MORPHOLOGICAL AND GENETIC CHARACTERIZATION OF BAMBARA GROUNDNUT (*Vigna subterranea* (L.) Verdc.) LANDRACES IN KENYA

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DECLARATION AND RECOMMENDATION

DECLARATION

This research thesis is my original work and has not been submitted for examination in any other institution.

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RECOMMENDATION

This thesis has been submitted for examination with our approval as university supervisors according to Egerton University regulations.

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DEDICATION

This thesis is dedicate to The Almighty God from whom all grace and wisdom comes, my loving wife Mrs. Gemana Akumu, sons James Otieno and Christopher Okoth, my parents Mr. Richard Odongo and Mrs. Monica Odongo.

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ABSTRACT

The existence of genetic diversity in germplasm collections is crucial for cultivar development. The objectives of this study were to determine genetic diversity of bambara groundnut (*Vigna subterranea* (L) Verdc.) landraces from Kenya using genetic markers and characterize bambara groundnut landraces from Kenya using morphological markers. Genetic and morphological relationships among bambara groundnuts accessions, were evaluated using morphological and microsatellite markers. Twelve Simple Sequence Repeat (SSR) markers were used to analyse the genetic diversity among 105 bambara groundnut germplasms collected from Western Kenya and the Genetic Resources Research Institute (GeRRI) of Kenya. In the genetic diversity twenty four alleles were revealed with a mean of 2 alleles per locus. The polymorphic information content (PIC) and gene diversity values averaged 0.28 and 0.35 respectively indicating low genetic diversity among the evaluated bambara groundnut germplasm. Genetic distance based on Jaccard's similarity coefficient from the SSR marker analysis ranged from 0.08 to 1.16 among the landraces. Cluster analysis distinctly grouped the 105 accessions into three major clusters. The Analysis of Molecular Variance (AMOVA) revealed that 98% of the total genetic variation was within accessions whereas variation among accessions accounted for 2% of the total genetic variation. Quantitative traits were all statistically significant at ($p \leq 0.05$) except for seed weight, seed number per plant and number of stems per plant. The first four principal components accounted for 33.28, 18.39%, 13.32% and 8.17 %, respectively of the morphological variations among the landraces. The landraces were grouped into two distinctive clusters with the second cluster sub-divided into four sub-clusters. Qualitative traits however accounted for less of the variations. This study is useful for germplasm management and utilization into crop improvement in future breeding efforts.

Keywords: Bambara groundnuts, cluster analysis, genotype, landraces, principal component analysis.

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LIST OF ABBREVIATIONS

cM	centiMorgan
dNTP	Deoxynucleotide Triphosphates
EDTA	Ethylene Diamine Tetracetic Acid
MAS	Marker Assisted Selection
MVSP	Multivariate Statistical Package
NTSYS	Numerical Taxonomy and Multivariate Analysis System
PCoA	Principal Coordinates Analysis
PCA	Principal Component Analysis
UPGMA	Unweighted Pair Group Method with Arithmetic means

CHAPTER ONE

INTRODUCTION

1.1 Background Information

The evaluation of available genetic diversity is a pre-requisite for genetic improvement in crops for example in bambara groundnut (Olukolu *et al.*, 2012). Investigation of genetic diversity in both wild and domesticated species is equally important. Wild populations are known to be a potential source of useful genes and traits which could be introduced into the domesticated gene pool; in particular, genes responsible for adaptation to stressful environments such as those providing a particular resistance to a pathogen or to arid conditions (Cattan-Toupance *et al.,* 1998). Wild populations in centers of diversity or domestication constitute the initial gene pool of crops species. Crop failures and dispersal of germplasm within the centre of origin or limited introduction or isolated locations ('Founder Effects') could lead to reduced genetic diversity in particular breeding populations, which could have long-term negative consequences for production (Trethowan and Mujeeb-Kazi, 2008). By focusing on commercial and elite germplasm the breeder may further reduce the genetic diversity of the domesticated gene pools (Yi *et al.,* 2008; Rauf *et al.*, 2010). Studies of genetic diversity can help to guide the exploitation of wild relatives in a breeding program to retrace or enhance gene flow between wild and domesticated populations which may increase the genetic diversity in domesticated gene pools (Gepts and Papa, 2002).

Estimating the genetic diversity of crop species can be achieved using different marker methods, including; morphological trait, biochemical and molecular. The latter has several advantages over conventional phenotypic markers, as they can be used efficiently regardless of the developmental stage of the plant under investigation (Mondini *et al.*, 2009). Genetic diversity of Bambara groundnut from Tanzania were assessed with 49 polymorphic bands of 11 informative AFLP primers which revealed that bambara groundnut had two major groups in line with their putative geographic origins (Ntundu *et al.*, 2004). The results of this study agreed with a previous study that used RAPD markers on 25 African accessions from the collection in IITA (Ibadan and Nigeria) showing two main groups of accessions corresponding to their geographic distribution (Amadou *et al.*, 2001). High genetic identity between wild and domesticated accessions was detected in an isozyme diversity study of bambara groundnut, Ht= 0.087 with 14 polymorphic loci and Ht= 0.052 with only 7 loci for the wild and domesticated, respectively (Pasquet *et al.*, 1999). The study suggested that wild bambara

groundnut is likely to be the true progenitor of domesticated bambara groundnut. Besides the high value of intra-population diversity in both wild and domesticated accessions, the study also suggested that self pollination is the major mode of sexual reproduction for both accession types. Two hundred and forty single plant accessions of bambara groundnut were assessed using 22 SSR markers. Higher gene and allelic diversity were obtained in the West African and Cameroon/Nigeria regions than others (East African, Central African, and Southeast Asian) with 6.68 and 6.18 alleles per locus, and 0.601 and 0.571, respectively (Somta *et al.*, 2011).

An extensive and diverse range of germplasm was investigated to study genetic diversity of bambara groundnut in the study by (Olukolu *et al.*, 2012). Morphological and quantitative descriptors, alongside DArT markers that represent wide genome coverage, were used and a high genetic diversity was observed for the Cameroon/Nigeria region relative to other regions (Olukolu *et al.*, 2012). The available literature reveals a number of studies of genetic diversity in bambara groundnut in the wild and domestication material. They offer a reasonable start to understanding the genetic basis of the domestication event(s) in this crop, potentially enabling parents with a wide genetic base to be identified for developing mapping populations and subsequent QTL analysis.

1.2 Statement of the Problem

Despite bambara groundnuts being grown in various parts of Kenya, its genome is not well understood. The Kenyan bambara groundnut germplasm has not been characterized both at molecular and morphological levels making its exploitation for breeding purposes uncertain. This has hindered efforts of plant breeders to breed for superior cultivars through crossing of accessions with genetic distances. This has also led to increased poverty and food insecurity among the small scale farmers growing the crop. This study will therefore characterize bambara groundnuts based on morphological and molecular diversity which will be useful for germplasm management and utilization into future efforts in bambara groundnut improvement programs. This will enhance food security and poverty alleviation among bambara groundnut small scale farmers which is a great concern for the Kenyan government.

1.3 Justification.

Knowledge of the existence and extent of genetic diversity in crop species is of prime importance in plant breeding programmes for the development of improved cultivars. Traditionally, morphological traits coupled with reactions to pest, diseases and other stresses have long been used to determine the genetic diversity existing within and between germplasm collections and characterizing them into varieties. However, such phenotypic associations tend

to vary according to environment and are most useful for traits that are controlled by only a small number of genes. As such classifying germplasm collections based on phenotypic differences alone may not provide an accurate indication of genetic diversity. Bambara groundnut breeders will benefit from the knowledge generated on genetic distances or similarity estimates for various landraces from East Africa since they will be potential source of parents for hybridization breeding of the crop. The use of morphological and molecular markers provide some complementary information, especially where morphological markers fail to differentiate some landraces, therefore the use of molecular markers may be unavoidable. The use of these techniques is important in crop breeding since markers can be used in prediction of variability, estimation of heterosis and for selecting the best lines for crosses and these may make breeding more efficient and effective. Since bambara groundnut is an underutilized crop, studies of its genetic and morphological diversity are scarce. No work has been done to characterize bambara landraces held at the Genetic Research Institute of Kenya and farmers fields. This study will help in characterization of bambara groundnut germplasm based on similarities and dissimilarities of traits which will be exploited for future breeding efforts.

1.4 Objectives

1.4.1 Broad Objective

To contribute to Bambara groundnut breeding in Kenya by providing information on their genetic and morphological diversity.

1.4.2 Specific Objectives

i. To determine genetic diversity of bambara groundnut landraces from Kenya, using SSR markers.
ii. To characterize bambara groundnut landraces from Kenya, using morphological markers.

1.5 Hypotheses

i. There is no genetic diversity among the bambara groundnut landraces collected from different regions of Kenya.

ii. There is no morphological diversity among the bambara groundnut landraces collected from different regions of Kenya.

1.7 References

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CHAPTER TWO
LITERATURE REVIEW

2.1 Origin of bambara groundnut.

Bambara groundnut, (*Vigna subterranea* (L) Verdc.) is an indigenous African leguminous crop and one of the most important pulses grown on the continent (Doku and Karikari, 1969). In addition to Africa, it is now found in many parts of South America, Asia and Oceania (Baudoin and Mergeai, 2001). The centre of origin of bambara groundnut is probably north - eastern Nigeria and northern Cameroon since it is found in the wild from central Nigeria eastwards to southern Sudan, and is now cultivated throughout tropical Africa and to a lesser extent in tropical parts of America, Asia and Australia (Brink *et al.,* 2006). Bambara groundnut was domesticated in the semi-arid zone of West Africa, around the headwaters of the Niger River, from where it spread in ancient times to Central Africa, and more recently to the Malagasy Republic, Asia and South America (Tweneboah, 2000). Among the pulses, bambara groundnut is a hardy plant particularly well suited to the growing conditions found in the savanna regions with a Sudanese and Sudano–Guinean climate (Baudoin and Mergaei, 2001). It has been cultivated throughout tropical Africa for many centuries. It was taken at an early date to Madagascar, probably by Arabs and reached Brazil and Surinam early in the seventeenth century and was later taken to the Philippines and Indonesia (Purseglove, 1992).

2.2 Taxonomy of bambara groundnut.

Bambara groundnut (*Vigna subterranea* (L) Verdc.) is an herbaceous, self-pollinating plant with an indeterminate growth habit. The domesticated bambara groundnut landraces have quite a distinct tap root and numerous short lateral stems on which the trifoliate leaves are borne, while the wild forms have a limited number of elongated lateral stems with no clear tap root. The petiole is long, stiff and grooved with a base of a wide range of colours such as green, purple or brown (Swanevelder, 1998). The species *subterranea* is further divided into two groups: var. *spontanea,* comprising the wild forms, found in a small area around northern Cameroon and Nigeria, and var. *subterranea* comprising the cultivated forms in parts of the tropics, mostly in sub-Saharan Africa (Basu *et al.,* 2007). The chromosome number in both wild and cultivated plants is $2n = 2x = 22$ (Forni-Martins, 1986). The wild bambara groundnut landraces usually have a spreading growth habit, compared to the compact type of domesticated landraces (Swanevelder, 1998). The other major difference between the two types is that of pod size, with domesticated landraces having bigger seeds which do not wrinkle upon drying,

compared to the wild type (Pasquet, 2003; Basu *et al.*, 2007;). The germination of cultivated forms is rapid and uniform while in the wild forms it is erratic and takes longer, approximately 15 to 30 days (Basu *et al.,* 2007). Generally, the domestication of crops involves a number of major steps, with the development of altered plant architecture and also of harvest ability traits, so that a wild form plant can be domesticated and made more amenable to intensive agriculture (Basu *et al.,* 2007). Morphological and isozyme analysis has shown that wild bambara groundnut (*spontanea*) is the true progenitor of domesticated bambara groundnut (*subterranea*) by (Pasquet *et al.,* 1999). Bambara groundnut is related to cowpea and has a podding habit similar to that of peanut (*Arachis hypogaea*) in that the pale yellow flower stalk bends downward after fertilization. The relationships between crop legumes including vigna species was reported by Choi *et al.*, 2004b (Fig 1). This pushes the young pod into the soil, where it develops and matures (Doku and Karikari, 1969; Uguru and Ezere, 1997), however, it is not believed to require complete coverage with soil for the pods to develop.

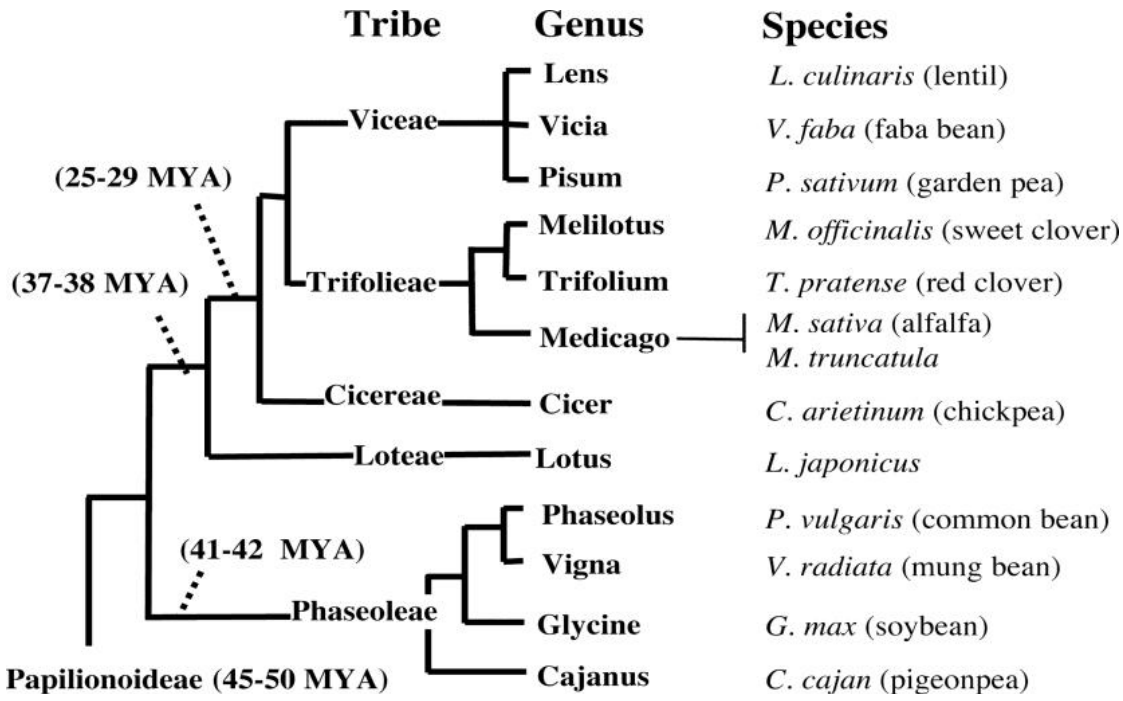

Figure 1: Taxonomic relationships between crop legumes (Choi *et al.*, 2004b).

2.3 Reproduction of bambara groundnut

Bambara groundnut produces perfect flowers, it is self-pollinating and the fertilization of the ovule occurs at the day of anthesis (Linnemann, 1994). Its difficult to undertake artificial

hybridisation, with several attempts unsuccessful (Suwanprasert *et al.,* 2006) and a few reported cases achieved (Massawe *et al.,* 2003). Therefore, relatively few studies have been undertaken on the inheritance of yield and related traits in bambara groundnut (Basu *et al.,* 2007), hence no breeding programme aimed at improving bambara groundnut has so far been initiated to develop cultivars or varieties (Oyiga *et al.,* 2010).

2.4 Adaptability of bambara groundnut

Through many years of successive cultivation, farmers have selected for desirable traits of bambara groundut such as growth habit and seed colour (Linnemann and Azam-Ali, 1993). Farmers prefer the stable, reliable and low yield of bambara groundnut to high yields of groundnut, which has been associated with more yield volatility (Linnemann, 1994). Bambara groundnut is adapted to wide climatic zones, it can be cultivated from sea-level up to 1600 m altitude, and an average temperature of 20-28 ^{o}C is considered ideal for the crop. A growth period of 110 to 150 days is required for the crop to develop, although a reduced growth cycle of approximately 90 days observed in Ghana (Berchie *et al.,* 2010) and it is usually grown in mixed intercropping systems with no application of fertilizers (Karikari *et al.,* 1995). The crop does well on poor soils which are low in nutrients; however the application of phosphorus results in better nitrogen fixation, increase in stover and kernel yield (Ellah and Singh, 2008). It grows well on well-drained soils, but sandy loams with a pH of 5.0 to 6.5 are most suitable (Swanevelder, 1998). The seed makes a complete food as it contains sufficient protein, carbohydrate, fat and micronutrients (Poulter and Caygill, 1980). Nutritional composition undertaken by several researchers revealed that on average the seeds contain 63 % carbohydrates, 19% protein, and 6.5 % oil (Ijarotimi and Esho, 2009). The protein is of high quality having a good balance of the essential amino acids and a relatively high lysine (6.8%) and methionine (1.3%) content (Ellah and Singh, 2008). The seeds are consumed in a variety of ways, as fresh pods or boiled with salt and pepper, or eaten as a snack or mixed with maize seeds or with maize flour as a relish. The gross energy is higher than cowpea, lentils and pigeon pea (Poulter, 1980). The high nutritional value of bambara groundnut provides a cheap source of protein to poorly-resourced farmers in semi-arid areas (Amarteifio *et al.,* 2006) making it a good supplement to a cereal-based diet. According to the Food and Agriculture Organisation of the United Nations: FAOSTAT (2009) most of bambara groundnut production takes place in West African countries with Burkina Faso, Mali, Cameroon and Democratic Republic of Congo producing 44712, 25165, 24000 and 1000 metric tonnes respectively.

Figure 2: Bambara groundnut (*Vigna subterranea* (L.) Verdc.). (a) A botanical sketch (Maesen and Somaatmadja, 1989), (b) freshly harvested plant.

2.5 Morphological characteristic of bambara groundnut

Bambara groundnut is a herbaceous, intermediate, annual plant and believed to be mainly self-pollinating (Heller *et al.*, 1997). The morphological structure of the crop largely matches that of the groundnut (*Arachis hypogaea*), in that the pale yellow flower stalk bends downwards after fertilization bearing its pods below the ground (Uguru and Ezeh, 1997). It has two main contrasting growth habits; the branched form and the bunched habit, with a reproductive cycle of usually 90 to 150 days, depending on environment and landraces (Goli, 1997; Berchie *et al.*, 2010). The tap root is well developed with many profuse geotropic lateral roots of around 20 cm long on the lower part (Akpalu, 2010). Nodules formed on the roots fix atmospheric nitrogen through symbiosis with *Rhizobium* bacteria, which makes them useful for crop rotation and intercropping (Linnemann and Azam-Ali, 1993; Karikari *et al.*, 1999). Bambara groundnut is believed to be autogamous and floral reproduction starts 30 to 35 days after sowing and may continue until the end of the plant's life (Swanevelder, 1998; Directorate Plant Production, 2009). Flowers are normally carried in pairs on short peduncles by a pedicle which arises from the axis formed by the petioles and the stem (Doku, 1968). Flowers produced on the same peduncle do not open synchronously, although they will open within a 24 hours interval. Delayed flower opening may be caused by low temperatures and cloudy skies (Massawe *et al.*, 2003). It has been reported that fertilization in bambara groundnut takes place on the same day as anthesis (Linnemann and Craufurd, 1994). After fertilization, the flower stem elongates initiating the physiological processes leading to the formation of pods (Heller

et al., 1995). The sepal enlarges and the fruit develops above or just below the soil surface. Pod development lasts up to 30 days after fertilization and the seed develops over a further 10 days (Swanevelder, 1998). The pod is small, round or slightly oval shaped and wrinkled. Generally a single seed is produced in the pod, although two seeds per pod have been reported (Pasquet and Fotso, 1997). Seeds are mature when the parenchymatous layers surrounding the embryo have disappeared and the pods become light brown (Toungos *et al.*, 2009). The seeds are round, smooth and very hard when dried, with highly variable testa colors, including cream, brown, red and blotched (Stephens, 2003).

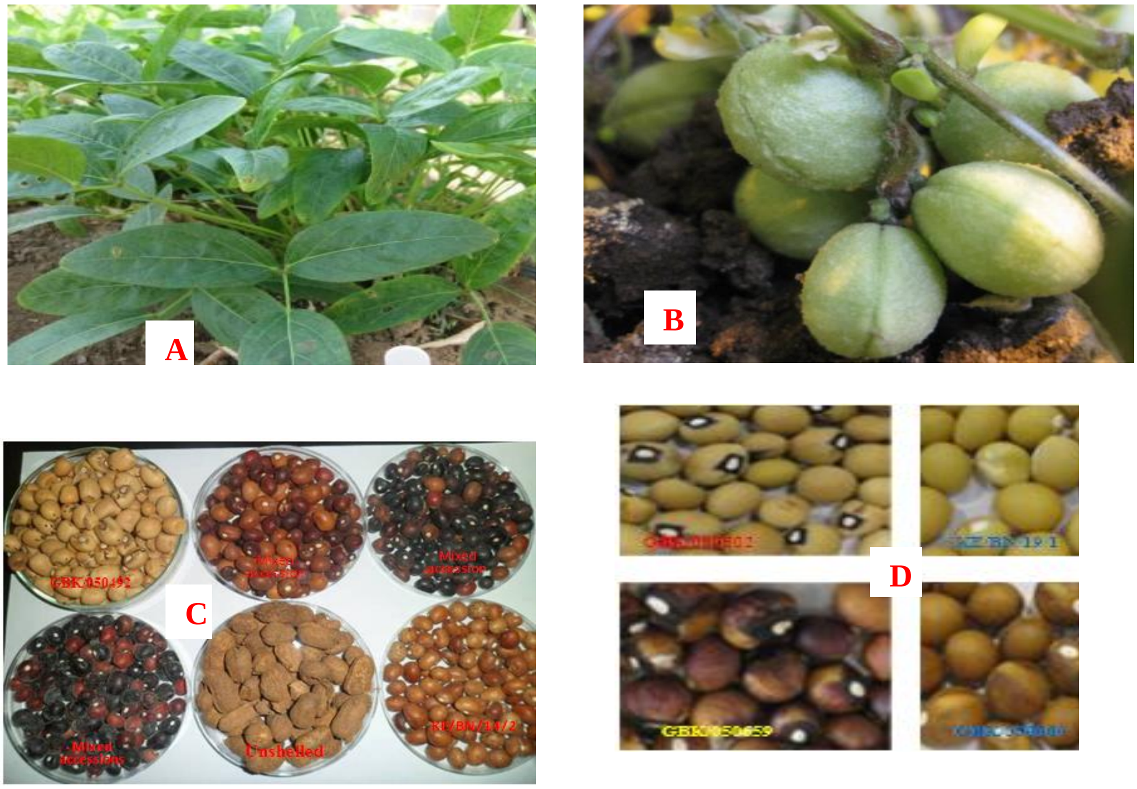

Plate 1: Bambara groundnut; (A) plant, (B) flowers and pods (C, D) seeds with different colours of testa.

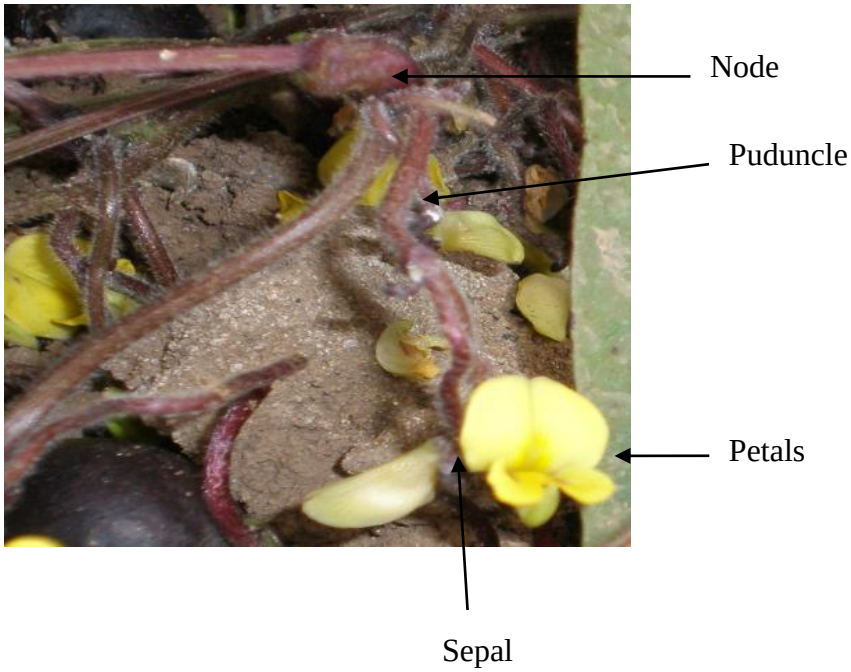

Figure 3: An illustration of the morphology of floral structure of bambara groundnut flower

2.6 Production potential of bambara groundnut

Most African countries rely on rainfed agriculture, but such agriculture is particularly vulnerable to climate change. In addition, there are usually other concerns such as poverty, soil degradation and recurring drought (Mendelsohn, 2000). In most countries in sub-Saharan Africa that are prone to drought, unreliable rainfall, poor soils and poor crop productivity, the production of more drought tolerant, indigenous crops, such as bambara groundnut are encouraged. There is evidence that the crop is more resilient to adverse environmental conditions as it tolerates low soil fertility soils and low rainfall.

Bambara groundnut landraces have been shown to tolerate drought as they can sustain leaf turgor pressure by employing a combination of osmotic adjustment, leaf area reduction and effective stomatal regulation of water loss (Collinson *et al.*, 1997). Some changes in the leaf orientation, which assist the crop to reduce incident radiation on the leaf surface, are reported in drought tolerant landraces such as DipC from Botswana and DodR from Tanzania, reducing water loss through transpiration (Collinson *et al.*, 1999). The crop is endowed with the advantages of being relatively resistant to pests and diseases, and has substantial morphological diversity, with good adaptation to marginal areas and poor conditions (Azam-Ali *et al.*, 2001). It also contributes to the soil fertility through biological nitrogen fixation making it beneficial in crop rotations and intercropping (Karikari *et al*, 1995), hence farmers do not normally apply chemical fertilizers to bambara groundnut (Mkandawire, 2007).

2.6.1 Yield potential of bambara groundnut

In a controlled environment, the crop is more adaptive than groundnut since it forms pods even where groundnut fails which is a clear indication of the crop potential (Azam-Ali *et al,* 2001). Bambara groundnut landraces produced as much as 4 tonnes per hectare (Collinson *et al.,* 1999). In the field in Swaziland, Sesay *et al.,* (2008) seed yield of 2.6 tonnes per hectare was obtained while in Cote d' Ivoire (Kouassi and Zoro, 2009) seed yield as high as 4 tonnes per hectare was obtained. If these landraces are developed further to produce cultivars and varieties they could possibly produce even greater yields. The fresh seed of bambara groundnut often have a high market price, with demand outweighing supply in many areas (Coudert, 1984). In Kenya, the price ranges from Ksh 450 to 600 per kilograme of shelled nuts.

2.6.2 Genetic diversity resources

There are substantial amount of genetic resources held by the International Institute for tropical Agriculture (IITA) approximately 2000 seed accessions are held and further 972 accessions are held in the various gene banks in the Southern Africa Development Community (SADC) countries (Massawe *et al.,* 2005). Despite these abundant genetic resources, at the moment there is no Consultative Group on International Agricultural Research Institution (CGIAR) that has a mandate undertake bambara groundnut research (Mayes *et al.,* 2009). IITA lists its legume crops as cowpea and soybean (http://www.iita.org) while International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) considered their legume crops as chickpea, pigeonpea and groundnut (http://www.icrisat.org). The genetic potential of bambara groundnut is not yet fully exploited, however, with the introduction of biotechnology, novel techniques such as molecular markers will assist researchers to better understand the genetics of bambara groundnut.

2.6.3 Potential areas of expansion for global Bambara groundnut production

Bambara groundnut has wide adaptability, since it is able to grow in ecological zones of varying climates, ranging from areas with annual rainfall as low as 300 mm annually in Botswana to high annual rainfall of 1250 mm in Swaziland (Azam-Ali *et al.,* 2001). Using Geographic Information Systems (GIS) technology, Azam-Ali *et al.,* (2001) identified some areas in America, Australia, Asia, as well as in Africa, where bambara groundnut could produce significant pod yields, and some areas in the Mediterranean where it is predicted to have the potential of producing yields as high as 8.5t h^{-1}

2.7 Importance of bambara groundnut

The legume is a rich source of protein and along with other local sources of protein could help to alleviate nutritional problems in areas where staple foods are predominantly carbohydrate sources (Massawe *et al.*, 2005; Okpuzor *et al.*, 2010). This legume is a useful ingredient for different bevarages, infants and children milk food (Eltayeb *et al.*, 2011). Bambara groundnut seed makes a 'complete food', as on average the seed contains sufficient protein (19%), carbohydrate (63%) and fat (6.5%) for a nutritionally balanced diet (Ijarotimi and Esho, 2009). Mineral content was also estimated for 100g seed, giving; iron 59 mg, potassium 1240 mg, phosphorus 296 mg, sodium 3.7mg and calcium 78 mg (Amarteifio and Moholo, 1998). In addition it has high protein quality with a good balance of essential amino acids, compared to most of other grain legumes, with relatively high lysine (6.8%) and methionine (1.3%) (Ellah and Singh, 2008; Okpuzor *et al.*, 2010) which are often only available at low levels in legumes. In a cream testa bambara groundnut a methionine content of 2.84% (of total crude protein) was reported (Olaleke *et al.*, 2006). Some anecdotal medicinal uses of bambara groundnut seed and leaves mixed with other crops in North Eastern Nigeria have also been noted (Atiku, 2000; Directorate plant production, 2011). Symbiosis of bambara groundnut with *Rhizobium* bacteria to fix atmospheric N_2 enhances the value of this crop for crop rotation and intercropping, as it contributes to the supply of soil nitrogen for other crops (Karikari *et al.*, 1999). Additionally, naturally-occurring NO^{3-} ion tolerant symbioses in bambara groundnut have been identified. These compare well to tolerance of artificial nitrate in other legumes, where there is a strong inhibitory effect on symbiosis. This potentially allows Nitrogen fertilization in intercropping systems without inhibiting N_2 fixation in the associated legumes (Dakora, 1998).

2.8 Morphological diversity of bambara groundnut

The morphological method is the oldest and considered the first step in description and classification of germplasm (Hedrick, 2005). However, morphological estimations are more dependent on environment and are more subjective than other measurements (Li *et al.*, 2009). Morphological variability depends on a limited number of genes, and may not access much of the potential variability for the agronomic traits present in a crop (Mayes *et al.*, 2009). The use of morphological and agronomic traits is a standard way of assessing genetic variation for many species, especially under-researched crops such as bambara groundnut (Azam-Ali *et al.*, 2001). Substantial morphological diversity was revealed in the characterized and evaluated approximately 1400 bambara groundnut accessions at the International Institute of Tropical

Agriculture (IITA) in Nigeria based on 38 characters, which included both quantitative and qualitative traits which they recommended to be confirmed using molecular markers (Goli *et al.,* 1995), Ntundu *et al.,* (2006) identified some vegetative traits that had prominent loadings in principal components analysis, and these are useful in distinguishing bambara groundnut landraces. Similar traits, like seed weight, internode length, petiole length, leaflet length, leaflet width, were identified as important traits in distinguishing between wild and domesticated bambara groundnuts when analysed with isozyme markers (Pasquet *et al.,* 1999). In addition, morphological characters which can be highly correlated to grain yield give breeders the choice to make decisions as to which traits to select for in bambara groundnut landraces (Karikari, 2000). Several numerical taxonomic techniques have been successfully employed to classify and measure the patterns of genetic diversity in the germplasm collection by other researchers working on crops such as black gram (*Vigna mungo*) and Mungbean (*Vigna radiata*) (Ghafoor *et al.,* 2001) and wheat (*Triticum aestivum*) (Bechere *et al.,* 1996). The comparison of phenotypic and genotypic variation within and between several other crops has been examined to provide accurate taxonomic and genetic differentiation in cowpeas (*Vigna unguiculata*) (Omiogui *et al.,* 2006) and sorghum (*Sorghum bicolor*) (Can and Yoshida, 1999). Agronomic and morphological traits have been used to identify traits contributing to important traits such as yield in crops like bambara groundnut (Makanda *et al.,* 2009) and soybean (Malik *et al.,* 2007).

2.9 Genetic diversity of bambara groundnut

Various methods are available for use in estimating the genetic diversity of crops, such as morphological, biochemical and molecular markers. Measurements of genetic diversity can be generated using conserved accessions in gene banks (Parzies *et al.,* 2000). DNA-based molecular markers have several advantages over the conventional phenotypic markers since their presence is not dependent on the growth stage of the crop and can be found in all tissues (Mondini *et al.,* 2009). Breeding a new variety with conventional methods takes many years especially when there are effects of trait pleiotropism and or when there is a multifactorial basis to morphological traits. Hence breeders are interested to try new techniques to make this process more efficient. Developments in molecular marker technology offers such a possibility by adopting a wide range of novel approaches which have altered the way plant breeding is being undertaken, allowing the breeder to use them potentially in estimating the genetic diversity and the level of heterozygosity among plants and animals (Dani *et al.,* 2008; Kumar *et al.,* 2008) as a first step to determining the best parents and best strategies for breeding. DNA

markers are not usually affected by the age, physiological condition of the cell or environmental factors and are generally held to have no pleiotropic or epistatic effects (Mondini *et al.*, 2009).

Extensive use of molecular markers derived from different technical approaches allows the segregation patterns of different alleles to be scored easily and construction of genetic maps from them. Construction of linkage maps is one of the main uses of DNA markers in research on crop species (Collard *et al.*, 2005). Such genetic maps serve several purposes, including detecting association between the genes and traits studied in QTL analysis, with the aim to use the markers to tag those traits, allowing the application of marker assisted selection of these target traits in subsequent breeding programs (Semagn *et al.*, 2006). DNA-based markers have been established in many agricultural crops and the availability of reliable molecular markers is of great importance for plant breeding as molecular markers linked to desirable traits have been used to accelerate plant breeding programs (Ribaut and Hoisington, 1998). The ideal molecular marker technique should generate many markers that cover the entire genome in a single, simple and reliable experiment (Luikart *et al.*, 2003). DNA markers are divided based on the method of their detection into three classes, hybridization-based; polymerase chain reaction (PCR) based and DNA sequence-based (Gupta *et al.*, 1999; Joshi *et al.*, 1999).

Some of commonly used markers are; Restriction Fragment Length Polymorphism (RFLPs), Amplified Fragment Length Polymorphism (AFLPs), Random Amplification of Polymorphic DNA (RAPDs), Variable Number Tandem Repeat (VNTRs), Simple Sequence Repeat, (SSR), Single Nucleotide Polymorphism (SNPs), Short Tandem Repeat (STRs), Single Feature Polymorphism (SFP) and Diversity Arrays Technology (DArT). No single technique fulfills all research needs and it is difficult to predict the emergence of new standard techniques (Semagn *et al.*, 2006). Different aspects of cost-effectiveness, accuracy, sensitivity and reproducibility in addition to the availability of markers specific to an organism and their limitations should be taken into account to determine the best suitable technology for a specific genotyping purpose and approach.

2.9.1 Microsatellites or Simple Sequence Repeats (SSR)

Microsatellites, or simple sequence repeats (SSR), are nucleotides sequence motifs flanked by sequences and are present in most eukaryotes genomes (McCouch *et al.*, 1997). They arise due to slippage-like events occurring randomly in stretches of repetitive sequence (Tautz, 1989). This makes microsatellite a more powerful genetic maker and because of their high reproducibility and co-dominance they are the marker of choice (Gupta and Varshney, 2000; Reusch, 2001). Microsatellites are mostly useful in comparative and association studies,

genetic diversity, marker-assisted selection, population, evolutionary studies (Nunome *et al.*, 2006; Shi *et al.*, 2011) and QTL analysis (Oyoo *et al.*, 2010). Because of their high variability they are especially good at distinguishing closely related individuals (Kumar *et al.*, 2009). A number of microsatellites are now available for a wide range of crops, such as groundnut (*Arachis hypogaea*) (He *et al.*, 2003; Cuc *et al.*, 2008), pigeonpea (*Cajanus cajan*) (Odeny *et al.*, 2007; Saxena *et al.*, 2010), bambara groundnut (Basu *et al.*, 2007), chickpea (*Cicer arietinum*) (Sethy *et al.*, 2003) and common bean (*Phaseolus vulgaris*) (Blair *et al.*, 2011). The major problem with microsatellites is that they need to be isolated *de novo* from each species (Zane *et al.*, 2002). In addition, there is poor transferability of markers developed for one taxon to another (Ellis and Burke, 2007). They are commonly used as molecular markers. SSRs are highly mutable loci which could be present at various sites in a genome (Tautz, 1989). The application of next-generation sequencing (NGS) technology (Illumina and 454 sequencing) for genome sequencing led to the discovery of a large number of genome-wide and gene based microsatellites in plant much more efficiently (Wang *et al.*, 2012; Zalapa *et al.*, 2012).

2.9.2 Single Nucleotide Polymorphism (SNP)

SNPs are a marker system that can differentiate individuals based on variation detected at the level of a single nucleotide base in the genome and such variation represents all sequence differences between individuals (Kumar *et al.*, 2009). Although SNPs can be used as a powerful and high throughput automated marker system in different applications of linkage disequilibrium and QTL analysis of plant species, they are only amenable in major crops which have been already sequenced (Park *et al.*, 2009). SNP can now be developed in coding sequence through Next Generation Sequencing approaches at reasonable cost, but they are more common in non-coding regions of the genome as coding sequences are often under selective constraints (Mondini *et al.*, 2009). On an average, one SNP every 170 bp was identified comparing the sequences from two different rice cultivars, which makes this marker system an attractive tool in plant genomes in constructing linkage maps, QTL analysis and marker assistant selection (Gupta *et al.*, 2001; Rafalski, 2002). Generally, the frequency of SNPs in plant species is estimated to range from 1 in 30 bp to 1 in 500 bp (Park *et al.*, 2009). It is anticipated that SNP markers will play an increasingly important role in the genetics and breeding of wheat (Chao *et al.*, 2009). SNPs are known to contain the highest level of molecular markers in the genome.

2.10 A comparison of morphological and DNA markers

Polymorphism is defined by McDonald (2004) as the presence of two or more variants of the DNA at a given locus and is often applied to variants of an expressed gene. The ease of detection of DNA polymorphisms depends on both frequency and form of sequence variation (Nakitandwe *et al*., 2007). Molecular markers are plenty, independent of tissue or environmental effects, and allow cultivar identification in the early stages of plant development (Manifesto *et al*., 2001). Microsatellite markers for example have been used successfully to determine the degree of relatedness among individuals or groups of accessions, and to clarify the genetic structure, or partitioning of variation among individuals, accessions, population and species of rice (Gupta, 1999). According to Rajendrakumar *et al* (2007), molecular markers can be used to accurately detect contaminants in cytoplasmic male sterile (CMS) seed stocks of rice. However, the development of reliable molecular markers is very important especially where there is a narrow genetic background of close relatives and inbreds (Nakitandwe *et al*., 2007). Morphological markers have high dependency on environmental factors such as the conditions that a plant is grown which influences the expression of these markers and often lead to false determination (Akhtar *et al*., 2010). These markers are time consuming, labour intensive and require large populations of plants and plots of land to be grown in while used in breeding experiments (Stuber *et al.*, 1999). DNA-Markers on the other hand are phenotypically neutral and literally unlimited in number and allow scanning of the whole genome and assigning landmarks in high density on every chromosome (Bhat *et al*., 2010; Shiwa *et al*., 2011).

2.13 References

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CHAPTER THREE

Genetic diversity of bambara groundnut (*Vigna subterranea* (L.) verdc.) landraces in Kenya using microsatellite markers

3.1 Abstract

Bambara groundnut (*Vigna subterranea* (L.) verdc.) is one of the important but underutilized legumes in the African continent. The existence of genetic diversity in germplasm collections is crucial for cultivar development. The objective of this study was to characterize bambara groundnut landraces from Kenya using SSR markers. Genetic relationships among 105 Bambara groundnuts (*Vigna subterranea* (L) Verdc.) accessions from Kenya were analysed using twelve microsatellite markers. The bambara landraces were collected from farmers in the Western region and the Genetic Resources Research Institute (GeRRI) of Kenya. Twenty four alleles were revealed with a mean of 2 alleles per locus. The polymorphic information content and gene diversity values averaged 0.28 and 0.35, respectively indicating low genetic diversity among the evaluated Bambara groundnut germplasm. Genetic distance based on Jaccard's similarity coefficient from the SSR marker analysis ranged from 0.08 to 1.16 among the landraces. Cluster analysis distinctly grouped the 105 accessions into three major clusters. The Analysis of Molecular Variance (AMOVA) revealed that 98% of the total genetic variation was within accessions whereas variation among accessions accounted for 2% of the total genetic variation. The genetic diversity observed in this study provides the basis for selection of appropriate parental genotypes for breeding programmes and mapping populations to further broaden the genetic base of Bambara groundnut germplasm in Kenya.

Keywords: Genetic analysis, *Vigna subterranean*, PIC, germplasm, gene diversity, cluster analysis

3.2 Introduction

Evaluation of available genetic diversity is a pre-requisite for genetic improvement in crop plants, especially in underutilized like Bambara groundnut (Olukolu *et al.*, 2012). Investigation of genetic diversity in both wild and domesticated species is equally important. Wild populations of different crop species are known to be a potential source of useful genes and traits which could be introduced into the domesticated gene pool (Cattan-Toupance *et al.*, 1998). Crop failures and dispersal of germplasm within the centre of origin or limited introduction or isolated locations could lead to reduced genetic diversity in particular breeding populations (Trethowan and Mujeeb-Kazi, 2008). The genus *Vigna* (Family *Leguminosae*) is an important legume taxon. It comprises about 90 described species of which seven species are cultivated as economic crops in various regions. Several species are cultivated as minor crops and some wildly grown species are harvested for food and feed. Bambara groundnut is the third most important food legume of Africa after peanut and cowpea. The crop is a very important source of dietary protein for poor people who cannot afford expensive animal protein (Baryeh, 2001). Thus it has high potential for food security in unpredictable drought regions.

Average yield of Bambara groundnut is rather low compared with other cultivated *Vigna* crops. This is due mainly to the fact that all of Bambara groundnut cultivars grown are landraces. No improved cultivars were developed by a selective breeding program because an efficient hybridization technique has just been developed (Suwanprasert *et al*., 2006). Before setting up a breeding program for Bambara groundnut, a thorough understanding on its genetic diversity is necessary. Like many other orphan crops, there are only a few studies on genetic diversity in a large set of Bambara groundnut germplasm. Diversity studies based on seed patterns in 1,384 and 1,973 accessions found that Bambara groundnut from Cameroon/Nigeria region had a higher diversity than those from the other geographical regions Goli *et al*. (1997) and Olukolu *et al*. (2012). Diversity study in 124 accessions using 28 quantitative traits and in 40 accessions using 554 Diversity Arrays Technique (DArT) markers revealed the highest diversity in Cameroon/Nigeria region (Olukolu *et al.*, 2012). The results supported the view of Hepper (1963) that center of origin/domestication of Bambara groundnut is in the Cameroon/Nigeria region. In contrast, Somta *et al*. (2011) studied diversity in a collection of 240 Bambara groundnut accessions using 22 simple sequence repeat (SSR) markers found highest diversity in West African (excluding Cameroon and Nigeria) (Rungnoi *et al*., 2012). These studies suggest that the center of diversity and origin of Bambara groundnuts is still inconclusive and more evidence is needed to elucidate them.

In this work, genetic diversity was conducted in 105 Bambara groundnut collections. Accessions from several geographical origins in Kenya were analysed using simple sequence repeat (SSR) markers. The objective was of the study was to determine genetic diversity among different Bambara groundnut accessions in Kenya.

3.3 Materials and Methods

3.3.1 Plant materials and DNA isolation

A total of 105 Bambara groundnuts accessions (Table 2) from Busia (0.4347° N, 34.2422° E) (44), the National Genebank of Kenya (32), Kakamega (0.2837° N, 34.7515° E) (21), Bungoma (0.8479° N, 34.7020° E) (6) and Vihiga (0.0816° N, 34.7229° E) (2) were planted in pots of 12cm in diameter in the greenhouse at the Kenya Agricultural and Livestock Research Organization (KALRO) Njoro, Kenya. The pots were filled with a ration of 4:1 soil to manure. Young leaf sample (2 weeks old) from four plants per accession were collected for genomic DNA isolation and analysis using a modified CTAB protocol described by Doyle and Doyle (1990). The modifications involved omission of Ammonium acetate stage and longer hours (12 hours) for DNA precipitation. DNA Quantification was carried out by 0.8% agarose gel and Nanodrop 200c spectrophotometer (Thermo scientific corp.) and was diluted to 10ng μl^{-1} for PCR.

Table 2: Names, sources and seed coat colour of one hundred and five Bambara groundnut landraces used for the phenotypic study

Accession	County	Seed coat colour	Accession	County	Seed coat colour
KE/BN/1/1	Busia	Black	KE/BN/23/2	Kakamega	Dark Red
KE/BN/1/2	Busia	Dark Red	KE/BN/23/3	Kakamega	Light Red
KE/BN/2/1	Kakamega	Cream entire	KE/BN/24	Vihiga	Dark Red
KE/BN/2/2	Kakamega	Cream spotted	KE/BN/25/1	Kakamega	Black
KE/BN/3/1	Bungoma	Red	KE/BN/25/2	Kakamega	Light Red
KE/BN/4/1	Kakamega	Black	KE/BN/26/1	Busia	Light Red
KE/BN/4/2	Kakamega	Brown	KE/BN/26/2	Busia	Dark Red
KE/BN/4/3	Kakamega	Light Red	KE/BN/27	Busia	Dark Red
KE/BN/5/1	Busia	Cream entire	KE/BN/28	Busia	Brown red black spotted
KE/BN/5/2	Busia	Cream one side spotting	KE/BN/29	Busia	Light Red
KE/BN/8/1	Busia	Lght Red	KE/BN/30/1	Busia	Brown
KE/BN/8/2	Bungoma	Dark Red	KE/BN/30/2	Busia	Red
KE/BN/9	Kakamega	Dark Red	KE/BN/31/1	Busia	Black
KE/BN/10	Vihiga	Brown	KE/BN/31/2	Busia	Dark Red
KE/BN/12/1	Kakamega	Black	KE/BN/32/1	Busia	Black
KE/BN/12/2	Kakamega	Light Red	KE/BN/32/2	Busia	Light Red
KE/BN/12/3	Kakamega	Brown spotted	KE/BN/34/1	Busia	Brown
KE/BN/13/1	Busia	Black	KE/BN/35/1	Busia	Black
KE/BN/13/2	Busia	Light Red	KE/BN/35/2	Busia	Dark Red
KE/BN/13/3	Busia	Dark Red	KE/BN/36	Busia	Cream Red spotted
KE/BN/13/4	Busia	Brown	KE/BN/37/1	Bungoma	Light Red
KE/BN/13/5	Busia	Brown Black spotted	KE/BN/37/2	Bungoma	Dark Red
KE/BN/14/1	Kakamega	Brown entire	KE/BN/38/2	Busia	Light Red
KE/BN/14/2	Kakamega	Brown spotted	KE/BN/39/1	Kakamega	Cream
KE/BN/15/1	Kakamega	Black	GBK/050490	Genebank	Cream entire white eye

Table 2:cont

KE/BN/16/1	Busia	Dark Red	GBK/050491	Genebank	Cream entire white eye
KE/BN/16/2	Busia	Light Red	GBK/050492	Genebank	Cream spotted white eye
KE/BN/16/3	Busia	Black	GBK/050493	Genebank	Red brown white eye
KE/BN/17/1	Kakamega	Dark Red	GBK/050494	Genebank	Red Brown spotted white eye
KE/BN/17/2	Kakamega	Light Red	GBK/050495	Genebank	Orange brown white eye
KE/BN/18/1	Kakamega	Black	GBK/050496	Genebank	Orange brown white eye
KE/BN/19/1	Busia	Cream entire	GBK/050499	Genebank	Brown white eye
KE/BN/19/2	Busia	Cream spotted	GBK/050501	Genebank	Cream white white eye
KE/BN/20/2	Busia	Light Red	GBK/050502	Genebank	Cream white white eye
KE/BN/21/1	Bungoma	Black	GBK/050649	Genebank	Black white eye
KE/BN/21/2	Bungoma	Light Red	GBK/050650	Genebank	Black white eye/red brown white eyes
KE/BN/22/2	Busia	Dark Red spotted	GBK/050653	Genebank	Dark brown white eye
KE/BN/22/3	Busia	Light Red	GBK/050654	Genebank	Red brown white eye
KE/BN/23/1	Kakamega	Brown	GBK/050655	Genebank	Black white eye
GBK/050656	Genebank	Black white eye	GBK/050671	Genebank	Black white eye
GBK/050657	Genebank	Light red white eye	GBK/050672	Genebank	Black white eye
GBK/050658	Genebank	Black white eye	GBK/050673	Genebank	Black white eye
GBK/050659	Genebank	Black brown white eye	KE/BN/40	Busia	Black white eye
GBK/050660	Genebank	Cream spotted white eye	KE/BN/41	Busia	Black white eye
GBK/050661	Genebank	Black white eye/ Brown spotted white eye	KE/BN/42	Busia	Black white eye
GBK/050663	Genebank	Light red white eye	KE/BN/43	Busia	Black white eye
GBK/050664	Genebank	Black white eye	KE/BN/44	Busia	Black white eye
GBK/050665	Genebank	Black white eye/light red white eye	KE/BN/45	Busia	Black white eye

Table 2:cont

GBK/050666	Genebank	Black white eye/light red white eye	KE/BN/46	Busia	Black white eye
GBK/050667	Genebank	Light red white eye	KE/BN/47	Busia	Black white eye
GBK/050668	Genebank	Brown white eye/black white eye	KE/BN/48	Busia	Black white eye
GBK/050669	Genebank	Light red white eye	KE/BN/49	Busia	Black white eye
GBK/050670	Genebank	Black white eye			

3.3.2 Microsatellite marker analysis

Twelve microsatellite primers (Mosiwa, 2012) (Table 3) were used to assess the genetic diversity of the 105 Bambara groundnuts accessions. The PCR amplification was performed in a 10µl volume mix consisting of 5U Dreamtaq polymerase enzyme (Thermo scientific corp, Lithuania), x6 Dreamtaq buffer (Thermo scientific corp, Lithuania), 2.5mM of each dNTPS (Bioneer corp, Republic of Korea), $MgCl_2$, 5µM of each primer (Inqaba biotec, S.A) and 30ng DNA template in an Applied Biosystems 2720 thermocycler (Life Technologies Holdings Pte Ltd, Singapore). The PCR thermocycler regime consisted of initial denaturation at 94 °C for 5 minutes followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 54-59.7 °C (depending on the primer) extension at 72 °C for 1 minute followed by one cycle of final extension at 72 °C for 10 minutes. The amplicons were mixed with 6x Orange DNA loading dye (Thermo scientific corp, Lithuania) and separated on a 2% agarose gels (Duchefa, Netherlands) stained with ethidium bromide at a concentration of 0.5 µg/mL (Invitrogen corp, U.S.A) in a 0.5x Tris Boric ETDA (TBE) buffer. The separated amplicons were visualized on an Ebox-VX5 gel visualization system (Vilber Lourmat inc, France). The alleles were scored as absent or present based on the size of the amplified product using a 100bp O'geneRuler ready to use DNA Ladder (Thermo Scientific Corp, Lithuania).

3.4 Data analyses

Molecular data evaluated was in binary fashion for SSR marker loci analysed and scoring was based on presence (1) or absence (0) of band for each primer set. The summary statistics on major allele frequency, allele number, gene diversity, PIC values (Botstein *et al.,* 1980) were calculated using Power Marker version 3.25 (Liu and Muse, 2006) based on the following formular:

$$Dsa = \frac{1}{m} \sum_{j=1}^{m} \sum_{i=1}^{aj} \min(pij, qij) \qquad 3.1$$

Where, pij and qij are the frequencies of the i^{th} allele at the j^{th} locus, m is the number of loci examined, aj is the number of alleles at the j^{th} locus. Shannon's information index (*I;* Lewontin, 1972) of each locus was calculated using software popGene32 version 1.32 (Yeh *et al*., 2000). Analysis of molecular variance (AMOVA) was performed using Arlequin v.3.1 (Excoffier *et al*., 2005). Genetic dissimilarities between all the accessions was calculated using DARwin version 5.0 (Perrier and Jacquemoud-Collet, 2006; Perrier *et al*., 2003) using simple matching coefficient based on the following formula:

$$GSij = \frac{2Nij}{(Ni+Nj)} \quad 3.0$$

Where *GSij*- Observation of fragments shared by accessions *i* and *j*, *Nij*- the number of fragments shared by accessions *i* and *j*, *Ni*- amplified fragments in sample *i* and *Nj*- amplified fragments in sample *j* (Nei and Li, 1979)

The dissimilarity coefficients were then used to generate an unweighted neighbour-joining tree (Saitou and Nei, 1987) with Jaccard's Similarity Coefficientwith a bootstrapping value of 1,000 using the same software (DARwin 5.0).

Table 3: Primer information for twelve SSR markers used for amplification of genomic DNA isolated from 105 accessions of Bambara groundnut germplasm.

Marker	**Sequence(5´-3´)**	**Product size (bp)**	**Annealing Temperature (^{0}c)**
PRIMER 1F	AGGCAAAAACGTTTCAGTTC		55.3
PRIMER 1R	TTCATGAAGGTTGAGTTTGTCA	273	55.3
PRIMER 2F	AGGAGCAGAAGCTGAAGCAG		55.3
PRIMER 2R	CCAATGCTTTTGAACCAACA	212	55.3
PRIMER 3F	TTCACCTGAACCCCTTAACC		57.6
PRIMER 3R	AGGCTTCACTCACGGGTATG	247	57.6
PRIMER 4F	ACGCTTCTTCCCTCATCAGA		57.6
PRIMER 4R	TATGAATCCAGTGCGTGTGA	197	57.6
PRIMER 5F	TCAGTGCTTCAACCATCAGC		55.3
PRIMER 5R	GACCAAACCATTGCCAAACT	260	55.3
PRIMER 6F	CCGGAACAGAAAACAACAAC		57.6
PRIMER 6R	CGTCGATGACAAAGAGCTTG	189	57.6
PRIMER 7F	TGTGGGCGAAAATACACAAA		59.7
PRIMER 7R	TCGTCGAATACCTGACTCATTG	198	59.7
PRIMER 8F	CAAACTCCACTCCACAAGCA		57.6
PRIMER 8R	CCAACGACTTGTAAGCCTCA	250	57.6
G358B2-D15F	TGACGGAGGCTTAATAGATTTTTC		59.0
G358B2-D15R	GACTAGACACTTCAACAGCCAATG	193	59.0
mBam2co80F	GAGTCCAATAACTGCTCCCGTTTG		59.0
mBam2co80R	ACGGCAAGCCCTAACTCTTCATTT	220	59.0
G180B2-D11F	GAGGAAATAACCAAACAAACC		59.0
G180B2-D11R	CTTACGCTCATTTTAACCAGACCT	198	59.0
G358B3-D15F	TGACGGAGGCTTAATAGATTTTTC		59.0
G358B3-D15R	GACTAGACACTTCAACAGCCAATG	196	59.0

3.5 Results

3.5.1 DNA quantification and analysis

The genomic DNA extracted was of good quality, with absorption ratio at 260/280nm wave length being in the range of 1.8 to 2.0, with very few samples falling below or above that range (Table 6). DNA of high quality should have A260/280 of 1.8 and above (Sambrook *et al*., 1989). Approximately 88.9% and 96.8% of the samples assessed for quality and quantity using Nanodrop were in the range of 1.8 to 2.0 in terms of ratio of absorption at 260/280nm and over 50ng in terms of DNA quantity.

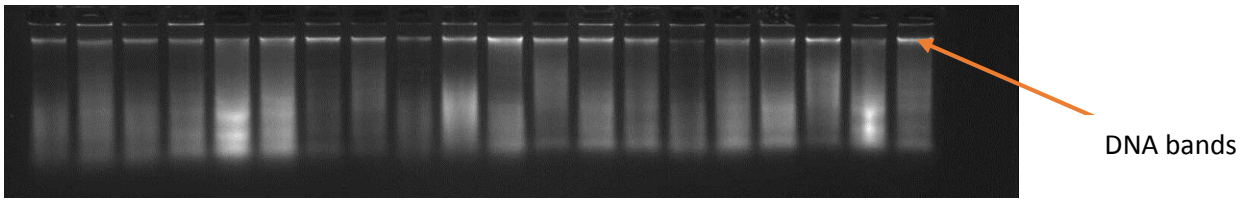

Plate 2: Representative samples of genomic DNA observed using 0.8% Agarose Gel

3.5.2 Marker polymorphism, diversity within accessions and genetic distance among acceessions

SSR analysis in the 105 Bambara groundnut accessions (Table 4) revealed that number of reproducible DNA bands per primer ranged from 70 (Primer 1) to 97 (mBam2co80) totalling to 958 with an average of 79.83 bands.

Table 4: Estimate of genetic diversity of Bambara groundnut germplasm collections using 12 SSR markers.

Locus	na*	ne*	h*	I*	Major allele frequency	PIC	No. of amplified bands
Primer 1	2.0000	1.8000	0.4444	0.6365	0.67	0.35	70
Primer 2	2.0000	1.2771	0.2170	0.3744	0.88	0.19	92
Primer 3	2.0000	1.8202	0.4506	0.6429	0.66	0.35	69
Primer 4	2.0000	1.4953	0.3312	0.5133	0.79	0.28	83
Primer 5	2.0000	1.8396	0.4564	0.6489	0.65	0.35	68
Primer 6	2.0000	1.5448	0.3527	0.5375	0.77	0.29	81
Primer 7	2.0000	1.9489	0.4869	0.6800	0.58	0.37	61
Primer 8	2.0000	1.5448	0.3527	0.5375	0.77	0.29	81
G358B2-D15	2.0000	1.5201	0.3421	0.5257	0.78	0.28	82
mBam2co80	2.0000	1.1638	0.1408	0.2694	0.92	0.13	97
G180B2-D11	2.0000	1.4213	0.2964	0.4728	0.82	0.25	86
G358B3-D15	2.0000	1.3725	0.2714	0.4428	0.84	0.23	88
Mean	**2.0000**	**1.5624**	**0.3452**	**0.5235**	**0.76**	**0.28**	**79.83**

na***** = Observed number of alleles, ne***** = Effective number of alleles, h*****= Nei's (1973) gene diversity and I***** = Shannon's Information index [Lewontin (1972)].

Polymorphic information content (PIC) ranged from 0.13 to 0.35, (marker 10 and 5, respectively) with an average of 0.28 (Table 4). Accessions from Kakamega, Bungoma and Vihiga counties had the highest gene diversity (H_E = 0.5) and Shannon's diversity index (I =0.6931), followed by those from the National Genebank of Kenya (H_E = 0.49, I =0.6928) and Busia (H_E = 0.47, I =0.6663). The National Genebank of Kenya accessions had the lowest H_E *and* I with 0.1023 and 0.2103, respectively followed by accessions from Busia county with 0.1420 and 0.2712. Kakamega and Bungoma counties accessions both had genetic diversity of 0.2778 and Shannon's diversity index of 0.4506.

Analysis of molecular variance (AMOVA) revealed that the highest proportion of the total variation (98%) was among individuals within accessions (Table 6). The variation due to accessions were 2%. Genetic distance based on Jaccard's similarity coefficient from the SSR marker analysis ranged from 0.08 to 1.17 among the landraces. Accessions from Bungoma county had the least genetic distance (0.41) indicating close genetic relationship while greatest genetic distance was observed in accessions from Busia county (1.11) indicating distance genetic relatedness. Accessions from the Resource Research Institute of Kenya (1.03) also had a high genetic distance.

Table 5: Concentration and quality of genomic DNA from Bambara groundnut quantified using ND 1000 Spectrophotometer

Genotype	ngµl^{-1}	260/280	Genotype	ng µl^{-1}	260/280	Genotype	ngµl^{-1}	260/280	Genotype	ngµl^{-1}	260/280
1	297.25	1.99	24	83.64	1.89	56	315.33	1.95	81	290.10	1.95
2	194.86	1.99	26	206.94	1.97	57	316.92	1.95	82	303.44	1.96
3	205.38	2.00	27	130.23	2.01	58	180.79	1.93	83	353.10	1.71
4	179.20	2.02	28	279.33	1.98	59	86.03	1.93	84	49.68	1.9
5	134.75	2.04	35	259.02	1.96	60	268.76	1.93	85	165.08	1.96
6	96.69	2.01	36	163.47	1.96	61	223.43	1.99	86	189.37	1.93
7	209.13	1.99	37	23.76	1.83	62	113.01	1.91	87	263.92	1.95
8	156.44	1.99	38	52.11	2.01	63	82.18	1.88	89	275.87	1.94
9	108.53	2.01	39	56.33	2.02	65	60.57	1.92	90	247.14	1.94
10	63.77	2.08	41	278.18	1.95	66	291.18	1.95	91	341.60	1.94
11	98.36	2.02	42	54.91	1.96	67	215.10	1.95	92	243.06	1.97
12	105.79	2.10	43	350.08	1.95	68	64.35	1.99	93	262.81	1.96
13	292.87	1.98	44	324.63	1.97	69	59.49	1.91	94	313.52	1.95
14	237.83	1.99	45	154.90	1.98	70	239.14	1.92	95	260.59	1.94
15	324.22	1.99	46	151.04	1.98	71	169.66	1.89	96	337.56	1.95
16	370.28	1.96	47	109.01	1.94	72	40.76	1.99	97	82.63	1.98
17	165.84	2.03	48	98.63	1.95	73	81.42	1.95	98	131.96	2.01
18	245.95	1.99	49	66.72	1.95	74	72.62	1.95	99	44.65	1.99
19	259.56	1.97	50	315.83	1.96	75	205.72	1.93	100	74.97	1.97
20	62.56	1.92	51	45.78	1.96	76	237.89	1.94	101	38.21	2.06
21	307.46	1.98	52	70.14	1.89	77	96.34	1.96	102	34.16	1.96
22	152.46	1.97	53	32.41	1.95	78	271.11	1.95	103	42.76	1.99
23	275.87	1.96	54	169.24	1.98	79	284.81	1.94	104	55.48	1.98
24	211.14	1.99	55	174.57	1.97	80	196.11	1.97	105	96.00	1.92

DNA Concentration in Nanogram per microlitre and Absorption ratio at 260/280 wave length of light

Table 6: Analysis of molecular variance (AMOVA) for 105 bambara groundnut genotypes.

Source of variation	Df	SS	MS	Variance components	variation
Among Accessions	4	15.275	3.819	0.065	2%
Within Accessions	100	263.772	2.638	2.638	98%
Total	104	279.048		2.703	100%

3.5.3 UPGMA, Principal coordinate analyses

Clear pattern of germplasm clusters based on their places of origin was not observed in this study (Fig. 5). In most cases, accessions from different regions or counties were clustered with one another. However, it demonstrated that accessions from Busia county and the National Genebank of Kenya tended to agglomerate together in cluster III. All the 105 individual genotypes were grouped into three (I, II, III) main clusters (Fig. 5). Except for cluster I all the remaining clusters had sub- clusters. There was a general trend as those accessions from the National Genebank of Kenya and Busia county tended to group together in cluster III while those from Kakamega county tended to cluster together in cluster I. Accessions from Vihiga and Bungoma counties were foundin clusters I and III. Two clusters with the highest number of genotypes were cluster II and III with 27 and 58 individual genotypes respectively. Grouping of the genotypes of these landraces into sub- clusters indicated substantial level of intra-landrace polymorphism. Similarly high level of intra-landrace polymorphism can be said of the landraces in cluster II and III all of which had their individual genotypes grouped into more than two sub-cluster units. Cluster I had all the individual genotypes clustered into only one unit suggesting lesser level of intra-landrace polymorphism within the cluster compared to the rest of the landrace clusters.

Principal coordinate analysis (PCoA) (Fig. 6, 7 and 8) revealed that genetic relationship among Bambara groundnut accessions. They accounted for 84.3% of the total variations (Table 7) with each axes explaining 63.58%, 12.21% and 8.24% variation in that order. The first three axes accounted for the highest variation (96.81%) for Bungoma county accessions followed by accessions from Busia county (71.4%), Kakamega county (59.59%) and Genebank (59.31%). Principal component analysis failed to differentiate accessions according to their area of origin. Most of the accessions overlapped demonstrating close genetic relationships.

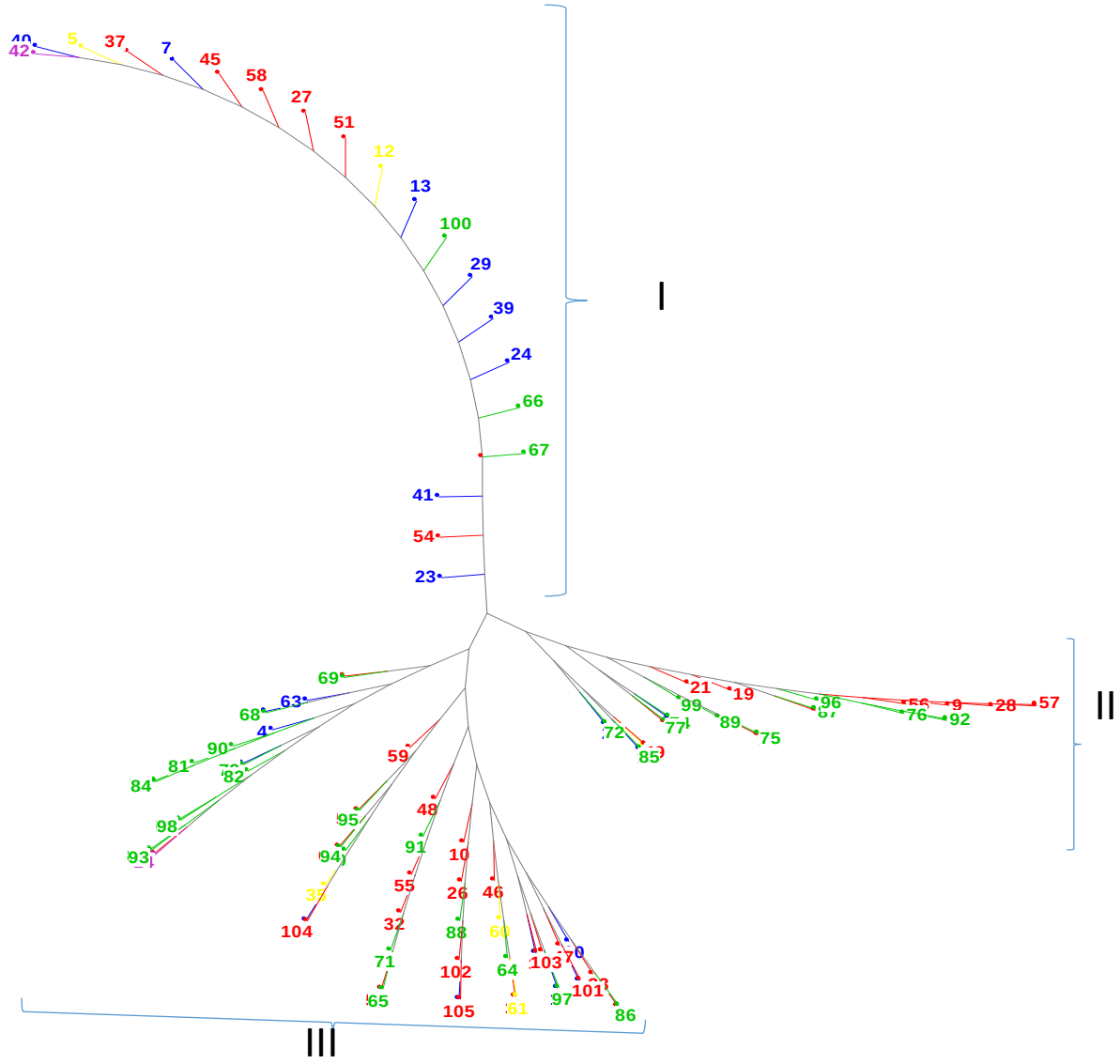

Figure 4: Genetic relationships generated by Jaccard's similarity coefficients among 105 Bambara groundnut accessions. Accessions given in red were from Busia, blue from Kakamega, purple from Vihiga, yellow from Bungoma and green from Genebank.

Table 7: Eigen value and percentage of total variation accounted for by the first three component axes.

Axis	Eigen value	Proportion (%)	Cumulative (%)
1	29.21	63.58	63.85
2	5.612	12.21	76.06
3	3.786	8.24	84.30

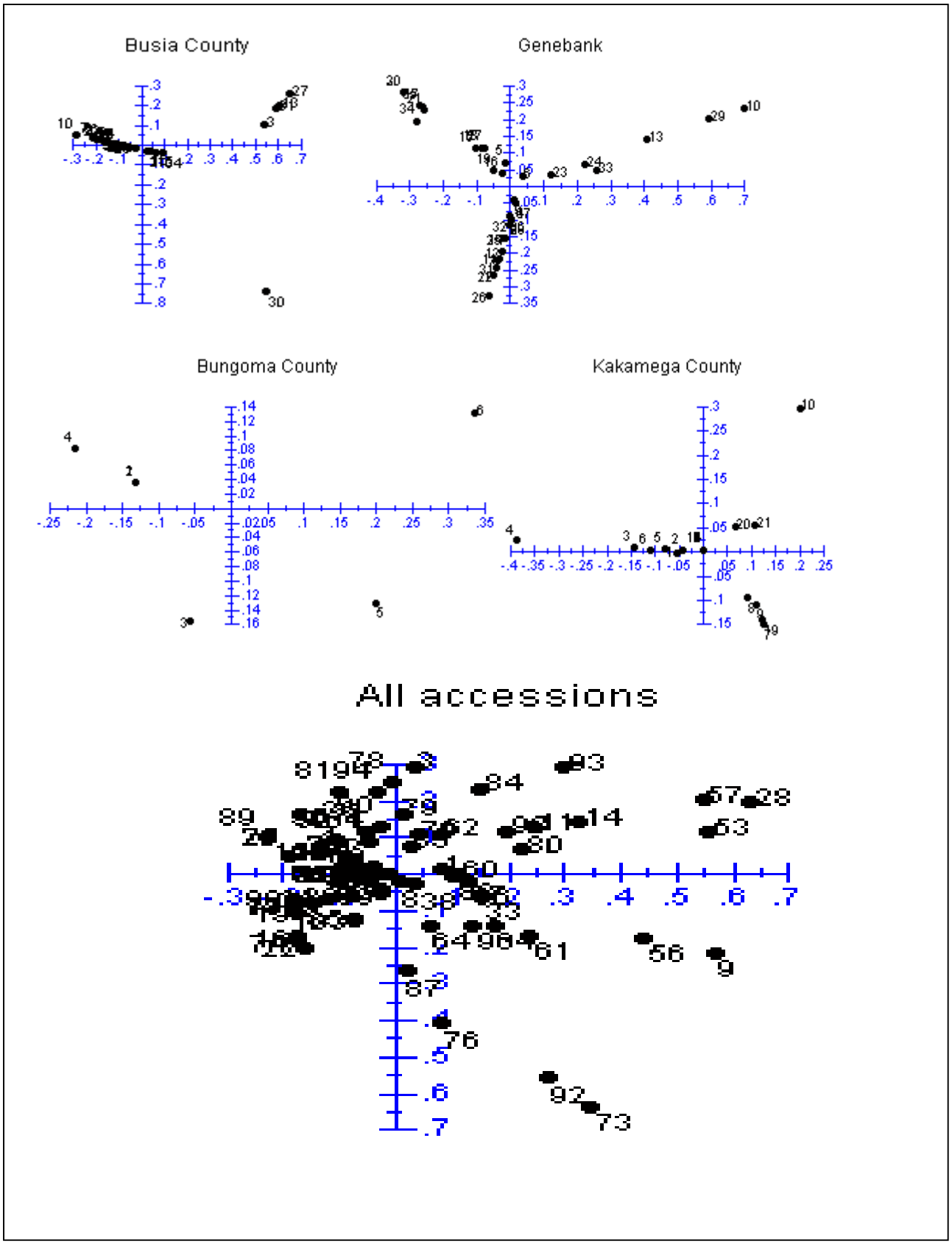

Figure 5: PCoA of axes 1 and 2 based on dissimilarity of 12 SSR markers across 105 Bambara groundnut landraces from different regions.

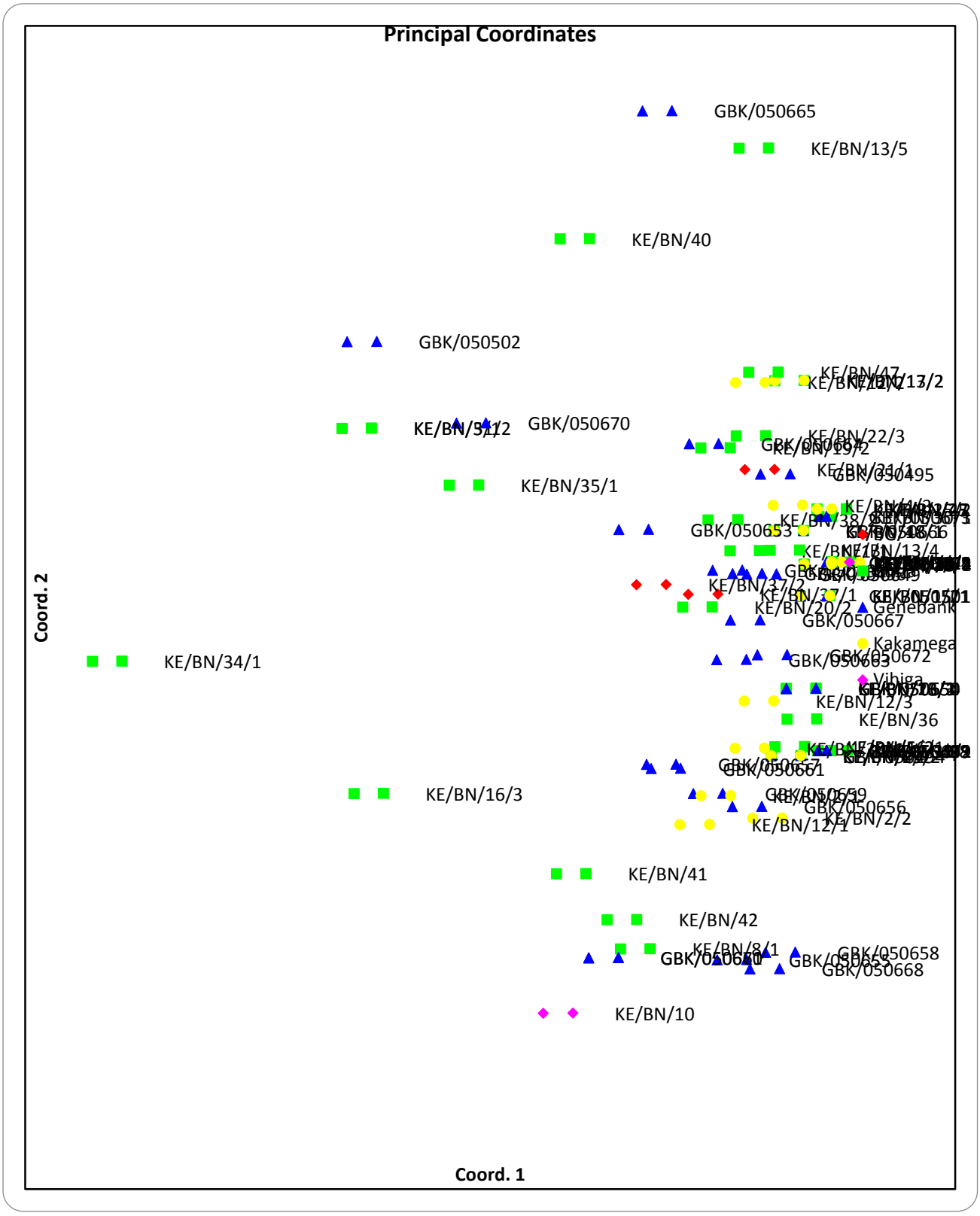

Figure 6: Configuration of bambara groundnut accessions under principal component axis 1 and 2. BG- Bungoma county

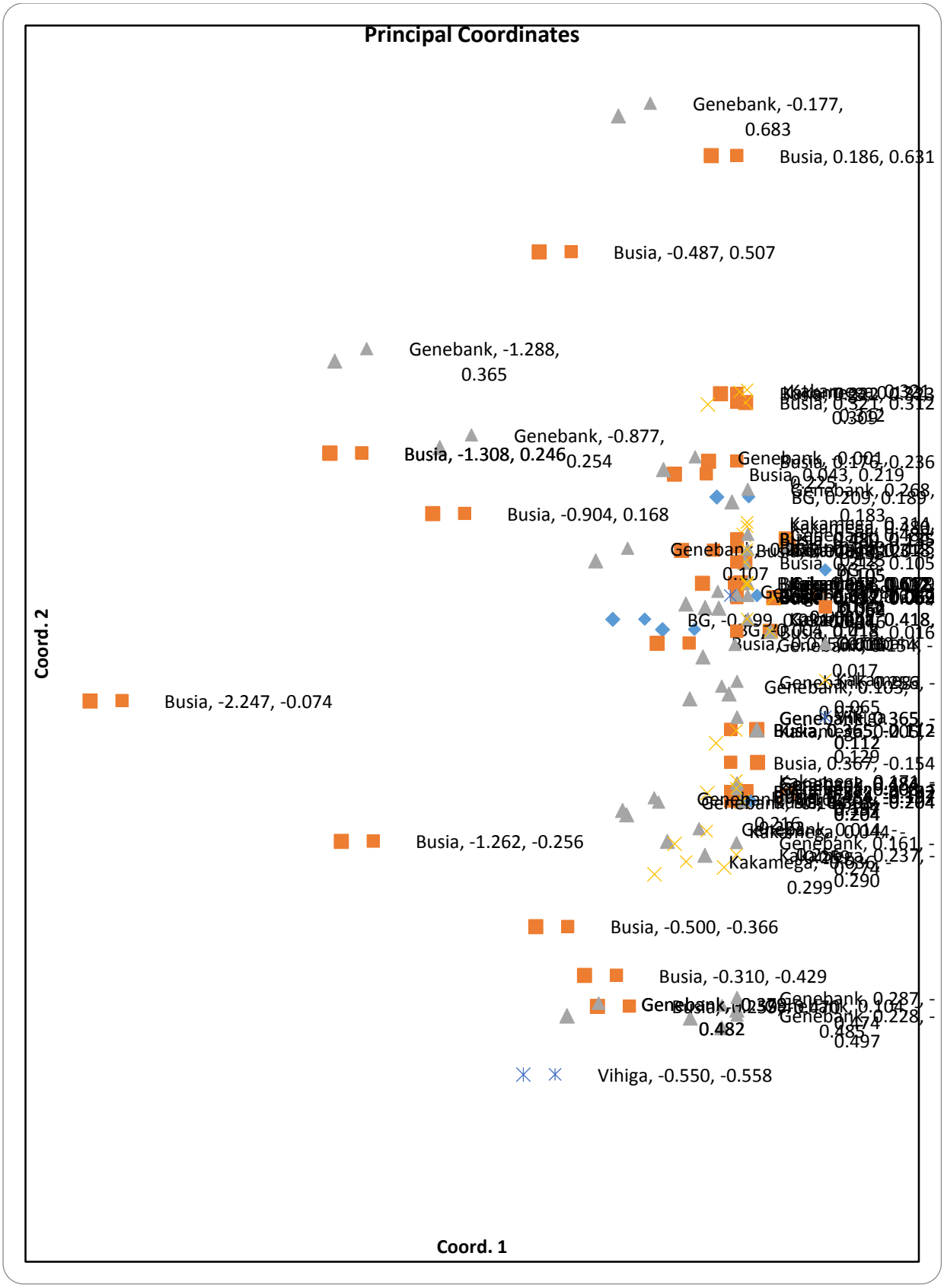

Figure 7: Distribution of bambara groundnut accessions by location under principal component axis 1 and 2. BG- Bungoma county.

3.6 Discussion

Genetic analysis of diversity is very critical as it gives more accurate measure of polymorphism compared to morphological characterizations.This is important in broadening genetic base. In the present study, extent and organization of genetic diversity within 105 accessions of bambara groundnut from Western Kenya and the Genetic Resources Research Institute of Kenya was assessed using 12 polymorphic SSR bands. The twelve SSR markers revealed the availability of polymorphism among the landraces of bambara groundnuts as evidenced in genetic distances and the cluster analysis (Fig. 5). Based on AFLP molecular marker analysis it was revealed that there was extensive genetic diversity between 12 African bambara groundnut landraces from diverse origin (Massawe *et al*., 2002). Amadou *et al*. (2001) also reported considerable genetic diversity among 25 African bambara groundnut accessions from International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria, using Random Amplified Polymorphic DNA (RAPD) markers. They demonstrated two main groups of accessions mainly along the lines of their origin. High genetic diversity among 240 bambara groundnut accessions from Africa and Southeast Asia using SSR markers as did (Somta *et al*., 2011; Olukolu *et al*., 2012; Aliyu *et al*., 2013). In contrast, based on isozyme analysis, Pasquet *et al*. (1999) observed that both wild and domesticated bambara groundnuts were characterized by low genetic diversity, indicating that wild bambara groundnut is the progenitor of the domesticated form. This is expected as isozymes are generally limited by the low levels of polymorphism detectable and may fail to discriminate cultivars differing only slightly in genetic make up.

In this work, genotypes were clustered into three clusters (I, II and III) with clusters II and III forming sub-clusters. There was substantial intra-landrace polymorphism as two of the three clusters had sub-clusters with distinct genotypes though from different regions. The high level of intra-landrace polymorphism could be attributed to seed exchange between farmers as well as the geographical proximity of the areas. Contrary to the high intra-polymorphism of most of the landraces, genotypes in cluster I appeared less heterogeneous. Accessions from Kakamega, Busia counties and the Genetic Resources Research Institute of Kenya tended to form a clear group (Cluster III). This was elucidated further by AMOVA, which partitioned the total genetic variation among and within accessions. This showed that the majority of genetic variation observed in the germplasm (98%) was due to the variation among individuals instead of being between specific accession groups. Divergent accessions may have good breeding value, which may be utilized for direct selection and as parents of crosses with

accessions from different clusters. The mixture of accessions in cluster I, II and III mainly from the counties of Busia, Kakamega and Genetic Resources Research Institute of Kenya indicated that bambara groundnut accessions in this group constituted a more heterogenic group, with variable genetic backgrounds. This can also be explained by the high frequency of bambara groundnut seed exchange by farmers over wide geographic-ethnic regions as well as the different informal names given to landraces from one region to another which may give room for genotype duplications as was suggested by Hudu and Saaka, 2011.

The low level of genetic diversity revealed in this work could be supported by the fact that small scale farmers in Eastern Africa generally tend to exchange seeds frequently. This arguemenet is supported by the studies of Ntundu, 2002, who conducted a survey on Bambara groundnut seeds pathway in Tanzania.

Principal Component Analysis (PCA) is a descriptive technique which reveals the pattern of character variation among genotype (Aremu *et al*., 2007). PCA failed to group accessions according to their areas of origin. This could have lead to a generally low coefficient of variation observed in bambara groundnut accessions, an indication of a high level of uniformity. This suggested that the source of these accessions could be same due to seed exchange among the farmers. From the PCoA plot of the accessions (Figure 7 and 8), principal axes 1 and 2 showed that KE/BN/34/1, KE/BN/13/5, KE/BN/16/3, KE/BN/40 from Busia county and GBK/050665 and GBK/050502 from the Genetic Resources Research Institute of Kenya were the most distinct from the other accessions studied.

3.7 Conclusion

This study shows that bambara groundnut landraces from Kenya, form a genetically diverse population and SSR markers can be effectively employed to assess genetic diversity and to measure the extent of genetic relationship among accessions. Knowledge of the degree of genetic relationships between bambara groundnut accessions will be of importance for crop improvement and may help to establish a core collection as part of the germplasm collection management to sample a maximum of genetic variation of accessions. This study revealed that bambara groundnut accessions from Western Kenya and the Genetic Resources Research Institute of Kenya constitute three major genetic clusters. The study revealed a low genetic variability among the accessions but a high genetic variability within them, thus a number of landraces could be identified which are relatively pure for use in the selection as pure lines in bambara groundnut breeding.

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CHAPTER FOUR

Morphological characterization of bambara groundnut (*Vigna subterranea* (L.) Verdc.) landraces in Kenya

4.1 Abstract

Bambara groundnut (*Vigna subterranean* (L) Verdc.) an indigenous crop of African origin is drought tolerant and is one of most important leguminous crop in Sub-Saharan Africa. It has comparable value to other legumes for food and nutritional security in Kenya. However, small-scale farmers continue cultivating unimproved landrace varieties over the production areas in Kenya. Bambara groundnut landraces exist as heterogeneous mixtures of seeds, which typically contain a few to several seed morphology types that may embrace wide genetic diversity The objective of this study was to characterize bambara groundnut landraces from Kenya using morphological markers. A field experiment was conducted at Kenya Agricultural and Livestock Research Organization (KALRO)-Alupe to assess the genetic diversity of bambara groundnut landraces based on morphological characters. Most of the landraces displayed pointed and round and yellowish pod colour, with grooved and oval seed shapes. Out of the 105 landraces accessed for leaf morphology, 49.4% had round leaves, while 21.5% had elliptical leaves, with 55.7% landraces that were heterogeneous and possessing more than one leaf shapes. The analyses of variance from the morphological characterization for all quantitative traits were statistically significant ($p \leq 0.05$) except for seed weight, seed number per plant and number of stems per plant. The first four principal components accounted for 33.28%, 18.39%, 13.32% and 8.17 %, respectively of the morphological variations among the landraces. The landraces were grouped into two distinctive clusters with the second cluster sub-divided into four sub-clusters. Qualitative traits however accounted for less of these variations. Leaf morphology could be a useful marker for strategic breeding and genetic conservation of Bambara groundnut. These results suggest that there exists qualitative and quantitative variation among Bambara groundnut accessions in Kenya, providing a baseline on morphological diversity information that can be utilized for further genetic crop improvement and core germplasm conservation.

Keywords: Morphological traits, bambara groundnut, breeding, landraces

4.2 Introduction

Knowledge on morphological variability of germplasm collections improves understanding of the relationship between the structural morphology of plants and their corresponding functional botany (Lauri and Normand, 2017). The morphological method is the oldest and considered the first step in description and classification of germplasm (Hedrick, 2005). However, morphological estimations are more dependent on environment and are more subjective than other measurements (Li *et al.,* 2009). Morphological variability depends on a limited number of genes, and may not access much of the potential variability for the agronomic traits present in a crop (Mayes *et al.,* 2009). The use of morphological and agronomic traits is a standard way of assessing genetic variation for many species, especially under-researched crops such as bambara groundnut (Azam-Ali *et al.,* 2001). Since bambara groundnut is an underutilised crop, studies of its genetic diversity are scarce. However, Goli *et al.,* (1995), characterized and evaluated approximately 1400 bambara groundnut accessions at the International Institute of Tropical Agriculture (IITA) in Nigeria based on 38 characters, which included both quantitative and qualitative traits. Substantial agro-morphological diversity was revealed, which they recommended to be confirmed using molecular markers. Ntundu *et al.,* (2006) identified some vegetative traits that had prominent loadings in principal components analysis, and these are useful in distinguishing bambara groundnut landraces. Similar traits, like seed weight, internode length, petiole length, leaflet length, leaflet width, were identified as important traits in distinguishing between wild and domesticated bambara groundnuts when analysed with isozyme markers (Pasquet *et al.,* 1999). In addition morphological characters which can be highly correlated to grain yield give breeders the choice to make decisions as to which traits to select for in bambara groundnut landraces (Karikari, 2000).

Morphological markers have been used for phenotypic diversity studies in a number of crops. Several numerical taxonomic techniques have been successfully employed to classify and measure the patterns of genetic diversity in the germplasm collection by other researchers working on crops such as black gram (*Vigna mungo*) and mungbean (*Vigna radiata*) (Ghafoor *et al.,* 2001), soybean (*Glycine max*) (Cater *et al.,* 2001) and wheat (*Triticum aestivum*) (Bechere *et al.,* 1996). The comparison of phenotypic and genotypic variation within and between several other crops has been examined to provide accurate taxonomic and genetic differentiation in *Musa* spp, (Crouch *et al.,* 2000), cowpeas (*Vigna unguiculata*) (Omiogui *et al.*, 2006) and sorghum (*Sorghum bicolor*) (Can and Yoshida, 1999). Agronomic and morphological characters have been used to identify traits contributing to important traits such as yield in crops like bambara groundnut (Makanda *et al.,* 2009) and soybean (Malik *et al.,* 2007). In a strategy to develop phenotypic similarity index (PS), Cui *et al*., 2001 conducted a study on morphological and agronomic traits to study the phenotypic diversity of Chinese and North American soybean and the results found more phenotypic diversity

among the Chinese cultivars, than the North American cultivars, they also found clear differences between the two groups. From the use of morphological markers they managed to come up with a strategic plan to broaden the North American germplasm by the introgression of Chinese cultivars, especially those from different clusters. A study on phenotypic diversity study which identified traits with higher loadings in principal component analysis (PCA) in a collection of Asian groundnut (*Arachis hypogaea*) (Swamy *et al*., 2003).

Morphological variability within bambara groundnut landraces have been reported (Ouedrago *et al*., 2008; Ntundu *et al*., 2006) which although useful is not an accurate measure of diversity as it is affected by the growth stage of the plant and environmental conditions. Moreover, reports on morphological evaluation of accessions from Kenya are scanty. Hudu and Saaka (2011) reported farmers evaluation of bambara groundnut landraces from the Upper East region of Ghana alongside some quantitative description of morphological features, however information on qualitative features were not captured in detail. Ntundu *et al*. (2006) also reported on morphological diversity of bambara groundnut form Tanzania of which quantitative traits contributed much of the diversity. Genetic diversity study is the foremost step in crop improvement and the efficient deployment of molecular markers to assess level of polymorphism is vital in order to harness the huge genetic pool of bambara groundnut landraces (Massawe *et al*., 2005). The present study analyses the level of diversity in a collection of 105 Kenyan bambara groundnut using morphological characterization.

4.3 Materials and methods

4.3.1 Plant material

A collection of 105 Bambara groundnut accessions were collected from varied agro ecological zones in Kenya. Accessions included in this study were 44 from Busia, 32 from the Genetic Resources Research Institute of Kenya, 21 from Kakamega, 6 from Bungoma and 2 from Vihiga (Chapter 3 Table 2).

4.3.2 Experimental site

The study was conducted at the Kenya Agricultural and Livestock Research Organization (KALRO) - Alupe approximately 12 km North East of Busia town (0.4347° N, 34.2422° E) and elevation of 1220m above sea-level and experiences mean annual temperature of 22.2^{o}C. In terms of agro-ecological zone, it belongs to Lower midland one (LM1) (Jaetzold, *et al*., 2007). The soil in the area is sandy clay loam to clay petroplinthite (murram) and strong acidic. Physical and chemical soil characteristics at the site are: texture sandy loam with Cation

exchange capacity (CEC) 14%, base saturation 39%, organic carbon 0.6%. Irrigation was not carried out as it relied on rainfed and all other environmental factors (temperature, humidity, light, CO^2 concentration) were not artificially controlled. All necessary cultural practices were carried out as and when required.

4.3.3 Experimental design

Seeds of one hundred and five landraces of bambara groundnut were planted in the field in a randomized complete block design (RCBD) with three replications for two seasons at a planting distance of 50 cm by 30 cm. Combined analysis of variance was conducted on all the agronomic traits considered using general linear model (SAS Institute NC, 2003). In the analysis genotypes were considered fixed. The statistical model used in the analysis is as follows:

$$Y_{ijkl} = \mu + E_i + R_j + G_k + GE_{ik} + \varepsilon_{ijkl} \quad 4.0$$

i = 1, 2. ; j = 1, 2,3. ; k= 1, 2, 3…….

Where; Y_{ijkl} –Observation , μ – Overall mean, G_k– Effect of the k^{th} Genotype, E_i– Effect of the i^{th} Environment, R_j– Effect of the j^{th} Replicates, GE_{ik}– Interaction between effects of the i^{th} Genotype on the $_k^{th}$ Environment and ε_{ijkl}– Random error component associated with each observation.

Means were separated using SNK-Test.Treatment means were assigned letters to indicate significant differences between them.

4.3.4 Data collection

Morphological data on the one hundred and five landraces of bambara groundnut were recorded for characterization purpose. Bambara groundnut descriptors note (IPGRI, 2000) was used as guideline for all data recordings. A total of nineteen quantitative traits and seven qualitative traits (Table 6) were evaluated. Data was recorded for individual plants at different growth stages and during harvesting as follows:

The quantitative field data included number of days to 50% seedling emergence (SDE) by counting number of days from planting to 50% seedling emergence. Plant height (PHT) was measured using measuring ruler and expressed in cm as the distance from the ground level to the longest terminal leaf of the plant. Canopy spread (CNS) was taken as the widest end of the plant, terminal leaf lenth (TLL), terminal leaf width (TLW) were measured as the distance from

the leaf tip to the point the leaf the leaf by the leaf blade ends on the leaf stalk and the widest ends across the leaf blade, respectively. Petiole length (PETL) was taken between the point of attachment to the stem and the leaf blade. These data were taken from 10 weeks after planting. Qualitative data included leaf colour (LCE) at emergence, terminal leaf shape (TLS), growth habit (GH), stem pigmentation (SPG), petiole colour (PCL), leaflet joint pigmentation (pigmentation at the point of attachment to the petiole), fresh pod colour (PC), Pod shape (POS), seed shape. The qualitative data were determined by visual observation at 8-10 weeks after planting.

Post harvest quantitative data were taken two months after harvest by which time all the seeds in the pod were dry. They include pod weight (PDW), seed weight (SDW), measured in grams (g) using measuring scale, while hundred (100) seed weight (SWT) was also measured in grams using measuring scale. Seed length (SDL), seed width (SDW) in micro metres were determined using Vernier calliper.

Table 8: Qualitative and quantitative traits observed in bambara groundnut diversity study.

Code	Qualitative traits	Phenotypic scale
POT	Pod texture	1= smooth, 2= little grooves, 3= much grooved, 4= much folded
POC	Pod colour	1= yellowish brown, 2= brown, 3= reddish brown, 4= purple, 5= black
POS	Pod shape	1= No point, 2= Ending in a point round one side, 3= pointed one side with a hook, 4= pointed each side
TLC	Terminal leaflet colour	1= green, 2= red, 3= purple
TLS	Terminal leaflet shape	1= round, 2= oval, 3= lanceolate, 4= elliptic
GTH	Growth habit	1= bunch type, 2= semi bunch type, 3= spreading type
SDS	Seed shape	1= round, 2= oval
	Quantitative traits	
PDL	Peduncle length	(mm)
NLP	Number of leaves per plant	(-)= Total number of produced by a plant
TLW	Terminal leaflet width	(mm)- Average width of four leaves at 4th internode
TLL	Terminal leaflet length	(mm)- Average length of four leaves at 4th internode
PTL	Petiole length	(mm)- Average length of 4 leaves petiole measured at 4th internode from the stem to the base of the leaf
PTS	Plant spread	(cm)- Widest point between the two opposite ends of the plant
PHT	Plant height	(cm)- Measured from the ground (base of plant) to the tip of the highest point (terminal leaflet inclusive)
INL	Internode length	(mm)- Average length of the 4th internode of four plants
NSP	Number of stems per plant	(-)- Total number of stems produced by a plant
POL	Pod length	(mm)
PDW	Pod width	(mm)
SDL	Seed length	(mm)
SDW	Seed width	(mm)
SWT	100-Seed weight	(g)
DTF	Number of days from sowing to 50% flowering	(d)- From date of sowing to the date of 50% flower emergence
DTM	Number of days from sowing to maturity	(d)- From date of sowing to the date of seedling emergence
NPP	Number of pods per plant	(-)

4.4 Data analyses

4.4.1 Multivariate analyses

Quantitative data analyses were performed using the generalized linear model analysis of variance in SAS statistical package software version 9.1. Quantitative features that showed statistical significant differences ($p \leq 0.05$) together with the qualitative features were subjected to principal component analysis using correlation matrix in DARwin statistical package version 5.0 (Perrier *et al*., 2003; Perrier and Jacquemoud-Collet, 2006). This is to account for the individual contribution of the various morphological traits (qualitative and quantitative) to the total amount ofvariation observed among the landraces. These qualitative and quantitative (statistically significant) data setswere then subjected to cluster analysis using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) with DARwin version 5 statistical parkage using simple matching coefficient. A dendrogram was constructed to cluster the genotypes into discrete groups and calculate the morphological distances between groups.

4.5 Results

4.5.1 Variability of qualitative traits

Frequency distribution of qualitative characters included in this study is summarized in Figure 8. Landraces showed wide range of differences on qualitative morphological features in growth habit pod shape and colour. Two leaflet shapes namely oval and round dominated with 83 % and 17%, respectively while terminal leaflet colour was green (85%) and purple (15%). Three classes of growth habit namely bunched, semi-bunched and spreading growth habits exist in bambara groundnut. Short stems and internodes characterize bunched growth type resulting in plants with tightly clustered leaves. Plants with spreading (open) growth habit type have stems with long internodes resulting in plants with a much larger diameter of the plant foliar crown. Plants of semi-bunched growth habit lie between the two extreme cases. In the germplasm under study the semi-bunched type was the most frequent (62%) followed by bunched types (30%) and only few (8%) accessions were classified as spreading type.

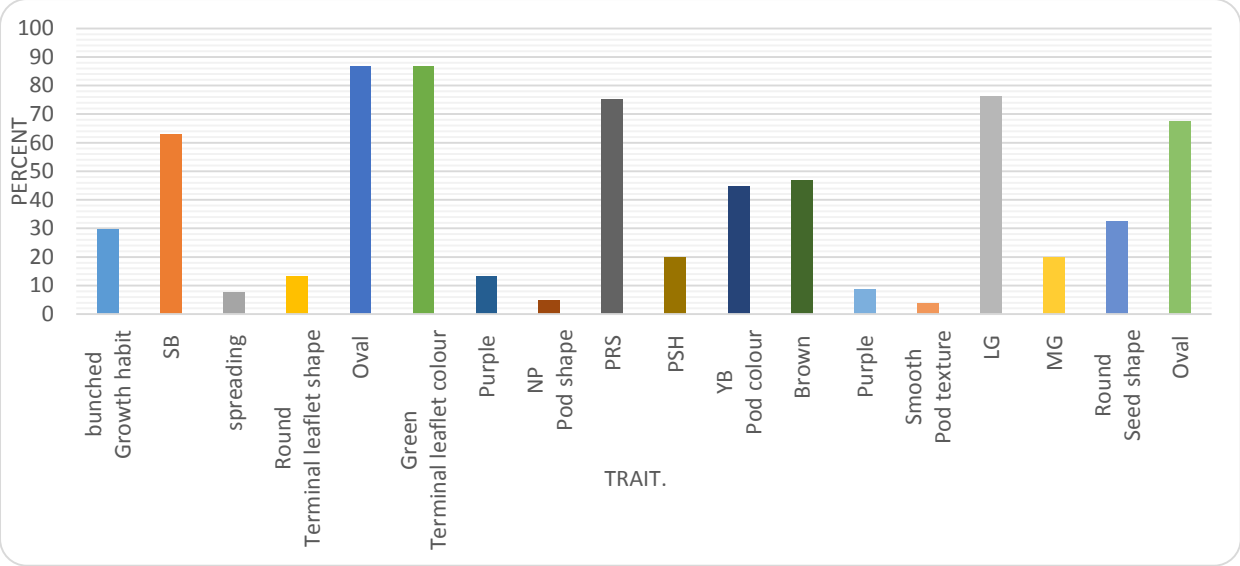

Fig. 7: Distribution of some qualitative morphological features among the Kenyan landraces of bambara groundnut

The pod shape and pod colour showed considerable variation among the accessions studied. A large number (77%) had a pod shape ending in a point at the upper side (dorsal) and round on the (ventral) other side, while 20% had a pod shape ending in a point with a hook on the other side. Only 3% of the accessions studied had a pod shape without a point. For pod colour, 44 and 46% of the accessions were yellowish-brown and brown coloured, respectively. About 10% of the accessions had pods with purple colour. Other qualitative characters, including terminal leaflet shape, terminal leaflet colour, pod texture and pod shape, displayed little variation. Two types of terminal leaflet shapes were observed in the germplasm studied namely, oval and round types. Accessions with oval leaflet shape were most common (87%), while few accessions (13%) had round terminal leaflet shape. Most accessions (88%) had green terminal leaflet colour while few accessions (12%) had purple terminal leaflet colour. Three types of pod texture were observed including smooth, little grooves and much grooved. Of the 105 accessions studied 78% had a pod texture with little grooves, 20% were much grooved and only 2% accession were smooth, indicating that this pod character was very rare in the germplasm. Among other qualitative characters observed, two seed shape types were noted, namely oval and round. Oval shaped seeds were more frequent (66%) seeds as compared to round shaped seeds, which constituted 34% of the total number of accessions studied. Grey seed colour were more predominant (61%) as compared to those of red seed coat colour (22%), black seed coat clour (15%) and spotted seed coat colour at (2%) (plate 4).

Plate 4: Testa colours of Bambara groundnuts showing variabilities

4.5.2 Analysis of quantitative morphological traits

The analyses of variance (ANOVA) for the nineteen quantitative morphological features among the one hundred and five landraces were all statistically significant at ($p \leq 0.05$) except for seed number per pod, number of stems per plant, 100-seed weight and number of days to maturity did not show significant difference (not statistically significant) among the accessions tested for the two seasons (Table 9). Number of seeds per pod for the accessions studied did not vary significantly for the accessions included herein. This is also supported by the narrow range and mean for number of seeds per pod for the materials included in this experiment (Table 10). This observation may be accounted for by the fact that genetically most of bambara groundnut pods on average contain two seeds (Goli *et al*., 1988; 1997). However, a few genotypes have three or even four seeded pods (Goli 1987).

Table 9.0: Mean of squares for quantitative morphological features of one hundred and five landraces of bambara groundnut evaluated over two seasons

Source of Variation	df	DAE	PDL	SWT	NLP	TLW	TLL	PTL	PTS	PHT
Replication	2									
Season	1	4.8**	1.37	3.21	54.91**	0.02	0.02	0.27	0.29	0.34
Genotype	104	23.27**	39.30**	509.12	873.08**	1.11*	2.91**	19.80*	271.45**	32.71*
Genotype*Season	104	0.66**	0.33	1.34	2.18	0.01	0.02**	0.02	0.072	0.04
Error		0.047	0.33	1.21	1.99	0.01	0.01	0.02	2.35	1.10
CV		2.15	15.82	26.73	22.97	13.18	9.69	16.32	17.40	13.56

Source of Variation	df	POL	PDW	SDL	SDW	SNP	DTM	INL	NSP
Season	1	0.57	0.85	0.16	0.00	0.01	1.63	0.55	120.91*
Genotype	104	52.33**	10.23**	9.62**	14.77**	0.55	154.07	17.93*	8.49*
G*S	104	1.73	0.21	0.06	0.002	0.01	0.75	0.07	1.4
Error		1.45	0.20	0.06	0.02	0.01	0.73	0.07	3.95
CV		9.63	5.67	14.19	13.82	4.21	1.50	14.97	19.49

**, * Significant at P≤0.001 and 0.05 respectively.

Other quantitative morphological traits including number of stems per plant, 100-seed weight, and days to maturity indicated no significant morphological variation among the accessions, and exhibited narrow ranges (Table 11). The reason that could be advanced here is that probably there is limited variation exhibited among the materials studied for these traits in question. The high coefficients of variation observed for seed weight among accessions studied could be due to heterogeneity existing among bambara groundnut landraces (Table 3). Local landraces of bambara groundnut are reported to be so heterogeneous that more than ten different genotypes can be found within a single accession (Madamba 1997; Ntundu *at al*., 2006).The ranges and means standard errors estimates for the 19 quantitative characters studied are presented in Table (11). In spite of the variations in the magnitudes of the ranges, means of the accessions generally displayed considered difference for most of the traits evaluated. Gene bank accessions, followed by Kakamega accessions, tended to be comprised of bigger plants reflected in larger plant spread, plant height, internode length, terminal leaflet length and petiole length, while Vihiga and Bungoma accessions were generally smaller plants (Table 11). Gene bank accessions were characterized by low 100-seed weight, pod length and pod width.

Across the locations, the quantitative traits such as days to emergence, peduncle length, number of leaves per plant, petiole length, internode length, number of stems per plant and pod width showed significantly differences across the seasons (Table 10 a, b). This observation could be due to other factors such as different environmental conditions, pest infestation and diseases. The rest of the traits did not show significant differences across the seasons.

Table 10.0: Mean separation of quantitative morphological features of Kenyan bambara groundnut landraces characterized in the present study

Season	DAE	PDL	NLP	TLW	TLL	PTL	PTS	PHT	INL	NSP
1	10.13^{a}	11.88^{b}	71.98^{a}	2.54^{a}	6.44^{a}	11.17^{b}	32.40^{a}	22.68^{a}	13.59^{a}	7.64^{a}
2	9.95^{b}	11.98^{a}	71.39^{b}	2.55^{a}	6.45^{a}	11.21^{a}	32.37^{a}	22.59^{a}	13.53^{b}	6.76^{b}

Season	POL	PDW	SDL	SDW	NPP	SNP	SWT	DTF	DTM
1	21.42^{a}	12.01^{b}	11.19^{a}	8.81^{a}	16.11^{a}	1.89^{a}	42.60^{a}	46.32^{a}	119.66^{a}
2	21.36^{a}	12.09^{a}	11.22^{a}	8.81^{a}	16.12^{a}	1.90^{a}	42.61^{a}	46.31^{a}	119.56^{a}

*Means followed by the same letters are not significantly different according to SNK at p≤0.05

Table 11: Mean values of quantitative traits for Bambara groundnut accessions

Descriptor	Bungoma	Busia	Gene bank	Kakamega	Vihiga
Days to emergence	10.36 ± 0.45	9.92 ± 0.13	10.55 ± 0.14	9.47 ± 0.13	9.58 ± 0.31
Peduncle length	10.39 ± 0.34	11.97 ± 0.17	12.15 ± 0.15	12.00 ± 0.27	11.46 ± 0.91
Number of leaves per plant	74.33 ± 1.67	71.82 ± 0.73	67.05 ± 0.82	77.48 ± 1.02	73.92 ± 3.36
Terminal leaflet width	2.45 ± 0.06	2.63 ± 0.03	2.43 ± 0.02	2.55 ± 0.05	2.53 ± 0.08
Terminal leaflet length	6.25 ± 0.08	6.36 ± 0.05	6.62 ± 0.05	6.51 ± 0.06	6.29 ± 0.06
Petiole length	10.22 ± 0.28	11.12 ± 0.13	11.82 ± 0.10	11.57 ± 0.16	11.06 ± 0.37
Plant spread	31.00 ± 0.99	31.53 ± 0.42	36.39 ± 0.44	31.78 ± 0.37	30.20 ± 1.81
Plant height	21.19 ± 0.27	22.84 ± 0.14	23.50 ± 0.14	23.02 ± 0.25	22.70 ± 0.60
Internode length	12.20 ± 0.12	13.30 ± 0.10	14.20 ± 0.12	13.95 ± 0.18	13.16 ± 0.30
Number of stems per plant	7.03 ± 0.25	7.15 ± 0.11	6.85 ± 0.15	7.86 ± 0.15	7.58 ± 0.48
Pod length	21.64 ± 0.71	22.08 ± 0.19	20.54 ± 0.17	20.92 ± 0.30	24.17 ± 1.20
Pod width	11.79 ± 0.25	12.10 ± 0.09	11.38 ± 0.09	11.47 ± 0.11	12.52 ± 0.23
Seed length	11.58 ± 0.16	11.15 ± 0.08	11.25 ± 0.08	11.06 ± 0.13	12.00 ± 0.30
Seed width	8.97 ± 0.20	8.68 ± 0.09	9.28 ± 0.12	8.43 ± 0.13	8.00 ± 0.00
Seed number per pod	2.00 ± 0.00	1.81 ± 0.02	1.94 ± 0.02	1.94 ± 0.02	2.00 ± 0.00
100-Seed weight	55.08 ± 5.74	44.65 ± 1.83	20.77 ± 0.24	44.45 ± 2.17	79.95 ± 5.62
No.of days to 50% flowering	51.42 ± 1.53	46.59 ±0.40	45.75 ± 0.42	45.65 ± 0.45	41.17 ± 0.54
Number of days to maturity	123.92 ± 0.63	123.04 ±0.31	117.45±0.37	121.02 ±0.37	117.08 ±1.08
Number of pods per plant	13.83 ± 0.94	13.23 ± 0.51	11.66 ± 0.53	12.81 ± 0.41	16.50 ± 0.75

†† The values are the means ± SE of the three replicates. Figures within a column whose SE values do not overlap are statistically different at (p≤ 0.05

4.5.3 Principal component analysis

Principal coordinate analysis (PCoA) (Figures 9 and 10) was performed to reveal morphological relationship among bambara groundnut accessions. The four principal components (PCs) accounted for 73.16% of the total variations (Table 12) with each axes explaining33.28, 18.39, 13.32 and 8.17% of the morphological variations among the landracesin that order.The first four traits with the highest loadings for both PC1 and PC2 are all quantitative morphological features an observation which implies that qualitative features accounted for less of the variation among the landraces.Principal component analysis failed to differentiate accessions according to their area of origin with most of the accessions overlapped demonstrating close morphological relationships. This suggests that these accessions could have originated from the same source but given different local names. From the PCoA plot of the accessions (Figure 9), principal axes 1 and 2 showed that KE/BN/2/2 from Kakamega, KE/BN/10 (14) from Vihiga, KE/BN/8/1 (11), KE/BN/16/3 (28), KE/BN/30/2 (51) and KE/BN/48 (105) from Busia county, GBK/050491 (65) from the National Genebank of Kenya and KE/BN/3/1 (5) from Bungoma were the most distinct from the other accessions.

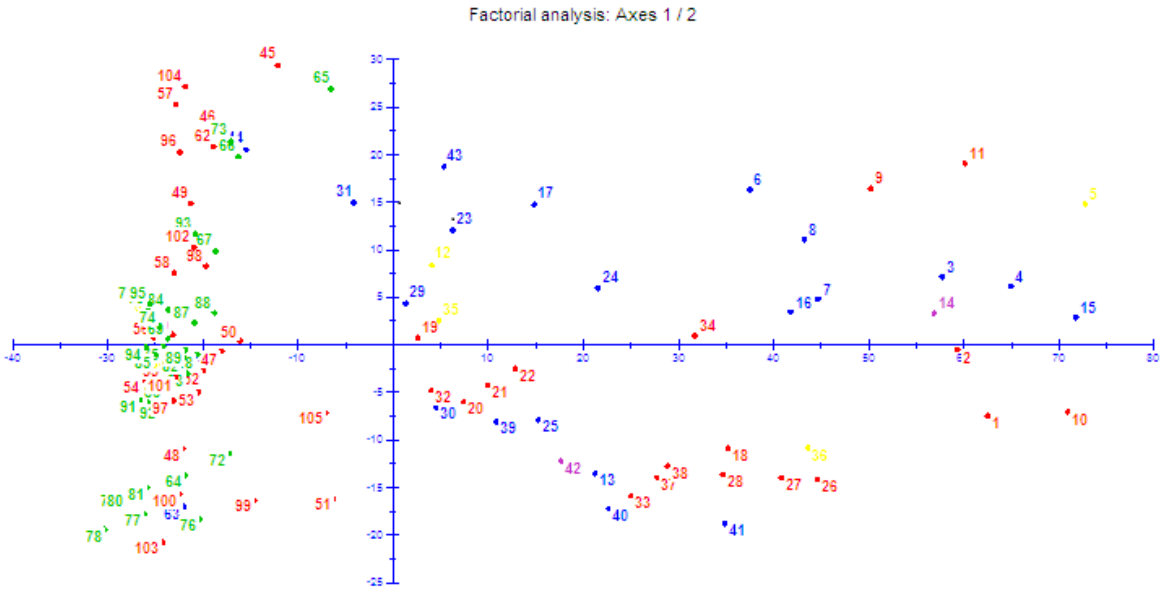

Figure 9: PCoA of axes 1 and 2 based on the dissimilarity of 105 bambara landraces. Accessions given in red were from Busia, blue from Kakamega, purple from Vihiga, yellow from Bungoma and green from Genebank.

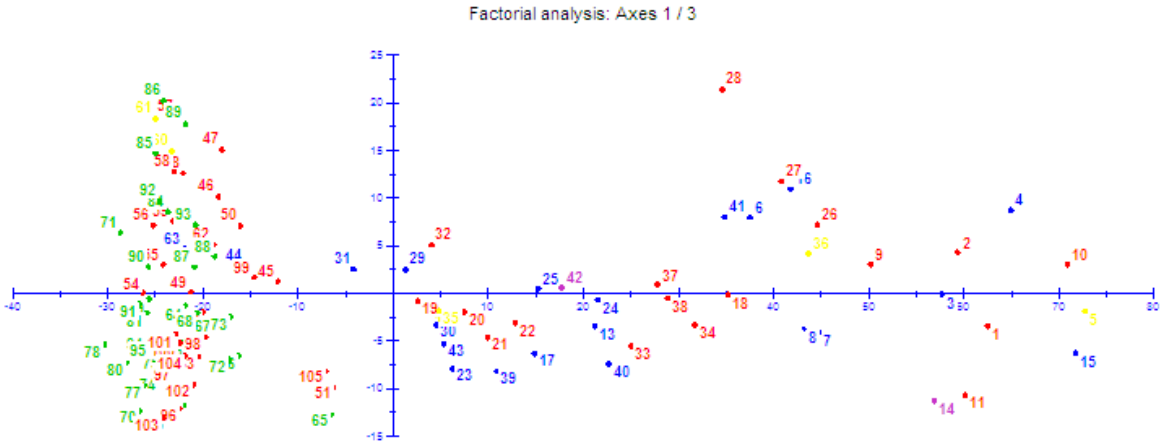

Figure 9: PCoA of axes 1 and 3 based on the dissimilarity of 105 bambara landraces. Accessions given in red were from Busia, blue from Kakamega, purple from Vihiga, yellow from Bungoma and green from Genebank.

Table 12: Principal component analysis for quantitative and qualitative morphological traits of bambara groundnut landraces.

Traits	PC 1	PC 2	PC 3	PC 4
Latent roots (Eigen values)	5.63	2.41	1.79	1.23
Percentage variation	33.28	18.39	13.32	8.17
Cumulative % variation	33.28	51.67	64.99	73.16
Peduncle length	0.24	0.06	0.23	-0.48
Number of leaves per plant	0.31	-0.09	0.37	-0.23
Terminal leaflet width	0.29	0.14	-0.03	0.26
Terminal leaflet length	0.42	0.04	-0.06	0.24
Petiole length	0.36	0.38	-0.29	0.12
Plant spread	0.41	-0.68	0.14	0.03
Plant height	0.34	0.36	-0.21	0.07
Internode length	0.25	0.05	-0.02	-0.29
Pod length	0.25	0.51	-0.22	-0.35
Pod width	0.09	0.50	0.03	0.08
Seed length	0.21	0.42	0.35	-0.2
Seed width	-0.06	0.58	0.35	-0.05
Seed number per pod	-0.08	0.61	0.47	0.02
Number of days from sowing to 50% flowering	-0.17	-0.36	0.25	-0.21
Number of pods per plant	-0.05	0.54	0.42	0.02

4.5.4 Unweighted Pair Group Method with Arithmetic Mean (UPGMA)

A dendogram generated by UPGMA cluster analysis failed to illustrate clear pattern of germplasm clusters based on their regions (Figure 10). In most cases, accessions from different regions or counties were clustered with one another. However, it demonstrated that accessions from Busia county and the National Genebank of Kenya tended to agglomerate together in cluster II in all the sub-clusters. All the 105 individual phenotypes were grouped into two (I, II) main clusters (Figure 11). Cluster I and II had sub- clusters with all the accessions from different regions present in sub-cluster I except those from the National Genebank of Kenya. There was a general trend as those accessions from the National Genebank of Kenya and Busia county tended to group together in cluster II while those from Kakamega county tended to cluster together in cluster I with only two accessions grouped in cluster II. Vihiga county were found only in cluster I. Cluster II had the highest number of genotypes with 65 individual. Grouping of the genotypes of these landraces in to sub- clusters indicated substantial level of intra-landrace polymorphism. Similarly high level of intra-landrace polymorphism can be said of the landraces in study which had its individual genotypes grouped into four sub-cluster units in each clusters.

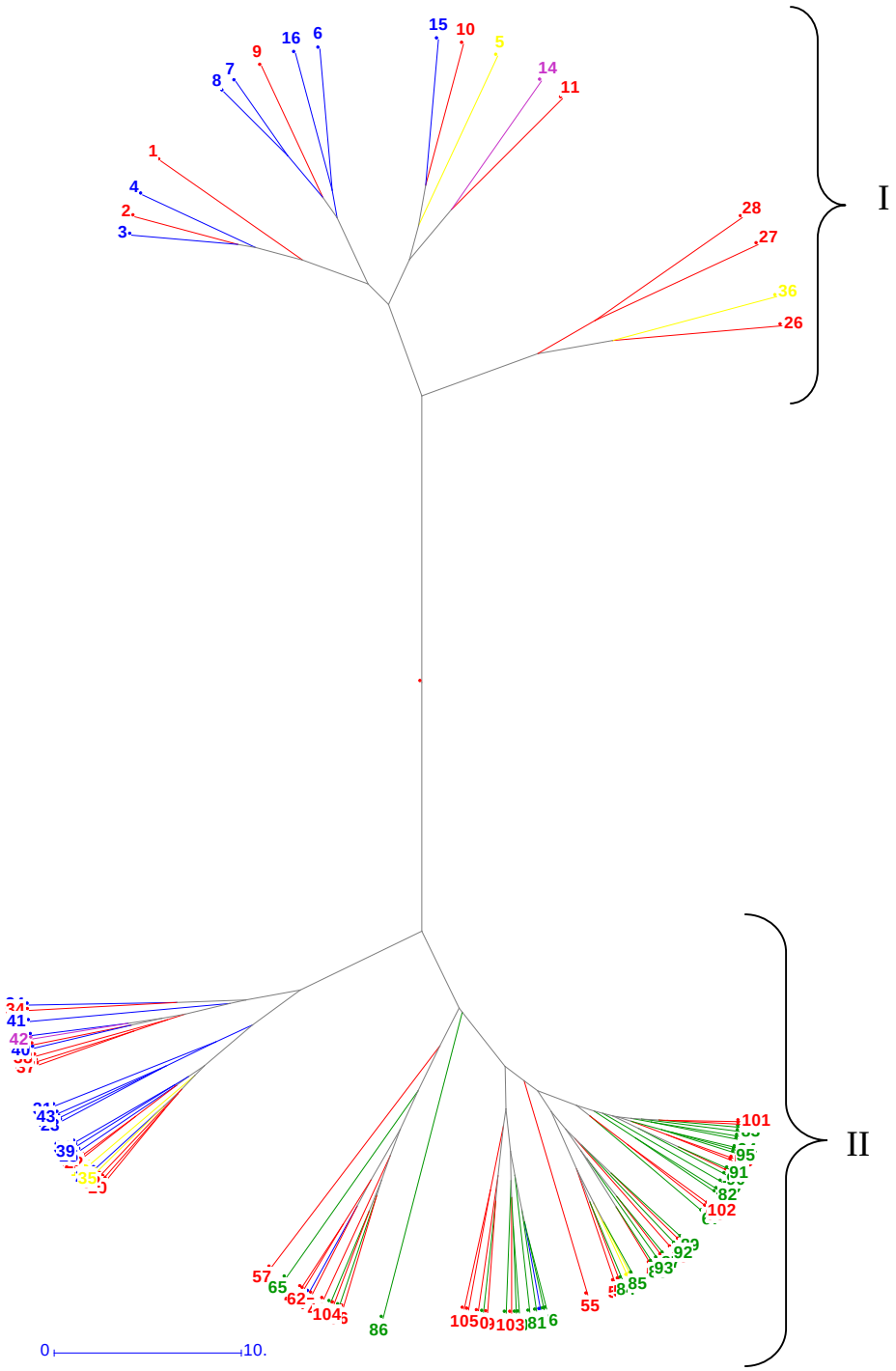

Fig. 10: Morphological relationships generated by Jaccard's similarity coefficients among 105 Bambara groundnut accessions. Accessions given in red were from Busia,blue from Kakamega, purple from Vihiga, yellow from Bungoma and green from Genebank.

4.6 Discussion

Evaluation of morphological variability and its characterization are the first step in the assessment of genetic diversity. Previous studies have shown that the choice of 15 to 20 agronomic characteristics is very useful to assess bambara groundnut genetic diversity (Goli *etal*., 1997; Ouedraogo *et al*., 2008). The one hundred and five bambara groundnut accessions studied displayed a considerable level of variability for qualitative characteristics such as growth habit, pod shapes, pod colours and pod texture. For example, all three types of bambara groundnut growth habits, namely bunch, semi-bunch and spreading (open) were observed, however with a low proportion of the spreading type. This may suggest that farmers have selected against this type of growth habit dominant in wild population. The explanation for selecting against spreading types could be that the stretching stems with usually long internodes give much larger diameter to the plant foliar crown, thereby causing difficulties during harvesting and increase yield losses as many of the mature pods may remain in the ground.

The more frequently observed growth habits were bunched and semi-buncheds types indicating that both are popular among farmers. Short stems and internodes that produce plants with tightly clustered leaves are typical characteristics of these plants (Goli *et al*. 1988; Ntundu *et al*. 2006). Such a configuration could be an advantage to farmers during harvesting because most of the pods remain attached to the stem crown after the plant is pulled up. In addition both bunched and semi-bunched alleles could have been selected by farmers during the course of domestication as it appears easy to manage in mixed cropping systems that is very common in the low input subsistence farming system under which the crop is mostly grown in Kenya. However, landraces with spreading growth habit could be used strategically in intercropping situations where they would form a more rapid ground cover and help suppress weed growth. In contrast, the bunched and semi-bunched types would be preferred in monocultures where optimum yield would be achieved at higher plant populations (Ntundu *et al*. 2002). This difference in growth habit between the wild types and the domesticated landraces is generally considered as one of the domestication syndrome of *Vigna subterranea* (Basu *et al*. 2007).

Similar to our findings, Goli *et al*. (1997) in the characterization of 1384 bambara groundnut accessions at the International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria, reported that 45 and 47% of accession were of bunch and semi-bunchy conformation with few accessions (8%) being of spreading type. In the characterization of 100 accessions of bambara groundnut from Tanzania Ntundu *et al*. (2006) reported that 30 and 63% of accessions were bunch and semi-bunch with few accessions (7%) being of spreading type. Plants with narrow leaves are characteristic for adaptation to drought (Ghafoor *et al*. 2001). In this study

intermediate oval leaved types were dominating, as could be expected considering the relative high rainfall prevailing in the areas of collection. The small lanceolate leaf types found less frequently may however be exploited for adaptation to the most drought-prone areas of the country.

Partitioning the variance into its components assists the genetic resources conservation and their utilization. In the present study, a significant variation among accessions was displayed in characters such as peduncle length, number of leaves per plant, terminal leaflet width, terminal leaflet length, petiole length, plant spread, plant height, internode length, pod length, pod width, seed length, seed width and number of pods per plant. These traits thus would be the most useful for the characterization of bambara groundnut landraces in Kenya.

Multivariate analysis for 19 quantitative characters that showed significant variation for most of the morphological variations for bambara groundnut landraces were accounted by the first four PCs. The main quantitative characters which accounted for more variability in both PC 1 and PC 2 during the two seasons included terminal leaflet width, terminal leaflet length, petiole length, plant spread, plant height, pod length, pod width, seed length, seed width, number of pods per plant. This suggests that accessions with high PC 1 and PC 2 values for both growing seasons had high vegetative characters measurements and large seed. These could be considered important for the accessions under investigation. The main loadings for the vegetative and seed characteristics observed in this study for PC 1 and PC 2, respectively, confirm the fact that farmers emphasize on leaf size and shape, Seed size and seed colour during selection (Marandu and Ntundu 1995; Ntundu *et al*. 2002; 2006).

Cluster analysis for qualitative and quantitative characters for the two test seasons failed to distinguish accessions according to their area of origin. This supports the findings in the principal component analysis. This can also be explained by the high frequency of bambara groundnut seed exchange by farmers over wide geographic-ethnic regions as well as the different informal names given to landraces from one region to another which may give room for genotype duplications as was suggested by Hudu and Saaka (2011). PCA failed to group accessions according to their areas of origin. This could have lead to a generally low coefficient of variation observed in bambara groundnut accessions, an indication of a high level of uniformity. This suggested that the source of these accessions could be same due to seed exchange among the farmers.

In this work, genotypes were clustered into two clusters (I and II) with cluster II forming sub-clusters indicating substantial intra-landrace polymorphism with distinct genotypes though from different regions. The high level of intra-landrace polymorphism could be attributed to

seed exchange between farmers as well as the geographical proximity of the areas. Contrary to the higher intra-polymorphism of most of the landraces, genotypes in cluster I appeared less heterogeneous. All the accessions except from the National Genebank of Kenya tended to form a clear group (Cluster I). Divergent accessions may have good breeding value, which may be utilized for direct selection and as parent stocks for hybridizations. The mixture of accessions in clusterII indicated that bambara groundnut accessions in this group constituted a more heterogenic group, with variable genetic backgrounds. This can also be explained by the high frequency of bambara groundnut seed exchange by farmers over wide geographic-ethnic regions as well as the different informal names given to landraces from one region to another which may give room for genotype duplications as was suggested by Hudu and Saaka (2011).

The low level of genetic diversity revealed in this work could be supported by the fact that small scale farmers in Eastern Africa generally tend to exchange seeds frequently. A farmers field survey (Ntundu, 2002) indicated that at least 44% of farmers in Tanzania obtain their bambara groundnut seeds from others farmers within (39%) and outside (5%) of their regions, annually. In their survey on seed market assessment in Dodoma, Iringa and Morogoro regions in Tanzania, Ashimogo and Rukulantile (2000) reported that 35.4% of farmers obtained maize (*Zea mays* L.) seeds from their fellow farmers, while 60.1% use only their own seeds. Further studies showed that sources of seed for planting of bambara groundnut in Ghana include farmer saved seed, exchange and market purchase (Berchie *et al*., 2010).

4.7 Conclusion

Based on the results of the present study, bambara groundnut germplasm from Kenya displayed a considerable range of diversity for most of the morphological and agronomic traits studied. Cluster analysis has proved as an effective method in grouping landraces that may facilitate the management and utilization in crop improvement by selecting a workable collection. A further collection to target the under-represented counties such as Vihiga and Kakamega is suggested for maximum diversity sampling. In future, it is suggested that a comprehensive comparative study between the collections of bambara groundnut from Kenya conserved at IITA, the Southern African Cooperation Plant Genetic Resources Centre (SPGRC) and the new collections included in this study would be important to assess the genetic variation of conserved germplasm collected from Kenya for conservation and use in crop improvement. Joint research on bambara groundnut with IITA in future would benefit Kenya as this institution has accumulated a lot of data, which are included in the International bambara groundnut data base.

4.8 References

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6.0 APPENDICES

Appendix 1: Preparations of standard solutions

1.0M Tris pH 8.0

12.11 g Tris base dissolved in 1.0M HCl until pH 8.0

Final volume is adjusted to 100 mL with water.

0.5 EDTA PH 8.0

168.1 g of Di-sodium EDTA dissolved in 800 mL water

20 g of NaOH pellets dissolved pH adjusted to 8.0 with 1.0M NaOH solution

Final volume adjusted to 1L with water.

5.0M NaCl

29.22 g NaCl dissolved in 70mL water

Final volume adjusted to 100 mL.

5 x TBE DNA Electrophoresis buffer

54 g Tris base

27.5 g Boric acid

20 mL 0.5M EDTA pH 8.0

Final volume brought to 1Litre. Stir until dissolved.

1 x TBE Buffer

200 mL 5x TBE buffer

Final volume adjusted to 1Litre.

6 x loading buffer (for DNA gels)

To make 30% glycerol (15 mL + 35 MQ water)

0.025 g Bromophenol Blue

0.025g Xylene Cyanol.

CTAB (2% EB):

2% Cetyltrimethylammonium bromide (CTAB) extraction buffer (1 liter)

100mM Tris-HCL (100 µLl of 1M Tris-HCL, pH 8.0)

20mM EDTA (40 µLof 0.5M EDTA, pH 8.0)

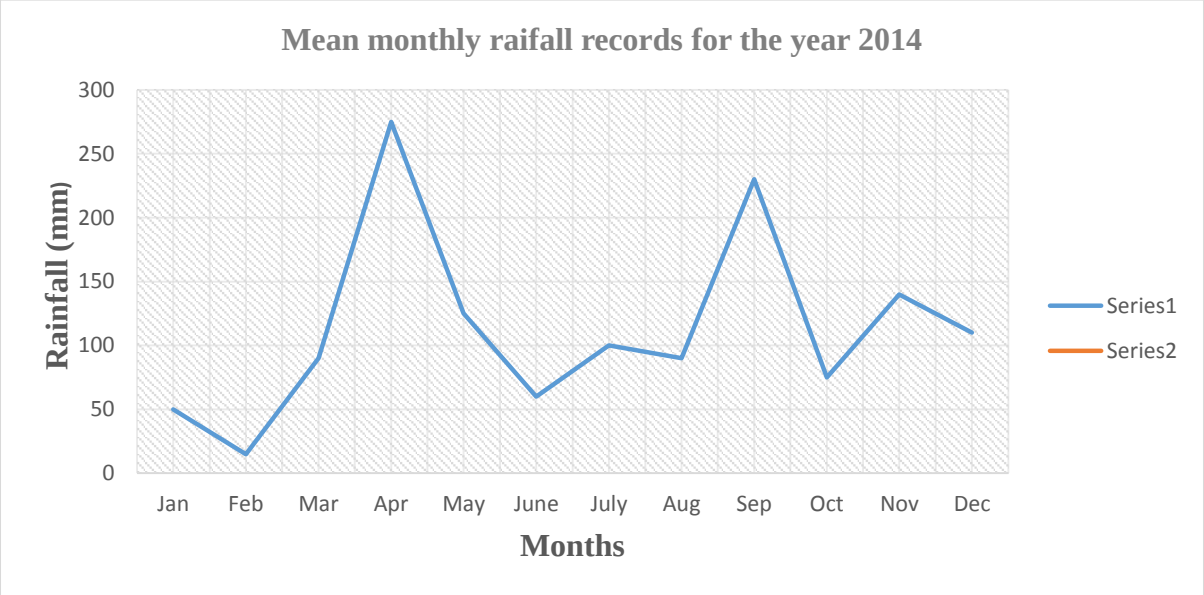
1.4M NaCL(280 µLl of 5M NaCL)

2% (w/v) CTAB (30g)

1% (w/v) PVPP (Polyvinyl polypyrrolidine)

Make up to 950ml with de-ionized water and adjust to pH 8.0 using HCL and adjust total volume to 1 liter with de-ionized water.

Appendix 2: Mean monthly rainfall records at KARI-Alupe in 2014



Appendix 2: (a) Authors own Publications (Odongo *et al*., 2015).

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Full Length Research Paper

Genetic diversity of Bambara groundnut (*Vigna subterranea* (L.) verdc.) landraces in Kenya using microsatellite markers

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The existence of genetic diversity in germplasm collections is crucial for cultivar development. Genetic relationships among 105 Bambara groundnuts (*Vigna subterranea* (L) Verdc.) accessions from Kenya were evaluated using 12 microsatellite markers. The Bambara landraces were collected from farmers in the western region and the National Genebank of Kenya. A total number of 24 alleles were revealed with a mean of 2 alleles per locus. The polymorphic information content and gene diversity values averaged 0.28 and 0.35, respectively indicating low genetic diversity among the evaluated Bambara groundnut germplasm. Genetic distance based on Jaccard's similarity coefficient from the simple sequence repeat (SSR) marker analysis ranged from 0.08 to 1.16 among the landraces. Cluster analysis distinctly grouped the 105 accessions into three major clusters. The analysis of molecular variance (AMOVA) revealed that 98% of the total genetic variation was within accessions whereas the genetic variation among accessions accounted for 2% of the total genetic variation. The genetic diversity observed in this study provides the basis for selection of appropriate parental genotypes for breeding programmes and mapping populations to further broaden the genetic base of Bambara groundnut germplasm in Kenya.

Key words: *Vigna subterranean*, accessions, Kenya, microsatellite markers, gene diversity, cluster analysis.

INTRODUCTION

Evaluation of available genetic diversity is a pre-requisite for genetic improvement in crop plants, especially in underutilized crops such as Bambara groundnut (Olukolu et al., 2012). Investigation of genetic diversity in both wild and domesticated species is equally important. Wild populations are known to be a potential source of useful genes and traits which could be introduced into the domesticated gene pool (Cattan-Toupance et al., 1998). Wild populations in centers of diversity or domestication constitute the initial gene pool of crops species. Crop

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(b) Conference presentation

Odongo, F.O., Maurice E. Oyoo and Victor Wasike. (2015). Morphological characterization of Bambara groundnut (*Vigna subterranean* (L.) verdc.) landraces in Kenya. *2nd International Conference On Bidiversity for Food and Nutrition and National Food Fair*. Held at Safari Park Hotel, Nairobi Kenya on 23rd November 2015.