 9.1. Treatment means were separated using LSD at $P \leq 0.05$ and Simple correlation coefficient (r) was carried out using Pearson's correlation.

Molecular analysis was done by fluorescence thereafter visualized using a computer programme Gene Mapper version 4.0 as relative fluorescent unit (RFU) against fragment length in base pairs. An allele was scored for each data point as length in base pairs at the highest RFU peak. Alleles were called and scored using the Gene Mapper 4.0 (Applied Biosystems, USA) and allelic data for each marker analyzed with Power Maker V3.25 (Liu and Muse, 2005).

Power Marker was used to generate summary statistics such as allele number, gene diversity, polymorphic information content, heterozygosity and number of major alleles. Polymorphism information content (PIC) measures usefulness of each marker in distinguishing one individual from another. Polymorphic information content was calculated using the formulae;

$$PICt = 1 - \sum_{u=1}^{k} Plu2 - \sum_{u=1}^{k-1} \sum_{v=u+1}^{k} 2\ \mathrm{P}^2 lu \mathrm{P}_{lv}$$

Where P*lu* is the allele population frequency at the *l*th locus and P*lv* is the genotype population frequency at the *l*th locus.

DARwin software calculated dissimilarity using the formulae below

$$dij = 1 - \frac{1}{L}\sum_{l=1}^{L} m1/\pi$$

Where *dij* is the dissimilarity between units *i* and *j*, *L* is the number of loci, π is the ploidy and *ml* is the number of matching alleles for locus *l*. The software was also used to display dendograms using the dissimilarity matrix calculated by simple matching. The distances were computed for microsatellite data and trees constructed using the neighbor- joining method.

CHAPTER FOUR

RESULTS

4.1: Yield performance and adaptation of 16 pigeonpea genotypes

Grain yields varied significantly ($P \leq 0.01$) among genotypes in different sites. In Marigat, the mean grain yield for the genotypes ranged from 0.2-0.8 t/ha with an average yields of 0.4 t/ha (Table 6). Genotype ICEAP 01147 recorded the highest yield of 0.8t/ha .The genotypes also varied significantly ($P \leq 0.05$) in days to 50% flowering, height at maturity and 100 seed weight (Table 6). Similarly, significant difference ($P \leq 0.01$) was recorded in days to physiological maturity, number of secondary branches and number of pods per plant as shown in (Table 6). However, there was no significant difference in number of seed per plant. ICEAP 01147 flowered earlier just like the resistant (ICEAP 00850) and susceptible (KAT 60/8) check. ICEAP 00902 recorded the highest number of pods per plant while ICEAP 00557 had highest number of secondary branches. Four genotypes ICEAPs 1147-1, 00911, 00557 and 00850 (the resistant check) matured late (135-141 days) while ICEAP 0079-1 matured early (125 days) as KAT 60/8 (susceptible check) at 127 days as presented in (Table 6).

Table 6: Table of means for yield and yield components for 16 pigeonpea genotypes in Marigat during April - October 2014 cropping season

Genotype	GY (t/h)	D50% F	DPM	HM	Branches	PPP	SPP	100 SW
ICEAP 01147	**0.8a**	105.7c	126.7ef	135b-d	12.3b	152a-d	4a	12.8a-c
ICEAP 01179	0.7b	107.7a-c	128c-f	129.7b-d	9b	163.7a-d	4a	13.4a
ICEAP 1147-1	0.6b	116ab	135a-c	157ab	12.0b	197.7a	3.7a	13.3ab
ICEAP 01159	0.3c-e	**117a**	133.3b-e	128cd	12.0b	68.7e	4a	12.5a-c
ICEAP 00554	0.2e	109.3a-c	128.7b-f	148.3a-c	11.3b	127cd	3.7a	11.6bc
ICEAP 01541	0.2e	105.7c	132.3b-e	128.7b-d	10.3b	143.3a-d	3.7a	11.5c
ICEAP 00540	0.3c-e	110.7a-c	134b-d	124cd	10.7b	107.7de	3.3a	12.4a-c
ICEAP 00911	0.4c	108.3a-c	135.3a-c	118.3d	11.7b	133b-d	3.7a	12.1a-c
ICEAP 00902	0.3cd	115a-c	130.3b-f	127.7cd	**13.0b**	**198a**	4a	12.3a-c
ICEAP 01150	0.3de	109.3a-c	131 b-f	133.7b-d	10. 3b	180a-c	3.7a	12.8a-c
ICEAP 00068	0.4c	114.3a-c	130.7b-f	134.3b-d	9.7b	137.7b-d	4a	**13.8a**
ICEAP 00557	0.3c-e	115a-c	135.7ab	131.7b-d	23a	188.3ab	3.7a	13.2ab
KAT 60/8^{S}	0.6b	107.3bc	126.7ef	149a-c	8.7b	138.3b-d	4a	13.2a-c
ICEAP 00850^{R}	0.3cd	115a-c	**141.3a**	**174a**	11.3b	131.3b-d	3.3a	12.4a-c
ICEAP 0979-1	0.4c	106.7bc	125f	128.3cd	10.0b	130.7b-d	3.7a	13.6a
ICEAP 1154-2	0.2de	106.3bc	127.7d-f	136b-d	11.0b	119.3de	3.7a	13.5a
Genotype	****	*	****	*	****	****	**NS**	*
grand mean	**0.4**	**110.55**	**131.35**	**136.48**	**8.7**	**144.79**	**3.75**	**12.78**
CV%	**16.9**	**5**	**3.2**	**12.4**	**11.4**	**23.9**	**11.7**	**8.3**
LSD	**0.11**	**9.37**	**7.21**	**2.04**	**8.01**	**57.9**	**0.73**	**1.79**

Key: Means followed by a different letter within a column are significantly different ($p \leq 0.05$) where $p \leq 0.05$=*; $p \leq 0.01$=** and $p \leq 0.001$=***. Genotypes with superscript (S and

R) are susceptible and resistant checks. NS=not significant; CV= Coefficient Variation and LSD- least significant difference.GY-grain yield per plot, D50% F-days to 50% flowering, DPM- days to physiological maturity , HM- height of the plants at maturity , Branches- number of secondary branches, PPP- number of pods per plant, spp- number of seeds per pod and 100 SW- weight in grams for 100 seeds.

Genotypes varied significantly among the traits tested in Koibatek and their results are presented in (Table 7). Genotypes exhibited significant variations ($P \leq 0.01$) in grain yield, days to 50% flowering and not significantly different in number of seed per plant and 100 seed weight but significant variation ($P \leq 0.01$) in days to physiological maturity, height at maturity, number of secondary branches and number of pods per plant was also recorded (Table 7). Grain yield means ranged from 1.7 to 3.2 t/ha with an average yield of 2.45t/ha. Eight genotypes (ICEAPs 1147-1, 01159, 00554, 01541, 00911, 00850, 0979-1 and 1154-2) recorded higher yields ranging from 2.6-3.2 t/ha (Table 7). ICEAP 00850- resistant check differed significantly from the rest by flowering late after 111 days while ICEAP 0979-1 flowered earlier after 99 days resembling the susceptible check- KAT 60/8 (103 days). The genotypes matured within a range of 131-138 days with ICEAPs 00540 maturing early (131 days) and 01179 maturing late after 138 days (Table 7). ICEAP 00902 recorded the highest number of branches (12.3) similar to the resistant check- ICEAP 00850 (10) while ICEAP1147-1 recorded the highest number of pods per plant as shown in (Table 7).

Table 7: Table of means for yield and yield components for 16 pigeonpea genotypes in Koibatek during April - October 2014 cropping season

Genotype	GY(t/h)	D50% F	DPM	HM	Branches	PPP	SPP	100 SW
ICEAP 01147	2.1e-h	102.3fg	131.7bc	145.7a	8.7a-c	230bc	5a	15.3a
ICEAP 01179	1.7h	102.3fg	**138a**	144.3ab	9.7a-c	170.3c	4.3a	14.4a
ICEAP1147-1	2.9a-c	102.3fg	135.3a-c	152.7a	12.0ab	**313a**	4.3a	15.4a
ICEAP 01159	2.7a-e	106.3b-f	137.3ab	143.7ab	7.7c	169.7c	4.7a	15.1a
ICEAP 00554	2.6a-f	108a-d	133.3a-c	145a	8.7a-c	227.7bc	5a	15.4a
ICEAP 01541	2.6a-f	105.3b-f	133.3a-c	142.3ab	9.3a-c	227.7bc	5a	14.5a
ICEAP 00540	2.3c-h	108a-d	131.3c	129.3b	7.0c	255.3ab	5a	15.1a
ICEAP 00911	2.9ab	105c-f	132bc	143.3ab	8.3bc	245a-c	4.3a	14.4a
ICEAP 00902	2.2d-h	108.3a-c	133.3a-c	140.7ab	**12.3a**	215bc	5a	14.3a
ICEAP 01150	1.9gh	103.7d-g	132.7a-c	145.3a	9a-c	199bc	4.3a	15a
ICEAP 00068	2.1f-h	107.7a-e	131.7bc	139ab	6.7a-c	252a-c	4.7a	14.3a
ICEAP 00557	2.5b-g	109.7ab	131.3c	142.7ab	8.3bc	190bc	4.3a	15.5a
KAT 60/8^{S}	1.7h	103.3e-g	132bc	**147a**	8.7bc	225.3bc	5a	14.4a
ICEAP 00850^{R}	2.9a-c	**111a**	132.7a-c	144.7a	10.0a-c	269.3ab	4.7a	14.8a
ICEAP 0979-1	2.8a-d	99.3g	131.7bc	138.7ab	9.7a-c	197.7bc	5a	15.1a
ICEAP 1154-2	**3.2a**	102.7fg	136a-c	145a	9.3a-c	239.7a-c	4.3a	15.6a
Genotype	****	**	*	*	*	*	NS	NS
grand mean	2.45	105.33	133.35	143.08	9.06	226.67	4.69	14.93
CV%	16.2	2.4	2.4	6.4	24.78	21.9	8.8	5.6
LSD	0.66	4.4	5.52	15.63	5.05	80.26	0.69	1.41

Key: Means followed by a different letter within a column are significantly different ($p \leq 0.05$) where $p \leq 0.05$=*; $p \leq 0.01$=** and $p \leq 0.001$=***. Genotypes with superscript (S

and R) are susceptible and resistant checks. NS=not significant; CV= Coefficient Variation and LSD- least significant difference.GY-grain yield per plot, D50% F-days to 50% flowering, DPM- days to physiological maturity , HM- height of the plants at maturity , Branches- number of secondary branches, PPP- number of pods per plant, Spp- number of seeds per pod and 100 SW- weight in grams for 100 seeds.

Fluorspar genotypes also differed significantly in parameters measured as shown in (table 8)**.** Significant variations ($p \leq 0.01$) among the genotypes was noticed in grain yield, days to 50% flowering and days to physiological maturity while pods per plant and 100 seed weight showed no significant difference among the genotypes. Also the genotypes varied significantly ($p \leq 0.05$) in height at maturity, number of secondary branches and number of seeds per plant (Table 8). The grain yield recorded a range of 0.1- 0.4 t/ha with three genotypes recording the highest yield (ICEAPs 01159, 0979-1 and 00850-resistant check) as presented in Table 8. ICEAP 00554 flowered early after 113 days while ICEAP 00540 flowered late after127 days and reaching physiological maturity late after 147 days. ICEAP 1154-2 matured at a height of 94cm and is the shortest among all the genotypes but closer to the resistant check (107 days) Table 6. ICEAP 00902 recorded higher number of branches (3.7) and ICEAP 1154-2 recording the lowest number of 1.7 (Table 8).

Table 8: Table of means for yield and yield components for 16 pigeonpea genotypes in Fluorspar during April - October 2014 cropping season

Genotype	GY(t/h)	D50% F	DPM	HM	Bran	PPP	SPP	100SW
ICEAP 01147	0.2cd	117d-g	137d-g	122a-c	3abc	32a	4.7a-c	12.4a
ICEAP 01179	0.2bc	116fg	132.7gh	124a	3.3ab	33a	5ab	13.2a
ICEAP1147-1	0.3ab	117.3d-g	131hi	118a-d	2.7a-c	35.3a	5ab	14.1a
ICEAP 01159	**0.4a**	116.7e-g	127i	120a-c	3a-c	36a	4.7a-c	12.9a
ICEAP 00554	0.1d	**113g**	135e-h	102.3de	2.7a-c	23.3a	4.3bc	12.4a
ICEAP 01541	0.1d	124ab	144a-c	111.7a-d	2.7a-c	33a	4.7a-c	12.7a
ICEAP 00540	0.1d	**127.7a**	**147.7a**	107.3b-e	3.3ab	33.7a	4.7a-c	13.7a
ICEAP 00911	0.1d	120.7b-f	140.3b-d	118.7a-c	2.7a-c	37.7a	4.7a-c	12.5a
ICEAP 00902	0.1d	123ab	140b-e	115.3a-d	**3.7a**	40a	4.7a-c	12.5a
ICEAP 01150	0.2bc	118e-f	138e-f	**123a**	2bc	32.7a	5ab	12.7a
ICEAP 00068	0.1d	123ab	140.3b-d	109.3a-e	2.7a-c	38a	**5.3a**	13.4a
ICEAP 00557	0.1d	121.7b-d	137.7d-g	109.3a-e	2.3a-c	18a	4.7a-c	13.1a
KAT 60/8^{S}	0.2bc	117d-g	133.7f-h	113.3a-d	1.7c	29a	4.7a-c	13.2a
ICEAP 00850^{R}	**0.4a**	122.3bc	139.3c-e	107c-e	3ac	40.7a	5ab	13a
ICEAP 0979-1	**0.4a**	121.3b-e	144.7ab	117.3a-d	2.7bc	27.7a	4.7a-c	12.5a
ICEAP 1154-2	0.1d	119.3b-f	137d-g	94.3e	1.7c	20.7a	4c	13.5a
Genotype	**	**	**	*	*	NS	*	NS
grand mean	0.2	119.88	137.83	113.31	2.67	31.92	4.73	12.99
CV%	24.08	2.3	2.3	8.3	30.58	43.12	10.1	9.9
LSD	0.08	4.71	5.3	15.93	1.36	22.95	0.8	2.16

Key: Means followed by a different letter within a column are significantly different ($p \leq 0.05$) where $p \leq 0.05$=*; $p \leq 0.01$=** and $p \leq 0.001$=***. Genotypes with superscript (S and R) are susceptible and resistant checks. NS=not significant; CV= Coefficient Variation and LSD- least significant difference.GY-grain yield per plot, D50% F-days to 50% flowering, DPM- days to physiological maturity , HM- height of the plants at maturity , Bran- number of secondary branches, PPP- number of pods per plant, spp- number of seeds per pod and 100 SW- weight in grams for 100 seeds.

Genotypes across the three sites showed high significant difference ($P \leq 0.01$) in grain yield, days to 50% flowering, days to physiological maturity and number of pods per plant. Similarly, significance at ($P \leq 0.05$) was revealed in height at maturity, number of branches and 100 seed weight but there was no significant difference in number of seeds per pod among the genotypes as presented in (Table 9).The variations of the traits measured attributed to differences in genotype and their interactions with the environment except for number of seeds per pod which showed no significant difference (Table 9).

Table 9: Table of means for Yield and yield components across the three sites 2014 cropping season

Genotypes	GY (t/ha)	D50%F	DPM	HM	Bran	PPP	SPP	100 SW
ICEAP 01147	1.03 a-d	108.3f	131.8de	134.2a-c	8bc	138b	4.6a	13.5a-c
ICEAP 01179	0.87 d	108.7f	132.9c-e	132.7a-c	7.3bc	122.3bc	4.4a	13.7a-c
ICEAP1147-1	**1.27a**	111.9b-f	133.8b-e	142.6a	8.8a-c	182a	4.3a	14.3a
ICEAP 01159	1.13 a-c	113.3a-e	132.6c-e	130.6b-d	7.6bc	91.4c	4.4a	13.5a-c
ICEAP 00554	0.98b-d	110.2ef	132.3de	131.9a-c	7.6bc	126b	4.3a	13.1bc
ICEAP 01541	0.98b-d	111.7c-f	136.6ab	127.6cd	7.4bc	134.7b	4.4a	12.9c
ICEAP 00540	0.9cd	115.4ab	137.7a	120.2d	7.0bc	132.2b	4.3a	13.8a-c
ICEAP 00911	1.17ab	111.3d-f	135.9a-c	126.8cd	7.6bc	138.6b	4.2a	13bc
ICEAP 00902	0.89 cd	115.4ab	134.6a-d	127.9cd	9.7ab	151ab	4.5a	13.1bc
ICEAP 01150	**0.81d**	110.3ef	133.9b-e	134a-c	7.1bc	137.2b	4.3a	13.5a-c
ICEAP 00068	0.87d	115a-d	134.2b-d	127.6cd	6.3bc	142.6b	4.6a	13.9a-c
ICEAP 00557	0.98 b-d	115.1a-c	134.9a-d	127.9cd	11.2a	132.1b	4.2a	14ab
KAT 60/8^{S}	0.86d	109.2f	130.8e	136.4a-c	6.2c	130.8b	4.6a	13.6a-c
ICEAP 00850^{R}	1.21ab	116.1a	137.8a	141.9ab	8.1bc	147.1b	4.3a	13.4a-c
ICEAP 0979-1	1.18ab	109.1f	133.8b-e	128.1cd	7.4bc	118.7bc	4.4a	13.7a-c
ICEAP 1154-2	1.18ab	109.4f	133.6b-e	125.1cd	7.3bc	126.6b	4.3a	14.2a
Genotype	**	*	**	*	*	**	NS	*
Site	**	**	**	**	**	**	**	**
Grand mean	1.02	111.91	134.18	130.96	7.79	134.45	4.39	13.57
Gen*Site	**	*	**	*	*	*	NS	*
CV%	27.1	3.6	2.7	9.5	40.8	27.3	10.2	7.9
LSD	0.25	3.76	3.4	11.68	2.97	34.39	0.42	1

Key: Means followed by a different letter within a column are significantly different ($p \leq 0.05$) where $p \leq 0.05$=*; $p \leq 0.01$=** and $p \leq 0.001$=***.

Genotypes with superscript (S and R) are susceptible and resistant checks. NS=not significant; CV= Coefficient Variation and LSD- least significant difference.GY-grain yield per plot, D50% F-days to 50% flowering, DPM- days to physiological maturity , HM- height of the plants at maturity , Branches- number of secondary branches, PPP- number of pods per plant, spp- number of seeds per pod and 100 SW- weight in grams for 100 seeds.

Grain yields significantly varied among genotypes. The mean grain yield for the three sites was 1.01 t/ha as summarized in Table 9. Six genotypes (ICEAPs 01147, 1147-1, 01159, 00911, 0979-1 and 1154-2 produced over 1.01 t/ha while the rests including the susceptible check averaged less than 1.01 t/ha. ICEAP 1147-1 recorded high and consistent grain yield a cross the three sites (Table 9). Sites varied significantly with Koibatek, recording the highest yield of 2.5 t/ha (Table 7), then Marigat 0.4 t/ha (Table 6) and lastly Fluorspar 0.2 t/ha (Table 8). The yield variations are also revealed in (Plate 7).

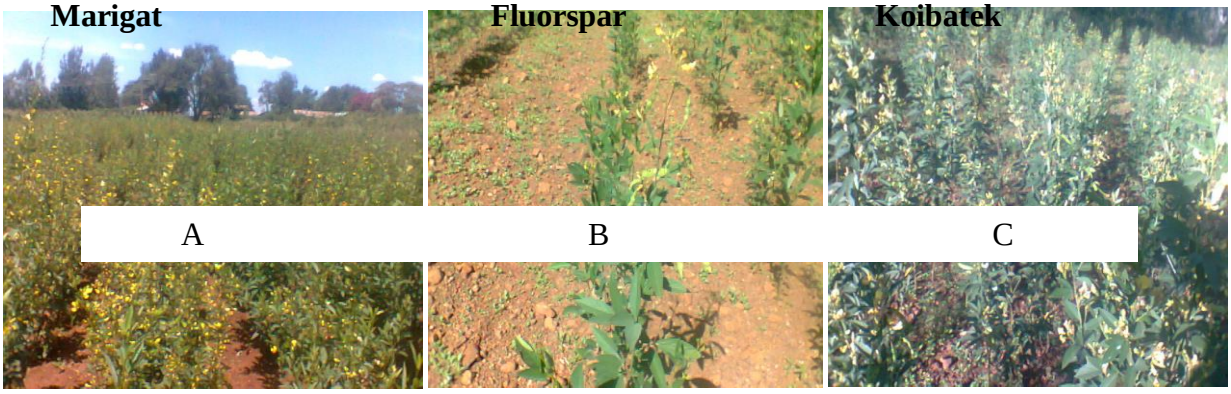


Plate 7: Field evaluation of 16 genotypes at Marigat (A), Fluorspar (B) and Koibatek (C)

Source: Author (2014). Marigat, Fluorspar and Koibatek field experimental sites

Days to 50% flowering varied significantly among the 16 genotypes. Genotypes flowered at a range from 108 to 116 days with average days of 111days. 9 genotypes flowered between 109-111 days inclusive of the susceptible check (KAT 60/8) and 5 flowering later (113-116 days) similar to ICEAP 00850 (resistant check) as shown in Table 9. The sites varied significantly ($p \leq 0.01$) with Koibatek flowering early after105 days (Table 7), 110 days in Marigat (Table 6) and late (119 days) in Fluorspar (Table 8). The mean days to physiological maturity were 134.18 days**.** Variations were observed among the genotypes. The susceptible check- KAT 60/8 matured early after 130 days while the resistant check- ICEAP 00850 with ICEAP 00540 maturing late after137.8 days. Significant difference was also observed between sites. Fluorspar recorded the longest period of 137.83 days (Table 8), followed by Koibatek 133.35 days (Table 7) and Marigat 131.35 days (Table 6). Genotypes revealed significant variations in height at maturity. ICEAP 1147-1 was the tallest with a height of 142.6 cm (Table 7) while ICEAP 00540 was the shortest (120.2 cm). The average height at maturity was 130.96 cm (Table 9) but Fluorspar genotypes matured at a shorter height of 113.31 cm (Table 8) while Marigat (Table 6) and Koibatek (Table 7) matured at relative height of 136.48 and 143.08 cm respectively.

The mean number of pods per plant was 134. 45. Genotype variation was significant and ICEAP 1147-1 recorded the highest number of pods of 182 while ICEAP 01159 the least (91.4) compared to the susceptible (130) and resistant (147) checks. There was significant variation between sites with highest number in Koibatek 226.67 (Table 7), then Marigat 144.79 (Table 6) finally Fluorspar 31.93 (Table 8). Number of seeds per pod was not significantly different among the genotypes (Table 9). The converse was however true

between sites with Marigat recording 3.8 (Table 6) and Fluorspar with Koibatek recording similar number of 4.7 as shown in Table 8 and 7 respectively. There was low significant difference among the genotypes on 100 seed weight but sites varied highly significantly (Table 9). Koibatek recorded the highest weight of 14.9g (Table 7) while Marigat recorded 12.7g (Table 6) and Fluorspar 12.9g (Table 8). Number of secondary branches per plant exhibited low variation ($p \leq 0.05$) among the genotypes with ICEAP 00557 recording the largest number of 11.2 (Table 9) and KAT 60/8 lower number (6.2). However, high significant variations between the sites were revealed (Table 9). Marigat scored higher number of 11 (Table 6), then Koibatek 9 (Table 7) and Fluorspar 3 (Table 8), culminating to an average mean of 7.79 (Table 9).

4.1.1: Correlation analysis for grain yield and its yield related traits

Correlation analysis was undertaken fitting yield and its components to determine the effect of each component. Some of the associations were significant at 5% while others at 1% probability as tabulated in Table 10. Positive significant correlation was found in height at maturity (r=0.48***), branches (r=0.23**), pods per plant (r=0.73***), seeds per pod (r=0.26*) and 100 seed weight (r=0.66***) with yield. There was also negative significant correlation on days to 50% flowering (r=-0.60***) to yield. Similarly, Positive association between days to 50% flowering with days to physiological maturity (r=*0.54****) was noted but a negative one between height at maturity (r=-*0.49****), number of secondary branches (r=-*0.43****) number of pods per plant (r=-*0.69****) and 100 seed weight (r=-*0.39****) was also observed among these traits. Negative significant correlation was reported in height at maturity (r=-,-0.211***) number of secondary branches (r=-0.292***) and number of pods per plants (r=--0.349***) with physiological

maturity. Similarly number of secondary branches had positive correlation with number of pods per plant (r=-0.517***) but negative with number of seeds per pod (r=--0.394**). There were also non -significant correlation between yield with days to maturity (-0.12ns), 100 seed weight with days to physiological maturity (-0.134ns) and 100 seed weight with number of secondary branches (0.050ns).

Table 10: Correlation analysis for grain yield and its components among the 16 pigeonpea genotypes

	GY	**D50%F**	**DPM**	**HM**	**Bran**	**PPP**	**SPP**	**100 SW**
GY	1							
D50%F	-0.60***	1						
DPM	-0.12ns	0.54***	**1**					
HM	0.48***	-0.49***	-0.211*******	**1**				
Bran	0.23**	-0.43***	-0.29*******	0.43*******	**1**			
PPP	0.73***	-0.69***	-0.35***	0.63******	0.52***	**1**		
SPP	0.26*	0.11ns	0.18*	-0.12ns	-0.39**	-0.04ns	**1**	
100 SW	0.66***	-0.39***	-0.13ns	0.30******	0.05ns	-0.04***	0.23**	1

Key: GY-grain yield per plot, D50% F-days to 50% flowering, DPM- days to physiological maturity, HM- height of the plants at maturity, Bran- number of secondary branches, PPP- number of pods per plant, spp- number of seeds per pod and SW- weight in grams for 100 seeds indicates significance at $p \leq 0.05$=*; $p \leq 0.01$=**, $p \leq 0.001$=*** and ns- not significant.

4.2. Assessing resistance level to insect pest complex among 16 pigeonpea genotypes in three sites.

4.2.1. Incidence and severity of the insect pest complex

Genotypes responded differently to the pests in different sites. In Marigat, Significant difference ($P \leq 0.01$) among the genotypes was detected for number of pod borer eggs at 50% podding, pod borer damage at 50% podding, pod fly damage at 50% podding and seed damage by pod fly, sucking bug and pod borer. Likewise, the genotypes differed significantly ($P \leq 0.05$) in number of pod borer larvae at 50% podding as tabulated in (Table 11).

Table 11: Incidence and severity of insect pest complex among 16 genotypes in Marigat during April – October 2014 cropping season.

Genotype	PBET	PBLT	PBDATP	PFDATP	SDPFAT	SDSBAT	SDPBAT
ICEAP 01147	**7a**	0.3b	11.5g	2.9g	0.6h	50.7bc	30.2f-h
ICEAP 01179	5b-d	0.7b	24.2b-d	4.3de	1.7gh	15.9i	31.1fg
ICEAP1147-1	2i	0.3b	21.3c-f	5.1c	1.8fgh	36.7e-g	38.7cd
ICEAP 01159	4.7c-e	0.7b	**39.3a**	3.5fg	7.4cd	**59.1a**	57.7a
ICEAP 00554	3f-i	0.3b	15.8e-g	**7.8a**	10.2b	39.2de	35.3e
ICEAP 01541	3.3e-i	0.3b	16.1e-g	6.3b	2.4fgh	28.5h	40.0c
ICEAP 00540	2i	0.3b	21c-f	4.6cd	6.5d	37.1e-g	39.8c
ICEAP 00911	3f-i	0.0b	19.3d-g	3.8ef	4.1ef	45.3cd	27.4g-i
ICEAP 00902	4.3e-f	1ab	22.1c-e	1h	3.2fg	31.9f-h	25.7i
ICEAP 01150	6.3ab	0.3b	13.7gh	4.7cd	1.8fgh	38ef	42.7c
ICEAP 00068	2.3ih	0.3b	20.3d-f	4.1d-f	10.4b	57.1ab	26.9hi
ICEAP 00557	6a-c	1ab	28.6bc	4.7cd	5.9de	27.8h	33.5ef
KAT 60/8^{S}	4d-g	0.3b	25bcd	6.7b	9.1bc	49.9c	**55.8a**
ICEAP 00850^{R}	3.7d-h	0.0b	15.2e-g	3.5fg	**24.4a**	39.2de	33.7ef
ICEAP 0979-1	2.7g-i	**2.7a**	30.5b	4.2de	2.1fgh	41.5de	47.1b
ICEAP 1154-2	4d-g	0.7b	22.3c-e	3.9ef	3.4fg	30.7gh	29.9f-h
Genotype	**	*	**	**	**	**	***
grand mean	**3.9**	**0.58**	**21.64**	**4.46**	**5.9**	**39.28**	**37.2**
CV%	**22.65**	**169.5**	**21.7**	**9**	**24.2**	**10.5**	**6.4**
LSD	**1.49**	**1.65**	**7.83**	**0.67**	**2.39**	**7.1**	**4**

Key: Means followed by a different letter within a column are significantly different ($p \leq 0.05$) where $p \leq 0.05$=*; $p \leq 0.01$=** and $p \leq 0.001$=*** as analyzed by least significant different test (lsd). Genotypes with superscript (S and R) are susceptible and resistant

checks. NS=not significant; CV= coefficient variation and LSD- least significant difference. PBET- number of pod borer eggs at 50% podding, PBLT- number of pod borer larvae at 50% podding, PBDATP-pod borer damage on pods at 50% podding, PFDATP- pod damage by pod fly, SDPFAT-Seed damage by pod fly after threshing, SDSBAT- Seed damage by sucking bug after threshing and SDPBAT- Seed damage by pod borer after threshing.

The average number of eggs was 3.9 with ICEAP 01147 recording the highest number (7) while ICEAPs 1147-1 and 00540 recording the lowest number of 2 (Table 11). Pod borer caused pod damage of (21.64%) while that of pod fly is (4.46%). Pod borer caused high damage to ICEAP 01159 (39.3%) and low damage to ICEAP 11.5%. Similarly, Pod fly damage was higher to ICEAP 00554 (7.8) and lower to ICEAP 00902 (1%).The percentage seed damage by pod borer ranged from 25.7 to 57.7 % while that of pod fly was 0.6-24.4% and that of sucking bug was 15.9-59.1 % (Table 11). Similarly in Koibatek, significant variation among the genotypes was observed at different levels as shown in (Table 12).

Table 12: Incidence and severity of insect pest complex among 16 genotypes in Koibatek during April – October 2014 cropping season.

Genotype	PBET	PBLT	PBDATP	PFDATP	SDPFAT	SDSBAT	SDPBAT
ICEAP 01147	1ab	0a	0d	2.8e	4.7d-f	5.2fg	2.5b-d
ICEAP 01179	0b	0a	0d	5.1b	8.6b	6.9ef	2.3c-e
ICEAP1147-1	0b	0.3a	1.1c	4.8bc	9.8ab	8.8de	1.8e-g
ICEAP 01159	0b	0a	0d	5.9a	8.2bc	11.6c	0.7jk
ICEAP 00554	1.3ab	0a	0d	1.5f	5.4de	5.2fg	1.6gh
ICEAP 01541	0b	0a	0d	2.4e	1.6g	4gh	2.2c-e
ICEAP 00540	0b	0a	0d	5b	4.6d-f	1.7i	1.6gh
ICEAP 00911	0b	0a	0d	2.8e	4.8d-f	2.9ih	1.7fg
ICEAP 00902	0.7ab	0.3a	2.5b	2.9e	3.1fg	1.6i	2.7bc
ICEAP 01150	0b	0a	0d	1.2f	3.8ef	2.7ih	1.1h-j
ICEAP 00068	0b	0a	0d	3.7d	3.1fg	5.5fg	0.9ij
ICEAP 00557	0.7ab	0a	0d	4.9bc	5.1d-f	19.7b	1.4h-j
KAT 60/8^{S}	**1.7a**	0a	**3.4a**	**6.1a**	11.2a	18.9b	**4.6a**
ICEAP 00850^{R}	0b	0.3a	0d	5.8a	**11.5a**	**24.3a**	1.3h-j
ICEAP 0979-1	0b	0a	0d	4.4c	6.4cd	6.3f	0.2k
ICEAP 1154-2	0b	0a	0d	2.6e	3.5efg	9.7cd	3b
Genotype	*	NS	**	**	**	**	***
grand mean	**0.3**	**0.06**	**0.44**	**3.89**	**5.9**	**8.44**	**1.9**
CV%	**271.7**	**400**	**34.1**	**8.2**	**20.2**	**15.4**	**16.2**
LSD	**1.51**	**0.42**	**0.25**	**0.53**	**2.01**	**2.09**	**0.5**

Key : Means followed by a different letter within a column are significantly different ($p \leq 0.05$) where $p \leq 0.05$=*; $p \leq 0.01$=** and $p \leq 0.001$=*** as analyzed by least significant

different test (lsd). Genotypes with superscript (S and R) are susceptible and resistant checks, .NS=not significant; CV= coefficient variation and LSD- least significant difference. PBET- number of pod borer eggs at 50% podding, PBLT- number of pod borer larvae at 50% podding, PBDATP-pod borer damage on pods at 50% podding, PFDATP- pod damage by pod fly, SDPFAT-Seed damage by pod fly after threshing, SDSBAT- Seed damage by sucking bug after threshing and SDPBAT- Seed damage by pod borer after threshing.

The genotypes varied significantly ($P \leq 0.01$) in number of pod borer larvae, pod damage by pod borer and pod fly, seed damage by pod fly and sucking bug. Variations at ($P \leq 0.05$) was observed in number of pod borer eggs and at ($P \leq 0.001$) for pod borer seed damage. Five genotypes (ICEAPs 01147, 00554, 00902, 00557 and KAT 60/80-susceptible check) recorded higher number of eggs ranging between 0.7-1.7 while the rest recorded no eggs inclusive of the resistant check. Pod borer caused an average damage of 0.44% on the pods while that of pod fly was 3.89%. The damage by pod borer was high in the susceptible check- KAT 60/8 (3.4%) but pod fly caused similar damage to both checks, resistant -ICEAP 00850(5.8%) and susceptible- KAT 60/8 (6.1). Sucking bug caused high seed damage of 8.4% compared to pod fly (5.9%) and pod borer (1.9) as represented in (Table 12). However genotypes in Fluorspar differed significantly as displayed in (Table 13).

Table 13: Incidence and severity of insect pest complex among 16 genotypes in Fluorspar during April October 2014 cropping season.

Genotype	PBET	PBLT	PBDATP	PFDATP	SDPFAT	SDSBAT	SDPBAT
ICEAP 01147	0.0d	0.0b	31b	9.5a-d	3.8fg	0.0f	0.0f
ICEAP 01179	0.7dc	1ab	14.9d	**12.8a**	1.6h	0.0f	2.8d--f
ICEAP1147-1	0.0d	0.0b	12.6d	12.1a	4.9ef	12.9a	0.0f
ICEAP 01159	3.3b	0.7ab	0.0e	11.9ab	7.8cd	1.6e	1.8d-f
ICEAP 00554	0.0d	0.0b	0.0e	5.2e	**18.7a**	**13.4a**	4.9cd
ICEAP 01541	0.0d	0.0b	0.0e	10.5a-c	3.5fg	4.2bc	4c-e
ICEAP 00540	0.0d	0.0b	22c	6.9c-e	9.1c	0.7ef	1.8d-f
ICEAP 00911	0.0d	0.0b	0.0e	5.4de	3.7fg	4.8b	**11.9a**
ICEAP 00902	0.0d	0.0b	0.0e	9.7a-c	3.5fg	0.7ef	2.7d-f
ICEAP 01150	1.7c	0.7ab	0.0e	12.7a	6.6de	0.9ef	2.9d-f
ICEAP 00068	0.0d	0.0b	0.0e	8b-e	6.5de	0.3f	11.4ab
ICEAP 00557	0.0d	0.0b	0.0e	10.3a-c	7.7cd	0.0f	3.9c-e
KAT 60/8^{S}	**5.3a**	1.7a	29.7b	11.9ab	7.9cd	3.3cd	7.6bc
ICEAP 00850^{R}	0.0d	0.0b	0.0e	8b-e	15.5b	0.0f	0.7ef
ICEAP 0979-1	1.7c	**1.7a**	30.9b	11.6ab	3.2gh	0.0f	1.1d-f
ICEAP 1154-2	0.0d	0.0b	**43.9a**	10.5a-c	5.6e	2.9d	0.0f
Genotype	**	*	**	**	**	**	***
grand mean	**0.79**	**0.35**	**11.57**	**9.82**	**6.8**	**2.85**	**3.6**
CV%	**95.5**	**197.1**	**14.8**	**25.1**	**14.4**	**25.6**	**65.5**
LSD	**1.26**	**1.16**	**2.8**	**4.1**	**1.65**	**1.19**	**3.9**

Key: Means followed by a different letter within a column are significantly different ($p \leq 0.05$) where $p \leq 0.05$=*; $p \leq 0.01$=** and $p \leq 0.001$=*** as analyzed by least significant different test (lsd). Genotypes with superscript (S and R) are susceptible and resistant checks, .NS=not significant; CV= coefficient variation and LSD- least significant difference. PBET- number of pod borer eggs at 50% podding, PBLT- number of pod borer larvae at 50% podding, PBDATP-pod borer damage on pods at 50% podding, PFDATP- pod damage by pod fly, SDPFAT-Seed damage by pod fly after threshing, SDSBAT- Seed damage by sucking bug after threshing and SDPBAT- Seed damage by pod borer after threshing.

High variations at ($P \leq 0.01$) was detected among the genotypes in number of pod borer eggs, pod damage by pod borer and pod fly, and seed damage by the three pests. Conversely low variations ($P \leq 0.05$) were revealed in number of pod borer larvae among the genotypes. KAT 60/8 was exposed to higher number of pod borer eggs (5.3) and larvae (1.7). Mean Pod damage by pod borer was high (11.5%) compared to pod fly (9.82%).The damage by pod borer range from 0-43.9% while for pod fly was between 5.2-12.8%. Seed damage by pod fly (6.8%) was high compared to (3.6%) caused by pod borer and 2.85% by Sucking bug. The three sites differed significantly in the parameters measured at different probability levels as presented in (Table 14).

Table 14: Incidence and severity of insect pest complex among the 16 genotypes in three sites during April – October 2014 cropping season

Genotype	PBET	PBLT	PBDATP	PFDAPM	SDPFAT	SDSBAT	SDPBAT
ICEAP 01147	2.7b	0.1b	14.2b	1f-h	3ih	18.6cd	13.4b-c
ICEAP 01179	1.9bcd	0.6b	13b	7.4ab	3.9gh	7.6i	13.8b-c
ICEAP 1147-1	0.7f	0.2b	11.7bc	7.4ab	5.5f	19.5b-d	12.9b-c
ICEAP 01159	2.7b	0.4b	13.1b	7.1a-c	7.8d	24.1a	21.1a
ICEAP 00554	1.4cd-f	0.1b	5.3f	4.8gh	11.4b	19.2b-d	14.5b-c
ICEAP 01541	1.1d-f	0.1b	5.4f	6.4b-e	2.5i	12.2gh	14.9bc
ICEAP 00540	0.7f	0.1b	14.3b	5.5d-g	6.8de	13.2gh	14.5b-c
ICEAP 00911	1d-f	0.0b	6.4ef	4h	4.2g	17.6de	14.5b-c
ICEAP 00902	1.7c-e	0.4b	8.2de	4.5gh	3.3i-h	11.4h	10.4d
ICEAP 01150	2.7b	0.3b	4.6f	6.2b-f	4.1gh	13.9fg	15.2bc
ICEAP 00068	0.8ef	0.1b	6.8d-f	5.3e-h	6.6d-f	20.9bc	12.2cd
ICEAP 00557	2.2bc	0.3b	9.5cd	6.7b-d	6.2ef	15.8ef	14.4b-d
KAT 60/8	3.7a	0.7b	19.4a	8.2a	9.4c	24.1a	22.3a
ICEAP 00850	1.2d-f	0.1b	5.1f	5.8c-g	17.1a	21.2b	12.5b-d
ICEAP 0979-1	1.4c-f	1.4a	20.5a	6.8b-d	3.9gh	15.9ef	16.6b
ICEAP 1154-2	1.3c-f	0.2b	22.1a	5.7d-g	4.1gh	14.4fg	11.4cd
Genotype	**	*	**	**	**	**	***
Site	**	**	**	**	**	**	***
Grand mean	1.69	0.33	11.22	6.06	6.2	16.86	14.5
Gen*Site	**	*	**	**	**	**	***
CV%	60.7	216	26.3	23.4	19.5	15.1	30.2
LSD	0.96	0.68	2.77	1.33	1.13	2.39	4.1

Key: Means followed by a different letter within a column are significantly different ($p \leq 0.05$) where $p \leq 0.05$=*; $p \leq 0.01$=** and $p \leq 0.001$=*** as analyzed by least significant different test (lsd). Genotypes with superscript (S and R) are susceptible and resistant checks, .NS=not significant; CV= coefficient variation and LSD- least significant difference. PBET- number of pod borer eggs at 50% podding, PBLT- number of pod borer larvae at 50% podding, PBDATP-pod borer damage on pods at 50% podding, PFDATP- pod damage by pod fly, SDPFAT-Seed damage by pod fly after threshing, SDSBAT- Seed damage by sucking bug after threshing and SDPBAT- Seed damage by pod borer after threshing.

Significant difference ($P \leq 0.01$) among the genotypes was observed in number of pod borer eggs, pod damage by pod borer and pod fly and seed damage by pod fly and sucking bug. Variations at ($P \leq 0.05$) in number of pod borer larvae and seed damage by pod borer at ($P \leq 0.001$) were also recorded. Sites varied highly significantly ($P \leq 0.01$) in number of pod borer eggs, larvae, pod damage and seed damage by the insect pests (Table 12). Significant variations ($P \leq 0.01$) in genotype* site interaction was also observed in number of pod borer eggs, pod damage and seed damage by the three pests. Low variation was revealed in number of pod borer larvae at ($P \leq 0.05$) as presented in (Table 14). The average number of pod borer eggs was 1.69 (Table 12). The susceptible check (KAT 60/8) recorded the highest number of eggs (3.7) while ICEAP 1147-1 recorded lowest number (0.7) as shown in Table 12. Sites differed also significantly, the number was high in Marigat 3.9 (Table 11), then Fluorspar 0.79 (Table 13) and low in Koibatek 0.3 (Table 10). The pod borer larvae mean was 0.33 with ICEAP 0079-1 reporting the highest (1.4).

The incidence was more in Marigat with a mean of 0.58 (Table 11), followed by Fluorspar 0.35 (Table 13) and less in Koibatek 0.06 (Table 12). Pod damage by pod borer was 11.22% (table 12). ICEAP 01150 was least attacked (4.6%) while ICEAP 1154-2 (22.1%) and ICEAP 0079-1 (20.5% were highly exposed similar to the susceptible check (KAT 60/8) at 19.4% (Table 14). Marigat (21.64%) reported the highest damage (Table 11), then Fluorspar 11.57% (Table 13) and finally Koibatek 0.4% (Table 12). Pod fly was more damaging on pods in Fluorspar with mean of 9.8% (Table 13) than in Marigat 4.4% (Table 11) and Koibatek 3.9% (Table 12).

Seed damage was profound in sucking bug (16.86%) then pod borer (14.5%) and finally pod fly (6.2%) as summarized in Table 14. The seed damage by pod borer was great in Marigat (37.2%) then Fluorspar (3.6%) and least in Koibatek (1.9%). Seeds from ICEAP 01159 were highly (17.7%) damaged like both checks. Seed damage by sucking bug varied significantly between sites. Marigat (39.28%) reported the highest damage (Table 11), then Koibatek 8.4% (Table 12) and fluorspar 2.85% (Table 13). ICEAP 01159 (24.1%) just like the susceptible check KAT 60/8 (24%) was highly attacked by sucking bug while ICEAP 01179 (7.6%) was the least (Table 14). Seed damage by pod borer was cross cutting among the genotypes and between sites. ICEAP 01159 (21.1%) and susceptible check KAT 60/8 (22.3%) were heavily damaged (Table 14). Marigat had the highest seed damage by pod borer of 37.2% (Table 11), Fluorspar 3.6% (Table 13) and Koibatek 1.9% (Table 12). The percentage seed damage for pod fly was high in Fluorspar 6.8% (Table 13) and similar in Marigat 5.9% (Table 11) and Koibatek 5.9% (Table 12). ICEAP 00850 was more susceptible to pod fly (17.1%) in all the three sites as shown in (Table 14).

4.2.2: Scoring for resistance based on 1-9 rating scale by Lateef and Reed, 1990

The pod and seed damage means in the Tables 9-12 above, were used to score for resistance based on 1-9 rating scale by Lateef and Reed, 1990. Pigeonpea is among the crops attacked simultaneously by several insect pests. The resistance to one particular insect may enhance the population of the other pests and cause enough damage to negate the effect of resistance. This therefore warrants scoring pest effect independently. For example ICEAPs 01179 and 01541 is highly susceptible to pod fly but resistant to pod borer (Table 15). This finding is corroborated by negative correlation as shown in (Table 17). The genotypes responded differently to the three pests in the different sites. For instance, three genotypes inclusive of the resistant check (ICEAPs 00902, 01541 and 1154-2) showed some levels of tolerance to pod borer across the three sites by rating 5, 4 and 3 respectively. Similarly, three genotypes (KAT60/8, ICEAPs 01159 and 0979-1) rating 9, 7 and 7 as shown in Table 16. On the other hand, seven genotypes (ICEAPs 01147, 01179, 01541, 00902, 01150, 0979-1 and 1154-2) were least attacked by pod fly compared to the resistant check. Nine genotypes were least attacked by sucking bug (ICEAPs 01179, 01541, 00540, 00911, 00902, 01150, 00557, 0979-1 and 1154-2).

Table 15: Scoring Pod damage by pod borer and pod fly at 50% podding using Reed and Lateef 1990 scale

	Pod borer						Pod fly					
Genotype	**Marigat**		**Koibatek**		**Fluorspar**		**Marigat**		**Koibatek**		**Fluorspar**	
ICEAP 01147	7.8	6	0	6	100	1	17.1	5	51.7	3	-18.8	7
ICEAP 01179	13.6	5	28	5	23.3	5	-22.9	7	-12.1	7	-60.0	9
ICEAP 1147-1	12.2	5	40	4	100	1	-45.7	8	17.2	5	-51.3	9
ICEAP 01159	-44.0	8	52	3	-111	9	0.0	6	-1.7	6	-48.8	8
ICEAP 00554	-11.2	7	40	4	-456	9	-122.9	9	74.1	3	35.0	4
ICEAP 01541	12.4	**5**	24	**5**	36.7	**4**	-80.0	**9**	-58.6	**8**	-31.3	**8**
ICEAP 00540	-9.2	7	40	4	-100	9	-31.4	8	13.8	5	13.8	5
CEAP 00911	15.8	5	16	5	12.33	5	-8.6	6	51.7	3	32.5	4
ICEAP 00902	22.6	**5**	16	**5**	21.1	**5**	71.4	**3**	50.0	**4**	21.3	**5**
ICEAP 01150	-3.2	6	52	3	-222	9	-34.3	8	79.3	2	-58.8	9
ICEAP 00068	32.8	4	60	3	-1189	9	-17.1	7	36.2	4	0.0	6
ICEAP 00557	8.5	6	-8	6	-356	9	-34.3	8	15.5	5	-28.8	8
KAT60/8	-35.5	8	-112	9	-767	9	-91.4	9	-5.2	6	-48.8	8
ICEAP 00850	0.0	**6**	0	**6**	0	**6**	0.0	**6**	0.0	**6**	0.0	**6**
ICEAP 0979-1	-44.8	8	72	3	-44	9	-20.0	7	24.1	5	-45.0	8
ICEAP 1154-2	**13.1**	**5**	**18**	**5**	**100**	**1**	**11.4**	**5**	**55.2**	**3**	**15.9**	**5**

Table 16: Scoring Seed damage by the three pests in the three sites using Reed and Lateef 1990 (1-9 rating scale)

	Pod borer						Pod fly						Sucking bug					
Genotype	**Marigat**		**Koibatek**		**Fluorspar**		**Marigat**		**Koibatek**		**Fluorspar**		**Marigat**		**Koibatek**		**Fluorspar**	
ICEAP 01147	10.4	5	-92.3	9	100	1	97.5	1	59.1	3	75.5	2	-29.3	8	78.6	2	100	1
ICEAP 01179	7.7	6	-76.9	5	-300	9	93.0	1	45.2	4	89.7	2	59.4	3	71.6	3	100	1
ICEAP 1147-1	-14.8	7	-38.5	8	100	1	92.6	1	14.8	5	68.4	3	6.4	6	63.8	3	-1190	9
ICEAP 01159	-71.2	9	46.2	4	-157	9	69.7	3	48.7	4	49.7	4	-50.8	9	52.3	3	-60	8
ICEAP 00554	-4.7	6	-23.1	7	-600	9	58.2	3	53.0	3	20.6	7	0.0	6	78.6	2	-1240	9
ICEAP 01541	-18.7	**6**	-69.2	**5**	-471	**6**	90.2	**1**	86.1	**2**	77.4	**2**	27.3	**4**	83.5	**2**	32	**5**
ICEAP 00540	-18.1	7	-23.1	7	-157	9	73.4	3	60.0	3	41.3	4	5.4	6	93.0	2	30	4
ICEAP 00911	18.7	5	-30.8	8	-1600	9	83.2	1	58.3	3	76.1	2	-15.6	7	88.1	2	-380	9
ICEAP 00902	23.7	**5**	67.7	**4**	28.6	**5**	86.9	**1**	73.0	**3**	77.4	**2**	18.6	**5**	93.4	**2**	30	**4**
ICEAP 01150	-26.7	8	15.4	5	-314	9	92.6	1	67.0	3	57.4	3	3.1	6	88.9	2	10	5
ICEAP 00068	20.2	5	30.8	4	-1529	9	85.7	1	73.0	3	58.1	3	-45.7	8	77.4	2	70	3
ICEAP 00557	0.6	6	-7.7	6	-457	9	75.8	2	55.7	3	50.3	4	29.1	4	18.9	5	100	1
KAT 60/8	-65.6	9	-253.8	9	-986	9	62.7	3	2.6	6	49.0	4	-27.3	8	22.2	5	-230	9
ICEAP 00850	0.0	**6**	0.0	**6**	0	**6**	0.0	**6**	0.0	**6**	0.0	**6**	0.0	**6**	0.0	**6**	0	**6**
ICEAP 0979-1	-39.8	8	84.6	1	-57	9	91.4	1	44.3	4	79.4	2	-5.9	6	74.1	3	100	1
ICEAP 1154-2	11.3	**5**	30.8	**4**	100	**1**	86.1	**1**	69.6	**3**	63.9	**3**	21.7	**5**	60.1	**3**	29.0	4

4.2.3: Correlation analysis of grain yields and incidence/severity of insect pest complex

The different insect pest complex parameters were correlated to establish any possible significant relationship with yield. The coefficient correlation results are summarized in (Table 17). There was significant correlation in some traits (<0.01) but not in others. Seed damage by pod fly (r=-0.08) and number of pod borer larvae at 50% podding (0.19) showed a negative correlation with yield but not significant. In contrast, number of pod borer eggs at 50% podding (r=-0.35***), pod borer damage on pods at 50% podding (r=-0.53***), seed damage by sucking bug (r=-0.24**) and seed damage by pod borer after threshing (r=-0.41***) were negatively correlated to yield. High negative significance was revealed between yield with pod damage by pod borer and pod fly and seed damage by pod borer while a low negative correlation was also revealed on pod borer eggs and seed damage by sucking bug with yield.

Table 17: Correlation analysis for grain yields and incidence/severity of insect pest complex

	YIELD	**PBET**	**PBLT**	**PBDATP**	**PFDATP**	**SDPFAT**	**SDSBAT**	**SDPBAT**
YIELD	1							
PBET	-0.35***	*1*						
PBLT	-0.19ns	0.42***	*1*					
PBDATP	-0.53***	0.50***	0.37 ***	***1***				
PFDATP	*-0.46****	-0.12ns	0.14ns	0.12ns	***1***			
SDPFAT	-0.08*ns*	*-0.08ns*	-0.13 *ns*	-0.10ns	0.05ns	***1***		
SDSBAT	-0.24**	*0.60****	*0.15ns*	0.47***	-0.36***	0.09ns	***1***	
SDPBAT	-0.41***	0.68***	*0.19ns*	0.55***	-0.26**	-0.05ns	0.85ns	***1***

Key*:* PBET- number of pod borer eggs at 50% podding, PBLT- number of pod borer larvae at 50% podding, PBDATP-pod borer damage on pods at 50% podding, PFDATP- pod

damage by pod fly, SDPFAT-Seed damage by pod fly after threshing, SDSBAT- Seed damage by sucking bug after threshing and SDPBAT- Seed damage by pod borer after threshing. Correlation significance at $p<0.05$=*; $p<0.01$=** and $p<0.001$=***; ns= not significant.

4.3 Genetic diversity assessment using 20 SSR markers among 16 pigeonpea genotypes

DNA Quality

Based on CTAB protocol, all the samples extracted produced good quality, high molecular weight DNA as illustrated in (Figure 3).

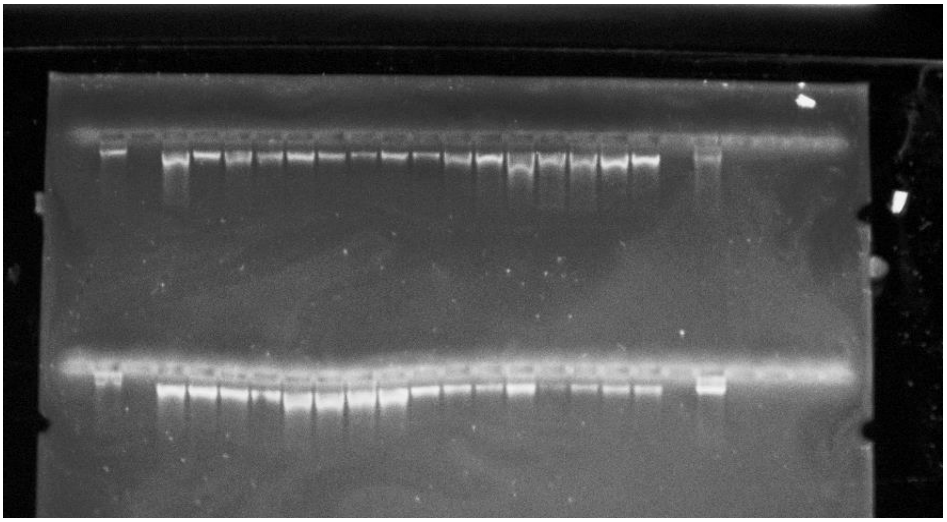


Figure 3: Agarose gel (0.8% w/v) image of extracted DNA obtained from a 13-day old leaf material.

Source: Author (2015). ICRISAT Nairobi Molecular laboratory

Polymerase Chain Reaction

All the 24 primer pair (numbered 1-24) optimized using touch down procedure of 60-52^{o}C had bands showing successful amplification by PCR (Figure 4). They were then taken for fragment analysis (capillary electrophoresis) to reveal the allelic sizes. The DNA ladder (L) used was 100bp.

Figure 4: Agarose gel (2%w/v) image of 24 primer pair across three randomly selected genotypes Source: Author (2015)**. ICRISAT Nairobi Molecular laboratory**

Allele scoring and analysis.

The amplified DNA fragments were successfully separated by capillary electrophoresis on an ABI3730 automatic sequencer and this allowed the allele (fragment) sizes to be scored using Gene Mapper® software as presented in (Figure 5).

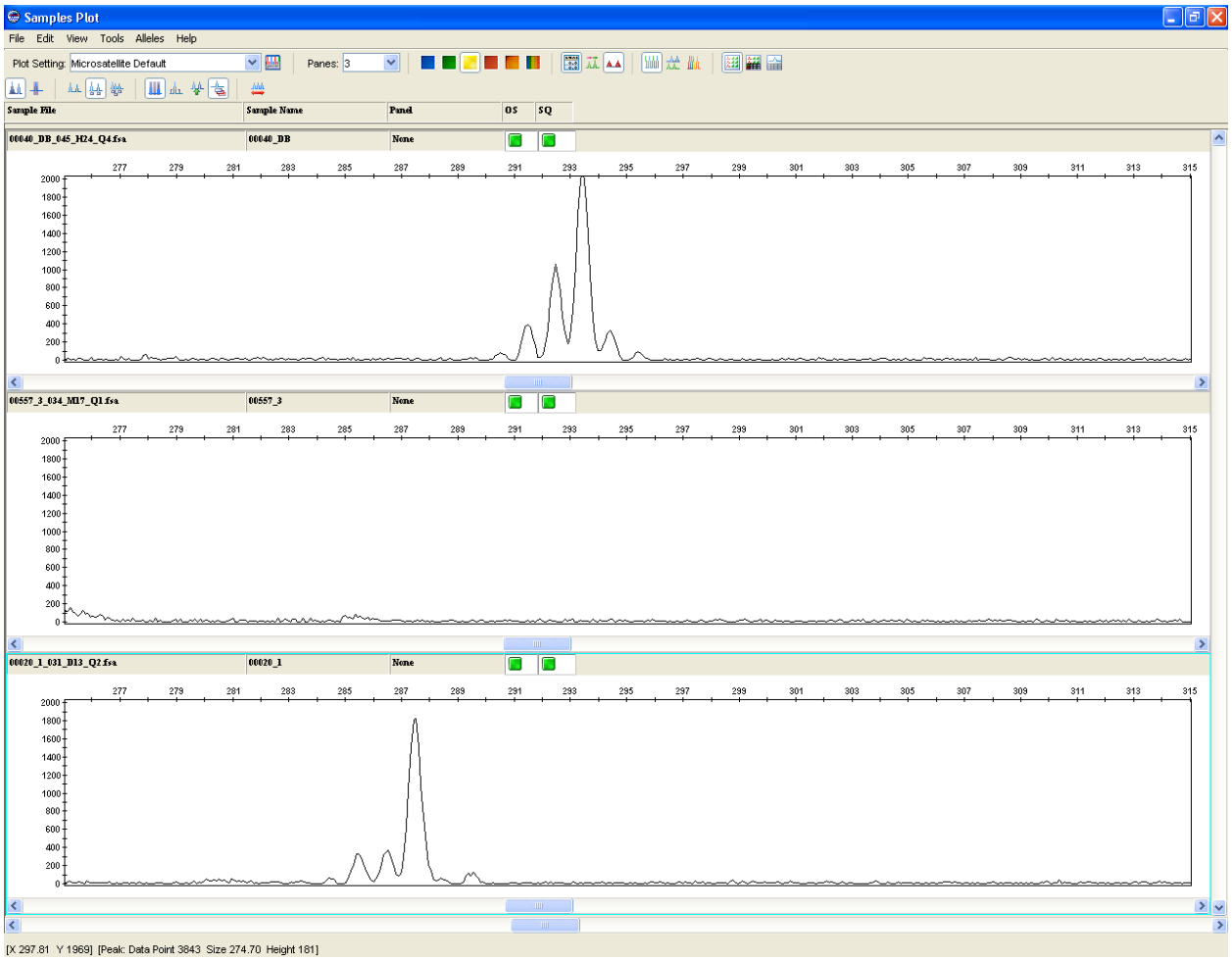


Figure 5: Computer screen shot of Gene Mapper® peaks. The first and third samples showed different sizes (alleles) for the same SSR primer pair while the second sample amplified no peak at this locus.

Source: Author (2015)

Power Marker® version 3.25 was used to analyze the data generated by Gene Mapper by giving summary statistics of polymorphic information content (PIC) values, number of alleles, heterozygosity, availability, genetic diversity and major allele frequency. Out of the 24 markers, 20 of them were used for further analysis as they showed polymorphism

(Appendix III). The level of polymorphism among the genotypes was evaluated by calculating allelic number and PIC values for each of the 20 SSR primer pairs. 12 SSRs were found to be highly polymorphic among the population with polymorphic information content (PIC) values ranging from 0.40 to 0.70. However, 8 of the markers showed low polymorphism with PIC values below 0.40 (Table 18). The markers generated 78 alleles in total with a mean of 3.67 alleles per marker. Marker CcM1825 revealed the highest number of alleles (8) with a PIC value of 0.66; similarly, CcM1348 showed 7 alleles but with the highest PIC value of 0.70 while CcM2332 had the lowest PIC value (0.13) and the lowest number of alleles (2.00).The average PIC value was 0.39 as shown in (Table 18).

Table 18: Power Marker® Summary statistics output for the selected 20 markers across 16 individual DNA samples.

Primers	Major Allele Frequency	Allele. no	Gene Diversity	Heterozygosity	PIC
CcM0603	0.48	3.00	0.59	0.34	0.50
CcM1232	0.50	5.00	0.63	0.81	0.56
CcM0047	0.69	2.00	0.43	0.25	0.34
CcM1139	0.69	4.00	0.46	0.25	0.40
CcM1373	0.40	4.00	0.66	0.06	0.59
CcM1582	0.75	3.00	0.39	0.22	0.34
CcM0602	0.88	4.00	0.23	0.13	0.22
CcM1506	0.73	5.00	0.43	0.31	0.40
CcM1820	0.39	5.00	0.68	0.50	0.62
CcM1611	0.53	2.00	0.50	0.31	0.37
CcM1825	**0.45**	**8.00**	**0.70**	**0.56**	**0.66**
CcM1348	**0.40**	**7.00**	**0.74**	**0.32**	**0.70**
CcM1447	0.56	4.00	0.54	0.84	0.45
CcM2044	0.58	3.00	0.57	0.60	0.50
CcM2332	0.92	2.00	0.14	0.16	0.13
CcM0444	0.88	3.00	0.22	0.13	0.21
CcM2049	0.41	4.00	0.66	0.31	0.59
CcM0594	0.72	4.00	0.43	0.03	0.37
CcM2060	0.91	3.00	0.17	0.13	0.17
CcM1014	0.63	3.00	0.52	0.28	0.45

DARwin software was used to produce principle coordinate analysis, dissimilarity matrix, which was displayed in a neighbor-joining tree or dendogram, illustrated in (Figure 6).

The genotypes were grouped into three main distinct clusters (cluster I, II and III). Cluster I and II were further grouped into several sub groups. All the genotypes that showed some levels of tolerance were grouped in cluster I. The resistant and susceptible check was grouped in different distinct clusters (cluster I and II) respectively as indicated in (Figure 6). Susceptible genotypes identified in this study clustered in cluster II and III except for two genotypes (ICEAPs 00054 and 0979-1) that were clustered in cluster I.

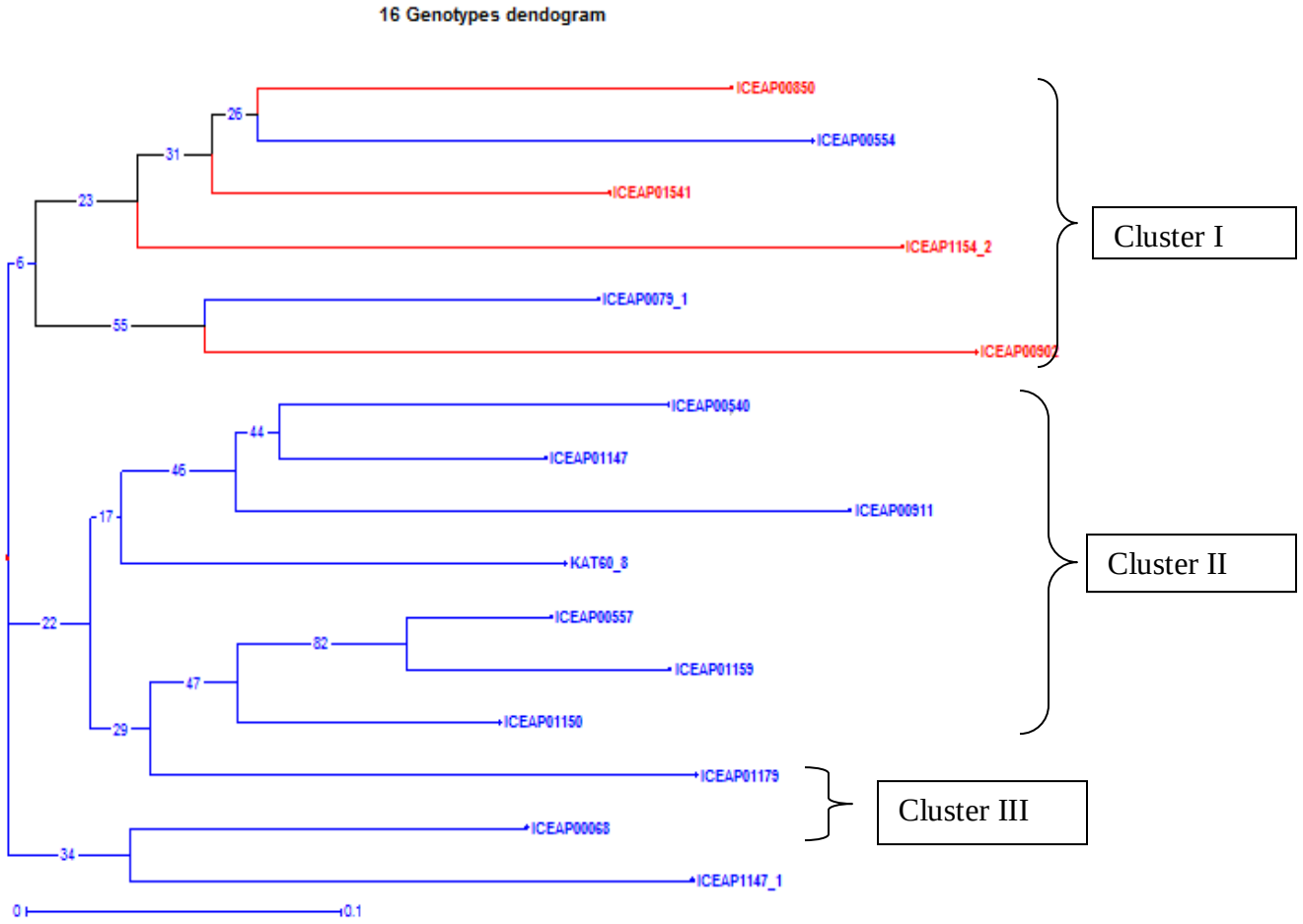


Figure 6: Dendogram showing different Pigeonpea clusters of 16 pigeonpea genotypes based on 20 SSR markers

CHAPTER FIVE

DISCUSSION

5.1 Yield performance and adaptation among 16 pigeonpea genotypes

The sixteen genotypes differed significantly in grain yield. The variations observed showed adequate variability in the germplasm which is contributed by variations in yield components having direct impact on yield. These yield components include; number of secondary branches, number of pods per plant, 100 seed weight and number of seeds per pod. The mean grain yield for the genotypes across the three sites was 1.01 t/ha. These results is similar to Gwata & Shimelis, 2013 in evaluation of medium pigeonpea germ plasm for important agronomic traits in southern Africa which had 1.0 t/ha. Egbe, & Vange (2008) in a study on yield and agronomic characteristics of 30 pigeonpea genotypes at Otobi in Southern Guinea Savanna of Nigeria reported a mean of 1.37 t/ha.

Six genotypes (ICEAPs 01147, 1147-1, 01159, 00911, 0979-1 and 1154-2) similar to the resistant check (ICEAP 00850) produced high yield of more than 1.0t.ha. This indicates the potential adaptability of these six genotypes to the study areas and their high production may be due to their potential of producing higher number of secondary branches leading to higher number of pods. This may also be attributed by resistance to the insect pest complex by having higher number of pods so that even if there is a larger pest incidence the severity is not felt as the plant is still able to yield more. ICEAP 1147-1 recorded high and consistent grain yield a cross the three sites. It yielded higher number of pods per plant (182), high 100 seed weight (14.3) and was the tallest among the genotypes. This might have contributed to its high ranking. This genotype was stable, adaptable to the three

environments and could therefore be recommended for use in north Rift valley Kenya as a background selection for yield as reported by Manyasa, 2008.

Yield variations were observed between sites. Koibatek recorded the highest yield of (2.5 t/ha), then Marigat (0.4 t/ha) and lastly Fluorspar (0.2 t/ha). A variation in yield production between these sites is an indication of differences in environmental conditions. These environmental factors include amount of rainfall, temperature, type of soil, humidity patterns and insect pest complex infestation. The amount of rainfall during the cropping season was higher in Koibatek compared to Marigat and Fluorspar (Table 19). This rainfall variation together with moderate temperature values might have affected the growth and development of the genotypes especially during flowering and podding stage due to differences in moisture and fertility levels.

Days to 50% flowering differed significantly between genotypes and in sites. The flowering and maturity of pigeonpea is influenced by combination of rainfall and temperature (Singh & Oswalt, 1992). Genotypes in Koibatek flowered earlier (105 days), then Marigat (110 days) and late (119 days) in Fluorspar. The early flowering in Koibatek may have been contributed by moderate rainfall during flowering period.There was less rain in Marigat and Fluorspar in June which was the flowering initiation period (Table 19) and this might have affected flower initiation. This site variation is similar to results by Silim *et al.* (2006) who reported 119, 94, 125 and 122 days for Kabete, Katumani, Kiboko and Mtwapa respectively. This variation suggests site ecological variation and individual trait adaptability. Similar results were reported by Das and Fakir, (2010) Manyasa *et al.* (2009) and Changaya (2007) who reported 105.18 days, with a range from 93 to 181days and 79-164 days respectively. Variations in days to physiological maturity among the genotypes in this study can be explained by the differences in seed filling duration. The

period ranged between 126-148 days with a mean of 134.18 days which compared favorably with range of 121 -170 days and 144 days reported by Minja (2002) and Manyasa *et al.* (2009). It however contrasted with Gwata & Shimelis, (2013); Das and Fakir, (2010); Egbe & Vange , (2008) who reported 161days, 164.0 and 151 to 180 days respectively. This early maturity is probably because the trial area is along/near the equator. Maturity was significant between sites with Fluorspar maturing late after 137.83 days relative to Koibatek 133.35 days (cool) and Marigat 131.35 days (warm). Similar results were also reported by Manyasa, *et al*. (2009) who recorded early maturity in warm areas and late in cool places. This is basically because genotypes in cool environment tend to have long number of vegetative growth compared to warm environment. The hormones tend to concentrate on vegetative growth than the reproductive stage hence taking time in grain and pod filling.

Height at maturity varied highly between sites but was lower among genotypes. This indicates that environment influences the height of most crops due to influence by Genotype*environment interaction. Amount of rainfall and temperature influences height. Crops in cooler environments tend to grow tall because of higher vegetative growth that result to higher photosynthetic ability, growth and dry mater production of a plant. The average height at maturity was 130.96 cm. Changaya (2007), Das and Fakir, (2010) and (Khaki, 2014) also reported similar results of less than 2m for medium maturing genotypes. The results however disagrees with Egbe & Vange (2008) who reported mean hieght of over 3 metres (3.15-3.27). Genotypes at Fluorspar matured at a shorter height (113.31 cm) while for Marigat and Koibatek matured at relative height of 136.48 and 143.08 cm respectively. This is possibly due to variation in amount of rainfall and temperature across

the three sites as shown in Table 19. This might have influenced the general crop development.

Number of pods per plant differed significantly among the genotypes. ICEAP 1147-1 had the highest number of pods (182) while ICEAP 01159 the least (91.4). This is attributed to its higher efficacy in translocating photosynthate to the reproductive parts hence higher yields. These findings concur with Khaki, (2014) who reported significant difference in mean number of pods between genotypes in Uganda experiment. It does however contrast, with Egbe & Vange (2008) who reported no significant difference in pod number per plant on medium duration pigeon genotypes. The mean number of pods per plants was 134.45 with a range of 31.93 to 226.6. These results agrees with 111.8 reported by Khaki, (2014) within the range of (41-278 pods per plant) but varies with Manyasa *et al.* (2009), Egbe, & Vange (2008) who reported 190-952; 195.25-204.45 respectively and Upadhyaya *et al*. (2010) who reported 81.3.The differences in these results is attributed to differences in agro-ecological zones and the genotypes tested.

There was no variation in number of seeds per pod among genotypes. This is expected because the genotypes are elite lines for yield that were developed through selection based on the number of seeds. This contrasts with Changaya (2007) who reported highly significant variation but similar range of 1 to 5.7 seeds per pod. Similar results were reported by Upadhyaya, *et al*. (2010) and Manyasa *et al.* (2009) who both reported 5. The number of seed per pod in this study was however more compared to 3.7 reported by Das & Fakir, (2010). The converse was however true between sites with Marigat differing (3.8) from Fluorspar and Koibatek which recorded similar number of 4.7. The less number of seeds per pod in Marigat is probably due low amount of rainfall and higher temperatures which affected the biomass accumulation.

Biomass accumulation and photosynthate translocation are key factors to 100 seed weight .This is governed by yield components like plant height at maturity and number of seeds per pod. The mean 100 seed weight was 13.57g/100 seeds with low significant difference among the genotypes .This finding disagrees with Changaya (2007) who found higher significant variation among genotypes. However, the mean seed weight reported in this study correspond to a range of 7.6-11.2; 12-16g; 14.1 -23.4g/100 seeds reported by Manyasa *et al.* (2009), Khaki, (2014) and Changaya, (2007). It is higher compared to 6.75/100 seeds reported by Das & Fakir, (2010) but lower than 30.1g/100 seeds reported by Upadhyaya *et al*. (2010). Koibatek recorded the highest weight (14.9 g) while Marigat and Fluorspar had the lowest (12.7g and 12.9g) respectively. The high vegatative growth contributed by high moisture and soil fertility level in Koibatek relative to the other two sites may explain this results. Similar results were reported by Manyasa, *et al.* (2009) who reported different 100 seed weight in Kabete relative to Kampi ya Mawe and attributed the results to excessive vegetation growth in Kabete.

Number of secondary branches per plant did not significantly differ among the genotypes but differed in sites. The average mean of number of secondary branches was 7.79. Large variability was reported in Koibatek (9) and Marigat (8) relative to Fluorspar (3), comparably with results reported by Manyasa, *et al.* (2009) but lower compared to 17.40 reported by Egbe & Kalu, (2009). The diversity in branching may be explained by Singh & Oswalt, 1992 who stated the branching pattern in pigeonpea depends on genotype, amount of rainfall, temperature and spacing between rows and plants. This therefore attributes to the lower number in Fluorspar which received very low rainfall throughout the cropping season.

5.1.2: Correlation analysis for grain yield and its components

Grain yield and its components exhibited varying associations. Positive significant correlation was found in height at maturity, number of secondary branches, pods per plant, seeds per pod and 100 seed weight with yield. This explains that under favorable conditions, the taller the plant, the higher the branches and higher number of pods hence higher yields. This has been revealed by ICEAP 01147-1 which recorded high yield across the three sites. Similar results were reported by Manyasa (2007) and Khaki (2014) who found positive correlation in number of pods per plant and number of secondary branches with yield and reported that direct relationship among these traits indicates that they are important yield components and therefore should be considered when selecting for yield (Vange and Egbe, 2009).

Days to 50% flowering and grain yield recorded high and negative correlation. The negative correlation is due to lack of enough time for the plant to accumulate biomass which is a precursor to yield. Also high temperatures, low rainfall and high pest infestations may have increased flower abortion and podding which in turn affected number of pods per plant and 100 seed weight that indirectly reduced yield. Negative significant correlation has also been reported in green grams (Pandey 2013). This contrasts with Khaki (2014), Vange and Egbe (2009), Atta *et al*. (2008), who found out consistent, strong and positive correlations among these quantitative traits. Days to 50% flowering contributed positively to days to physiological maturity but a negatively to height at maturity, number of secondary branches, number of pods per plant and 100 seed weight. This is basically because, when a plant reaches 50% flowering earlier, the physiological processes will be altered to reproduction rather than vegetative hence contributing to low yields.

Negative significant correlation was reported in height at maturity, number of secondary branches and number of pods per plants with physiological maturity. This implies that as the plant mature early, it will yield lower number of secondary branches and lower number of pods because the plant will not be able to accumulate enough biomass required for the development of pods and branches. Number of secondary branches revealed positive correlation with number of pods per plant. This shows that the number of secondary branches determines the number of pods and this is because branches yield leaves and flower buds which leads to development of pods.

5.2: Assessing resistance levels to insect pest complex among 16 Pigeonpea genotypes

Genotypes responded differently to the insect pest complex depending on the sites. Significant variations were observed in number of pod borer eggs per plant, number of larvae per plant, pod damage and seed damage. The variations in incidence and severity among the genotypes may be attributed to the genetic makeup of the plant and environmental factors favoring the distribution and existence of the pests in different environments as reported by Minja, (1999).

The average number of pod borer eggs per plant was 1.7, but more in Marigat (3.9), then Fluorspar (0.79) and Koibatek (0.3). The variations can be attributed to differences in amount of rainfall and temperature during this time of pest build up. Koibatek exhibited high amount of rainfall in the month of June (podding time) which may have washed away the eggs. On the other hand, Fluorspar received low rainfall and exhibited high temperatures that could not allow survival of the eggs. The susceptible check (KAT 60/8) recorded the highest numbers of pod borer eggs (3.7) while ICEAP 1147-1 the lowest number (0.7). This may be due to oviposition preference of the pod borer on KAT/8 and not

on ICEAP 1147-1.Similar trend applied to number of pod borer larvae. This concurs with Khaki, (2014) who found significant differences in response to pest damages during flowering and maturity stages across seasons.

The pests differed significantly on their attack on pods and seeds at different levels at different sites. Marigat (21.64%) reported the highest pod damage by pod borer, then Fluorspar (11.57%) and Koibatek (0.4%). This means that pest pressure was high in Marigat, then Fluorspar and Koibatek. This may be explained by preference of pod borers to warm areas compared to cool areas (Koibatek). It may also be explained by the nature of pod borer undergoing diapause during cool and dry weather conditions as reported by Minja (1999). The mean percentage pod damage was 11.22%, this percentage is lower than 25-70% reported by Sharma, *et al*. (2010) in review of pests of pigeonpea and their management. Similar pod borer results were reported by Singh *et al*.(2006) on a study on Evaluation of pigeonpea genotypes for resistance to pod borer complex where he recorded an average pod damage of 17 -26%.

Pod damage by pod fly (4.46%) was low compared to that of pod borer (21.64%) in Marigat. This may also be attributed to environmental factors within the hot semi-arid study site which favored pod borer and not pod fly. This is confirmed by Subharani and Singh. (2007) who reported that pod fly infestation is not influenced significantly by other environmental factors, except for relative humidity, which exerts significant negative effect with the pest infestation.

There was contrasting trend of attack by the three pests. Sucking bug (39.4%) and pod borer (38.4%) were very infectious in Marigat while pod fly (6.4%) and sucking bug (6.3%) were high in Koibatek then pod fly was more pronounced (6.9%) in Fluorspar. This may be attributed by high amount of rainfall in Koibatek which washed away pod

borer eggs and larvae. The high temperatures recorded in Fluorspar did not favor growth and development of pod borer larval stages. Seed damage by the insect pests varied significantly between sites. The damage by pod borer (37.2%) and sucking bug (39.2) was high in Marigat compared to 8.4% sucking bug, 1.9% pod borer in Koibatek and 2.8% sucking bug, 3.6% pod borer in Fluorspar. Similar greater variations in seed damage were observed between locations in Kenya and other regions as reported by Minja (1999) who reported that warm and dry locations had less seed damage than warm and humid, cool and dry, or cool and humid locations in Kenya, Malawi, and Tanzania.

5.3 Genetic diversity analysis of 16 Pigeonpea genotypes based on 20 SSR markers

The markers generated 78 alleles in total with a mean of 3.67 alleles per locus. The low number of alleles and low number of alleles per locus indicate narrow genetic diversity among the pigeonpea genotypes studied which is also reported by Odeny *et al*. (2009) whose study revealed 110 total numbers of alleles with an average of 3.1 alleles per locus. These results differ with results reported by Songok *et al*. (2010 who recorded a total number of 47 alleles with a mean of 8 alleles per locus. The high number of alleles per locus recorded by Songok might have been contributed by wild relatives and landraces included in the study which have been reported to have wide genetic diversity compared to this study that assessed elite lines that were selected specifically for yield. Odeny used higher number of SSR markers (113) in her study which lead to the higher number of alleles (110). The PIC values range from 0.13 to 0.7 with Marker CcM1825 revealing the highest number of alleles (8) with a PIC value of 0.66; however CcM1348 showed 7 alleles but with the highest PIC value of 0.70 while CcM2332 had the lowest PIC value (0.13) and the lowest number of alleles (2.00). PIC values measures the usefulness of each marker in

distinguishing one individual from another. Therefore, marker CcM1348 showed to be highly polymorphic and informative by providing 70% genetic diversity information among the genotypes tested.

The markers grouped the genotypes into three major distinct clusters (cluster I, II and III) based on their resistance/susceptible level. Cluster I and II were further grouped into several sub groups. The genotypes that showed some levels of tolerance were all grouped in cluster I. The resistant and susceptible check was grouped in different distinct clusters (cluster I and II) respectively. The genetic differences observed between these genotypes could indicate possible different sources of resistance to the insect pest complex.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

1. Genotypes performed differently in diverse locations. Four genotypes (ICEAPs 01147, 01179, 1147-1, KAT 60/8) in Marigat, Eight (ICEAPs 1154-2, 00911, 1147-1, 0979-1, 00850) in Koibatek and Four (ICEAPs 01159, 00850, 0979-1, 1147-1) in Fluorspar recorded higher yields and are promising yield potential.
2. Six genotypes (ICEAPs 01147, 1147-1, 01159, 00911, 0979-1 and 1154-2) are suitable for the three sites.
3. Three (ICEAPs 01154-2, 01541 and 00902) tolerant genotypes were identified in this study
4. The 16 genotypes revealed narrow genetic diversity based on SSR markers
5. Two markers (CcM1825 and CcM1348) are highly polymorphic
6. Koibatek recorded the highest yield (2.45t/ha) compared to Marigat (0.4t/ha) and Fluorspar (0.2) hence high yield potential area.

6.2 Recommendations

1. Six promising genotypes (ICEAPs 01147, 1147-1, 01159, 00911, 0979-1 and 1154-2) recording high yields identified in this study need to be further evaluated for stability of the traits for another season.
2. Mechanisms of resistance to this insect pest complex need to be identified to aid in breeding for resistance to insect pests in Pigeonpea.

3. Future genetic diversity studies should include wild relatives in the study as they could reveal new genetic resource.
4. There is need of developing molecular markers for insect pest resistance using Single Nucleotide Polymorphism (SNP) markers through Genome Wide Association Studies (GWAS).

REFERENCES

Allen ON, Allen EK. (1981) The Leguminosae: A source book of characteristics, uses, and nodulation. The University of Wisconsin Press, Wisconsin, USA, pp 812

Atta, B.M., Ahsanul Haq, M. and Shah, T.M. (2008). Variation and Inter-Relationships of Quantitative Traits in Chickpea (*Cicer Arietinum* L.). *Pak. Journal of Botany*. 40(2):637-647.

Bohra, A., Saxena, R. K., Gnanesh, B. N., Saxena, K., Byregowda, M., Rathore, A.,Varshney, R. K. (2012). An intra-specific consensus genetic map of pigeonpea [*Cajanus cajan* (L.) Millspaugh] derived from six mapping populations. *TAG. Theoretical and Applied Genetics. Theoretische Und Angewandte Genetik*, *125*(6), 1325–1338.

Bramel, P.J., Ford-Lloyd, B.V., Chandra, S. and Newbury, H.J. (2004). Assessment of degree and distribution of genetic diversity in pigeonpea landraces using SSR markers. A case study of pigeonpea in Andhra Pradesh. *International Crops* Pg75.

Changaya, A.G. (2007). *Development of high yielding pigeonpea (Cajanus cajan) germplasm with resistance to Fusarium wilt (Fusarium udum) in Malawi,* Pietermaritzburg,: African Centre for Crop Improvement School of Biochemistry, Genetics, Microbiology and Plant Pathology Faculty of Science and Agriculture University of KwaZulu-Natal pg1-59.

Charcosset A., Moreau L. (2004). Use of molecular markers for the development of new cultivars and the evaluation of genetic diversity. *Euphytica* 137: 81-94.

Chitra, U., Singh, U., Venkateswara Rao, P. (1996). Phytic acid, *in vitro* protein digestibility, dietary fiber and minerals of pulses as influenced by processing methods. *Plant Foods for Human Nutrition*, 49: 307–316.

Choudhary A. K., R. Sultana, A. Pratap, N. Nadarajan and U. C. Jha. (2011) "Breeding for Abiotic Stresses in Pigeon- pea," *Journal of Food Legumes* 24.(3) 165-174.

Das S.S. and Fakir M.S.A. (2010). Morphological features and pod growth in late sown indeterminate Pigeonpea Department of Crop Botany, Bangladesh Agricultural University, *Bangladesh Journal. Agroforestry &. Environment*. 4 (1): 55-58.

Edwards J, Barker A, Daly A, Jones C, Karp A. (1996). Microsatellite libraries enriched for several microsatellite sequences in plants. *Biotechniques,* 20: 758-759.

Egbe , O. M. & Kalu, B. A. (2009). Evaluation of pigeonpea [cajanus cajan (l.) Millsp.] Genotypes for intercropping with tall sorghum (sorghum bicolor (l.) Moench) in southern guinea savanna of nigeria. *Arpn journal of agricultural and biological science,* 4(4). 54-65.

Egbe,, O. M. & Vange , . T. (2008). *Yield and agronomic characteristics of 30 pigeonpea genotypes,* nigeria: *Life Science Journal* 5(2) 20-80.

FAOSTAT. (2015). Pigeonpea production in Kenya 2000-2013. Retrieved from http://faostat3.fao.org/browse/Q/QC/E

Faujdar Singh and Oswalt D.L. (1992) Genetics and Breeding of Pigeonpea Skill Development Series no. 10 Human Resource Development Program International Crops Research Institute for the Semi-Arid Tropics Patancheru, Andhra Pradesh

Flower D.J. and Ludlow, (1987). Variation among accessions of pigeonpea (*Cajanus cajan*) in osmotic adjustment and dehydration tolerance of leaves *Field Crops Research*., 17 (3) 229–243

Green, p. W. C., Stevenson, p. C., Simmonds, m. S. J., and Sharma, H. C. (2002). Can larvae of the pod-borer, Helicoverpa armigera (Lepidoptera: Noctuidae), select between wild

and cultivated pigeonpea [Cajanus sp. (Fabaceae)] *Bull. Entomology. Research*. 92:45–51

Gupta PK, Varshney RK, Sharma PC, Ramesh B. (1999). Molecular markers and their applications in wheat breeding. *Plant breeding* 118:369-390

ICRISAT Newsletter. (2003). Crop improvement and management. International Crops Research for the Semi-Arid Tropics (ICRISAT) Newsletter October 2003, pp 3- 9.

ICRISAT, (2008). International Crops Research Institute for the Semi-Arid Tropics. Annual technical Report Nairobi, Kenya, Pp 105.

Jaetzold, R. and Schmidt, H.(1983). Farm management handbook of Kenya-natural conditions and farm management information, Vol .II/B: Central Kenya (Rift Valley and Central Provinces) Pp 8-408.

Joshi PK, Parthasarathy Rao P, Gowda CLL, Jones RB, Silim SN, Saxena KB and Jagdish Kumar. (2001). The world chickpea and pigeonpea economies: facts, trends, and outlook. Patancheru 502 324, Andhra Pradesh, India: *International Crops Research Institute for the Semi-Arid Tropics*.. 31–62.

Kanyam- Phiri GY, Snapp SS, Minae S. (1998). Partnership with Malawian farmers to develop organic matter technologies. *Outlook Agriculture*. 27: 167-175.

Khaki, Nolipher (2014). Evaluation of malawi pigeonpea (*Cajanus cajan* (l) accessions for tolerance to moisture stress and superior agronomic traits in uganda; a thesis submitted to the directorate of research and graduate training in partial fulfilment for the award of a masters, kampala: makerere university Pp.1-110.

Kumar Rao JVDK. (1990). Pigeonpea: nitrogen fixation. In: Nene YL, Hall SD, Sheila VK (eds) The pigeonpea. CAB International, Wallingford, Oxon, UK, pp 233–256

Kumari, A. D., Reddy, D. J. and Sharma, H. C. (2006). Effect on grain yield in different pigeonpea genotypes with different levels of resistance to pod borer, *Helicoverpa armigera*. *Indian Journal of Plant Protection* 34, 184-187.

Lateef, S S. and Reed, W. (1990). Insect pests of pigeonpea, in S. R. Singh (ed.). Insect Pests of Tropical Food Legumes. John Wiley & Sons, New York. pp. 193–242.

Le Pelley R.H. (1959). Agricultural insects of East Africa. East African high commission, Nairobi Kenya pp 307.

Li, Y. C., Roder, M. S., Fahima, T., Kirzher, V. M., Beiles, A., Korol, A. B. and Nevo, E., (2000), Natural selection causing microsatellite divergence in wildemmer wheat at the ecologically variable microsite at Ammiad, Israel. *Theoretical and. Applied . Genetics*. 100: 985-999.

Litt, M. and Luty, J. A., 1989, A hypervariable microsatellite revealed in vitro amplification of dinucleotide repeat within the cardiac muscle actin gene. *American. Journal of. Human. Genetics*, 44: 397- 401.

Liu, K., and S. V. Muse, 2005: PowerMarker: integrated analysis environment for genetic marker data. *Bioinformatics* 21, 2128-2129.

Mace ES, Buhariwalla KK, Buhariwalla HK, Crouch JH. (2003). A high-throughput DNA extraction protocol for tropical molecular breeding programs. *Plant Molecular Biology Reporter* 21(4):459-460.

Mander, M., Mander, J., Breen, C., (1996). Promoting the cultivation of indigenous plants for markets: Experiences from KwaZulu-Natal, South Africa. In: *Domestication and Commercialisation of Nontimber Forest Products in Agroforestry Systems*. Non-wood Forest Products, 9: 298.

Manyasa, E. O, Silim, S. N, Githiri, S. M, & Christiansen, J. L. (2008). Diversity in Tanzanian pigeonpea [*Cajanus cajan* (L.) Millsp.] landraces and their response to environments. *Genetic. Resource and Crop Evolution*. , 55, 379-387.

Manyasa, E.O., Silim, S.N. and Christiansen, J.L. (2009). Variability patterns in Ugandan pigeonpea landraces. *Journal of SAT Agricultural Research* 7 1-9.

Miller J. (1990). RFLP analysis of phylogenetic relationships and genetic variation in the genus Lycopersicon. *Theoretic and Appllied Genetics* 80:437–448.

Minja, E.M. (2001). Yield losses due to field pests and integrated pest management strategies for pigeon pea: Proceedings of a regional workshop, 12-15 Sept 2000, Nairobi, Kenya. pp. 48-54.

Minja, E.M., Shanower, T.G., Songa, J.M, Ong'aro, J.M., Kawonga, W.T., Mviha, P., Myaka, F.A., Slumpa, S., Okurut-Akol, H., Opiyo C. (1999). Surveys of insect pest damage and pest management practices on pigeonpea in farmers' fields in Kenya, Malawi, Tanzania and Uganda. *African Crop Science Journal* 7 (1): 59-69.

Minja , E., (1999). *Surveys in farmers fields during 1992 in Kenya to determine the incidence, distribution, and damage levels due to insect pest on pigeonpea,* Nairobi: ICRISAT.

Minja, E. M, Shanower, T. G, Silim, S. N, & Singh, L. (1999). Evaluation of pigeonpea Pod borer and pod fly tolerant lines at Kabete and Kiboko in Kenya. *African Crop Science Journal*, 7, 71-79.

Minja, E. M. (1997). Insects of pigeonpea in Kenya, Malawi, Tanzania and Uganda and grain yield losses in Kenya: a consultant’s report, Bulawayo, Zimbabwe: ICRISAT, 65

Minja, E. M, Shanower, T. G, Songa, J. M, Ongaro, J. M, Mviha, P, Myaka, F. A, & Okurut-akol, H. (1996). Pigeonpea seed damage from insect pests in farmers' fields in Kenya, Malawi, Tanzania and Uganda. Int. Chickpea Pigeonpea Newsletter, 3, 97-98.

Nadimpalli, B. G., Jarret, R. L., Pathak, S.C. and Kochert, G., (1992). Phylogenetic relationships of the pigeonpea (*Cajanus cajan*) based on nuclear restriction fragment length polymorphism. *Genome*, 36: 216–223.

Odeny, A.D. (2007). The potential of pigeonpea (*Cajanus cajan* (L.) Millsp.) in Africa. *Natural Resources Forum.* 31 297–305.

Odeny, D. A., Jayashree, B., Gebhardt, C. and Crouch, J., (2009), New microsatellite markers for pigeonpea (*Cajanus cajan* (L.) millsp.). *BMC Research.* 2**:** 35.

Okiror M.A. (1986). Breeding for Resistance to *Fusarium* wilt. PhD thesis, University of Nairobi pp. 1-65.

Okori, P., Fahleson, J., Rubaihayo, P. R., Adipala, E. and Dixelius, C., (2003), Assessmentof genetic variation among East African *Cercosporazeaemaydis* populations using AFLP and RFLP. *Journal of. African. Crop Science.*, 11:75-86.

Osman, A. N., Ræbild, A., Christiansen, J. L., & Bayala, J. (2011). Performance of cowpea (Vignaunguiculata) and Pearl Millet (*Pennisetum glaucum*) Intercropped under Parkiabiglobosain an Agroforestry System in Burkina Faso. *African Journal of Agricultural Research*, 6(4), 882-891.

Panguluri DK, Janaiah K, Govil JN, Kumar PA, Sharma PC. (2006). AFLP fingerprinting in pigeonpea (*Cajanus cajan* (L.) Millsp and its wild relatives. *Genetic Resources and Crop Evolution* 53: 523–53.

Paul, S., Wachira, F.N., Powell, W. and Waugh, R. (1997). Diversity and genetic differentiation among populations of Indian and Kenyan tea (*Camellia sinensis* (L.) Kuntze) revealed by AFLP markers. *Theoretical of Applied Genetics*, 94: 255–263.

Pillen, K., Binder, A., Ramsay, K. L., Waugh, R., Forster, J. and Leon, J. (2000). Mapping new EMBL derived barley microsatellites and their use in differentiating German barley cultivars. *Theoretical of Applied Genetics* 101: 652-660.

Polanco, C. and Ruiz, M. L., (2002). AFLP analysis of soma clonal variation in *Arabidopsis thaliana* regenerated plants. *Plant Science*., 162: 817–824.

Prasannalatha, C. H., Parampreet Kaur and Bhalla, J. K., (1999). Molecular characterization of somaclonal variants in pigeonpea. *Current. Science*, 76 (5): 693-695.

Rao, S. C., Coleman, S.W. and Mayeux, H. S., (2002). Forage production and nutritive value of selected pigeonpea ecotypes in the southern great plains.*Crop Science.,* 42**:**1259-1263.

SAS Institute Inc. (1998). SAS/STAT user's guide, release 6.03 ed. SAS Institute, Cary, USA.

Saxena KB. (2008) Genetic improvement of pigeonpea – a review. *Tropical Plant Biology* 1:159–178

Shanower, T.G., Romeis, J., Minja, E.M., (1999). Insect pests of pigeonpea and their management. *Annual Review of Entomology*, 44: 77–96.

Sharm, O. P. (2011). Management of pigeonpea pod borers with special reference to pod fly. *Indian Journal of Agricultural Sciences* , 6: (81). 539–43,.

Sharma, H. C., Green, P. W. C., Stevenson, P. C., and Simmonds, M. S. J.(2001). What makes it tasty for the pest? Identification of Helicoverpa armigera (Hubner) feeding stimulants and location of their production on the pod-surface of pigeonpea [Cajanus

cajan (L.) Millsp.]. Competitive Research Facility Project R7029 C, Final Technical Report. DFID, London, United Kingdom pp: 1-13.

Sharma, H.C., Pampapathy, G., Reddy, L.J., (2003). Wild relatives of pigeonpea as a source of resistance to the pod fly (*Melanagromyza obtuse* Malloch) and pod wasp (*Tanaostigmodes cajaninae* La Salle). *Genetic Resources and Crop Evolution*, 50: 817–824.

Sharma, S. K., Knox, M. R. and Ellis, T. H. N., (1996), AFLP analysis of the diversity and phylogeny of Lens and its comparison with RAPD analysis. *Theoretical. Applied. Genetics.,* **93**: 751–758.

Silim S.N, Coe R, Omanga Pa and Gwata E.T (2006). The response of pigeonpea genotypes of different duration types to various temperature and photoperiod under field conditions in Kenya. *Journal of food, agriculture & environment vol.4 (1); 209-214*

Singh , F. & Oswalt, . D. L., (1992). *Pigeonpea Botany and Production Practices,* Andhra Pradesh: International Crops Research Institute for the Semi-Arid Tropics,pp 5-33.

Snapp SS (1998). Phosphorus and sustainability of Sub Saharan Africa smallholder farms, In: J. P. Lynch and J. Deikman (eds.). Phophorus in plant biology: Regulatory roles in molecular, cellular, organismic and ecosystem processes. Am. Soc. Plant physiol., Madison, Wis., pp. 59-72

Shuelke M. (2000). An economic method for the fluorescent labeling of PCR fragments. *Nature Biotechnology*.18:233-234

Songok, S, Ferguson, M, Muigai, A. W, & Silim, S. (2010). Genetic diversity in pigeonpea [*Cajanus cajan (*L.) Mill sp.] Landraces as revealed by simple sequence repeat markers. *African. Journal.of Biotechnology*. , 9, 3231-3241.

Speranza, C.I., Kiteme, B., Wiesmann, U., (2007). Droughts and famines: The underlying factors and the causal links among agro-pastoral households in semi-arid Makueni district, Kenya. *Global Environmental Change*. 18(1) 220-233

Subbarao, G.V., Chauhan, Y.S., Johansen, C., (2000). Patterns of osmotic adjustment in pigeonpea — its importance as a mechanism of drought resistance. *European Journal of Agronomy*, 12: 239–249.

Subharani Tekeoglu, M , S. & Singh., . T. . K., (2007). Influence of meteorological factors on population dynamics of pod fly, Melanagromyza obtusa Malloch (Diptera: Agromyzidae) in pigeonpea under agro-climatic conditions of Manipur.. *Indian Journal of entomology*, 1:(69) 78–80.

Tyagi, A. P., (1997), Investigation of quantitative trait loci (QTLs) in two diverse strains of pigeonpea (*Cajanus cajan*) using RAPD markers. *Journal of South Pacific Agriculture* 4:92-98.

Upadhyaya, H. D., Reddy, K. N., Gowda, L. C. & Singh, S., (2010). identification and evaluation of vegetable type pigeonpea (cajanus cajan (L.) Millsp.) in the world germplasm collection at ICRISAT genebank. *plant genetic resources: characterization and utilization* , 2621. 1-9.

Valenzuela, H., Smith, J., (2002). Pigeonpea. *Sustainable Agriculture Green Manure Crops*, Aug. 2002, SA-GM-8.

Vange, T. and Egbe M. O. (2009)*; Studies* on Genetic Characteristics of Pigeonpea Germplasm at Otobi, Benue State of Nigeria. *World Journal of Agricultural Sciences* 5 (6):714-719

Varshney, R. K., Close, T. J., Singh, N. K., Hoisington, D. A. and Cook, D. R.,(2009 *)* Orphan legume crops enter the genomics era**.** *Current. Opinion. Plant Biology.*, 11**:**1-9.

Vos P, Hoger R, Bleeker M, Reijans M, Van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M *et al* (1995) AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research* 23:4407–4414

Wallis ES, Byth DE, Saxena KB (1981). Flowering responses of thirty-seven early maturing lines of pigeonpea. In: International Workshop on Pigeonpeas, 2, 15–19 Dec. 1980. ICRISAT, Patancheru, AP, India, pp.143–150

Wenzel P, Li H, Carling J, Zhou M, Raman H, Paul E, Hearnden P, Maier C, Xia L, Caig V, Ovesná J, Cakir M, Poulsen D, Wang J, Raman R, Smith KP, Muehlbauer GJ, Chalmers KJ, Kleinhofs A, Huttner E, Kilian A (2006). A high-density consensus map of barley linking DArT markers to SSR, RFLP and STS loci and agricultural traits. *BMC Genomics*. ;7: 206.

Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A. and Tingey, S.V., (1990), DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research*, 18: 6531–6535.

Yang, S., Pang, W., Ash, G., Harper, J., Carling, J., Wenzl, P., Huttner, E., Zong, X. and Kilian, A., (2006). Low level of genetic diversity in cultivated pigeonpea compared to its wild relatives is revealed by diversity arrays technology. *Theoretical. Applied. Genetics*., 113: 585 – 595.

Young, N.D., Mudge, J., Ellis, T.N., (2003). Legume genomes: More than peas in a pod. *Current Opinion in Plant Biology*, 6: 199–204.

APPENDICES

Appendix I: Rainfall and Temperature from January to December 2014 (period of the study) for the three sites

Rainfall and Temperature readings						
Month	**Marigat**		**Fluorspar**		**Koibatek**	
Mon	Rainfall	temp	Rainfall	Temp	Rainfall	temp
Jan	100	33	14.00	30.2	100	27
Feb	90	34	25.90	26.46	60	28
Mar	150	34	87.80	29.89	80	28
Apr	200	32	46.80	28.25	180	26
May	145	33	130.00	21.88	220	25
Jun	**120**	**32**	**83.20**	**30.6**	**300**	**25**
Jul	115	31	243.80	25.12	200	24
Aug	160	31	170.60	27.2	250	24
Sep	**90**	**33**	**65.10**	**34.45**	**360**	**26**
Oct	145	33	314.80	26.79	330	25
Nov	100	32	123.70	28.22	410	25
Dec	125	33	85.00	29.36	550	26
Total	**1540**	**391**	**1390.7**	**328.42**	**2940**	**309**
Ave	**128.3**	**32.58**	**115.89**	**27.37**	**245**	**25.75**

Sourced from www.worldweatheronline.com and weather-average/rift valley/ke.aspx

Appendix II: Qubit® readings for 32 genomic DNA samples in ng/ µ

Sample No.	Readings (ng/µ)	Sample No.	Readings (ng/µ)
Std.1	<0.010	Std.1	<0.010
Std. 2	90.8	Std. 2	90.8
1	263	17	215
2	274	18	255.6
3	251	19	214
4	203	20	177.6
5	253	21	236
6	251.4	22	278
7	213	23	212
8	271	24	186.1
9	209	25	234
10	182	26	218
11	132	27	208
12	232	28	192.8
13	179	29	211
14	248.5	30	176.3
15	279.1	31	176.2
16	310	32	193.4

Appendix III: Power Marker® Summary statistics output for the selected 24 markers across 32 individual DNA samples.

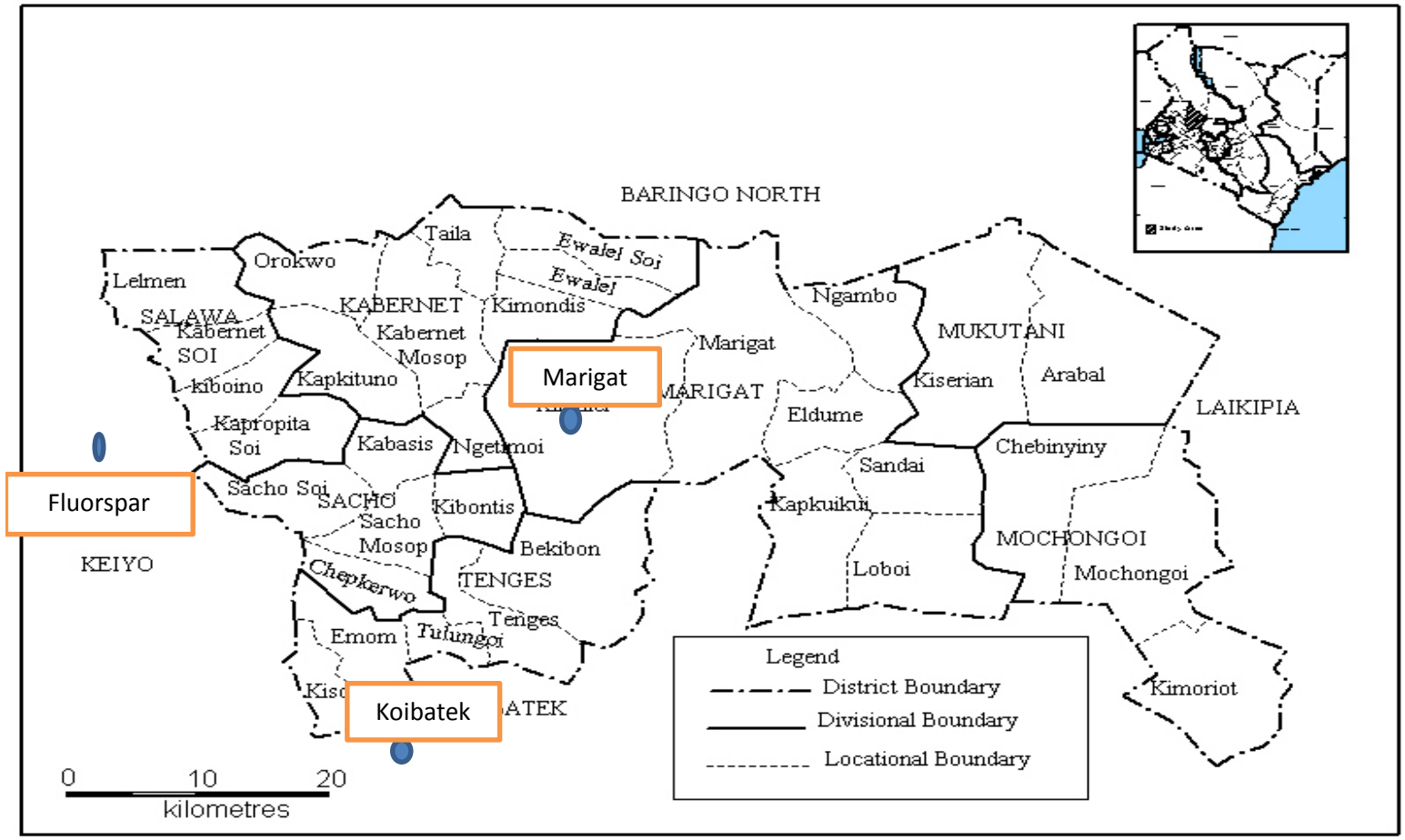
Marker	MajorAllele Frequency	Sample Size	No. of obs.	Allele No	Availability	Gene Diversity	Heterozy gosity	PIC
CcM0603	0.48	32.00	32.00	3.00	1.00	0.59	0.34	0.50
CcM1232	0.50	32.00	32.00	5.00	1.00	0.63	0.81	0.56
CcM0047	0.69	32.00	32.00	2.00	1.00	0.43	0.25	0.34
CcM1139	0.69	32.00	32.00	4.00	1.00	0.46	0.25	0.40
CcM1373	0.40	32.00	31.00	4.00	0.97	0.66	0.06	0.59
CcM1582	0.75	32.00	32.00	3.00	1.00	0.39	0.22	0.34
CcM0602	0.88	32.00	32.00	4.00	1.00	0.23	0.13	0.22
CcM1110	0.94	32.00	32.00	2.00	1.00	0.12	0.00	0.11
CcM1493	1.00	32.00	32.00	1.00	1.00	0.00	0.00	0.00
CcM1598	0.95	32.00	32.00	3.00	1.00	0.09	0.09	0.09
CcM1506	0.73	32.00	32.00	5.00	1.00	0.43	0.31	0.40
CcM1820	0.39	32.00	32.00	5.00	1.00	0.68	0.50	0.62
CcM1611	0.53	32.00	32.00	2.00	1.00	0.50	0.31	0.37
CcM1825	0.45	32.00	32.00	8.00	1.00	0.70	0.56	0.66
CcM1348	0.40	32.00	31.00	7.00	0.97	0.74	0.32	0.70
CcM1447	0.56	32.00	31.00	4.00	0.97	0.54	0.84	0.45

CcM2044	0.58	32.00	30.00	3.00	0.94	0.57	0.60	0.50
CcM2332	0.92	32.00	32.00	2.00	1.00	0.14	0.16	0.13
CcM0444	0.88	32.00	32.00	3.00	1.00	0.22	0.13	0.21
CcM2049	0.41	32.00	32.00	4.00	1.00	0.66	0.31	0.59
CcM0594	0.72	32.00	32.00	4.00	1.00	0.43	0.03	0.37
CcM2060	0.91	32.00	32.00	3.00	1.00	0.17	0.13	0.17
CcM1014	0.63	32.00	32.00	3.00	1.00	0.52	0.28	0.45
CcM1277	0.48	32.00	21.00	4.00	0.66	0.61	0.05	0.54
Mean	**0.66**	**32.00**	**31.33**	**3.67**	**0.98**	**0.44**	**0.28**	**0.39**

Appendix IV: List of 20 SSR primer pairs used in the study

S.No	Primer name	Repeat motif	PIC value	Forward primer (5'-3')	Reverse primer (5'-3')
1	CcM0047	(A)12n(TC)5	0.76	TGTCTTTTGGATGAAAGTAGGGA	GTTGGGGATGGGAAGAGAAT
2	CcM0602	(AT)14	0.86	TTTGCTCTATAACAAGGGATTCA	TGCTCTAATTCATGTCAAAACCC
3	CcM0603	(A)21(AG)9	0.80	TGAGAGAGGATGTGTGGTGC	GTTGCACACACTGGCAAATC
4	CcM0444	(TA)7	0.79	TGTCATGAGTGGCTGATCCT	TCAACCAAAATCCAAACCAA
5	CcM0594	(GA)9n(TC)9	0.77	GGCTTGGTTCTTTCTTGGTG	AAGTCCCTGACTTTCCCCAT
6	CcM1014	(AT)10	0.60	ACCCGATCATTTTGGACAGA	TGATGATCGATATTTGTAATATGAAGT
7	CcM1139	(ATG)6	0.69	CAGGGACAAATGCCTGAAGT	CCCACCAAGCTCTCAAAGAC
8	CcM1348	(T)10n(AT)18	0.64	TGAAGTCATTGCACCTTGACA	AAAGTGGCTAAACCAATTGCATA
9	CcM1373	(TG)7	0.73	AATCAACTCGGCCATTCTTG	TCCTTTCAAGCGGAAAACAT
10	CcM1232	(GA)6	0.68	GCTTGAGGCCTTGAGCTAGA	GCCCTCAGCAATTCTCATTC
11	CcM1447	(AT)10	0.82	CTTTCCACGGTCCAGTGAAT	TCCATTTGACATGTAAACAATGAAC
12	CcM1582	(AAT)5n(TGT)8	0.69	CCAACACCTTTCCTTAGCCT	CCTAACCAAGGATGACGTGG
13	CcM1506	(A)10n(A)10(TA)11	0.68	TGTTTTTGCAAAGGTTTCCC	CAACACAATGAAAAAGTAAACATCA
14	CcM1611	(AT)22	0.81	AAGAAAAATTCCTAAACCCGTG	GGAAGATCGTCCCTACGACA
15	CcM1820	(AT)14	0.75	GGCAGAAAATGGAGGGTACA	TTGGCTATCGGAAATAACGG
16	CcM1825	(G)13n(TG)5	0.69	TGAAGTTGGCGGAAAAACAT	TCGGACGAAAAACATACTTGC

17	CcM2044	(TAT)9	0.84	ATCACTCCAAGCACCCAAAC	TGCAAATGGAAGGGAATAGC
18	CcM2049	(TAT)9	0.72	GCGACCAGGTACTTTCAAGC	CGAAAAGCGATTTCAGAATTT
19	CcM2060	(AT)21n(AT)6	0.60	TGGAGAAGAGATTCATTTATTTGATG	AGAAGCCAAACTTCCTTCGG
20	CcM2332	(ATA)13	0.72	TCCATTTTTCTTCTGCGGTG	TGCTATCAATCCAAAAACACAAA



Appendix V: A map for the three sites (Marigat, Koibatek and Fluorspar) Source: Baringo District Development plan (2003-2008)