UNIVERSITY OF CAPE COAST

MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF SWEET POTATO ACCESSIONS

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BY

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THESIS SUBMITTED TO THE DEPARTMENT OF CROP SCIENCE, SCHOOL OF AGRICULTURE OF THE COLLEGE OF AGRICULTURE AND NATURAL SCIENCES, UNIVERSITY OF CAPE COAST, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR AWARD OF MASTER OF PHILOSOPHY DEGREE IN CROP SCIENCE.

MAY 2015

DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my own original research and that no part of it has been presented for another degree in this university or elsewhere.

Candidate's Signature:…… Date:……

Name: Peter Appiah-Danquah

Supervisors' Declaration

We hereby declare that the preparation and presentation of this thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

Principal Supervisor's Signature:…….. Date:…….

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Name: Dr. Marian Docas Quain

ABSTRACT

For any good breeding work and its subsequent improvement, a knowledge in genetic diversity is very necessary. Genetic diversity of eighty seven Sweetpotato accessions assembled from various potential growing areas and two research institutions in the country, Crops Research Institute at Fumesua in Ashanti Region and Plant Genetic Resources Institute in the Eastern Region were put together and investigated using Morphological and Molecular characterization approaches.Both qualitative and quantitative traits were employed to identify differences in the accessions used for the investigations. Twenty one Simple Sequence Repeats (SSR) primers selected were used to screen and detected 107 polymorphisms and 5 monomorphisms. Principal Component Analysis clustered the accessions into 5 groups for quantitative traits, combination of quantitative and qualitative traits produced 6 groups and qualitative traits produced 6 groups respectively. Cluster analysis based on the Unweighted Paired Grouped Arithmetic Average (UPGMA) grouped the accessions into 13 clusters. Genetic distances resulting from the analysis of the dendrogram showed genetic diversity within the sweet potato accessions. However, hierarchical and non-hierarchical analyses identified Zambezi and Gweri as the same. Sauti showed the highest genetic distance of 82% as compared to Zambezi which had lowest distance of 7%. With the Core collection for future breeding and other agronomic programmes, twenty two accessions were selected for conservation *in vitro* and *ex situ* in order not to them. Breeders can depend on the selected high quality materials future breeding work.

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DEDICATION

To my late mother, Madam Akua Brempomaah, (alias, Akua Pitre) and my late wife, Felicia Antwiwaah.

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CHAPTER ONE

INTRODUCTION

Background of the Study

Sweetpotato (*Ipomoea batatas* (L.) Lam is the seventh most important food crop in the world based on total production (FAOSTAT, 2003). In many tropical countries sweetpotatoes are highly valued by resource poor farmers because they provide a highly nutritious staple food for humans and feed for livestock. The roots can be stored, transported and sold to supplement their income.

Sweetpotato is an important and valuable food crop worldwide. It was originally domesticated in the New World (Austin, 1988). The hypothesized center of origin, based on numerical analysis of key morphological features of the crop and the wide Ipomoea is between Central America and Northern South America in the region between the Yucatan Peninsula of Mexico and the Orinoco River in Venezuela (Austin, 1988) Sweetpotato was subsequently spread throughout the world due to its value as a nutritious and plentiful food source for animals and humans as it can be grown in a wide spectrum of soils, yielding relatively well in poor soils. Sweetpotato is grown for its starchy roots and other rich food substances e g, Beta-carotene which is a precursor for vitamin A. This naturally endowed food substance makes it more preferred crop in terms of food value as compared with other root and tuber crops and even grains and cereals. In the United States of America and the Republic of

China and Brazil sweetpotato is used as a substitute for the production of ethanol in the absence and scarcity of maize and cassava.

Sweetpotato has gained an important position in the food crop production system and many farmers are seriously engaged in its production especially farmers in the coastal savanna and the transitional eco zones of Ghana grow the crop on marginal soils to meet both the local and export markets (Dapaah *et al*., 2005; FAOSTAT, 2003). Germplasm resources are the heredity materials in plants that carry genetic information by which traits are transmitted from parents to their offspring from one generation to another.

Problem Statement

Like other food crops, sweetpotato is genetic resources in Africa are under threat of genetic erosion due to the fact that agricultural biodiversity is being threatened due to the context of current yield trends, predicted population growth and pressure on the environment. Traits relating to yield stability and sustainability which also include durable disease tolerance, abiotic stress, tolerance and nutrient and water use efficiency (Mackill *et al*., 1999).

Rationale of the Study

Farmers current method of selection based on physical appearances of materials could result in genetic erosion. Some of the rejected materials might possess essential traits for crop improvement through molecular breeding. Sweetpotato is a crop which has been kept in low profile even from research institutes. In Africa and for that matter Ghana and it thus belongs to crops tagged ‘Orphaned’ crops which have not attracted much research attention for

a long period of time and very little improvement has been made to this crop (Otoo, 2007). Knowledge of genetic variation and relationships between germplasm is important to understand the variability available and its potential use in breeding programs. There is therefore, the need to do collection, characterization and evaluation of the genetic materials (both local and exotic) to have a thorough knowledge of the key trait which are of immediate value to research, agronomic and breeding potential of the materials (Thormann *et al*., 1994).

Morphological trait characterization will enable breeders access to genetic diversity in the crop, form base population and help in the assemblage of good attributes to the germplasm. Morphological trait characterization of genetic resource collections which are based on the physical attributes have got its own limitations like low polymorphism, late expression and effect of the environment on the crop (Smith & Smith, 1992). With the limitations associated with the morphological trait characterization, therefore there is the need to employ molecular marker system which will enable breeders to develop genotypes with specific agronomic traits that will resist any change that the organisms may undergo due to environmental and climatic changes.

For example, fungal and insect pest continually evolve and overcome most plant resistance among others (Evans, 1997) molecular genetic diversity studies are often used to augment estimates of physiotymic analysis based on genetic diversity. To date there are several molecular genetics techniques that have been used to analyse genetic diversity. The most common marker systems used to assess molecular diversity present in plant populations include:

1. RFLP-Restriction Fragment Length Polymorphism markers which were among the first molecular markers used to characterize germplasm (Paull *et al*., 1988).
2. RAPD-Random Amplified Polymorphism DNA markers were developed shortly after the RFLP marker (Zhang *et al*., 1996).
3. AFLP- Amplified Fragment Length Polymorphism markers represent the fission of RFLP and RAPD technologies (Vos *et al*., 1995).
4. SSR-Simple Sequence Repeat or Microsatellite method is becoming one of the most important molecular markers for population genetic analysis of both animals and plants, but SSR require time, effort and money to implement due to the need for developing specific primers which require extensive sequencing and screening work (Jarret & Bowen, 1994).

Exchange of sweetpotato planting materials among farmers has led to duplication of planting materials whose local and exotic names have changed in different localities. To enable farmers to continue to increase productivity, there is the need to ensure purity of varieties, higher yield and other important attributes.

General Objective

The general objective of the study is to assemble, characterize and establish sweetpotato germplasm using both morphological and molecular techniques (SSRs) for selection and conservation.

Specific Objective

Specifically, the objectives of the study seek to:

1. investigate genetic diversity and relationships among 87 sweetpotato germplasm using morphological characters and SSRs markers.
2. assess the correlation between genetic distance estimates of sweetpotato based on morphological traits and molecular markers selection.
3. identify distinct genotypes and eliminate obvious duplicates from the germplasm.
4. select core collection of sweetpotato accessions for conservation and future breeding work.

CHAPTER TWO

REVIEW OF LITERATURE

Botany

Sweetpotato is a plant with chromosome number 2n = 6x = 90. This indicates that it is a hexaploid plant with a basic chromosome number x = 15 (Huaman & Zhang, 1997). It is a dicotyledonous plant belonging to the family convolvulaceae. There are over 400 *Ipomoea* species distributed throughout the tropics and although some of the species have fleshly roots, they are usually unpalatable, *Ipomoea batatas* is the only species of major economic importance (Hall & Phatak, 1993). A very large number of sweetpotato cultivars exist due to domestication and artificial hybridization and selection by man, natural hybridization and mutations over time have all resulted in a number of cultivars.

Woolfe (1992) reported that the level of diversity in the sweetpotato is higher than in root crops like cassava and yams. Cultivars differ from one another in the colour of the root skin and flesh, in the size and shape of the roots and leaves and in the depth of rooting, maturity duration, texture of cooked roots and tolerance to biotic and abiotic stress. On the basis of root texture after cooking, sweetpotato cultivars fall into three groups (Onwueme, 1978). First, those with firm dry mealy flesh. Second, those with soft moist gelatinous flesh.and those with very coarse tubers which are suitable only for animal feed

and industrial use. The cultivated species, *batatas* include plants that are very variable in their morphology. Thousands of cultivars have been selected and cultivated in Latin America since time immemorial. It is now grown between latitudes 40^{o}N and 40^{o}S of the equator and from sea level up to an altitude of 2000 m, (Haln, 1977). It contains latex in all its parts. Most sweetpotato cultivars possess serious problem in breeding especially when many of the desirable parents belong to the same incompatible group during self and cross pollination Charles *et al*., (1973). However, a few cultivars are self-compatible and the genes for this characteristic can conveniently be introgressed into other cultivars. Sweetpotatoes express an extremely diverse range of phenotypes and their foliage and storage roots demonstrate a myriad of shapes, colours and sizes. The storage roots exhibit a wide range of flesh colours, dry matter contents and nutrient profiles in addition to many other traits such as resistance to biotic and abiotic stress (Woolfe, 1992).

Currently, there are two primary sweetpotato germplasm repositories that are set up in the world. The larger of the two is established at the International Potato Centre (CIP) in Lima, Peru, with roughly 7,000 accessions. The second, which has roughly 750 accessions *in vitro* is managed by the United States Department of Agriculture-Agricultural Research Service (USDA-ARS) and located at the Plant Genetic Resources Conservation Unit (PGRCU) in Griffin, Georgia. Scientists collaborating with CIP and the PGRCU have carried out numerous sweetpotato-collecting expeditions in Latin America, the Caribbean, and Papua New Guinea since 1985 according to (Huaman & Zhang, 1997). The sweetpotato germplasm collections have also been expanded and benefited by germplasm transfers and donations from other

institutions around the world such as the Asia Vegetable Research and Development Centre in Taiwan. The CIP and PGRCU collections have been characterized for many phenotypic traits of potential economic significance

Growth Habit

The sweetpotato is a herbaceous perennial plant. However, it is grown as an annual by vegetative propagation using either storage roots or stem cuttings. Its growth habit is predominantly prostrate with the shoot system that expands rapidly horizontally on the ground. The growth habits of sweetpotatoes are erect, semi-erect, spreading, and very spreading (Bartolini, 1985; Hayward, 1967; Kays, 1965).

Stem

The sweetpotato plant has a creeping stem above ground which is called a 'stolon' sometimes twining thin, 3-10 mm in diameter. At intervals, the nodes along the stem give rise to adventitious roots and shoot while its terminal shoot creeps along horizontally. Sweetpotato has internodes 2-10 cm long, glabrous or pubescent when young. It is light green to purple in colour, angular and has bundles bi-collateral (Purseglove, 1988).

Leaves

The leaves of sweetpotato occur spirally on the stem in a 2/5 phyllotaxy, simple, estipulate, petiole 5-30 cm long, grooved on upper surface with two small nectarines at the base. The petiole has the ability to grow in a curved or twisted manner so as to expose the lamina to maximum light. The leaves are

simple and may have entire margin, digitated lobed. The lamina is green in colour and sometimes with a purple coloration especially along the veins. (Purseglove, 1988).

According to Dahniya (1979), stomata are present on both the upper and lower leaf surfaces but are comparatively numerous on the lower surface. Lamina is extremely variable in size and shape even for leaves on same plant ranging from toothed, triangular, semi-circle, semi-elliptic.elliptic, lanceolate, oblanceolate and linear (CIP, AVRDC, IBPGR, Descriptors for sweetpotato 1991) by Huaman *et al* 1991.

Flower

Sweetpotato has regular flower with atypically large bell or funnel-shaped corolla of five fused petals. The large, reddish-purple flowers are solitary or cymose inflorescence on peduncle of 3-15cm long. The calyx-tube is made up of five sepals free from each other except at the base. The corolla tube is a funnel of five united petals up to 6cm long and 5cm across. There are five free epipetalous stamens inserted on the base of the corolla with anthers and filaments of varying length and are white, purple or white and purple in colour. The relative position of the stigma as compared to the highest anther may be inserted shorter than the longest anther, same height as highest anthers slightly exerted and exerted that is longer than the longest anther. The spherical pollen grains are covered with numerous minute papillae. The superior, two or four located ovary has two ovules in each locale and is surrounded at the base by an orange nectar. The flowers open in the morning and wither a few hours later. The Flowers are cross-pollinated by bees but

pollen tube growth and cross-fertilization occur only after pollinations between cross-compatible cultivars (Bartolini, 1985).

Fruit

The fruit is a globular dehiscent capsule in which false septa may develop. It contains up to four small, black flat-sided seed with one smooth and one angular surface and with a deep micropylar hollow just above the helium on the flat surface (McDonald & Austin, 1990) The testa is thick, very hard and almost impermeable to water, so that the seeds germinate at irregular intervals, depending upon the time taken for them to absorb water. Germination can be improved by scarifying the seed either by mechanically clipping the testa or by treating with dilute sulphuric acid.

The Root System

When sweetpotato is planted from stem cuttings, adventitious roots arise from the cuttings in a few days and grow quickly to form the fibrous root system of the plant. These roots can penetrate the soil to depths of 2m or more depending on the soil conditions. As the vine grows along the surface of the soil, roots are produced at the various nodes. Such roots grow into the soil and increase the effective feeding area of the plant.

The Storage Roots

The storage roots are formed by secondary thickening of the adventitious roots, either those produced by the original stem cutting or those from nodes of the creeping stem. Sweetpotato cultivars differ from one another and the

storage root skin colour usually white, cream, yellow, orange, brownish orange, pink, red, purple-red and dark purple. The storage root flesh colour also ranges from white, cream, dark cream, pale yellow, dark yellow, pale orange, intermediate orange, dark orange and strongly pigmented with anthocyanins have been reported by (Onwueme, 1978). Different scores of percentages were recorded for different traits of storage root parts as was reported by (Ritschel *et al*. 1998; Daros *et al*. 2002; Martin & Rhodes, 1983; Ruberte & Martin, 1983).

Genetic Diversity Studies

According to Hoogendijk and Williams (2001), genetic diversity (studies and analysis) employs the following steps:

1. Description of variations within and between populations, regions or areas.
2. Assessment of relationships between individuals, populations, regions, area and
3. Expression of relationships between results obtained from different sets of characters.

Genetic Diversity Assessment of Sweetpotato

Plant genetic resources are a valuable resource in agriculture, food security and forestry because they provide genetic diversity necessary for both farmers and breeders to obtain new cultivars (Laurentin, 2009). The ability to identify genetic variations is indispensable to effective management and utilization of genetic resources in a breeding programme as a proper analysis of the genetic

variation and relationships between accessions or genotypes is important to: (1) understand the variability available and its potential use in breeding programmes, (2) estimate any possible loss of genetic diversity; (3) offer evidence of the evolutionary forces shaping the genotypic diversities and (4) choose priority genotypes for conservation (Smith & Duvick, 1989).

According to Beeching *et al*. (1993), a prerequisite for any genetic improvement programme is knowledge of the extent of genetic variation present between genotypes and the genetic distance between all closely related species with which hybrids could be produced. This can be achieved through the characterization of the germplasm using either morphological, biochemical or genetic markers. Genetic diversity analysis and characterization allows evaluation of genetic variability which is a fundamental element in determining breeding strategies and genetic conservation plans. As such, knowledge is even necessary before the breeding materials are exploited further.

Genetically, broad-based breeding programme provides ideal response to environmental variability, disease and economic trends. In contrast, a narrow-based programme would provide slow response to selection and increase the likelihood of crises triggered by outbreaks of diseases and pests. A lack of genetic variability across breeding programmes could exacerbate these deficiencies nationally and internationally, conceivably threatening the usefulness of breeding stocks (Smith & Duvick, 1989).

Genetic Diversity

Genetic variability and genetic diversity of a taxon is of great importance for plant geneticists, breeders and taxonomists (Prince *et al.*, 1994). In populations, the genetic composition and genetic diversity are originated from wild progenitors and it has been influenced by evolutionary processes such as mutation, recombination, genetic drift, migration, natural selection Hartl and Clark, (1997) and adaptation to different environments. Frankel and Brown, (1984) defined genetic diversity as the product of interplay of biotic factors, physical environment, artificial and plant characters such as size, mating system, mutation, migration and dispersal.

In general, the knowledge of genetic diversity and relationship among sets of germplasm as well as the potential merit would be beneficial to all phases of crop improvement (Lee, 1995; Geleta, 2003). Evaluation of genetic diversity among adapted or elite germplasm provides the estimation of genetic variation among segregating progeny for pure line development (Manjarrez Sandoval *et al.*, 1997) and the degree of heterosis in the progeny of certain parental combinations (Cox and Murphy, 1990; Geleta, 2003). The information about genetic diversity in available germplasm is important for the optimal design of a breeding programme (Geleta, 2003) and the nature of genetic relationships among lines, population or species has been an important tool for effective management of genetic diversity in a given gene pool (Manjarrez-Sandoval *et al.*, 1997).

The study of genetic diversity has been of interest to plant breeders and germplasm curators. It is a process where variation among individuals or groups of individuals is analyzed using specific methods of combination

(Mohammadi & Prasana, 2003). In plant species, it can assist in the evolution of germplasm as possible sources of genes that can improve the performance of cultivars (Yang *et al.*, 1996; Geleta, 2003). More recently, breeding efforts started to also contribute to the generation of genetic variability.

Genetic Distance

Genetic distance is the extent of the gene differences between cultivars, as measured by allele frequencies at a sample of loci (Nei, 1987) while the genetic relationship among individuals and populations can be measured by similarity of any number of quantitative characters (Souza & Sorells, 1991). Genetic distance measures are indicators of relatedness among populations or species and are useful for reconstructing the historic and phylogenetic relationships among such groups.

Genetic distance has been measured using two approaches, the parsimony analysis and the cluster analysis, and they represent the phylogenetic and genetic relationship, respectively. The data used in this analysis involve numerical or a combination of different variables provided by a range of markers that can be used to measure the genetic distance. They include pedigree data, morphological traits, isozymes and, recently, Deoxy ribonucleic acid (DNA)-based markers, such as restriction fragment length polymorphism (RFLP), random amplified polymorphism (RAPD), simple sequence repeats (SSR), amplified fragment length polymorphism (AFLP), and others. The molecular markers are recognized as significant tools to orient plant genetic resource conservation management, providing means to accurately estimate

the genetic diversity and genetic structure for a species of interest (Hamrick & Godt, 1996).

Diversity Measurement

There are four methods for measuring genetic diversity, namely farmers view point and traditional classification, morphological characterization, biochemical characterization and molecular characterization (Hoogendijk & Williams, 2001). In this study, morphological and molecular characterization would be given prominence.

Morphological Characterisation

The use of molecular technique for analysis of genetic diversity and the structure of germplasm, the first conceptual step in marker-assisted breeding, has been fruitful for many species (Naylor *et al*., 2007). However, before the advent of molecular markers, morphological descriptions were used for germplasm management. A morphological description provides special identification for specific cultivated varieties. The application of morphological descriptors list is the simplest of the formal, standardized, repeatable methods of measuring crop genetic diversity (Hoogendijk & Williams, 2001). Even though morphological traits were amongst the earliest markers used in germplasm management, they have a number of limitations, including low polymorphism, low heritability, late expression and vulnerability to environmental influences (Smith & Smith, 1992).

The main advantages of conducting morphological characterization are that published descriptor lists are readily available for most major crop species,

and it can be carried out directly on-farm. It is relatively cheap and easy to carry out (Hoogendijk & Williams, 2001). The following are some of the identified limitations associated with the use of morphological characterization for diversity studies:

1. The first limitation is the difficulty in taking environmental influences into account in the case of quantitative characters.
2. Unavailability of descriptors for many neglected and underutilized crops.
3. Lastly, in most morphological traits, the genetic control is unknown, although it is known that multiple genotypes can produce similar phenotypes. (Smith & Smith, 1992).

Definition of Plant Morphology

Plant morphology deals with plant form, including its development and evolution. It can be defined in the narrow sense as referring only to external form, in contrast to anatomy that refers to internal form. But plant morphology can be defined in a wide sense that includes both internal and external form at all levels of organization from the molecular and cellular level to the organism level (Sattler, 1978). Theoretically, plant morphology introduction is relevant to all fields of plant biology such as molecular genetics, physiology, ecology, and evolutionary biology. Therefore, to some extent these fields are based on or reflect morphology (Sattler & Rutishauser, 1997).

Morphological characterization of cassava has been performed in other countries such as Cote d'Ivoire. In such characterization, 14 external and root qualitative descriptors were applied to 340 accessions which were made up of

both landraces and exotic collections (N'Zue *et al*., 2009). Elsewhere in Brazil cassava was characterized to evaluate morphological characteristics and yield of two local varieties and seven introduced materials. Some of the characters evaluated were the petiole colour, the root cortex colour and the stem colour.Some of the agronomic data considered were yield of the harvested plants, starch content of the roots and diseases and pests attack were observed.

Molecular Characterisation

Morphological traits, isoenzyme and protein markers have been used in sweetpotato germplasm studies (Kokubu & Hira, 1998, Huaman *et al.*, 1991). However, these markers are often subject to development and environmental variations (Bailey, 1983; Berntzky & Tanksley, 1989). Molecular techniques have proved to be powerful tools and have been widely utilized for genetic manipulation in many crop plants, in the areas of germplasm characterization, variety identification, phylogenetic study and diversity analysis (Maughan *et al*., 1996; Ellis *et al*., 1997; Barker *et al.*, 1999; Degani *et al.*, 2001; Lefebvre *et al*., 2001). The two markers; random amplified polymorphic DNA Williams *et al*., (1990) and amplified fragment length polymorphism Vos *et al*., (1995) have been employed to study the genetic diversity in sweetpotato (Jarret & Austin 1994, Gichuki *et al*, 2003, Zhang *et al*., 1998, 2000).

Molecular markers have been used to analyze many crops for different reasons including genetic diversity assessment Ipek, *et al*; (2003); Xu, *et al*., (2003); Zhang *et al*.,(2000), genetic variability assessment Shim & Jorgenson, (2000); Van Truren, (2001), genetic linkage map construction (Klein *et al,* 2000; Kriegner *et al.*, 2003), Core collection establishment and

collection management McGregor *et al*., (2002); Zhang *et al*., (2000), trait identifications and associations Ghislain *et al*., (2001) and sequencing (Kim, *et al*., 2003). Molecular genetic diversity studies are often used to augment estimates of phenotypic analysis based on diversity and has been applied to many crops to understand the molecular genetic differences between novel phenotypes. Analysis of a combination of molecular genetics and phenotypic data provide a powerful tool for establishing core collections at Crop Research Institute that will facilitate more efficient yet critical durable collections.

There are many molecular genetics methods available to assess the genetic composition of germplasm that help to establish a core collection and efforts have been made on a number of plants including cassava Olsen and Schaal, (2001), rye grass Roldan – Ruiz *et al.*, (2001); potato McGregor, *et al.*, (2002) and sorghum Klein *et al.* (2000).

Restriction Fragment Length Polymorphism Markers

Restriction Fragment Length Polymorphism is one of the first molecular markers used to characterize germplasm (Paull *et al.*, 1988). This technique uses differences in cleavage site for specific end nucleases that produce different lengths of DNA strands. The advantage of this method is that they are highly reproducible. The drawbacks include the use radioactive rays and the amount of useful or scoreable markers per reaction is very low.

Random Amplified Polymorphic DNA (RAPD)

Random amplified polymorphism DNA markers are based on the polymerase chain reaction (PCR). It is relatively easy to use and produces a larger number

of polymorphism per reaction (Zhang *et al*, 1996). The RAPD-PCR products can be analysed using both agarose and acrylaminde gels. They are cheap and can produce a lot of polymorphism in very few reactions.

Studies using RAPD Markers in Sweetpotato

Due to the importance of sweetpotato in the diet of many communities in the developing countries, there is the need to fast track the development of the crop but this will require the use of novel breeding technologies (FAOSTAT, 2000). In recent years the development of Marker Assisted Selected (MAS) protocols has attracted a lot of attention from plant breeders (Young, 1999). Young further suggested that the development of marker systems which involve DNA manipulation has offered promise in the construction of highly accurate DNA marker maps and quantitative trait loci (QTL) analysis. The lack of complete genetic map for the sweet potato (Labonte *et al*., 1997) has presented greater challenges in identification of QTLs for the crop's improvement. Although polymerase chain reaction (PCR) based marker systems like random amplified polymorphic DNA's (RAPDs) Williams *et al*., (1990), amplified fragment length polymorphism (AFLP) Vos *et al.*, (1995) and simple sequence repeats

Akkaya, *et al*., (1995) have provided easier protocols for genome analysis. A study on the development of technique for trait marker linkage reveals two major break-throughs which have been useful but are plagued with many disadvantages. These protocols include the use of near isogenic lines (NILs) (Young, *et al.*, 1999) and bulked segregant analysis (BSA) (Michelmore, *et al.*, 1991).

Amplified Fragment Length Polymorphism (AFLP)

Amplified Fragment Length Polymorphism (AFLP) markers represent the fusion of RFLP and RAPD technologies. Vos *et al.* (1995) reported that the AFLP technique is based on the selective PCR amplification of restriction fragments from a total digest of genomic DNA. The AFLP technique involves three steps: (1) restriction of the DNA and ligation of oligonucleotide adapters, (2) selective amplification of sets of restriction fragments and (3) gel analysis of the amplified fragments. AFLPs like RAPDs represent a dominant marker type, which is one drawback of this method (Vos, *et el.,* 1995). It is based on the EcoR and Msel restriction sites, and the ability of selected primers to bind to those sites. The resolution and reproducibility of this method is very high and can be used with very little DNA to distinguish between closely related organisms and it is relatively more cost effective.

The Simple Sequence Repeats (SSR)

Simple sequence repeats (SSR) have been well established over the last decade in mammalian systems. This technique has been developed for a number of species Dib *et al,* (1996) and a variety of plants species including important crops (Weising *et al.,* 1989, Condit & Hubbed, 1991). However, a number of limitations are associated with SSR discovery in plants. This include a lack of DNA sequence in databases a perceived low abundance of SSRs (compared to mammals) and differences in the most common types of repeats found. SSR markers require time, effort and money to implement due to the need for developing specific primers with extensive sequence and

screening work.SSRs are stretches of 1 to 6 base pair nucleotides and distributed across the entire genome Powell *et al*., (1996).

Hamada *et al.,* (1982) demonstrated the large number and widespread occurance of short tandem repeats in eukaryoptic genomes.The finding was verified by Tautz & Renz (1994). SSRs markers have been used in studies and have generally developed by three routes: (1) transfer from closely related species by Provan *et al., (*1996), (2) search sequence database by Swamwell *et al*., (2001) and repeated oligonucleotides and sequencing candidate clones by Powell *et al.,* (1996).

Studies using SSR Marker in Sweetpotato

Sweetpotato and its related wild species have been analysed using RAPDs, RFLPs and ISSRs (Jarret *et al.,* 1992; He *et al*., 1995; Dhillon & Ishki, 1999; Huang & Sun, 2000; Hu *et al*., 2003). At present only a few useful microsatellite loci have been identified for sweetpotato (Jarret & Brown, 1994; Buteler *et al*., 1999). These markers have been used in genetic inheritance analysis (Buteler, 1999), percentage analysis (Buteler *et al*., 2002) and in the assessment of genetic diversity and fingerprinting (Zhang *et al*., 2000 Hwang *et al*., 2002). To promote practical genetic analysis and breeding programs in sweetpotato, the number of microsatellite markers should be significantly increased to cover the entire genome.

Calculation of Genetic Distances

There are two ways that data from the DNA fingerprinting can be used. The first approach is the parsimony analysis where phenogram representing

phylogenetic relationship are constructed on the basis of the lowest number of characteristic state transformation that yields a particular phenogram. The second is the dendrogram which is often used. Diagrams of genetic relationships are constructed using the cluster analysis based on the pair wise genetic distance. An input data matrix containing absence (0) and presence (1) value after the running and analysis of the random amplified polymorphic DNA and is directly used to calculate pairwise genetic distance.

All the possible pairwise grouping of individuals have their pairwise distance values calculated and grouped in a table of pairwise distance matrix. The index of genetic similarity (F) of Nei & Li (1979), among other formula has been used in most of the studies and is stable to calculate the pairwise distance matrix from RAPD data and SSR. Jaccard's similarity coefficient Jaccard (1908); simple matching (SM) and Rogers' distance Roger (1972) are other coefficients which have frequently been used to calculate genetic distance.

Computer software programs include Numerical Taxonomy and Multivariate Analysis program package (NTSys-Pc) Rohlf (1993), RAPD distance, phylogeny inference package and phylogenetic analysis using parsimony (PAUP) (Swafford, 1991). These programs can be used to calculate distance matrixes using the formula $F = 2(nxy)/nx + ny$. The resultant data are processed with cluster analysis using methods such as unweighted pair group arithmetic average analysis (UPGMA), Sneath & Sokal (1973) and then plotted in dendrogram representing the genetic relationship among the pairwise genetic matrix.

Core Collection

Core collections were first defined as a limited set of accessions representing the genetic diversity of a crop species and its wide relatives with a minimum of repetitiveness (Frankel, 1984). This early definition described an idea that has been utilized by the genebank community and adapted in the day-to-day genebank management.

Most core collections are created to represent a portion of an existing collection or of several existing collections, but some are newly created collections; some represent the diversity in a complete genus, including wild species. While others represent only a small part of the known gene pool, some core collections contain as much diversity as possible, but most give higher priority to certain types of materials reducing the amount of diversity captured but increasing utility. This conflicts with the original definition of core collection.

This methodology of core collection allows the selection of "a set of accessions, which optimally represents specific genetic diversity". It is up to the user to determine what genetic diversity should be represented and what is considered an optimal representation. If optimally is interpreted as with a minimum of repetitiveness and if "specific genetic diversity is interpreted as the genetic diversity of a crop species and its wild relatives" this corresponds to the original definition of core collection. However, other interpretations have often been given to "optimally" and its wild genetic diversity, since the original definition is not always that useful for everyday genebank management practices. As a result, the term "core collection" has in some cases been applied more broadly than given in the original definition. This

paper will also use the wider interpretation of the term for the selection of the set of accessions for the genebank.

CHAPTER THREE

MATERIALS AND METHODS

Characterization of Sweetpotato

An experiment was conducted to characterise 87 sweetpotato accessions using both morphological and molecular traits. The experiment covered two areas of studies. The first phase was morphological characterization carried out at Crops Research Institute at Fumesua–Kumasi. The molecular characterization constituted the second phase and was done at the Molecular and Biotechnology laboratory also at Fumesua.

Sites of Accessions Collection

Collection of the sweetpotato accessions was done throughout the country with focus on potential production areas. Sweetpotato cultivars were collected in the form of cuttings from farmers' fields. Collection was done at a selected distance within an area where the accessions were collected to include all possible diversity but at wider distance from each selected area to avoid collection of identical or very closely related clones due to the possibility that planting materials can exchange hands of farmers. Some of the cultivars were also obtained from Plant Genetic Resource Research Institute (PGRRI) at Bunso in the Eastern Region of Ghana. Exortic accessions in the form of vines and seeds that were brought to Crops Research Institute were added to the collection.

Site of collection of the Germplasm

Volta Region Ketu, Abor, Ohawu & Akatsi
Brong Ahafo Region Fiaso &Wenchi
Central Region Jukwa
Ashanti Region Fumesua & Kwadaso
Eastern Region Bunso & Asuogyama-Ehiamankyene
Western Region Aboadzi
Key I: The above key shows the sites in the map of Ghana where the germplasm collections were done.

Figure 1: Sites of the sweetpotato germplasm collection in Ghana

Site of collections
Uganda
Kenya
Malawi
Tanzania
Nigeria
South Africa
Key II above shows countries where sweetpotato germplasm were collected.

Figure 2: An African map showing the site of the sweetpotato germplasm collection

Methods of Collections of the Accessions

Collected materials of each accession were first fastened together with a twine. These were put in a collecting bags and labelled both within the bag and outside it. Each accession was given a unique collection number for easier

identification. Besides the collection number, other passport data collected with the materials were names of donors, locations or place of collection (village, towns, district and region) and the date of collection. Names of accessions were also recorded. These exercises were performed in order to prevent the accessions or materials collected from being mixed up. Collection sources, sites and donors are shown in Table 1.

Table 1: Sweetpotato Accessions used for the Morphological and Molecular Characterization and Sites of Collection

Local Name	Donor/Source	Country/Region/Town
TIS 86/0350	IITA(CIP)	Nigeria
Tek Santom	IITA	Nigeria
Faara	IITA	Nigeria
Santom Pona	IITA	Nigeria
Okumkom	IITA	Nigeria
Sauti	CIP	Malawi
Zambezi	CIP	Kenya
Beauregard	CIP	Kenya
Otoo	CIP	Tanzania
NCSU 1560	CIP	Kenya
Carrot C	CIP	Kenya
K118	CIP	Kenya
Ogyefo	CIP	Kenya
TIS 83/0138	CIP	Kenya
Hi-Starch	CIP	Kenya
Naspot 1	CIP	Uganda

Santo Amaro	CIP	Kenya
Jonathan	CIP	Kenya
199062.1	CIP	Kenya
Gweri	CIP	Uganda
Brondal	CIP	Kenya
MOHC	CIP	Kenya
Tanzania	CIP	Kenya
Ningshu 1	CIP	Kenya
Cemsa 74/228	CIP	Kenya
Xushu 18	CIP	Kenya
SPK 004(441768)	CIP	Kenya
Yanshu 1	CIP	Kenya
Humbachero	CIP	Kenya
Ejumula	CIP	Nigeria
Pipi	CIP	Kenya
Excel	CIP	Kenya
Wagabolige	CIP	Kenya
Kemb 37	CIP	Kenya
Ukerewe	CIP	Kenya
Apomuden	CIP	Kenya
Jewel	CIP	Kenya
K135	CIP	Kenya
Zapallo	CIP	Kenya
Naveto	CIP	Kenya

Resisto	CIP	Kenya
Blessbok	CIP	Kenya
Kayia White	CIP	Kenya
KayiaWhiteXantroli	CIP	Kenya
CIP 13 Beauregard	CIP	South Africa
Kayia Red	CIP	Kenya
Mayai	CIP	Kenya
AOA 98/066(Y)	PGRRI	Bunso, E/R-Ghana
BAD 03-021	PGRRI	Bunso, E/R-Ghana
BAD 03-032	PGRRI	Bunso, E/R-Ghana
BD 96 -072	PGRRI	Bunso, E/R-Ghana
BOT 03-020	PGRRI	Bunso, E/R-Ghana
AOAWhite 98/066	PGRRI	Bunso, E/R-Ghana
BAD 03-037	PGRRI	Bunso, E/R-Ghana
BAD 03-027	PGRRI	Bunso, E/R-Ghana
BAD 03-110	PGRRI	Bunso, E/R-Ghana
BOT 03-036	PGRRI	Bunso, E/R-Ghana
BOT 03-028	PGRRI	Bunso, E/R-Ghana
Jukwa Orange	-	Jukwa, C/R Ghana
EA 08-001	John Armah	Aboadzi,W/R-Ghana
DAAK08/008	Ketu	Ghana
DAAK 08/002	Akatsi	Akatsi, Ghana
DAAK 08/003	Fafa Avorkpo	Xantroli Ketu
DAAS 07/001		Xantroli, Ketu Ghana

Kokrozitor	-	Ketu Ghana
DAAK 08/005	Acolatsey	Xantroli Ketu, Ghana
DAAK 08/007	-	Ketu-North-Ghana
DAD 08/001	-	Ketu-North-Ghana
DAAK08/004	Acolatsey	Ketu-North-Ghana
AB 07/001	-	Ohawu, Ghana
AB 07/002	-	Ohawu, Ghana
DAAK 09/006	-	Ohawu, Ghana
ADA 06/001	-	Ohawu, Ghana
Gintor	F. Nanewortor	Ehi-Homeglobo, Ketu
Hitor Asiator	Fred Danku	Kporkuve, Ketu, Ghana
DAK 08/001	-	Kporkuve, Ketu Ghana
ADA 06/001	-	Kporkuve, Ketu Ghana
DAAK 08/001	Yawvi	Abor, Ghana
AB 07/002	-	Abor, Ghana
DAAK 08/006	Ben Acolatsey	Kporkuve, Ketu Ghana
Fiaso Local	Farmers	Fiaso B/A-Ghana
K566632	-	Wenchi-Ghana
AOB 09/001	Kwasi Owusu	Fumesua, Ash Reg.Ghana
AS 07/001	Salifu	Kwadaso-Kumasi-Ghana
AS 07/001	Salifu	Kwadaso-Kumasi-Ghana

Field Establishment and Operations

The field experiment was conducted on clayey loam soil at the Crops Research Institute experimental plots at Fumesua. The site of the experiment

falls under forest ecological zone in the Ashanti region of Ghana. The area is characterized with an annual rainfall ranging between 1000 mm to 1500 mm and mean day temperature of 25 ^{o}C. Land area used for the experiment was 5808 m^2 for both major and minor seasons. The experiment was repeated to verify the accuracy of the rated morphological traits. Each accession was planted on 3 ridges of 5 meters long each. The height and width of ridges were (60×60) cm. The planting distance was 30 cm between each vine on the ridge and 16 cut vines of approximately 15 cm each were planted on each ridge. This gave a total number of 48 vines for each replication. There were three replications and therefore, a total number of 144 vines for each accession were planted for the first season. Similar work was done for the second season.

Cultural Operations

The field was slashed on the 5th of May, and ploughed on 12th of May, 2009 respectively. The field was then harrowed, plots marked out and ridges were constructed from the 13th to the 17th of May 2009. Planting was done on the 18th May, 2009. First weeding was done on the 5th week after planting and the second weeding was also done the 10th week after planting. Harvesting was done after twenty-one weeks. The minor season experiment commenced in August, 2009. Activities carried out included: slashing, ploughing, harrowing, field layout and ridging from the 12th to the 22nd of August, 2009. Planting was on the 24th of August 2009. Harvesting was done twenty-one weeks after planting on 2nd January, 2010. The parameters of the plants shoot were taken from the 12th-16th week after planting. The roots parts were also assessed in the 21 st week which was the harvesting time.

Identification of Morphological Characters and the Agronomic Assessment

Eighty seven (87) accessions were cultivated at Fumesua.The morphological characterization comprised three areas which were; the vegetative parts which constituted; the leaf shape, mature, immature leaf colour, the vines, the vine length, vine diameter, vine colour, hairiness. The second part was the storage roots which constitute: cortex thickness, root shape, secondary skin colour, distribution of secondary skin colour were taken. Flesh colour, viral disease, *Cylas spp*, *Alcidodis* and Millipede pest scoring were also recorded recorded. Other harvesting data like plant stand at harvest, roots weight and vegetative weight were also recorded.

Morphological characterization

The characterization was performed using sweetpotato descriptors produced by CIP and approved by the International Board for Plant Genetic Resources Rome, Italy. The morphological assessment comprises three areas namely; the vegetation and the storage parts and the agronomic assessment or evaluation

Aerial characteristics

The vegetative data was taken 90 days after planting (DAP) starting with the immature leaf colour and ending with the ground cover or canopy of the plant. These were scored as follows:

Immature Leaf Colour

The immature leaf which is the newly developed leaves produced by the plants normally have different colour as they have not developed much chlorophyll. With the help of Munsell colour chart and the sweet potato

descriptor brought nearer to the immature leaves, the colour that matches that leaves were picked as the colour of the immature leaves. There were different colours that the colours were chosen from in line with the parameter produced in descriptor: (A) Purple both surfaces, (B) Green upper lower, (C) Mostly purple, (D) Slightly purple, (E) Green with veins on upper surface, (F) Greyish-green (due to heavy pubescence), (H) Green and (I) Yellow-green

Mature Leaf Colour

The under mentioned parameters were chosen from with the help of Munsell colour chart. Here again the colour chart was brought closer to the plant and the following parameters were looked for comparison and the ones were picked.The colours that picked from were: (A) Purple both surfaces,(B) Green upper and purple lower,(C) Mostly purple,(D) Slightly purple,(E) Green with purple veins on upper surface,(F) Greyish-green,(G) Green with purple edge,(H) Green,(I) yellow-green.

Predominant Vine Pigment Colour

The Munsell colour chart was used to show the pigment colour of the vine (stem) of the sweetpotato plants.The following parameters produced in the descriptor were compared to the colour of the vines of the accessions that were under study: (A) Totally dark purple, (B) Totally purple, (C) Mostly dark purple (D) Mostly purple, (E) Green with many dark purple spots, (F) Green with many purple spots, (G) Green with many purple spots, (H) Green with few purple spots and (I) Green.

Leaf Shape

The sweetpotato descriptor which described different types of leaf shapes was used to select the right leaf shape of the accession under investigation. Parameters that were measured under the leaf shape include: (A) Almost divided. (B) Lobed, (C) Hastate (trilobular and spear-shaped with the basal lobes more or less divergent), (D) Triangular, (E) Cordate (heart shaped), (F) Reniform (kidney shaped and (G) Rounded.

Ground Cover

The vegetation ground cover measurements were done 35 -40 days after planting. Measuring tape and string were used to measure the peripheries of the plant's vegetative coverage.Then a measurable shape was made out of the string to calculate the area covered by the plant vegetation. Bare grounds within vegetation cover were measured and when added up were subtracted from the total area obtained from the area produced by the string. The measurements were recorded as follows: (A) Total [>90%] (C) High [75-90%] (E) Medium [50-74%] (G) Low [<50%].

Hairiness

The degree of hairiness was measured at the apex of immature leaves using the following scale: (A) Heavy (C) Moderate (E) Sparse (G) Absent. This measurement was done with the help of a hand lens which magnified the hairs if they are present for good observations.

Mature Leaf Size

Leaf size was taken from the length of the base lobes to the tip of the leaf. The average expression of at least three leaves located in the middle section of the main vine of the plants were selected for the measurement. These measurements were performed with the electronic calipers.The data were recorded as follows: (A) Very large (>25 cm), (C) Large (16-25 cm), (E) Medium (8-15 cm), (G) Small (<8 cm).

Vine Internode Length

The **v**ine internode measurements were taken as follows: The average expression of three internodes located in the middle section of the main vine of the plants. This was made possible by use of electronic venier calipers. The measurements recorded were compared and grouped as directed by CIP descriptors and the vine internodes length were grouped as follows: (A) Very long (>12 cm), (C) Long (10-12 cm) (E) Intermediate (6-9cm), (G) Short (3-5 cm) (H) Very short (<3 cm).

Vine Internode Diameter

The vine internode diameter measurements were taken with the help of electronic callipers. These data were grouped as follow: (A) Very thick (>12 mm) (C) Thick (10-12 mm) (E) Intermediate (7-9 mm) (G) Thin (4-6 mm).

Vine Colour

These are Anthocyanin pigment action present in the vines besides the green colour. This was done with the use of the Munsell colour chart and the CIP

descriptors. The colour chart was brought near the vine and the colour that corresponds the vine colour was selected. The predominant colour was evaluated from the base of the main plant to the tip of the plant. The colours were selected from: (A) Totally dark purple (B) Totally purple (C) mostly dark purple (D) Mostly purple, (E) Green with many dark purple spots (F) Green with many purple spots (G) Green with few purple spots and (H) Green.

Petiole Length

The lengths of three petioles were measured and the average taken to represent the actual petiole length. These lengths were compared and grouped according to the following scales: (A) Very long (>40 cm), (C) Long (31-40 cm), (E) Intermediate (21-20 cm), (G) Short (10-20 cm), (I) Very short (<10 cm). These measurements were taken with calipers.

Petiole Colour

Some of sweetpotato plants have anthocyanin pigmentation which are distributed on the leaf petioles. The Munsell colour chart was used to select the colours by bringing the colours chart near the plant. The selected colour was picked from CIP descriptor grouping as follows: (A) Totally or mostly purple, (B) some petiole purple others green, (C) purple with green near leaf, (D) Green with purple stripes, (E) Green with purple spots throughout petiole (F) Green with purple at both ends, (G) Green with leaf purple near leaf (H) Green with purple near stem and (I) Green Some of the characteristics measured from potato leaves are shown in Plates 1 A to 1 F.

Plate 1A shows hastate leaf shape and yellowish-green leaf colour

Plate 1B shows lobed leaf shape and green with purple edge colour

Plate 1C shows lobed leaf shape and yellowish green colour at immature stage, green leaf at mature stage and green green vine at mature stage

Plate 1D shows triangular leaf shape with purple leaf colour at immature stage and green leaf colour at mature stage

Plate 1 E shows hastate leaf shape and green leaf colour at mature stage

Plate 1F shows cordate leaf shape, green leaf and green petiole colour at maturity stage

Storage Root Characteristics

The following storage root characteristics were recorded four months after planting (MAP).

Storage Root Shape

CIP descriptors were used as a guide to determine the shapes and measurements of the root shapes of the harvested plants. These measurements were taken on longitudinal section of the roots and data were taken as follows: (A) Long irregular or curved, (B) Long elliptic-elliptic outline with a (L/B) ratio of more than 3 to1 (C) Long oblong - oblong outline with a (L/B) ratio of more than 3:1,), (D) Oblong –almost rectangular outline with sides nearly parallel and corners rounded (L/B) ratio about 2:1 (E) Obovate - inversely ovate outline. The broadest part is at the proximal end (that is close to the root stalk, (F) Ovate- outline resembling the longitudinal section of an egg.The broadest part is at the distal end (i.e,away from the root stalk), (G) Elliptic -symmetrical outline with the maximum breath at equal distance from both ends which are slightly acute (L/B) ratio not more than 3:1 , (H) Round ecliptic – a slightly circular outline with acute ends (L/B) ratio not more than 2:1,(I) Round- almost a circular outline with a length to breath (L/B) ratio of about 1:1

Root surface Defect

With the root surface defect, the roots were harvested and washed in clean water to give a good observation. The observed root surfaces were thoroughly examined.These were then compared with CIP descriptors which is givimg as

follows: (A) Other (as the observer has seen it), (B) Deep constrictions and deep grooves, (C) Deep longitudinal grooves, (D) Shallow grooves, (E) Deep horizontal constrictions, (F) Shallow horizontal constrictions, (G) Veins, (H) Alligator-like skin (I) No defect.

Storage Root Cortex Thickness

These were measured as follows: (A) very thick (>4) mm, (C) Intermediate (2-3) mm, (D) =Thin (1-2) mm and (E) = Very thin (<1) mm.This was done after removal of the root cotexes of 3 plants of the same accession and each measured with the electronic calipers.

Storage Root Skin Colour

The evaluation was done when roots were freshly harvested. Storage roots were washed with water to get a clean skin. Munsell colour chart was brought near the tubers to pick the colour that matches the storage root skin colour. The storage root skin colour was divided into 3 groups as: (i) Predominant skin colour, (ii) intensity of predominant skin colour and (iii) secondary skin colour.

Predominant Root Skin Colour

Sweetpotato roots skin con possess one or more colours of which one of them dominates and the other representing the secondary colour.The CIP roots skin colour descriptor and Munsell colour chart were used to pick the predominant colour of the accessions under investigations.The predominant root skin colour of the accessions used include: (A) Dark purple., (B) Purple - red, (C)

Red, (D) Pink , (E) Brownish orange, (F) Orange, (G) Yellow, (H) Cream and (I) white.

Intensity of Predominant Root Skin Colour

This parameter grouped accessions into 3 and they are: (A) Dark. (B) Intermediate (C) Pale With this investigation the CIP descriptor and Munsell colour chart were used to select the right intensity colour the skin possessed.

Secondary Root Skin Colour

This parameter grouped the accessions into: (A) Dark purple, (B) Purple - red, (B) Purple - red, (C) Red (D) Pink (D) Pink, (E) Brownish orange, (E) Brownish orange, (F) Orange, (G) Yellow, (H) Cream (I) White and (0) Absence of secondary colour. The secondary root skin colours of the accessions were picked with the help of the CIP roots skin descriptors. This was performed by comparing the root skins to the descriptor colours shown in the CIP guide book and the munsell colour chart as was done with the predominant root skin colour.

Storage Root Flesh Colour

This description started from cross sections and longitudinal sections made at the middle of the selected freshly harvested storage roots. This description was done by using the CIP flesh colour description and the Munsell colour chart to pick the right colour for the root flesh.This was divided into three sections including:

(i) **Predominant Flesh Colour:** Consisted of the following characteristics: (A) Strongly pigmented with authocyanin. (B) Dark orange, (C) Intermediate orange, (D) Pale orange, (E) Dark yellow (F) Pale yellow (G) Dark cream, (H) Cream and (I) White. This selection was done as described above.

(ii) **Secondary Flesh Colour:** This was consisted of the following traits**:** (A) Dark purple. (B) Purple (C) Purple-Red (D) Red (E) Pink (F) Orange (G) Yellow (H) Cream (I) White and (O) Absent. The CIP descriptor and the munsell colour chart were then and again used to select the colour that comes next after chosing the predominant colour.

(iii) **Distribution of Secondary Flesh Colour:** The CIP descriptor was used to determine the presence and the nature of the secondary colour distribution in the tuber flesh. The observed colour was compared and grouped as follows: (A) Covering all flesh.(B)) Covering most of the flesh, (C) in longitudinal sections,(D) Ring and other areas in flesh , flesh ,(E) Broad ring in flesh, (F) Narrow ring in G) Scattered spots in flesh, (H) Broad ring in cortex, (I) Narrow ring in cortex and (0) Absence of secondary flesh colour.

Storage Root Relative Range of Dispersal

This is the relative arrangements of the roots on the underground stems. These observations were determined by assessment of the clustering nature and compared them with the CIP descriptors. This was done after watering the selected plant for the observation.Absolute care was taken and the soil aroud roots were removed. Further washing away of remaining soils on the roots were also done to remove the interference from the soil. These were then compared with the CIP descriptor for the scoring.They were scored as the

follows: (A) Very dispersed. (B) Dispersed. (C) Open cluster. (D) Closed cluster.

Plate 2A shows white colour of root flesh

Plate 2B shows dark cream colour with pink spots scattered in the root flesh

Plate 2C shows cream root flesh colour with orange scattered spots in the flesh

Plate 2D shows orange root flesh colour

Plate 2E shows long oblong root shape and cream root skin colour

Plate 2F shows elliptic root shape and purple root skin colour

Harvesting Components

The harvesting components that were considered were plant stands at harvest, number of tubers obtained at harvest, weight of tubers at harvest, vine weight and dry matter content all taken from the 87 accessions of the crop.

Other areas that were also considered were the pest and disease attack. These were *Cylas spp, Alcidodes*, Millipede, and Viral infection.

With regards to the molecular characterization, harvested young leaves from the eighty seven accessions were sent to the molecular or biotech laboratory for the extraction of the DNAs from the leaves.

Deoxyribonuclic Acid (DNA) Extraction

Young leaf tissue sample of weight 100-120 mg from each sweet potato accession were placed into 2ml eppendorf tube. Liquid nitrogen was put on it and ground into a fine powder. An 800 µl of Buffer A (lysis) was put on the ground powder. This was incubated at 90^{o}C for 10 mins and vortex strongly the cell to lyse. This was then incubated at 65% for 10 min and inverted 3 times during the incubation. The lyse was cooled at room temperature for 2 min and 400 µl of 5M potassium acetate was added and mixed gently by inversion 5 to 6 times. This was incubated for 30 min with shaking. The content was centrifuged at 13,000 mm for 10 min. The upper phase of the content was transferred into a new eppendorf tube. One volume of cold isopropanol 1/10th of 3M sodium acetate, were added to the content and was mixed 10X by inverting. This was incubated at -20^{o}C for 1hr and was then centrifuged at 13,000 rpm for 10 min.

The supernatant was poured off and the pellets were washed with 800 µl of 80% ethanol. The content was centrifuged at 14,000 rpm for 5 min. The alcohol and the pellets were dried. 500µl IX TE Buffer was added to the pellets to dissolve them. About 4µl RNase enzyme was added and incubated at 37^{o}C for 30 min. About 250 µl of 7.5M ammonium acetate was added to

the content and was incubated on ice for 30mm min, this was then centrifuged at 13000 ssrpm for 5 min. The supernatant was transferred into a new 1.5 ml tube and another 700µl of Isopropanol and was mixed by inversion. This was kept on ice for 3 min. After the 3 min, it was then centrifuged at 13,000 rpm for 15 min. The supernatant was discarded and the pellets were washed with 1 ml 80% ethanol. This was then centrifuged at 14,000 rpm for 5 min. The supernatant was discarded and the pellets were dried at room temperature. The DNA pellets were dissolved in 200µl IXTE Buffer. After this procedure the DNA quality was checked on 0.8% agarose gel.

PCR Conditions

The PCR reaction mixture consisted of 50 mg template DNA, 1 XPCR buffer and 1.5mM $MgCl_2$ 0.2 mM dNTP, 250mM each of forward and reverse minors was used per 10µl PCR reaction. PCR amplifications were carried out in a BIO-RAD MyCycler ^{TM} Thermal Cycler. The SSR profiles have an initial denaturation at 95^{o}C for two min, 30 cycles of denaturation at 95^{o}C for 1 min, annealing at 55^{o}C for 1 min, extension at 72^{o}C for one min and a final extension for 10 min at 72^{o}C holding at 4^{o}C. The gels were scored and data entered in excel as present or absent bands. Genetic similarity among genotypes was evaluated unweighted pair grouping with arithmetic average (UPGMA) cluster method of genetic Darwin-jaccard distances (Sneath & Sokal, 1973).

Table 2: Sweetpotato SSR-Marker and their Sequences used to study Diversity in Sweetpotato

Primer name	Forward primers	Reverse primers	Motif	Temp°C
IB242	**5-gcggaacggacgagaaaa-3**	**5-atggcagagtgaaaatggaaca-3**	**(ct)3ca(ct)11**	**58**
IB297	**gcaatttcacacacaaacacg**	**cccttcttccaccactttca**	**(ct)3**	**58**
IB316	**caaacgcacaacgctgtc**	**cgcgtcccgcttatttaac**	**(ct)3c(ct)8**	**58**
IBCIP-1	**cccacacccttcattccattact**	**gaacaacaacaaaaggtagagcag**	**(acc)7a**	**63**
IB-R 03	**gtagagttgaagagcgagca**	**ccatagacccattgatgaag**	**(gcg)5**	**58**
IB-S10	**ctacgatctctcggtgacg**	**cagcttctccactccctac**	**(ct)12**	**60**
IB-S11	**ccctgcgaaatcgaaatct**	**ggacttcctctgccttgttg**	**(ttc)10**	**58**
IB-S17	**cagaagagtacgttgctcag**	**gcacagttctccatcctt**	**(gga)4**	**58**
IB-S18	**ctgaacccgacagcacaag**	**gggaagtgaccggacaaga**	**(tagc)4**	**58**
IBJ67	**cacccatttgatcatctcaacc**	**ggctctgagcttccattgttag**	**(gaa)5**	**58**
IBC12	**tctgagcttctcaaacatgaaa**	**tgagaattcctcggcaaccat**	**(ttc)6**	**56**
IBJ175	**atctatgaaatccatcactctcg**	**actcaattgtaagccaaccctc**	**(aatc)4**	**58**
IBJ10A	**tcaaccacttcattcactcc**	**gtaattccaccttgcgaagc**	**(aag)6**	**58**
IB-R16	**gacttccttggtgtagttgc**	**agggttaagcgggagact**	**(gata)4**	**60**
IB-R12	**gatcgaggagaagctccaca**	**gccggcaaattaagtccatc**	**(caag)5a**	**60**
IB-R21	**gacagtctccttctcccata**	**ctgaagctcgtcgtcaac**	**(gac)5**	**58**
J116A	**tcttttgcatcaaagaaatcca**	**cctcagcttctgggaaacag**	**(cct)6**	**58**
IB3/24	**tttggcatgggcctgtatt**	**gttcttctgcactgcctgattc**	**-**	**56**
IB-S01	**tcctccaccagctctgattc**	**ccattgcagacccatacttg**	**(aga)10**	**56**
IBCIP-2	**gtaacctgtcagccatctgt**	**cctagtgggtatttgcagag**	**(acc)2+6**	**63**
IB-R19	**ggctagtggagaaggtcaa**	**agaagtagaactccgtcacc**	**(cag)5b**	**60**

Data Analysis

Morphological and Molecular markers were used to detect genetic diversity in the sweetpotato cultivars assembled for the study and which are planted by the farmers and the Research institutions in Ghana. Morphological data was subjected to both univariate and multivariate analyses. Univariate analysis was

performed where each trait was tested using the analysis of variance by general linear model procedure. The multivariate analyses performed were carried out using Principal component analysis. A hierarchical clustering was done and dendrogram was constructed using Darwin-jaccard distance similarity and dissimilarity matrix and UPGMA clustering method. Prior to that the morphological data was coded into binary matrix where presence or absence was given trait (1) or (0) respectively.

CHAPTER FOUR

RESULTS

Storage Root Shape

Storage root shape separated the 87 accessions into 9 distinct groups. Group A had 32 accessions which were long irregular and accounted for 36.8% of the total accessions used. Group B with long elliptic roots had 23 accessions and accounted for 26.4% of the accessions. These accessions were long with elliptic storage root shape. Group C had 4 accessions which represented 4.6% were and mainly long oblong. Group D had 2 accessions and accounted for 2.3%. They had oblong root shape. Group E had 8 accessions and they were obovate in shape and accounted for 9.2%. Group F had 3 members and represented 3.4%. It had ovate root shape. Group G had 3 accessions and represented 3.4%. The storage root shape of this group was elliptical. Group H had 7 accessions and accounted for 8.0%. They had round elliptic root shape. The last Group I had 5 accessions and represented 5.7% of the total accessions. They were round shaped. The result showed that each group is significantly different from the other as shown in Figure 3.

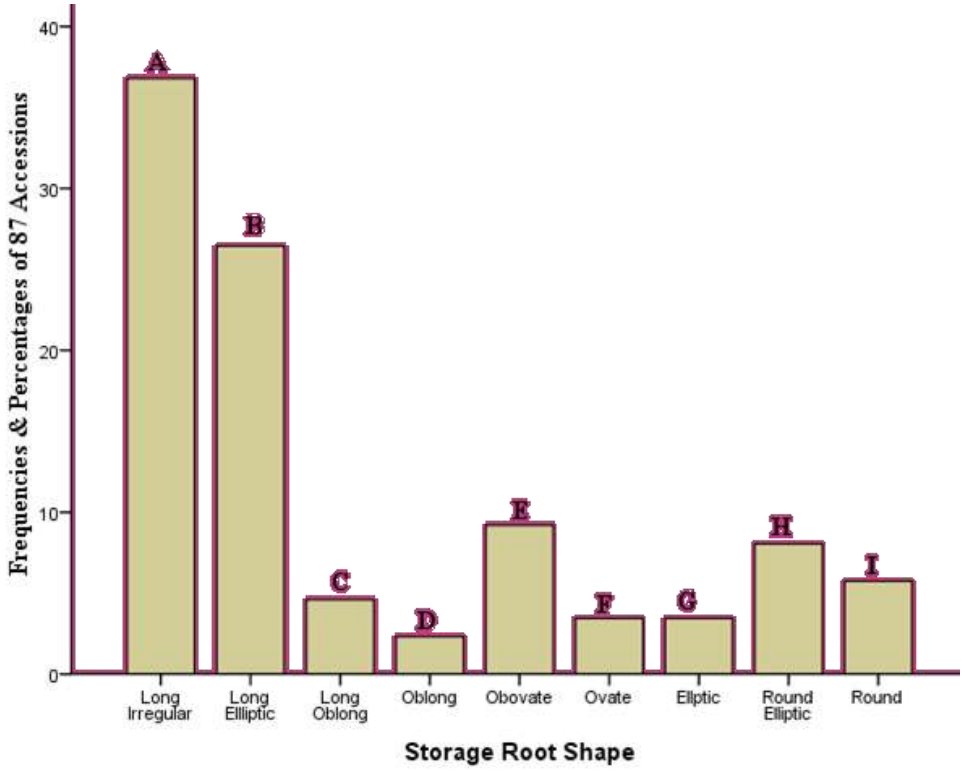


Figure 3: Distribution of storage root shape of sweetpotato accessions

Root Surface Defect

On the basis of root surface defects, five different groups were observed (Fig. 4). Group A had 12 accessions and accounted for 13.8% of the accessions. These accessions had shallow longitudinal grooves on the root surface. Group B had 2 members and accounted for 2.3%. They had shallow horizontal shape constrictions. Group C had 4 accessions and accounted for 4.6%. They had veins on the storage root surface. Group D had 2 accessions and accounted for 2.3% and they had alligator-like skin storage root surface. Group E had the largest number of the accessions (67), and represented 77% of the total. These roots showed no surface defects on their storage roots.

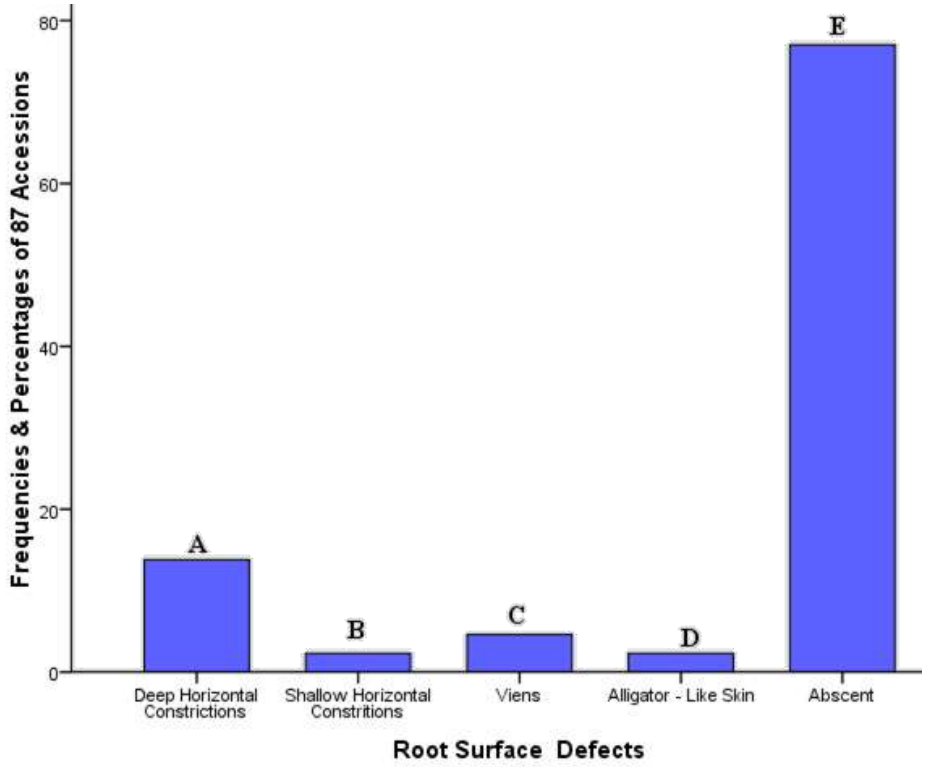


Figure 4: Distribution of root surface defects of sweetpotato accession

Root Cortex Thickness

The skin cortex thickness analysis put the accessions into four distinct groups. Group A has six accessions and made up 6.9% of the total accessions. This Group was far smaller than Group B which contained 33 accessions and represented had 37.9% of the total accessions. Groups C had 24 accessions and represented 27.6%.Group D also had 24 accessions and represented 27.6% as were depicted in Figure 5.

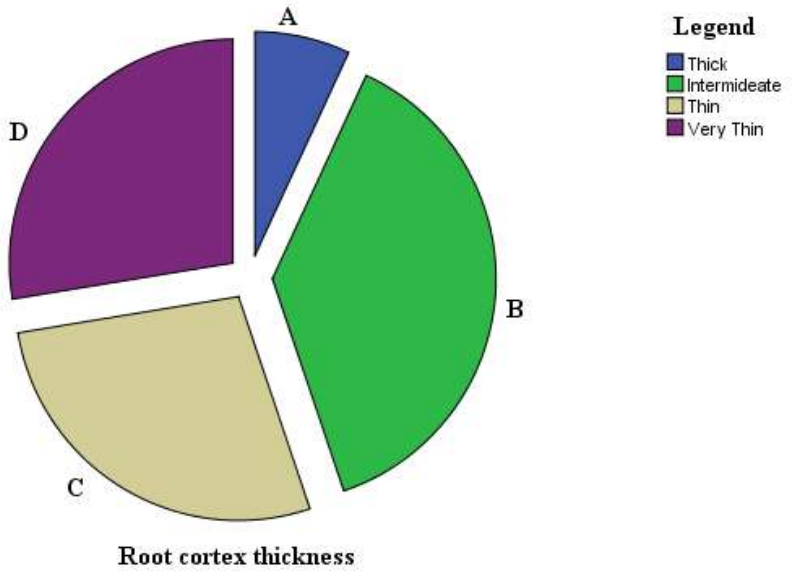


Figure 5: Distribution of root cortex thickness of sweetpotato accessions

Predominant Root Skin Colour

Various variations in skin colours were observed and were grouped as shown in Figure 6. Group A had only one accession a predominantly dark purple skin colour and accounted for 1.1% of the total population of the sweet potato accessions. Group B had 4 accessions of predominantly purple red skin colour and accounted for 4.6% of the total accessions used. Group C recorded 7 accessions of predominantly red skin colour which accounted for 8.0% of the accessions used. Group D had only one accession with predominantly pinkish red skin colour. It accounted for 1.1% of the total accessions used. Group E recorded 11 accessions and that made up 12.6% of the total accessions used with predominantly pink colour. Group F had only one accession and accounted for 1.1% of the total accessions. It poccessed brownish orange

colour. Group G had 11 accessions and accounted for 12.6% of the accessions. They had orange colour. Group H had 19 accessions of predominantly yellow skin colour and accounted for 21.8% of the total accessions. Group I had 24 accessions of predominantly cream skinned colour and accounted for 27.6% of the total accessions used. Group J had 8 accessions of predominantly white skin colour. It accounted for 9.2% of the accessions used.

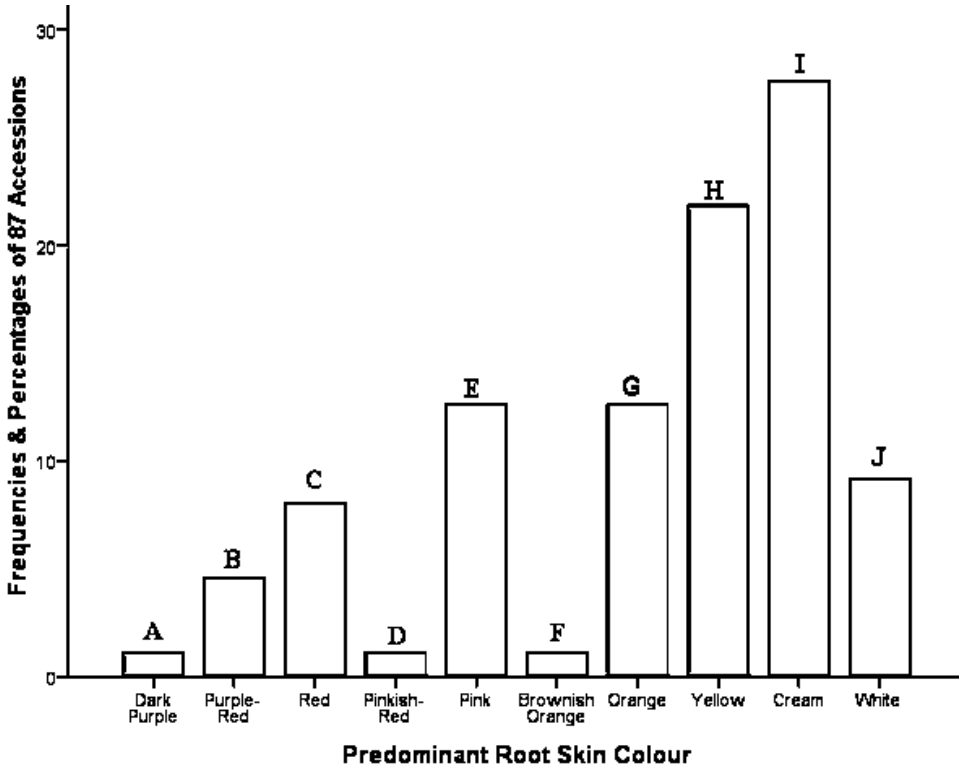


Figure 6: Distribution of predominant root skin colour of sweetpotato accessions

Secondary Root Skin Colour

Secondary skin colour was used to differentiate two accessions where the predominant skin colour could not clearly show the variations. From the results 6 groups of secondary skin colour were identified as shown in Figure

7. Group A had 4 accessions with dark purple secondary skin colour and accounted for 4.6% of the total accessions used. Group B, had one accession of purple red secondary skin colour which accounted for 1.1% of the total accession used. Group C which comprised 14 accessions had red secondary skin colour and constituted 16.1% of the total accessions used. Group D which recorded one accession also had pink secondary skin colour and accounted for 1.1% of the total accessions. Group E also had one accession with brownish orange secondary skin colour and accounted for 1.1% of the total accessions. Sixty-six accessions were registered under group F and accounted for 75.9% of the total accessions used. This group did not show any secondary skin colour.

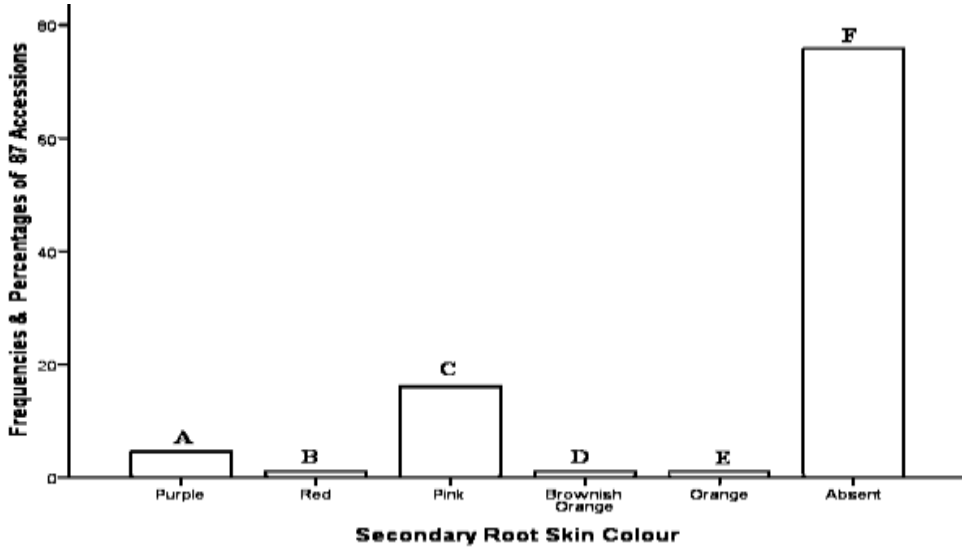


Figure 7: Distribution of secondary root skin colour of sweetpotato accessions

Predominant Root Flesh Colour

Eight groups of predominant root flesh colour were observed (Figure 8), Group A had 11 accessions and accounted for 12.6% of the total accession

used. They had a predominant flesh colour of dark orange. Group B which had 5 accessions accounted for 5.7% of the total accessions and had intermediate orange colour. Group C had 4 accessions and accounted for 4.6% of the accessions used. They had pale orange colour. Group D had 15 accessions and accounted for 17.2% and had dark-yellow colour. Group E had 16 accessions which represented 18.4% and were pale-yellow coloured. Group F had 4 accessions and accounted for 4.6%. They also had dark-cream flesh colour. Group G had 22 accessions and had cream flesh colour and accounted for 25.3%. Group H had 10 accessions and accounted for 11.5% and has white flesh colour.

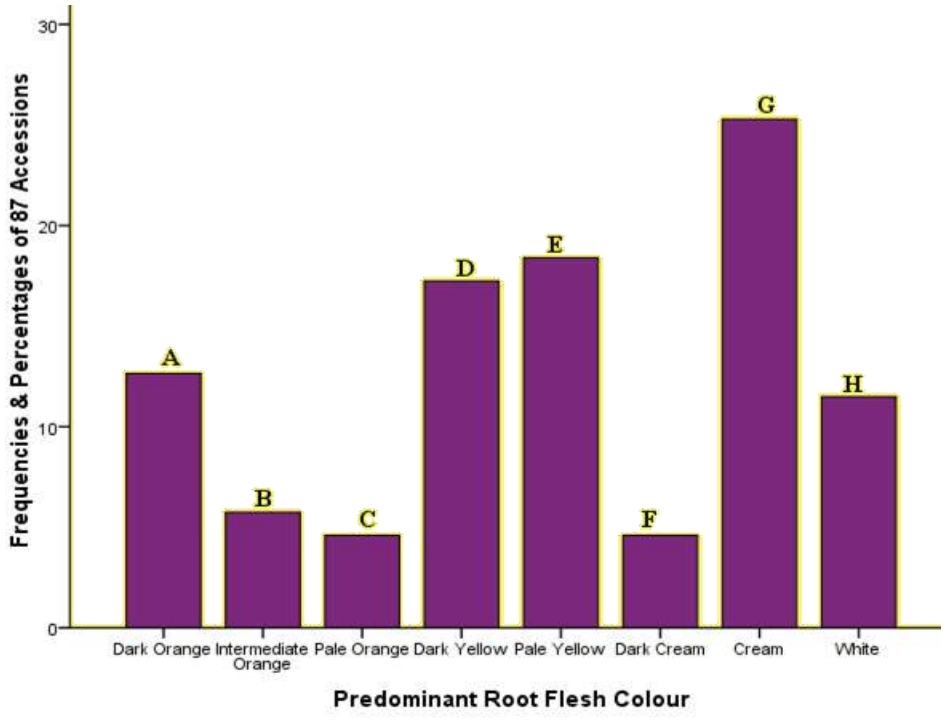


Figure 8: Predominant colour of root flesh of sweetpotato accessions

Secondary Colour of Root Flesh

Secondary flesh colour can be employed to differentiate between the accessions where predominant colour of root flesh cannot show distinct differences. Secondary flesh colour separated accessions into 5 distinct groups (Figure 9).The first group, A had orange secondary flesh colour and contained 13 accessions which represented 14.9% of the total. The second group B, had yellow secondary flesh colour had 12 accessions and accounted for 13.8% of the total accessions used. The third, fourth and fifth groups were white, cream and no colour and had 9, 7 and 46 accessions and represented 10.3, 8.0 and 52.9% of total respectively.

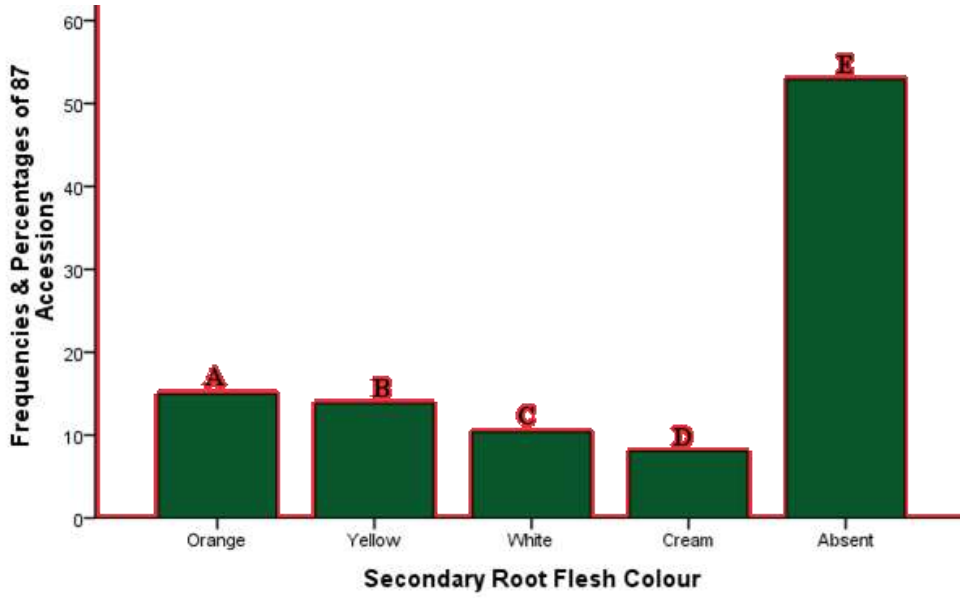


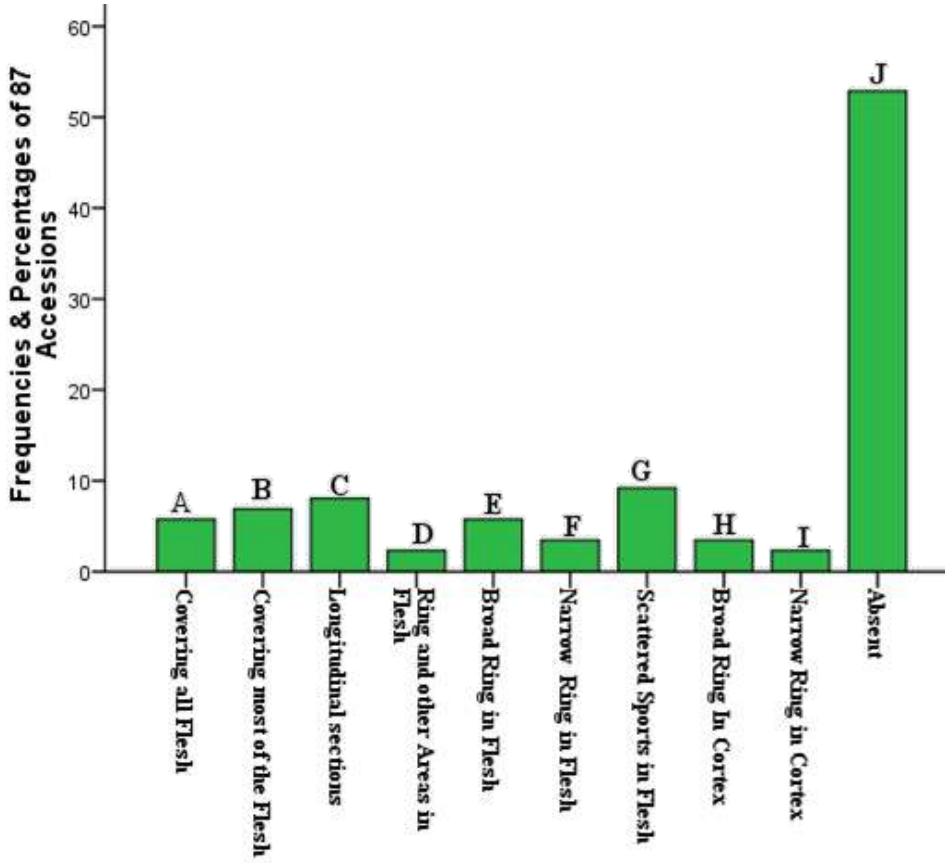
Figure 9: Distribution of secondary colour of root flesh of sweetpotato accessions

Distribution of Secondary Colour of Root Flesh

The distribution of secondary flesh colour also revealed 9 groups (Figure10). Group A had 5 accessions and constituted about 5.7% of the total accessions

with the colour distributed colour all over the flesh. Group B had 6 accessions and accounted for 6.9% of the total accession used with distribution of the colour covering most of the flesh. Group C had 7 accessions which accounted for 8.0% of the total accessions used. Their colour distribution was in longitudinal sections. Group D had 2 accessions and accounted for 2.3%. This group had secondary flesh colour in the form of rings in the flesh. Group E had 5 accessions and accounted for 5.7% of the total accessions and had their secondary flesh colour distributions in the form of broad ring. Group F had 3 accessions and accounted for 3.4% and had secondary colour in the form of narrow rings distributed in their flesh. Group G has 8 accessions and accounted for 9.2%.

Their secondary flesh distribution was scattered spots in flesh. Group H had 3 accessions and accounted for 3.4%. This group had broad ring in cortex. Group I had 2 accessions and accounted for 2.3% and had the secondary colour distribution in form of narrow ring in their flesh cortex. Group J had 46 accessions and accounted for 52.8% of the total number of accessions. There is no secondary flesh colour in this group.



Distribution of Secondary Colour in Root Flesh

Figure 10: Distribution of secondary colour in root flesh of sweetpotato accessions

Root Dispersal in Soil

In the root distribution in the soil four groups were realized (Figure 11). Group A had 2 accessions, 2.3% of the total accessions with very dispersed root formation. Group B had 37 accessions and accounted for 42.53% of the total accessions. They had dispersed root formation. Group C had 47 accessions which accounted for 54.02%. They had open clustered root formation. Group D had only one and accounted for 1.15%. They had closed clustered root formation.

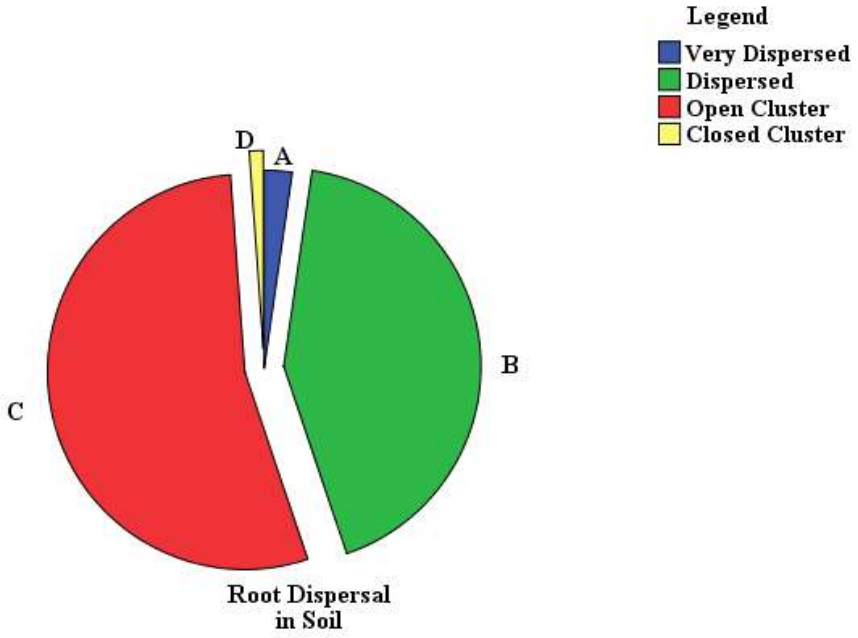


Figure 11: Distribution pattern of root dispersal of sweetpotato accessions in the soil

Ground Cover of Sweetpotato

Ground cover analysis separated the 87 accessions into 4 groups (Figure 12). In Group A, 6 accessions which represent 6.9% out of the total gave the maximum ground cover of more than 90%.Group B, comprising 23 accessions representing (26.4%) of the total had ground cover of between 75-90%. Group C made up of 42 accessions (48.3%) of the total had medium ground cover while 16 accessions(18.4%) of the total,classified as low ground cover had a range below 50%, constituted group D.

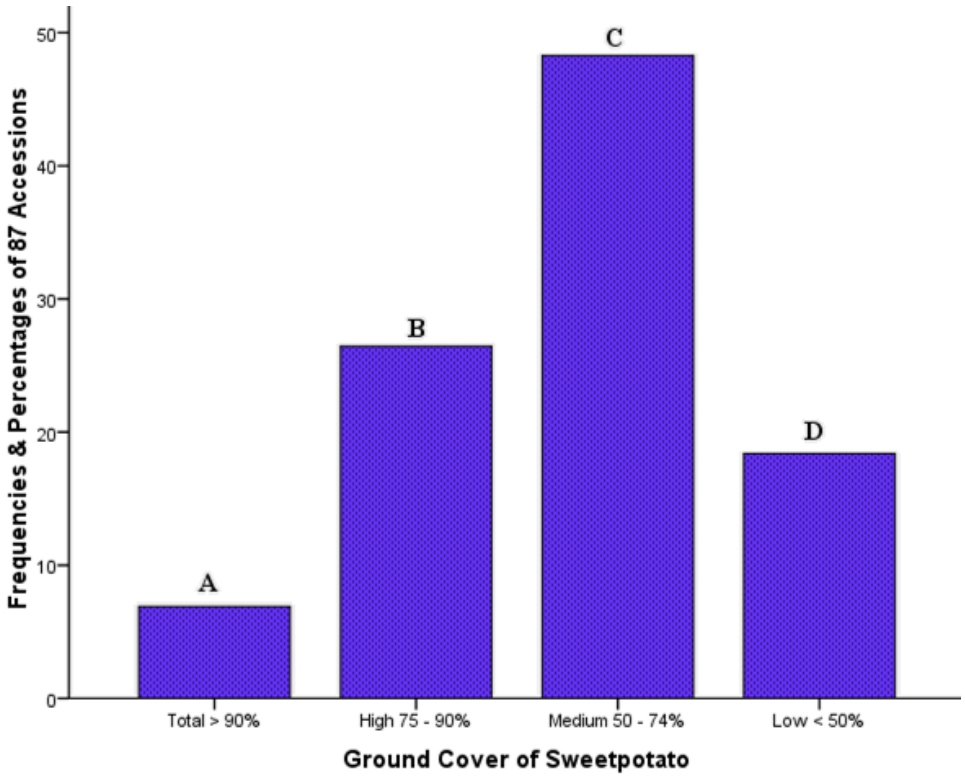


Figure 12: Distribution percentage ground cover of sweetpotato accessions

Immature Leaf Colour

Five distinct immature leaf colours occurred among the 87 accessions (Figure 13). Group A had 2 accessions and had purple upper surfaces. Group B had almost purple at both leaf surfaces. Group C had green with purple veins on the upper surface Group D had green with purple edge whilst Group E had completely green colour on both surfaces.

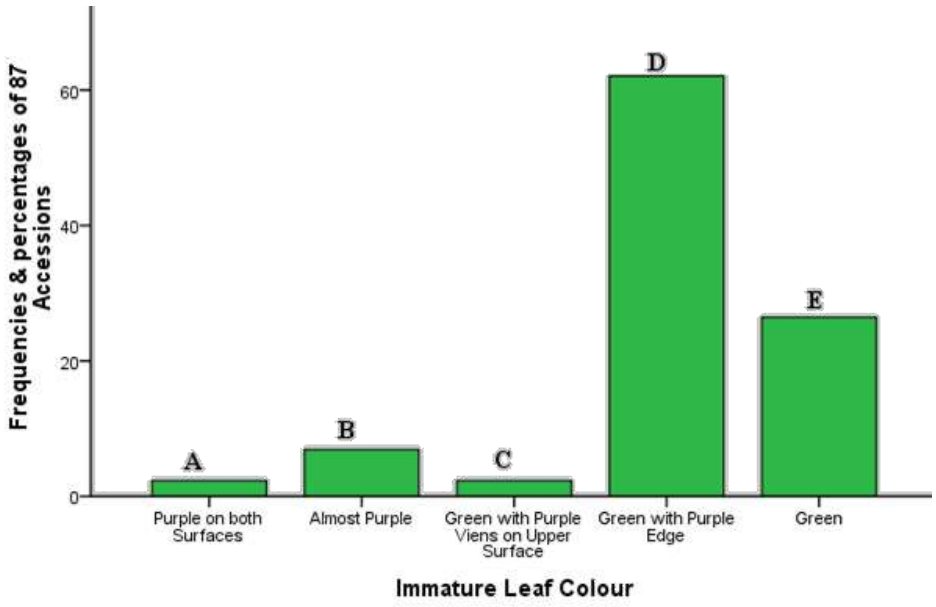


Figure 13: Distribution of immature leaf colour of sweetpotato accessions

Mature Leaf Colour

From Figure 14 mature leaf colour segregated the accessions into 3 categories. Group A (10.92 %) had purple colour on both surfaces. Group B (78.16%) of the accessions had green with purple veins on upper surface at the mature stage. Group C (10.92%) had green with purple edge.

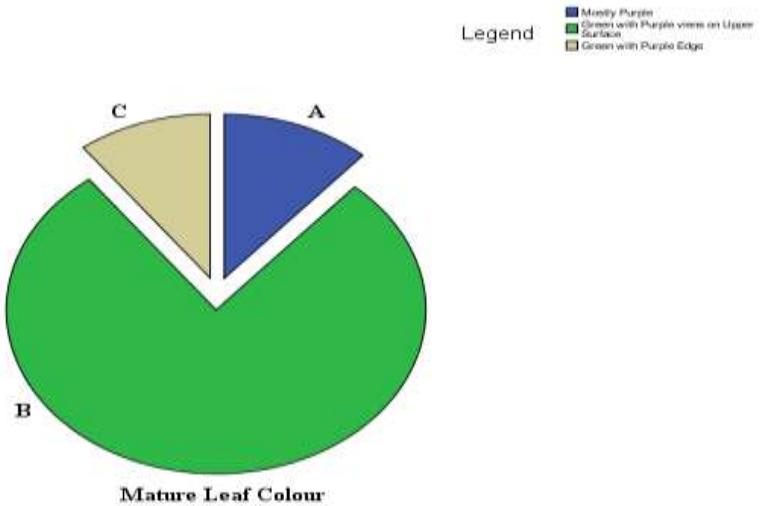


Figure 14: Distribution of mature leaf colour of sweetpotato accessions

Hairiness of Vine Tip

The analysis of vine tip pubescence put the accessions into three categories (Figure 15) Group A had 12 accessions and accounted for 13.79% of the total. These accessions produced 'heavy pubescence'. The second group comprising 45 accessions (51.70%) showed moderate vine tip pubescence. The third group with 30 (34.48%) accessions did not have hair on their vine tips.

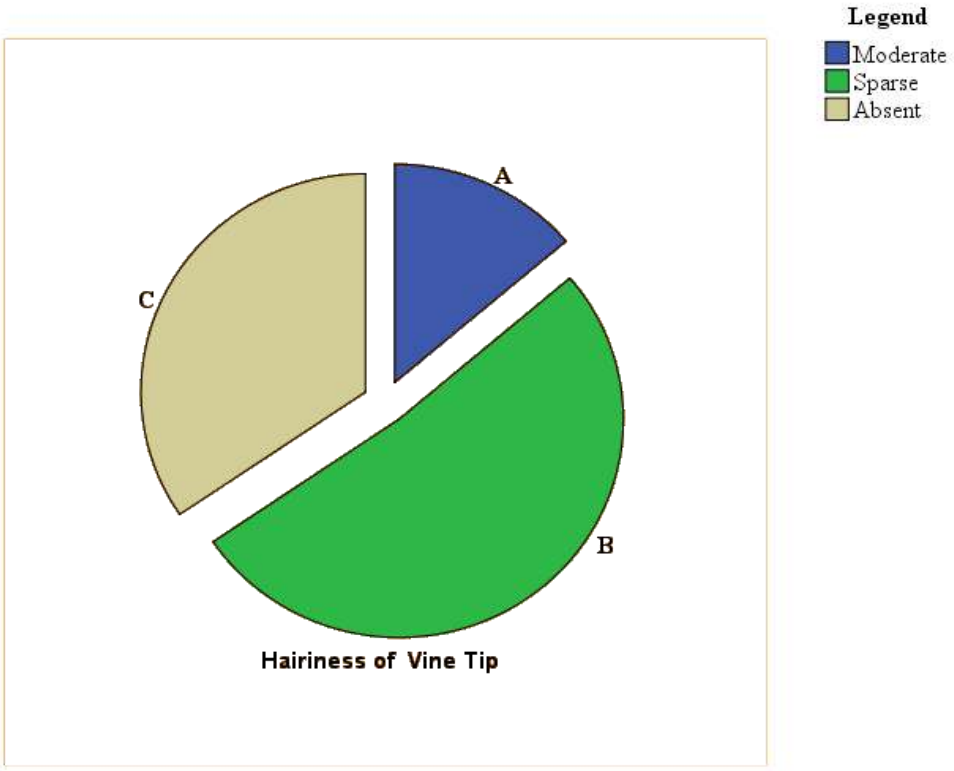


Figure 15: Distribution of hairiness of vine tip of sweetpotato accessions

Mature Leaf Shape

Due to high diversity in sweetpotato accessions, many different types of leaf shapes were observed. Based on the analysis of mature leaf shape of the 87 characterized accessions, five different types leaf shapes were identified. Group A represented Triangular leaf shape had 51 accessions which

accounted for 58.6% of the total accessions studied. Lobed leaf shape had 21 accessions and produced 24.1% .Group C had Hastate leaf shape comprised of 11 accessions and represented 12.6%. Cordate shape had 3 accessions and accounted for 3.4% One accession had its leaf Almost divided shape (deep lobes) resulted in 1.1% of the total (Fig.15).

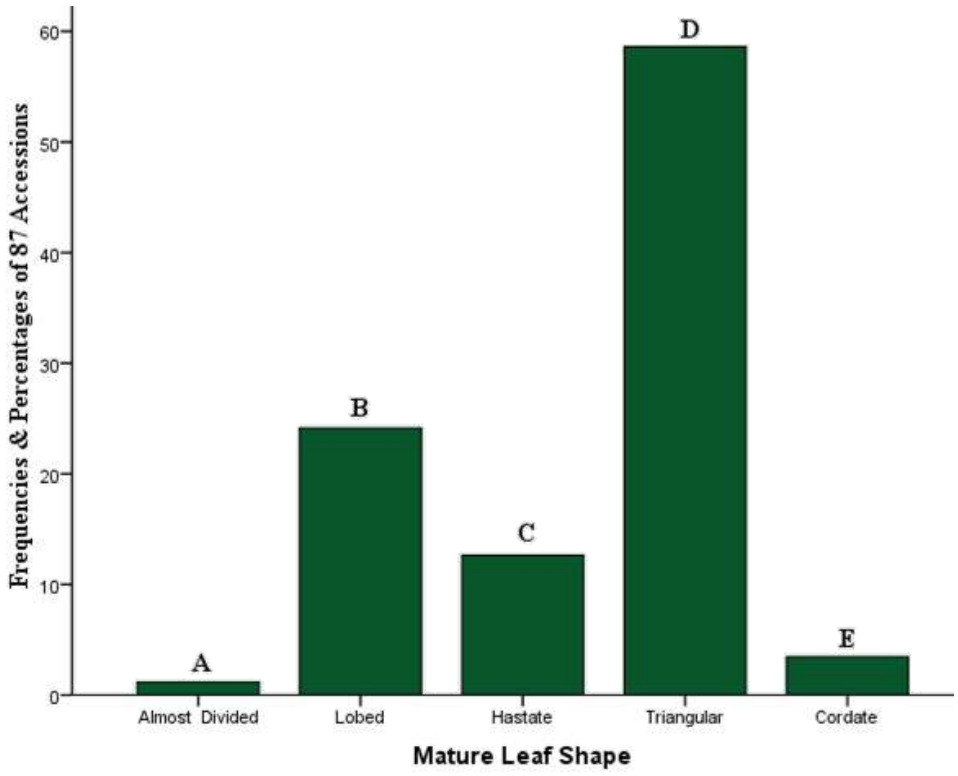


Figure 16: Distribution of mature leaf shapes among sweetpotato accessions

Vine Internode Diameter

Three groups of vine internode diameter were observed. (Figure.17) Group A had 3 accessions (3.45%) representing a diameter of 10-12 mm. Group B contained 31 accessions and made up 35.63% of the total. This group had a diameter of 7-9 mm and the third group C contained 53 accessions representing 60.92% of the total and had a diameter of 4-6 mm.

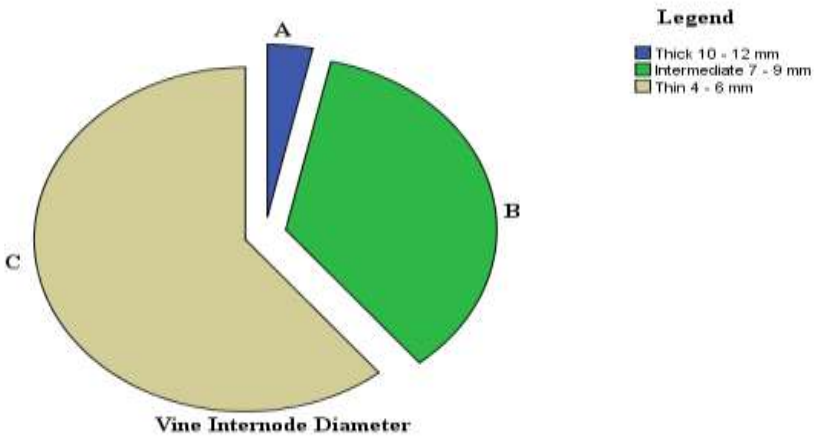


Figure 17: Distribution of vine internode diameter of sweetpotato accessions

Vine Internode Length

Four different groups of vine internode lengths were distinguished amongst the 87 accessions. The first group had 8 accessions, representing 9.2% of the total which measured 10-12 cm long. The second group of intermediate lengh (6-9 cm) comprised 20 accessions which accounted for 23% of the total. The third group had 39 accessions (44.8%) with short internode length of 3-5cm and the last group which were very short (<3 cm) comprised 20 accssions which represented 23% of the 87 accessions used for the studies.

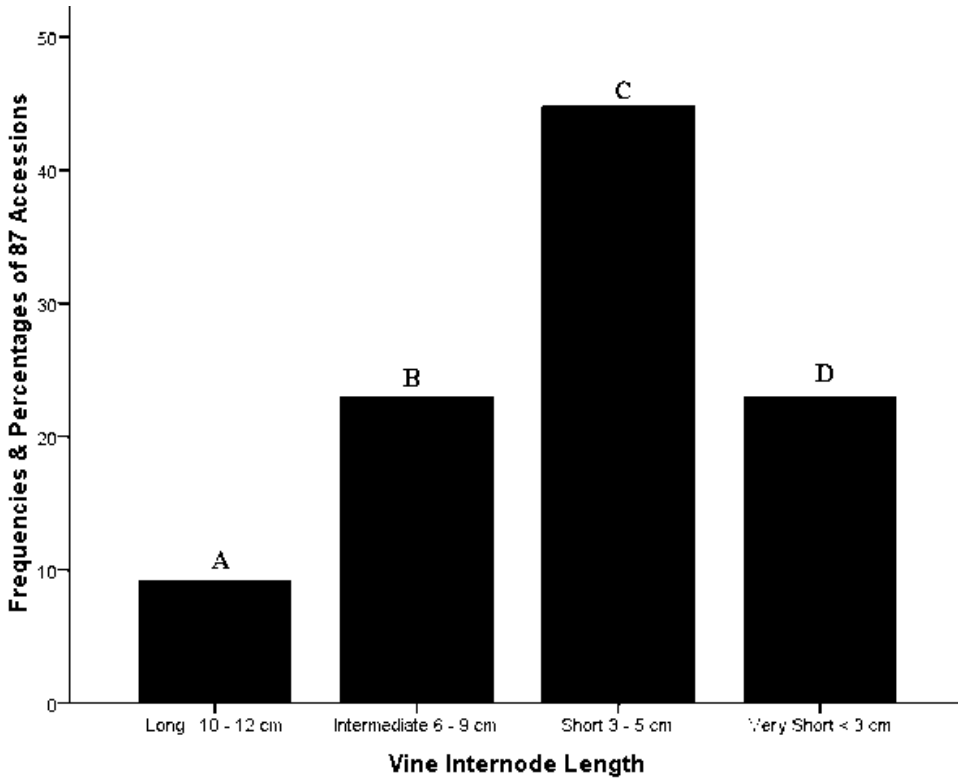


Figure18: Distribution of vine internode length of sweetpotato accessions

Vine Colour

From the analysis six kinds of vine colours were identified (Figure 19). Group A and B had one accession each and showed totally dark purple and mostly purple colours respectively and each represented 1.1% portion of the total accessions used. Group C had 5 accessions and showed green with many dark purple spots and represented 5.7% of the accessions used for the studies. Group D had 26 accessions and they were green with many purple spot on the vines. They represented 29.9% of the accessions. Group E had 51 accessions. These showed green with few purple spots on the vines and constituted 58.6% of the total number of accessions used. Group F had three accessions which had green vine colour and they constituted 3.4% of the population.

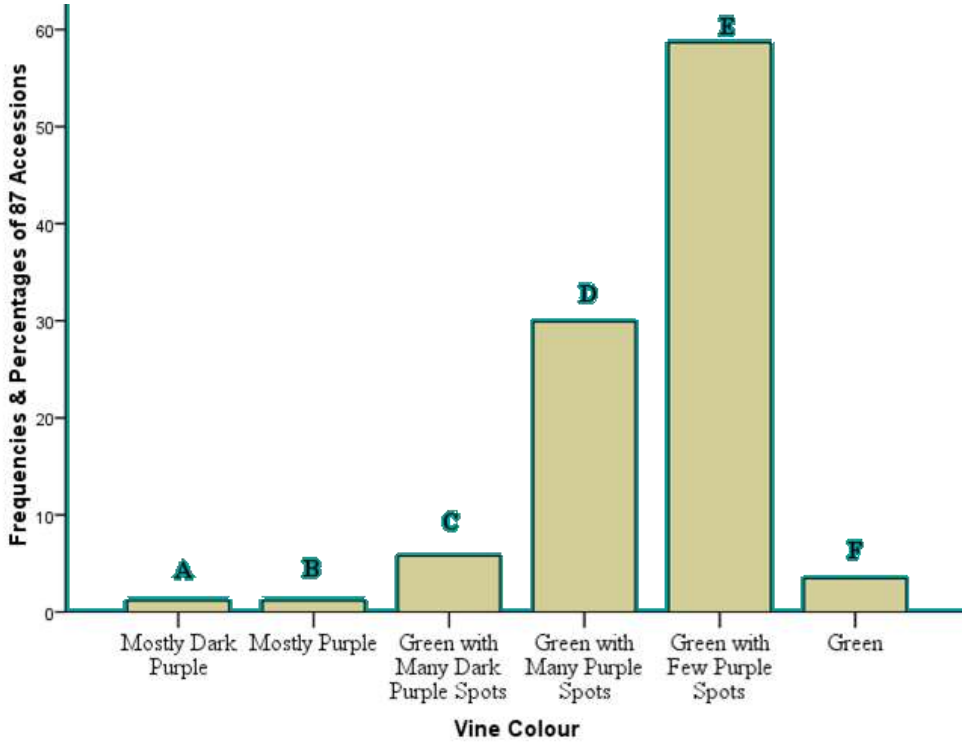


Figure 19: Distribution of vine colour of sweetpotato accessions

Petiole Length

The petiole length analysis produced 4 groups (Figure 20). Group A had only one accession with a measurement range of (31 - 40 cm) which was long (according to CIP sweet potato descriptor).This represented 1.1% portion of the whole population. Group B with 7 accessions constituted 8.0% of the total number accessions produced a measurement range of (21 -30 cm) which represented intermediate length described by the CIP descriptor. Group C had 55 accessions and represented 63.2% of the population. It had a measurement range of (10-20 cm) which was short according to the CIP descriptor. Group D had 24 accessions and represented 27.6% of the population. It gave measurements of (<10 cm) and was described as very short by the CIP descriptors.

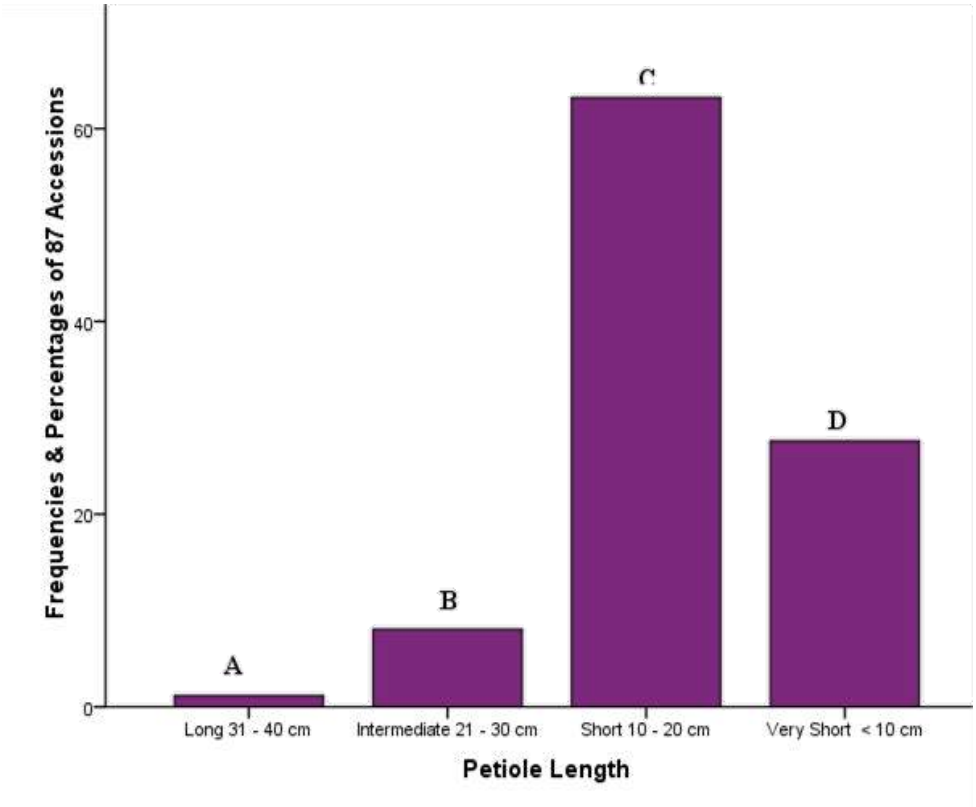


Figure 20: Distribution of petiole length of sweetpotato accessions

Petiole Colour

Petiole colour analysis resulted in 6 groups. Group A had 4 accessions and represented 4.6% of the total accessions, the petioles were mostly purple. Group B had 3 accessions which was 3.4% of the total. They had purple with green near their leaves. Group C were 37 accessions and produced 42.5%. These accessions had green petiole colour with purple stripes. Group D had only one accession. It was 1.1% of the population and exhibited green with purple spots on the petiole. Group E had 4 accessions and was 4.6% of the population.These also had green petiole with purple near the leaves. Group F had 38 accessions with green petiole colour. They represented 43.7% of the total (Figure21).

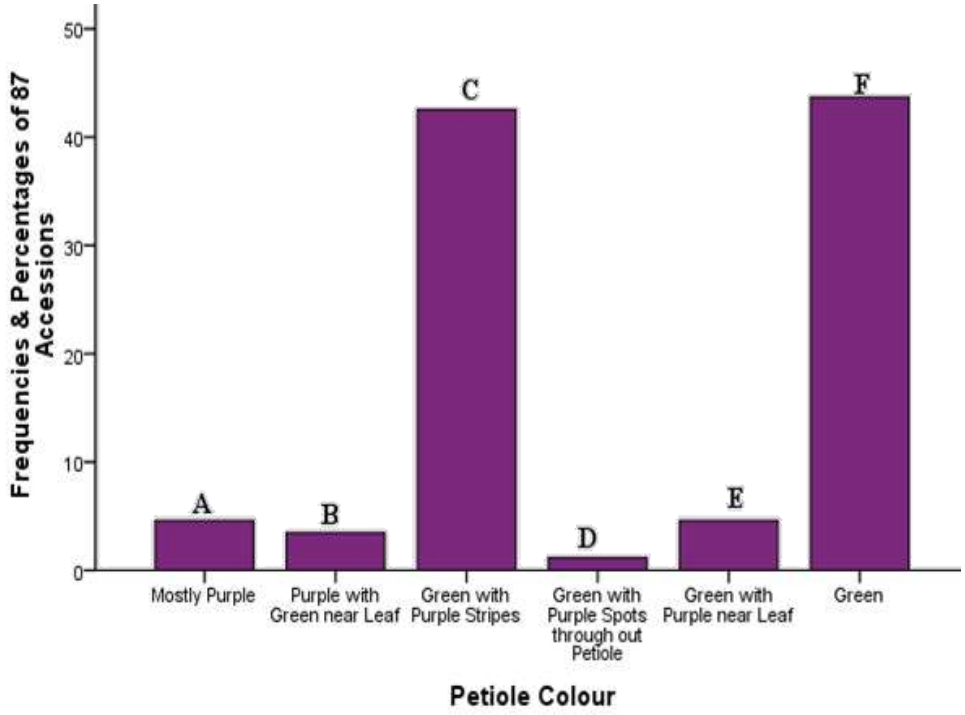


Figure 21: Distribution of petiole colour of sweetpotato accessions

Principal Components Analysis (PCA) of the Qualitative Traits of the 87 Sweetpotato Accessions

The first 10 principal components (PCA) with coefficient values greater than 1.0 together explained 35.75% of the total variance present in the Table 3. Scores on the first principal component (PC-1) which accounted for 4.52% of the total variation were highly correlated to characters related to secondary colour of the flesh, intensity of predominant colour of the skin, vine colour.The second principal component (PC-2) explained 4.45% of the total variation and was highly associated with Secondary colour of the skin, Petiole length, immature leaf colour. The third component (PC-3) which explained 4.06% of the variation was mainly correlated to characters related to the secondary colour of the flesh, Distribution of secondary colour of the flesh and Cortex thickness. The fourth component (PC-4) explained 3.72% of the total variation and was determined by

the matured leaf shape, vine colour and storage root shape. The fifth component (PC-5) was related the distribution of secondary colour of the flesh and secondary which accounted for 3.61% of the total variables. Principal component six (PC-6) explained 3.34% of the total variation and was associated with vine internode length and pubescence. Principal component seven (PC-7), principal component eight (PC-8), principal component nine (PC-9) and principal component (PC-10) explained an additional 3.19%, 3.03%, 2.96% and 2.87% of the total variation respectively. PC -7 was determined by characters such as the storage root surface defect, vine internode diameter, petiole length, root relative dispersal and storage root shape. PC -8 was determined by vine internode diameter and vine internode length characters. PC-9 was determined basically by vine internode length. PC-10 was controlled by characters such as storage root shape, immature leaf colour, and predominant skin colour.

Table 3: Eigen Value and Loading from Principal Component Analysis of Qualitative Traits in Sweetpotato

Characters	PCA 1	PCA 2	PCA 3	PCA 4	PCA 5	PCA 6	PCA 7	PCA 8	PCA 9	PCA 10
Cortex skin thickness	0.02	0.05	**0.12**	0.06	0.06	-0.05	0.01	-0.08	-0.12	-0.11
Distribution. of secondary colour flesh	**0-30**	**0.19**	**0.18**	0.08	**0.18**	0.06	-0.01	0.07	-0.01	0.09
Ground cover	**0.10**	0.03	0.03	0.01	-0.03	-0.09	0.00	0.01	-0.08	-0.04
Immature leaf colour	**0.11**	**0.13**	-0.13	-0.12	-0.05	-0.10	-0.06	-0.05	-0.14	**0.18**
Intensity of predominant colour Skin	**0.19**	0.04	0.05	-0.09	-0.13	0.09	0.01	-0.08	-0.10	-0.12
Mature leaf colour	**0.11**	0.04	0.02	-0.15	0.09	-0.09	0.06	0.07	0.03	-0.16
Mature leaf shape	0.04	0.02	0.09	**0.26**	-0.02	-0.03	0.05	-0.02	-0.01	0.07
Predominant colour flesh	**0.11**	0.01	0.01	0.08	-0.15	0.06	0.03	0.01	0.05	-0.10
Petiole length	**0.13**	**0.15**	-0.06	0.04	-0.05	-0.08	**0.12**	-0.11	0.04	-0.07

Predominant colour skin	**0.15**	0.05	0.08	-0.17	-0.07	0.06	0.06	-0.01	-0.10	**0.11**
Pubescences/Hairiness	**0.11**	0.03	0.09	0.03	-0.17	**0.16**	-0.02	0.06	0.00	-0.05
Root relative dispersal	**0.12**	0.00	0.01	0.06	-0.02	-0.01	**0.12**	0.01	0.06	-0.22
Secondary colour flesh	**0.22**	0.19	**0.18**	0.08	**0.18**	0.06	-0.02	0.08	-0.04	0.07
Storage root surface defect	**0.11**	0.06	-0.01	0.00	-0.02	-0.10	**0.15**	0.02	0.02	-0.24
Storage root shape	0.17	0.10	0.02	**0.12**	-0.07	-0.07	**0.11**	0.05	-0.02	**0.11**
Secondary colour skin	0.02	**0.15**	0.02	-0.01	-0.07	0.01	0.09	-0.08	-0.16	-0.04
Vine colour	**0.18**	0.05	-0.92	**0.15**	-0.05	-0.18	-0.09	-0.08	0.05	-0.02
Vine internode diameter	**0.17**	0.09	-0.87	-0.08	-0.07	-0.03	**0.13**	**0.13**	0.09	-0.13
Vine internode length	**0.14**	0.01	0.01	0.10	-0.23	**0.23**	-0.05	**0.19**	**0.10**	0.05
Eigen value	5.34	5.25	4.80	4.39	4.26	3.94	3.76	3.58	3.49	3.38
%variability	4.52	4.45	4.06	3.72	3.61	3.34	3.19	3.03	2.96	2.87
Cumulative variability	4.52	8.97	13.03	16.75	20.36	23.70	26.89	29.92	32.88	37.75

Distribution of 87 Sweetpotato Accessions into Different Clusters Based on Their Qualitative Characters

The 87 accessions were grouped into six clusters from qualitative traits analysis as depicted in Ttable 4. Cluster V had the highest accessions of 28. Cluster I had the least of eight accessions. Generally, the accessions were collected from Ghana, Nigeria, Kenya, Malawi, Tanzania, South Africa and Uganda with most of the accessions obtained from Sweet potato International Center (C I P) sub-station in Kenya.

Table 4: Distribution of 87 Accessions of Sweetpotato into Different Clusters based on their Qualitative Characters

Cluster Number	Number of Accessions	Name of Accessions	Source
I	8	BOT03036,BOT03028	GHANA
		K118, 199062.1, Kayia red, MOHC,	KENYA
		Zapallo	MALAWI
		Sauti	
I I	16	DAAK09/006, DAAK08/003, EA 08/001,	GHANA
		TA08/002	
		Beauregard, Ogyefo, Kayia white, Mayai,	KENYA
		Hi-Starch, Kayia white- Xantroli, Ukerewe,	
		Resisto	
		TIS86/0350, Okumkom, Ejumula	NIGERIA
		Naspot 1	UGANDA
III	13	DAAK08/008, Gintor, Hitor Asiantor,	GHANA
		AS07/001, DAAK08/007,	
		EA07/002	KENYA
		Ninshu !, Xushu18,Wagabolige, Excel,	

		Kemb 37,K135	
		CIP 13 Beauregard	S. AFRICA
I V	13	DAAK08/002, DAAK08/003, BD96072,	GHANA
		BOT03020, DAK08/007, Jukwa orange	
		Zambezi, Jonathan, Gweri, Tanzania,	KENYA
		Apomuden, Naveto	
		Santom Pona	NIGERIA
V	28	DAAS07/001, AB09/001, DAAK08/005,	GHANA
		DAAK08/004, AB07/001, AB07/002,	
		BAD03032, BAD03021, BAD03937,	
		BAD03027, AOA98/066white, Fiaso Local,	
		DAAK08/006, DAAK08/001, BAD03110,	
		ADA06/001, Kokrozitor, AOB09/001,	
		AOA98/066Y	
		NCSU1560, Santo Amaro, brondal,	KENYA
		SPK004, Jewel, Blesbok, TIS83/0138	
		Faara, Tek Santom	NIGERIA
V I	9	DAD06/01,DAD08/001	GHANA
		Carrot C, Huambachero, PIPI. K566632,	KENYA
		Yanshu 1, Cemsa 74228	
		Otoo	TANZANIA

Cluster Frequencies, Nearest Clusters and Distances between Cluster Centroids of 87 Sweetpotato Accessions on Qualitative Traits Using Principal Component Analysis

From the Table 5, cluster V had the highest number of accessions (28) and its nearest cluster was cluster II. Cluster I had the smallest number (8) of accessions. Cluster III was the nearest cluster to cluster I and cluster V farthest cluster to cluster I.Cluster VI had the farthest distance between centroids.

Table 5: Cluster Frequencies, Nearest Clusters and Distances between Cluster Centroids of Sweetpotato Accessions on Qualitative Traits using Principal Component Analysis

Cluster number	Number of accessions	Nearest cluster	Distance between cluster centroids
I	8	III	6.639
II	16	V	5.576
III	13	I	6.639
IV	13	II	6.995
V	28	II	5.526
VI	9	V	7.374

Cluster Means of Sweetpotato Accessions Associated According to Qualitative Agronomic Characters in Sweetpotato

From the cluster mean scoring (Table 6), there were differences seen in the qualitative agronomic parameters such as ground cover, immature leaf colour, petiole colour, storage root shape, secondary colour skin and the distribution of secondary colour flesh. However, within these selected clusters, there were

some similarities found between some of the clusters. Distribution of secondary colour flesh associated with cluster I, III, IV each showing differences among themselves and against cluster II, V and VI. Similar situation was observed from agronomic character of storage root shape which associated with clusters II, III, IV showing differences among themselves and also against clusters I, V, VI which had similar scoring effect. Agronomic characters suh as the intermediate predominant skin colour, mature leaf shape, mature leaf colour had no strong effect on the cluster differences.

Table 6: Cluster Means of Sweetpotato Accessions Associated According to Qualitative Characters in Sweetpotato

Agronomic character	Cluster Number /Cluster Mean					
	I	II	III	IV	V	VI
Mature leaf shape	5	5	5	5	4	5
Mature Leaf colour	2	3	3	3	4	3
Groundcover	4	6	6	5	6	4
Hairiness	2	2	2	3	3	1
Immature leaf colour	2	3	4	3	3	4
Vine diameter	4	4	4	4	4	4
Vine length	4	5	5	6	6	5
Vine colour	2	2	2	2	2	2
Petiole length	3	2	2	3	3	2
Petiole colour	2	1	5	2	6	5
Storage root shape	8	9	5	2	8	8
Root surface defect	2	1	0	1	1	1

Cortex skin thickness	5	6	6	6	5	5
Predominant skin colour	2	4	4	3	5	3
Intermediate Predominant skin colour	2	2	2	2	2	2
Secondary skin colour	3	0	2	1	0	6
Predominant flesh colour	5	4	4	4	4	4
Secondary flesh colour	3	1	1	2	1	1
Distribution Secondary flesh colour	8	1	7	2	1	1
Roots relative dispersal	4	4	4	3	4	4

Principal Component Analysis (PCA) of Morphological Quantitative Traits

Eigen values and loadings showing the relative contribution of the quantitative character in determining the overall variability among the accessions used in the diversity studies. The first 9 principal components accounted for 100% of the total variations observed. Each principal component or new variable comprised of a linear combination of the original variables recorded. The first variable accounted for 28% and had weight of storage roots, number of roots obtained at harvest and plant stand at harvest showing positive influence on variations among the accessions. The number of storage roots obtained at harvest had positive effect on the ninth new variable with 64% variable captured. The second new variable accounted for 17% of the total variation observed. This was highly associated with the *Alcidodes spp*, *Cylas spp* and Virus scores. The third principal component (PC-3) which explained 12.76% of the variation was mainly correlated to characters related to the *Cylas spp*

and weight of fresh vine. The fourth principal component (PC-4) explained 11.88% of the variation and was determined by *Cylas spp*. The fifth principal component (PC-5) was related to the fresh vine weight and accounted for 9.16% of the total variability. Principal Component seven (PC-7) explained 6.32% of the variation and was determined by Virus attack. Principal component eight (PC-8) explained 5.31% of the variation and was determined by *Cylas spp* and vine fresh weight. Principal component nine (PC-9) was controlled by number of storage roots obtained at harvest and accounted for 2.04% of the total variability which was less than 4% and therefore, considered to be of less significance to the overall variability.

Table 7: Eigen Values and Loadings from Principal Component Analysis of Quantitative Agronomic Traits in Sweetpotato

Principal component	P C1	P C 2	P C 3	P C 4	P C 5	P C 6	P C 7	P C 8	P C 9
Alcidodes spp	-0.21	**0.48**	-0.38	-0.18	**0.32**	-0.26	-0.47	-0.39	-0.03
Cylas spp	-0.29	**0.46**	0.21	**0.35**	-0.22	-0.10	-0.30	**0.61**	-0.12
Millipede *spp*	-0.16	0.35	**0.69**	-0.04	-0.29	-0.13	**0.24**	-0.51	**0.08**
Virus scores	-0.31	**0.38**	-0.32	**0.20**	**0.24**	**0.32**	**0.64**	0.04	0.06
Plant stand @ harvest	**0.40**	0.24	0.02	**0.23**	-0.17	-0.72	-0.34	-0.25	-0.10
Vine fresh weight (kg/ha)	0.31	0.20	**0.39**	-0.32	**0.64**	-0.32	-0.05	**0.32**	**0.28**
No of roots obtained @ harvest	**0.47**	0.27	-0.25	0.19	-0.29	-0.32	0.11	0.03	**0.64**

Weight of roots (Kg/ha)	**0.52**	0.29	-0.05	-0.07	0.01	-0.29	**0.29**	0.06	-0.69
Dry matter (%)	-0.09	0.20	-0.14	-0.79	-0.48	**0.22**	0.04	**0.19**	0.04
Eigen value	**2.54**	**1.53**	**1.15**	**1.07**	**0.82**	**0.67**	**0.57**	**0.48**	**0.18**
Variability percentage	**28.20**	**17.06**	**12.76**	**11.88**	**9.16**	**7.28**	**6.32**	**5.31**	**2.04**
Cum. Percentage	**28.20**	**45.26**	**58.02**	**66.90**	**79.06**	**86.34**	**92.66**	**97.97**	**100.0**

Distribution of 87 Accessions of Sweetpotato into Different Clusters Based on their Quantitative Characters

The eighty seven accessions were grouped into 5 clusters showed in Table 8. Cluster V had the largest group with 26 accessions. Out of these accessions, 16 of them were obtained from Kenya, 8 collected from Ghana and one each from Nigeria and Uganda. The second largest group had 25 accessions which was cluster I. The smallest group was cluster II and had 2 accessions and these came from Ghana and Nigeria. Generally, all the clusters had accessions from Ghana and Kenya.

Table 8: Distribution of 87 Accessions of Sweetpotato into Different Clusters Based on Their Quantitative Characters

Cluster Number	Number of accessions	Name of accessions	Source of collection
I	25	Jukwa orange, AOA 98/066white, ADA06/001	GHANA
		DAAK08/007, AS 07/001, DAAK08/006, BOT 03028	
		TA 08/002	KENYA
		TIS 83/0138, Kayia white, MOHC, Tanzania, Excel, Kayia white Xantroli, Cemsa 74 228, Apomuden, Kemb 37,	
		K566632	S. AFRICA
		CIP 13 Beauregard	NIGERIA
		Okumkom	
II	2	Fiaso Local	GHANA
		Ukerewe	KENYA

III	13	AOA 98/066Y, DAAK 09/006,	GHANA
		DAK 08/003, EA08/001,	
		DAD 08/001, BOT 03-036,	
		Hitor Asiator	
		Ogyefo, Santom pona, Naspot	KENYA
		1, Kayia red, Jewel	
		Faara	NIGERIA
IV	21	BAD 03-027, DAK08/002,	GHANA
		DAAK08/003, DAAS 07/001	
		AB09/001, DAAK 08//001,	
		DAAK08/002, DAAK08/004,	
		DAD06/001, BAD 03-110	
		NCSU 1560, Huambachero,	
		Hi-starch, Jonathan,	KENYA
		Otoo	TANZANIA
		Sauti	MALAWI
		TIS 86/0350,Tek Santom	NIGERIA
V	26	EA 07/002, DAK08/007, BAD	GHANA
		03-037, Gintor,	
		BOT03020 ,Kokrozitor,	
		BAD03021, AB07/002	
		K135, Zapallo, Naveto,	KENYA
		Resisto, Pipi, Yanshu 1	
		Wagabolige, SPK 004 441768,	
		Xunshu 18, Brondal	
		Santo Amaro, Mayai, K118,	

	Carrot C, Beauregard, Zambezi	
	Ejumula	NIGERIA
	Gweri	UGANDA

Cluster Frequencies and Distances between Centroids of Sweetpotato Accessions Based on Quantitative Traits Using Principal Component Analysis

Cluster frequency, nearest cluster and distance between cluster centroids are presented in Table 9. Cluster V had the largest number of accessions (26) with a nearest distance of 18.18 between cluster IV. Cluster II had the least number of frequencies, 2 and with cluster I as the nearest cluster centroid and the distance in between them was 18.47 one of the highest distance.

Table 9: Cluster Frequencies and Distances between Centroids of Sweetpotato Accessions Based on Quantitative Traits Using Principal Component Analysis

Cluster Number	Accessions	Nearest Cluster	Distance between cluster centroids
I	25	II	18.47
II	2	I	18.47
III	13	IV	12.42
IV	21	III	12.42
V	26	IV	18.18

Cluster Means of Sweetpotato Accessions Associated According to Quantitative Agronomic Characters in Sweetpotato

There were a considerable difference in plant stands at harvest, number of roots obtained at harvest, their weights and fresh vine weight at harvest (Table 9). The dry matter had higher effect in distinguishing between clusters and characters like incidence of *Cylas spp.*, millipede *spp.* and *Alcidodes*. Virus scores on the other hand, had low effect in differences shown between the clusters. This results support the results of the principal component analyses.

Table 10: Cluster Means of Sweet Potato Accessions Associated According to Quantitative Agronomic Characters in Sweet Potato

Parameter	I	II	III	IV	V
Plant stand at harvest/plot of $9m^2$	13	13	13	13	13
Number of makertable tubers obtained at harvest/plot of $9m^2$	15	15	15	15	15
Weight of roots(kg)	15.4	16.4	14.7	13.8	15.6
Fresh vine weight(kg)	5.9	6.5	8.8	5.7	5.9
Cylas spp scores at 3 M A P	2	2	2	1	2
Millipede scores at 3 M A P	1	2	2	2	2
Alcidodes scores at 3 M A P	2	2	2	2	2
Virus scores at 3 M A P	2	2	3	3	3
Dry matter	38.0	32.6	28.5	40.3	39.0

Correlation Analysis of Quantitative Characters in Sweet Potato

Table 11 shows phenotypic analysis of quantitative traits in sweetpotato accessions. The result of the correlation analysis of the quantitative traits

showed that there was a significant and positive correlation (r =0.73; P<0.01) between number of storage roots obtained at harvest and the weight. *Cylas* scores, Virus scores, *Alcidodes* scores showed significant and positive correlation. However, there was significant but negative correlation (r = -0.22; P<0.5) between *Cyla*s scores and weight of storage roots.

Table 11: Correlation Analysis of Quantitative Character in Sweetpotato

Parameter	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9
Plant stand at harvest									
Number of roots obtained at harvest	.47**								
Wt. of root(kg/ ha)	.41**	.73**							
Vine fresh Wt (kg/ha)	.26*	.13	.44**						
Cylas spp scores at	-.07	.10	-.22*	-.14					

3MAP								
Milliped e scores at 3MAP	-.06	-.17	-.07	.098	.37**			
Alcidodes Scores at 3MAP	-12	-04	-06	-03		05		
Virus scores at 3MAP	-12	-.16	-.20	-.18	-.33**	.08	.37**	
Dry Matter	-0.8	-0.7	-0.1	-01	.01	.08	.19	.05

****** Correlation is significant at the 0.01 level

*Correlation is significant at the 0.05 level

Table 11: Legend

X1 = Plant stand at harvest

X2 = Number of roots obtained at harvest

X3 = Wt. of roots (kg/ha)

X4 = Vine fresh weight (kg/ha)

X5 = *Cylas spp*. scores at 3MAP

X6 = Millipede scores at 3MAP

X7 = *Alcidodes* scores at 3MAP

X8 = Virus scores at 3MAP

X9 = Dry Matter

Analysis of Morphological Dendrogram Construction

Generally, accessions used for the morphological analysis show a high level of variation among the accessions under the Darwin 5.0v-Jaccard method, UPGMA method of dissimilarity matrix. The accessions were grouped according to where they originated. Most of the accessions came from Kenya and therefore, the accessions from Kenya dominated each cluster generated. Accessions in cluster F were collected from Ghana and Kenya. In cluster D 10 accessions were collected from Ghana, eleven originated from Kenya and two came from IITA Nigeria and one each was collected from Tanzanian and Uganda.Cluster B had 9 accessions and out of this number four came from Ghana 1 came from Malawi and the rest 4 came from Kenya. With cluster C, there were 17 accessions. One came from Nigeria, 9 came from Ghana and the rest 7 came from Kenya.Cluster D had 25 accessions 13 came Kenya, 10 came from Ghana and 1 each came from Nigeria and Tanzanian respectively.Within Cluster E, the following number of accessions are obtained from these countries; Kenya had 12, Ghana had 14 and Nigeria, Uganda and South Africa had 1 each.

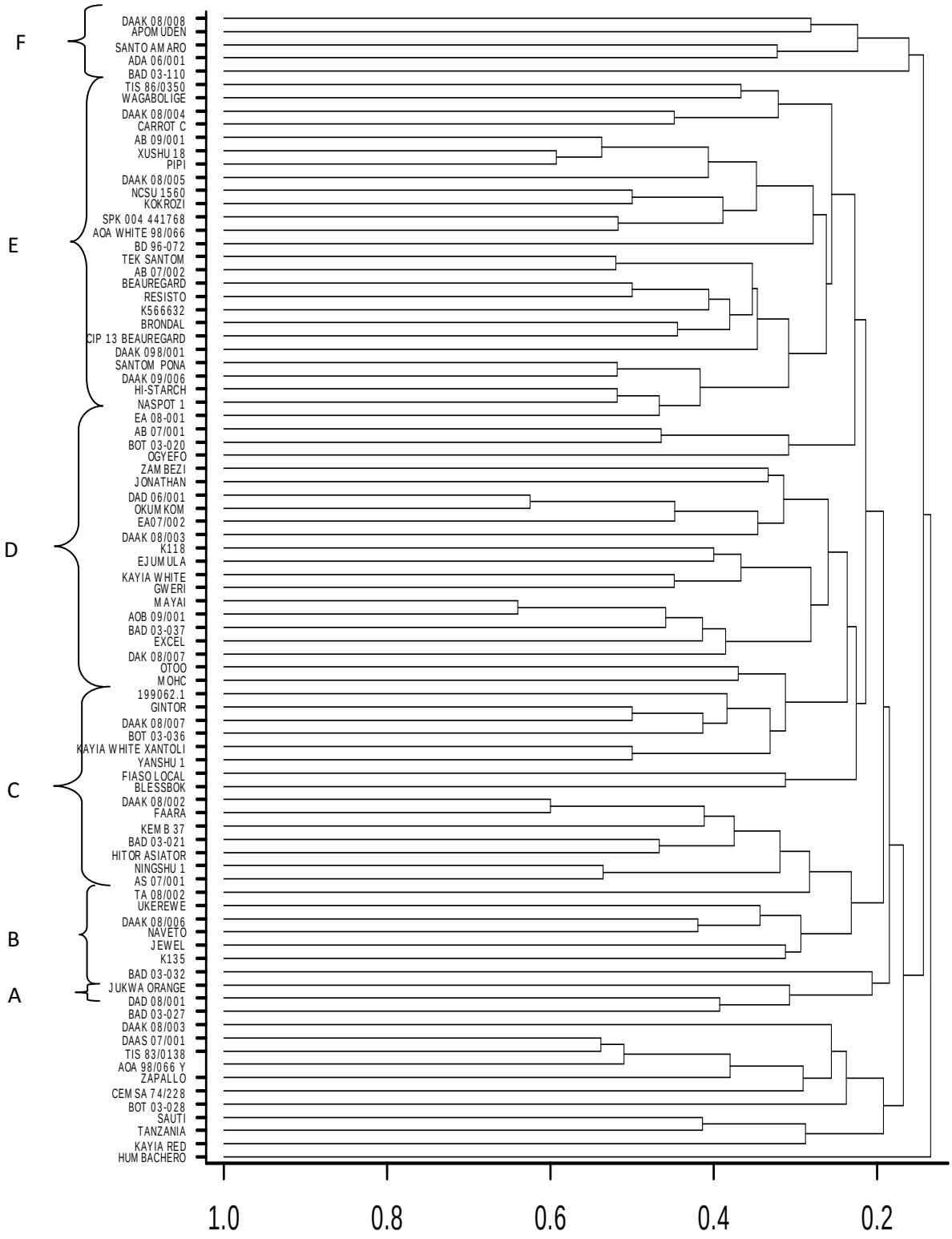


Figure 22: Genetic diversity dendrogram of sweet potato based on morphological data using DarwiJaccard's Index genetic dissimilarity matrix

Molecular Cluster Analysis (Dendrogram) based on Molecular data

Fifty eight sweetpotato accessions were used in the molecular studies shown in Figure23.Dendrogram constructed based on SSRs markers revealed 6 main clusters at 70% Darwin-Jaccard distance, UPGMA dissimilarity coefficient level. Cluster A, B, C, D, E and F were formed (Figure 23). Cluster A had only one accession that is Sauti. Cluster B had 8 accessions which were Blesbok, TIS 86/0350, Naveto, Brondal, NCSU 1560, Jewel, K566632 and Resisto. Cluster C contained 15 accessions and they were Xushu 18, Yanshu 1, Excel, Santom Pona, BAD 03-032, AOA98/066, Beauregard, CIP 13 Beauregard, Cemsa 74, Okumkom, Ninshu 1, Apomuden, Jonathan, Zapallo, and 199062.1. Cluster D had 4 accessions and they were MOHC, BOT 03 036, Otoo and Ogyefo. Cluster E had 15 accessions and they were; Huambachero, Santo Amaro, BAD 03-010, AOA 98/066(white), AOA 96/066, BOT 03-028, Tek Santom, BAD 03-021, Kemb 37, BOT 03-020, BD 96 072, Wagabolige, TIS 83/0138, BAD 03-027 and ADA 06 001.The last Cluster F had 15 accessions and they were PIPI, Ukerewe, Faara, K 118, K 135, Jukwa Orange, SPK 004, Naspot 1, Mayai, Carrot C, Ejumula, Tanzania, Gweri, Zambezi and Hi-Starch.

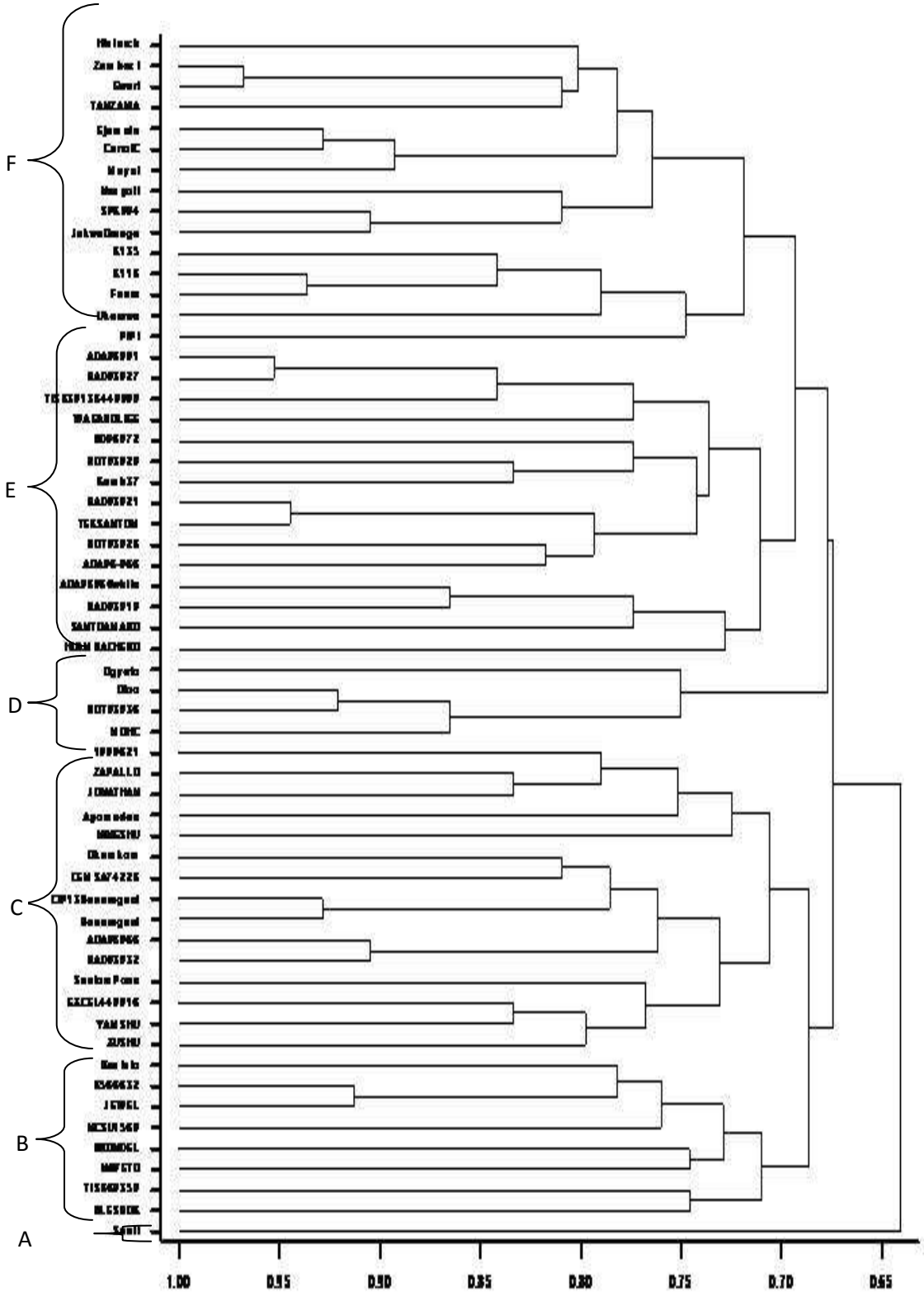


Figure 23: Genetic diversity dendrogram of sweet potato based on molecular data using Jaccard's index genetic dissimilarity matrix

Comparison of Morphological and Molecular characterization

Clustering of 58 sweetpotato accessions based on morphological and molecular data and UPGMA clustering using the Darwin 5.0v-Jaccard dissimilarity coefficient matrix (Fig.24). The combined morphological and molecular dendrogram constructed using Darwin 5.0v –Jaccard, UPGMA method of dissimilarity matrix.This is presented in Fig. 24. The dendrogram constructed produced 7 clusters at coefficient level of 38% dissimilarity matrix. Sauti was outlier and formed cluster A. Cluster B had 9 accessions which were BOT03028, Zapallo, BAD03110, AOA98/066Y, Santo Amaro, Jonathan, Huambachero, Apomuden and 199062.1.Cluster C had three accessions and these were MOHC, BOT03036 and Otoo. Cluster D had eleven accessions which were: Cemsa 74, Xushu 18, Ninshu 1, Yanshu 1, Excel, Kemb 37, Tek Santom, BAD03032, AOA98066Y and Santom Pona. Cluster E contained sixteen accessions and these were these: Blesbok, NCSU 1560, Naveto, Brondal, Beauregard, CIP13 Beauregard, Faara, K118, Gweri, Zambezi, Okumkom, Jewel, K566632, Resisto, TIS 86/0350 and Ogyefo. Cluster F had seven accessions including: Wagabolige, AOA 96066, BOT03020, BD96072, TIS 83/0138, BAD03027 and ADA 06 001. Cluster G had the following eleven accessions. These were PIPI, Ukerewe, K135, Mayia, Carrot C, Ejumula, Tanzania, Jukwa Orange, SPK004, Naspot 1 and Hi-Starch.

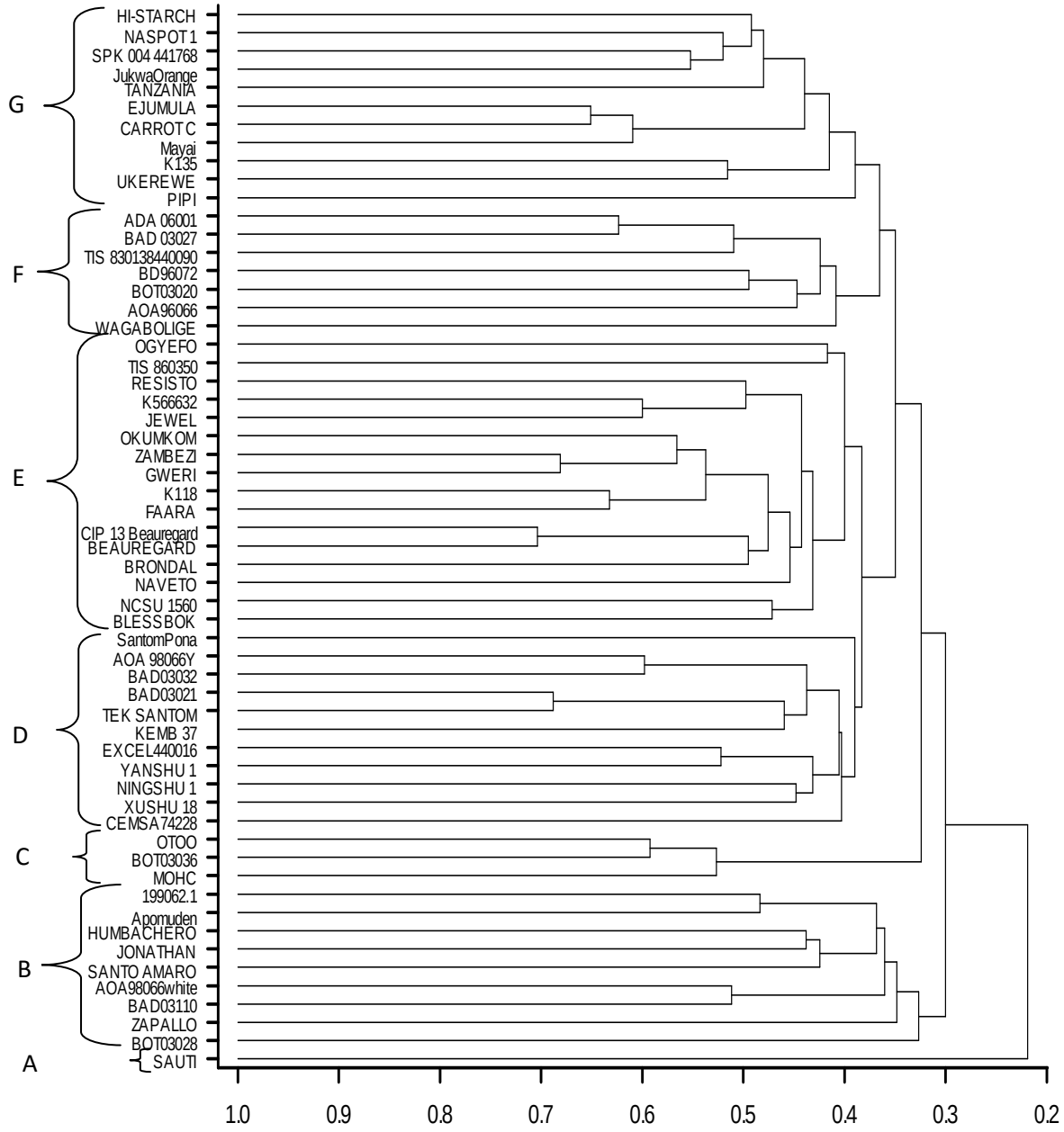


Figure 24: Genetic dissimilarity matrix of combined morphological and molecular data of sweet potato

Molecular Characterization

Twenty-one primers were used for the study. Table 12 shows the sequence of the 21 primers used and the results of the SSR. The twenty one primers detected polymorphism among the fifty eight sweetpotato accessions used.

The primers discovered a total of 112 clear and easily readable and scorable bands with 95.5% polymorphic low and 4.5% monomorphic was the number of bands per primers ranged between two and seven. The mean allelic number is 146.9.Each of the following bands; 152, 201, 243, 175, 222 produced one allele (1). Band that produced the highest number of alleles is 211 which is one hundred and six (106) alleles. The least number of alleles monomorphic (1) were produced by primers Ib324 and it is associated with Yanshu 1 accession, primer IbS18 and is found in Sauti accession, primer IbS01 and accession Apomuden, primer 297 and it is seen in TIS83/0138 accession and primer IbS17 and it is connected with Resisto gernotype. On the other hand, highly polymorphic primers were IbR19, IbJ10a and IbJ116a.The rest of these accessions were associated with these primers.

Table 12: Twenty One Primers Used for the Microsatellite Analysis of the 58 Accessions Showing Range of Allele Sizes, Number of SSR loci and the number of Mono and Polymorphics

SSR Maker Name	Allele Sizes Identified	Total No. of SSR loci	No. of Polymorphic	No. of Monomorphic	Min. size detected	Max. size detected
Ib242	149,151,153, 159,161	5	5	0	149	161
Ib316	152,156,160, 166,168	5	5	0	152	168
IbC12	111,108,144, 117,120, 123,129	7	7	0	111	129
IbJ175	132,134,136, 138,140,142,	9	9	0	132	150

	144,147 ,150					
IbJ67	191,193,197, 213	4	4	0	191	213
IbR16	221,224,227, 230	4	4	0	221	230
IbR19	209,215,217, 225,227	5	5	0	209	227
IbS11	236,239,242, 245,248,251, 254	7	7	0	236	254
Ib324	138,144,146, 148 150,152	6	5	1	138	152
IbCIP1	155,161,164, 167	4	4	0	155	167
IbJ10a	193,203,205, 211 215,219,221	7	7	0	193	221
IbS17	180,183,195, 198,221	5	4	1	180	221
IbR12	337,346,358, 361	4	4	0	337	361
IbR21	203,206	2	2	0	203	206

IbS10	291,294,297, 300 303,309,312	7	7	0	291	312
IbS18	243,247,251	3	2	1	243	251
Ib297	145,151,157, 159 173,175,183	7	6	1	145	183
IbCIP2	280,283,286 289,295,298	6	6	0	280	298
IbJ116a	205,208,211, 214 217,223,229	7	6	0	205	229
IbR03	260,266,269, 275	4	2	0	260	275
IbS01	222,225,228, 231234,237,2 40	7	4	1	222	240
Total		112	10 (95.5%)	5 (4.5%)		

Amplified Polymorphic Fragments

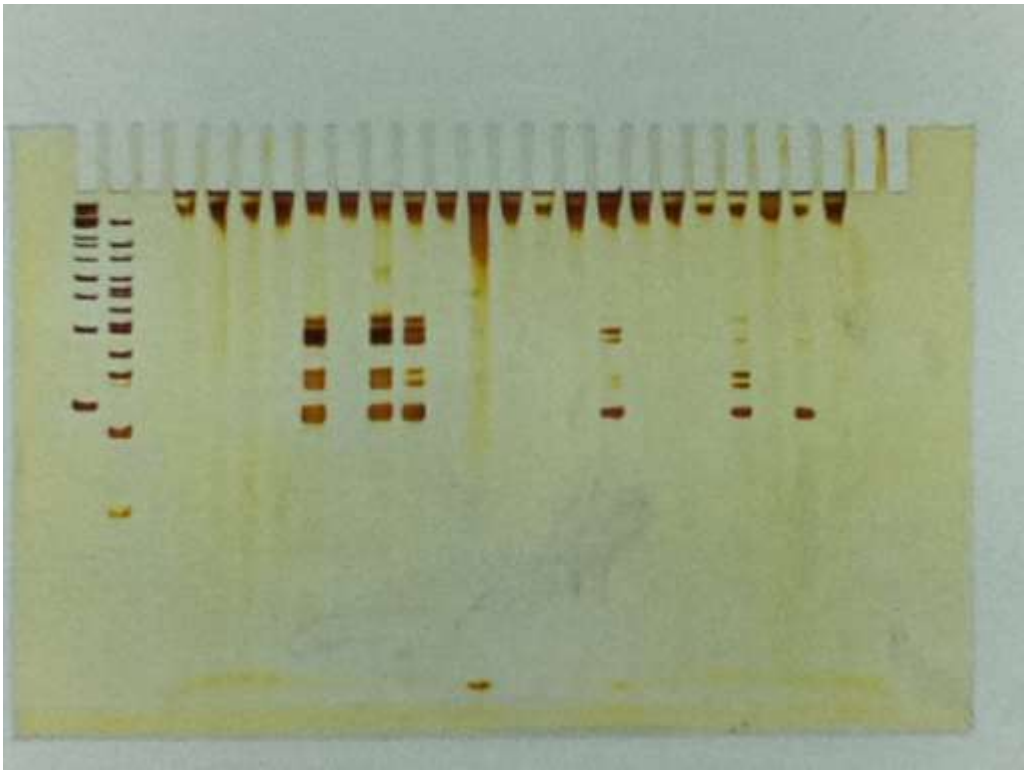


Plate 3A showing SSR primer IBS 11 image on visual gel

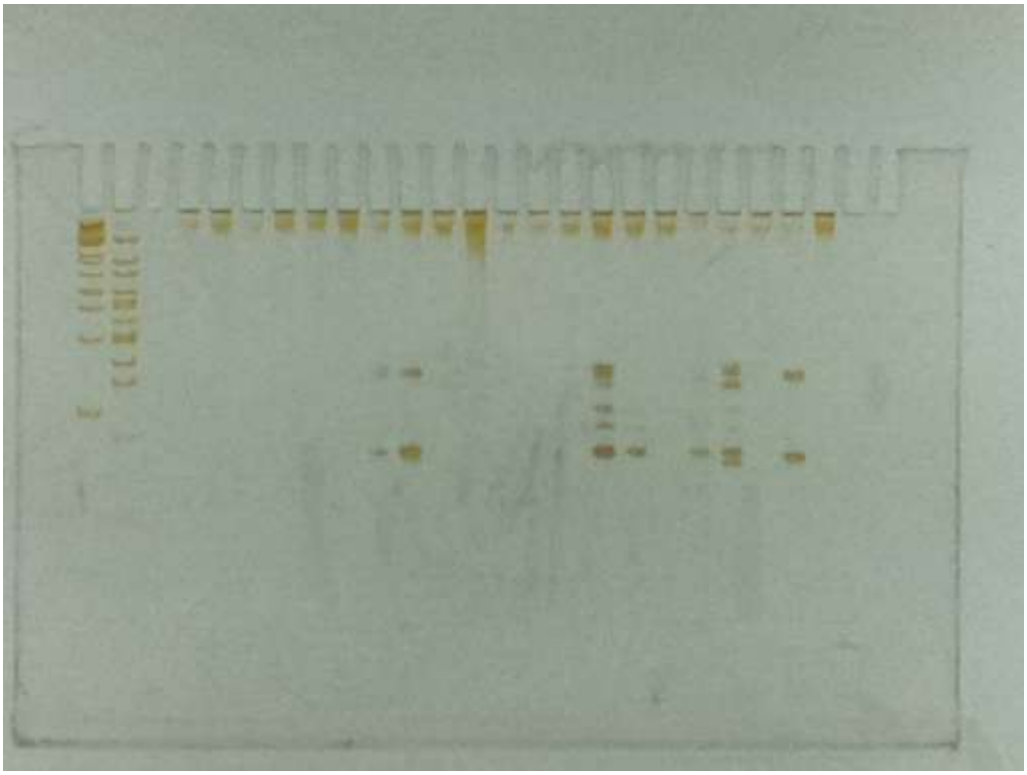


Plate 3B showing SSR primer IBC 17 image on visual gel

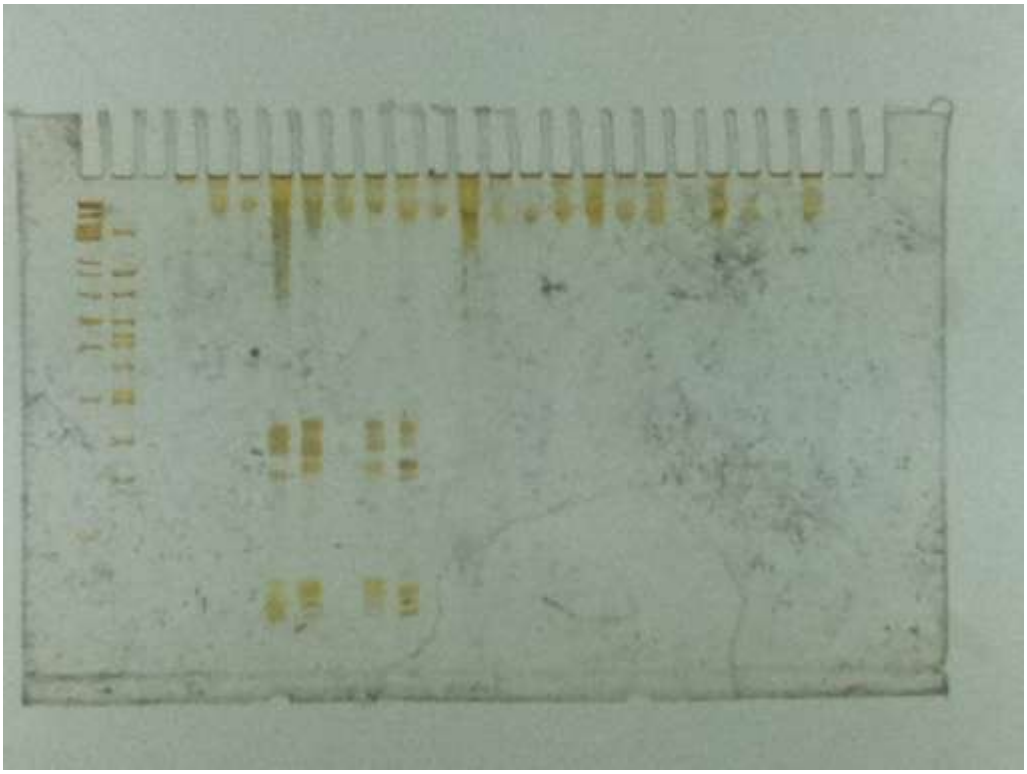


Plate 3C showing SSR primer IBJ 10A image on visual gel

PAGE analysis on 21 accessions using SSR primers IBS 11, IBC 17 and IBJ 10A

Core Collection Determination and Mode of Selection

The combined morphological and molecular dendrogram, their clusters realized were taken into consideration. Distantly apart shows that they are morphological and molecular different and materials are selected from such relativeness whereas closeness shows that they are much same and the materials are selected from such relativeness. Besides that agronomic properties were also one of the criteria used for selection of the conservation materials.

Selection based on clusters

The selected accessions were picked from the following clusters: Cluster A comprised of only 1 accession that is Sauti. Cluster B was made up of Zapallo, BOT 03 028, Apomuden and BD 96 072. . Cluster C also comprised of MOHC, Otoo and BOT 03 036, cluster D was made up of MOHC and Otoo. Cluster E made up of Gweri CIP Beauregard, K566632 and Zambezi. Cluster F produced BOT 03 020. The last cluster G comprised of Jukwa Orange, Hi-Starch and Carrot C.

Selection based on Agronomical properties

1. Genetic distance-closely related accessions were selected. Eg.Sauti and Hi-Starch.

2. Accessions tolerant to *Cylas spp.* Eg.Otoo

3. Beta carotene (Vit.A) root fresh colour. Eg, Jukwa Orange and Apomuden.

4. High yielding accessions.Eg Tek santom and Sauti.

5. High starch content. Eg Hi Starch and Ejumura.

6. Draught tolerance due to their leaf shapes (hastate).Eg.Huambachero and 199062.1

In all they are 22 accessions. The accessions selected will be conserved in a growing medium (in-vitro) tissue culture systems that will ensure that they do not become extinct under field gene bank conditions.

Table 13: List of Core Collections of Sweetpotato considered for Conservation was picked from Genetic Dissimilarity Matrix of Combined Morphological and Molecular Data of Sweetpotato Accessions

	Accession Name	Accession Origin	Cluster Number
1	Sauti	Malawi	A
2	Zapallo	Kenya	B
3	BOT 03 028	Ghana	B
4	Apomuden	Kenya	B
5	B D 96 072	Ghana	B
6	Huambachero	Kenya	B
7	199062.1	Kenya	B
8	Otoo	Tanzania	C
9	BOT 03 036	Ghana	C
10	MOHC	Kenya	C
11	AOA 98 066Y	Ghana	D
12	Excel 440016	Kenya	D
13	BAD 03 021	Ghana	D
14	Gweri	Uganda	E
15	CIP Beauregard	S. Africa	E
16	Zambezi	Kenya	E
17	K566632	Kenya	E
18	BOT 03 020	Ghana	F
19	Ejumula	Nigeria	G
20	Jukwa Orange	Ghana	G
21	Carrt C	Kenya	G
22	Hi-Starch	Nigeria	G

CHAPTER FIVE

DISCUSSION

Morphological Variation and Heritability Estimates

Qualitative and quantitative characters produced the clearest indicators of the intra and inter specific variation considering those with similar quantitative characters, seemed well grouped in the clusters derived from principal component analysis but qualitative description showed little intra specific variation. The efficiency of the use of qualitative descriptors was low and might be due to environmental influence such as soil fertility, weather and human judgement on the character concerned under observation. Daros *et al* (2002) observed high morphological variability while evaluating 14 sweet potato accessions which confirmed the morphological high variation were observed within the 87 accessions used.

From the field observations fifty nine of the accessions which accounted for sixty eight percent did not produce flowers. This was in consonance with Rajendra and Amma (1996) who reported that 13.9% of 764 sweetpotato accessions did not flower. Though some of the accessions do not produce flowers at all, it may be the influence of the environment and climatic control especially photoperiod. Example is Ejumula which produced flowers in Nigeria but could not produce flowers during the periods data was being taken. The major problems in sexual reproduction of sweetpotato are non-flowering and seeds infertility.

Storage Root of Sweetpotato

With storage root shape, it was observed that irregular or curved shape dominated followed by elliptic shape. Hammett (1966) reported that tuber was controlled by additive effect in the absence of dominant genes and female parent was found to exert a greater influence than the male parent. Yield of sweetpotato was not influenced by accessions.

Root Cortex Thickness

With root cortex thickness, Intermediate thickness cortex of (2-3 mm) represented 37.93% were the highest in number of the accessions whilst very thick cortex accessions (>4 mm) which represented 6.90% were the least. This is in sharp contrast with the observation by Oliveira *et al*. (2000) in which all the 51 accessions used in diversity studies had very thin (>1 mm) cortex thickness. This character contributed highly in the divergence studies of this work.

Root Skin Colour

Root skin colours of cream, yellow, pink and orange colours were one of the significant traits that contributed in genetic diversities observed within the accessions used in the studies.These variables (colours) were also mainly responsible for the genetic divergence in the results obtained by Oliveira *et al,* (2000). Similar results were obtained by Hernandez *et al.* (1967) in the studies of controlled crosses between parent of rose and copper, rose and purple as well as cream and copper. They reported that coloured skin is incompletely dominant over white or cream skin colour. Appearance of white colour in the

progeny may be due to transgressive segregation. Characters controlled by 2 pair of genes include root formation, root skin colour, root flesh colour and nature of leaf margin in complementary action (Poole, 1952). Hammett (1966) reported that the uniformity of root colour was controlled by few genes with partial dominance. Constantin (1965) and Hernandez *et al.* (1965, 1967) observed skin colour as a quantitative character which was controlled by several genes in complementary action. High heritability for root shape and flesh colour has been reported (Jones, 1986).

Root Flesh Colour

Different flesh colours were observed from the root flesh surfaces. Cream flesh colour dominated in the studies, followed by pale yellow, then dark yellow, dark orange and white respectively. Similar studies conducted by Huaman *et al*., (1991) was dominated by cream root flesh colour. On the other sweetpotato studies undertook by Hernandez *et al.* (1965) it was reported that white flesh colour dominanted the accessions used in his studies. He further asserted that white flesh colour was incompletely dominant over orange and total carotenoid pigments and these pigments appeared to be controlled by several genes, that are additive.

Ground Cover of Sweetpotato Accessions

Ground cover might be influenced by the genotype, rainfall patten, soil fertility and temperature. Accessions that were classified as group A had maximum, while group D had least ground cover. An accessions ability to effectively cover the ground has major agronomic benefits. These accessions

with high canopy prevent soil erosion, help in water perculation, maintain soil temperature and aid soil microbial activities. Accessions with maximum ground cover can trap significant solar energy to aid photosynthetic activity which ultimately improves yield of sweetpotato.

Leaf Colour

Sweetpotatoes change leaf colouration during their growth period. Whilst five colours were identified in the immature stage, only 3 colours (mostly purple, green with purple veins and green with purple edge) were observed in the mature plants.

Immature Leaf Colour

On immature leaf colour, as many as 62% of the accessions possessed green with purple edge. In similar study, Vimalar & Nair (1988) observed that majority of sweetpotato accessions had green with considerable amount of purple pigmentation along the veins.

Mature Leaf Colour

Almost 78% of the accessions had green with purple vein colour .Leaves that had mostly purple and green with purple edge were observed. In a similar studies conducted by Austin *et al.* (2000) green leaves predominated by (65.8%) and the rest were represented by green leaves with purple edges and purple veins on the lower surface. During same studies Austin *et al*, (2000) had 90% of the mature leaves to be green with purple veins on the lower surface. This different leaf coloration contributed significantly in this diversity

studies. These observations made confirmed the results obtained in this work. Accessions with high green leaf colouration produced good yield. Examples are Hi-Starch and Otoo which have green leaf colouration during maturity produced high yield compared with those with purple colouration.

Leaf Shape

The mature leaf shape were observed as follows: triangular shape 58.6% followed by lobed shape 24.1%, hastate shape 12.6% as shown in Figure 16.In a similar studies done Daros *et al*. (2002) analyzed 14 sweetpotato accessions and observed lobed leaf shape as predominant corresponding 93.0%. Ritchel and Huaman (2002) assessment of sweetpotato germplasm observed predominance of the cordate shape of 49.6%. It was observed that accessions with triangular, cordate and lobed shapes had large leaf area index and therefore can help in photosynthesis processes due to their higher sun energy absorption and therefore can produce larger and many roots with optimal climatic and other conditions being equal.

Hairiness of Sweetpotato Vine Tip

Of the three categories of vine tip hairiness identified, only 13.79% of the accessions produced heavy pubescence, while 34.48% of the accessions did not produce pubescence. In a similar experiment conducted by Ritschel *et al*. (1998) found out that 33% of 51 accessions of sweetpotato did not produce hairs at all. This supported the results obtained in this studies. This trait is contributory fact in this diversity studies and helps to do initial gouping in the

absence of readily molecular assessment.Hairy vine tips have the advantage of protecting the plant against insect attack.

Vine Internode Length

Thirty-nine out of the 87 accessions had intermediate length (3- 5cm) of vine internode length dominated. The accessions which had longer internode length were few. The intermediate and moderately short internode dominated the accessions used for the studies.The different internode lengths of the plants may have been caused by hereditary, the heterogeneous nature of the soil and also the climatic state when the accessions were planted.

Vine Colour

Fifty-nine percent (59%) of the accessions had green vine colour.This was followed by green with few spots. Vimala & Nair (1988) reported similar variability in studies conducted in India in which approximately 43% were purple, 40% were totally green and green with few spots (5.2%) when studying segregation pattern of some sweetpotato progenies.In this diversity studies the colour of the vine plays an important role in clustering similarities and dissimilarities and therefore helps in eliminating of duplicates.

Petiole Length

Petiole lengths were observed to have effect on the conopy formation potential of accessions and the level to which the associated crop in an intercrop can be shaded. The prevalence of cultivars with short petiole length in this study suggest their suitability for intercropping and also use as cover

crop. Analysis of petiole length measurement implicated 55 accessions as short (10-12) cm. Leurie & Nierderwieser (2004) the other hand observed petiole length ranges from 30 -50 cm.

Petiole Colour

Petiole colour of accessions had green-purple pigmentation as predominant from the studies. Generally, petiole colours were green with purple near stem, at both ends of leaf, spots throughout and strips.In a related study, Ritschel *et al*. (1998) identified different petiole colour in sweetpotato landraces. Daros *et al*. (2002) also reported great petiole colouring variability and detected higher heterogeneity of this characteristics.

Quantitative Trait

Quantitative traits cover parameters like plant stand at the time of harvest, roots harvested and roots weight after harvest. Plants used for the research were on the average 13 stands per accession. The number of roots per stand ranged from 3 to 60 storage roots. The accession which did not perform well showed similar performances across all the replications. This problem might have been caused by virus disease attack, coupled with erratic and low rainfall at the time the field experiments were conducted.But Germplasm (Huambachero) yield performance was very poor showing across all the 3 replicates.

Root weight of accessions recorded ranged from 0.35g to 1.70 kg per root. Fresh vine weight ranged from 0.40g to 27.00 kg per plant. The accession, Fiaso local a landrace produced 51 roots per stand. Roots weight was 1.3kg.

Fresh vine weight was 27kg and dry matter was 27%. On the other hand, NCSU 1560 produced 36 roots and total root weight of 14kg, Fresh vine weight is a function of yield. Generally, accessions with high vine weight yield are comparatively higher. Compared to other root crops, sweetpotato has high moisture content resulting with relatively low dry matter content. Hamilton *et al.* (1986) observed a positive correlation between light fresh colour of the root and dry matter content while Dai *et al*. (1988) suggested that an additive gene was responsible effect for the starch content and dry matter content of accessions. The similarity which also showed up within the group members could be attributed to admixture, mislabeling and duplications as accessions were taking from one region to another region or area and different names were given to them. Mislabeling creates duplicates more especially as most farmers are illiterate to handle such situations perfectly and in some cases research workers and agricultural frontline staff during material storage and planting time.

Morphological quantitative Variation and Heritability Estimates

Sweetpotato cultivars from 7 different countries were classified into in five different clusters using the principal component axes. Both qualitative markers put the accessions into clusters without any unique cluster relationship with the collection points or countries.These observations are consistent with reports from the studies in Uganda (Elameen *et al*., 2008). All the clusters had at least an accession from the countries where these materials were collected from.

Accessions with common names like DAAK 08/001, DAAK 08/003, DAAS 07/001, DAAK 08 / 004, DAAK 05/002 were collected from the Akatsi district in the Volta region of Ghana where sweetpotato is predominantly cultivated.It is most probable that planting materials were passed on from 1 farmer to another, which resulted in the different names of accessions.

Different farmers might have given different names to the same accession. For example, Kokrozitor was similar in morphological traits to AB 07/001. But dissimilar in petiole colour.Kokrozitor has some purple strips in its petioles while AB 07/001 is totally green.This may be caused by somatic mutation originated from asexual propagation results from cuttings.This report is in consonance with studies by Zhang *et al*.,(2000). Similarly, accessions like AOB 09/001 and AS 07/001 were all collected from the Eastern region of Ghana which also share commom traits such as leaf shape, vine colour and petiole colour but differ significantly in their flesh colour.This suggested that they might be the same accession which was given different names as it passed through different hands of farmers.

In comparing the dispersion of accessions in a given cluster, It was noted that accessions from Kenya dominated in cluster V from the qualitative and quantitative principal component axes. Ghisslain *et al*., (2000) also reported in similar studies where sweetpotato germplas from North America group together with minimal interference from other regions. However, Kokrozitor was collected from Volta region whereas AB 07/001 was collected from Eastern region. Kokrozitor being one of the highly rated accessions in the Volta region could have been brought to Eastern region for cultivation by a farmer who could not name it or vice verca (McGregor *et al*, 2001).

In cluster I, K566632 and Apomuden share similar traits like yellow flesh colour and hairiness of vine tip. K566632 is a well known accession that arrived in Ghana earlier than Apomuden through exchange materials from foreign research institutions but by then sweetpotato was not popular in the country and such accessions got lost through the state of disinterest, but some of the research materials had already got into the hands of the farmers on farm trials and farmers own curiosity. The early arrival of most of the accessions might have been the cause of its new name since they were all brought from Kenya.

Morphological Markers Observations

In this study, morphological markers employed for the qualitative and quantitative traits depicted that maximum inter cluster distance existed between cluster I and IV. Such accessions would be expected to produce larger variability in segregating generation (Sindhu, 1985). However the qualitative inter cluster distance analysis depicted a minimum existence. The farthest distance was found between cluster II and VI. Since most of the (parental) materials or accessions came from CIP sub-station in Kenya and had passed through Crop Research Institute, Nyanpkala Savana Research Institute and Plant Genetic and Resource Research Institute, it is therefore, assumed that planting materials spread to farmers either via on - farm trials, materials that were given to agricultural extension agents for distribution, during farmers day or the farmers themselves took them from any other places they can get them out of curiousity. It is believed that parental materials from both internal and external breeding institution are from distantly separated

and distinct geographical regions like South Africa, East Africa, Nigeria, Ghana, Kenya and Uganda get most of their germplasm from International Potato Centers in Lima - Peru and Griffin- Georgia in the United States of America (Huaman & Zhang,1997).

The direction of selection practiced in the different geographical locations may determine genetic variation of the accessions. For instance, in Ghana farmers at different farming locations with different farming systems may select for culinary qualities, plant shape and stature (architecture), canopy or ground cover, yield, root colour, root sizes and dry matter content. With the factors mentioned above, farmers mostly restrict their selection for desirable economic traits and discard some unrealized genetic potential and could cause low genetic variability in the germplasm at hand. This suggested that materials used as parents for hybridization should be considered depending on the strength of genetic divergence and not geographical distances.

Correlation analysis showed traits with combine variation. There was a significant correlation between harvested roots obtained and root weight in the accessions. Root weight and fresh vine weight showed high positive correlation. However, negative significant correlation between *Cylas spp* incidence and roots weight was observed. Sweetpotato is a crop or plant that establishes well two to three weeks after planting and matures within 3.5 months to 5 months depending on the varieties (Dapaah *et al.*, 2005). Plant vigour expressed in healthy and vigorous canopy is an index of yield. Selection for high yielding potentials like starch for industrial purposes and the different dishes that could be processed from sweetpotato have boost it as food security crop.

Selection of accessions for conservation and for future breeding work should not be based on a single factor. According to Grafius (1959) increasing total yield would be made easier by selecting for components of yield because they are more often easily inherited than total yield itself. Correlation studies enable the breeder to understand the mutual component characters in which selection can be based for genetic improvement. Adebisi *et al*, (2001) studied the relationships between different traits in different crops such as soyabean, cassava, sweetpotato amongst other crops and can be used by plant breeders to to enhance their quest for new but economic potential varieties that will meet the ever increasing world population. Stathers *et al*, (2003) and Islam *et al*. (2002) showed that vine length, number of roots and root diameter were positively and significantly correlated with root yield (total root weight) in sweetpotato while Tsegaye *et al* .(2006) reported positive yet significant root girth among thirty sweetpotato genotypes.

Investigating into the diversity of sweetpotato is complicate, polyploiy (2n=6x=90) nature. The connection genetic studies have proposed that cultivated sweetpotato could be either allopolyploid (Jones, 1965) or autopolyploidy (Shiotani, 1988). The complex genome of sweetpotato and it's heterozygocity make it to exhibit multiple combinations of chromosomes and genes due to its ploidy.This contributes to the complexity of the crop as well as its molecular diversity. The morphological dendrogram had one outlier which was the Huambachero accession therefore, this shows that selection based on morphological analysis Huambachero stands out not closely related to rest of the accessions used for the studies (figure 22) cluster A. At branch length 0.28 Kayia Red, Tanzania and Sauti had some connection which are

green leaf colour, leaf shape and petiole colour. The molecular dendrogram analysis had Sauti accession as an outlier (figure 23) cluster A. In cluster D all the accessions were collected from Kenya with the exception of Otoo accession which was collected from Tanzania. Other clusters did not contain accessions that were collected from only one country and therefore, the morphology and the molecular dendrogram analysis did not group accessions according to country of origin. Zhang *et al*., (2000) reported similar observation when undertaking diversity studies on 113 genotypes. That is no cluster has only accessions from same site of collections. This also meant that the Sauti is genetically distant from the rest of the accessions used in the studies. With the combined dendrogram, Sauti was an outlier. Significantly high variations existed in the molecular and the combined molecular and morphological analysis.

Morphological Characters in Diversity Studies

High level of variation was reported in diversity studies of Kenyan sweetpotato accessions (Karuri *et al.,* 2009). The variations observed in the accessions in the current study might be attributed to natural mutations affecting traits like root and skin colour, leaf and vine characteristics. Accumulation of random mutations from asexual propagation of sweetpotato via stem cuttings and adventitious buds rising from storage roots have been reported (Villordon & Labonte, 1990). Mogie, (1992) also reported that the high level of ploidy in sweetpotato might be responsible for the variability in qualitative traits due to the increased mutation rate associated with polyploidy.

From the dendrogram constructed with the different statistical approach in assessing the diversity, the accessions MOHC and Otoo were collected from Kenya and B0T 03036 from Ghana and were grouped together in cluster D in the molecular cluster analysis. However, in the combined morphology and molecular dendrogram analysis they were together in Cluster C.

In the Darwin-Jaccard method for the molecular, morphological and combined method, accessions B0T03028 and Zapallo were grouped in cluster B of the morphological and the combined analyses. In general, the molecular and the morphological analysis did not have very close relationship in terms of settlement of the various accessions in the clusters. On the other hand, the molecular and the combined morphological and molecular analyses showed that most of the accessions positions in the various clusters were almost similar except the presence of a few odd accessions used in the morphological studies were observed within the clusters.

For example, the accessions found in cluster F of the molecular analysis were all found in cluster G of the combined data. Schneider *et al,* (2000) acknowledged that combined molecular and morphological data produce best estimate to information on genetic variation. Zannou *et al*, (2006) emphasized that characterization that is only based on morphological traits is known to mask or front important genetic information. However the employment of predominant and stable agro-morphological traits can provide fundamental information and stratification earlier to systematic molecular characterization. Soller & Beckmann (1983) underscored the importance of molecular markers as extra tool for germplasm characterization and description aid efficiency to the whole process.

In considering the Jacaard dissimilarity dendrogram and the principal component clusters set ups, there were a few accessions occupying similar positions in the clusters. Considering the principal component analysis, CIP 13 Beauregard and Excel accessions appeared in cluster I while they also appeared in same cluster in the Jacaard molecular dissimilarity dendrogram analysis. Gweri, Jonathan and Zambezi were in cluster D of the morphological qualitative dendrogram but Gweri and Zambezi are found in cluster IV of the principal component analysis so also they appeared in the cluster F of the molecular dengrogram. This grouping of accessions under different data analysis did not give any definite grouping as regards to the origin of the accessions as they seen in Table 4, Figure 22 and Figure 23.

The amplification analysis of the PCR - RAPD of study presented 112 loci, with 107 polymorphic Loci (95.5%). Jaccard dissimilarity coefficient ranged from 7% to 82% for the distant accessions. Gweri and Zambezi were closely related as shown in all the Genstat-jacaard dissimilarity dendrogram and the Principal Component analyses.The two accessions originated from Uganda and Kenya respectively. From the quantitative characters both accessions exhibited similar characters. They have high yield and high dry matter content, Zambezi exhibited high Cylas *spp* resistance whilst Gweri was highly attacked by Cylas pest. The 7% dissimilarity might have been caused by somatic mutation and transposable elements that have been detected in sweet potato and reported by (Tahara *et al.*, 2004 and Love *et al*., 1978) which resulted from asexual propagation and movement of genetic elements through the genome during the diffenciatial that occurs in tissue culture. The most distantly related accessions showed 82% Jaccard dissimilarity coefficient

between Sauti and Tek Santom. They were collected from Nigeria and Malawi. Both accessions exhibited high yield, diseases and pest resistance and good shape of tubers. They both have high dry matter content. Each of them was found at the ends of the dendrogram and in different clusters.

The 95.5% polymorphic loci realised in this study was relatively higher than 69% and 7% found among sweetpotato cultivars in Taiwan and Tanzania respectively Tseng *et al.,* (2002) Elameen *et al.,* (2008). This showed a greater genetic variation in the accessions. A large volume of the accessions from a wide range of geographical areas is very essential for genetic distance estimation (Nei, (1987). Marmey *et al* (1994), identified Manihot species and 19 cassavas, collected from many countries using RAPDs.

Thompson *et al,* (1997) reported that self- incompatibility in the flowers of sweetpotato results in allogamy increasing genetic heterozygosity. Accessions were collected from both internal and external centre for sweetpotato conservation in Africa.The principal centres of collection were:

Kenya, Uganda, Tanzania, Malawi, Nigeria, South Africa and Ghana.The different sources resulted in a larger loci which increased the genetic variation in accessions.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

1. Significant genetic diversity occurred amongst the eighty-seven accessions assessed.The extent of diversity was great to permit rigorous breeding activity for the selection of the most desired materials.

2. Yield an important quantitative traits showed a positive correlation among accessions. However, the PCA revealed a negative correlation between the weight of roots and *Cylas* incidence.

3. Both the molecular and the morphological traits used for the characterization, grouped the accessions into six distinct clusters. Since the morphological traits normally mask the molecular traits, most of the accessions selected for conservation was based on the molecular traits. In all twenty-two accessions were selected from the eighty-seven accessions used for the study. They were all distantly related and ideal materials for future breeding and other related programs.

4. Sauti and Hi-starch were identified as the most genetically distant related accessions. The two accessions were therefore included in the core collection for conservation.

Recommendations

1. Core collected materials should be preserved both in-vitro and ex-situ in order not to lose them.

2. Breeders can depend on the selected high quality materials for breeding work.
3. Sweetpotato materials which were not included in this studies should be brought in to assess their genetic diversity (molecular and morphological) traits to augment available materials for breeding programs.
4. Breeders and agronomists should collaborate and use the selected materials to produce new and acceptable varieties for farmers and other stakeholders.
5. Breeders should concentrate on sweetpotato traits that possess high and stable starch content, high beta carotene (precursor of vitamin A) and high dry matter content for farmers which can be used for the production of industrial starch and confectionary.

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