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GENETIC CHARACTERIZATION OF EXOTIC AND LANDRACES OF CASSAVA IN GHANA

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BY

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DECLARATION

I hereby declare that this work is a direct result of my original field and laboratory research undertakings and are supported by cited references in relation to other previous and similar work performed, and therefore this thesis has not been presented anywhere for a degree.

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DEDICATION

I make this special dedication to my parents, Mr. Henry Karim and mother Mrs. Fatmata Karim. This is in remembrance of their numerous financial sacrifices, prayers and moral support to ensure the realization of my academic achievements.

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To God be the glory, honour and power forever more, Amen!

ABSTRACT

Cassava cultivars grown in Ghana are often misrepresented due to improper characterization of the genetic materials available. The genetic diversity of 45 accessions which consist of 24 exotic and 21 landraces (selected as a core of 150 cassava accessions) collected from Crops Research Institute of Ghana were assessed and characterized using agro-morphological and molecular means. Both quantitative and qualitative traits such as plant height, angle at first branching, canopy spread, petiole length, root yield, dry matter content, root shape, petiole colour, and pubescence on apical leaves were used to assess their variability for phenotypic characterization. Genomic DNA of the accessions were extracted and used as template for Polymerase Chain Reaction (PCR) amplification involving nine Simple Sequence Repeat (SSR) markers. The recorded gel bands as well as the agro-morphological traits were subjected to cluster analysis and development of dendrogram to show the corresponding similarity coefficients. Agro-morphological characterization grouped 2 accessions into cluster A, whilst 43 accessions were grouped in cluster B. Molecular characterization also grouped 16 accessions in cluster A and 29 in cluster B. Overall, both molecular and agro-morphological characteristics put 3 accessions in cluster A and 42 in cluster B. The genotype identification showed that, the phenotypic characters have a similarity coefficient range of 0.80%-1.00% whilst the molecular also had a wider coefficient range of 0.2-1.0%. These features showed the extent of diversity present in the accession evaluated and served as a basis for efficient management and utilization of germplasm in breeding programme. Therefore, the application of morphological descriptors in characterization of germplasm should be backed by the use of molecular markers.

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LIST OF ABBREVIATIONS

ACMD	Africa Cassava Mosaic Disease
AFLP	Amplified Fragment Length Polymorphism
Bp	base pair
CBB	Cassava Bacterial Blight
CAD	Cassava Anthracnose Disease
CRI	Crop Research Institute
CSIR	Council for Scientific and Industrial Research
CIAT	Centro International de Agriculture Tropical
CTAB	Cetyl Trimethyl Ammonium, Bromide
DNA	Deoxyribo Nucleci Acid
dNTP'S	deoxy Nitro Triphosphates
EDTA	Ethylene Diamine Tetraceti Acid
FAO	Food and Agriculture Organization
IITA	International Institute of Tropical Agriculture
IBPGR	International Board Plant for Genetic Resources
Kb	Kilobases (lkb=10^3 base pair)
L	Liter
MAP	Months After Planting
MOFA	Ministry of Food and Agriculture

M	Meter
Mg	Milligram
$Mgcl_2$	Magnesium Chloride
PCR	polymerase Chain Reaction
PAGE	Polyacrylamide Gel Electrophoresis
QTL	Quantitative Trait Loci
RAPD	Random Amplified Length Polymorphism
RCBD	Randomized Complete Block Design
RELF	Restriction Fragment Length Polymorphism
SSR	Simple Sequence Repeats
SAHN	Sequential, Agglomerative, Hierarchical and Nested
TAE	Tris-Acetate-EDTA buffer
Tris	Tris (hydroxymethly)-aminoethana
UPGMA	Unweighted Pair Group Arithmetic Means Algorithm

CHAPTER 1

1.0 INTRODUCTION

Cassava (*Manihot esculenta* Crantz), currently is the sixth most important world food crop (FAO, 2008). Cassava has an edible starchy root tuber, which provides more than half of the calories consumed by more than 800 million people in Sub-Saharan Africa (SSA), Latin America and Asia (Shore, 2002). It has become the most important source of dietary energy in SSA (Scott *et al*., 2000) as it provides more dietary energy per hectare and working hours than any other staple crop (Akoroda, 1995; Fregene *et al*., 2000; Nassar, 2005). The main nutritional component of cassava is carbohydrate, which is derived from starch accumulated in the tuberous storage roots. The storage roots also contain small amounts of proteins ranging from 1-2% on fresh weight basis (IITA, 1990).

Cassava's advantage over other food crops includes flexibility in planting time, harvesting time, and its drought tolerance ability. Moreover, it is also able to grow and produce on low nutrient soils, where cereals and other crops do not grow well, and is well suited for incorporation in various cropping systems (Onwueme, 1978; Fregene *et al*., 2000; Nassar, 2005). The leaves and tender shoots are consumed as vegetable in many part of Africa and are a cheap but rich source of proteins, vitamins A, B. C, and other minerals (Hahn, 1988; FAO, 1993; Fregene *et al*., 2000; IITA, 2001).

In Ghana, cassava is grown across all agro-ecological zones and ranks first in the area under cultivation and utilization; and contributes 22% of Ghana's Agricultural Gross Domestic Product (AGDP) (Parkes, 2009). Cassava is produced by over 70% of

Ghanaian farmers and consumed by more than 80% of the population (Parkes, 2009), indicating its importance as food security crop. However, the average crop yield in Ghana is 12 mt ha^{-1} against an achievable yield of 28 mt ha^{-1} (MOFA, 2005). The low yield of the crop is attributed to many factors. This included the farmers' use of unimproved planting materials alongside few improved varieties which are seldom planted in pure stand and farmers also lack of good agronomic practices, such as late planting, non- row planting, poor weed control at the wrong time as well as lack of knowledge in controlling diseases and pests.

Although cassava is well integrated into the diverse traditional farming systems very little genetic improvement has been achieved, because cassava planting materials have been selected and distributed by subsistence farmers (Beeching *et al.*, 1993). Farmers have selected genotypes that best fit their needs and, thus generate a large number of traditional varieties. In addition, different ethnic groups have contributed to selection, thus leading to numerous vernacular names to the same varieties according to ethnic groups (Mignouna *et al.*, 1998). This nomenclature has lead to confusion in the exact numbers and identity of cassava varieties under cultivation in Ghana. There is the need to characterise the national collection of cassava, to remove possible duplications and establish the diversity of the cassava varieties to enhance genetic improvement of the crop.

A number of DNA marker techniques are available and are important tools for genetic identification in plant breeding and germplasm management (Mba *et al.*, 2001). These DNA markers used in diversity analysis include Random Amplified Polymorphic DNA (RAPDs), Restriction Fragment Length Polymorphisms (RFLPs), Amplified Fragment

Length Polymorphisms (AFLPs) and Simple Sequence Repeats (SSRs). Molecular characterization of argonomically important traits within the cassava germplasm, using different markers will be useful in molecular breeding programmes. Among the recently developed molecular markers, SSR markers are considered as the markers of choice as they are able to detect variation in allele frequency at many unlinked loci (Moyib *et al.*, 2007). SSR markers are particularly attractive to study because they have high level of polymorphism (Tauz and Renz, 1984; Gupta *et al*., 1996; Powell *et al.*, 1996; Mba *et al*., 2001).

Current advances in the development of methods using DNA polymorphisms as molecular markers provide alternative methods for characterization of cultivars and hold a promise for revealing genetic variation and resolving the ambiguities in cassava collection. Valuable attributes of all SSR markers are co-dominance (multiple allelic forms that reveal heterozygosity), technical simplicity, sensitivity, analytical simplicity (data are unambiguously scored, and highly reproducible) and high abundance (markers are uniformly dispersed throughout genomes as frequently as every 10 Kb and therefore are ideal tools for many genetic applications.

Genetic improvement of cassava is, to a larger extent, limited by inadequate knowledge of genetic diversity within the species. A prerequisite for any programme of genetic improvement of cassava is knowledge of the extent of genetic variation present within the cassava germplasm with which hybrid could be produced (Beeching *et al.*, 1993). The more genetic diversity that can be available to the breeder, the wider the range of choice they will have in selecting the appropriate kinds of diversity for their programme.

MAIN OBJECTIVE

This study was, therefore, carried out with the main objective of characterizing, exotic and landraces of cassava by phenotypic and molecular means.

SPECIFIC OBJECTIVES

- To distinguish the cassava collections based on their morpho-agronomic traits.
- To identify the differences between the various genotypes using molecular markers.
- To group the genotypes based on their phenotypic and molecular characters.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 **Importance of cassava**

Cassava (*Manihot esculenta* Crantz) is a staple food for over 800 million people in sub-Saharan Africa, South America and Asia. More than half of the world's cassava is produced in Africa, where it is a cheap and major source of calories for about 40% of the population. The crop is efficient in production of carbohydrates and is adapted to a wide range of environments. At present, cassava is grown almost exclusively on an estimated area of 10.8 million ha in 42 African countries (FAO, 2000).

The crop is preferred by most resource-poor farmers because of its low input requirement, tolerance to low rainfall and poor soils and ease of propagation by use of vegetative stem cuttings compared to most other crops. The roots of sweet cassava varieties are eaten raw, roasted, fried or boiled. Fresh cassava roots may be sliced, grated, fermented or pounded, then dried and further processed into dried chips and balls. The bitter type of cassava (cyanogenic) can only be used after fermentation. The dried chips are milled into flour which can be used alone or as a composite with millet, sorghum, maize flour to make a pasty product (Smith, 1988). Fresh sweet cassava is used to feed swine, cattle, sheep and goat (Okeke and Oti, 1998). Pellets and chips processed from cassava are a source of energy in animal feeds (Asiedu, 1989). Besides the cassava storage roots which are rich in energy, the leaves are edible and provide nutritive values similar to other green leaf vegetable that are good sources of vitamins A and C, iron, calcium, and protein. In Africa, there exists a need for increased production of cassava to

meet food requirements and have surplus for industry, feed and export. Processing adds value at farm level and reduces deterioration and bulkiness, thereby facilitating the sale of cassava products in the off-season and in distant markets (Chiwona-Karltun, 2001)

Industrial uses of cassava starch include the production of adhesive and glue for use in paper and in the production of ethyl alcohol (Asiedu, 1989). Cassava exports account for less than 0.1% of the total production, in form of meal or more generally, pellets account for 10-15 % of world production (Silvestre, 1989). The bulk of world trade in dried cassava is conducted between Thailand (with annual exports of 13.6 million tonnes) and Europe, which imports about 12.2 million tons annually (Silvestre, 1989).

2.2 Genetic composition of cassava

Cassava belongs to the euphorbiaceous family and genus *Manihot.* The genus includes 98 other species that are useful as genetic resources in cassava improvement (Rogers and Appan, 1973; Chavez *et al*., 1989). Members of the Euphorbiaceae family are characterized by vessels composed of sector cells and include several commercially important plants. Some of these are rubber trees (*Hevea brasiliensis*), castor oil (*Ricinus comunis*), root crops (*Manihot spp*.) and ornamental plants (*Euphorbia spp*.) (Osiru *et al*., 1996).

Cassava is widely distributed in the tropics and subtropics and is the only species from the genus that is widely cultivated. A few other *Manihot* species have had minor uses, especially as alternative sources of latex for rubber production (M. *glaziovii* and M. *caerulescens)* (Franche *et al*., 1991)

2.3 Cassava plant morphology and growth

Cassava is a dicotyledonous shrub, with intermediate growth habit, and possessing a wide spectrum of variability (Mandal, 1993). It is a perennial crop, although farmers usually harvest it during the first or second year. Abandoned stands of cassava may continue to grow for several years (Onwueme, 1978) and most often in association with other crops (Hershey, 1993a).

Different cultivars have been distinguished by morphological characteristics, such as colour and shape, branching habit, plant height, colour of stem and petiole, root shape and root skin colour, time of maturity, yield and the cyanogenic glycosides content in the roots (Onwueme, 1978; Osiru *et al.*, 1996).

It is propagated mainly from stem cuttings; however, under natural conditions, as well as in plant breeding, propagation by seed is quite common. When cuttings are planted in moist soil under favourable conditions, they produce sprouts and roots within a week.

When propagated by seed, plant establishment is considerably slower. The seedlings genetically segregate into different types (Osiru *et al*., 1996). A few weeks after emergence or sprouting, the shoot lengthens and the root extends downwards and spreads. The cassava plant can be divided into a shoot and root system. The shoot system consists of the leaves, stem inflorescence and the root system consists of feeder roots and storage roots (Osiru *et al*., 1996).

2.4 Leaf production

Cassava leaves, leaf longevity and whole plant products are determined by genotype and environmental conditions. Cassavas leaves are arranged alternately in a spiral order on the stem. The arrangement of leaves on the stem is 2/5 spiral. Leaves are simple, with lobed lamina and petiole. Each leaf is subtended by three to five stipules, each about one cm long. The number of lamina lobes varies between three and nine. Most cassava varieties grown in Africa have lobes that are elliptical or lanceolate (Onwueme, 1978).

2.5 Flowering

Cassava plants are monoecious, producing separate male and female flowers on the same plant. Male and female flowers are borne on the same branched panicle, with female flowers at the base, and male flowers toward the tip. The flowers are small, with the male flower being about 0.5 cm in diameter, and the female flower slightly larger. In a given inflorescence, female flowers open first and the male flowers follow from one to a few weeks later. Time of flowering varies by genotype and environment and can range from 1 month to more than 2 years. Flowering is also dependent on plant habit, with more highly branched genotypes flowering more prolifically than those with a sparsely-branched habit. Therefore, apical branching is a good visual indication of plants that are about to start flowering.

Environment can have a major impact and some cassava clones will flower profusely in one environment and not flower at all in another environment. Flowering is frequent and

regular in some cultivars, while in others it is rare or non-existent. The flowers are borne in terminal panicles, with the axis of the branch being continuous with that of the panicle inflorescence. The male flowers occur near the tip, while the female flowers occur closer to the base. Each flower, whether female or male, has five yellowish or reddish perianths. The male flower has ten stamens arranged in two whorls of five stamens each. The filaments are free and the anthers small. The female flower has an ovary mounted on a ten-lobed glandular disc.

Cross-pollination is usually a rule, no incompatibility system has been observed so far that would completely prevent crossing among species of the genus Manihot (Rogers, 1963). Hybridisation in cassava is relatively easy (Kawano, 1978) and open pollination schemes are used extensively to increase the amount of hybrid seeds.

2.6 Breeding of cassava

The goal in cassava breeding in various research centers has been directed towards developing varieties that combine the largest number of desirable traits associated with high yield, disease and pest resistance, good root quality and stability of production across environments (Cock, 1984). Plant breeders have recognized the important role of local cultivars in providing naturally occurring resistance to pests and diseases and tolerance to sub-optimal growing condition (e.g. drought, cold, and salt). When these traits are incorporated into economically important cultivars, large losses in commercial production can be avoided (Simmonds, 1983).

Another reason for the introduction of new traits is to meet the demand for desired characteristics, which improve crop quality for specific end-user requirements. A prerequisite for the introduction of these new traits is the existence of a genetically diverse pool of cassava germplasm. In order to be able to determine levels of genetic diversity, techniques for the identification and classification of genotypes are required. Assessments of the genetic diversity of elite crop germplasm have been sought and used by plant breeders for numerous reasons – genetic relationships, parent selection, germplasm management, sampling, and germplasm protection (Millan and Cubero, 1995).

The major conventional cassava breeding methods include introduction, hybridization (intra and interspecific) and selection (Magoon, 1970; Hahn *et al*., 1979; Bueno, 1985).

The most common procedure is the identification of superior parents, hybridization between selected parents and selection within progenies (Hahn and Williams, 1973). Since cassava is heterogeneous at all loci and most traits are under additive gene action (Iglesias and Hershey, 1993), the use of recurrent selection has been recommended to increase the frequency of favorable alleles (Hahn *et al.*, 1977; Hahn *et al*., 1978; Byrne, 1984). The success of this method depends on the genetic diversity between and among selected parents (Kawano, 1978; Hahn *et al*., 1979; Fehr, 1987).

2.7 Germplasm conservation and maintenance

The method used in conserving germplasm varies according to several factors, including the species, their geographic distribution, breeding systems involved and seed behaviour. However, two main methods of conservation have so far been identified, in situ and ex

situ conservation (Pirreno, 1992). In cassava generally, the accessions are conserved in the field where they are regenerated year-by-year, and maintained by vegetative cultivation in the field. New plantings are often made from stem cuttings in old-field. In addition to the high-cost of field maintenance, valuable germplasm is often exposed to pest and disease attacks, and soil and climatic stress. The main danger of this method of conservation is the loss of accessions due to the effect of biotic factors like pest and disease. The other method used for preserving cassava is in vitro conservation (Ng, 1992). This is an expensive method and is practised when facilities are available.

2.8 Gains from genetic diversity in cassava improvement

One consequence of modern agricultural practices, which generally emphasize maximum productivity with acceptable quality and uniformity, has been a reduction in the genetic diversity of the primary gene pool under cultivation, with similar fates for the secondary and tertiary gene pools of most major crops (Lee, 1995). The consequences of a narrow genetic base of major crops have been experienced sporadically throughout history, often with significant human and economic costs (Franche *et al.*, 1991).Therefore, an awareness of genetic diversity and management of crop genetic resources have been important components of plant improvement programs.

Considerable progress has been reported in cassava breeding through genetic improvement from various research centres. Genetic improvement has also been reported to contribute a great deal to the development of new and better types in terms of providing lines with resistance to disease and other useful agronomic traits (IITA, 1993a).

Crossing cassava with *Manihot glaziovii* derivative has yielded success in the incorporation of resistance to African Cassava Mosaic Virus (ACMV) and Cassava Bacterial Blight (CBB) in cassava (Hahn *et al.*, 1977). The importance of local landraces in improving cassava has also been shown in the broadening of genetic base of the existing germplasm and creating a large genetic variability in source population to facilitate selection of desirable genotypes (IITA, 1993b).

The importance of an existing collection of these local germplasm in enhancing the levels of resistance and improving stability and food quality traits has been evaluated in various studies. Belloti *et al.* (1987) reported that locally adapted varieties of cassava used in traditional farming systems in the tropics show considerable tolerance to indigenous pests and disease. In addition, notable degree of stability has been observed in them and this is because the native varieties have been selected over a long period of time (Dixon *et al.*, 1992).

2.9 Classification of cassava

Cassava varieties exist in each locality where the crop is grown. The cultivars have been distinguished by morphological characterisation such as leaf characteristics, colour and shape, branching habit, plant height, colour of stem, root shape and flowering and time to maturity (Dixon *et al.*, 1994). Genetic studies have shown potential benefit to breeding for the improvement of quality traits (Beeching *et al*., 1993). Landraces of cassava offer rich source of genetic diversity and these provide a valuable source of genetic materials

for crop improvement. The fast developments of high-yielding cultivars of cassava for their preservation should be of the highest priority (Attere, 1997).

Cyanogenic glucoside content as a trait has been used to place cassava cultivars into three groups. (1) cassava with high potential to generate HCN-10 mg per 100g fresh weight or more (ii) Intermediate types in which the levels of HCN range between 5 and 10 mg per fresh weight (iii) cassava with low potential to generate HCN- less than 5 mg per 100g fresh weight. The cyanogenic glucosides are often concentrated in the peel (IITA, 1990). The breeding strategies in cassava are strongly influenced by its vegetative propagation, allowing the fixation of heterozygous genotypes at any stage of selection (Hershey, 1993b).

2.10 Morphological characterisation

Since Mendel, breeders and geneticists have used morphological characteristic such as leaf and flower attributed to follow segregation of genes and hybrids, but most agronomic traits are not associated with easily observed phenotypic markers (Kochert, 1990). Phenotypic identification of plants is commonly based on the morphological traits assessed and recorded in the field. In cultivar classification, characterization has been a powerful tool. Usually the certification of new cultivars is based on the genetic purity of a particular crop (Stegeman, 1984).

Morphological characterisation has also been used for purposes like the identification of duplicates, studies of genetic variation patterns, and correlation with characteristics of

agronomic potential. These may involve a lengthy survey of plant growth that may be costly, labour intensive and vulnerable to environmental conditions. However, in cassava breeding programmes the major emphasis has been on the collection and conservation of gene pools (CIAT, 1993) and characterization to eliminate duplicates.

Plant characterisations are grouped according to either variable or constant characteristics. The variable characteristics are those associated with large genotypes by environment interaction. The constant characteristics typify the species or cultivar, for instance, the branching types in cassava cultivars. Because cassava grows in several different ecological environments, it is difficult to describe the morphological characteristics. Therefore, the influence of the environment in the genotype is always important.

The study of taxonomy and genetic relationships for the identification of genotypes in many crops has become complicated by a large number of spontaneous as well as man-made crosses. Their use in crop improvement has being restricted to genes affecting morphological traits, such as leaf morphology and dwarfism. Moreover, many morphological traits, belonging to all developmental stages are required in order to assign an individual to a specific tax on (Millan and Cubero, 1995).

Traditionally, the characterization and classification of cassava germplasm has been accomplished by the use of morphological descriptors. IBPGR (1998) has defined a set of relatively stable morphological traits useful for cassava genotype characterization. They

include shoot and root parts characterization of cassava with quantitative and qualitative measurements. This important taxanomic method has been extended by molecular approaches.

Cassava cultivars are generally distinguished based on morphological traits. They have a wide variability of botanical characteristics. Numerous cultivars are distinguishable by morphological characteristics such as plant height; size, shape and leaf colour can also be of importance. An obstacle to the reliable identification of cultivars is the existence of considerable linguistic polymorphism. As in most parts of the world, the cultivated forms of crops have been unequivocally named. Each site has its own unique series of names for different cultivars with specific meaning.

Cassava morphological classification has played an important role in rectifying ambiguities of cultivars. Researchers have tried to characterize them using the adapted International Board for Plant Genetic Resource (IBPGR) scale. In some distinct cultivars, the plants architecture can be very different with presence or absence of branches as well as level of branching, immature leaves varying from light green to purple and with different number of leaf lobes. Generally, the accessions are made up of large numbers, sometimes with lack of definitive identification by the influence of changing environmental conditions (Waycott and Fort, 1994). Streekumari *et al.* (1988) used fifty cassava genotypes to evaluate tolerance for shade conditions based on morphological and agronomic traits. They identified seven genotypes tolerant to shady conditions, based on root yield characteristics.

The majority of landraces cultivated in Sierra Leone are typically, late branching, with varying plant heights. Petiole colour is mostly pink and so also is root skin. There is apparent variability in stem colour. Arbitrary naming of landraces frequently based on name of person giving out cutting may have resulted in the same landrace having several names while different landraces can have the same name. Identifying broad groups of genotypes with a view to further distinguish those sub groups using advanced biotechnology tools. The situation will not be different from that of Ghana and hence duplicate collections need to be sorted out.

2.11 Importance of characterization

Conservation of genetic resources entails several activities, activities related to the acquisition of germplasm (location and describing the diversity), its conservation (using effective procedures) and evaluation for useful traits. In all, the availability of sound genetic information ensures that decision made on conservation will improve germplasm management. Of the activities related to genetic resources, those involving germplasm evaluations and the addition of value to genetic resources are particularly import- ant as they help identify genes and traits, and thus provide the foundation on which to enhance use of collection.

De Vicente *et al*. (2005); and Rubenstein and Heisey (2003) reported descriptor list are a vital tools for ensuring that those who are documenting the characteristics of conserved species are using the same language and standards. In the agreed terminology of gene banks and germplasm management, the qualitative traits stand for the description of

character that are usually highly heritable, easily seen by the eye and equally expressed in all environments (IPGR\CIP, 2003). Similarly, Anon (2010) reported the quantitative traits are measurable characteristics which exhibit continuous variation (height, weight) and are the result of the interaction between two or many genes and their environment.

In genetic terms, characterization refers to the detection of variation as a result of difference in either DNA sequences or specific genes or modifying factors. Standard characterization and evaluation of accessions may be routinely carried out by using different methods, including traditional practices such as the use of descriptor lists of morphological characters.

They also involve evaluation of agronomic performance under various environmental conditions. In contrast, genetic characterization refers to the description of attributes that follow a Mendelia inheritance or that involve specific DNA sequences. In this context, the application of biochemical assays such as those that detect differences between isozymes or protein profiles, and the application of molecular markers. (De Vicente *et al*., 2005).

2.12 DNA- based molecular techniques

DNA fingerprinting is a technique, which has been widely adopted to differentiate among organisms at the species and subspecies levels (McClean *et al*., 1994). The techniques used for cultivar identification are designed to detect the presence of specific DNA sequences or combination of sequences that uniquely identify the plant. Cultivar identification can be achieved more accurately using DNA fingerprinting data, especially in materials characterized by low genetic variation between cultivars.

The most closely related cultivars are usually distinguished with the DNA fingerprinting method (Beckman and soller, 1986). Another advantage of DNA fingerprinting over morphological markers is the dominance and the absence of environmental effects. The application of DNA fingerprinting could be very valuable in the identification of cultivars and species and could help identify agriculturally important quantitative trait loci (QTL). The high variability of DNA fingerprinting described in humans, animals and plants allows the identification of different individuals, genotypes, and species (Lin *et al.*, 1993).

2.13 Random Amplified Polymorphic DNA (RAPD)

The invention of PCR (Polymerase Chain Reaction) was a milestone in the development of molecular techniques. PCR results in the selective amplification of a chosen region of a DNA molecule. Random amplification of DNA with short primer by PCR is a useful technique in Phylogenetics. The important point is the banding pattern seen, when the products of PCR with random primers are electrophoreses as a reflection of the overall structure of the DNA molecule used as the template. If the starting material is total cell DNA then the banding pattern represents the organization of the cell's genome. Differences between the genomes of two organisms can be measured with RAPD. Two closely related organisms would be expected to yield more similar banding patterns than two organisms that are distant in evolutionary terms (Miesfeld, 1999). Moreover, this technique requires only small piece of tissue as the extracted DNA can be amplified million times using PCR.

2.14 Amplified Fragment Length Polymorphism (AFLP)

AFLP analysis is able to detect high levels of polymorphism and has high repeatability and speed of analysis. These markers have a very high diversity index, resulting in a limited number of primer combinations required to screen a whole genome and has been applied to develop a system for the fingerprinting of an organism (Faccioli *et al*., 1999) and for map expansion (Castiglioni *et al*., 1998). Vos *et al*. (1995) described the AFLP technique as being based on the detection of restriction fragments by PCR amplification and argued that the reliability of the AFLP technique is combined with the power of the PCR technique. AFLPs provide high levels of resolution to allow delineation of complex genetic structures. AFLPs are fragments of DNA that have been amplified using directed primers from restriction digested genomic DNA (Matthes *et al*., 1998; Karp *et al*., 1997).

The major advantage of the AFLP technique is the large number of polymorphisms that the method generates. Its ability to differentiate individuals in a population makes the technique useful for paternity analyses (Krauss, 1999), gene-flow experiments, and also for Plant Variety registration (Law *et al*., 1998). Other advantageous features of the AFLP techniques are: i) no sequence information is required; ii) the PCR technique is fast; and iii) a high multiplex ratio is possible (Rafalski *et al*., 1996).

2.15 Microsatellites

Microsatellites, alternatively known as simple sequence repeats (SSRs) are usually 1-6 base pair repeat motifit (example TA, CA, GTG, TAAA and GGTA) repeats. Simple

Sequence Length Polymorphisms (SSLPs), are tandem repeats of sequence units generally less than 5 bp in length (Bruford and Wayne, 1993). These markers appear to be hypervariable, in addition to which their co-dominance and reproducibility make them ideal for genome mapping, as well as for population genetic studies (Dayanandan *et al.*, 1998). They are ubiquitous in eukaryotic genomes and their study has been greatly facilitated in PCR technology. These markers often present high levels of inter- and intra-specific polymorphism, particularly when tandem repeats number ten or greater. nucleotide repeats are very frequent in human and other genomes, and present every few thousand base pairs. Inter-SSRs are a variant of the RAPD technique, although the higher annealing temperatures probably mean that they are more rigorous than RAPDs. Chloroplast microsatellites (SSRs) are similar to nuclear microsatellites but the repeat is usually only 1bpn (Proven *et al*., 1999).

Conserved flanking sequences of SSR, are a unique characteristic that enable the design and synthesis of universal primers for routine amplification of the SSR regions of choice across many laboratories engaged in genotyping work. In this study, 9 SSR primer pairs were employed for characterizing of 45 cassava collection in Ghana.

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Location of study

The field experiments were conducted at the research fields of CSIR-Crops Research Institute at Fumesua in Ashanti Region (01° 36°W; 06° 43°N) from May to August, 2011. Fumesua is in the semi-deciduous forest zone with elevation of 186 m above sea level. The average annual rainfall is about 1700 mm and has a bimodal rainfall distribution. The major rainfall season is from March to July while the minor rainfall season is from August to November. The mean minimum and maximum temperatures are 21°C and 31°C, respectively. The mean annual relative humidity is 95% in the morning and 61% at noon. The soil at the experimental site at Fumesua belongs to the Asuansi series and is classified as Ferric Acrisol (FAO/UNESCO, 1988). It has 16-20cm thick layer of sandy loam and slope of 1-5 percent.

3.2 Source of cassava accessions

Hundred and fifty cassava accessions from Crop Research Institute (CRI) Ghana were investigated to determine their genetic diversity based on agro-morphological characters. The cassava accessions evaluated were 24 exotic and 21 landraces of cassava, selected as representatives of clusters for a dendrogram based on morpho-agronomic characterization of 150. Dice coefficient similarity was calculated and used to construct a dendrogram, based on UPGMA and SAHN clustering. Because the 150 accessions were too many and produced a very congested for dendrogram, three sets of dendrogram were produced for each group of 50 accessions. From the clusters of each of the three dendrograms, 15

accessions were selected to constitute a core collection. Thus 45 accessions were selected as a core collection. The molecular and the agro-morphological characterisation was therefore carried out on 45 accessions.

3.3 Land preparation

The land was slashed, and later sprayed with a pre-emergent herbicide (glyphosate) to control early weeds emergence and to ensure better crop establishment.

3.4 Experimental design and plot layout

The design was a randomized complete block. The plot size was 10 m x50 m, plant distance was 1m x 1m between and within rows laid out in three blocks, 10 within each replication with 500 plants per each plot giving a plant population of 1500.

3.5 Planting and cultural practices

Planting was done in May, 2011, cuttings of about 20-25cm were planted. Manual weeding was done at 1, 2, and 3 months after planting. No fertilizer was applied. Harvesting was done 12 month after planting.

Table 3.1: List of the accessions from the core collection and their respective groups from morpho-agronomic characterization.

EXOTIC	CODE	LANDRACE	CODE
ABASAFITTA	K1	DABODABO	K5
01/0040	K8	MM96/5280	K6
BANKYE BRONI	K10	UCC	K13
01/1097	K19	AK DEBOR	K18
00/0338	K23	AHWENGYANKA	K21
01/0093	K31	BD96/141	K28
98/0510	K36	ESSIABAYA	K49
01/0220	K38	BD96/075	K54
AMPONG	K55	TECK BANKYE	K73
01/0265	K57	BD96/136	K85
01/0061	K59	BD96/154	K88
94/006	K66	KSI2000/092	K103
01/011	K70	BD96/093	K110
01/0046	K76	KW2000/053	K112
96/0603	K80	NKABOM	K115
01/0104	K81	UCC2001/111	K118
01/0140	K82	OFF2000/145	K127
BANKYE HEMMA	K89	AFS2000/131	K130
00/0346	K99	AFS2000/043	K139
97/0730	K122	AFS2000/071	K147
TME693	K125	AW2000/053	K146
97/2236	K128		
00/0354	K136		
ESSAM	K148		

3.6 molecular diversity assessment

3.6.1 DNA extraction and purification

The youngest leaves were harvested at 14 days after planting for DNA extraction. DNA extraction was carried out at the Crop Research Institute (CRI) Molecular Biology Laboratory, Fumesua, Ashanti Region, Ghana. DNA extracted was done using Egnin *et al*. (1998) protocol. This protocol consisted of cell lysis, precipitation and purification, as follows:

200mg of tissue was weigh into 2 ml eppendorf tube, grind to fine power with liquid nitrogen, 800µl of buffer A (lysis powder) was added then, incubated at 90°C for 10minutes and vortex every 5minutes. The solution was cooled at room temperature for 2 minutes, then 400 µl 5m potassium acetate was added mixed gently by inversion 5-6X and incubate on ice for 30 minutes with shaking and centrifuge at 13,000 rpm for 10 minutes, The supernatant was transfer the upper phase to a new eppendorf tube and 1 value of cold isopropanol, 1\10th of 3M sodium acetate and mixed 10X by inverting. The DNA was precipitated at 20°C for 1hour and centrifuged at 13,000 rpm for 10 minutes to pellet the DNA. The supernatant was poured and pellets washed with 800 µl. 80 % ethanol, centrifuge at 13,000 rpm for 5 minutes. The alcohol was discarded and DNA pellets air dried. DNA was dissolved in 500µl 1x TE Buffer to and treated with 4Nl RNase A at 37°C for 30 minutes. Two hundred and fifty milliliters of 7.5M ammonium acetate was added and incubated on ice for 3 minutes, centrifuged at 13,0 00 rpm for 5 minutes. The supernatant was transferred into a new 1.5 ml tube and 700 µl of

isopropanol added and mixed by inversion and centrifuged at 13,000 rpm for 15 minutes. The supernatant was discarded and pellets washed with 1ml of 80 % ethanol, and centrifuge at 14,000 rpm for 5 minutes. The supernatant was discarded and pellet dried at room temperature. DNA pellets was dissolved in 200 µl 1XTE Buffer. DNA quality was checked on 0.8. 4% agarose gel.

3.6.2 Microsatellite amplification

3.6.2.1 PCR amplification of SSRs

9 highly polymorphic SSR markers, procured from Integrated DNA Technologies (Crolville, Iowa, USA) which are widely distributed in the cassava genome (Chavarriaga-Aguirre *et al*., 1998; Mba *et al*., 2001) were used in genotyping the accessions.PCR reactions were conducted in a Techne Thermocycler (TC-512) in a 10 µl reaction mixture in 96-well plates. The mixture contained 10x PCR Buffer 1µl, 20mM dNTPs 0.2 µl, 0.25 µl each of the forward and reverse primers, 0.125 µl of Super-Therm Taq polymerase, 6.275 µl sterile distilled water (SDW) and 1 ng genomic DNA. The PCR programme consisted of an initial denaturation for three min at 94°C and then 35 cycles of denaturation for 30 sec at 94°C, annealing at the appropriate temperature for each pair of primers at 45°C for 30 sec, and extension at 72°C for 1 minutes and then put on hold at 4°C was included. a 2 µl loading dye was added to the reaction mixture, mixed and span down prior to being loading on Polyacrylamide Gel Electrophoresis (PAGE). The amplified products were stored at 20°C until they were needed to run gels.

3.6.2.2 Primers

Nine primers were used for the molecular analysis to characterize the cassava accessions. Their primers sequences are shown in Table 3.2

Table 3.2 Primer Sequences

SSR Locus	Left primer sequence	Right primer sequence
SSRY 4	ATAGAGCAGAAGTGCAGGCG	CTAACGCACACGACTACGGA
SSRY 21	CCTGCCACAATATTGAAATGG	CAACAATTGGACTAAGCAGCA
SSRY 51	AGGTTGGATGCTTGAAGGAA	GGATGCAGGAGTGCTCAACT
SSRY 52	GCCAGCAAGGTTTGCTACAT	AACTGTCAAACCATTCTACTTGA
SSRY 59	GCAATGCAGTGAACCATCTTT	CGTTTGTCCTTTCTGATGTTC
SSRY 63	TCCAGAATCATCTACCTTGGCA	AAGACAATCATTTTGTGCTCCA
SSRY 64	CGACAAGTCGTATATGTAGTATTCAG	GCAGAGGTGGCTAACGAGAC
SSRY 146	TCAAACAAGAATTAGCAGAACTGG	TGAGATTTCGTAATATTCACTT
SSRY 179	CAGGCTCAGGTGAAGTAAAGG	GCGAAAGTAAGTCTACAACTTTT CTA

3.6.2.2 Running of gel

Horizontal Polyacrylamide Gel Electrophoresis (hPAGE) was used for running the samples. 6X Orange DNA loading dye (10 mM Tris- HCl (pH 7.6), 0.15% orange G, 0.03% xylene cyanol FF, 60%glycerol, 60 mM EDTA) was used for visual tracking of DNA migration during electrophoresis. The presence of glycerol in the solution was to ensure that the samples were properly laid at the bottom of the well, while the EDTA was to bind any divalent metalions and inhibit metal dependent nucleases from degrading the DNA samples.

One volume of the dye was added to 5 volumes of DNA sample. After initial denaturing at 95°C for 5 min using the PCR machine, 6 µl of sample (or DNA ladder in the first well) was loaded in each well of a 41-well 5% polyacrylamide gel. The DNA ladder used was a 1kb and100 bp ladder. Gels were run at 300V for 2 h using a Baid and Tatlock Nucleic Acid Electrophoresis Cell and power pack and 1x TBE as running buffer.

3.6.2.3 Sliver staining

The gel was fixed in 10% acetic acid (a fixation solution) for 10 minutes. This was followed by washing with de-ionised water for 2 minutes. The water was poured out, after which the gel was oxidised in 1.55 units of nitrate acid for 5 minutes and again washed with de-ionised water. Silver nitrate solution was added for 20 minutes (for best results, the tray was covered as light affects the sliver nitrate solution), and the gel washed with distilled water. A cold developer solution was added for the appearance of

the bands, this was followed by stop solution, (10% glacial acetic acid). The gel was stored in a bowl containing distilled water.

3.7 Data collection

3.7.1 Leaf and Canopy Characteristics.

Colours of apical leaves, pubescence on apical leaves were recorded at (3 MAP). Petiole colour, shape of central leaflet, flowering, petiole colour was assessed at (6 MAP). Growth habit on stem was assessed at (9 MAP). Height to first branching (cm), angle of branching and canopy spread (cm), were recorded at harvesting (12 MAP).

3.7.2 Diseases assessment

The genotypes were evaluated for their reaction to Africa Cassava Mosaic Disease (ACMD), Cassava Bacterial Blight (CBB) and Cassava Anthracnose Disease (CAD) by recording the symptom expressions using a scale of 1 to 5 were 1- No Symptoms, 2-Mild Symptoms, 3-Moderate Symptoms, 4-Severe Symptoms and 5- Very Severe Symptoms. Disease severity symptoms were recorded for ACMD at 6 months after planting (MAP), for CBB at 3 MAP and CAD at 9 MAP.

3.7.3 Storage Roots Characteristics.

Fresh storage root number, storage root weight and storage root dry matter percentage of five (5) tagged plants were determined by counting, weighing (kg) and expressed as a percentage of the ratio of the dry weight over the wet weight respectively. Root shape, and texture of root epidermis were assessed at harvest.

3.8 Dry matter

The dry matter percentage of storage roots was determined from a random bulk sample of roots selected from each accession. One hundred (100) gram sample was weighed and dried for 24 hours in a forced air drying oven at 105 °C for 24 hours. The dry samples were re-weighed to obtain the dry weights, and the dry matter percentage were calculated as the ratio of the dry weight over the fresh weight and multiplied by 100.

3.9 Scoring of bands from agarose

Scoring of bands on agarose was done with the alpha imager connected to the computer.100-bp DNA ladder from invitrogen was used as a molecular-weight size marker for each gel alongside the DNA samples. The scoring was done visually to ascertain the presence or absence of bands.

3.10 Statistical analysis

All data collected were entered into excel. The data were statistically analyzed using Gen-Stat package 9th edition. Mean, standard error and coefficient of variations were calculated. The significance was tested at 5%, standard errors was use to compare the means. Frequency distribution was used to compute and categorize the accessions into classes. Dendrogram were prepared. For PCR amplification products, the bands were scored as presence (1) or absence (0) for each of the cultivars with the 9 primers. Only SSRs bands with good distinctiveness were recorded.

3.11 Framework for characterization of the accessions

Characterization of the accessions was based on qualitative and quantitative data for agro-morphological traits as well as molecular differentiations. For morphological characterization, the following traits and scores were used,

Table 3.3. Descriptors of qualitative traits

Qualitative traits	Score or scale
Petiole colour:	
Light green	3
Dark green	5
Green purple	6
Red	7
Purple	9
Root shape:	
Conical	1
Conical-cylindrical	2
Cylindrical	3
Irregular	4
Shape of central leaflet:	
Elliptic-lanceolate	1
Obovate-lanceolate	2
Oblong-lanceolate	3
Lanceolate	4
Growth habit on stem:	
Straight	1
Zig-Zag	2
Pubescence on apical leaves:	
Absent	0
Little pubescence	3
Moderate pubescence	5
High pubescence	7
Flowering:	
Flowering	1
Non-flowering	0
Texture of root epidermis:	
Smooth	3
Intermediate	5
Rough	7
Colour of apical leaves:	
Light green	3
Dark green	5
Purplish green	7
Purple	9

Table 3.4. Range for quantitative traits and the scale used.

Quantitative traits	Range	Scale
Plant height	64.7 – 114	1
	115 – 164	2
	165 – 213	3
	214 - 262	4
	> 262	5
Canopy spread (cm)	20.67-25	6
	26-31	7
	32-37	8
	> 37	9
Height at first branching(cm	26.7-42	10
	43-58	11
	59-74	12
	75-90	13
	91-106	14
	107-122	15
	123-138	16
	139-154	17
	155-170	18
	> 170	19
Angle at first branching()	63.3-104	20
	> 104	21
Petiole length (cm)	8.5-16	22
	17-23	23
	24-30	24
	> 30	25
Tuber yield (T/ha)	0.27-10	26
	11-21	27
	22-32	28
	> 32	29
Dry matter (%)	20.67-25	30
	26-31	31
	32-37	32
	> 37	33

For molecular characterization clustering was done using the scoring on PAGE to visually ascertain the presence or absence of bands.

CHAPTER 4

4.0 RESULTS

4.1 Core collection establishment for morpho-agronomic characterization

A core set with a limited diversity reduction and higher number of sample reductions was implemented, separately for the 150 cassava accessions, a core set of 45 accessions were identified .In the core collection, the cassava cultivars were divided into three groups of 50 for each to obtained a dendrogram for selection, 15 were selected from each dendrogram in other to avoid duplicates.

Cluster analysis for morphological characterization for the first group revealed two major clusters (A and B) and four sub-clusters. Sub cluster I contained only one exotic cultivar (00/0338). Sub cluster II contained fourteen cultivars and four were selected: three exotic (01/0040, Bankye Broni and UCC) and one landrace cultivars (Ak Debor). Sub-cluster III contained ten cultivars. Three exotic cultivars (01/0093, 01/0220 and 98/0510) were selected. Sub-cluster IV contained four cultivars two were selected one exotic (01/1097 and one landrace cultivar Ahwengyanka). Sub-cluster V contained 21 cassava accessions five cultivars were selected two exotic (Abasafitaa and Essiabaya) and three landraces (Dabodabo, MM96/5280 and BD96/1414). The germplasm covered a narrow range of genetic similarity (GS) values from 0.89 to 0.98 (Appendix 1).

In the second dendrogram, analysis for morphological characterization revealed two major clusters (A and B), with the first cluster (A) containing only three accessions. Cluster B contained most of the accession with four sub-clusters. Sub-cluster I and II contained 3 cassava cultivars each. One and one local cultivar was selection for each cluster (Teck

Bankye and 01/10061). Sub-cluster III contained nine accession, two exotic cultivars were selected (94/006 and 01/011). Sub-cluster IV contained the rest of the accessions and eleven; were selected three local (BD96/075, BD96/145 and BD96/136) the rest were exotic accession. The germplasm covered a narrow range of genetic similarity (GS) values from 0.89 to 0.98 (Appendix 2).

In the third dendrogram, analysis for morphological characterization revealed two major clusters (A and B), with the first cluster (A) contained only one accession (OFF 2000/145). Cluster B consisted of the rest of the accessions with four sub-clusters (Appendix 3). Sub-cluster I and II contained two cassava accessions each and one was selected for each cluster Essam (exotic) and AFS2000/131 (local). Sub-cluster III contained eight cultivars two exotic were selected (97/0730 and TME 693). Sub-cluster IV contained the rest of the cultivars and ten were selected; two exotic (97/2236 and 00/0353) the rest were landrace accessions. The germplasm covered a narrow range of genetic similarity (GS) values from 0.89 to 0.99 (Appendix 3).

4.2 **Genetic differences in Morpho-agronomic traits**

4.2. **1 Morpho-agronomic analysis of quantitative traits**

There were highly significant differences ($p<0.001$) among the cultivars for plant height, canopy spread, height at first branching, angle at first branching, petiole length, root yield and dry matter. (Appendix 4). The range of values produced were 64.70-276.60 cm, for plant height. 20.67-40.23cm for canopy spread, 26.70-183.30 cm for height at first branching, 6.70-183.30 for angle at first branching. 8.50-33.33 cm for petiole length. 0.27-44.00 for root yield and 40.20-16.38 for dry matter content, (Table 1).

Table 4.1 Mean, standard error, range, standard deviation (SD) and coefficient of variation were analysed for seven quantitative traits.

Traits	Mean ± S.E	Range	SD	CV (%)
Plant height (cm)	118.72 ± 7.28	64.70-276.70	48.88	26.88
Canopy spread (cm)	29.01 ± 0.71	20.67-40.23	4.75	16.38
Height at first branching (cm)	84.65 ± 6.15	26.70-183.30	15.07	48.76
Angle at first branching (degree)	100.62 ± 2.25	63.30 -130.00	41.28	14.97
Petiole length (cm)	19.78 ± 0.90	8.50 -33.33	6.03	30.48
Tuber yield (T/ha)	12.35 ± 1.54	0.27- 44.00	10.35	83.79
Dry matter content (%)	1 29.01 ± 0.71	20.67-40.23	4.74	16.38

4.3 Genetic variation in qualitative traits

4.3.1 Root shape

There were very highly significant differences ($p < 0.001$) among the cultivars for root shape (appendix5). The proportion of cassava accessions show that 38% of the accessions were irregular, 36% cylindrical, 24% conical cylindrical and 2% conical(figure 4.1).

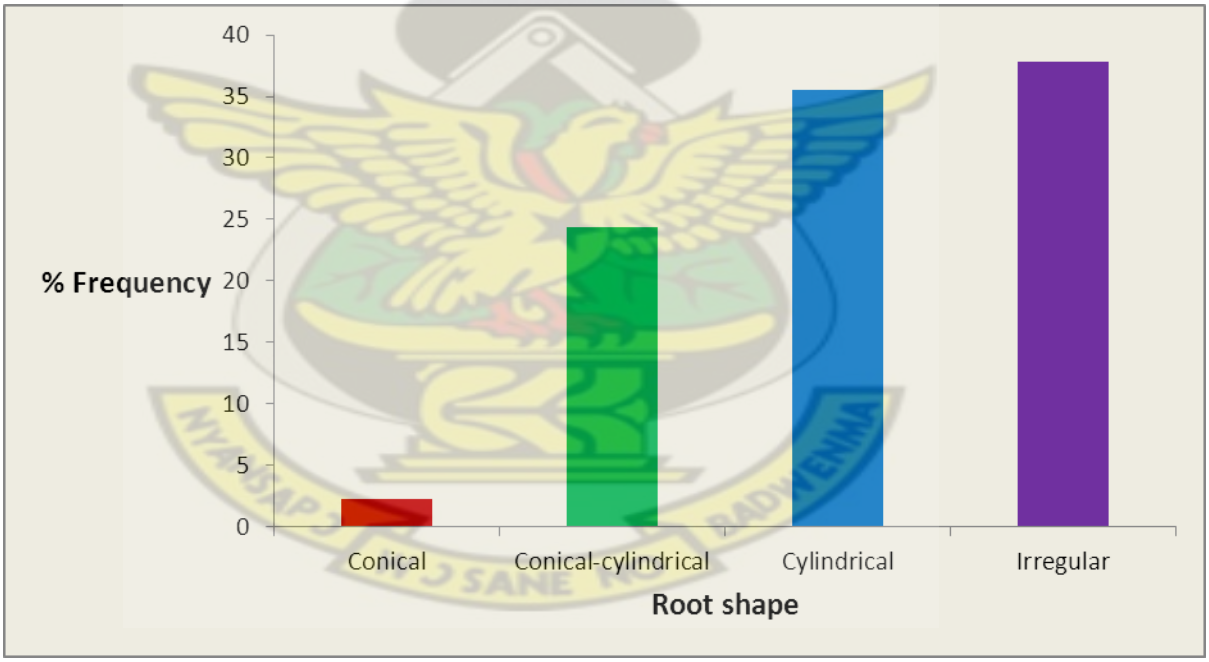

Figure 4.1. The distribution of root shape in 45 accessions of cassava

4.3.2 Petiole colour

The differences in petiole colour among the cultivars were highly significant ($p < 001$) (Appendix 6). 18% of the cassava accessions had purple petiole, 33% had red, 31% had dark green, 9% green- purple color and 9% light-green (Figure 4.2).

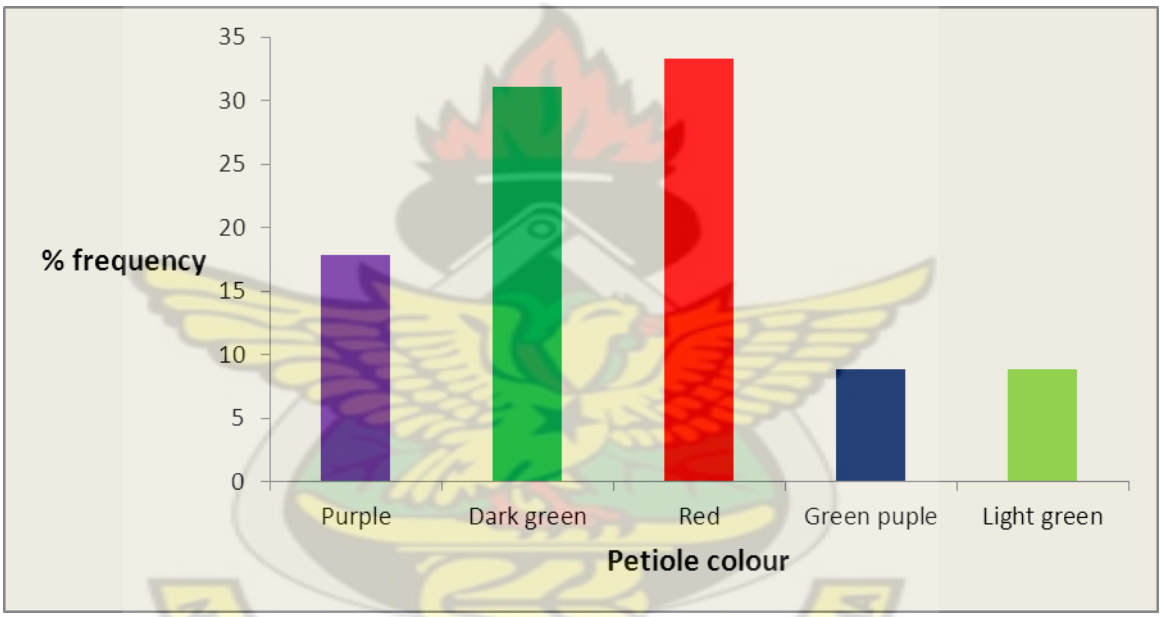

Figure 4.2. The distribution of petiole colour for 45 accessions of cassava

4.3.3 Shape of central leaflet

There were highly significant differences ($p < 001$) among the cultivars for the shape of central leaflet (Appendix7). Half of the cultivars had lanceolate (50%), 44% had Elliptic-lanceolate, 4% had oblong-lanceolate and 2% had obovate-lanceolate (Figure 4.3).

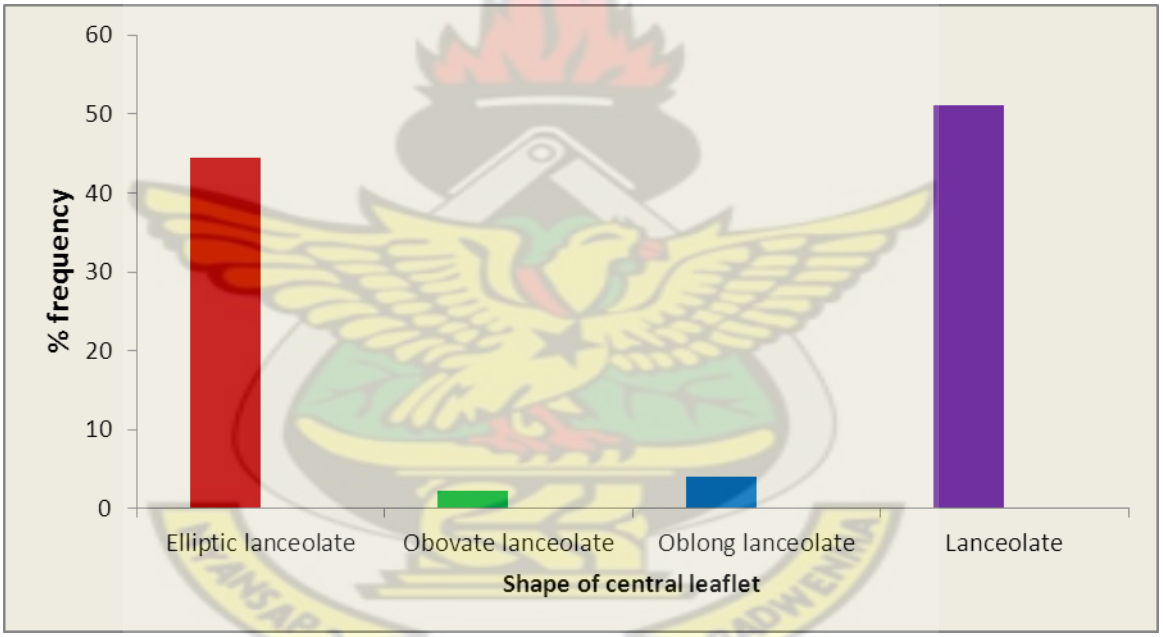

Figure 4.3. The distribution of shape of central leaflet of 45 cassava accessions

4.3.4 Growth habit of stem

Results of the growth habit of stem showed highly significant differences ($p < 0.001$) (Appendix 8) among the accession 31% were Zig-Zag and 69% were straight (figure 4.4).

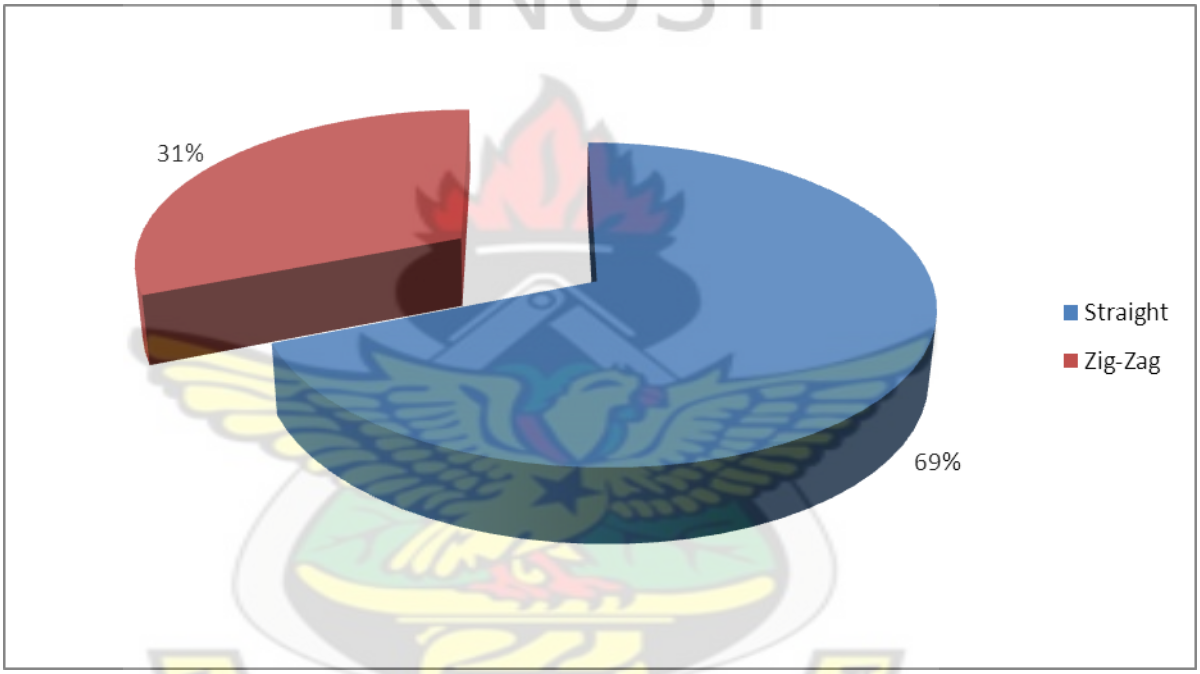

Figure 4.4: The distribution of growth habit of stem for 45 accessions of cassava

4.3.5 Pubescence on apical leaves

Differences in pubescence on apical leaves among the accessions were highly significant ($p < 0.001$) (Appendix 9). Pubescence on apical leaves showed that 20% were absences, 38% little pubescence, 20% showed moderate pubescence and 22% had high pubescence (Figure 4.5).

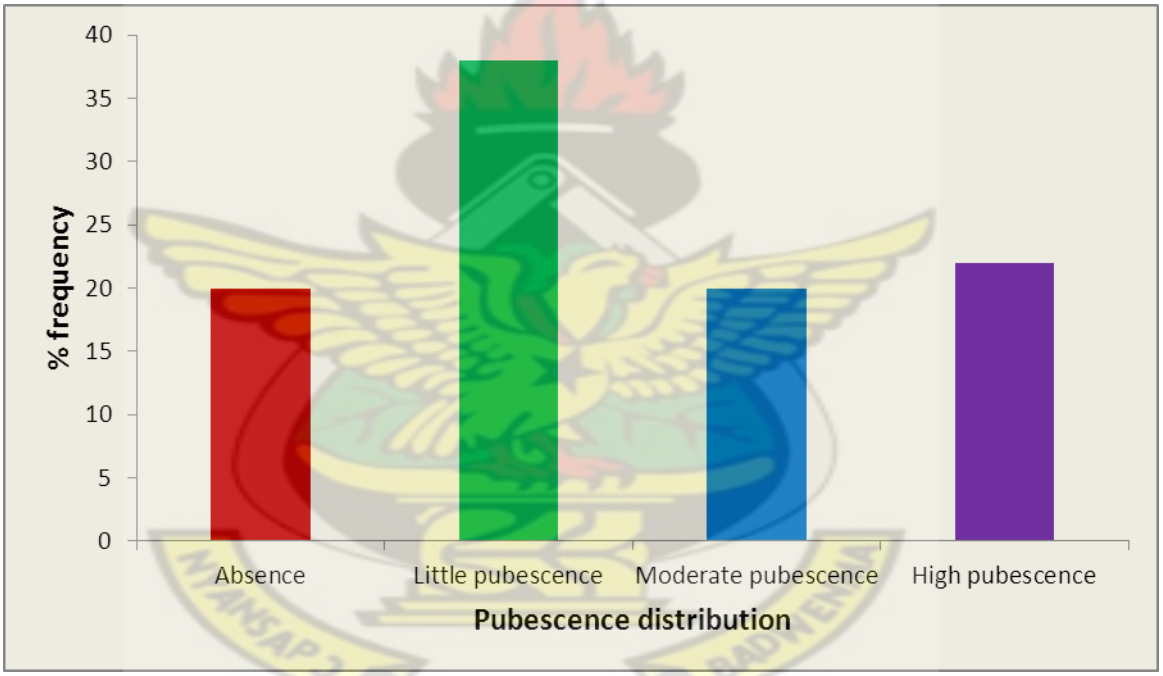

Figure 4.5: The distribution of pubescence on apical leaves for 45 accessions of cassava

4.3.6 Flowering

Differences in flowering among the cultivars were highly significant ($p < 001$) (Appendix10). Seventy-six percent of the accession produced flowers while 24% did not (figure 4.6).

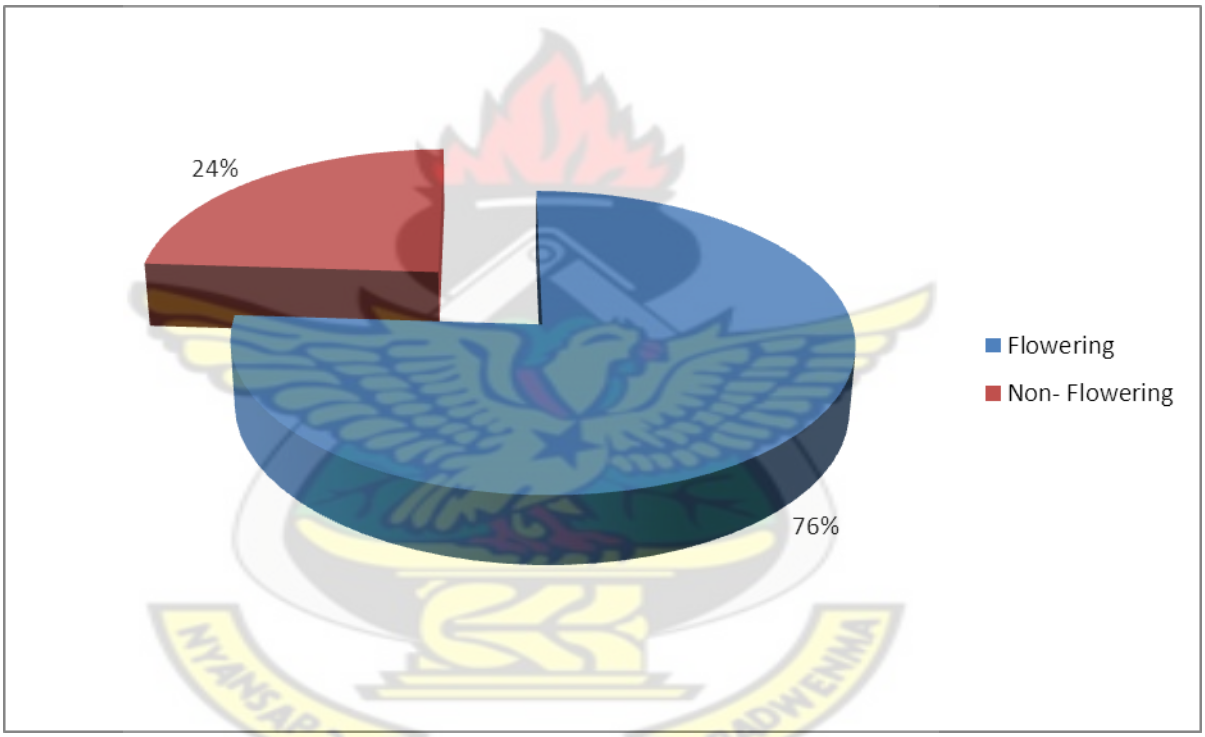

Figure 4.6: The distribution of flower production among the 45 accessions of cassava

4.3.7 Texture of root epidermis

There were highly significant differences ($p < 0.001$) among the accession for root shape (Appendix11). The results shows that among the cassava accessions that more than half of the accessions (53%) had rough root epidermis, 11% o f the accession had smooth root texture, and 36% intermediate root epidermis (Figure 4.7).

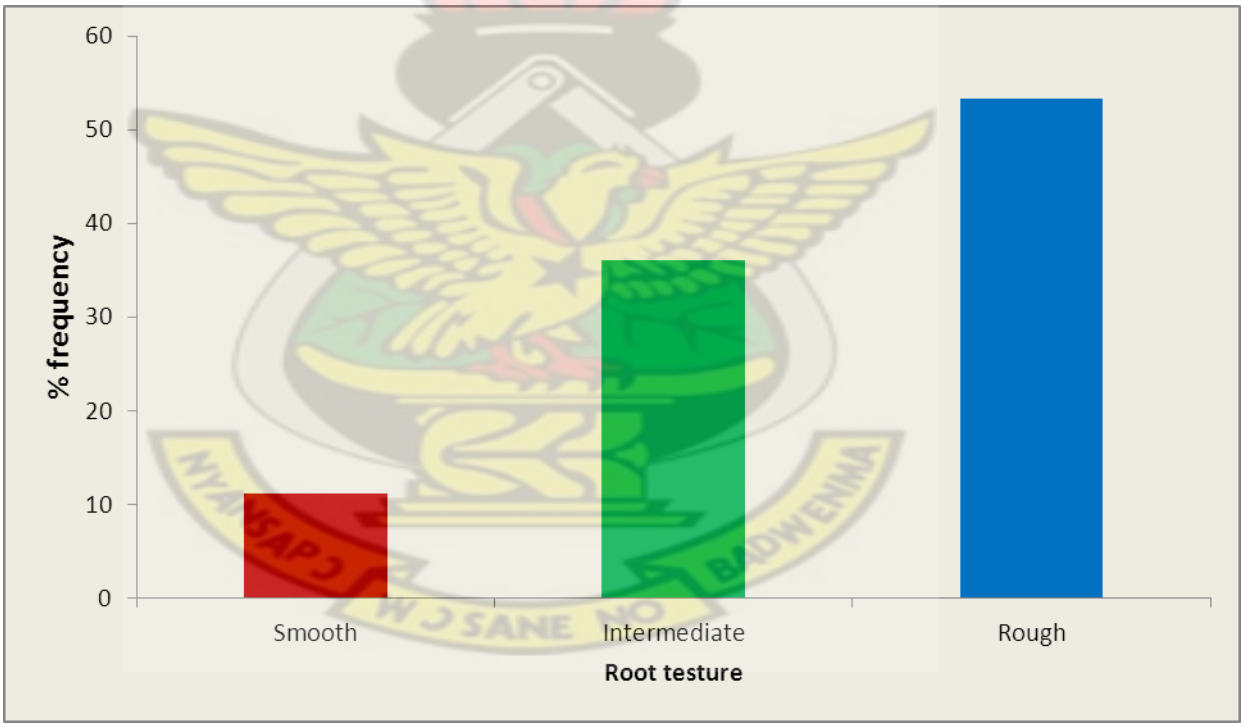

Figure 4.7. The distribution of texture of root epidermis for 45 accessions of cassava

4.3.8 Colour of apical leaves

There were highly significant differences ($p < 0.001$) among the cultivars for apical leaf colours (Appendix12). Twenty percent of the accessions had light green, 4% purple color, 51% dark green, and 25% were purplish green (Figure 4.8).

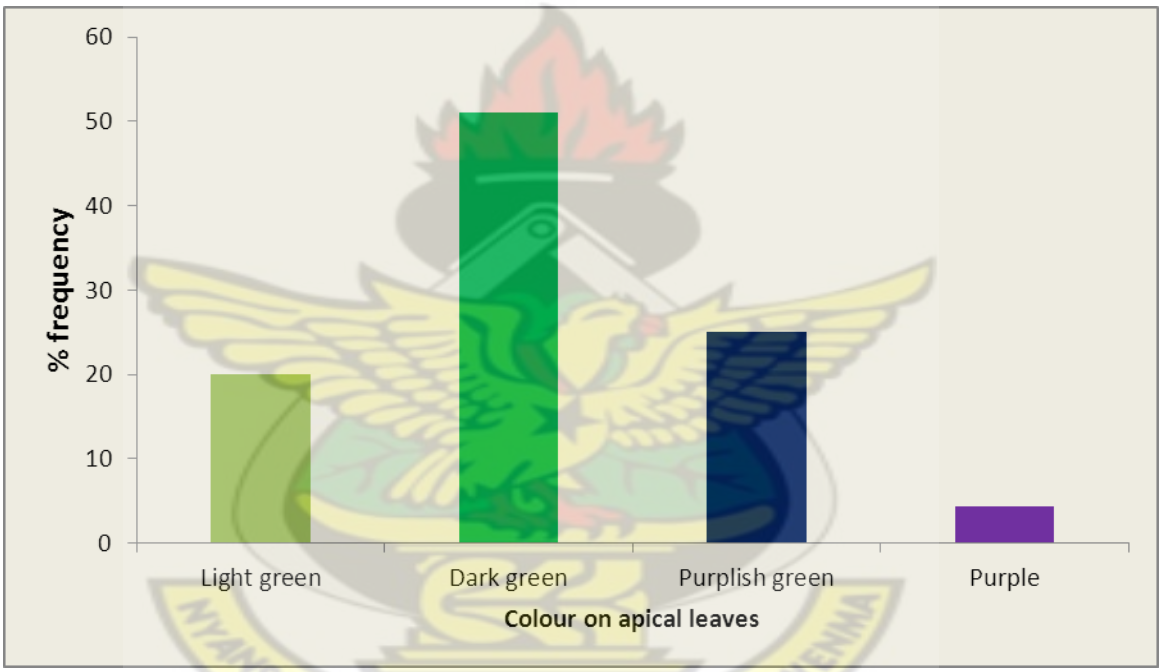

Figure 4.8. The distribution of the colour of apical leaves for 45 accessions of cassava

4.4 Severity of diseases

4.4.1 Cassava Bacterial Blight (CBB)

The severity of CBB scores at 3 MAP showed significant differences ($p < 0.001$) (Appendix13). Nine percent had no symptoms, 50% of the accessions had mild symptoms, 30% had moderate symptoms, 4% had severe blight symptoms and 7% very severe blight symptoms (Figure 4.9).

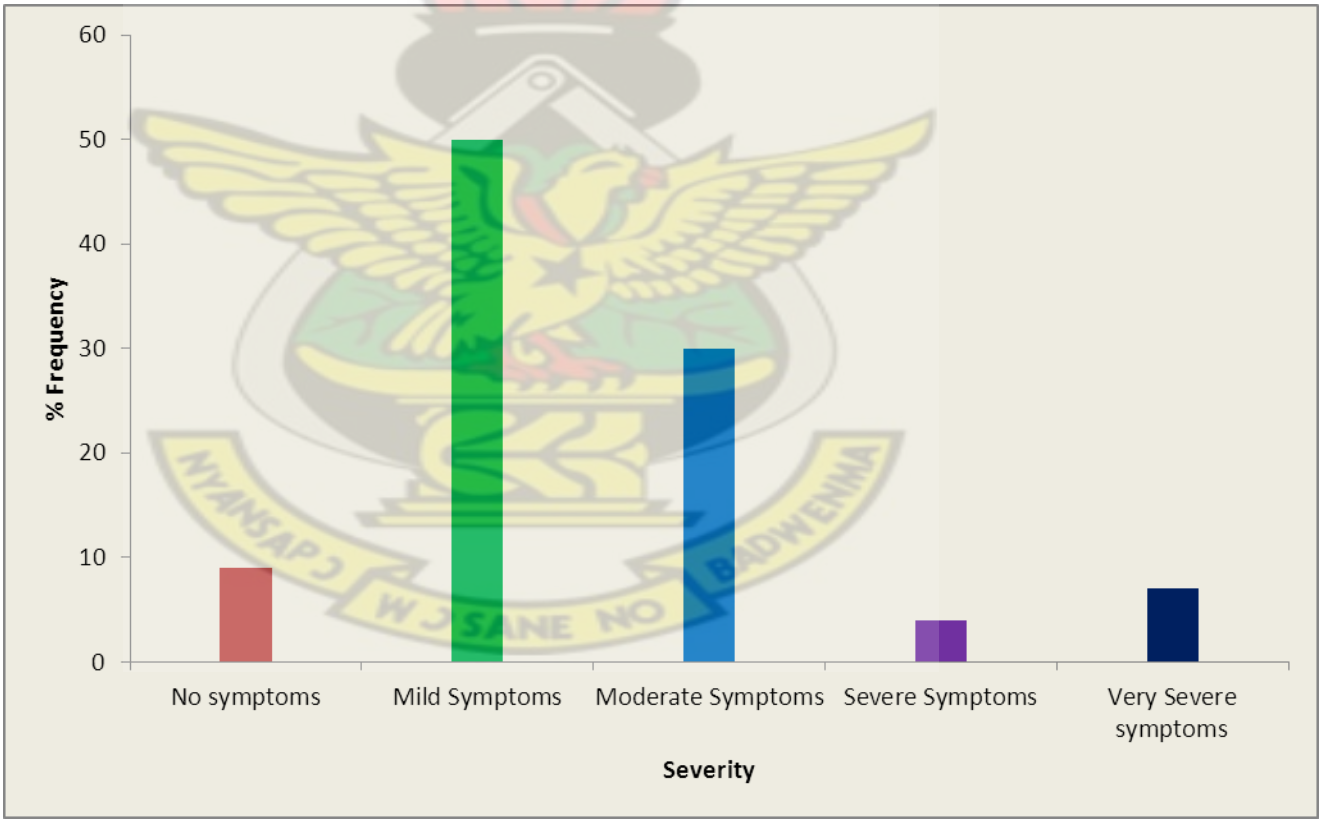

Figure 4.9. Severity of CBB for 45 accessions of cassava

4.4.2 Cassava Anthracnose Disease (CAD)

The severity scores of CAD at 9 MAP showed highly significant difference ($p < 0.001$) among the accessions (Appendix14). More than half of the accessions 60% had no symptoms, 4% had mild symptoms, 16% had moderate symptoms 11% had severe symptoms and 9% had very severe symptoms (Figure 4.10).

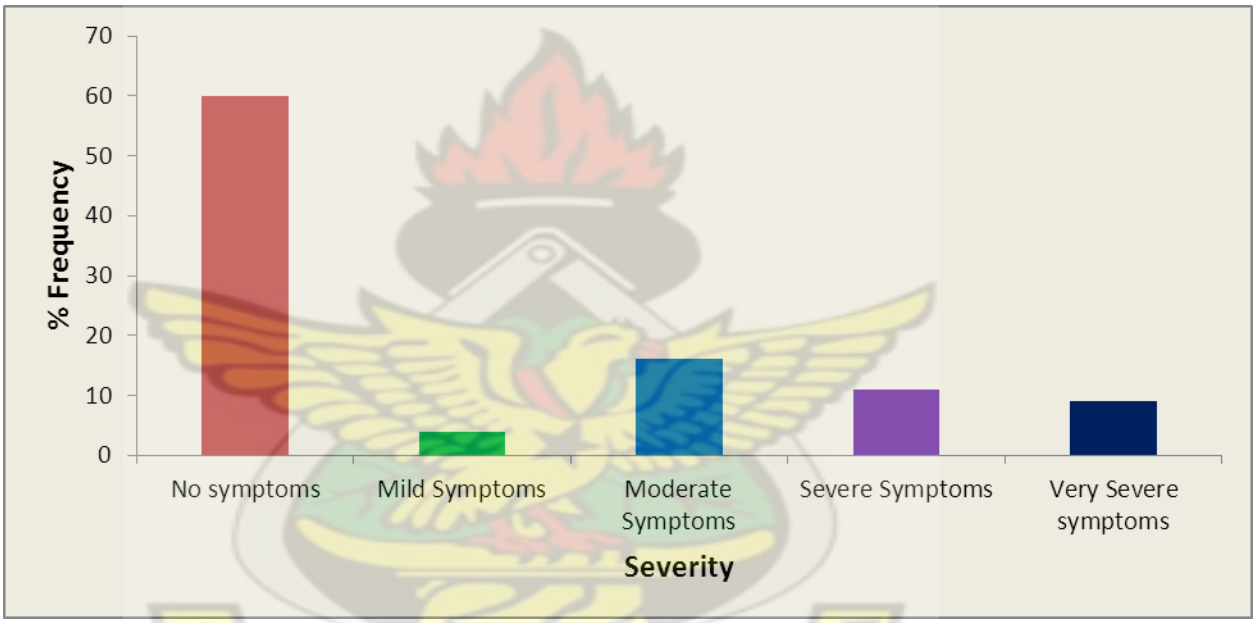

Figure 4.10. Severity of CAD for 45 accessions of cassava

4.4.3 Africa Cassava Mosaic Disease (ACMD)

The severity of the ACMD scores at 6 MAP showed significant differences ($p < 0.001$) among the accessions (Appendix15). Half of the accessions had no symptoms 50%, 13% had mild symptoms, 18% were moderate symptoms, 10% had severe symptoms and 9% had very severe symptoms (Figure 4.11).

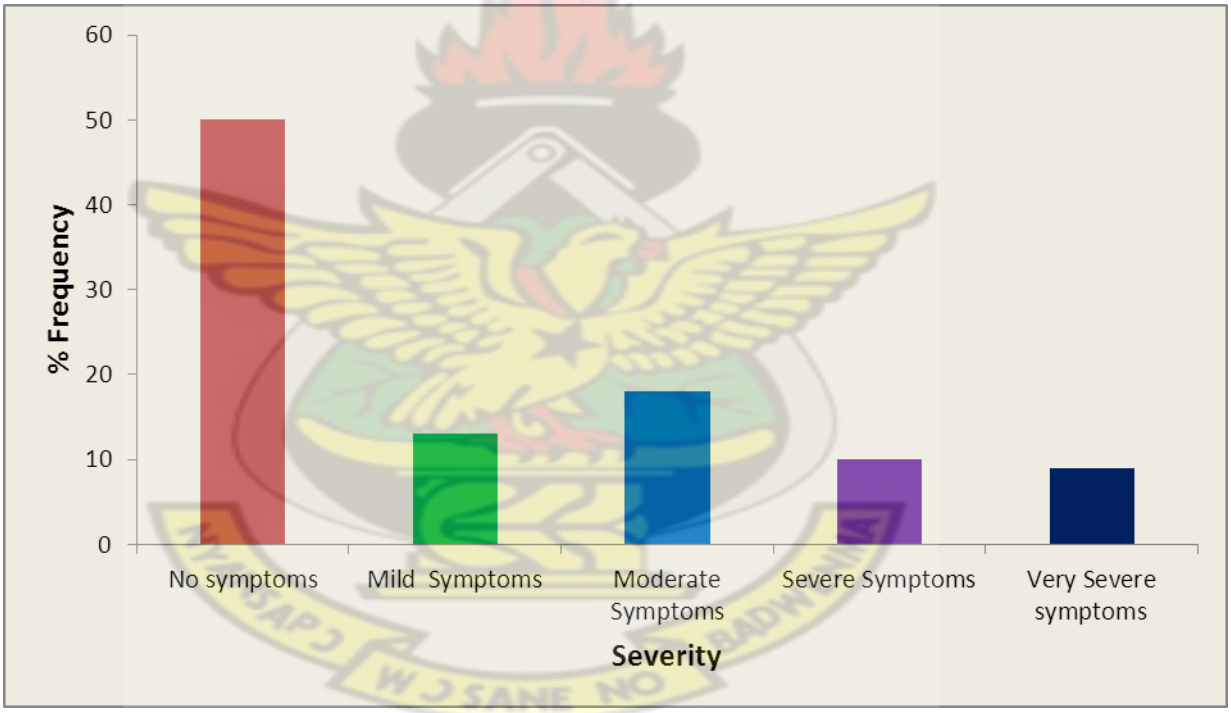

Figure 4.11. Severity of ACMD of 45 accessions of cassava

4.5 Morphological characterization using Dendrogram

Cluster analysis for morphological charaterisation of the of 45 cassava accessions revealed two major clusters (A and B), with the first cluster (A) containing only two accessions. Cluster B contained of most of the genotypes (Figure. 12). Sub-cluster I contained two landraces (MM96/528 and Dabodabo) and one exotic cultivar (01/0040). Accessions in this cluster were differentiated as having, dark green petiole colure, irregular root shape and smooth texture of root epidermis.

Sub-cluster II contained two exotic accessions and one landrace (Figure 12). Accessions in this cluster were characterised by expanded apical leaves. Sub- cluster III one exotic cultivar (00/0338), sub- cluster IV contained 6 landraces cultivars (BD96/075, KW2000/030, BD96/154, AFS2000/043, AFS2000/131 and AFS2000/071 and one exotic cultivar (Ampong). Sub-cluster V contained 29 accessions, 11 landraces accessions (OOF2000/145, UCC2001/111 ,BD96/093, AW2000/053, KSI2000/092, BD96/141, Ak Debor, Teckbankye, UCC, Nkabom, Essiabaya, and BD96/136) and 18 exotic cultivars (01/097, 94/0006, 01/0264, Essam, 97/2236, 97/0016, 00/0104, TME693, 00/0354, 98/0510, 01/0220, 01/0093, 01/0346, 01/0111, 01/0061, 01/0046 and 00/01040. These cultivars had green apical leaves, purple petioles and strength growth habit which made them unique from the rest of the accessions.

Although, some morphologically similar accessions were identified, the morphological indicators revealed narrow genetic diversity for landraces and exotic germplasm. The germplasm range between genetic similarity values from 0.08-1.0%.

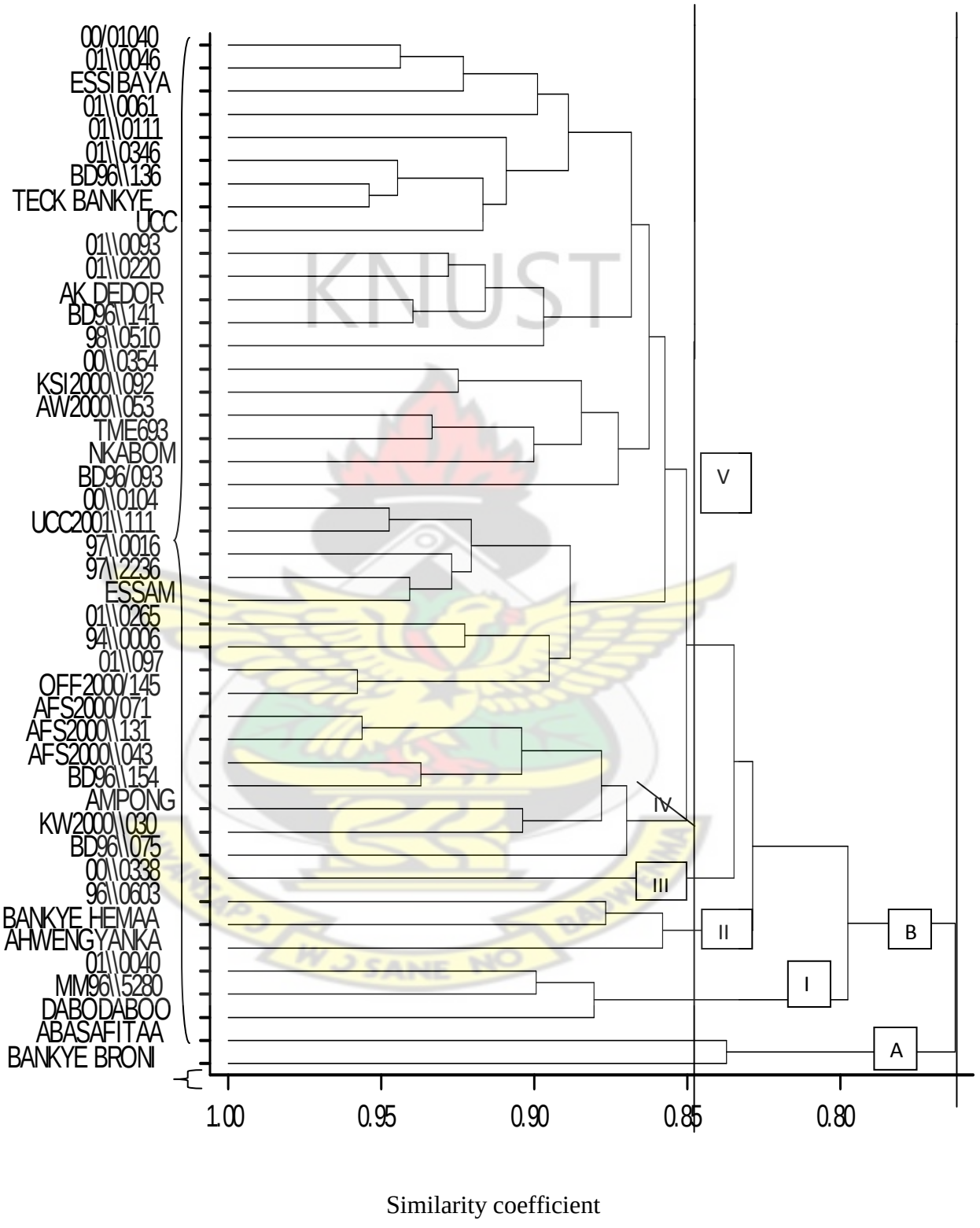

Figure 4.12. Dendrogram for morphological characterization of 45 cassava accessions.

4.6 Genetic diversity analysis using SSR markers.

Nine out of the 19 primers gave polymorphic bands whiles the remaining primers were either monomorphic or failed to amplify any product and therefore were not considered for further analysis. The number of alleles ranged from 10 (in SSR164) to 41(in SSR51) primers per locus with a mean value of 23 alleles per locus. The Polymorphic Information Content (PIC) values also ranged from 0.78 (in SSR164) to 0.96 (in SSR51) with an average of 0.91. The most polymorphic primers were SSR51, SSR52, SSR4, SSR82, SSR63 and SSR21 based on PIC values (Table 4.2). The allele frequency of all the primers indicate that they were all polymorphic in character. Gene diversity was high ranging from 0.80 in SSR164. However, SSR21, SSR82 and SSR4 had the same Gene diversity of 0.93 with a mean value of 0.92 for all.

Table 4. 2. Allele frequency, Number of Allele, Gene Diversity, Heterozygosity and Polymorphic Information content (PIC) values generated from SSR data.

Marker	Allele frquency	AlleleNo	Genediversity	Heterozygosity	PIC
SSR179	0.1667	14.0000	0.9047	0.9556	0.8970
SSR164	0.3571	10.0000	0.8039	0.6190	0.7831
SSR21	0.1071	28.0000	0.9388	0.4048	0.9354
SSR63	0.1000	38.0000	0.9563	0.9778	0.9546
SSR82	0.1310	21.0000	0.9308	0.5952	0.9266
SSR4	0.1143	21.0000	0.9331	0.2857	0.9291
SSR52	0.1000	26.0000	0.9462	1.0000	0.9435
SSR51	0.0610	41.0000	0.9697	0.6585	0.9688
SSR59	0.1429	12.0000	0.9005	0.2143	0.8919
Mean	0.1422	23.4444	0.9204	0.6345	0.9145

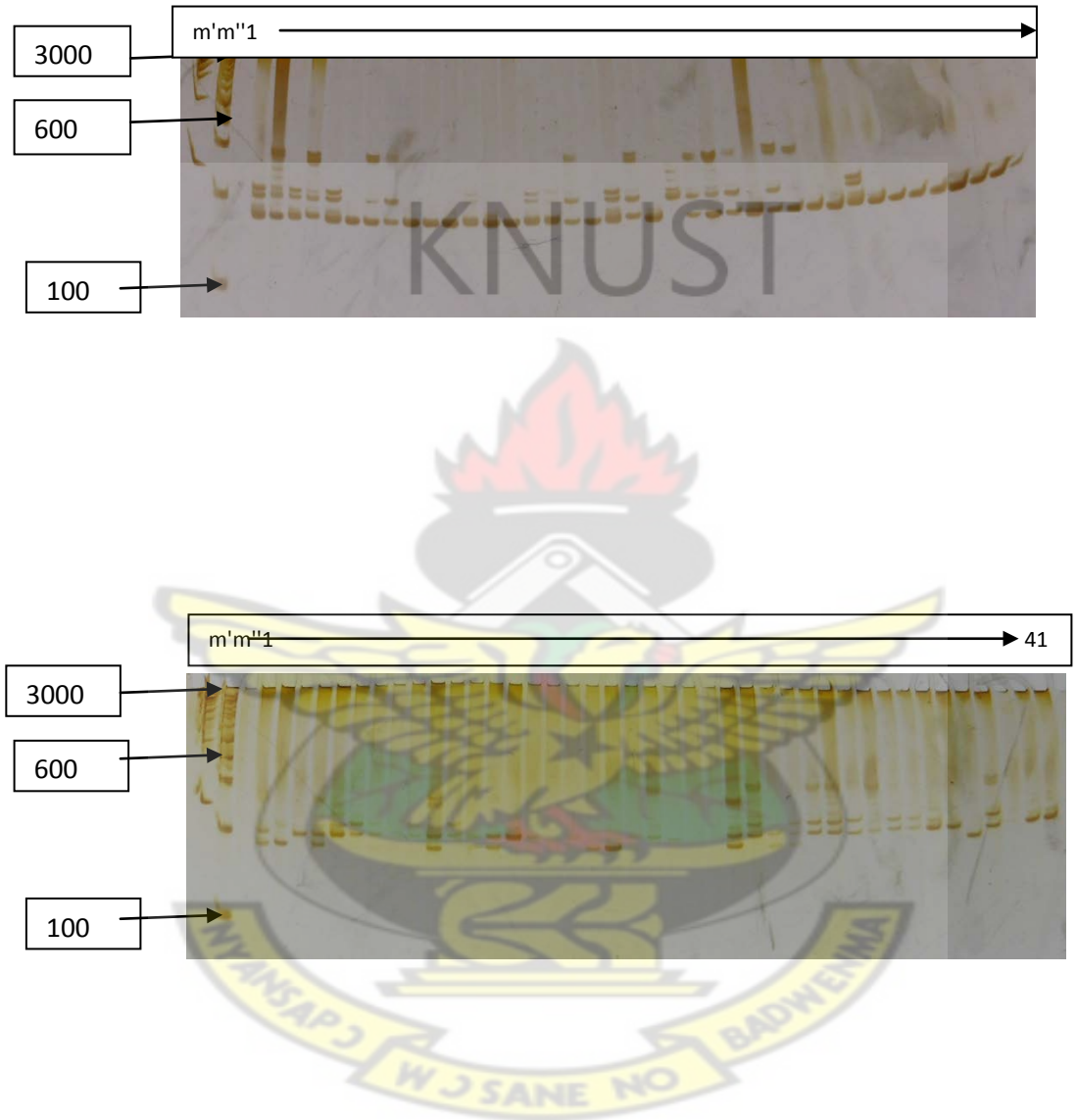

Fig 4.13. Amplification products obtained after separation of SSR products on Acryl amide gel obtained from the cassava accessions.

The dendrogram for the subset of the 45 accessions revealed two main clusters A and B (Figure.4.14). 16 of the accessions belonged to cluster A, while the rest were in cluster B. Sub-cluster I (within cluster A) contains two exotic accessions (97/0016 and TME693). Accessions in this cluster were characterised by lanceolate shaped, central leaf lobes and irregular root shapes.

Sub-clusters II and III (within cluster A) contain one landrace each (AFS2000/131 and OFF 200/145) while sub-cluster IV (within cluster A) contains one landrace (AFS2000/043) and one exotic cultivar (97/2236). Sub-cluster V (within cluster A) contains two landraces (AFS2000/043 and AW2000/053). Sub-cluster VI (within cluster A) contains one landrace (KW 2000/030). Sub-cluster VII (within cluster A) contains one exotic cultivar (00/035). Sub-cluster VIII (within cluster A) contains one landrace (BD96/093). Sub-cluster IX (within cluster A) also contains one landrace (KSI2000/092) and one exotic cultivar (01/0346). (Figure.4.14)

Sub-cluster I (within cluster B) contained two exotic cultivars (00/01040 and 00/104) (Figure.4. 14). The similarity coefficient range of 0.2-1.0 showed wide genetic diversity within this cluster. Accessions in this cluster were characterised by non-flowering, moderate pubescences and irregular root shape.

Sub-cluster II (within cluster B) contained one landrace (BD96/136) and one exotic cultivar (Bankyehemaa) (Figure.4. 14). These cultivars were characterised by dark green apical leaves and straight growth. Sub-cluster III had two cultivar one landrace and one exotic. Accessions in this cluster were characterised by white green petiole colour. In sub-cluster I, II and III (within cluster A), Essam, Nkabom and UCC2001/111 were separated from the rest of the accessions by which was an indication of uniqueness.

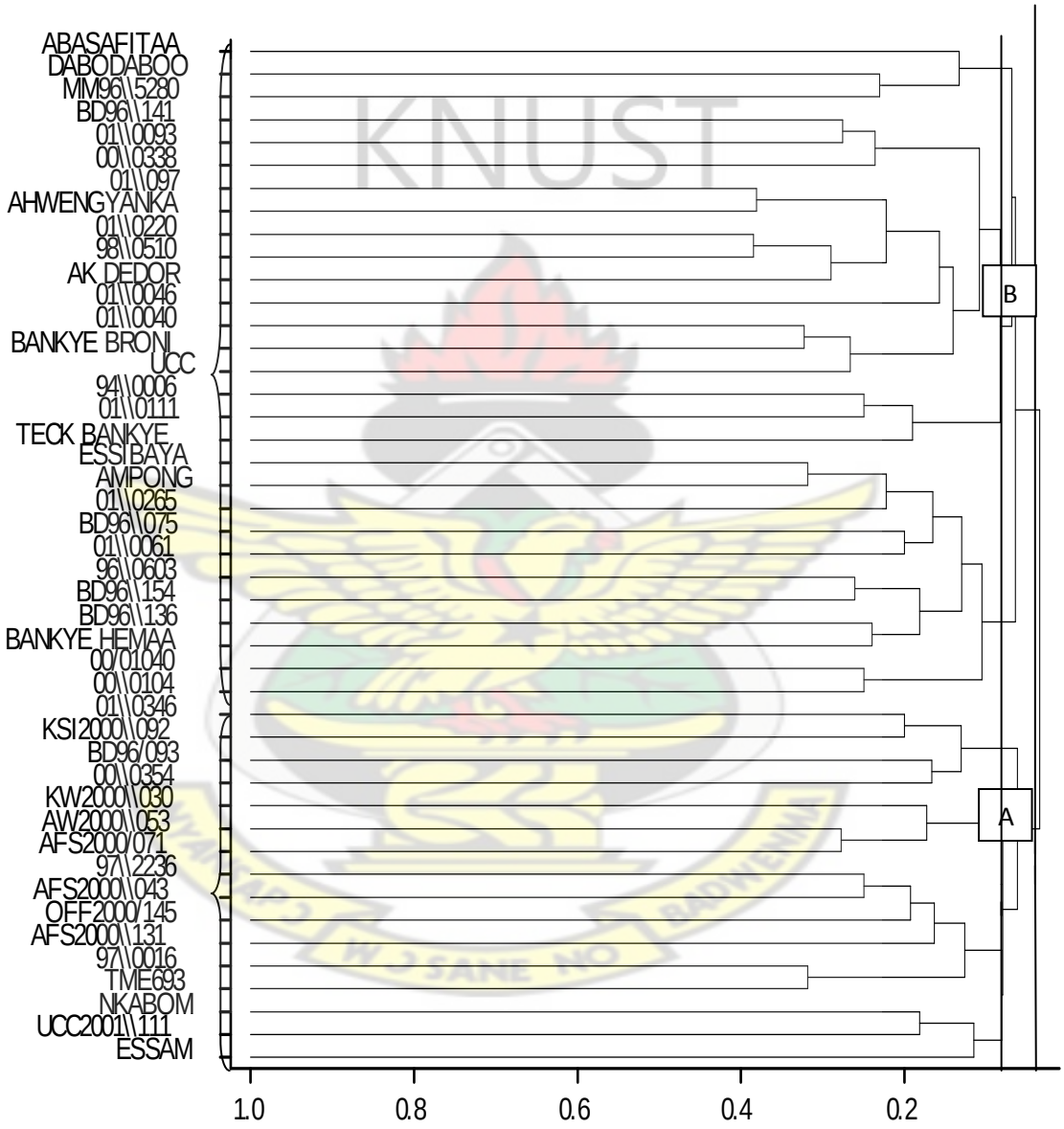

Figure 4.14 Similarity coefficient subset of 45 cassava accessions characterised using nine SSR primers.

4.7 Comparison of morphological and molecular characterization

Dendrogram generated using morphological and molecular data for the subset of 45 accessions are shown in (Figure. 4.15). This grouping shows two main clusters, A and B. Cluster A contained three accessions with one sub-cluster. Most of the accessions belonged to cluster B which further formed ten major sub-clusters (Figure.4.15) .

However, the grouping of both Morphological and SSR dendrogram were similar for clusters and genetic distances**.** TME 693 and 97/0016 clustered together. Dabodaboo and MM96/5280 also clustered together in both dendrogram and they were the most similar cultivars. Moreover, Ahwengyanka, Bankye hemaa and 96/0603 which were 100% similar based on morphological data (Fig4.12) clustered separately based on SSR data (Figure 4.14) dendrogram. These cultivars are morphologically and genetically similar. Therefore, SSR markers analysis confirmed morphological characterization.

Some differences were observed between morphological and molecular characters for some clusters and genetic distances**.** Teckbankye, AK Debor and 01/0046 clustered separately from other accessions in the SSR dendrogram in sub-cluster, but within sub-cluster B of the morphological dendrogram they cluster together. Accessions in cluster B of the morphological dendrogram clustered separately from other accessions but randomly within the SSR dendrogram.

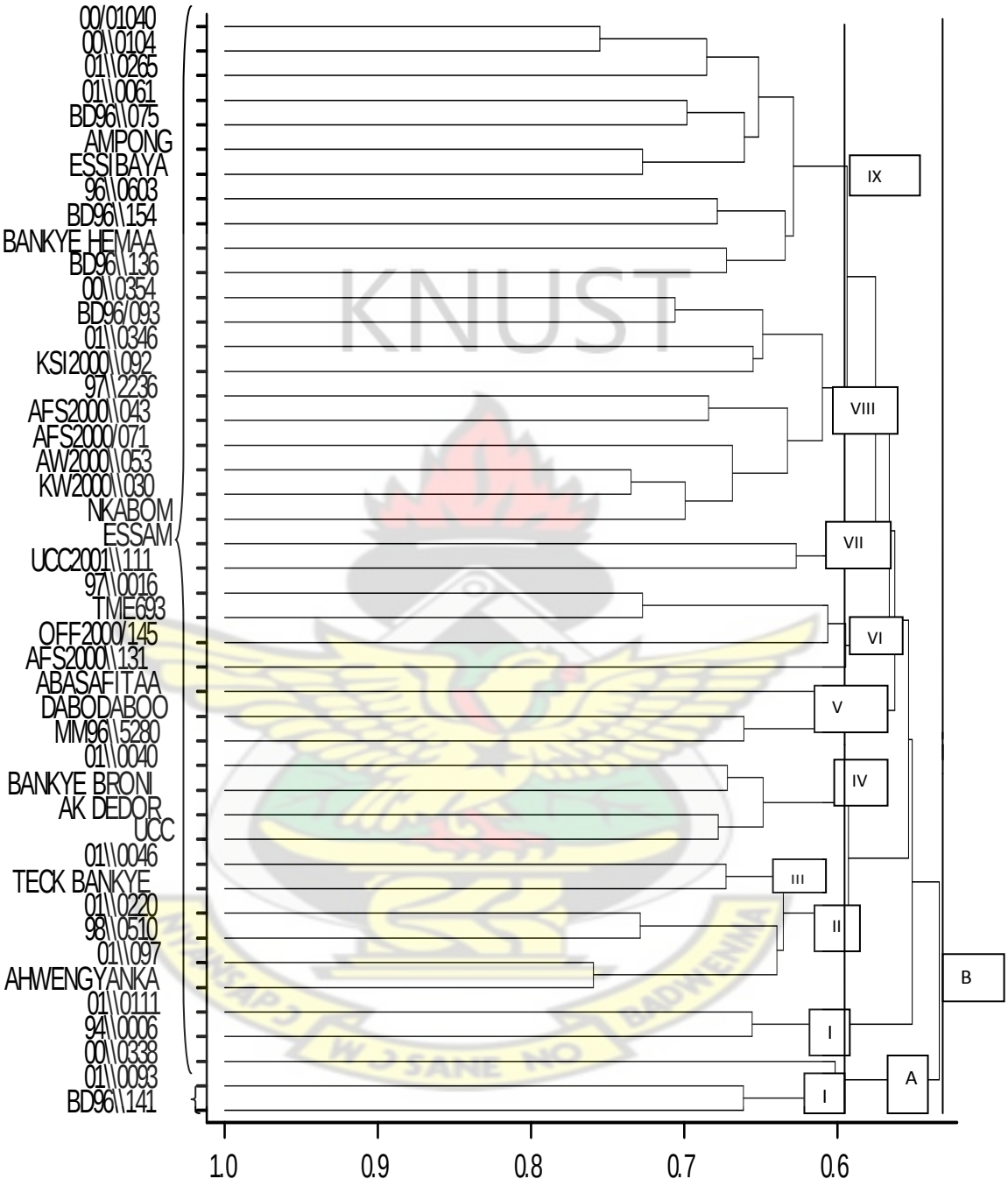

Figure 4.15. Grouping of the subset of 45 cassava accessions based on phenotypic and molecular characters using nine SSR Primers and eighteen morphological traits.

CHAPTER 5

5.0 DISCUSSION

5.1 Morphological differences in quantitative and qualitative traits

Different cassava cultivars are often given the same name or a single cultivar may be given different names depending on where they are found and used for production. This could over or underestimate the diversity available for use by farmers (Manu-Aduening, *et al*., 2005). Several workers including, Agyare-Tabi *et al*. (1997) and Fregene *et al*. (2001) have used either phenotypic or molecular characterization separately to address this challenge in cassava improvement programmes in Ghana and elsewhere. However, this concern has not been adequately addressed in Ghana. The present study was thus carried out to characterise exotic and landraces of cassava in Ghana using defined agro-morphological and molecular characterization. The results obtained revealed a significant amount of variation among the accessions for all the eighteen morpho-agronomic and molecular traits evaluated.

Root characters (root yield, root shape, texture and colour) gave the clearest indications of variation. In this respect, accessions with similar characters appeared in the same cluster. This supports similar findings reported by Luis *et al*. (1998), on passion flora.

Agro-morphological characterization indicated that two accessions composed of cluster A, whilst 43 accessions were grouped in cluster B. On the other hand, molecular characterization also grouped 16 accessions in cluster A and 29 in cluster B. Overall, the cluster analysis using both molecular and agro-morphological characteristics put 3 in cluster A and 42 in cluster B.

Morphological parameters such as levels of branching, angle of branching, height at branching, and plant height varied significantly among cultivars. The landrace AFS2000/071 branched the most, while Abasafitaa (exotic cultivar), 96/0006, 01/097 and UCC branched the least (Appendix 4). The landrace KSI2000/092, produced a fresh storage yield of 44.00 T/ha, a level considered high for landraces and comparable to that of the exotic cultivar, Ampong (30.00 T/ha). Some landraces cultivars used in this study (BD96/136, Dabodabo, and AW2000/053) were either low branching or had a wide angle of branching comparable to that of 00/0104, 01/0093 and 94/0006 which are exotic cultivars. This is significant as it helps in suppressing weeds, by forming a wide canopy spread that prevents the growth of non-shade tolerant weeds (Melifonwu *et al*., 2000). Early branching type with wide canopy spread will however not be suitable for intercropping.

The dry matter percentage ranged from 20.67 to 36.03%. Four landraces (Ahwengyanka, BD96/141, AK Debor and UCC) had high dry matter content while Essam, 96/0603, 98/0510, 94/0006, and Bankye Broni were on the lower side of the range (Appendix 4). The collections differed significantly for all the characters. This significant differences for the characters is an indication of wide genetic variability among the collection, which could be exploited in selection and breeding programmes.

Colour distribution, shape of central lobes and branching habit typified the diversity of the accessions, hence are important salient characters used by farmers to identify cultivars. Elias *et al*. (2001) reported that colour variables played a crucial role in differentiating cassava varieties. Morphologically close cultivars have a greater possibility of being confused. The confusion can happen either when one farmer acquires

cuttings from another farmer or even in the farmer's own field as he or she selects plants to be planted in the next field (Elias *et al*., 2001). This could be one of the reasons for the high number of morphologically similar accessions observed in this study.

The highly significant differences among petiole colour, plant height, root shape, pubescence on apical leaves, flowering, colour on apical leaves indicate that these characters are heritable among the accessions. Height at first branching was also evenly spread throughout the growing period as some have wide lower plant height and others with tall plant height. This can be attributed to the genetic makeup among the cultivars. There was a significant difference in the severity of ACMD, CBB and CAD in the accessions.

Morphological analysis on the dendrogram showed a narrow range of genetic similarity 0.80-1.0 among the tested genotypes (Figure 4.12). This was in agreement with a study by Fregene *et al*. (2000) who reported a genetic similarity (GS) range of 0.80-1.00. The narrow genetic similarity in this study could be due to the fact that morphological characters are controlled by a few major genes and may be caused by changes in a few loci as reported by Halward *et al*. (1992) that might be subjected to intense selection pressure. As a result, morphological variation is likely to decrease during domestication while molecular markers which are not subjected to direct selection, often increase as reported by Gepts (1991). These results agreed with the findings of cassava genetic diversity studies of Roa *et al*. (1997) and Wong *et al*. (1999) who found a narrow genetic similarity (GS) range in cassava analysis. Most of the accessions collected were closely related as revealed by morphological characterization, while the SSR primers revealed

wide genetically diverse among the accessions. Similar results were reported by Chiwona-Karltun *et al*. (2000) and Mkumbira (2002) .

5.2 Genetic characterization using SSR Markers

Genetic characterization in cassava has been previously studied using DNA molecular primers such as isozyme markers (Sarria *et al*., 1992), RFLP (Angel *et al.*, 1992), RAPD (Tonukari *et al*., 1997; Ugorji, 1998) and SSR (Fregene *et al*., 2001) with either low or medium observable genetic diversity (Moyib *et al*., 2007). In Nigeria, a medium genetic diversity was observed between exotic cassava cultivars and commonly grown Nigerian landraces (Moyib *et al.*, 2007). This revealed that the exotic and the landraces cultivars may have distantly related parents.

In the present study, the number of alleles produced by different primers range from 10-41 with an average of 23.4 alleles per locus. Similarly, Raghu *et al*., (2007) studying India cassava accessions with 15 SSR primers recorded a means number of 4 alleles with a range of 2 to 4 allele. Moyib *et al*. (2007) also obtained a range of 2 to 4 alleles among 31 exotic cassava cultivars and landraces. Thus the highest number of alleles in this study was higher than those reported by Moyib *et al*. (2007) and Raghu *et al*. (2007). A total of 211 clear and scorable DNA fragment were detected among the 45 cultivars using 9 SSR primers. SSRs generally have high levels of polymorphism in many important crops including *M. esculenta* (Chavarriaga-Aguirre *et al.*, 1999; Mba *et al.*, 2001, Zacarias *et al.*, 2004; Raghu *et al.*, 2007).

The mean PIC value recorded in the current study compared favorably with results obtained from another study by Tams *et al*. (2004), where 128 accessions of Tritical with 28 SSR markers gave a mean PIC value of 0.54. Similarly, Moyib *et al*. (2007) reported a mean of 0.91 to 0.66 in the current study. The Polymorphic Information Content (PIC) range from 0.78 to 0.96 in this study suggests that most of the SSRs used were highly informative and can be used for genetic diversity studies.

SSR analysis on the dendrogram showed a high range of genetic similarity (GS) values of 0.2-1.00 compared to morphological data with genetic similarity (GS) range of 0.80-1.0. Similar results were obtained when DNA techniques were used by Gepts (1991) on common beans, Miller and Tanksley (1990) on tomato and Keim *et al*. (1990) on soybean. However, the result showed that the SSR markers were efficient and the germplasm was diverse. Exotic accession clustered separately except for Abasafitta, which clustered together with local landraces in sub cluster XII (Figure4. 14).

A breeding programme between the exotic cultivars and landraces and among cultivars therefore has the potential to widen the genetic base of Ghanaian cassava germplasm and also provide new varieties with higher agronomic value. These results showed that a high degree of relationship existed between SSR and morphological traits diversity analyses methods. Nemera (2003) reported a significant correlation between SSRs and morphological genetic diversity analyses on sorghum. The correspondence between morphological and SSR analyses found in the current study might be due to the fact that mainly salient traits were used as recommended by Berthaud (1997) and Elias *et al.* (2001) since they are less affected by the environment and developmental stage of the plant. SSR uses the same principle of revealing salient fragments, which typify

individuals or populations. Salient traits should be compared with SSR analysis (Elias *et al*., 2001), while polygenic traits should be compared with Quantitative Trait Loci (QTL). Traditionally**,** genetic diversity estimates and segregation of genes and hybrids in crop species were based on differences in morphological characters and quantitative traits (Schut and Stam, 1997) which have been accurately done.

On the other hand, DNA markers are perceived as reliable, since it is not influenced by environmental factors and give rise to a high number of polymorphic loci (Karp *et al*., 1997). However, DNA markers require specialized knowledge, laboratory equipment and chemical supplies making them more expensive than morphological descriptors. Genetic characterization is mostly neglected, leading to little or no contribution to the formal breeding schemes, which has resulted in low adoption of exotic varieties.

During the germplasm collection farmers reported diverse characters which assisted them in the identification and selection of preferred cultivars and for economic gains (Dr. Joe Manu-Aduening, personal communication). These characters range from canopy spread, root tuber, resistant to cassava disease and pests and processing amenability and suitability in intercropping with various crops and cropping systems. Cassava accessions were diverse in each of these characters. This result therefore, showed that there would be easy formation of hybrids, and introgression of useful genes among the improved cassava cultivars and Ghana landraces assessed.

5.7 Characterization of genotypes based on their phenotypic and molecular characters

When the morphological data and the molecular data were grouped together, the similarity matrix coefficient of the dendrogram ranged from 0.6 to 1.0 and that of the SSR analysis ranged from 0.2 to 1.0. This indicated that the genetic diversity is higher in SSR analysis than the grouping of the genotypes based on their phenotypes and molecular characters and lower in morpho-agronomic evaluation range of 0.80-1.0 that was observed in all the dendrogram of 45 accessions of cassava. This might stem from the fact that the Ghanaian landraces and exotic cultivars were domesticated in the same ecological zones with narrow genetic base. This lack of consistency between different primer techniques was also observed in cowpea (Chen-Dao *et al.*, 2001). This might be due to the fact that different primer techniques detect different components of DNA variation when subjected to different evolutionary mechanisms. The results of this study showed that each of the 9 primers detected polymorphisms among the 45 cassava accessions studied.

The results from this study indicate that there is enough genetic diversity among Ghanaian cassava germplasm, which could be exploited for breeding and selection programmes for improved genotypes for cassava production. The current findings also suggest that morphological descriptors, even though easy to use and readily available, may lead to mislabeling particularly in the case where certain genotypes were identified based on morphological descriptors as the same accessions.

CHAPTER 6

6.0 CONCLUSION

Assessment of genetic diversity in any germplasm is essential for selection of parents for inclusion in breeding programs. Identification of superior genotypes by assessing genetic diversity is an important prerequisite for a successful crop improvement program. Genetic diversity in crop plants can be measured using various tools such as morphological, biochemical and molecular markers. Therefore, the findings of this study were.

- Morpho-agronomic characterization and SSRs primers were effective in assessing phenotypic and molecular diversity within the cassava germplasm collection.
- Morpho-agronomic characterization separate the germplasm into 2 main cluster (A and B) with A having only 2 accessions and B 43 accessions
- SSR markers were more powerful than morphological traits in distinguishing accessions because they are not affected by the environment.
- The differences observed in clusters were as the results of genetic variability in terms of morpho-agronomic and molecular characters.
- Molecular markers also grouped the accessions into 2 clusters A and B. However the clusters here contained different accessions because they are not affected by the environments.

6.1 RECOMMENDATIONS

- The application of morphological descriptors in management of germplasm should be backed by the use of molecular markers, because the former alone does not reveal much diversity due to the effects of the environment on quantitative traits.
- Because cassava is highly affected by the environment future work should consider other locations.
- Future work should increase the number of SSR markers for better results.

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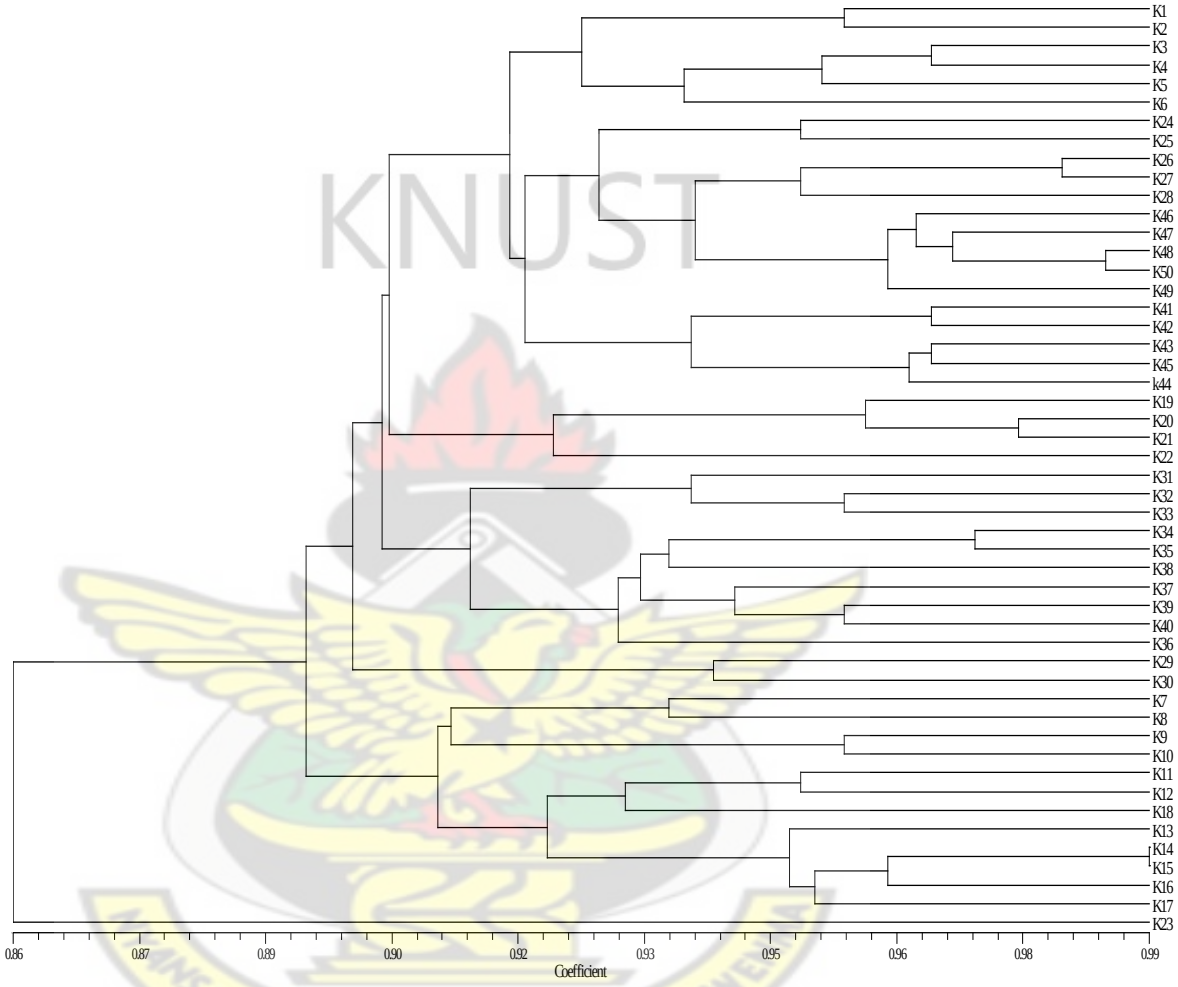
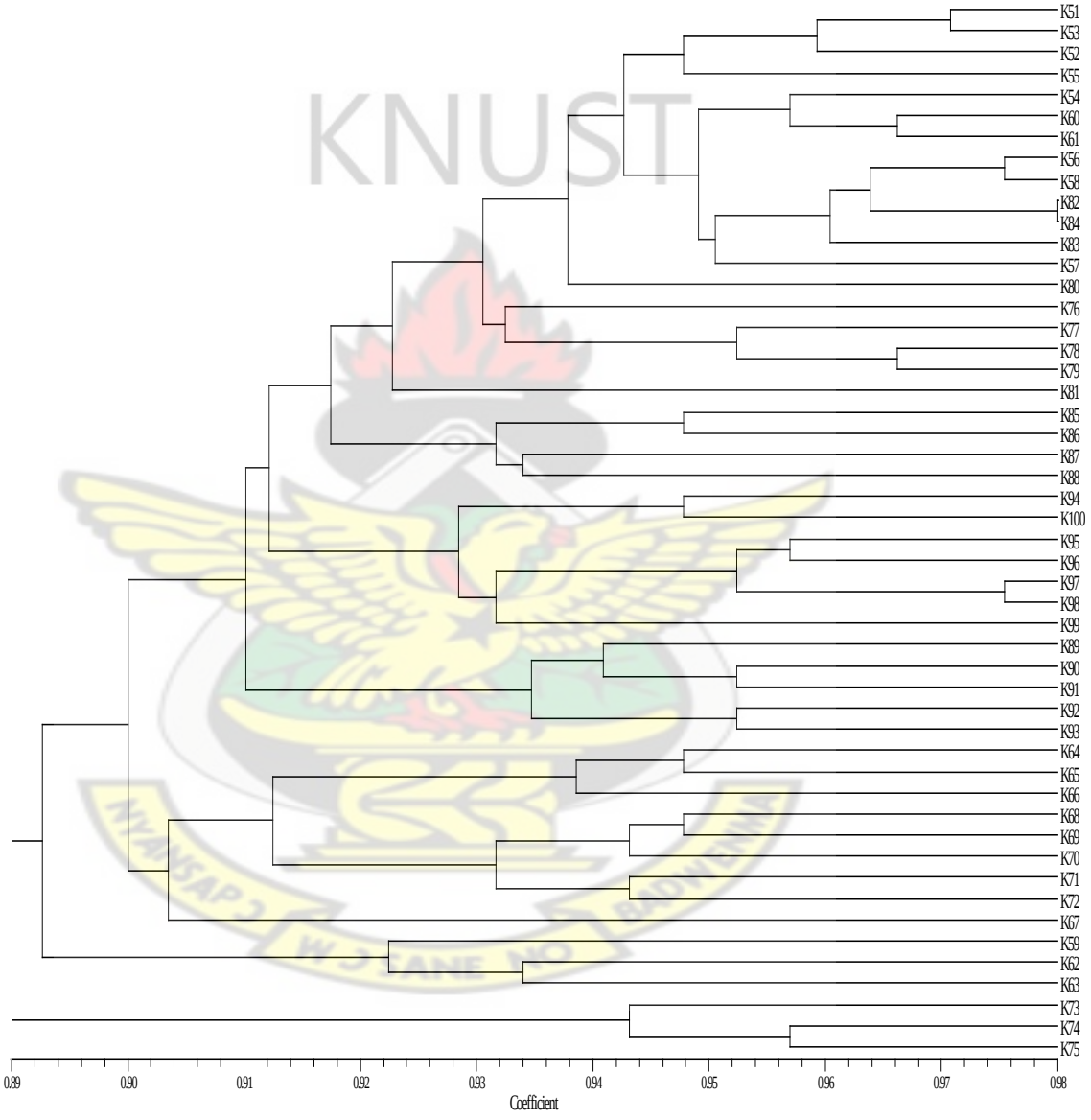
APPENDICES

Appendix 1: Dendrogram for morpho-agronomic charaterisation of 50 cassava accessions



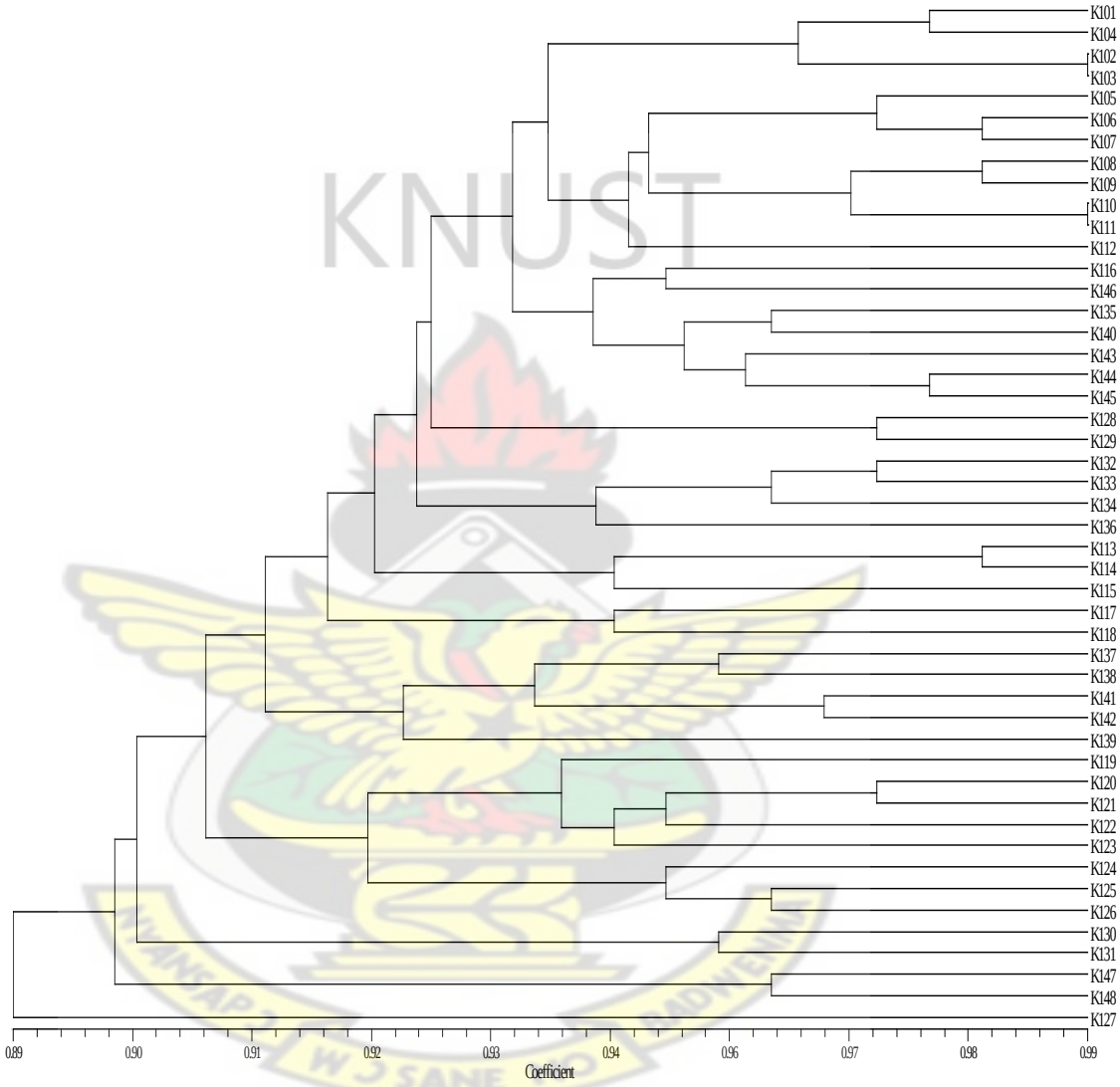
Similarity coefficient

Appendix 2: Dendrogram for morpho-agronomic charaterisation of 50 cassava accessions



Dice similarity coefficient

Appendix 3: Dendrogram for morpho-agronomic charaterisation of 50 cassava accessions



Dice similarity coefficient

Appendix 4 Table of means

Traits	Plant height (cm)	Canopy spread (cm)	Height at first branching (cm)	Angle at first branching((^{0})	Petiole length (cm)	Root yield (T\ha)	Dry matter (%)
00/01040	210.0	30.44	113.3	116.7	25.67	1.40	30.44
00/0104	143.3	23.91	31.7	113.3	19.67	17.33	23.91
00/0338	182.3	36.07	83.3	103.3	11.57	2.33	36.07
00/0354	146.7	36.66	71.7	90.0	17.50	7.40	36.66
00/0040	216.7	40.23	76.7	96.7	28.33	18.13	40.23
01/0046	166.7	27.37	73.0	130.0	27.17	9.33	27.37
01/0061	236.7	27.02	96.7	123.3	23.33	9.33	27.02
01/0093	200.0	24.27	35.0	63.3	17.17	5.67	24.27
01/0111	150.0	29.37	50.0	123.3	22.50	3.40	29.37
01/0220	176.7	25.18	103.3	110.0	21.17	4.93	25.18
01/0265	173.3	28.57	45.0	93.3	16.83	5.33	28.57
01/0346	133.3	32.02	58.3	106.7	17.50	10.67	32.02
01/097	176.7	32.43	43.3	83.3	11.67	7.53	32.43
94/0006	143.3	23.04	36.7	116.7	12.67	0.27	23.03
96/0603	250.0	22.60	153.3	110.0	33.33	4.13	22.60
98/0016	126.7	24.30	85.0	120.0	18.33	8.13	24.30
97/2236	126.7	26.33	65.0	113.3	19.00	6.80	26.33
98/0510	186.7	23.47	53.3	100.0	21.17	8.13	23.47
ABASAFITAA	64.7	34.00	26.7	63.3	10.37	15.60	34.00
AFS2000\071	210.0	29.04	166.7	100.0	31.00	0.27	29.04
AFS2000/043	173.3	26.11	96.7	83.3	22.50	8.20	26.11
AFS2000/131	236.7	24.47	183.3	90.0	24.00	7.80	24.47
AHWENGYANKA	246.7	36.03	157.3	96.7	27.37	18.87	36.03
AK DEBOR	190.0	35.09	120.0	93.3	15.40	32.00	35.09
AMPONG	190.0	24.03	56.7	113.3	24.00	30.00	24.03
AW2000/053	156.7	34.32	51.7	113.3	15.17	8.20	34.32
BANKYE BRONI	130.0	23.17	61.7	70.0	10.67	7.80	23.17
BANKYEHEMAA	190.0	32.43	120.0	96.7	27.00	4.53	32.43
BD96/093	180.0	25.35	136.7	86.7	20.33	25.53	25.35
BD96/075	140.0	29.52	61.7	103.3	8.50	18.00	29.52
BD96/136	163.3	32.52	51.7	106.7	13.00	5.67	32.52
BD96/141	273.3	35.32	146.7	80.0	18.00	10.67	35.32
BD96/154	126.7	29.77	63.3	88.3	22.13	16.00	29.77
DABODABOO	166.7	24.31	52.7	96.7	15.00	34.87	24.31

ESSAM	120.0	20.67	55.0	113.3	19.00	3.93	20.67
ESSIBAYA	233.3	25.11	101.7	96.7	26.00	9.33	25.11
KSI2000/092	276.7	30.57	155.0	100.0	23.00	44.00	30.57
KW2000/030	276.7	27.47	153.3	96.7	30.33	11.33	27.47
MM96/5280	213.3	31.03	65.0	90.0	24.17	38.07	31.03
NKABOM	123.3	23.81	71.7	113.3	9.83	0.33	23.81
OFF2000/145	143.3	32.34	61.7	93.3	17.00	9.67	32.34
TECK BANKYE	176.7	33.40	60.0	106.7	16.00	19.40	33.40
TME 693	226.7	26.33	118.3	110.0	20.67	19.60	26.33
UCC	140.0	35.51	46.7	100.0	15.33	8.00	35.51
UCC2001/111	183.3	30.27	96.7	133.3	19.77	17.67	30.27
F-Test	**	**	**	**	**	**	**
SE	19.58	17.43	18.71	11.35	1.956	6.345	0.975

Appendix 5 Root shape

Source	DF	SS	MS	F	P
Rep	2	19.2000	9.6000	43.40	
Genotypes	44	91.3333	2.0758	9.38	<.001
Error	88	19.4667	0.2212		
Total	134	130.0000			

Appendix 6 Petiole color

Source	DF	SS	MS	F	P
Rep	2	1.126	0.563	0.48	
Genotypes	44	1052.415	23.919	20.46	<.001
Error	88	102.874	1.169		
Total	134	1156.415			

Appendix 7 shape of central leaflet

Source	DF	SS	MS	F	P
Rep	2	0.5333	0.2667	0.59	
Genotypes	44	243.6000	5.5364	12.34	<.001
Error	88	39.4667	0.4485		
Total	134	283.6000			

Appendix8 Growth habit of stem

Source	DF	SS	MS	F	P
Rep	2	1.9704	0.9852	7.21	
Genotypes	44	18.5926	0.4226	3.09	<.001
Error	88	12.0296	0.1367		
Total	134	32.5926			

Appendix 9 Pubescence on apical leaves

Source	DF	SS	MS	F	P
Rep	2	10.844	5.422	2.49	
Genotypes	44	270.267	6.142	2.82	<.001
Error	88	191.822	2.180		
Total	134	472.933			

Appendix 10 Flowering

Source	DF	SS	MS	F	P
Rep	2	1.7333	0.8667	5.35	
Genotypes	44	11.7333	0.2667	1.64	<.00l
Error	88	14.2667	0.1621		
Total	134	27.7333			

Appendix 11 Texture of root epidermis

Source	DF	SS	MS	F	P
Rep	2	11.2000	5.6000	6.42	
Genotypes	44	229.3333	5.2121	5.97	<.001
Error	88	76.8000	0.8727		
Total	134	317.3333			

Appendix 12 Colour of apical leaves

Source	DF	SS	MS	F	P
Rep	2	2.311	1.156	0.37	
Genotypes	44	249.600	5.673	1.80	0.010
Error	88	277.689	3.156		
Total	134	529.600			

Appendix 13 CBB at 3 after planting

Source	DF	SS	MS	F	P
Rep	2	8.9333	4.4667	23.03	
Genotypes	44	43.3333	0.9848	5.08	<.001
Error	88	17.0667	0.1939		
Total	134	69.3333			

Appendix 14 CAD 9 after planting

Source	DF	SS	MS	F	P
Rep	2	0.41481	0.20741	5.09	
Genotypes	44	37.43704	0.85084	20.88	<.001
Error	88	3.58519	0.04074		
Total	134	41.43704			

Appendix15 ACMD 6 after planting

Source	DF	SS	MS	F	P
Rep	2	1.6444	0.8222	4.09	
Genotypes	44	105.0667	2.3879	11.88	<.001
Error	88	17.6889	0.2010		
Total	134	124.4000			

Appendix 16

Table of accessions and their clusters as per using quantitative and qualitative traits (Fig 4.12).

QUANTITATIVE TRAITS AND QUALITATIVE

Cluster	Sub Cluster	Similarity Coefficient	Number of Accessions
A		0.75	ABASAFITAA and BANKYE BRONI
B	I	O.85	DABODABOO, MM96/5280 and 01/0040
	II	0.85	AHWENGYANKA, BHNYYEHEMM and 96/0603
	III	0.85	00/0338
	IV	0.58	AFS2000/071/AFS2000/031, AFS2000/043, BD96/154, AMPONG, KW2000/030 and BD96/075
	V	0.85	00/01040, 01/0046, ESSIBAYA, 01/0061, 01/0111, 01/0346, BD96/0346, TECK.BANKYE, UCC, 01/0093, 01/0220, AK DEBOR, BD96/141, 98/0510, 00/0510, KSI2000/053, TME693, NKABOM, BD96/093, 00/0104, UCC2001/111, 97/0016, 97/2236, ESSAM, 01/0265, 94/006, 01/097 and 0FF2000/145

Appendix 17

Table of accessions and their clusters as per molecular analysis using SSR data (Fig 4.13).

Cluster	Sub Cluster	Similarity Coefficient	Number of Accessions
A	I	0.2	Essam
	II	0.2	UCC2001\111
	III	O.2	Nkabom
	IV	0.2	TME 693 and 97/0016
	V	0.2	AFS 200/131
	VI	0.2	OFF 200/145
	VII	0.2	AFS 2000/043 and 97/2236
	VIII	0.2	AW 2000/053/AFS 2000/071 KW2000/030
	IX	0.2	00/035
	X	0.2	BD96/093
	XI	0.2	01/0346/ KSI 200/092
	XII	0.2	
B	I	0.2	00/01040 and 00/0104
	II	0.2	B796/136 and BANKYE HEMAA
	III	0.2	96/0603 and BD96/154
	IV	0.2	B796/075 and 01/0061
	V	0.2	ESSIBAYA, AMPONG and 01/0265
	VI	0.2	TECK BANKYE
	VII	0.2	94/006 and 01/011
	VIII	0.2	01/0040, BANKYE BRONI and UCC
	IX	0.2	01/0046
	X	0.2	01/097, AHWENGYANKA, 01/0220, 98/0510 and AK DEBOR
	XI	0.2	BD98/141, 01/0093 and 00/0338
	XII	0.2	DABODABOO and MM96\5258

Appendix 18

Table of accessions and their clusters as per morphological traits and molecular analysis data (Fig 4.14).

Cluster	Sub Cluster	Similarity Coefficient	Number of Accessions
A	I	0.6	BD96/141, 01/0093 and 00/0330 (3)
B	I	0.6	01/0111 and 94/0006(2)
	II	0.6	01/0046, TECK BANKYE, 01/0220, 98/0510, 01/097 and AHWENGYANKA (6)
	III	0.6	01/0040, BANKYE BRONI, AK DEBOR and UCC (4)
	IV	0.6	ABASAFITAA, DABODABOO and MM96/5280 (3)
			AFS 2000/131 (1)
	V	0.6	OFF 2000/145 (1)
	VI	0.6	97/0016 and TME693 (2)
	VII	0.6	ESSAM, and UCC 2001/111 (3)
	VIII	0.6	00/0354, BD96/093, 01/0346, KSI 2000/092, 97/2236, AFS 2000/043, AFS 2000/071, KW 2000/030, AW 2000/053 and NKABONI (10)
	IX	0.6	
	X	0.6	00/01040, 00/0104, 01/0265, 01/0061, BD96/075, AMPONG, ESSIBAYA, 96/0603, BD96/154, BANKYE HEMMA and BD96/093 (11)