MORPHOLOGICAL AND GENETIC CHARACTERIZATION OF PIGEON PEA (*Cajanus cajan* L) USING SIMPLE SEQUENCE REPEAT MARKERS

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DECLARATION

I declare that the work presented in this thesis is my original work and has not been presented in any other university or any other award.

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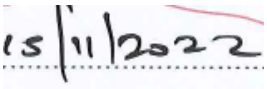
We hereby confirm that the candidate carried out the work reported in this thesis under our supervision.

Signature:……  ……. Date:… 

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DEDICATION

I dedicate this thesis to my dear wife and daughters for the support they accorded me during my study.

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ABBREVIATIONS AND ACRONYMS

AFLP	Amplified Fragment Length Polymorphism
AMOVA	Analysis of molecular variance
ANOVA	Analysis of Variance
CTAB	Cetyl Trimethyl Ammonium Bromide
DNA	Deoxyribonucleic Acid
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
EDTA	Ethylenediaminetetraacetic Acid
GBK	Gene Bank of Kenya
ICRISAT	International Crop Research Institute for Semi-Arid Tropics
KALRO	Kenya Agricultural Livestock Research Institute
KAT	Katumani
LD	Long Duration
MAS	Marker Assisted Selection
MD	Medium Duration
NJ	Neighbor Joining
NTSYSPC	Numerical taxonomy And Multivariate Analysis System
PC	Principal Component
PCR	Polymerase Chain Reaction
PCA	Principal Component Analysis
PCoA	Principal Co-ordinate analysis
PIC	Polymorphism Information Content
PTL	Plant Transformation Laboratory
RAPD	Randomly Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
SAS	Statistical Analysis Software
SYBR	Synergy Brands
SD	Short Duration
SMD	Sterility Mosaic Disease
TAQ	Thermus Aquaticus
TBE	Tris-Borate EDTA
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
USD	United States Dollar
UV	Ultra-Violet
XSD	Extra-Short Duration

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ABSTRACT

Pigeon pea [*Cajanus cajan* (L.) Millspaugh)] is a drought tolerant purse crop that is majorly cultivated for food and as a source of income. It is also used as a fodder crop in some countries. Narrow genetic variation caused by natural outcrossing and long-term artificial selection and cultivation of varieties possessing specific traits is a serious challenge in pigeon pea varietal development. Therefore, understanding the genetic diversity is a key precursor for pigeon pea genetic improvement. The objective of the present study was to establish the genetic diversity among 24 selected Kenyan pigeon pea accessions using nine agro-morphological traits and 12 SSR markers. Knowledge acquired on genetic and morphological variations as well as relationships among genotypes assist plant breeders to develop suitable breeding strategies aimed at resolving pigeon pea production constraints in Eastern and Coastal counties in Kenya. This can be done using parental lines index in the breeding programs. Planting materials (seeds) were collected from Dryland Agricultural Research Station-Katumani in Machakos. Morphological characterization was done by taking the 9 pigeon pea parameters among the 24 samples. On the other hand, for genetic characterization, DNA extraction was done using the CTAB method and the diversity done using 12 simple sequence repeat (SSR) markers. For analysis, MINITAB 17 software was used for construction of dendrogram and depicting of principal component. ANOVA results revealed a significant difference among the accessions for all 9 quantitative traits assessed. The stem diameter, number of branches per plant, 100-seed weight, which strongly associated with the first two PCs and pod length are the primary element this analysis identified. In cluster analysis, agro-morphological traits were utilized to characterize the accessions into four groups. A total of 33 alleles were observed with an average of 2.7500 alleles per marker. Polymorphic information content ranged between 0.2755 and 0.6036 with a mean of 0.4178. Pair-wise genetic dissimilarity coefficients ranged from 0.0750 to 0.9003. A neighbor-joining tree clustered the accessions into four groups. The analysis of molecular variance showed that 97% of genetic variation occurred within the populations and 3% existed among the populations. From the current study, both genetic and morphological characterization of the 24 accessions showed a significant difference among the germplasms and therefore genetic and morphological characterization clearly indicated that they are useful tools for characterization of *Cajanus cajan* The results obtained from the current study provides a basis for future genetic improvement of pigeon pea.

Keywords: agro-morphological traits, Kenya, pigeon pea polymorphism information content, SSR markers, varietal development.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Pigeon pea [*Cajanus cajan* (L.) Millspaugh] is one of the major pulse crops that is mostly grown in tropical and sub-tropical agricultural zones (Géofroy *et al.*, 2020). It belongs to the family *Leguminosae* and the genus *Cajanus*. In Kenya, pigeon pea is cultivated as a food and cash crop and is the 2nd most important source of legume food after field beans (*Phaseolus vulgaris*) (FAOSTAT, 2015). It is mainly grown in the eastern and coastal regions of Kenya (Cheboi *et al.*, 2016). The mean yield potential of pigeon pea in Kenya ranges between 0.40 to 0.70 t/ha; this is relatively low compared to the yields produced in India (the largest producer), that range between 1.5 and 2.5 t/ha (Hluyako *et al.*, 2017). Various abiotic and biotic stresses, as well as lack of quality seeds and poor crop husbandry, comprise the main factors contributing to the low grain yield of pigeon peas (Cheboi *et al.*, 2016).

Unlike other legume crops, pigeon pea is highly regarded for its multiple uses. Its grains are rich in minerals, proteins, and vitamins (Njung'e *et al.*, 2016). Besides, leaves are a good source of fodder and alternative medicine for curing various ailments, including malaria and fever (Zavinon *et al.*, 2018). Moreover, its stems provide fuel and materials for roofing (Kimaro *et al.*, 2020). Its root system is deep and extensive; it is able to fix atmospheric nitrogen and maintain optimal osmotic adjustment in leaves during dry periods. The combination of these adaptive features makes it survive in semi-arid tropics, which is characterized by high temperature and soils that are less fertile (Kwena *et al.*, 2019). In Kenya, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) pioneered pigeon pea genetic improvement. A number of novel cultivars possessing farmer-preferred traits such as dwarf, short and medium maturity duration have been released (Ojwang *et al.*, 2016). Majority of farmers within the pigeon pea growing areas have

predominantly adopted these improved cultivars, thereby boosting food security and livelihoods of farmers.

Several researchers have previously reported diversity analysis in pigeon pea (Nyirenda *et al.*, 2020). A few strategies are used in assessing diversity in plants. The utilization of agro-morphological characteristics is the traditionally and commonly used method for phenotypic diversity assessment of plant species (Géofroy *et al.*, 2020). The method is inexpensive and simple. However, the phenotypic characteristics can be highly affected by the environment (Kimaro *et al.*, 2021). The modern advancement in molecular techniques has resulted in the development of molecular markers for use in genetic diversity analysis. Among the commonly used molecular markers, simple sequence repeats (SSRs) are mostly preferred for use in diversity studies owing to relatively high abundance in the genome, co-dominance, and high polymorphism, in addition to being reproducible and easily detected using polymerase chain reaction (PCR) (Njung'e *et al.*, 2016).

Successful breeding and release of new cultivars mainly rely on the availability of a wider genetic base. Unfortunately, narrow genetic variation caused by long-term artificial selection and cultivation of improved cultivars possessing specific traits is a serious drawback in pigeon pea varietal improvement programs (Karmakar *et al.*, 2019). Diversity assessment is a key pre-breeding step for a successful pigeon pea breeding program. Therefore, there is an imperative demand to carry out a diversity assessment of key traits of pigeon pea germplasm accessions (wild, local landraces, breeding lines, and released cultivars) as a way of broadening germplasm resources and identifying superior genes for future use in pigeon pea genetic improvement programs.

1.2 Statement of the Problem and Justification of the Study

In Africa, breeding and encouraging farming of drought-tolerant crops are potential ways used to curtail the damaging effects of climate change. Pigeon pea is among the least studied legumes, although it is the most drought-tolerant crop compared with other legumes, including common beans, cowpea and soya beans. Diseases and pests have also been another major drawback in pigeon pea production in Africa and particularly in East Africa (Minja *et al*., 2000). The most important pests are pod borer, *Helicoverpa armiger*, through the flowers and pods are attractive to a wide range of insect pests. For diseases, the most common disease in East Africa is Fusanium wilt (*Fusarium udum*) though considerable effort has been devoted by ICRISAT to developing wilt-resistant pigeon peas, adapted to cultivation in the region. Other constraints are poor production practices that affect the yield, low soil fertility, inadequate or incorrect use of herbicides and fungicides and lack of appropriate storage facilities.

In many plants, diversity studies have previously been used as strong tools in grouping of cultivars and identification of superior qualities (Porter, 1983). Kumar (1999) showed that DNA markers are the most promising methods used to differentiate among genotypes at subspecies and species levels and particularly SSRs which are popular. SSRs disclose more dissimilarity among plant genotypes (Varshney *et al.*, 2010). Despite the benefits that might be realized as a result of carrying out such studies, knowledge of morphological and genetic diversity among the Kenyan pigeon peas is lacking. This will therefore delay the realization of the full potential of this legume as a food source and income-generating crop for poor households in Kenya. The pigeon pea has a big potential

to grow well in semi-arid areas of Kenya as well as keep improving soil fertility (Hillocks *et al.,* 2000).

Diversity studies have been shown to be powerful strategies used to group cultivars as well as in studies of taxonomic status. Porter, (1983) established that morphological variability in pigeon pea abounds in the tropics, thus giving light to the structure of germplasm in order to develop hybrids that are adapted to a specific ecology. Knowledge of genetic and morphological variation as well as relationships among genotypes will assist plant breeders to develop suitable breeding strategies aimed to resolve pigeon pea production constraints. It can be done using parental lines index in the breeding programs.

1.3 Research Questions

i. What are the genetic differences between the pigeon peas grown in Machakos, Makueni, Murang'a, Kitui, Meru, Embu, Tharaka-Nithi, Kwale and Kilifi counties in Kenya?

ii. What are the phenotypic dissimilarities between the pigeon peas grown in Machakos, Makueni, Murang'a, Kitui, Meru, Embu, Tharaka-Nithi, Kwale and Kilifi counties in Kenya?

iii. Is there a relationship between phenotypic yield attributes and genotypic traits in pigeon peas grown in Machakos, Makueni, Murang'a, Kitui, Meru, Embu, Tharaka-Nithi, Kwale and Kilifi counties in Kenya?

1.4 Objectives

1.4.1 General objective

To quantify genetic and phenotypic diversity among selected Kenyan pigeon pea (*Cajanus cajan*) genotypes.

1.4.2 Specific objectives

i. To determine phenotypic diversity among selected Kenyan pigeon pea genotypes using morphological traits.

ii. To determine the genetic diversity among selected Kenyan pigeon pea genotypes using simple sequence repeat (SSR) markers.

iii. To determine the correlation between the phenotypic yield attributes and genotypic traits in pigeon peas grown in Machakos, Makueni, Murang'a, Kitui, Meru, Embu, Tharaka-Nithi, Kwale and Kilifi counties in Kenya.

CHAPTER TWO

LITERATURE REVIEW

2.1 Pigeon peas (*Cajanus cajan*)

2.1.1 Classification

The taxonomic classification of Pigeon pea is the domain Eukaryota, which belongs to the Plantae (Kingdom), Spermatophyta (Phylum), Angiospermae (Subphylum), Dicotyledonae (Class), Fabales (Order), Fabaceae (Family), Faboideae (subfamily), Cajanus (Genus) and *Cajanus cajan* (species). It belongs to six genera, including *Cajanus*, *Eriosema*, *Dunbaria*, *Paracalyx*, *Flemingia*, and *Rhynchosia*, *Abortrifolia indica* was the initial scientific name that used to define pigeon pea. With reference to botanical classification international rules for crops, the conclusion regarding pigeon pea scientific name was then agreed to be *Cajanus cajan* as Linnaeus states (Smart, 1990).

2.1.2 Morphology

Pigeon pea is a perennial leguminous and erect shrub. It grows to an approximately 1- 2 m height but can grow up to 5 metres tall (Mula and Saxena, 2010). It also contains a deep poisonous taproot, whereas its stems are woody at the base and branching points. The leaves portray trifoliate and alternate features, whereas the leaflets resemble lanceolate. The flowers are also grouped at the axils racemes of the branches, papilionaceous and yellow. The fruit is a pubescent, straight and flat pod. It holds 2-9 seeds which can be black, red, cream-white, or brown in colour, and which are small and hard-coated.

2.1.3 Uses

As human food, pigeon pea seeds can be used in almost any imaginative form. The green pods and seeds are the most utilized form in Africa though dry seeds are increasingly gaining popularity. In Nigeria, for example, the dry seeds are cooked whole until tender then mixed with cooked yam, maize, dried cocoyam grits or freshly cooked cocoyam, sweet potatoes in addition to vegetables, palm oil, salt, pepper and other spices (Enwere, 1990). There are currently major efforts to promote the introduction of dehulling methods used in India in order to increase diversity of pigeon pea use in Africa (Agona and Muyinza, 2005). In many parts of Eastern Africa, dhal is becoming a popular meal. Some potential uses of pigeon pea for human consumption in Africa include the production of noodles (Singh et al., 1989), tempe (Lyimo, 2000) and other fermented products (Onofiok *et al*., 1996).

Elsewhere, pigeon pea is used as a flour additive to other foods in soups and with rice (Center for New Crops and Plants Products, 2002). Pigeon pea flour is an excellent component in the snack industry and has been recommended as an ingredient to increase the nutritional value of pasta without affecting its sensory properties (Torres et al., 2007). Millet/pigeon pea biscuits are reportedly highly nutritious and provide a cheaper alternative to wheat imports in Nigeria (Eneche, 1999). Although the medicinal value of pigeon pea in Africa has not been fully exploited, there seems to be great potential to that end. Pigeon pea leaves have been used to treat malaria (Aiyeloja and Bello, 2006) in Nigeria, while in Southern Africa, pigeon pea is currently one of the indigenous crops being promoted for potential medicinal use (Mander et al., 1996).

The stem of the pigeon pea is a good source of fuel because of their thriving habit. However, their energy value is almost half charcoal's energy (Cook *et al.,* 2005). Pigeon pea branches and stems are used for basketry. Some experiments have shown that it can be used as paper pulp raw material (Cook *et al.,* 2005). Additionally, it positively impacts the environment by involving alley cropping and acting as a windbreaker, cover crop, green manure, and shade plant (Kwesiga *et al*., 2003). It also has many applications in animal feeding in that the pods and leaves are some essential fodder rich in protein. The leaves of a pigeon pea can also be used as an alfalfa substitute for ruminant diets in cases where the growth of alfalfa is not possible (Saxena *et al*., 2002). The seeds may also be used in feeding poultry the same way they are fed to livestock (Wallis *et al*., 1986). Besides, pigeon pea acts as a good silkworm and lac insects' host (Cook *et al.*, 2005). Pigeon pea may alternatively be grown to produce seeds and forage management. Pure stands are grown or sown with molasses grass and Rhodes grass in Brazil and Hawaii. Mixtures can also be done using cereals like sorghum, millet and maize where possible. However, a mixture of pigeon pea and legumes is ineffective for fodder production as both are legumes and provide only protein (Orwa *et al*., 2009). Pigeon pea requires irrigation in the first two months, especially when rainfall is not sufficient, and also requires effective weed control (Duke, 1983).

Sowing pigeon pea can be done in either mixed pastures or pure stands. Once the establishment of pigeon pea is done, it has reached the flowering stage; grazing should remain rotational and light. This is because pigeon pea does not have the ability to withstand continuous and heavy grazing (Bekele *et al*., 2007).

2.1.4 Pigeon Pea Maturation

Time taken for the pigeon pea to mature is an essential factor in determining varieties' adaptation to cropping systems and different agro-climatic areas. The temperature controls the field duration of pigeon pea and how sensitive it is to the photoperiod. Pigeon peas are classified into four major groups of duration, as shown in table 2-1,

Table 2-0-1Pigeon pea Duration types and maturity days. (SN. Silim *et al*., 2006)

Grouping	Days to maturity (Approximate)
Extra-short duration (XSD)	<100 days
Short-duration (SD)	100-150
Medium- duration (MD)	151-180
Long- duration (LD)	>180

2.1.4.1 Extra- short duration (XSD)

A pigeon pea in this duration takes less than one hundred days, counting the duration between planting to flowering. Cooler temperatures may delay its growth to maturity from 94 days to 175 days; this delayed maturity reduces the late-season yield, and in return, it interferes with planting any other crop using a rotation approach (Snapp, 2003). XSD pigeon peas show that the yields experience little increase with a population increase from eight to sixty plants per meter square in an Indian tropical environment (Silim *et al*., 2007).

Extra-short duration in maturation of pigeon pea is an important factor in arid and semi-arid regions because it means the crop will utilize only the limited available moisture to mature within a very short time. If the traits controlling such character are identified, then breeders can use them to produce early maturing varieties of pigeon pea hence increasing production in arid and semi-arid regions.

2.1.4.2 Short-duration (SD)

The SD varieties can be grown in frost-free areas. Flowering in SD genotypes has the least sensitivity to photoperiod; hence one will find that they flower and mature during short summer. This group is more prone to pests, and those who grow them are mainly commercial farmers who got production inputs. Short-duration genotypes have a significantly smaller root system when compared with genotypes within long-duration (Singh & Oswalt, 1992).

The short-duration pigeon pea varieties were useful to my study since I expected them to harbour traits for early maturity which is a desirable characteristic for increasing production.

2.1.4.3 Medium duration (MD)

MD varieties are grown and intercropped in areas with warm temperatures. Maturity for MD varieties happens to experience delays in areas that are far from the equator, like Mozambique and Malawi. The MD types flower and they are photoperiod sensitive when a short-day period approaches. They have been developed through breeding and selection. As a result, medium altitudes act as their most preferred climate with 600 to 1500m with a mean temperature of twenty-three to twenty-five degrees Celsius (Walker *et al.*, 2015). Since they do well in medium altitude zones, they have traits that make them survive in these zones.

2.1.4.4 Long duration (LD)

They mostly grow in low-latitude or high elevated areas near the equator. Nevertheless, they can be intercropped in areas that are not near the equator so long there are warm

temperatures. They are photoperiod sensitive and flower in short days. The LD pigeon pea reserve soil moisture as the crop matures, especially in areas experiencing short rains (Njoroge *et al*., 2016).

Due to their survival in high altitude, they can provide traits that make them do well in these zones. Breeders, therefore, will be able to secure traits for almost all the altitudes.

2.2 Health benefits of a pigeon pea

Seeds of pigeon pea are consumed globally both in dried and fresh form. They are a rich in proteins, carbohydrates, minerals and fibre sources. Hence it is good for the production of fibre-fortified foods. These fortified foods are major nutrients supplier to the diet of children and adolescents. Pigeon pea has been an essential part of traditional medicine in South America, China, and India, where it is utilized in the treatment and prevention of various human illnesses. These diseases include measles, bronchitis, ulcers, hepatitis, some forms of cancer, yellow fever, diabetes, and pneumonia (Syed & Wu, 2018).

Besides being a source of fibre and proteins, it has a reasonable phytochemicals number that promotes health. The pigeon pea seed's phytochemicals include flavonoids, phenolic acids, tannins, phytic acid and saponins (Nix *et al*., 2015). Pigeon pea seeds are non-perishable with the food products' addition feasibility. Additionally, pigeon pea flour is suitable for food products, for example, nutritional bars, pasta, and bread making. It is a cereals substitute because of its gluten-free nature (Torres *et al*., 2007).

Table 2-0-2 Distribution of Nutrients in Mature Seed of Pigeon pea (Saxena *et al*., 2010)

Nutrient (g/100g)	**Mature seed**
Protein	21.7
Carbohydrates	62.78
Fibre	15
Lipid	1.49
Minerals (mg/100g)	
Calcium	130
Iron	5.23
Magnesium	183
Phosphorus	367
Sodium	1392
Zinc	17
Vitamins (mg/100g)	2.76
Vitamin c	0
Thiamin	0.643
Riboflavin	0.187
Niacin	2.965
Vitamin B-6	0.283
Amino acids (g/100g)	
Tryptophan	0.212
Threonine	0.767
Isoleucine	0.785
Leucine	1.549
Lysine	1.521
Methionine	0.243
Cystine	0.25
Phenylalanine	1.858
Tyrosine	0.538

2.3 Distribution of pigeon pea

Pigeon pea is cultivated all over the world, with the legume being highly distributed in the Asian continent in which India tops in production. The high production of pigeon pea in India has attracted numerous researchers to collect samples from the country for germplasm purposes. Research institutes like the ICRISAT, Regional Pulse Improvement Program and the Indian Agricultural Research Institute carry out breeding research for the improvement of the pigeon pea. In the Indian subcontinent, this legume is mainly intercropped with other crops during planting. For instance, it is intercropped with other crops like pearl millet, groundnuts, cotton, sorghum and many others. Pigeon pea was adopted in Australia nearly 1000 years ago, with the majority of the crop used as a fodder crop and currently grown for export (Mallikarjuna *et al.*, 2011). Other areas in the Asian continent, such as Indonesia, the pacific islands and Hawaii, pigeon pea is also grown in small scales. In total, the Asian and oceanic continent records about 28 countries growing pigeon pea which is reported to be grown at altitudes of up to 1800m.

In America, the crop was introduced in the 1500s and adapted in the semi-arid and Caribbean islands in the south and Central America. The legume is consumed as a fresh vegetable or as a canned vegetable in many areas of the continent. The USA is the leading importer of canned pigeon peas from the Dominican Republic and Puerto Rico, whose produce of pigeon peas is high, (Maesen, 1983). Throughout the years, the crop has been reported to be grown in 37 countries at 2300m altitudes, with flowering and fruiting happening all around the year, and the vegetable harvested between the month of February and April in most parts of the continent, (Johansen *et al*., 1993).

In Africa, the popularity of the Pigeon pea has been analysed to be grown through subsistence farming. More than 33 countries produce pigeon peas, although data on their production and specific areas where it is grown are grossly undervalued (Shanower *et al.*, 1999). The leading world producers of pigeon peas are India, Myanmar, Malawi and United Republic of Tanzania. Bulk production of pigeon peas is nevertheless concentrated in Eastern Africa (Shanower *et al.*, 1999), with its cultivation also reported in South Africa (Swart *et al.*, 2000), Nigeria (Aiyeloja and Bello, 2006), Zambia (Boehringer and Caldwell, 1989), Ethiopia, Zimbabwe (Kamanga *et al*., 2003), Botswana (Amarteifio *et al.*, 2002) and Niger, Benin, Mali (Versteeg and Koudokpon, 1993). Although most countries in Africa cultivate pigeon pea for subsistence use, some countries produce in large amounts both for use and export the rest (Shanower *et al.*, 1999). The overall production of pigeon pea in Africa is very small, amounting to 9.3%, compared to India, which contributes 74% of world production. Despite the low production, the high potential of pigeon pea in the African continent, specifically the semi-arid and arid areas of the region, the diversity in the continent is rendered to be much valuable plant material for programs of plant breeding such as in ICRISAT. Some morphological characteristics exhibited in this region are absent in other regions, for instance India, the plants' ability to survive in harsh conditions of very low rain and red soils, are key factors taken into consideration when coming up with improved cultivars, (Duke,1983).

Trends in crop production have shown an increase over the century with the reducing rainfall amount in the areas. The increased rate of production is factored in by increasing

the area under production rather than yields (Jones *et al.*, 2002). This legume is cultivated in a rain-fed setting whose latitude, temperatures and altitudes vary (Silim *et al.*, 2006). Its harvesting takes place only during dry seasons, even though flowering and fruiting can be all around the year. Pigeon pea adapts to an extensive range of climates and soils within the humid tropics and the semi-arid, with the exception of desert and Mediterranean climates in Africa, (Maesen, 1983).

Table 2-3: Pigeon pea cultivation area and production in different regions of the globe (Saxena et al., 2015)

Country	Area Harvested (Ha)	Production (tons)	Yield (tons/Ha)
Bahamas	135	180	1.33
Bangladesh	500	423	0.80
Burundi	4, 786	7,386	1.5
Myanmar	650, 000	800,000	1.2
Comoros	500	430	0.86
Dominican Republic	24, 103.21	26,855.12	1.1
Grenada	550	800	1.45
Haiti	108,633.63	86,906.91	0.8
India	4,650,000	3,022,700	0.65
Jamaica	832	957	1.15
Kenya	144,218	73,183	0.51
Malawi	217,068	287,983	1.3
Nepal	17,459	16,459	0.94
Panama	3,800	1,970	0.52
Philippines	514	858	1.67
Puerto Rico	360	320	0.89
Saint Vincent and the Grenadines	20	210	10.5
United Republic of Tanzania	287,182	247,387	0.86
Trinidad and Tobago	915	770	0.84
Uganda	105,000	93,930	0.89
Venezuela	4,286.35	3,227.63	0.75
Democratic Republic of Congo	11,000	7,000	0.64

2.4 Nutritional value of pigeon pea

Plant proteins are the main source of food proteins in underdeveloped and poor developed countries due to the unavailability and/or costly animal proteins. Plant proteins are obtained from plant products such as legumes, among which are pigeon peas. Pigeon pea is rated as one of the highly nutritive leguminous food and has plenty of food proteins. The legume is consumed as green seeds as vegetables, as decorticated splits, or as whole dry seeds, with the vegetable pigeon pea having higher fat, protein digestibility and crude fibre amount, (Saxena *et.al*., 2010). In terms of minerals, the green pigeon pea is considered better as it has 28.2% higher phosphorus, 48.3% zinc, 17.2% potassium, 14.7% iron and 20.9% copper, (Maesen, 1983). Pigeon pea dry seeds provide various nutrients. The seeds are made of embryo, cotyledon and seed coat. The cotyledon provides about 66.7% of carbohydrates, with the embryo providing 50% of the proteins contained in the seeds. The seed coat provides fibre and contains some amounts of calcium. The cotyledon and embryo also contain amino acids such as cysteine and methionine. Decorticated splits seeds of pigeon can be prepared as a thick soup known as *dal* whose nutritional concentration is higher compared to green pigeon pea as a vegetable. The protein and starch content of the *dal* is significantly higher and it is also reported to contain more 10.8% manganese and 19.2% calcium compared to green pigeon pea, (Maesen, 1983). Table 2.4 shows comparison of nutritional elements of the dal, dry seeds and the green seeds of the pigeon pea.

Table 2-4: Nutritional elements in dal, dry seeds and green seeds of pigeon pea

Nutrient	Dal	Green seed	Dry seed
Protein (%)	24.6	21.0	18.8
Protein digestibility (%)	60.5	66.8	58.5
Trypsin inhibitor (units mg-1)	13.5	2.8	9.9
Starch content (%)	57.6	48.4	53.0
Starch digestibility (%)	-	53.0	36.2
Amylase inhibitor (units mg-1)	-	17.3	26.9
Soluble sugars (%)	5.2	5.1	3.1
Flatulence factors (g 100g-1souble sugar)	-	10.3	53.5
Crude fibre (%)	1.2	8.2	6.6
Fat (%)	1.6	2.3	1.9
Minerals and trace elements (mg 100g-1)			
Calcium	16.3	94.6	120.8
Magnesium	78.9	113.7	122.0
Copper	1.3	1.4	1.3
Iron	2.9	4.6	3.9
Zinc	3.0	2.5	2.3
Cooking time (min)	18	13	53

With a large population in the underdeveloped and poor developed countries consisting of poor and low-income farmers, pigeon pea is an ideal supplement to tubers and cereal meal for a balanced diet. The availability and use of this legume in the developing countries fill the nutritional gap for proteins that is unable to get a supply of animal proteins. For a common pigeon pea, the percentage of the protein content is estimated to be 18% to 26%. This percentage of protein content has been significantly increased in the Indian pigeon pea to 32.5%, and the amino acids containing sulphur also raised through developing high protein lines by researchers at ICRISAT.

2.5 Economic importance of pigeon pea

Pigeon pea is commonly cultivated in subtropical and tropical areas because of its edible seeds. It grows fast and is resistant to drought (Tessema, 2007). It is regarded as an

essential food security crop in areas where drought is likely to occur because of its drought resistance nature. It also provides green forage with a high value when other ones are not available at the end of a dry season.

The crop is utilized as human food across Africa, with a special meal known as 'dhal' gaining popularity in many regions of Eastern Africa. Pigeon peas have been used in Africa to produce Tempe (Lyimo, 2000), noodles (Singh *et al.*, 1989), as well as other fermented foodstuffs (Onofiok *et al.*, 1996). In other parts of the world, it is used as an additive in flour form to rice and other kinds of soup food (Center for New Crops and Plants Products, 2002). Moreover, its flour is an exceptional element in the manufacturing of snacks. In addition, suggestions indicate that it is one of the ingredients that increase pasta's nutritional value without effects on its sensory characteristics (Torres *et al.*, 2007).

In Nigeria, pigeon pea/ millet biscuits are purportedly to be highly nutritive and are inexpensive as compared to imported wheat (Eneche, 1999). Although fully unexploited pigeon peas in Africa have great medicinal value, in Nigeria, the leaves are employed to treat and manage malaria (Aiyeloja and Bello, 2006). Pigeon peas native to South Africa are being researched to determine their medicinal value (Mander *et al.*, 1996). Besides being used for human consumption, pigeon pea forms part of animal feed and fodder (Saxena *et al.*, 2002). Moreover, its fodder has been proven to upsurge the consumption of lower-quality sage, leading to accelerating weight in animals (Karachi and Zengo, 1997). Its foliage has high nutritive value in addition to being an excellent fodder (Onim *et al.*, 1985), whereas its seeds are consumed as animal feeds (Wallis *et al.*, 1986). By-

products obtained from shrunken and fragmented seeds are also utilized as a feed that is a cheaper substitute to other sources, for instance, fish and bone meal (Chisowa 2002). Furthermore, the tall perennial pigeon peas are frequently used as a windbreaker, live fences, and for conserving soil in Africa (Phatak *et al.*, 1993).

2.6 Constraints to pigeon pea production in Africa

The production of Pigeon pea has remained static in Eastern and Southern Africa despite extensive breeding efforts applied in the past two decades (Souframanien *et al.*, 2003). The challenges faced in its production include poor production practices that greatly affect its yields (insufficient weeding and both plant densities), low soil fertility, inadequate or incorrect use of herbicides and fungicides. The single most important biotic factor is pests, especially insects that feed on seeds, flowers, and pods (Shanower *et al.*, 1999). These insect pests consist of *Clavigralla horrida* Germa and *Clavigralla tomentosicollis* Ståll (pod sucking bug), *Etiella zinkenella* Treitsch, *Helicoverpa armigera* Hübner, *Maruca vitrata* Geyer, (pod boring lepidoptera) and *Melanagromyza chalcosoma* Spence (pod fly) (Minja *et al.*, 2000).

Before harvesting the pigeon peas, some insects infest the crops. The infestations are from weevils (*Callosobruchus chinensis*) and bruchids (*Callosobruchus spp*.) which may end up causing damage to the yields and have grave consequences when storing (Nahdy *et al.*, 1998). Additionally, the lack of appropriate storage facilities as well as unsuitable dehulling techniques has enhanced cross-contamination while it is being stored (Agona and Muyinza, 2005). There is an alternative to counteract the problem; however, the chemical treatment used is expensive for small-scale farmers. Moreover, past experience

has shown that the pesticide used in developing nations is unfitting and hazardous (Shanower *et al.*, 1999), besides being a danger to the environment (Agona and Muyinza, 2005).

Furthermore, the green peas, which are the favourite form of pigeon peas locally, are highly perishable and lack technical facilities for processing it is a problem for farmers, yet its export market is rising (Onyango and Silim, 2000). The farmers lack a cold storage facilities and improper handling of yields. Reports of diseases that affected pigeon pea in Africa in the past include Fusarium wilt (*Fusarium udum* Butler), powdery mildew (*Leveillula taurica*), leaf spot (*Mycovellosiella cajani*) and sterility mosaic disease (SMD) (Hillocks *et al.*, 2000).

2.7 Implications of genetic diversity on crop improvement

Genetic diversity is the quantity of genetic variability among different entities or varieties of a given species (Brown, 1983). A number of factors can be discriminated during the assessment of genetic diversity, such as the number of alleles, their effect on performance, their distribution, and the general difference between populations (Saxena *et al.*, 1983). Identification of cultivars requires a deep understanding of genetic variation and the relations amongst genotypes. Understanding genetic variability enables one to identify the appropriate conservation strategies and gives evidence of evolutionary forces leading to diversities in cultivars, and also enables further improvements of the cultivars (Thormann *et al.*, 1994). Consequently, understanding genetic diversity in plant genetic resources is needed for proper use as well as conservation of germplasms. Throughout

history, landraces' farmers have been selecting landraces with desirable traits of pigeon peas to boost their systems of production and uses.

In the last decade, more than fifteen improved varieties of the crop were meant for Africa, however, they were only available in a few countries. Unless it is not reported, the rest of the African farmers use local landraces to cultivate pigeon pea. The varieties obtained by farmers from the breeding programs only served their immediate needs therefore; the shortage issue is still unresolved. As a result, there is a need for an urgent national call to breeding programs to organize schemes that would develop cultivars that combine the superior traits of the crop. Molecular marker technology (Wenzel, 2006) is projected to help in breeding pigeon pea by availing information on its genetic diversity (Sharma *et al.*, 1978). It also predicts and identifies promising genotypes used to develop cultivars. In addition, marker-assisted selection (MAS) along with molecular tagging of desired genes will improve the efficiency of breeding.

2.8 Genetic markers used for diversity studies in crops

Molecular genetic methods have been used to better understand the extent and distribution to which genes vary between and within species (Vikram *et al.*, 2011). Molecular markers are recognizable DNA sequences located at a precise site in the genome and are passed down from generation to generation (Varshney *et al.*, 2005). The main categories of molecular markers are SSRs, RFLPs, RAPDs, AFLPs, and SNPs.

2.8.1 Restriction Fragment Length Polymorphism (RFLP)

RFLP markers' main strengths are good ability to be transferred between laboratories, their highly reproducible, co-dominant inheritance, and the ease in scoring due to the large size difference between with lack of sequence information (Meksem *et al.*, 1995).

Nevertheless, RFLP analysis has many limitations since it needs high DNA quality and quantity to be present. It is dependent on the species-specific probe library development; the method used is not amenable for automation, low polymorphism level and detection of a small number of loci per assay, it is costly, consumes a lot of time and is laborious (Ahmed *et al.*, 2012). The RFLP probes have frequently been used genome mapping and in variation analysis like genotyping, forensics, paternity tests and hereditary disease diagnostics (Schaid DJ *et al*., 2018)

2.8.2 Randomly Amplified Polymorphic DNA (RAPD)

Random amplified polymorphic DNA is also known as multiple arbitrary amplicon profiling (Caetano-Anolles, 1994). This method was the first in amplifying fragments of DNA without previous information on the sequences from any species (Semagn *et al.*, 2006), therefore, it is determined by complex processes which are kinetic and thermodynamic. Amplification of nucleic acids with arbitrary primers primarily involves enzymes, primer interaction, and template annealing sites (Caetano-Anollés, 1997). RAPD markers have been applied in studies involving genetic identity and studies on closely-related species. They are also used in gene mapping studies where other markers have failed (Hadrys H., 1992)

2.8.3 Amplified Fragment Length Polymorphism (AFLP)

The AFLP technique involves binding adaptor (primer recognition sequence) to restricted DNA through the combination of RFLP power with PCR-based flexibility (Lynch and Walsh, 1998). AFLP main feature is its capacity to represent the genome through concurrent screening of representative DNA regions that are randomly distributed all over the genome (Meksem *et al.*, 1995). Generation of AFLP markers for any organism's DNA

can be without initial probe development/primer investment and sequence analysis. If the DNA is devoid of both restriction enzyme and polymerase chain reaction inhibitors, both the partially degraded and the good DNA can be used for digestion (Blears *et al.*, 1998). AFLP markers have been applied for accessing genetic diversity within species, to infer population-level phylogenies and biogeographic patterns to generate genetic maps and to determine relatedness among cultivars (Bleeker M. *et al.*, 1995)

2.8.4 Single Nucleotide Polymorphism (SNP)

Studies have shown that various species like plants have single nucleotide polymorphisms (SNPs), which are present and are scattered all over the genome (Semagn *et al.*, 2006). Therefore, the SNP marker system becomes a good tool to map map-based cloning and marker-assisted breeding. This is attributed to the fact that the plant genome has abundant polymorphisms (Batley *et al.*, 2003). SNP marker is a DNA sequence whose single base is changed by replacing possibly two nucleotides at a given position. Hence, alleles cannot be discriminated against according to their gel size differences (Gupta and Varshney, 2000). All SNP genotyping methods consist of two steps, the first being the combination of two elements that produce allele-specific products; secondly, its analysis (Caetano-Anolles, 1994).

2.8.5 Simple Sequence Repeats (SSRs)

These are sequences of one to six DNA base pairs that are repeatable. Also, they are known as Short Tandem Repeats or microsatellites. SSR markers are very informative because of their heritability, co-dominance, abundance, multiallelism and wide genome coverage. Its other advantage is conserving the flanking regions across generations, thus allowing repeated usage of the method (Emanuelli *et al.*, 2013). SSR polymorphism

between 2 varieties is attributable to the variances in the length of the repeats between the 2 preserved sequences. Simple sequence repeat makers are relatively cheaper for genotyping plants and can be used by small laboratories. SSR markers have been applied in fingerprinting, genetic diversity studies, population structure analysis, association mapping and linkage mapping (Sugita T *et al*., 2013).

CHATER THREE

MATERIALS AND METHODS

3.1. Plant materials

A total of 24 farmer-preferred pigeon pea varieties comprising 19 breeding line accessions and 5 local landraces were amassed from 9 major pigeon pea growing counties in Kenya, including, Kitui, Machakos, Murang'a, Meru, Makueni, Embu, Tharaka-Nithi, Kwale and Kilifi counties in Kenya. The main breeding lines and landraces grown in these mentioned areas were selected for my current study. The counties that I sampled were also mainly arid and semi-arid regions. The name of the pigeon pea accessions employed in this research and their description are shown in Table 3.1.

Table 3-0-1 List of pigeon pea accessions used in this study and their description.

Name	Description	County where Grown
GBK 034224	Breeding line	Makueni
GBK 038241	Breeding line	Makueni
GBK 038245	Breeding line	Makueni
GBK 041807	Breeding line	Machakos
GBK 041821	Breeding line	Kitui
GBK 041876	Breeding line	Machakos (Muthetheni)
GBK 041880	Breeding line	Machakos
GBK 041907	Breeding line	Kitui (Mwingi)
GBK 041914	Breeding line	Machakos
GBK 041941	Breeding line	Kwale
GBK 042014	Breeding line	Meru
GBK 042028	Breeding line	Embu (Ishiara)
GBK 042046	Breeding line	Murang'a
GBK 047027	Breeding line	Machakos
GBK 047047	Breeding line	Makueni (Kibwezi)
ICEAP 00902	Breeding line	Machakos
KAT 60/8	Breeding line	Kitui
KENDI TALL	Breeding line	Tharaka Nithi
MBAAZI 1	Breeding line	Machakos
KIONZA	Local landrace	Makueni (Wote)
MUKUNE	Local landrace	Makueni (Wote)
MUSUNGU	Local landrace	Makueni (Wote)
MUTERIKI	Local landrace	Machakos (Kangundo)
SYOMBONZE	Local landrace	Makueni (Wote)

3.2. Preparation of plant materials

Plastic pots measuring 60 cm by 60 cm by 60 cm were filled with soil obtained from the experimental agricultural fields of Kenyatta University whose GPS coordinates are Latitude $1^{O}10'36.19"$S Longitude $36^{O}55'46.34'$E, Altitude 1,572 meters above sea level. The seedlings were kept outside the plant transformation laboratory and not inside the greenhouse. They were therefore exposed to natural environmental conditions of the locality. The soil was well-watered, and 10 seeds of each variety were then sown. After 21 days, weak and growth-retarded seedlings were uprooted and 3 seedlings showing

vigorous growth were left in each pot for diversity studies. Normal agronomic practices approved for pigeon pea cultivation were followed. Three biological replicates for each variety were used. This experiment was carried out at the Plant Transformation Laboratory field of Kenyatta University.

3.3. Determination of phenotypic diversity

Agro-morphological traits measured in this study included leaf length (LL), leaf width (LW), stem diameter (StD), 100 seed weight (100 SW), seeds per pod (SP), seed diameter (SD), pod length (PL), plant height (PH), and branches per plant (BP) (Table 3.2) These traits were determined between the flowering stage and harvesting stage depending on individual traits (Table 3.2). The measurements for LL, LW, StD, SD, and PL were taken using a digital Vernier caliper and data was recorded either in centimeters or millimeters, depending on the trait measured. A tape measure was used in determining plant height and the measurement was recorded in centimeters. Evaluation of branches per plant and the number of seeds per pod was done by physical counting. An electronic weighing balance (Mettler Toledo) was used to determine the 100 SW from each accession and data recorded in grams.

Table 3-0-2 List of agro-morphological traits, their abbreviations, observation period and unit of measurement.

Trait	Code	Observation period	Unit
Leaf length	LL	At flowering stage	cm
Leaf width	LW	At flowering stage	cm
Stem diameter	StD	At flowering stage	cm
Number of branches per plant	BP	At maturity stage	unity
Plant height	PH	At maturity stage	cm
Pod length	PL	At maturity stage	cm
Grain diameter	GD	After seed harvest	mm
Number of seeds per pod	SP	At maturity stage	unity
100 seed weight	100 SW	At maturity stage	g

3.4. Determination of genetic diversity

3.4.1 DNA extraction

Extraction of genomic DNA from 14-day-old seedlings' leaves using slightly modified Cetyl Trimethyl Ammonium Bromide (CTAB) protocol (Doyle and Doyle, 1990). The fleshy young leaves were harvested from 1 individual seedlings of each accession in the Plant Transformation Laboratory's greenhouse, Kenyatta University and then placed in falcon tubes which were labeled according to the germplasm code that was used. The young fleshy leaves were put in a mortar and liquid Nitrogen was added to cover the leaves. Using a pestle, the leaves were gridded into very fine powder. Each powdered leaf sample was put in labeled 2mls micro centrifuge tubes and 1000µml of buffer containing 100µml tris-base, 20µml EDTA, 1.4m sodium chloride, 3% (w/v), CTAB, 1% beta mercaptol ethanol and 2% polyvinyl pyrrolidone was added, although this was varied with the amount of powder so that the buffer could cover the powdered leaf sample.

Each 2ml micro centrifuge tube containing the sample of each germplasm leaf powder and buffer was placed on a vortex shaker and then incubated in a water bath at 65^{O}C for thirty minutes. This was to provide optimum temperature, which is favorable for the buffer to open up the cells. 1000µm of Chloroform: isoamyl alcohol in the ratio of 24:1 was added to each sample to extract the DNA and then the samples were put in a centrifuge at 10000 revolutions per minute for 10 minutes. Then, 700µm of the supernatant fluid was transferred into 1.5ml micro centrifuge tubes and closed. Addition of 2/3 of 700µm of isopropanol precipitates the DNA. It was then incubated for one hour in a refrigerator. After one hour, the samples were centrifuged at 11000 revolutions per

minute for 30 minutes to separate the solids from the liquid. After this time, the supernatant material was carefully poured to leave the DNA material that had settled at the bottom inform of solid gel. To the solid DNA material, 1ml of 70% analaR ethanol was added to clean the DNA material in each micro centrifuge tube. The excess analaR ethanol was poured and this step was repeated twice and then the samples were incubated in a drier for one hour still in micro centrifuge tubes. After one-hour, 30µl of PCR water was added to dissolve each sample of pelleted DNA material for storage.

3.4.2 DNA quality determination

Determination of the isolated DNA quality was done on 5µl of genomic DNA through electrophoresis at 100V for thirty minutes in agarose (1%) gel prepared in 100 milliliters of TBE buffer. DNA quality was indicated by clear bands formation, which was then used to run PCR using simple sequence repeat markers. A spectrophotometer (Thermo Scientific Nano drop 2000 system, USA) was used to determine the concentration of isolated DNA at 260nM and nM and compared the ratio with the pure DNA concentration.

3.4.3 Primer selection

Previous publications aided in the selection of a total of 12 sets of SSR markers (Table 3.3) covering dissimilar genomic regions of pigeon pea (Njung'e *et al.*, 2016). They were used to amplify the extracted DNA. They were selected on the basis of their annealing temperature and the amplicon size.

Table 3-0-3 List of the twelve polymorphic SSR markers used in the present study.

Marker name	Marker sequence		Annealing temperature(°C)
	Forward (5'-3')	Reverse (3'-5')	
CCB 1	AAGGGTTGTATCTCCGCGTG	GCAAAGCAGCAATCATTTCG	46
CCB 10	CCTTCTTAAGGTGAAATGCAAGC	CATAACAATAAAAGACCTTGAATGC	45
CCB 7	CAACATTTGGACTAAAAACTG	AGGTATCCAATATCCAACTTG	43
PGM10	TCACAGAGGACCACACGAAG	TGGACTAGACATTGCGTGAAG	46
PGM102	ATCGGCTTTTGTCTTGATGA	AAGCTACAAGGGATACACATGC	45
PGM106	TGAAATGAACAAACCTCAATGG	TGTATTGCACATTGACTTGGCTA	45
PGM109	ATTCCCTCTCTATCTCAGACTTTT	TCGTGATGGAACTCAAGATACACT	46
PGM3	ACACCACCATGCTAAAGAACAAG	CCAAGCAAGACACGAGTAATCATA	45
CCttc008	TCACAGAGGACCACACGAAG	TGGACTAGACATTGCGTGAAG	48
CCttc006	TAGAGGAGGTTCCAAATGACATA	ATCTGTCTGGTGTTTTAGTGTGCT	46
CCtta011	TCAGGGGTAAATGCGGTATC	GAATTGCTTTTTGCTTCCTCA	45
CCtta015	AACACGCACCTCAATTCCA	GAATGAGGAATGAAGGGACAAA	45

3.4.4 (i) Amplification of DNA using Polymerase chain reaction (PCR)

Polymerase chain reaction (PCR) analysis was performed in a 25µl reaction volume comprising 1µl genomic DNA and 0.5 µl of each primer (10µM). One Taq 2X Master Mix with Standard Buffer (New England Biolabs®) was used. A negative control (contains PCR master mix without the DNA template) was run per every batch of PCR amplifications which were performed by a thermocycler, with each cycle involving an initial denaturation step of 94 ^{O}C for thirty seconds followed by thirty-five cycles of denaturation at ninety-four degrees Celsius for thirty seconds, each primer annealing at appropriate varying temperatures ranging from an average of 45 ^{O}C to 47 ^{O}C for thirty seconds, elongation at 72 ^{O}C incubation for one minute, a final extension for 5 minutes followed by a hold at 4^{O}C. Gel electrophoresis was used to check for amplification in the PCR products. This was done on a two percent agarose gel in 1X Trisacetic acid-EDTA (TAE) buffer stained with SYBR Green alongside a DNA ladder of 100 base pairs (Bioline®).

(ii) Agarose Gel Electrophoresis of PCR products

PCR products were separated on 2% of TAE agarose gel running for one hour at 80V. The agarose gel was prepared by dissolving 2 g of 100ml of 1×TAE and then a microwave was used to boil it until a clear homogenous boiling solution was formed. This gel was allowed to settle for 30 minutes after being carefully poured into a gel tray that was set with combs. The PCR products for each sample were prepared by adding onto a parafilm 2 µl of loading dye and mixed it well with 2 µl of SYBR Green dye to 10 µl of each sample. They were thoroughly mixed and loaded into gel wells. A 5 µl of 100 base pairs of DNA ladder (Bioline®) was loaded to the first gel well for estimation of the size of

amplicons. The power was turned on at the above stated voltage and time. Visualization of the genomic DNA was under a UV trans-illuminator, and a digital camera took photographs for the purpose of scoring.

3.5 Data management and statistical analysis

3.5.1 Phenotypic data

The quantitative data were first tabulated on a spread sheet. To assess the phenotypic diversity based on the 9 agro-morphological characters, the quantitative data was subjected to analysis of variance (ANOVA), succeeded by Tukey's posthoc to separate means at a significance level of 5%. In order to determine the phenotypic similarities or differences among the 24 varieties, a dendrogram was constructed using Average linkage-Euclidean distance. Furthermore, to establish the underlying source of agro-morphological variation and the major characters contributing to delineation, principal component analysis (PCA) was performed and a scatter plot generated. A software was used to perform all the analyses. Minitab software (State College Pennsylvania-USA) version 17.0 was used to compute all the analyses.

3.5.2 Molecular data

Genetic diversity at each locus was evaluated in terms of polymorphism information content (PIC), heterozygosity, gene diversity, and the number of alleles. The genetic analysis software package Power Marker version 3.25 was used to perform all calculations (Liu and Muse, 2005). Genetic dissimilarity coefficients for assessing the relatedness among the accessions were calculated presumably from the "C.S Cord 1967'' distance (Cavalli-Sforza and Edwards, 1967). In order to establish the evolutionary relationships among the 24 varieties, Power Marker software version 3.25 was used, and

the Neighbor-Joining (NJ) method was applied to reconstruct a phylogenetic tree (Liu and Muse, 2005). Moreover, principal coordinate analysis (PCoA) was carried out to further partition genetic variation among the varieties. Furthermore, analysis of molecular variance (AMOVA) was performed to unfold genetic variation within and among the pigeon pea varieties. GenALEx 6.5 statistical software was used to perform both PCoA and AMOVA (Peakall and Smouse, 2009).

CHAPTER FOUR

RESULTS

4.1. Agro-morphological diversity among selected pigeon pea varieties

4.1.1. Accession variation based on phenotypic traits

Significant variations were observed among the accessions for all the nine evaluated phenotypic traits in the current study ($p > 0.05$; Table 4.1). Observations made were; *GBK 038245*, a breeding line accession, had the highest value mean value of leaf length (14.6±0.74cm), whereas *SYOMBOZE,* a local landrace, had the lowest mean value of leaf length (8.88±2.01cm). In terms of mean leaf width, *GBK 038241*, *GBK 047047* and *GBK 038245*, three of which are breeding lines, had the highest mean value of leaf width (6.00±0.14cm), whereas ICEAP 00902, a released cultivar, had the lowest mean value of leaf width (3.70±0.15cm).

Furthermore, it was observed that *GBK 041876,* a breeding line, had the highest mean stem diameter value (1.68±0.08cm), whereas *GBK 042028,* also a breeding line, recorded the lowest mean stem diameter value (0.54±0.08cm). Moreover, *GBK 041876,* a breeding line, recorded the highest mean number of branches per plant with a mean value of 10.00±0.45, whereas *GBK 041907,* also a breeding line, was found to have the lowest mean number of branches per plant with a mean value of *1.00*±0.00.

In terms of plant height, *GBK 041807,* a breeding line, recorded the highest mean value of 220.00±7.62cm, whereas *MBAAZI 1,* a released cultivar, recorded the lowest mean value of 102.00±1.67cm. *GBK 038241,* a breeding line, exhibited the highest mean value

of pod length (10.08±0.34cm), whereas *GBK 041907*, a breeding line, recorded the lowest mean value of pod length (5.00±0.09cm). In terms of grain diameter, *KIONZA,* a local landrace, exhibited the highest mean value (9.00±0.42mm), whereas *GBK 041914,* also a breeding line, portrayed the smallest mean grain diameter with a mean value of (6.00±0.16mm). *GBK 038241*, a breeding line, had the highest number of seeds per pod with a mean value of (6.60±0.25) amongst all the accessions evaluated, whereas *KAT 60/8,* a released cultivar, produced the lowest mean number of seeds per pod with an average value of (4.00±0.00). Regarding 100 SW (yield), *MUSUNGU,* a local landrace, recorded the highest mean value of (23.00±0.32gm), whereas *GBK 041914*, a breeding line, produced the lowest yield with a mean value of (12.00±0.32gm).

Table 4-0-1: Analysis of variance of 9 quantitative traits evaluated in this study

Variety	LL(cm)	LW(cm)	StD (cm)	BP	PH (cm)	PL (cm)	GD (mm)	SP	100 SW(gm)
GBK 034224	13.5±0.43^{abc}	5.40±0.27^{abc}	1.00±0.08^{bcd}	6.00±0.45^{c}	201.00±3.03^{abc}	6.90±0.29^{defgh}	7.00±0.274^{bc}	5.00±0.00^{cd}	16.00±0.71^{ef}
GBK 038241	14.5±0.265^{ab}	6.00±0.29^{a}	0.76±0.02^{cdef}	1.20±0.20^{fg}	198.00±2.45^{abc}	**10.08±0.34^{a}**	8.00±0.35^{ab}	**6.60±0.25^{a}**	19.20±0.37^{bcd}
GBK 038245	**14.6±0.74^{a}**	**6.00±0.14^{a}**	0.68±0.02^{def}	1.40±0.25^{fg}	164.00±8.56^{cdefg}	9.56±0.22^{a}	7.00±0.16^{bc}	6.00±0.00^{b}	17.00±0.55^{de}
GBK 041807	12.68±0.44^{abcd}	5.50±0.50^{ab}	1.06±0.11^{bc}	5.00±0.45^{cd}	**220.00±7.62^{a}**	7.62±0.22^{cde}	7.00±0.16^{bc}	5.00±0.00^{cd}	15.00±0.32^{efg}
GBK 041821	12±0.65^{abcde}	5.30±0.49^{abcd}	0.86±0.02^{bcdef}	4.00±0.32^{de}	151.20±5.42^{efgh}	7.00±0.16^{defg}	7.00±0.27^{bc}	6.00±0.00^{b}	15.20±0.37^{efg}
GBK 041876	12.3±0.20^{abcde}	5.60±0.47^{ab}	**1.68±0.08^{a}**	**10.00±0.45^{a}**	176.00±8.15^{bcde}	5.90±0.09^{hij}	8.00±0.22^{ab}	5.00±0.00^{cd}	19.40±0.51^{bc}
GBK 041880	12.5±0.68^{abcd}	5.50±0.20^{ab}	0.82±0.09^{bcdef}	6.00±0.45^{c}	163.20±8.40^{cdefg}	7.00±0.00^{defg}	7.00±0.32^{bc}	5.40±0.25^{c}	18.40±0.51^{cd}
GBK 041907	12.4±0.33^{abcde}	4.50±0.49^{abcd}	0.56±0.05^{ef}	**1.00±0.00^{g}**	122.60±10.20^{hi}	**5.00±0.09^{j}**	7.00±0.16^{bc}	4.00±0.00^{e}	16.00±0.32^{ef}
GBK 041914	11±0.42^{bcde}	4.60±0.29^{abcd}	1.00±0.05^{bcd}	8.00±0.45^{b}	153.60±13.20^{efgh}	7.00±0.00^{defg}	**6.00±0.16^{c}**	5.00±0.00^{cd}	**12.00±0.32^{i}**
GBK 041941	12.2±0.25^{abcde}	4.6±0.36^{abcd}	0.88±0.02^{bcde}	2.00±0.32^{fg}	169.00±7.88^{cdefg}	6.64±0.39^{defghi}	6.00±0.00^{c}	5.00±0.00^{cd}	12.40±0.51^{hi}
GBK 042014	10.1±0.57^{cde}	3.80±0.24^{cd}	0.54±0.05^{f}	1.20±0.20^{fg}	139.40±8.10^{efghi}	8.38±0.18^{bc}	7.00±0.27^{bc}	6.00±0.00^{b}	15.40±0.68^{ef}
GBK 042028	11±1.12^{bcde}	4.10±0.11^{bcd}	**0.54±0.08^{f}**	1.00±0.00^{g}	137.00±9.80^{fghi}	6.50±0.22^{fghi}	6.50±0.32^{c}	5.00±0.00^{cd}	14.20±0.66^{fghi}
GBK 042046	13.5±0.48^{abc}	4.60±0.31^{abcd}	0.68±0.06^{def}	2.00±0.32^{fg}	212.00±9.88^{abc}	7.50±0.14^{cdef}	8.00±0.22^{ab}	6.00±0.00^{b}	16.00±0.32^{ef}
GBK 047027	12.6±0.34^{abcd}	3.80±0.20^{cd}	0.78±0.04^{bcdef}	2.00±0.32^{fg}	192.00±3.41^{abcd}	7.40±0.09^{cdef}	7.00±0.16^{bc}	5.00±0.00^{cd}	15.00±0.45^{efg}
GBK 047047	12±0.53^{abcde}	6.00±0.152^{a}	0.64±0.02^{ef}	1.20±0.20^{fg}	142.60±2.36^{efgh}	6.00±0.16^{ghij}	7.00±0.27^{bc}	5.00±0.00^{cd}	14.40±0.51^{fgh}
ICEAP 00902	11.2±0.83^{abcde}	**3.70±0.15^{d}**	0.58±0.04^{ef}	3.00±0.45^{ef}	137.00±6.53^{fghi}	6.80±0.20^{defgh}	7.00±0.42^{bc}	6.00±0.00^{b}	15.20±0.37^{egf}
KAT 60/8	10.3±0.48^{cde}	4.20±0.23^{bcd}	0.68±0.05^{def}	4.00±0.32^{de}	170.00±5.10^{cdefg}	5.60±0.19^{ij}	6.50±0.16^{c}	**4.00±0.00^{e}**	13.00±0.32^{ghi}
KENDI TALL	12±0.33^{abcde}	5.40±0.27^{abc}	1.10±0.09^{b}	8.00±0.32^{b}	165.80±3.50^{cdefg}	6.76±0.23^{defgh}	8.00±0.16^{ab}	4.80±0.20^{d}	17.20±0.49^{cde}
MBAAZI 1	10.6±0.29^{cde}	4.50±0.28^{abcd}	0.80±0.10^{bcdef}	9.00±0.71^{ab}	**102.00±1.67^{i}**	7.66±0.17^{cde}	6.50±0.16^{c}	5.00±0.00^{cd}	14.60±0.25^{fgh}
KIONZA	13.5±0.39^{abc}	5.90±0.19^{a}	0.80±0.07^{bcdef}	1.20±0.20^{fg}	154.60±8.85^{defgh}	9.76±0.17^{a}	**9.00±0.42^{a}**	6.00±0.00^{b}	22.00±0.32^{a}
MUKUNE	10.2±0.74^{cde}	4.10±0.29^{bcd}	0.98±0.06^{bcd}	5.00±0.32^{cd}	135.00±8.14^{ghi}	7.70±0.30^{cd}	8.00±0.16^{ab}	6.00±0.00^{b}	21.00±0.32^{ab}
MUSUNGU	12.5±0.69^{abcd}	5.00±0.16^{abcd}	0.6±0.03^{ef}	1.40±0.25^{fg}	175.00±3.33^{bcdef}	9.40±0.22^{ab}	8.00±0.27^{ab}	6.00±0.00^{b}	**23.00±0.32^{a}**
MUTARIKI	9.2±0.50^{de}	4.20±0.38^{bcd}	0.56±0.04^{ef}	1.20±0.20^{fg}	196.00±3.59^{abc}	5.62±0.10^{ij}	7.00±0.42^{bc}	5.00±0.00^{cd}	14.40±0.25^{fgh}
SYOMBONZE	**8.88± 2.01^{e}**	4.50±0.34^{abcd}	1.06±0.05^{bc}	9.00±0.32^{ab}	123.20±8.81^{hi}	6.60±0.19^{efghi}	8.00±0.22^{ab}	5.00±0.00^{cd}	19.00±0.32^{bcd}

The values are articulated as Mean±SEM. The values succeeded by similar superscript are not significantly different by ANOVA succeeded by Tukey's post hoc test (P>0.05).

4.1.2. Cluster analysis of morphological traits

The 24 studied varieties were classified into 4 main sub-clusters (C1, C2, C3 and C4), with the accessions in each cluster ranging from 3 to 8 (Figure 4.1). Sub-cluster C2 had the highest phenotypic diversity, comprising of 8 accessions, whereas sub-cluster C3 had the least phenotypic diversity, comprising of 3 accessions.

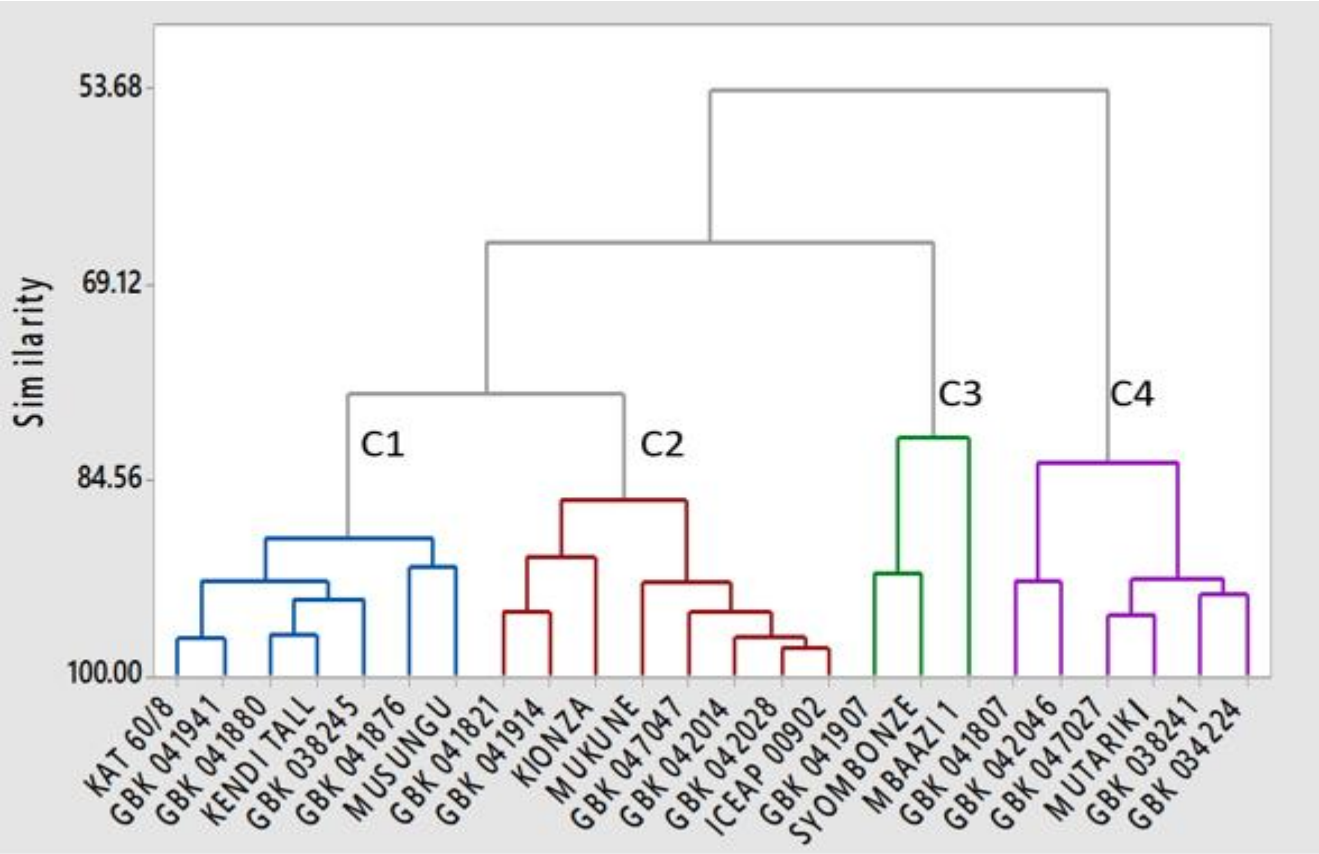

Figure 4.1 A Dendrogram showing the clustering of the 24 accessions based on mean values of the 9 phenotypic traits.

4.1.3. Principal component analysis (PCA)

Principal component analysis (PCA) is a mathematical method for reducing the size of the data sets, and increases interpretability but concurrently minimizes information loss. It acted by creating new uncorrelated variables that successively maximize variance (Ian

and Jorge, 2016). Using PCA can help the researcher to identify the correlation between variables. PCA is currently a very popular technique used by researchers when dealing with large volumes of data sets. In PCA, one extracts the significant information from the data and expresses it as a set of summary indices known as principal components.

Principal component analysis was executed on the correlation matrix of the 24 phenotypic traits. The eigen value (>1), the proportion of variance and cumulative percentages for the first three components are shown in Table 4.2. A score plot for the first two components was also generated, as shown in Figure 4.2. The first PC accounted for 38.5% proportion of variance, whereby it accounted for the highest percentage of variation, and with a positive correlation to leaf width, leaf length, stem diameter, pod length, plant height, seeds per pod, grain diameter and 100 seed weight. However, it was negatively correlated with branches per plant. In PC2, the proportion of variance was 23.0%, with the highest positive contribution from stem diameter (0.649) and the lowest negative contribution from number of seeds per pod (-0.215). The third PC accounted for 16.4% proportion of variance, with plant height contributing the highest positive correlation of 0.564% and 100 seed weight contributing the lowest negative contribution of -0.392. Based on loading plot results, pod length had the largest positive loading (0.438) on the first component, whereas stem diameter had the largest positive loading (0.649) on the second component (Figure 4.2).

Table 4-0-2: Principal Components Morphological Traits.

	PC1	**PC2**	**PC3**
Eigen value	3.4643	2.0699	1.4778
% Proportion of variance	**38.5**	**23.0**	**16.4**
% Cumulative	38.5	61.5	77.9
Traits	**Eigen vectors**		
LL	0.377	-0.066	0.474
LW	0.349	0.223	0.343
StD	0.063	**0.649**	0.102
BP	-0.113	0.620	-0.097
PH	0.202	0.001	**0.564**
PL	**0.438**	-0.182	-0.116
SD	0.401	0.184	-0.315
SP	0.398	**-0.215**	-0.230
100 SW	0.410	0.165	**-0.392**

KEY: SW- seed weight, LW- Leaf width, LL- Leaf length, StD- Stem diameter, PL- Pod length, SD- Grain diameter, SP- Number of seeds per pod, PH- Plant height, BP- Number of branches per plant

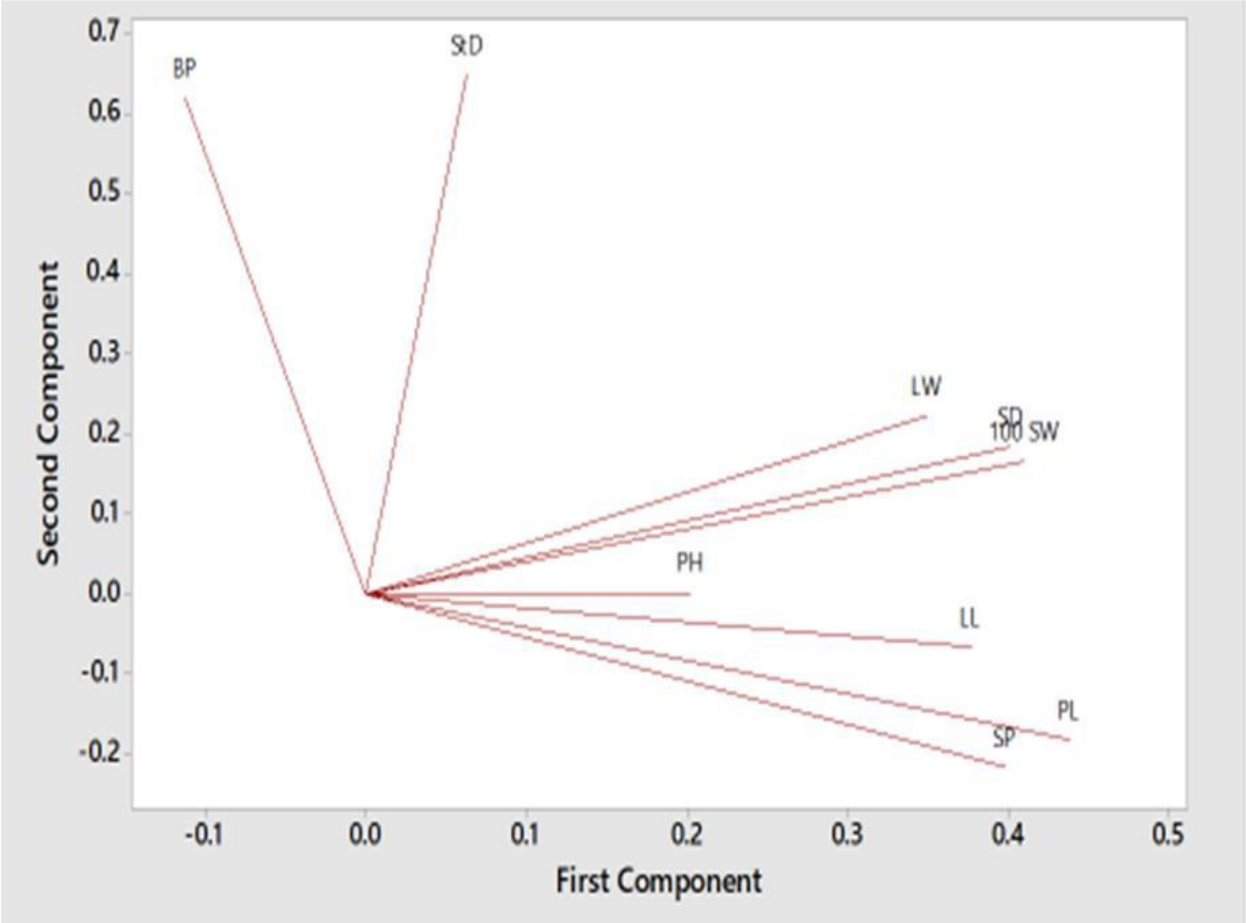

Figure 4.2 Projection of 9 agro-morphological traits on the first two components of the PCA

4.2. Genetic diversity analysis

4.2.1. Scoring of markers

PCR products were sized manually from the gel images against l00bp DNA ladder (Life sciences-USA). Samples that produced clear band(s) were scored as "1" whereas the absence of bands was scored as "0". A representative of the gel images is shown in image 4.1.

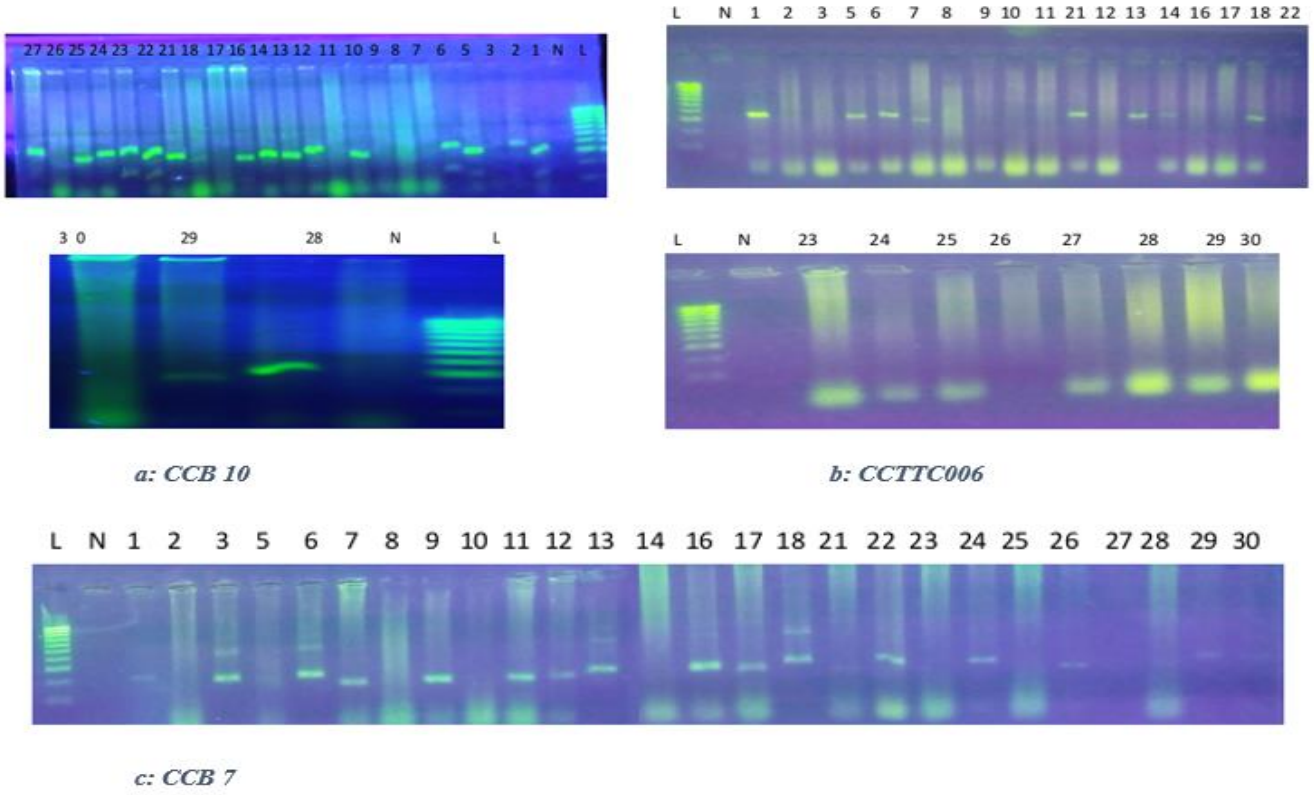

Image 4-1: Gel images, (a) CCB 10, (b) CCTTC006 and (c) CCB7

L-Ladder

N-Neutral

1- MBAAZI 1
2- KAT60/8
3- GBK041807
4- GBK041930
5- GBK042046
6- GBK038241
7- GBK034224
8- GBK043224
9- GBK042014
10- GBK041821
11- SYOMBONZE
12- GBK047027
13- MUKINE
14- GBK041941
15- GBK038227
16- KENDI TALL

17- GBK041914
18- GBK041876
19- GBK041899
20- GBK038254
21- GBK041907
22- MUTARIKI
23- GBK038245
24- GBK042028
25- MUSUNGU
26- GBK047047
27- ICCAP 00902
28- GBK041880
29- KIONZA
30- KATX 1311

4.2.2. Polymorphism of SSR markers

The present study used 15 SSR markers, out of this only 12 markers produced amplification products of the expected band size at different annealing temperatures and the remaining 3 were either monomorphic or did not yield any detectable band. Based on the 12 polymorphic markers, a total of 33 alleles were obtained from the 12 polymorphic markers, ranging between 2 and 4, with an average of 2.75 alleles per locus (Table 4.3). Gene diversity values ranged from 0.3299 to 0.6597 with an average of 0.4939 (Table 4.3). The observed heterozygosity values ranged between 0.0000 and 0.2917 with an average of 0.0868 (Table 4.3). PIC values ranged from 0.2755 (PGM 102) to 0.6036 (CCB 10) with an average of 0.4178 (Table 4.3).

Table 4-0-3 Summary statistics of the 12 SSR markers employed in the current study.

Marker	Number of alleles	Gene Diversity	Heterozygosity	PIC
CCTTC006	3.0000	0.5417	0.2500	0.4598
PGM 109	**2.0000**	0.4132	**0.0000**	0.3278
CCB 7	**4.0000**	0.5981	**0.2917**	0.5410
CCTTA011	**2.0000**	0.4965	0.0000	0.3733
PGM 106	3.0000	0.3793	0.2083	0.3475
CCTTC008	**2.0000**	0.4444	0.0000	0.3457
CCB1	**4.0000**	**0.6597**	0.0000	0.5972
PGM 3	**2.0000**	0.4861	0.0000	0.3680
PGM 102	**2.0000**	**0.3299**	0.0000	**0.2755**
CCB 10	**4.0000**	0.6554	0.2500	**0.6036**
CCTTA015	**2.0000**	0.4132	0.0000	0.3278
PGM 10	3.0000	0.5095	0.0417	0.4470
Mean	**2.7500**	**0.4939**	**0.0868**	**0.4178**

4.2.3. Pairwise genetic dissimilarity

In order to establish the level of relatedness among the 24 accessions, genetic distance was calculated using a dissimilarity matrix presumably from "C.S Cord 1967" shared SSR. The lowest genetic dissimilarity value of 0.0750 was recorded between accessions *KIONZA* and *MUTARIKI,* whereas the highest genetic dissimilarity was found between accessions *GBK 041876* and *SYOMBONZE; GBK 041880 and GBK 034224* with the two pairs recording a dissimilarity value of 0.8659 and 0.9003 respectively (Table 4.4).

Table 4-0-4 SC. Cord coefficients of dissimilarity among pairs of 24 pigeon pea **accessions**

OTU	GBK 038241	GBK 038245	GBK 041807	GBK 041821	GBK 041876	GBK 041880	GBK 041907	GBK 041914	GBK 041941	GBK 042014	GBK 042028
GBK 038241	0										
GBK 038245	0.675237237	0									
GBK 041807	0.375131798	0.450158158	0								
GBK 041821	0.525184518	0.300105439	0.600210877	0							
GBK 041876	0.450158158	0.525184518	0.450158158	0.750263597	0						
GBK 041880	0.71584121	0.340709412	0.531366105	0.450158158	0.531366105	0					
GBK 041907	0.265683052	0.525184518	0.340709412	0.525184518	0.340709412	0.565788491	0				
GBK 041914	0.640814851	0.525184518	0.415735772	0.525184518	0.565788491	0.565788491	0.525184518	0			
GBK 041941	0.300105439	0.375131798	0.375131798	0.375131798	0.450158158	0.565788491	0.150052719	0.600210877	0		
GBK 042014	0.490762131	0.300105439	0.190656693	0.600210877	0.415735772	0.490762131	0.375131798	0.375131798	0.375131798	0	
GBK 042028	0.340709412	0.375131798	0.340709412	0.525184518	0.490762131	0.565788491	0.300105439	0.375131798	0.300105439	0.150052719	0
GBK 042046	0.278130727	0.640814851	0.300105439	0.490762131	0.490762131	0.722022797	0.231260666	0.415735772	0.265683052	0.490762131	0.39376106
GBK 034224	0.415735772	0.675237237	0.450158158	0.525184518	0.565788491	0.900316316	0.490762131	0.375131798	0.490762131	0.450158158	0.450158158
GBK 047027	0.490762131	0.600210877	0.525184518	0.525184518	0.525184518	0.306287026	0.565788491	0.490762131	0.525184518	0.565788491	0.456339745
GBK 047047	0.640814851	0.525184518	0.490762131	0.525184518	0.640814851	0.565788491	0.450158158	0.375131798	0.600210877	0.375131798	0.375131798
ICEAP 00902	0.565788491	0.225079079	0.300105439	0.375131798	0.675237237	0.415735772	0.525184518	0.525184518	0.450158158	0.450158158	0.490762131
KAT 60/8	0.525184518	0.300105439	0.600210877	0.100543646	0.750263597	0.450158158	0.525184518	0.525184518	0.375131798	0.600210877	0.525184518
KENDI TALL	0.525184518	0.300105439	0.450158158	0.150052719	0.600210877	0.340709412	0.565788491	0.565788491	0.525184518	0.490762131	0.565788491
KIONZA	0.490762131	0.450158158	0.415735772	0.300105439	0.565788491	0.490762131	0.225079079	0.300105439	0.375131798	0.450158158	0.375131798
MBAAZI 1	0.3187347	0.490762131	0.340709412	0.490762131	0.531366105	0.531366105	0.190656693	0.375131798	0.265683052	0.450158158	0.353157086
MUKUNE	0.375131798	0.375131798	0.375131798	0.525184518	0.375131798	0.606392464	0.265683052	0.340709412	0.300105439	0.415735772	0.340709412
MUSUNGU	0.600210877	0.375131798	0.525184518	0.375131798	0.600210877	0.565788491	0.450158158	0.450158158	0.300105439	0.375131798	0.300105439
MUTARIKI	0.415735772	0.375131798	0.340709412	0.375131798	0.490762131	0.415735772	0.150052719	0.375131798	0.300105439	0.375131798	0.300105439
SYOMBONZE	0.565788491	0.525184518	0.565788491	0.225079079	0.86589393	0.375131798	0.600210877	0.450158158	0.600210877	0.525184518	0.525184518

Continuation

OTU	GBK 034224	GBK 047027	GBK 047047	ICEAP 00902	KAT 60/8	KENDI TALL	KIONZA	MBAAZI 1	MUKUNE	MUSUNGU	MUTARIKA	SYOMBONZE
GBK 034224	0											
GBK 047027	0.750263597	0										
GBK 047047	0.450158158	0.640814851	0									
ICEAP 00902	0.675237237	0.525184518	0.600210877	0								
KAT 60/8	0.525184518	0.525184518	0.525184518	0.375131798	0							
KENDI TALL	0.600210877	0.525184518	0.565788491	0.375131798	0.150053	0						
KIONZA	0.525184518	0.490762131	0.375131798	0.450158158	0.300105	0.340709412	0					
MBAAZI 1	0.490762131	0.490762131	0.600210877	0.375131798	0.490762	0.531366105	0.265683	0				
MUKUNE	0.415735772	0.525184518	0.565788491	0.450158158	0.525185	0.525184518	0.340709	0.156234	0			
MUSUNGU	0.450158158	0.525184518	0.300105439	0.600210877	0.375132	0.525184518	0.375132	0.565788	0.450158	0		
MUTARIKA	0.600210877	0.415735772	0.450158158	0.375131798	0.375132	0.415735772	0.075026	0.190657	0.265683	0.450158158	0	
SYOMBONZE	0.525184518	0.415735772	0.300105439	0.525184518	0.225079	0.265683052	0.375132	0.525185	0.640815	0.450158158	0.45015816	0

4.2.4. Genetic Structure analysis

Construction of a Neighbor-Joining (NJ) tree reveals the genetic relationship among the 24 accessions using the 12 SSR markers. As shown in Figure 4.3, the studied entities were placed into 4 clusters. Cluster III was the largest and comprised 13 accessions. This cluster was further sub-divided into 2 sub-clusters (IIIA and IIIB). On the other hand, cluster IV was the smallest, comprising 3 accessions. To visualize the genetic similarity among and within the populations clustered according to their species identity, the use of PCoA most probably from Nei's genetic distance (Nei 1972), was employed. The PCoA results revealed that the 24 accessions displayed uniform distribution across the two axes and that the first two axes explained 35.21% of the total observed variation (Figure 4.4). Based on AMOVA results, the maximum variation (97%) occurred within the populations, whereas the minimum variation (3%) was observed among the populations (Table 4.5).

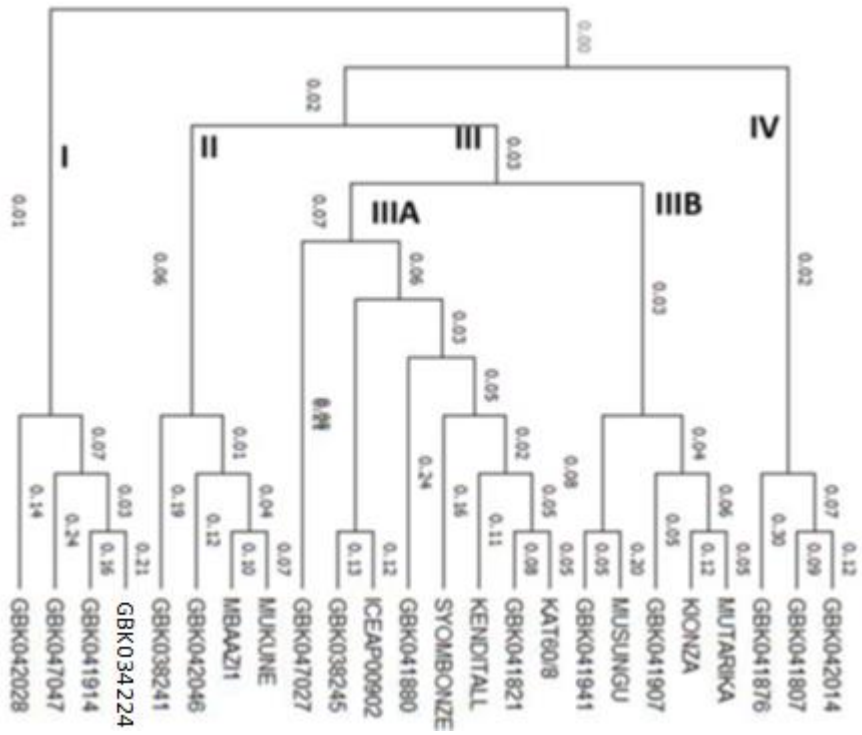

Figure 4.3: A Neighbour-joining dendrogram showing the genetic relationships among the 24 Kenyan pigeon pea accessions.

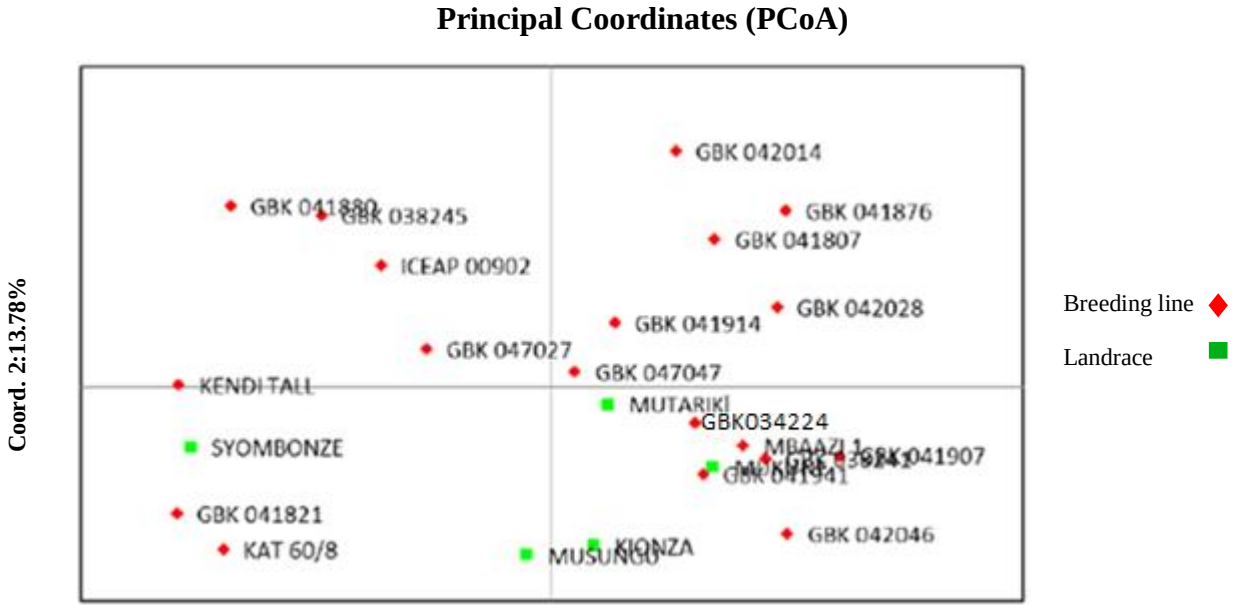

Figure 4.4 Principal coordinate analysis visualizing genetic relationship among the 24 pigeon pea accessions.

Table 4-0-5 Analysis of molecular variance within and among the 24 pigeon pea accessions. Df=degree of freedom, SS = sum of squares, MS = expected mean squares.

Source	df	SS	MS	Estimated Variance	%	P values
Among populations	1	13.845	13.845	0.308	3%	P<0.05
Within populations	22	250.905	11.405	11.405	97%	P>0.05
Total	23	264.750		11.713	100%	

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1.Discussion

Collection, preservation and diversity assessment of germplasm is a key precursor for crop genetic improvement (Shen *et al.*, 2019). ANOVA results in the present research showed significant levels of variation among the 24 accessions for the evaluated 9 agro-morphological traits. These outcomes are in congruence with those reported by Upadhyaya *et al.* (2007). These findings demonstrate the existence of a wider phenotypic diversity among the Kenyan pigeon pea germplasms and offer a valuable source of genetic diversity that can be useful in a pigeon pea breeding program.

The clustering of the 24 accessions into 4 major sub-clusters (Figure 4.1) suggested the presence of a high level of genetic variation among the accessions. Nevertheless, the mixture of landraces, breeding lines and released cultivars in each group could be ascribed to close genetic relatedness among the accessions since East Africa is recognized as a secondary source of pigeon pea diversity**.** PCA was utilized in this study to describe the relationships between the accessions.

Nine phenotypic traits were evaluated, which included leaf length (LL), pod length (PL), stem diameter (StD), plant height (PH), leaf width (LW), branches per plant (BP), seed diameter (SD), seed per pod (SP) and 100 seeds weight (100SW). These traits can be used in accelerating breeding programs for new varieties by taking advantage of these main factors.

The average leaf length (LL) of dissimilar varieties exhibited considerable significant differences, where GBK038245 recorded the highest mean leaf length of 14.6 ± 0.74cm,

whereas SYOMBONZE recorded the lowest mean leaf length of 8.88 ± 2.01cm. This attribute is a very important aspect in determining the yield of a crop. The leaf, being the photosynthetic area of a plant, determines how much the plant photosynthesizes hence the amount of food for the plant (Long *et al.*, 2006). This, therefore, determines the size and the number of seeds that the plant produces. This ultimately translates to the yield of the crop. It may translate that the longer the leaf, the higher the yield.

Leaf length could also be an adaptation for survival depending on the availability of moisture in different soils. Longer leaves increase the surface area of the leaf, which increases the area involved in transpiration hence the leaf losing more water to the atmosphere. Shorter and thin leaves loose less water into the atmosphere. Those crops that grow and are well adapted for dry areas have reduced leaf area to minimize water loss through evapotranspiration (Deblonde and Ledent, 2001).

The mean leaf width (LW) of the twenty-four varieties also recorded a significant difference, whereby GBK038241 had the highest mean leaf width of 6.00±0.29cm, whereas ICEAP 00902 recorded the lowest mean leaf width of 3.70±0.15cm. Width increase increases the leaf area. If both the width and the length increase, the leaf area increases hence the effect of increased or reduced leaf area is realized. Pigeon peas that grow in dry areas have thinner leaves than those growing in wet areas.

Assessment of the growth of woody vegetation is mostly done by measuring the diameter of the stem and also assessing the benefits provided i.e., environmental and commercial benefits, which include carbon sequestration, biomass or wood product and landscape remediation (Paul *et al.,* 2017). The mean stem diameter recorded significant differences, where GBK 041876 had the highest average stem diameter of 1.68±0.08cm, whereas

GBK042028 showed the lowest mean stem diameter of 0.54± 0.08cm. The increment in stem diameter resulting from secondary growth is attributed to the activity of lateral meristems. The lateral meristems include cork cambium and vascular cambium.

Diameter is a salient aspect in plant growth and development for it provides anchorage to the plant, conducts mineral transportation and translocation of food materials to all the parts of the plant. The stem also provides the frame that exposes the plant's photosynthetic area to sunlight. An increase in stem diameter, therefore, increases xylem and phloem vessels, thereby increasing mineral uptake and food translocation rate. This is evident considering the variety with the highest mean of branches per plant (10.00±0.45). This is as a result of having the highest mineral uptake and food translocation, which resulted in increased vegetative growth.

Increased vegetative growth translates to increased biomass, which is an important aspect for breeding *Cajanus cajan* varieties that are used for livestock feeding and production of the crop for green manure. The mean number of branches per plant (BP) also showed significant differences, where GBK041876 recorded the highest mean of 10.00± 0.45, whereas GBK041907 recorded the lowest mean of 1.00±0.00. Plant growth patterns are controlled by hormones within the plant. The growth of branches is controlled mainly by two kinds of hormones; auxins and cytokinins. Auxins are responsible for vertical stem growth, whereas cytokinins are involved in lateral branching (stem growth). Regular branching allows plants to expand and adapt to the environment (Evert, 2006). Increased branching increases the biomass of the crop, which results in increased forage material and wood fuel. Excessive branching also results in a well-established branching system

that exposes the photosynthetic area to direct sunlight, which translates to increased vegetative growth.

Apical dominance, which leads to vertical growth without branching, is undesirable due to decreased number of shoots for bearing. Increased branching, therefore, increases the number of shoots for bearing, which may increase the crop yield.

A substantial dissimilarity was further observed in plant height. GBK041807, a breeding line grown in Machakos, had the highest mean plant height of 102.00±1.67cm. Plant height correlates to lodging resistance, plant architecture, and yield performance. Gibberellins, growth-promoting phytohormones, are significant in the control of plant height. Plant height is influenced by mutations in gibberellins metabolism, biosynthesis, and signaling cascades. Furthermore, gibberellins interact with other phytohormones in the modulation of plant height (Wang *et al.*, 2017). Plant height has a lot of impact on crop yield. Tall crops are more vulnerable to wind and rain. They are at a higher risk of falling over, thus reducing the overall yield. To mitigate this risk, scientists have bred shorter crops using changes in a group of genes called DELLAs, (Hartweck, 2008). Though these mutations have led to improved yield, they have resulted in undesirable side effects like increased sensitivity to dry conditions during seed germination. There is, therefore, a need for more detailed understanding of the genes that control how tall a plant grows.

Pod length recorded a significant difference with GBK038241, a breeding line grown in Makueni, having the highest mean pod length of 10.08±0.34cm, whereas GBK041907, a

breeding line grown in Kitui (Mwingi), recorded the lowest mean pod length of 5.00±0.09cm. There was a direct correlation between the number of seeds per pod and the pod length GBK038241 had both the highest mean pod length and mean number of seeds per pod. Although yield is an expression of several parameters that are considerably influenced by the environment, grain yield has been reported to have a significant similarity with pod length. This indicates that an increase in pod length results in increased yield if all other factors are held contant.

Significant differences were also recorded in mean seed diameter, where KIONZA, a local landrace mainly grown in Makueni, had the highest mean seed diameter of 9.00±0.42mm, whereas GBK041914, a breeding line grown in Machakos, recorded the lowest mean seed diameter of 6.00±0.16mm. This showed a positive correlation with 100 seeds weight, where the variety with the lowest mean seed diameter also recorded the lowest mean 100 seeds weight. Seed size or seed diameter is related to the relative growth rate. Smaller seeds develop faster than larger seeds. In addition, smaller seeds have better chances for dispersal over a wide area when compared to larger seeds, thereby assisting in increasing the chances of some seedlings' survival. Larger seeds are better able to support themselves initially (especially before germination) (Deblonde and Ledent, 2001).

Many farmers and consumers prefer large pigeon pea seeds. Increased diameter, which translates to large seeds, therefore, increases the demand for the particular pigeon pea variety in the market. *Cajanus cajan* breeders should, therefore, focus on this aspect

together with the number of seeds per pod to breed improved varieties which meet the market demand for farmers and consumers. This would also drastically increase the yield per unit area of the crop.

The mean number of seeds per pod also recorded quite significant differences where GBK038241, a breeding line grown in Makueni, had the highest average number of seeds per pod of 6.60±0.25, whereas KAT 60/8, a breeding line grown in Kitui, recorded the lowest average number of seeds per pod of 4.00±0.00. A positive correlation of this aspect with the mean pod length was observed, where GBK038241 had both the highest mean number of seeds per pod and the highest mean pod length of 10.08±0.34. This positive correlation was earlier observed in Brassica and Sinapis (Li & Yang, 2014).

The mean seed weight, pod length and seed number per pod are considered the most significant components of yield (Yang *et al.*, 2017). Studies have made it possible to comprehend the genetic regulation of these traits and their significance in seed yield (Li *et al.*, 2019). An increased number of seeds per pod, especially if the seeds are large enough, will obviously lead to an increased number of seeds per plant hence increasing the overall yield of the crop. This is also an important aspect for the breeders of pigeon peas to consider in breeding for increased yield.

Seed-producing companies also consider the number of seeds per pod as a valuable attribute in seed multiplication process. Reduced number of seeds per pod in KAT 60/8 grown in Kitui region, which has reduced amount of rainfall and soils that have very little nutrients could be an adaptation for reduced moisture and soil nutrients. The variety is

known to mature within 135-150days (Kwena *et al.*, 2021). This is a drought escaping character in plants that grow in arid environments (Solbrig and Orians, 1977).

The mean of 100 seed weight also recorded significant differences with MUSUNGU, a local landrace grown in Wote-Makueni, recording the highest mean value of 23.00±0.32gm, whereas GBK041914, a breeding line grown in Machakos, recorded the lowest mean of 12.00±0.32gm. There was a positive correlation of this parameter with seed diameter, where the variety that recorded the lowest mean of one hundred seed weight also had the lowest seed diameter.

The weight of the grain is influenced by its width, length and thickness of the grain (Li *et al.*, 2021). These traits affect grain weight so profoundly that they all share potential trade-offs as well as coordinate controlling of grain quality and quantity. Endosperm development, ubiquitination, plant phytohormone regulation pathways, RNA-mediated regulation networks, photosynthetic product accumulation, chromatin modification, G-protein regulation pathways, and transportation processes are some of the processes identified in previous studies involved in regulating the weight of the grain (Li *et al.*, 2021). In general, increased grain weight translates to increased crop yield.

The morphological dissimilarity recorded under cluster analysis using a dendrogram was also recorded in genetic analysis using pairwise genetic dissimilarity in many of the varieties, for example, GBK 034224 and GBK 041880, in both genetic and morphological analysis showed high dissimilarity. Under cluster analysis of morphological traits using a dendrogram (Figure 4.1), GBK 034224 is in sub-cluster C4, whereas GBK 041880 is in

sub-cluster C1. On genetic relationship using a neighbour-joining dendrogram (Figure 4.3), GBK 034224 is cluster I, whereas GBK 041880 is in cluster III sub-cluster IIIA. Such dissimilarity is very important for crop breeders since the wider the diversity the more the characters are. Some of these could be very desirable characters in crop production. Cluster analysis of the twenty-four pigeon peas varieties also showed a morphological divergence with dendrogram classifying them into full sub-clusters (C1, C2, C3, and C4) (Figure 4.1).

Sub-cluster C2 had the highest phenotype diversity in eight accessions, whereas sub-cluster C3 had the least phenotypic diversity comprising three accessions. GBK 042028, which is a breeding line grown in Embu (Ishiara), and ICEAP 00902, also a breeding line grown in Machakos, recorded the highest similarity of 95%. Although several varieties showed dissimilarity, few varieties recorded a high similarity index, for example, those varieties in sub-cluster C1 (Figure 4.1) except GBK 041876, all of them were genetically found to be similar, hence, sharing many characteristics and were grouped using neighbour-joining dendrogram of genetic structure analysis in the same cluster III (Figure 4.3). This grouping may suggest these breeding lines may have hailed from landraces that have been used by farmers for many years in pigeon peas production. The genetic relationships are also useful tools in population genetics because they clearly show genetic variations of morphological traits of a plant in relation to the evolutional history story (Kisua *et al.*, 2015). However, the findings for morphological similarity observed in this study, to a large extent, match those of genetic similarity using SSR markers.

Principal components analysis performed on the twenty-four varieties indicated that the Eigenvalues of the 1st three components contributed highly to the variation and cumulatively accounted for 77.9% of total variation among the nine studied traits. The first principal component accounted for a 38.5% proportion of variance, which was the highest variation and it had a positive correlation to leaf width, stem diameter, leaf length, plant height, grain diameter, pod length, number of seeds per pod and one hundred seeds weight.

It was, however, negatively correlated to the number of branches per plant. In the second principal component, the proportion of variance was 23.0%, with the highest positive contribution from stem diameter (0.649) and the lowest contribution from the number of seeds per pod (-0.215). The third principal component recorded a 16.4% proportion of variance, with plant height contributing the highest positive correlation of 0.564 and 100 seeds weight contributing the lowest negative contribution of -0.392. For the loading plot, pod length had the largest loading (0.438) on the first component, whereas stem diameter had the largest positive loading (0.649) on the second component (Figure 4.2). Among the nine studied traits, those that contributed highly to the variation are yield determinants in pigeon peas and are the ones preferred by farmers.

The first Eigen value (3.4643) was very close though slightly higher than the one reported by Ngari *et al.* (2019), who recounted a Eigen value of 3.247 on phenotypic characteristics of *Catha edulis*. For the 2nd Eigen value (2.0699) was lower than that obtained by Mwangi *et al.* (2021) in seven Mung beans (*Vigna radiata*), which was 3.788.

The 3rd Eigen value (1.4778) was higher than the one reported by Mawia *et al.* (2015) of 0.38 in thirteen rice genotypes.

In the scatter plot, the twenty-four varieties indicated some wide existence of wide variations and few relatednesses among varieties. Varieties that were placed in the same plot showed morphological relatedness, whereas those far away from each other were phenotypically different. Mukune, a local landrace grown in Wote-Makueni, is closely related to GBK 041880, a breeding line grown in Machakos, GBK 041807, still a breeding line grown in Machakos, and GBK 034224, a breeding line grown in Makueni. This could suggest that the breeding lines could have the same ancestor, which is most likely to be Mukune, a local landrace grown in the same geographical region. It is however indicated that some varieties have a very wide phenotypic divergence; For example, GBK 041876, a breeding line grown in Muthetheni in Machakos, and GBK038241, a breeding line grown in Makueni, showed high phenotypic variation, indicating a very wide phylogenetic divergence between the two varieties.

In the current study, twelve simple sequence repeats (SSR) markers, also known as microsatellites, were employed to characterize the twenty-four pigeon peas accessions. Polymorphism information content (PIC) values were used in the determination of polymorphism levels. PIC is the assessment of polymorphism for a marker locus utilised in linkage analysis. It shows genotypic variation, including single base-pair variability and larger changes within a gene or genome. The PIC values range from zero to one. Values between 0 and 0.25 are slightly informative. The PIC values that ranged from 0.25

to 0.5 are reasonably informative, whereas those between 0.5 and 1 were highly informative (Patil *et al*., 2017).

Based on the current study, the markers were reasonably informative with a mean PIC value of 0.4178. The twelve markers produced amplification products of the expected band size. A total of thirty three alleles were obtained from the twelve polymorphic markers, ranging between 2 and 4 with an average of 2.75 alleles for every locus (Table 4.3). The gene diversity values ranged from 0.3299 to 0.6597, with a mean of 0.4939. The observed heterozygosity values ranged between 0.0000 and 0.2917, with a mean of 0.0868 (Table 4.3). PIC values ranged between 0.2755 (PGM 102) and 0.6036 (CCB10) with an average of 0.4178 (Table 4.3).

The mean number of alleles reported in the current study agreed with those reported by Mwangi *et al.* (2021), which also ranged between 2 and 4 and had an average of 2.4 in Kenyan Mung bean characterization. Sousa *et al.* (2011) obtained a higher mean number of alleles of 5.1 in genetic diversity analysis among pigeon peas genotypes adapted to South American regions according to microsatellites markers. This big difference in the mean number of alleles could have resulted from the different markers that were used, the accessions used and differences in geographical regions where these accessions were collected (Mwangi *et al*., 2021).

The genetic dissimilarity of the twenty-four pigeon pea accessions was determined using a dissimilarity matrix presumably from the “C.S Cord 1967” shared SSR. Based on this

method, genetic dissimilarity values ranged between 0.075026 and 0.900316. The highest dissimilarity was observed between varieties GBK 041880 and GBK 034224 as well as between varieties GBK 041876 and SYOMBONZE. There was a very wide variation among the varieties. In contrast, the lowest dissimilarity was observed between varieties KIONZA and MUTARIKI. Owing to the fact that the two are local landraces that have been grown by local farmers in the same geographical region, there has been a lot of crossbreeding between the two landraces hence sharing many of the genes.

The dissimilarity shown between GBK 041880, which is a breeding line grown in Machakos, and GBK 034224, a breeding line grown in Makueni, was also displayed under cluster analysis of morphological traits using a dendrogram based on the average values of the nine quantitative traits. These traits, therefore, could be used in determining the dissimilarities between the varieties. The results obtained concur with those obtained by Kusum *et al.* (2012) in the genetic diversity of pigeon peas (*Cajanas cajan (*L*)Mill sp.*) cultivars and their wild relatives using Randomly Amplified Polymorphic DNA (RAPD) markers, where they obtained a range of 0.29 to 0.88.

A Neighbour-joining (NJ) tree was also constructed to show the relationship among the twenty-four accessions using the twelve simple sequence repeat markers (Figure 4.3). The accessions were placed into 4 clusters (I, II, III and IV). Cluster III was further grouped into 2 sub-clusters (III A and III B). Cluster III had the largest number of accessions with thirteen varieties, whereas cluster IV had the smallest number with only three accessions. PCoA presumably from Nei's genetic distance (Nei, 1972), showed that

the twenty-four accession displayed uniform distribution across the two axes and the first two axes explained the 35.21% of the total observed variation (Figure 4.4). Accessions that cluster together in 1 quadrant are closely related. The first 2 PCoA axes accounted for 21.43% and 13.78% of the genetic variation among the twenty-four pigeon peas accessions. Coordinate one had the highest variability (21.43%) succeeded by coordinate two (13.78%). Mwangi *et al.* (2021), in the characterization of Mung bean germplasms, obtained higher coordinates of 37.52% and 28.53% in coordinates 1 and 2, respectively.

Analysis of molecular variance among and within the twenty-four pigeon peas accessions was performed using the Euclidean distance matrix (Schnider *et al.*, 2009). The results indicated that 3% of the total variation was among the populations, whereas 97% was within the populations. On the other hand, since most of the accessions appear to have originated from the local landraces or share the same ancestry, this could have contributed to low dissimilarity among the populations that have been identified in several accessions (Mwangi *et al.*, 2021). Most of the farmers have been planting a mixture of those local landraces and this could have led to cross-pollination hence sharing of the same genetic material among these populations.

Both mean grain diameter and mean of 100 seed weight are the two attributes I used to determine the yield. The weight of the grain is influenced mainly by thickness or diameter of the grain (Li *et al.*, 2021). KIONZA (9.00±0.42mm) and MUSUNGU (8.00±0.27mm) which are both local landraces grown in Makueni (Wote) recorded the highest mean of grain diameter. Both also recorded the highest mean of 100 seed weight with KIONZA having 21.00±0.32gm and MUSUNGU having 23.00±0.32gm. Genotypically both were

in the same cluster (III) and the same sub-cluster (IIIB). This indicated a positive correlation between phenotypic yield attributes and the genotypic traits.

The least mean of grain diameter was recorded by GBK042028, a breeding line grown in Embu (Ishiara) with a mean of 6.50±0.22mm and GBK041914 a breeding line grown in Machakos with a mean of 6.00±0.16mm. Both also recorded the lowest mean of 100 seed weight where GBK042028 had a mean of 14.20±0.66gm whereas GBK041914 had a mean of 12.00±0.32gm. The two are also genotypically in the same cluster (I). This also indicated a positive correlation between phenotypic yield attributes and genotypic traits.

From the results obtained in this study, there was a slightly strong relationship between phenotypic and genotypic characterization. This indicated there was slightly a strong association between phenotypic characteristics and the genetic background of the plant (Jordan *et al*., 2008).

5.2.Conclusions

i. Phenotypic characterization of the twenty-four selected pigeon pea germplasms studied indicated high diversity among all the germplasms.

ii. Genotypic characterization of the selected twenty-four pigeon pea germplasms using Simple Sequence Repeat (SSR) markers showed high genetic diversity among the twenty-four selected germplasms.

iii. The study also indicated a slight positive correlation between the phenotypic yield attributes and genotypic traits in the twenty-four studied germplasms.

5.3. Recommendations

i. SSR markers are reliable markers for establishing genetic variance in pigeon peas germplasms.

ii. Those primers with CCB codes are more reliable in showing polymorphism in pigeon pea germplasms grown in Kenya.

iii. Morphological characteristics can be used in determining variation among pigeon peas varieties.

iv. Pigeon peas landraces should also be conserved in gene banks as they have shown high genetic variation and they have high quality traits.

5.4. Suggestions for further research

i. Pigeon pea germplasms from other geographical regions apart from those grown in Kenya should be used to establish more traits among the pigeon pea varieties.

ii. The diversity of the studied pigeon pea varieties can be used to breed new crop hybrids with more desirable traits.

iii. To determine the exact variance between varieties of pigeon pea, DNA sequencing should be conducted to establish the best primers for genetic characterization.

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APPENDICES

Appendix I: Plant height (cm) after three months

NUMBER	NAME	SAMPLES				
01	MBAAZI(KARI)	96	105	103	101	105
02	KAT 60/80	160	172	186	174	158
03	GBK 041807	195	210	230	236	229
04	GBK 042046	193	210	204	215	203
05	GBK 038241	201	189	197	203	200
06	GBK 034224	196	206	199	210	194
07	GBK 042014	161	146	116	148	126
08	GBK 041821	165	136	160	153	142
09	SYOMBONZE	112	107	120	120	157
010	GBK 047027	200	184	190	186	200
011	MUKUNE	156	107	142	140	130
012	GBK 041941	175	150	195	156	169
013	KENDI TALL	171	153	173	165	167
014	GBK 041914	163	165	157	103	180
015	GBK 041876	162	190	158	170	200
016	GBK 041907	103	126	102	158	124
017	MUTERIKI	202	190	192	189	207
018	GBK 038245	137	167	186	176	154
019	GBK 042028	150	168	122	131	114
020	MUSUNGU	180	165	184	175	171
021	GBK 047047	145	142	136	150	140
022	ICEAP	153	114	144	140	134
023	GBK 041880	170	172	140	186	148
024	KIONZA	178	164	148	158	125

Appendix II: Pod length (cm)

NUMBER	NAME	SAMPLES				
01	MBAAZI(KARI)	7.3	7.2	8.0	7.8	8.0
02	KAT 60/80	6.0	5.5	5.5	6.0	5.0
03	GBK 041807	8.2	7.5	8.0	7.0	8.0
04	GBK 042046	7.5	8.0	7.5	7.2	7.3
05	GBK 038241	9.0	10.5	10.5	9.6	10.8
06	GBK 034224	7.5	7.5	6.2	7.0	6.0
07	GBK 042014	8.5	8.5	8.8	7.7	8.4
08	GBK 041821	7.0	6.5	7.0	7.5	7.0
09	SYOMBONZE	6.5	6.0	7.0	7.0	6.5
010	GBK 047027	7.0	7.3	7.2	7.5	7.6
011	MUKUNE	8.0	8.0	7.0	8.5	7.0
012	GBK 041941	6.0	7.0	6.2	8.0	6.0
013	KENDI TALL	6.5	7.4	7.4	6.5	6.0
014	GBK 041914	7.0	7.0	7.0	7.0	7.0
015	GBK 041876	5.6	6.0	6.0	5.8	6.1
016	GBK 041907	5.0	5.3	4.8	5.1	4.8
017	MUTERIKI	5.5	6.0	5.5	5.5	5.6
018	GBK 038245	9.0	9.5	10.0	10.1	9.2
019	GBK 042028	7.0	7.0	6.0	6.0	6.5
020	MUSUNGU	9.0	10.2	9.0	9.5	9.3
021	GBK 047047	6.5	6.0	5.5	6.0	6.0
022	ICEAP	7.5	6.5	7.0	6.5	6.5
023	GBK 041880	7.0	7.0	7.0	7.0	7.0
024	KIONZA	10	9.5	9.5	9.5	10.3

Appendix III: Number of grains per pod

As they appeared when they are ripe

Number of grains per pod						
NUMBER	**NAME**	**SAMPLES**				
01	MBAAZI(KARI)	5	5	5	5	5
02	KAT 60/80	4	4	4	4	4
03	GBK 041807	5	5	5	5	5
04	GBK 042046	6	6	6	6	6
05	GBK 038241	6	7	7	6	7
06	GBK 034224	5	5	5	5	5
07	GBK 042014	6	6	6	6	6
08	GBK 041821	6	6	6	6	6
09	SYOMBONZE	5	5	5	5	5
010	GBK 047027	5	5	5	5	5

011	MUKUNE	6	6	6	6	6
012	GBK 041941	5	5	5	5	5
013	KENDI TALL	5	4	5	5	5
014	GBK 041914	5	5	5	5	5
015	GBK 041876	5	5	5	5	5
016	GBK 041907	4	4	4	4	4
017	MUTERIKI	5	5	5	5	5
018	GBK 038245	6	6	6	6	6
019	GBK 042028	5	5	5	5	5
020	MUSUNGU	6	6	6	6	6
021	GBK 047047	5	5	5	5	5
022	ICEAP	6	6	6	6	6
023	GBK 041880	5	5	5	6	6
024	KIONZA	6	6	6	6	6

Appendix IV: Grain weight for 100 seeds (grammes)

After drying for one week

NUMBER	**NAME**	
01	MBAAZI(KARI)	15
02	KAT 60/80	13
03	GBK 041807	15
04	GBK 042046	16
05	GBK 038241	19
06	GBK 034224	16
07	GBK 042014	15
08	GBK 041821	15
09	SYOMBONZE	19
010	GBK 047027	15
011	MUKUNE	21
012	GBK 041941	12
013	KENDI TALL	17
014	GBK 041914	12
015	GBK 041876	19
016	GBK 041907	16
017	MUTERIKI	14
018	GBK 038245	17
019	GBK 042028	14
020	MUSUNGU	23
021	GBK 047047	14
022	ICEAP	15
023	GBK 041880	18

024	KIONZA	22

Appendix I: Grain Diameter (mm)

Grain Diameter (mm)						
NUMBER	**NAME**	**SAMPLE**				
01	MBAAZI(KARI)	6.0	6.5	6.5	7.0	6.5
02	KAT 60/80	6.5	7.0	6.0	6.5	6.5
03	GBK 041807	6.5	7.0	7.0	7.5	7.0
04	GBK 042046	7.5	8.5	8.0	7.5	8.5
05	GBK 038241	7.0	8.0	8.5	7.5	9.0
06	GBK 034224	6.5	7.0	6.5	8.0	7.0
07	GBK 042014	6.5	7.0	8.0	7.0	6.5
08	GBK 041821	8.0	7.0	6.5	6.5	7.0
09	SYOMBONZE	7.5	8.0	7.5	8.5	8.5
010	GBK 047027	7.0	6.5	7.0	7.5	7.0
011	MUKUNE	8.5	8.0	8.0	7.5	8.0
012	GBK 041941	6.0	6.0	6.0	6.0	6.0
013	KENDI TALL	8.0	7.5	8.5	8.0	8.0
014	GBK 041914	6.5	6.0	5.5	6.0	6.0
015	GBK 041876	7.5	7.5	8.0	8.5	8.5
016	GBK 041907	7.0	7.5	7.0	6.5	7.0
017	MUTERIKI	8.0	7.5	7.5	6.0	6.0
018	GBK 038245	7.0	7.5	7.0	6.5	7.0
019	GBK 042028	6.0	6.0	7.0	6.0	7.5
020	MUSUNGU	7.0	8.5	8.0	8.0	8.5
021	GBK 047047	8.0	7.0	6.5	7.0	6.5
022	ICEAP	7.0	6.0	6.5	7.0	8.5
023	GBK 041880	8.0	7.0	7.0	6.0	7.0
024	KIONZA	8.5	9.0	9.0	8.0	10.5

Appendix V: Stem diameter (cm)

After three months of age (90 days)

NUMBER	NAME	SAMPLE				
01	MBAAZI(KARI)	1.1	0.5	0.8	0.9	0.7
02	KAT 60/80	0.5	0.7	0.7	0.8	0.7
03	GBK 041807	0.9	1.0	1.2	0.8	1.4
04	GBK 042046	0.7	0.8	0.5	0.6	0.8
05	GBK 038241	0.8	0.7	0.7	0.8	0.8
06	GBK 034224	1.2	0.8	0.9	0.9	1.2
07	GBK 042014	0.6	0.5	0.5	0.4	0.7
08	GBK 041821	0.8	0.9	0.9	0.8	0.9
09	SYOMBONZE	1.2	1.0	0.9	1.1	1.1
010	GBK 047027	0.8	0.7	0.7	0.9	0.8
011	MUKUNE	1.1	0.8	1.0	0.9	1.1
012	GBK 041941	0.9	0.9	0.8	0.9	0.9
013	KENDI TALL	1.0	1.0	0.9	1.2	1.4
014	GBK 041914	1.2	1.0	0.9	1.0	0.9
015	GBK 041876	1.5	1.5	1.8	1.9	1.7
016	GBK 041907	0.6	0.6	0.4	0.5	0.7
017	MUTERIKI	0.5	0.5	0.6	0.7	0.5
018	GBK 038245	0.7	0.6	0.7	0.7	0.7
019	GBK 042028	0.4	0.3	0.7	0.6	0.7
020	MUSUNGU	0.7	0.6	0.5	0.6	0.6
021	GBK 047047	0.6	0.7	0.6	0.6	0.7
022	ICEAP	0.6	0.5	0.5	0.6	0.7
023	GBK 041880	0.9	1.1	0.7	0.8	0.8
024	KIONZA	0.9	0.8	0.7	1.0	0.6

Appendix VI: Number of branches after 6 months (180 days)

NUMBER	NAME	SAMPLES				
01	MBAAZI(KARI)	10	9	11	8	7
02	KAT 60/80	4	4	5	3	4
03	GBK 041807	5	4	4	6	6
04	GBK 042046	2	1	2	3	2
05	GBK 038241	1	1	1	1	2
06	GBK 034224	5	7	6	7	5
07	GBK 042014	1	1	1	2	1
08	GBK 041821	3	5	4	4	4
09	SYOMBONZE	8	10	9	9	9
010	GBK 047027	1	2	2	3	2

011	MUKUNE	5	6	5	5	4
012	GBK 041941	2	2	1	3	2
013	KENDI TALL	8	9	8	7	8
014	GBK 041914	9	7	9	8	7
015	GBK 041876	9	11	10	9	11
016	GBK 041907	1	1	1	1	1
017	MUTERIKI	2	1	1	1	1
018	GBK 038245	1	2	2	1	1
019	GBK 042028	1	1	1	1	1
020	MUSUNGU	2	1	1	1	2
021	GBK 047047	1	2	1	1	1
022	ICEAP	2	3	2	4	4
023	GBK 041880	7	5	6	5	7
024	KIONZA	1	2	1	1	1

Appendix VII: Leaf length (cm) three weeks after germination

NUMBER	NAME	SAMPLES				
01	MBAAZI(KARI)	10.4	10.0	11.0	10.1	11.5
02	KAT 60/80	9.0	10.2	9.8	10.6	11.9
03	GBK 041807	13.0	12.9	11.0	12.9	13.6
04	GBK 042046	15.1	12.6	13.4	12.5	13.9
05	GBK 038241	15.2	14.6	14.0	13.8	14.9
06	GBK 034224	14.1	13.7	13.9	14.0	11.8
07	GBK 042014	11.2	10.0	8.0	11.0	10.3
08	GBK 041821	10.0	13.0	12.6	11.0	13.4
09	SYOMBONZE	10.8	10.0	11.1	0.9	11.6
010	GBK 047027	13.1	11.3	12.6	13.0	13.0
011	MUKUNE	9.2	10.3	8.2	10.7	12.6
012	GBK 041941	11.9	12.5	12.0	13.0	11.6
013	KENDI TALL	12.4	12.0	11.4	13.0	11.2
014	GBK 041914	12.0	11.6	11.3	9.7	10.4
015	GBK 041876	12.7	11.8	12.0	12.2	12.8
016	GBK 041907	12.6	11.7	13.3	11.6	12.8
017	MUTERIKI	11.0	9.3	8.0	8.7	9.0
018	GBK 038245	15.6	12.0	13.9	15.8	15.7
019	GBK 042028	8.0	13.1	11.1	9.0	13.8
020	MUSUNGU	10.0	13.1	12.2	14.0	13.2
021	GBK 047047	12.8	13.0	10.0	12.0	12.2
022	ICEAP	10.0	11.0	9.0	13.7	12.3
023	GBK 041880	13.0	10.0	12.2	13.8	13.5
024	KIONZA	14.2	13.7	12.0	14.0	13.6

Appendix VIII: Leaf width (cm) three weeks after germination

NUMBER	NAME	SAMPLES				
01	MBAAZI(KARI)	4	3.8	4.5	5.3	4.9
02	KAT 60/80	4.0	5.1	3.8	4.0	4.1
03	GBK 041807	3.8	5.8	5.0	6.6	6.3
04	GBK 042046	4.5	5.0	3.7	5.5	4.3
05	GBK 038241	7.0	5.3	6.1	6.0	5.6
06	GBK 034224	6.0	5.1	5.0	4.8	6.1
07	GBK 042014	4.3	3.0	3.6	4.3	3.8
08	GBK 041821	6.2	4.1	6.1	4.1	6.0
09	SYOMBONZE	5.4	4.3	4.2	3.5	5.1
010	GBK 047027	3.5	4.1	3.2	4.3	3.9
011	MUKUNE	5.2	3.7	4.0	3.6	4.0
012	GBK 041941	5.1	3.5	4.0	5.3	5.1
013	KENDI TALL	5.0	4.8	5.2	6.3	5.7
014	GBK 041914	4.0	5.2	4.2	5.4	4.2
015	GBK 041876	6.1	3.8	5.8	6.5	5.8
016	GBK 041907	5.2	6.0	3.8	3.3	4.2
017	MUTERIKI	5.6	3.7	4.3	4.0	3.4
018	GBK 038245	5.6	6.0	5.8	6.2	6.4
019	GBK 042028	3.8	4.2	4.2	3.9	4.4
020	MUSUNGU	5.1	4.5	5.5	5.0	4.9
021	GBK 047047	6.2	5.5	6.2	6.3	5.8
022	ICEAP	4.0	3.3	4.1	3.6	3.5
023	GBK 041880	5.7	6.0	4.9	5.2	5.7
024	KIONZA	5.2	6.1	5.8	6.2	6.2

NUMBER	NAME	Leaf length 3 weeks after germination (cm)	Leaf width 3 weeks after germination (cm)	Plant height after 90 days s(cm)	Stem diameter at 90 days(cm)	Number of branches after 180 days
01	MBAAZI(KARI)	10.6	4.5	102	0.8	9
02	KAT 60/80	10.3	4.2	170	0.68	4
03	GBK 041807	12.68	5.5	220	1.06	5
04	GBK 042046	13.5	4.6	205	0.68	2
05	GBK 038241	14.5	6	198	0.76	1.2
06	GBK 034224	13.5	5.4	201	1	6
07	GBK 042014	10.1	3.8	139.4	0.54	1.2

08	GBK 041821	12	5.3	151.2	0.86	4
09	SYOMBONZE	8.88	4.5	123.2	1.06	9
010	GBK 047027	12.6	3.8	192	0.78	2
011	MUKUNE	10.2	4.1	135	0.98	5
012	GBK 041941	12.2	4.6	169	0.88	2
013	KENDI TALL	12	5.4	165.8	1.1	8
014	GBK 041914	11	4.6	153.6	1	8
015	GBK 041876	12.3	5.6	176	1.68	10
016	GBK 041907	12.4	4.5	122.6	0.56	1
017	MUTERIKI	9.2	4.2	196	0.56	1.2
018	GBK 038245	14.6	6	164	0.68	1.4
019	GBK 042028	11	4.1	137	0.54	1
020	MUSUNGU	12.5	5	175	0.6	1.4
021	GBK 047047	12	6	142.6	0.64	1.2
022	ICEAP	11.2	3.7	137	0.58	3
023	GBK 041880	12.5	5.5	163.2	0.86	6
024	KIONZA	13.5	5.9	154.6	0.8	1.2

NUMBER	**NAME**	**Pod length (cm)**	**Number of seeds per pod (when the pods are ripe)**	**Grain weight for 100 seeds After drying for one week (grammes)**	**Grain diameter after drying (mm)**
01	MBAAZI(KARI)	6.06	5	15	6.5
02	KAT 60/80	4.6	4	13	6.5
03	GBK 041807	6.14	5	15	7
04	GBK 042046	6.04	6	16	8
05	GBK 038241	7.92	6.6	19	8
06	GBK 034224	5.64	5	16	7
07	GBK 042014	6.7	6	15	7
08	GBK 041821	5.6	6	15	7
09	SYOMBONZE	5.3	5	19	8
010	GBK 047027	5.8	5	15	7
011	MUKUNE	6.3	6	21	8
012	GBK 041941	5.44	5	12	6
013	KENDI TALL	5.56	4.8	17	8
014	GBK 041914	5.6	5	12	6
015	GBK 041876	4.68	5	19	8
016	GBK 041907	4.04	4	16	7
017	MUTERIKI	4.5	5	14	7
018	GBK 038245	7.72	6	17	7

019	GBK 042028	5.2	5	14	6.5
020	MUSUNGU	7.54	6	23	8
021	GBK 047047	4.8	5	14	7
022	ICEAP	5.5	6	15	7
023	GBK 041880	5.6	5.4	18	7
024	KIONZA	7.7	6	22	9