

GENETIC DIVERSITY IN SOME LOCAL CASSAVA CULTIVARS IN GHANA

By

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the MASTER OF PHILOSOPHY degree in crop science
at the University of Ghana, Legon**

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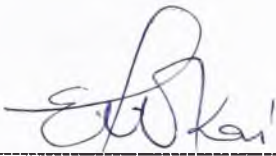


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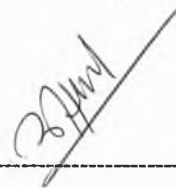
DECLARATION

I hereby declare that except for references to works of other researchers which have been duly cited, this work is my own original research and that neither part nor whole has been presented elsewhere for the award of a degree.



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DEDICATION

Dedicated to my late husband, Isaac Teiko Okai (Nuntso), who encouraged me to start the programme and would have wished to see its successful completion, and to my two sweet children, DOCIA and ZENAS who stood with me, sacrificed, prayed and showed great concern with the progress of my work at all stages until completion.



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LIST OF ABBREVIATIONS

ACMV	African Cassava Mosaic Virus
CIAT	Centro Internacional de Agricultura Tropical
CTAB	Cetyltrimethylammonium, bromide
DNA	Deoxyribonucleic acid
dNTP's	Deoxyribonucleotides Triphosphates
EDTA	Ethylenediaminetetracetic acid
IBPGR	International Board Plant Genetic Resources
IITA	International Institute of Tropical Agriculture
Kb	Kilobases (1kb=10 ³ base-pair)
CRI	Crop Research Institute
CSIR	Council for scientific and Industrial Research
Bp	bases pair
MAS	Marker-Assisted selection
PCR	polymerase Chain Reaction
QTL	Quantitative trait loci
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
SSR	Simple sequence repeat
TAE	Tris-acetate-EDTA buffer
Tris	Tris(hydroxymethyl)-aminoethane
U	One unit of <i>Taq</i> DNA polymerase enzyme that will incorporate 10 nmoles of dNTPs into insoluble material per 30 minutes at 74°C under standard analysis conditions.
PVP	Polyvinylpoly-pyrrolidone
M	Meter
L	Litre
SAHN	Sequential, agglomerative, hierarchical and nested
UPGMA	Unweighted pair group arithmetic means algorithm
MAP	Months after planting
WAP	Weeks after Planting

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ABSTRACT

The genetic diversity in 100 cassava accessions collected from eight regions of Ghana was assessed using morphological and molecular characterisation. Both qualitative and quantitative morphological traits were used to assess variability in the accessions. Canonical discriminant analysis of morphological data showed that quantitative traits detected more variability than qualitative traits. A set of 10 random sequence 10 mer oligonucleotide primers selected from among eighty that were screened detected polymorphisms and generated 63 amplified DNA bands. Cluster analysis based on the unweighted paired group method with arithmetic averages (UPGMA) categorised the accessions into eight groups. Some accessions with the same local name were put in the same cluster (Ankra, Bankye boodee, Bosomnsia and Steer bekum driver) and some duplicate accessions from source were clustered into different groups. RAPD markers revealed genetic variability and estimated the genetic distances, which facilitates identification of diverse parents in order to maximise the expression of heterosis. Accession Kav90004 'Trailasko' showed the highest genetic distance of 8% from Eop8903 'Katawire' and Dmgk05 'Bani bisa' 9% Jaccard similarity coefficient. Eop9802 'Yebesi' and Eop9801 were the most closely related of 87% Jaccard similarity coefficient. Seventeen representative accessions (15%) were selected and kept as core collection for future work. There was no relationship between geographical diversity and genetic diversity.

CHAPTER ONE

1. INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a major source of energy for more than 500 million people in Africa (Jennings and Hershey, 1985). It provides over 50% of the calorie requirement of over 200 million people in sub-Saharan Africa (Osiru *et al.*, 1996). 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. The leaves and tender shoots are consumed as a vegetable in many parts of Africa, and provides 7 g protein per 100 g edible portions with high lysine, minerals and vitamins (Hahn, 1989; IITA, 1990).

In Ghana, cassava ranks first in both the area under cultivation and utilisation (NARP, 1994). The crop plays an important role in Ghana's economy; it contributes 22% of the agriculture gross domestic product. Al-Hassan (1993) reported that cassava has a tremendous potential as feed for animal production. Other industrial uses are in food processing, pharmaceutical, paper, and textile. Its production, processing and utilisation offer jobs to large communities, especially women in the tropical countries (Thro *et al.*, 1995).

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, have generated a large number of traditional varieties. In addition, different ethnic groups have contributed to selection, thus leading to numerous vernacular names given to the same varieties according to ethnic groups (Mignouna *et al.*, 1998). This nomenclature has led 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.

A number of different approaches have been applied in characterising genotypes of crop plants. These include morphological, and molecular approaches. Characterisation based on morphological traits can be highly subjective and environmentally dependent. Molecular markers based on protein and isozyme analyses show low-degree of polymorphism and are influenced by the physiological status and environment of the plant. To avert these difficulties, advances in molecular biology have introduced an alternative for genotype identification that uses DNA markers (Paterson *et al.*, 1991). Biodiversity studies using Polymerase Chain Reaction (PCR) based tools have offered a versatile and reliable method of generating polymorphism.

The identification and characterisation of diversity can reveal the presence of very useful genetic markers which could be introgressed into the crop. Germplasm characterisation reveal duplicates, show genetic relationships among cultivars, therefore decreasing the years for improving or developing new cultivars.

Morphological methods have been used to characterise some of the cassava accessions collected and assembled in the gene banks in Ghana. Morphological characterisation is limited in the ability to reveal polymorphism. In order to generate useful and comprehensive information on the cultivars for future breeding work, morphological and molecular variation in the collection need to be studied.

The objectives for this study were to:

- (1) investigate genetic diversity among 100 cassava cultivars using morphological markers and Random Amplified Polymorphic DNAs (RAPDs) and
- (2) identify distinct cassava genotypes to conserve as core collection for future distribution and breeding programmes.

CHAPTER TWO



2. LITERATURE REVIEW

2.1 Origin and history of spread of cassava in Africa.

Fregene *et al.* (1994) reported that all species of the genus *Manihot* are native of the New World tropics and occur naturally only in the Western Hemisphere between the southern USA (33°N) and Argentina (33°S). Reports indicate that cultivated and wild species were introduced in the old world tropics in the 16th Century (Jones, 1959) by Portuguese explorers when they established forts, trading stations and settlements on African coastal and nearby islands (Carter *et al.*, 1992).

The first cultivation of cassava in Africa dates from 1558 (Carter *et al.*, 1992). The crop spread throughout Africa by various mechanisms of which the initial contacts with the Portuguese-Brazilian culture appear to be the most remarkable. Cassava spread became possible by river and overland trade by the merchandise, and by mass migration (Carter *et al.*, 1992). In the 19th and 20th centuries, the colonial administrators promoted its spread and increased cultivation. Cassava possesses botanical characteristics that enable it to compete well with weeds and therefore can survive under various conditions of neglect. The ability to regenerate from cuttings may have enhanced the spread of the crop to many new locations (Carter *et al.*, 1992). Figure 2.1 presents a summary of the diffusion of cassava from historical documents and travelogues. Cassava was introduced at a number of points along the West African Coast during the 17th Century from the Gambia River by the Portuguese. Cassava has been cultivated around Accra since 1785. In Benin, cassava was at first used as a medicine for the cure of tuberculosis (Carter *et al.*, 1992).

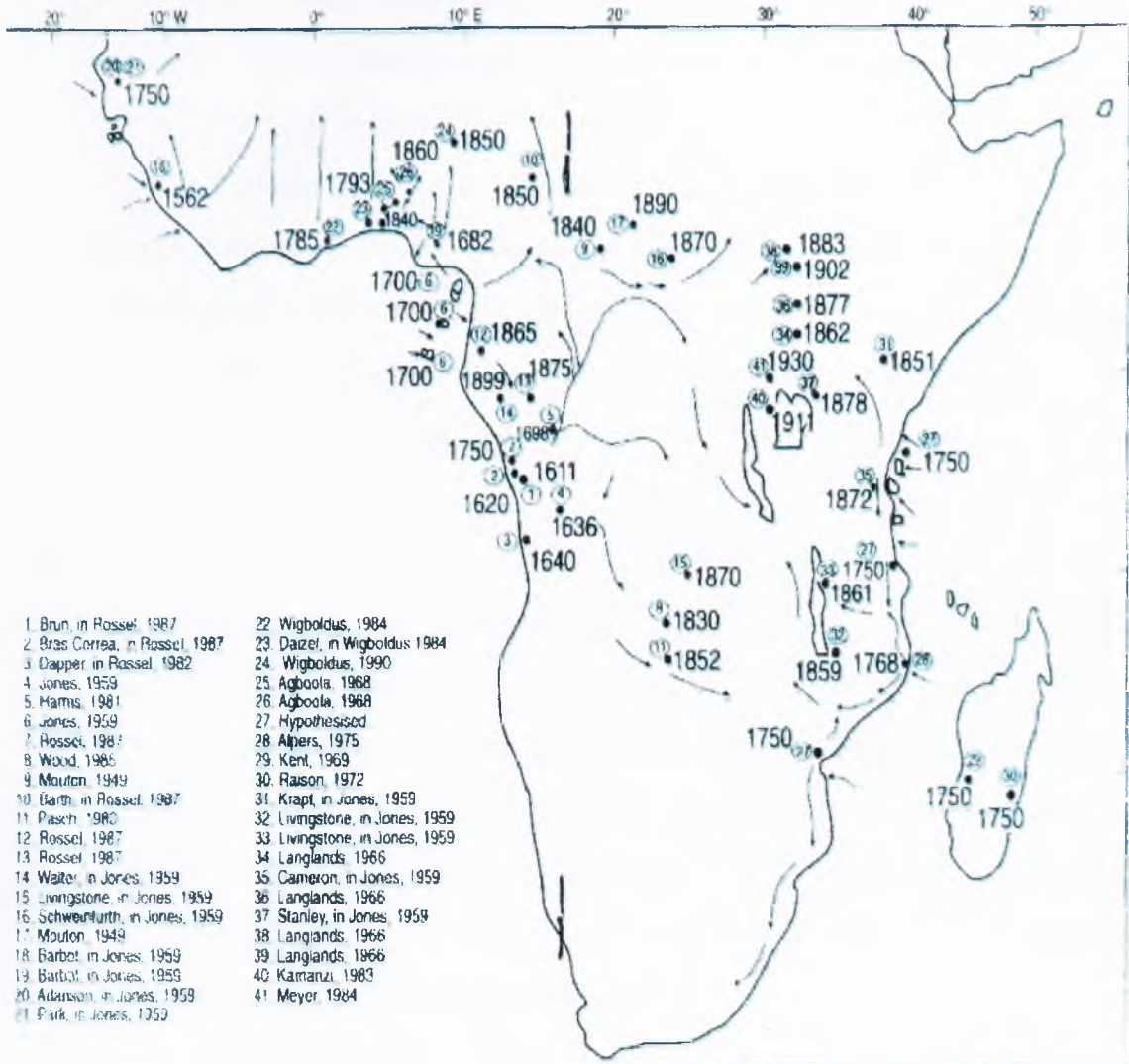


Fig 2.1 Summary of cassava diffusion in Africa (Carter *et al.*, 1992)

Initial attempts at improving cassava in Ghana were made by the Crops Research Institute at Kwadaso, Kumasi, where introduced cultivars, particularly accessions from East Africa were screened and elite clones such as K357, K102 and K680 were hybridised with local cultivars to generate high yielding and disease resistant clones (Doku, 1969). In collaboration with the International Institute of Tropical Agriculture (IITA), a number of introductions have been tested and screened to identify cultivars of high and stable yield across different agro-ecological zones. These include ‘Abasafitaa’, ‘Blemoduade’ and ‘Afisiafi’. Several genotypes are being evaluated for future release (NARP, 1994).

2.2 Cassava Plant Botany

Cassava is a perennial crop. Most farmers usually harvest within the first two years. Abandoned stands of cassava may continue to grow for several years (Onwueme, 1978) and most often in association with other crops (Hershey, 1993a). Cassava is propagated from stem cuttings, however, under natural conditions it can be propagated from seeds. Under favourable conditions cuttings planted in moist soil, sprout and root within one week. Seed propagation result in relatively slower plant establishment with smaller and weaker plants. The seedlings genetically segregate into different types (Ekanayake *et al.*, 1997).

A few weeks after sprouting, the shoot lengthens and the roots extend downwards and spread. Flowering may begin as early as the sixth week after planting, but this depends upon the cultivar and the environment. Storage roots formation begins about eight weeks after planting. Leaf area approaches its maximum size between 4-5 months depending on planting time. The average height of a cassava plant ranges from one to two meters; some cultivars however reach four meters (Ekanayake *et al.*, 1997).

2.2.1 Root and Shoot Systems

The cassava plant can be divided into the shoot and root system (Figure 2.2). The shoot system consists of the stem, leaves, inflorescence and the root system with feeder roots and storage roots. The cassava propagated by stem cutting (hard wood), develop adventitious roots at the base of the cuttings during the first two to three weeks. The adventitious roots subsequently develop into a fibrous root system, which absorbs water and nutrients from the soil. Nodal roots, which are adventitious roots that develop at the base of the auxillary buds on the cuttings, are at the nodes. The fibrous root system may reach 200 cm or more in length (IITA, 1990).

Cassava plants propagated by seeds first develop a tap root system. After 30 to 60 days, some roots increase in diameter and become tuberous storage roots. As tuberisation proceeds, the tuberous roots are developed as the result of cambium activity and starch accumulation. Tuberous roots are physiologically inactive and cannot absorb water or nutrients, however, the rest of the fibrous roots continue to function and absorb water and nutrients. The number of roots which form storage tuberous roots depends on several factors including genotype, assimilate supply, photoperiod and temperature (Ekanayake *et al*, 1997).

Cassava stems may grow up to four meters in height, whereas dwarf varieties may be one meter tall. The stems vary considerably in colour and usually are woody with very large pith. The older parts of the stem consist of prominent knob-like scars, which indicate nodal position where leaves were originally attached. Each nodal unit consists of a node, which subtends a leaf and an internode. The rate of node production on each stem is about one node per day during early and active growth stages, and about one node per week in the older plants. Internodes vary considerably depending on varieties and environmental conditions. They tend to be long under favourable conditions, and short under drought stress, and with insufficient light, they are usually abnormally long (Ekanayake *et al.*, 1997). Two types of branching patterns exist in most

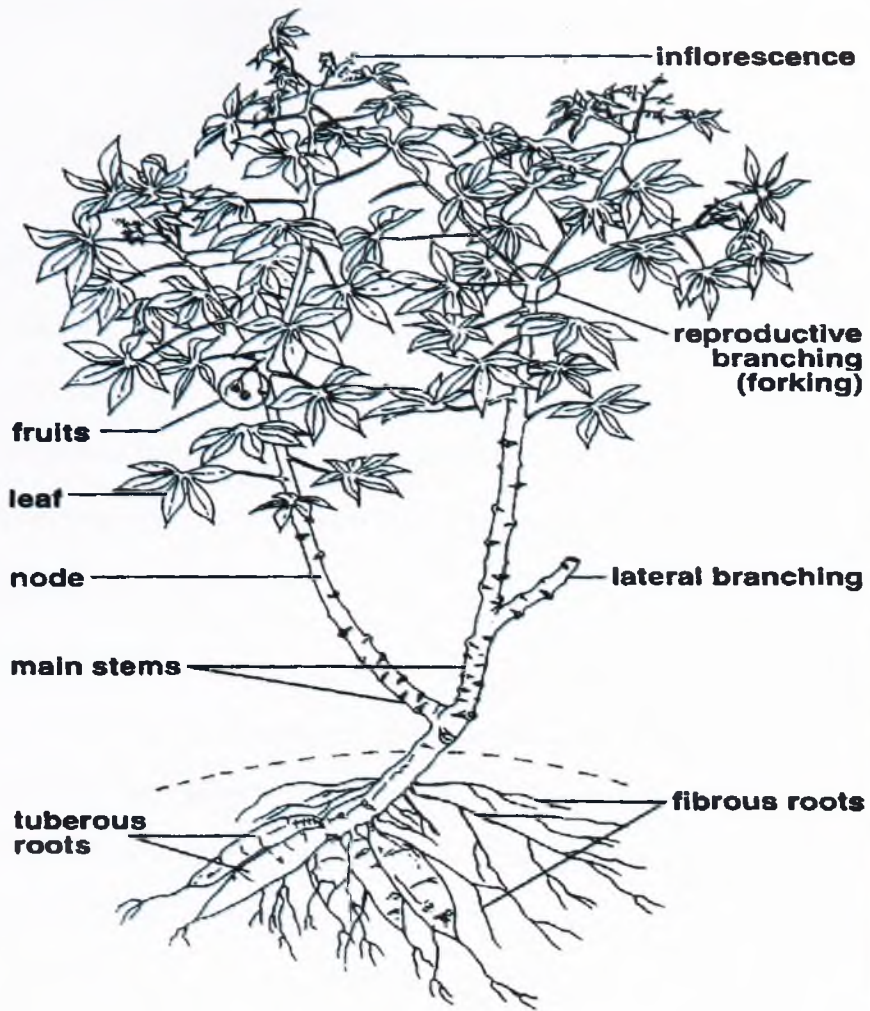


Fig 2.2: Cassava plant (Ekanayeke *et al.*, 1997)



varieties growing under normal conditions, namely, forking and lateral branching. The type of branching is influenced by several factors, including genotype and physical damage.

2.2.2 Leaves

The number of cassava leaves, leaf longevity and whole plant products are determined by genotype and environmental conditions. Cassava leaves are arranged alternately in a spiral order on the stem. The phyllotaxis or arrangement of leaves on the stem is $2/5$ spiral. Cassava 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 lanceolated (Onwueme, 1978).

2.2.3 Inflorescence

Cassava is a monoecious plant. 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 female flower opens first, while the male flower opens about a week later. Cross-pollination is usually the rule. Self pollination can occur when female and male flowers, located on different branches of the same plant open at the same time (Ekanayake *et al.*, 1997).

After pollination and fertilisation, the ovary develops into a fruit that matures in 70 to 90 days. The mature fruit is a globular capsule (diameter 1 to 1.5 cm) with six narrow longitudinal wings along which it naturally splits open when dry. The woody endocarp contains three locules, each with one seed. When the fruit is dry, the endocarp splits explosively to release the seed. The cassava seed shape is ellipsoidal and about 1.5 cm long. It has a brittle testa that is grey and mottled with dark blotches.

2.3 Classification of Cassava Varieties

Numerous cassava varieties exist in each locality where the crop is grown. The cultivars have been distinguished by morphological characteristics such as leaf characteristics, colour and shape branching habit, plant height, colour of stem, tuber shape and colour, time to maturity yield and the cyanogenic glucoside content in the roots (Dixon *et al.*, 1994) Genetic studies on the genus *Manihot* has shown potential benefit to breeding for the improvement of quality traits (Beeching *et al.*, 1993). Local landraces of crops offer a rich source of genetic diversity and these provide a valuable source of genetic material for crop improvement. However, the rapid development of high-yielding cultivars threaten such crops and efforts for their preservation should be of the highest priority (Attere, 1997).

Cyanogenic glucoside potential or content as a trait has been used to place cassava cultivars into three groups: (i) cassava with high potential to generate HCN-10 mg per 100 g fresh weight or more; (ii) Intermediate types in which the levels of HCN range between 5 and 10 mg per 100 g fresh weight (iii) cassava with low potential to generate HCN – less than 5 mg per 100 g fresh weight. The cyanogenic glucosides are often concentrated in the peel (Rao and Hahn, 1984; 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).

Cassava, *Manihot esculenta* Crantz (Synonymous with *Manihot ultissima* Pohl) (Onwueme, 1978) is a dicotyledonous crop, belonging to the Euphorbiaceae family and genus *Manihot*. The genus includes 98 other species that are useful as gene resources in cassava improvement (Rogers and Appan, 1973; Fregene *et al.*, 1994). The Euphorbiaceae family has members characterised by lactiferous vessels composed of secretory cells and include several commercial plants. Some of these are rubber trees (*Hevea brasiliensis*), oil plants (*Ricinus*

comunis), root crops (*Manihot spp*) and ornamental plants (*Euphorbia spp.*) (Ekanayake *et al.*, 1997).

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

2.3.1 Cytology of Cassava

Cytogenetics has been used as a basis for understanding the organisation of genetic diversity in many genera including *Manihot*. Chromosome morphology and behaviour has shown cassava as a diploid species with $2n=2x=36$ chromosomes (Hershey, 1993b).

There is paucity of information on the synapctic behaviour of chromosomes in *Manihot esculenta*, wild *Manihot* species or interspecific hybrids. On the basis of the observed number of satellite chromosomes and their behavior during the division stages, it has been postulated that cassava is a segmental allotetraploid with a basic chromosome number of $X=9$ (Perry, 1943; Magoon *et al.*, 1969a). However, analysis with ten isozyme loci revealed predominantly disomic inheritance suggesting cassava to be a diploid (Hussain *et al.*, 1987; Lefevre and Charrier, 1993). Gomez *et al.* (1994) confirmed disomic inheritance in cassava based on recent studies using arbitrary PCR markers. Cassava is genetically the least understood among the major staple crops that are used for food production (Gomez *et al.*, 1996).

Apparently less than fifty percent of the species within the genus *Manihot* have been examined. Bai's (1987) account on meiotic studies, and information on the family *Euphorbiaceae*, suggest irregularities at cell division stage. Polyploidy manipulation in cassava has been possible using colchicine to produce artificially induced somatic tetraploids ($2n=4x=72$) Hahn *et al.*, (1990). They did not show much variation and had undesirable characteristics such

as low yield and stunted growth. Hahn *et al.* (1990) reports on occurrence of spontaneous sexual tetraploids and triploids resulting from diploid interspecific crosses with cassava.

2.4 Morphological Characterisation

Morphological traits have been used as a powerful tool in the classification of cultivars as well as study their taxonomic status (Roger and Appan, 1973). Certification of new cultivars is usually based on the genetic purity of a particular crop. However, traditionally those assessments depend on the botanical traits. Breeders and geneticists have used morphological characteristics such as leaf and flower attributes to follow segregation of genes and hybrids, but most agronomic traits are not associated with easily observable phenotypic markers (Kochert, 1994).

Most of the descriptors are ambiguous, and have limited use for cultivar identification (Stegemann, 1984). Such characteristics are often controlled by multiple genes and subject to varying degrees of environmental modifications and interactions. Many of these traits are also difficult to analyse because they do not have the simple genetic control assumed by many populations in genetic models and they are of very little use (Tanksley *et al.*, 1989). Morphological characterisation has been used to identify duplicates, study genetic variation patterns and correlation with characteristics of agronomic potential. The use of morphological traits involve a lengthy survey of plant growth over time that is costly, labour intensive and vulnerable to environmental conditions.

Variation in traits do not reflect only the genetic constitution of the cultivar, but also the interaction of the genotype with the environment (G x E) within which it is expressed (Dixon and Nukenine, 2000). In cassava breeding programs most emphasis has been on the collection and conservation of gene pools (CIAT, 1993) and characterisation of the collections.

Morphological data on cassava have been useful in correcting ambiguities of cultivars. The IBPGR (International Board for Plant Genetic Resources, 1983) descriptors have been used to

characterize cultivars. The IBPGR has identified a set of relatively stable morphological traits useful for characterisation of cassava genotypes. The descriptors include qualitative and quantitative measurements for cassava root and shoot characters.

Since the past decades, a number of alternative laboratory methods have been successfully developed, such as isozyme analysis (Nienhuis *et al.*, 1995), seed storage protein electrophoresis and high performance liquid chromatography (Buehler *et al.*, 1989).

2.5 Molecular characterisation

The main constraint to morphological characterisation is the limited amount of polymorphism they are able to detect among closely related genotypes (Hu and Quiros, 1991). Isozymes often show low levels of polymorphism and problems with reproducibility arise due to tissue type and conditions. A large number of polymorphic markers are required to measure genetic relationships and genetic diversity in a reliable manner (Soller and Beckmann, 1983; Mignouna, 1994). This limits the use of morphological characters and isozymes. Advances in molecular biology have introduced DNA based procedures for cultivar identification. DNA sequences show greater variation than amino acid changes in isozymes. The composition of DNA is also consistent between tissues and is not affected by environmental changes (Beeching *et al.*, 1993). Target genes in a segregating population can be identified with the assistance of DNA markers so as to accelerate traditional breeding. (Thottappilly *et al.*, 2000).

One extensive use of these molecular markers is for development of detailed genetic and physical chromosome maps in a variety of organisms among animal and human systems and among plant systems. Molecular markers in plant systems have been found very useful in conventional breeding by carrying out indirect selection through molecular markers linked to the traits of interest. Molecular markers have been used for both simple and quantitative trait loci (QTL) because the environment does not influence these markers and can be scored at all stages

of plant growth. In addition to these two major applications, DNA markers can also be used in plant system for germplasm characterisation, genetic diagnostics, characterisation of transformants, study of genome organisation and phylogenetic analysis, (Rafalski *et al.*, 1993). Although each marker system has some advantages and disadvantages, the choice of any marker system is dictated to a large extent by the intended application, convenience, cost and time consideration, number of samples, how quickly the data is needed and the technique that will best yield the maximum data (Gupta *et al.*, 1999; Thottappilly *et al.*, 2000).

Molecular markers can be broadly grouped into:

- 1) Hybridization-based DNA markers such as restriction fragment length polymorphisms (RFLPs) and oligonucleotide fingerprinting.
- 2) PCR-based DNA markers such as random amplified polymorphic DNAs (RAPDs), which can also be converted into sequence characterised amplified regions (SCARs), simple sequence repeats (SSRs) or microsatellites, sequence-tagged sites (STS), amplified fragment length polymorphisms (AFLPs), inter-simple sequence repeat amplification (ISA), cleaved amplified polymorphic sequences (CAPs) and amplicon length polymorphisms (ALPs).
- 3) DNA chip and sequencing-based DNA markers such as single nucleotide polymorphisms (SNPs)
- 4) In addition to the above three groups of markers, microsatellite-primed polymerase chain reaction (MP-PCR), arbitrarily- primed PCR (AP-PCR), allele-specific PCR (AS-PCR) and DNA amplification fingerprinting (DAF) have also proved useful in the detection of polymorphism (Gupta *et al.*, 1999; Thottappilly *et al.*, 2000).

2.5.1 DNA-based molecular techniques

DNA fingerprinting is a technique, which has been widely adopted to differentiate among organisms at the species and subspecies levels (McClellan *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 characterised by high genetic variation between cultivars. The most closely related cultivars are usually distinguished with the DNA fingerprinting methods (Beckmann and Soller, 1986). Another advantage of DNA fingerprinting over morphological markers is the dominance and the absence of environmental effects that are shared. The application of DNA fingerprinting could be very valuable in the identification of cultivars and species and could help to create more efficient breeding programs through the detection of genetic linkages between DNA fingerprinting bands and 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).

2.5.2 PCR Technique

The polymerase chain reaction (PCR) Saiki *et al.* (1988) has been the basis of a growing range of new techniques for genome analysis based on the selective amplification of genomic DNA fragments. Williams *et al.* (1990) reported on the use of PCR with short oligonucleotide primers of arbitrary (random) sequence to generate markers, the basis of the random amplified polymorphic DNA (RAPD). Welsh and McClelland (1990) also reported on arbitrarily primed polymerase chain reaction (AP-PCR) while Caetano-Anolles *et al.* (1991) report on DNA amplification fingerprinting (DAF).

The PCR reaction requires deoxynucleotides to provide both energy and nucleotides for synthesis of DNA, DNA polymerase primer, template and buffer containing magnesium (Taylor, 1991). Typical PCR amplification utilises oligonucleotide primers that hybridise to opposite strands. The product of DNA synthesis of one primer serves as template for another primer. The PCR process requires repeated cycles of DNA denaturation, annealing and extension by DNA polymerase leading to amplification of the target sequence. The result is an exponential increase in the number of copies of the region bounded by the primer (Saiki *et al.*, 1988; Mullis, 1990). The technique can be applied to detect polymorphism in various plants, animals, bacterial species and fungi (Williams *et al.*, 1990).

The introduction of the PCR technique has revolutionized standard molecular techniques and has allowed for the proliferation of new tools serving to detect DNA polymorphism (Hu and Quiros, 1991). The electrophoresis pattern of fragments generated by each primer for one isolate can be used as DNA fingerprints for assaying diversity (Tommerup *et al.*, 1995) Polymorphism between two individuals is generally scored as a presence or absence (non-amplification) of a particular DNA fragment. The absence may result from deletion of a priming site or insertion rendering site too distant for successful amplification. Insertion can change the size of a DNA fragment without preventing its amplification (Williams *et al.*, 1990). PCR is simple, fast, specific, sensitive and relatively low cost.

The main advantages of this technique over other techniques are its inherent simplistic analysis (a single reaction can contain all reagents) and the ability to conduct PCR test with extremely, small quantities of tissue for DNA extraction (Welsch *et al.*, 1991). On the other hand PCR is limited in its usefulness because of the time and cost required to obtain the DNA sequence information required for primer design (Samec and Nasinec, 1995; Thottappilly *et al.*, 2000).



2.5.3 Simple Sequence Repeats (SSR) technique

Simple sequence repeats are usually 1-6 base pair repeat motifs (example TA, CA, GTG, TAA GATA) repeats. They are ubiquitous in eukaryotic genomes and their study has been greatly facilitated by recent advances in PCR technology. The first report of microsatellites in plants was made by Condit and Hubbel (1991), who suggested their abundance in the plant system, and subsequently confirmed by Gupta *et al.* (1996). Later Akkaya *et al.* (1992) reported on the length of sequence and polymorphisms of SSRs in soybean. Simple or short sequence repeats also known as short tandem repeats STRs or ‘microsatellites’ or incomplete amplification using primers to the sequences flanking these repeats can be used to generate polymorphism because of frequent variation in the length of the repeat regions. The simple sequence repeats has been reported as useful markers in many plant species including cassava (Maroof *et al.*, 1994; Roder *et al.*, 1995). Chavarriaga-Aguirre *et al.* (1999); Agyare-Tabbi *et al.*(1997) reported the presence of some useful repetitive and microsatellite DNA in cassava. The main limitation with the application of this technique in cultivar identification is the difficulty in cloning and sequencing the regions flanking the SSR. This must be done for each species since the flanking regions are relatively species specific and are not usually useful for application to even closely related species (Agyare-Tabbi *et al.*,1997). In some instances SSR primers may not reveal any or detect low levels of polymorphism (Taylor *et al.*, 1992; Agyare-Tabbi *et al.*,1997). Notwithstanding, the reliability and reproducibility of the markers, especially between laboratories, makes them attractive alternatives to other techniques such as RAPD and PCR. The SSR sequences are mostly found in introns and the 5’ flanking regions of plant genes (Roder *et al.*, 1995).

2.5.4 RAPD technique

Another PCR-based technique in use is the random amplified polymorphic DNA. RAPD markers are generated by the use of short (10-mer) synthetic oligonucleotides in single strand primer (Williams *et al.*, 1990). In this technique a decamer primer of arbitrary sequence is allowed to anneal at a relatively low temperature priming the amplification of DNA fragments distributed at random in the genome (Williams *et al.*, 1990). Amplification products are visualised by separation on agarose gel and stained with ethidium bromide. They usually result in DNA fragment patterns that are polymorphic between genotypes, therefore detecting diversity within them (Tommercup *et al.*, 1995). The main issue associated with its use is ensuring reproducible amplification profiles. The nature of amplification process with short primers is that many sites in the genome are potential templates and the profile may be influenced by any variation in the method used to prepare DNA template and the exact reaction composition (Muralidharan and Wakeland, 1993). However, a key requirement for reliable and reproducible RAPD results is a consistent approach to sample preparation and DNA isolation.

There are several advantages of RAPDs compared to other DNA based techniques. It is simple, rapid and does not involve radioactivity and costs less (Varghese *et al.*, 1997). Moreover it does not require target DNA sequence information and can provide markers in genomic regions not accessible by other analysis (Williams *et al.*, 1990). The technique uses a very small amount of genomic DNA (few hundred nanograms) which allows the analysis of single seeds or young seedlings (Hu and Quiros, 1991). Another advantage of the RAPD method is that a universal set of random primers can be used for genomic analysis of any organism (Welsch and McClelland, 1990)

Short random primers have been used to reproducibly amplify segments of genomic DNA from a wide variety of species including plants (Williams *et al.*, 1990; Quiros *et al.*, 1991, Fregene *et al.*, 1997). Polymorphism detected by RAPDs are inherited in a mendelian fashion as

dominant markers (William *et al.*, 1990; Welsch *et al.*, 1991). This polymorphism has been proved to be useful for identifying variation at different levels. RAPD analysis enables differentiation between very closely related organisms due to the high resolution of the technique (Tommerup *et al.*, 1998). The polymorphic fragments generated by RAPDs are useful as genetic markers to identify organisms (Williams *et al.*, 1990) and the relative degree of similarity between individual populations and species (Yang and Quiros, 1993; Tonukari *et al.*, 1997).

In cassava, RAPD technology has been used to assess genetic diversity in collections, relationship between *Manihot* species, develop linkage maps and for studies of evolutionary relationships (Marmey *et al.*, 1994; Fregene *et al.*, 1995)

In studies comparing molecular markers there have been similarity in results using other DNA based techniques and RAPDs in *Manihot esculenta* (Beeching *et al.*, 1993; Marmey *et al.*, 1994), thus concluding that any of the techniques can be used for the evaluation of genetic diversity.

2.5.5 Use of RAPD in marker assisted selection (MAS)

Molecular markers used as a tag for various traits have been proposed and used in breeding programs. This approach relies upon the establishment of a linkage between a molecular marker and the characteristic to be selected. Once this has been achieved, breeding selection can be conducted in the laboratory and does not require the expression of the associated phenotype (e.g. disease resistance). In tomato (*Lycopersicum esculenta*) RAPD markers for genes conferring resistance to mosaic virus were identified from the wild relative *L. peruvianum* located on the long arm of chromosome 9 (Ohmori *et al.*, 1995).

RAPDs has been used effectively for cultivar identification and screening for resistance in a variety of plant species including cassava and yam (Tonukari *et al.*, 1997; Mignouna and Dixon, 1997). RAPDs have been used on crops such as apple (*Malus domestica Borki.*) Landry

et al., 1994), asparagus (*Asparagus officinalis* L.), (Khand ka *et al.*, 1996), lemon (*Citrus lemon*) (Deng *et al.*, 1995), celery (*Aipum agraveolens* L.) (Yang and Quiros, 1993), pepper (*Capsicum annum*), (Prince *et al.*, 1994), raspberry (*Rubus* spp), (Parent *et al.*, 1993), sweet potato (*Ipomea batatas* L.), (Commoly *et al.*, 1994), yam (*Dioscorea* spp), (Asemota *et al.*, 1996), peas (*Pisum sativum* L.), (Samec and Masinec, 1995), wheat (*Triticum aestivum*), (Myburg *et al.*, 1996) and cocoa (*Theobromin cacao* L.) (Russel *et al.*, 1993).

2.5.6 Studies using RAPD markers in cassava

Tools for traditional linkage analysis and morphological markers are few in cassava. There exist no classical genetic map for cassava (Ocampo *et al.*, 1995). Linkage analysis using isozymes has been carried out in cassava (Lefevre and Cherrier, 1993). Seventeen isozymes loci from enzyme systems produced 59 alleles organised into three linkage groups.

Fregene *et al.* (1995) have used 200 RFLP and RAPD markers in a F1 population derived from intraspecific cross between two elite cassava clones to construct a preliminary linkage map. Eight hundred primers were screened with single dose fragments in a RAPD analysis, and 42 per cent polymorphisms identified. Five restriction enzymes were employed in the parental survey, 30 per cent polymorphisms were identified. Results of this study revealed about 5 per cent of all RFLP markers and 3 percent RAPD markers which did not segregate. About 30% were found to be lined in repulsion for both RFLP and RAPD markers. Cassava ancient polyploid, but not behaving as a diploid linkage map of cassava constructs from RFLPs microsatellites, isozymes and RAPD markers, 30% of the markers were found to be limited in repulsion, suggesting preferential paring among some chromosomes and random pairing among others (Gomez *et al.*, 1996).

RAPD markers have been used to measure genetic relationship within the genus *Manihot*. Reports on studies on the origin of cassava, the diversity and evolution of *Manihot* genus have

been carried out using different species including cassava germplasm. Genetic improvement of cassava is limited by poor knowledge of genetic diversity within the species (Marmey *et al.*, 1994). From 98 *Manihot* species reported by Rogers and Appan (1973), 80 occurred in Brazil, hence an important genetic resource centre for the genus. There are two hypotheses on the origin of cassava. Rogers (1963) considers cassava as a cultigen, that is a species selected by humans and not occurring naturally in the wild form. Therefore, cassava is thought to have originated through a series of hybridisation and introgression events involving several *Manihot* species. Allem (1987) however, supports the alternative hypothesis of wild progenitor species, having reported that wild cassava has been found in Brazil.

The use of RAPDs to detect phylogenetic relationship is controversial. However, RAPDs have detected fine-scale variation in *Manihot*, and can be used to distinguish *Manihot esculenta* cultivars which can help resolve ambiguous relationships such as was observed in some Brazilian *Manihot* species and *M. esculenta* (Schall *et al.*, 1995).

Tonukari *et al.* (1997) studied genetic polymorphism of cassava within the Republic of Benin, Laminski *et al.* (1997) also did a similar study for cassava elite lines in South Africa and RAPD markers were found useful in both studies.

2.5.7 Calculation of genetic distances

Data obtained from DNA fingerprinting can be used in two ways. The first approach in 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. Dendrogram is the second which is most widely used. Diagrams of genetic relationships are constructed using the cluster analysis based on the pairwise genetic distance. An input data matrix containing absence (0) and presence (1) value of all RAPD markers is directly used to calculate pairwise genetic distance.

Generally the fraction of bands shared between any two observed taxonomic units is used to calculate a similarity coefficient (S) from which genetic distance (D) is derived.

The genetic distance, $D=1-S$ or $D= - \ln (S)$. All 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 and Li (1979), among other formula has been used in most of the studies and is suitable to calculate the pairwise distance matrix from RAPD data and SSR. This formula $F=2(n_{xy})/n_x+n_y$ was developed for RFLP data where F (similarity) is the ratio of shared bands between individuals X and Y, $2n_{xy}$ is the number of shared bands, and n_x and n_y are the numbers of bands observed in cultivars x and y respectively. The F ratio is used to calculate the distance value ($D=1-F$) for a pairwise combination.

Jaccard's similarity coefficient (Jaccard, 1908), simple matching and Rogers' distance (Rogers, 1972) are other coefficients which have frequently been used to calculate genetic distance. Computer software programs include Numerical Taxonomy and Multivariate Analysis program package (Rohlf, 1993), RAPD distance, phylogeny inference package and phylogenetic analysis using parsimony (Swafford, 1991). These programs can be used to calculate distance matrixes using the formula $F=2(n_{xy})/n_x+n_y$. The resultant data are processed with cluster analysis using methods such as unweighted pair group mean arithmetic analysis (Sneath and Sokal, 1973) and then plotted in dendrograms representing the genetic relationship among the genotypes in the pairwise genetic matrix.

CHAPTER THREE

3. MATERIALS AND METHODS

An experiment was conducted to characterise 100 cassava accessions using morphological and molecular traits. There were two phases of the study, the first part being morphological characterisation at Pokuase, Ghana and the second phase, molecular characterisation at the Biotechnology Unit, of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

3.1 Morphological characterisation

3.1.1 Planting materials

One hundred cassava accessions used for the study were obtained from four institutions, namely, Plant Genetic Resources Centre (PGRC) Bunso, University of Cape Coast (UCC), Cape Coast, Savanna Agriculture Research Institute (SARI), Nyankpala and Crop Research Institute (CRI) out station, Pokuase. The name of each accession, region of collection, and source/institution are presented in Table 3.1.

3.1.2 Field establishment

The experimental area was prepared by ploughing and harrowing. Cassava cuttings were planted in the field in October 1998. A 10 x 10 simple lattice design with two replications was used. Each of the accessions was represented by two plots, with eight plants per plot. A spacing of 1m by 1m was used. Supplementary irrigation was provided by watering during the initial four weeks. Weeding was done at 1 MAP (month after planting), 4 MAP, and 9 MAP. No fertilisers were applied, and no pest or disease control was practiced..

Table 3.1. Cassava accessions used for morphological and molecular characterisation

NO.	ACCESSION	LOCAL .NAME.	SOURCE.	REGION
1	Jk90016 D1	STEER BEKUM DRIVER* 1	PGRC	Western
2	Jk90013 D2	ABISH	PGRC	Western
3	Acw86	KWESI TUTU	UCC	Western
4	Jk90006	KOFI ASEM	PGRC	Western
5	Jk90022	ETIBIRE	PGRC	Western
6	Acw157	ANKRA * 2	UCC	Western
7	Acw91	STEER BEKUM DRIVER* 1	UCC	Western
8	Acw82	ADZO CONGO	UCC	Western
9	Acw19	AWONA CONGO	UCC	Western
10	Acw11	DUA FRA	UCC	Western
11	Jk90021	ESI PANYIN	PGRC	Western
12	Jk90009	KASA FANTI	PGRC	Western
13	Jk90026	OKWADWOFO	PGRC	Western
14	Jk90001	APPIAH	PGRC	Western
15	Jk90012	ADUANE ASA	PGRC	Western
16	Jk90005	AKWADA PE	PGRC	Western
17	Acw6	KWESI TU	UCC	Western
18	Jk90008 D3	MANOA	UCC	Western
19	Acw5	BESREBEMA* 5	UCC	Western
20	Acw23	NIMME	UCC	Western
21	Acw125	ESI.PANYA	UCC	Western
22	Acw126	KWESI ITU	UCC	Western
23	Acw10	TABU	UCC	Western
24	Acw9	ADUO BILE	UCC	Western
25	Jka90013 D2	ABISH	PGRC	Western
26	Acw2	CONGO	PGRC	Western
27	Acw37	ADWOFUFULE	UCC	Western
28	Acw36	ANUONLA BEDE	UCC	Western
29	Acw75	BESERESBEMA* 5	UCC	Western
30	Jka90016 D1	STEER BEKUM DRIVER* 1	PGRC	Western
31	<i>Acw107</i>	ESSABAYEM	UCC	Western
32	Jka90008* D3	MANOA	PGRC	Western
33	Acw111	BANKYE SANTUM	UCC	Western
34	Acw16	AGRIC TABOO	UCC	Western
35	Acw29	NEME	UCC	Western
36	Acw71	ADROBLE	UCC	Western
37	Acw24	BESERABEMA* 5	UCC	Western
38	Acw62	NDA-AYA	UCC	Western
39	Acw61	ADAKOLA	UCC	Western
40	Jk90033	NSAWAM	PGRC	Central
41	Jk90031	HALF ASSIN	PGRC	Central
42	Mc90028	BANKYE MPAPRO	PGRC	Central
43	Mc90033	EFUA BEYAW	PGRC	Central
44	Mc90015	ADJOA BEESIWA	PGRC	Central
45	Mc90013	BANKYE SANTUM	PGRC	Central
46	Mc90012	AWONA BANKYE	PGRC	Central
47	Mc90019	SANTUM	UCC	Central
48	Mc90021	KETEKE WUSIE	UCC	Central

Table 3.1 continues

49	Mc90029	BANKYE BODEE* 3	PGRC	Central
50	Mc90022	GYANOA	PGRC	Central
51	Mc90031	SAA KWA	PGRC	Central
52	Mc90034	STEER BEKUM DRIVER* 1	PGRC	Central
53	Mc90020	SAAKWANYA	PGRC	Central
54	Mc90030	GARI BANKYE	PGRC	Central
55	Mc90036	PAAFIO	PGRC	Central
56	Mc90010 D4	KWAKU SAM	PGRC	Central
57	Mca90010 D4	KWAKU SAM	PGRC	Central
58	Acc497	OHYEWKAW	UCC	Central
59	Mc90006	ADIMADIN	PGRC	Ashanti
60	Kaa90060	ASONA	PGRC	Ashanti
61	Kaa90830	NO LOCAL NAME	PGRC	Ashanti
62	Kaa90067	BETEA	UCC	Ashanti
63	Kaa90087	BASARE BANKYE	PGRC	Ashanti
64	Kaa90062	BANKYE BODEE* 3	PGRC	Ashanti
65	Mc90005	ANKRA TUAKA * 2	PGRC	Eastern
66	Kae90050	BANKYE SONO	PGRC	Eastern
67	Kae90033	BOSOM NSIA	PGRC	Eastern
68	Btl97006	BTl97006	PGRC	Eastern
69	Mc90004	TUAKA* 4	PGRC	Eastern
70	B89003	B89003	PGRC	Eastern
71	B89037	B89037	PGRC	Eastern
72	Kae90034	AGEGE	PGRC	Eastern
73	B89031	B89031	PGRC	Eastern
74	Kae90046	BANKYE TUNTUM	PGRC	Eastern
75	B89013	B89 013	PGRC	Eastern
76	Kaa90063	AKOSUA TUMTUM	PGRC	Eastern
77	B89029	B89029	PGRC	Eastern
78	B89015	B89 015	PGRC	Eastern
79	B89001	IPGRU	PGRC	Eastern
80	B89032	B89032	PGRC	Eastern
81	Eop984	QUARANTINE	CRI	Greater Accra
82	Eop9801	BOSOM NSIA	CRI	Greater Accra
83	Eop9802	YEBESI	CRI	Greater Accra
84	Eop9803	KATAWIRE	CRI	Greater Accra
85	Kav90004	TRAILASKO	PGRC	Greater Accra
86	Kav90002	AKPANYA	PGRC	Greater Accra
87	Kav90007	KATAWIA	PGRC	Volta
88	Kav90028	SAKPA	PGRC	Volta
89	Kav90013	SOKLA	PGRC	Volta
90	Kav90008	TUAKA* 4	PGRC	Volta
91	Kav90006	HWIAKPO	PGRC	Volta
92	Kav90022	ANKRA* 2	PGRC	Volta
93	Kav90010	KPENYIVIA	PGRC	Volta
94	Kav90005	ADUSE	PGRC	Volta
95	Eop9805	AFISIAFI	IITA/CRI	Volta
96	Dmgk05	BIAN BASI	SARI	Northern
97	Dmgk12	KPLASO	SARI	Northern
98	Dmgk11	AGRIC	SARI	Northern

Table 3.1 continues

99	Dmgk02	"MADE IN DOZEN	SARI	Northern
100	So96002	BANKE	PGRC	Upper East

Source ;UCC University of Capecoast,CRI;Crop Research Institute,SARI; Savanna Agriculture Research Institute,PGRC Plant Genetic Resource Centre (D1,2,3,4 : Duplicate accessions from source *1,2,3,4,5: share common local names) Accessions in italics 5,31,39 and 77 were not used for the molecular (DNA) work.

3.1.3 Assessment of morphological characteristics

Morphological data were collected from all plants of each cultivar. Data were recorded using the International Board for Plant Genetic Resources (IBPGR) descriptor list for cassava as a guide. The descriptors used for morphological characterisation are listed in Table 3.2. Data were taken from the first month after planting (MAP). Data recorded included qualitative and quantitative measurements. Qualitative traits evaluated using different scales included, colour of unexpanded leaf, pubescence of young leaves, number of leaf lobes, petiole colour, distribution of anthocyanin pigmentation, growth habit of young stem, pubescence on young stem, stem colour, and flowering characteristics. At harvest, other traits measured included, storage root peduncle, storage root form, root constriction, position of roots, storage root surface colour, root surface texture, storage root length, storage diameter, ease of root periderm removal (outer-skin) ease of root cortex removal (inner skin), colour of outer surface of storage root cortex (inner skin) and storage root pulp colour.

Quantitative traits were measured at different stages of growth of the cassava plants. Data on the severity of African cassava mosaic virus (ACMV) and cassava bacterial blight CBB were taken at 3MAP and 6MAP. Incidence was scored as the number of infected plants over the total number of plants, severity was assessed based on a scale of 1 to 5 in increasing severity (IBPGR). Other quantitative traits measured included; length of petiole, height at first apical branch, height of plant, number of levels of branching, number of nodes to first apical branching and number of leaf lobes. At harvest time, root yield characteristics measured were recorded as

average number of roots, average fresh root weight per plant, and top weight. Hydrogen cyanide potential or content was determined using picrate acid test (Almazan,1988).

Table 3.2 Descriptor list of some morphologic traits and their scales

Trait code	Description	Scale
T1_1	Germination of stakes	Actual proportion
T1_2	Initial vigour	3-Low 5-Intermediate 7-High
T1_3	Colour of unexpanded Apical leaf	3-Light green 5-Dark green 7-Green purple 9-Purple
T1_4	Colour of first fully expanded apical leaf	3-Light green 5-Dark green 7-Green purple 9Purple
T1_6	Leaf vein colour	3-Light green 5-Dark green 7-Green purple 9Purple
T1_7	Pubescence of young leaves	0-Absent 3-Little pubescence 5-Moderate pubescence 7-High Pubescence
T1_11	Position of young leaves	1-Erect 2-Horizontal 3-Deflexed 4-Retorse
T1_15	Angle of petiole insertion	0-Not branching 3- (15-30°) 5- (45-60°) 7- (75-90°)
T1_17	Petiole length	0-Absent 3-Short 5-Medium 7-Long
T1_18	Petiole colour	3-Light green 5-Dark green 7-Green purple 8-Red 9-Purple
T1_22	Stem colour	1-Silver green 2-Light brown 3-Dark brown 4-Dark green
T1_27	Number of levels of branching	Actual levels (at harvest)
T1_28	Angle of branching	0-Not branching 3- (15-30°) 5-(45-60°) 7-(75-90°)
T1_29	Height of first apical branching	In cm ((months after planting)
T5_6	Storage root surface texture	3-Smooth 5-Medium- 7-Rough
T5_9	Number of storage root	Actual number

Abridged from (International Board for Plant Genetic Resources) (IBPGR) descriptor list for cassava, 1983.

3.2 Molecular characterisation

3.2.1 Lyophilised leaf samples

Leaf samples from the 100 cassava accessions were sent from Ghana as lyophilised leaf samples. The DNA extracted from the lyophilised leaf samples was of poor quality and degraded and hence was not suitable for DNA analysis and hence was discarded.

3.2.2 Screen house establishment

Stem cuttings were made from all the 100 accessions and packaged in labelled polythene and sent to IITA, Ibadan Nigeria. The stem cuttings were raised in nursery bags with topsoil in the screen house. Out of the 100 cassava accessions raised in the screen house, 96 survived and these were used for the genetic characterisation. Accessions 5,31,61 and 77 (Table 3.1) were the entries that did not survive in the screen house. Young fresh leaves were harvested and used for the laboratory work.

3.2.3.1 DNA Extraction using Cetyltrimethylammonium bromide (CTAB)

DNA extractions from fresh leaf tissues were prepared using the modified DNA isolation method described by Rogers and Bendich (1985). The DNA extraction buffer contained 2% CTAB, w/v, 100 mM of Tris-HCl (pH 8.0), 20 mM of EDTA (pH 8.0) 1.4 mM NaCl, 1% PVP (Poly Vinyl Pyrrolidone) and 0.3 β -mercaptoethanol added just before use. The leaf samples were ground with mortar and pestle in liquid nitrogen into fine powder. The powdered tissue was transferred into 50 ml falcon tubes and 15 ml of preheated (at 65°C) extraction buffer added. The extract was mixed thoroughly by gentle shaking, and incubated in a water bath at (60°C-65°C) for 1hr with occasional mixing. The mixture was extracted with phenol/chloroform (chloroform:isoamyl 24:1 v/v) and centrifuged for 10 minutes at 10,000 rpm in a Sorval RC-5C automatic super speed refrigerated centrifuge (Beckman, USA). The supernatant was transferred into 30 ml corex tubes and an equal volume of ice cold isopropanol, and a tenth volume of 3M sodium acetate (pH 7.0) added. The DNA was then precipitated at -20°C for 2 hours and centrifuged at 10,000 rpm for 10 min to pellet the DNA. DNA was air dried and dissolved in IxTE (10 mM Tris pH 8.0, 0.1 mM EDTA) and treated with 10 ng of RNase at 37°C for 30 mins. The DNA was extracted with phenol chloroform and precipitated with ice cold isopropanol and 3 M sodium acetate at -20°C for 2 hrs. The mixture was centrifuged for 5 mins

and the pellet washed with 500 μ l cold 70% ethanol by centrifugation at 10,000 rpm for 5 mins. The DNA was air-dried and the pellet resuspended in 1ml T.E.

The extracted DNA was examined on 1% agarose in 1xTAE buffer (45 mM Tris -HCl, 1 mM EDTA, 3% Glacial acetic acid, pH 8.0). The DNA concentration was measured photospectrometrically using DU-65 UV spectrophotometer (Beckman Instruments USA) at 260 nm. The DNA samples were diluted to a final concentration of 10 ng/ μ l and stored at -20°C for subsequent experiments. The CTAB protocol was used to extract DNA from the 96 accessions that survived at the screen house in IITA. Seventy-two (72) of the samples gave good quality DNA that could be amplified with RAPD primers.

3.2.3.2 DNA extraction using Qiagen kit

DNA was extracted with the Qiagen Kit according to the manufacturer's protocol. The constitutions of the accompanying buffers were not disclosed. Each fresh leaf sample (about 1 g) was ground in 1.5 ml eppendorf tube with plastic pestle in liquid nitrogen into fine powder. 400 μ l of preheated (65°C) extraction buffer AP1 and 4 μ l of Rnase was added to the powdered tissue and vortexed vigorously for cells to be lysed. The mixture was incubated at 65°C in water bath for 10 min, with occasional mixing by inverting 2 or 3 times. Buffer AP2 (130 μ l) was added to the lysate, mixed and incubated for 5 min on ice to precipitate detergent, proteins, and polysaccharides. The lysate was introduced into the QIAshredder spin column sitting in a 2 ml-collection tube and centrifuged for 2 min at maximum speed to remove precipitates or cell debris. The flow-through lysate (usually 450 μ l) was introduced into a new eppendorf tube and 0.5 volume of Buffer AP3 and 1 volume of ethanol added and mixed by pipetting. The mixture was then introduced into Dneasy mini spin column in a 2 ml collection tube, centrifuged for 1min at (8,000 rpm). Flow through was discarded and the step repeated for the rest of the sample. The Dneasy column membrane with the DNA was washed with 500 μ l of

Buffer AW by centrifugation for 1 min (8,000 rpm). The step was repeated at maximum speed (11,000 rpm) for 2 min to dry the column membrane. Since residual ethanol could interfere with subsequent reactions, the column was dried at 50°C for 15 min. The mini column is supported in a 1.5 ml eppendorf tube and 100 µl of preheated Buffer AE (65°C) directly dispensed into the Dneasy column membrane, incubated for 5 min at room temperature and then centrifuged for 1 min at (8,000 rpm) to elute the DNA. This was repeated with another 100 µl of Buffer AE and collected into a second tube.

RNase was added to eliminate RNA contamination. DNA yield and purity was determined by measuring the absorbance at 260 nm (A260) and (A280) in a spectrophotometer. The DNA was checked on 1% agarose in 1x TAE buffer (40 mM Tris – acetate and 1 mM EDTA, pH 8.0). The DNA samples were diluted to a final concentration of 10 ng/µl and stored at -5°C for the PCR experiments.

3.2.4 Primers

A total of eighty random 10-mer oligonucleotides primers from the A, O, P, Q, R, S, J, K, and E set kits (Operon Technologies Inc. Alameda CA, USA) were screened (see appendix 3). Ten of the primers, OPR-9, OPR-2, OPK-1, OPK-11, OPK-16, OPQ-18, OPS-18, OPJ-20, OPJ-5, and OPJ-14 were selected to amplify the DNAs. The primers were selected on the basis of their ability to generate highly unambiguous scorable bands.

Table 3.3 Oligonucleotide primers used for the RAPDs analysis of the Cassava accessions

Serial no	Operon code	Nucleotide sequence
1	OPJ-14	CACCCGGATG
2	OPJ-20	AAGCGGCCTC
3	OPJ-5	CTCCATGGGG
4	OPK-1	CATTCGAGCC
5	OPK-11	AATGCCCCAG
6	OPK-16	GAGCGTCGAA
7	OPQ-18	AGGCTGGGTG
8	OPR-2	CACAGCTGCC
9	OPR-9	TGAGCACGAG
10	OPS-18	CTGGCGAACT

3.2.5 RAPD-PCR conditions

Polymerase Chain Reaction amplification reactions were performed in 25 μ l reaction volumes. The reaction mixture composition was 5 ng template DNA, 2.5 μ l of 5% Tween 20, 2.5 μ l of 10 x Taq buffer, 1 μ l of 2.5 mM of each deoxyribonucleotide (dNTPs), 1 μ l of Primer and 2 units of Taq DNA polymerase, (Promega Corporation Madison, WI, USA) and 10.6 μ l of sterile distilled water. The reaction mixture was overlaid with a drop of mineral oil to avoid evaporation, PCR amplification was carried out in Perkin Elmer 9600 thermocycler. The amplification profiles programmed involved an initial denaturation step of 94°C for 3 mins followed by 45 cycles at 94°C for 1 min, 36°C for 1 min for primer to anneal, and extension at 72°C for 2 min. A final elongation step at 72°C for 7 mins was included. Amplification products were maintained at 4°C until electrophoresed. The PCR amplification products were separated by electrophoresis on 1.5% agarose gel in 1xTAE buffer (40 mM Tris-acetate and 1mM EDTA, pH 8.0), with a 1-kb DNA ladder (Promega Corporation, Madison, WI, USA) used as molecular size marker. The gels were stained with ethidium bromide (0.5 μ g/ml) and viewed under ultra-violet transilluminator and photograph taken using the computer soft ware for gel documentation called Grab-it.

3.3 Data analysis

3.3.1 Analysis of morphological data

Morphological and genetic markers were used to detect genetic diversity in the cassava cultivars from 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. Multivariate analysis included principal component analysis and canonical variate analysis were performed on all the traits measured. A non-hierarchical clustering was done using results from the canonical discriminant analysis. Phenotypic correlation analysis for the morphological characters was carried out. Heritability estimates were calculated on only quantitative traits. Broadsense heritabilities were calculated using the formula:

$$h_b^2 = V_g/V_p = V_g / (V_g + V_e) \quad (\text{Wricke } et al., 1986)$$

3.3.2 Analysis of RAPD data

RAPD markers that were consistently reproduced were used for the analysis. The position of scorable RAPD bands and sizes were estimated from the gel photograph by a comparison with the 100bp and 1-KB ladder marker. Scoring was done using the gel photograph system.

The entire set of reproducible RAPD bands generated in the 96 cassava accessions by the 10 primers was used for the RAPDs analysis. The bands were scored as present (1) or absent (0). A binary character matrix was generated with fragments in column and cultivar in rows. Pairwise distance matrices were compiled by the NTSYS-PC 2.02j software package (Rohlf, 1989) using the Jaccard similarity coefficient (Jaccard, 1908). The similarity index of Jaccard between cultivars (i and j) was given by:

$$S_{ij} = a / (a + b + c)$$

Where, a is the number of bands present in both i and j , b is the number of bands present in i but not in j , c the number of bands present in j but not in i . Clustering was done using the unweighted pair group method with arithmetic means (UPGMA) algorithm (Sneath and Sokal, 1973). The resultant data was used to generate a dendrogram showing the phylogenetic relationships.

The RAPD results analysed was subjected to similarity analysis using Jaccard (1908) coefficient. Hierarchical clustering was done using the sequential, agglomerative, hierarchical and nested (SAHN) method with UPGMA values. A phylogenetic tree or dendrogram was then generated.

CHAPTER FOUR

4.0 RESULTS

4.1 Variability and heritability of morphological traits

4.1.1 Variation in qualitative traits

Figure 4.1 shows the distribution of leaf petiole colour among the cassava accessions. More than half of the accessions (53%) had dark purple petiole; 22% had red, 14% had green and 11% light green colour. Stem colour distribution showed that 84% of the accessions were dark brown, 14% brown and 2% silver green (Figure 4.2). Thirty percent of the accessions had light green purple leaf colour, 24% dark green, 15% green and 14% light green (Figure 4.3). Variation in storage root surface colour is shown in Figure 4.4. Fifty-nine percent of the accessions had dark brown tuber colour, 26% light brown and 15% cream colour. Anthocyanin pigmentation showed considerable variation among the accessions with 49% of the accessions showing anthocyanin pigmentation on the entire petiole, 11% ventral portions, 26% patches on petiole and 14% did not show any anthocyanin pigmentation (Figure 4.5). Fifty-six percent of the cassava accessions flowered and 44% of the accessions did not flower (Figure 4.6).

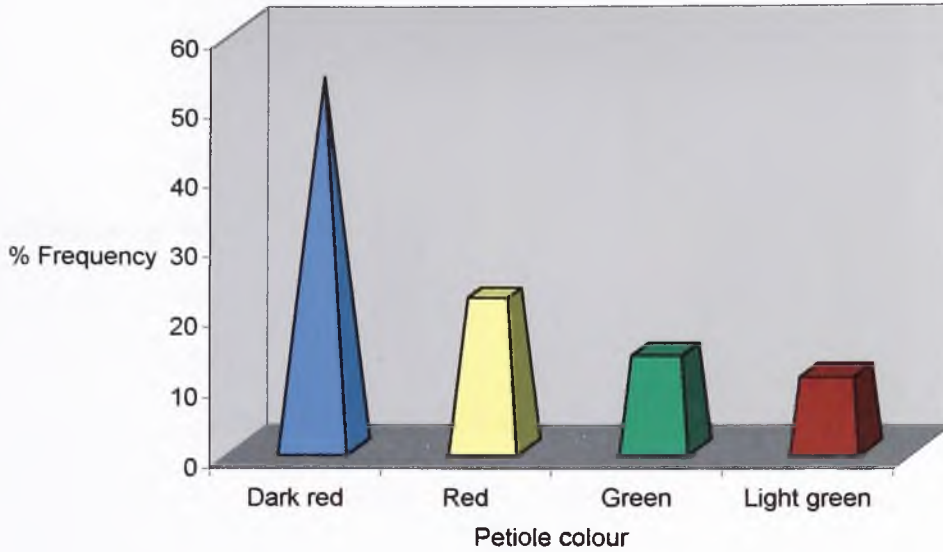


Figure 4.1: The distribution of petiole colour in 100 accessions of cassava.

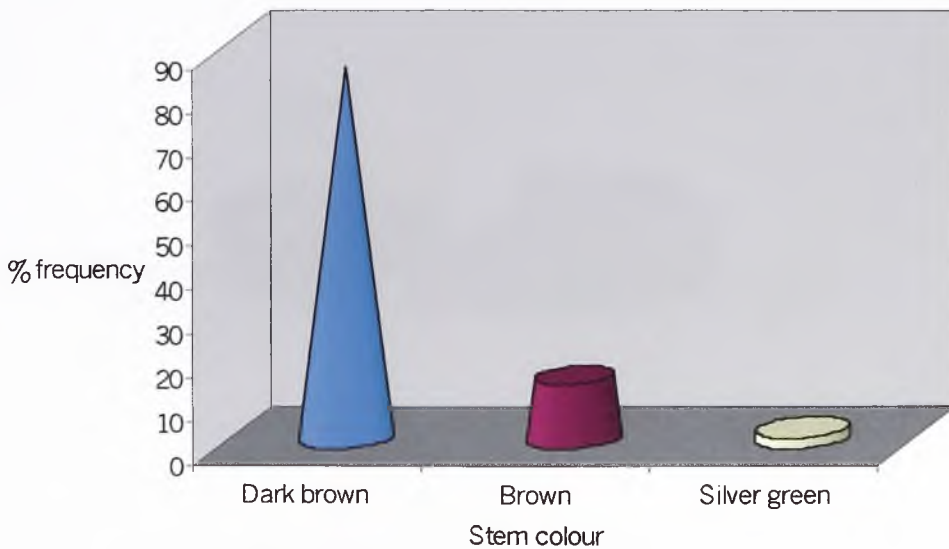


Figure 4.2: The distribution of stem colour in 100 accessions of cassava.

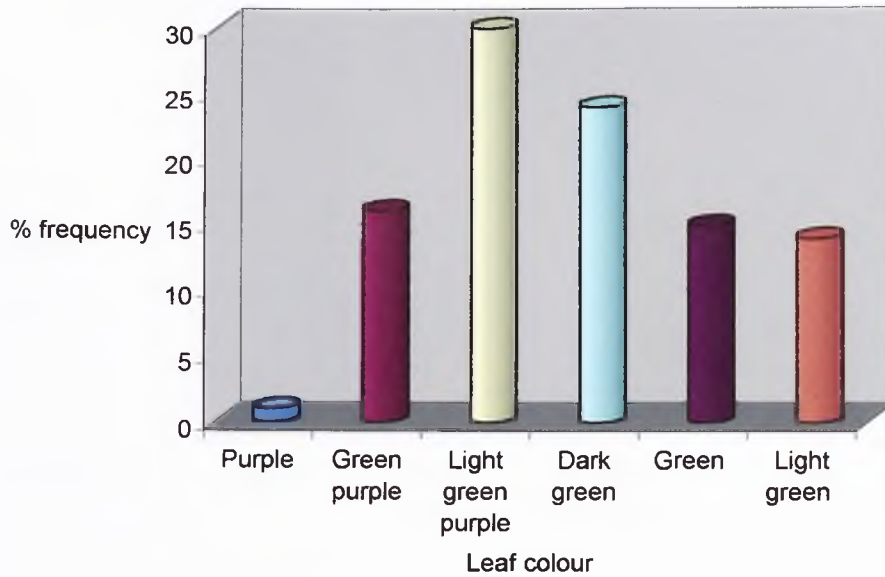


Figure 4.3: The distribution of colour of fully expanded leaf in 100 cassava accessions.

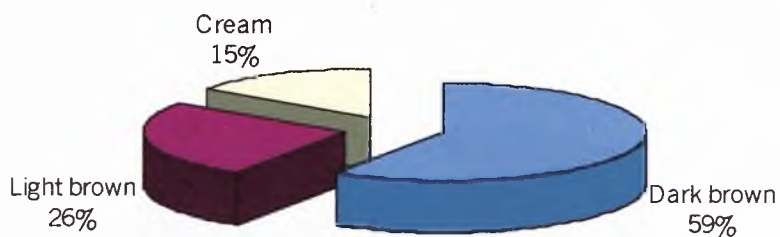


Figure 4.4: The distribution of root surface colour in 100 cassava accessions.



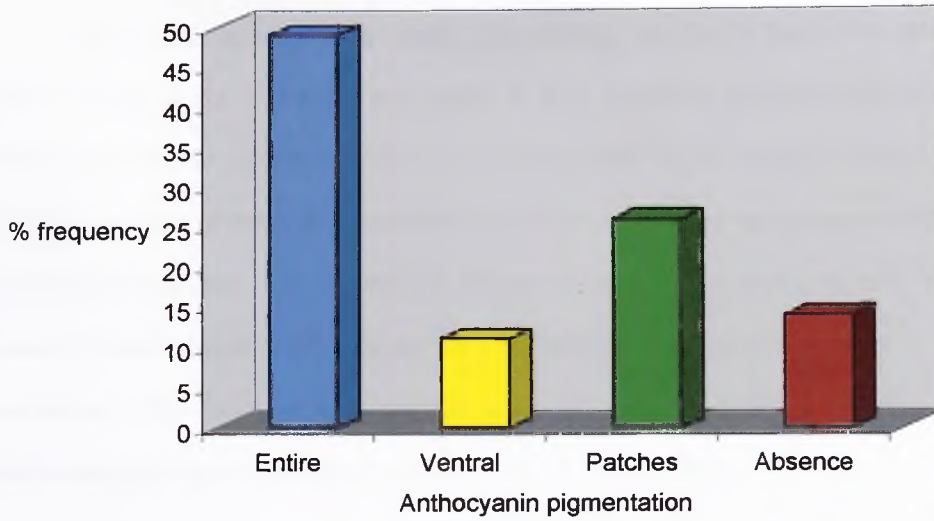


Figure 4.5: The distribution of anthocyanin pigmentation in 100 cassava accessions.

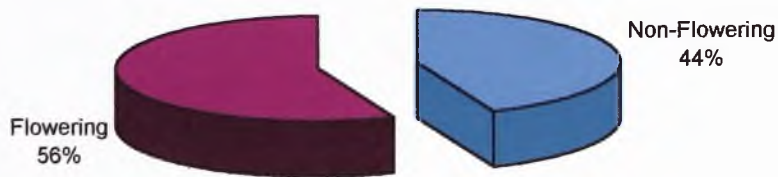


Figure 4.6: The distribution of flowering and non-flowering types among 100 cassava accessions.

4.1.2 Variation in quantitative traits

Plant establishment at four weeks after planting, number of leaf lobes, plant stand at harvest, plant height at harvest and height at first branching showed significant ($p < 0.01$) variation among the accessions (Table 4.1). All the yield traits measured showed significant ($p < 0.01$) variation (Table 4.2). There were significant variation in the accessions with regard to the diseases evaluated. The severity of African cassava mosaic virus (ACMV) disease and cassava bacterial blight (CBB) disease both showed significant ($p < 0.01$) variation among the accessions (Table 4.3). Hydrogen cyanide content and percentage foliage retention (Table 4.3) also showed significant ($p < 0.01$) variation among the 100 accessions.

Table 4.1: Mean squares for plant establishment, number of leaf lobes, plant stand and plant height (quantitative characters) measured in 100 accessions of cassava

Sources of variation	Degrees of Freedom	Plant establishment at 4 weeks	Number of leaf lobes	Plant stand at harvest	Plant height at harvest	Height at first branching (cm)
Replication	1	271	0.2	88	40	10503
Block (Replication)	18	250	0.21	198	986	3219
Accession	99	599**	0.61**	586**	3472**	8199**
Error	76	127	0.18	204	612	3165

** Significant at 5% and 1% respectively

Table 4.2: Mean squares for size of storage root, number of tubers per plot, yield and harvest index among 100 cassava accessions

Sources of variation	Degrees of Freedom	Storage root length (cm)	Storage root diameter (cm)	Number of fresh tuber per plot	Weight of fresh tuber yield tons/ha	Harvest index
Replications	1	6.51	4.13	139	119	0.01
Block (Replication)	18	1.35	1.14	124	42	0.01
Accession	99	2.68**	1.58**	866**	321**	0.03**
Error	76	0.77	0.56	97	41	0.01

** Significant at 5% and 1% respectively

Table 4.3: Mean squares for number of nodes to first branching, foliage retention, HCN content, ACMV and CBB severity scores at 6 MAP among 100 accessions of cassava

Sources of variation	Degrees of freedom	Number of nodes to first branching	% foliage retention	Hydrogen cyanide content (mg)	ACMV severity scores at 6 MAP	CBB severity scores at 6 MAP
Replications	1	5008	52	15.6	3.25	0.12
Block (Replication)	18	1923	22	1.96	0.29	1.69
Accession	99	4207**	60**	2.99*	0.75**	2.10**
Error	76	1683	22	1.81	0.17	0.58

** Significant at 5% and 1% respectively

4.1.3 Heritability of quantitative traits

Genotypic and phenotypic variances and ratios of genotypic and error variances to the phenotypic variance are shown in Table 4.4. A large proportion of the phenotypic variance of the length of stipules (84%), plant height at harvest (82%), number of fresh tubers (89%) and fresh tuber yield (87%) were attributable to genotypic differences among the accessions. Hydrogen cyanide (HCN) content had a low heritability estimate of 0.39, indicating that differences in HCN content were mostly conditioned by the environment.

Table 4.4 Genotypic (σ^2_g), phenotypic (σ^2_p) variances and variance ratios of quantitative agronomic characters in cassava

Character	Variance			
	(σ^2_g)	(σ^2_p)	($\sigma^2_g/(\sigma^2_p)$)	(σ^2_e/σ^2_g)
Length of stipules	2.13	2.52	0.84	0.16
Petiole length	0.6	1.105	0.54	0.46
Plant establishment at 4 weeks	213.6	299.6	0.71	0.29
Number of leaf lobes	0.215	0.305	0.70	0.30
Plant stand at harvest	191.22	293.4	0.65	0.35
Storage root length (cm)	0.955	1.34	0.71	0.29
Storage root diameter (cm)	0.51	0.79	0.65	0.35
Hydrogen cyanide content	0.59	1.49	0.39	0.61
Nodes to first branching	1261.8	2103.7	0.60	0.40
Height at branching (cm)	2517.3	4099.9	0.61	0.39
Plant height at harvest (cm)	1430	1736	0.82	0.18
Number of fresh tuber (yield/ha)	384.9	433.4	0.89	0.11
Fresh tuber yield (tons/ha)	140.2	160.7	0.87	0.13
Harvest index	0.01	0.015	0.67	0.33

4.2 Associations among quantitative morphological traits

Table 4.5 shows phenotypic correlation coefficients among quantitative traits in cassava accessions. There was a significant high and positive correlation ($r=0.49$; $p<0.01$) between storage root length and storage root diameter. Storage root diameter however showed significantly high positive correlation with all characters except for CBB and ACMV scores at 6 MAP, which showed negative correlation. There was a high positive correlation ($r=0.36$; $p<0.01$) between ACMV and CBB. ACMV and storage root length showed significantly high positive correlation ($r=0.53$; $p<0.01$). CBB showed high positive correlation with plant establishment at four weeks after planting. However CBB had negative significant correlation with all the morphological traits measured. The number of nodes at first branching correlated positively with storage root length ($r=0.43$; $p<0.01$), storage root diameter ($r=0.31$; $p<0.01$) and percentage foliage retention ($r=0.42$; $p<0.01$). Weight of fresh tubers and number of fresh tubers produced showed the highest positive correlation ($r=0.87$; $p<0.01$). Although the r values were significant the r^2 values were rather low.

4.3 Clustering of cassava accessions

4.3.1 Morphological characterization using quantitative traits

Table 4.6 shows the relative contribution of each of the different quantitative characters in determining the overall variability among the accessions. The first 10 Principal Components accounted for 93% of the total variation present. Each principal component or new variable was made up of a linear combination of the original variables scored. The first new variable, which accounted for 38%, had number of fresh root tubers and fresh tuber weight, storage root length and percentage foliage retention showing large positive influence on variation among accessions.

Table 4.5: Correlation Analysis of quantitative characters in cassava

Character	1	2	3	4	5	6	7	8	9	10	11	12	13
1 Plant establishment at 4 WAP	-												
2 Number of leaf lobes	0.08	-											
3 CMVD scores at 6 MAP	-0.11	-0.02	-										
4 Storage root length	0.28**	0.11	0.53***	-									
5 Storage root diameter	0.30**	0.21*	-0.30**	0.49**	-								
6 Hydrogen cyanide content	0.19	0.11	-0.07	0.22*	0.26*	-							
7 Percentage foliage retention	0.29**	0.08	-0.52***	0.67***	0.38***	0.18	-						
8 CBB scores at 6 MAP	0.01	-0.26*	0.36***	-0.31**	-0.34***	-0.16	-0.35**	-					
9 Number of nodes at first branching	0.20*	0.18	-0.15	0.43***	0.31**	0.13	0.42***	-0.32**	-				
10 Height at first branching	0.21*	0.11	-0.2*	0.44***	0.31**	0.11	0.47***	-0.35***	0.96***	-			
11 Plant height at harvest	0.41***	0.43***	-0.33**	0.40***	0.39***	0.07	0.39***	-0.44***	0.36***	0.38***	-		
12 Number of fresh tuber / ha	0.61***	0.11	-0.54***	0.52***	0.48***	0.28**	0.57***	-0.19	0.29**	0.30**	0.46***	-	
13 Weight of fresh tuber yield in tons/ha	0.44***	0.13	-0.63***	0.66***	0.59***	0.30**	0.65***	-0.38***	0.35***	0.38***	0.42***	0.87***	-
14 Harvest index	0.26*	0.03	-0.39***	0.41***	0.41***	0.37***	0.25*	-0.2*	0.10	0.10	0.20*	0.52***	0.53***

*, ** Significant at 5% and 1% respectively

Table 4.6: Eigen values and loadings from principal component analysis of quantitative agronomic Traits in cassava

Character	Principal component axes (loading of components)									
	1*	2	3	4	5	6	7	8	9	10
Plant establishment at 4 weeks	0.23	0.15	-0.38	-0.33	0.04	0.23	0.15	0.08	0.27	0.02
Number of leaf lobes	0.05	0.39	0.19	0.12	0.18	-0.43	0.53	0.20	0.17	0.18
Plant stand at harvest	0.25	0.13	-0.34	-0.31	0.01	0.26	0.10	0.10	0.30	0.08
Storage root length (cm)	0.27	0.08	0.10	0.18	0.06	-0.08	-0.38	-0.09	0.45	-0.17
Storage root diameter (cm)	0.25	0.01	0.02	0.32	0.08	0.08	0.37	0.15	0.17	0.44
Hydrogen cyanide content	0.12	0.02	0.20	0.27	0.52	0.07	0.17	0.75	-0.01	0.02
Percentage foliage retention	0.27	-0.16	0.11	-0.03	0.09	-0.38	-0.33	0.12	0.32	0.04
Nodes to first branching	0.21	-0.01	0.46	-0.19	0.30	0.30	0.05	-0.10	-0.11	-0.11
Height at branching (cm)	0.22	-0.05	0.46	0.21	0.23	0.30	0.01	-0.01	-0.12	-0.06
Plant height at harvest (cm)	0.24	0.40	0.13	-0.03	-0.16	-0.06	0.18	0.16	-0.06	0.27
Number of fresh tuber/ha	0.30	0.02	0.25	0.03	0.11	-0.15	-0.11	-0.19	-0.30	-0.17
Fresh tuber yield (tons/ha)	0.31	-0.06	0.11	0.14	0.12	-0.24	-0.09	-0.19	-0.23	-0.23
Harvest index	0.17	0.03	0.23	0.44	0.22	0.25	-0.13	-0.33	-0.27	0.46
Eigen value	7.23	2.51	1.83	1.62	1.27	0.87	0.76	0.62	0.53	0.39
% variability	38.0	13.0	9.60	8.54	6.67	4.61	3.99	3.25	2.81	2.05
Cumulative variability	38.0	51.0	60.60	69.14	75.81	80.42	84.41	87.66	90.47	92.52

* Refers to coefficient for first, second, third, fourth.....tenth principal component equations

Table 4.6: Eigen values and loadings from principal component analysis of quantitative agronomic Traits in cassava

Character	Principal component axes (loading of components)									
	1*	2	3	4	5	6	7	8	9	10
Plant establishment at 4 weeks	0.23	0.15	-0.38	-0.33	0.04	0.23	0.15	0.08	0.27	0.02
Number of leaf lobes	0.05	0.39	0.19	0.12	0.18	-0.43	0.53	0.20	0.17	0.18
Plant stand at harvest	0.25	0.13	-0.34	-0.31	0.01	0.26	0.10	0.10	0.30	0.08
Storage root length (cm)	0.27	0.08	0.10	0.18	0.06	-0.08	-0.38	-0.09	0.45	-0.17
Storage root diameter (cm)	0.25	0.01	0.02	0.32	0.08	0.08	0.37	0.15	0.17	0.44
Hydrogen cyanide content	0.12	0.02	0.20	0.27	0.52	0.07	0.17	0.75	-0.01	0.02
Percentage foliage retention	0.27	-0.16	0.11	-0.03	0.09	-0.38	-0.33	0.12	0.32	0.04
Nodes to first branching	0.21	-0.01	0.46	-0.19	0.30	0.30	0.05	-0.10	-0.11	-0.11
Height at branching (cm)	0.22	-0.05	0.46	0.21	0.23	0.30	0.01	-0.01	-0.12	-0.06
Plant height at harvest (cm)	0.24	0.40	0.13	-0.03	-0.16	-0.06	0.18	0.16	-0.06	0.27
Number of fresh tuber/ha	0.30	0.02	0.25	0.03	0.11	-0.15	-0.11	-0.19	-0.30	-0.17
Fresh tuber yield (tons/ha)	0.31	-0.06	0.11	0.14	0.12	-0.24	-0.09	-0.19	-0.23	-0.23
Harvest index	0.17	0.03	0.23	0.44	0.22	0.25	-0.13	-0.33	-0.27	0.46
Eigen value	7.23	2.51	1.83	1.62	1.27	0.87	0.76	0.62	0.53	0.39
% variability	38.0	13.0	9.60	8.54	6.67	4.61	3.99	3.25	2.81	2.05
Cumulative variability	38.0	51.0	60.60	69.14	75.81	80.42	84.41	87.66	90.47	92.52

* Refers to coefficient for first, second, third, fourth.....tenth principal component equations

The number of fresh tubers produced, had a negative effect for the ninth new variable with only 3% variation captured. Hence the large positive effect of this trait from the initial first new variable was not cancelled out. Storage root length also showed a large positive effect for the ninth new variable. The second new variable accounted for 13% of the total variation observed. The number of leaf lobes and the plant height at harvest showed large positive influence on performance.

The 100 accessions were grouped into six clusters (Table 4.7). Cluster III was the smallest with seven accessions. The seven accessions were from western and central regions only. Cluster II had 15 accessions with the entries representing five of the regions. The 39 and 19 accessions from the Western, Central regions respectively, were distributed in all the six different clusters. The five accessions from the northern part of Ghana were found in clusters IV and V.

Table 4.8 shows the cluster means for the various quantitative characters studied. There were considerable differences in the number and weight of fresh tubers produced, plant stand at harvest, storage root length and percentage foliage retention.

The number of nodes to first branching and height at branching for the various clusters had for each cluster both non-branching and branching accessions. These characters had higher effects on distinguishing between clusters than the other characters. This supports the results of the principal component analysis.

Table 4.7: Distribution of 100 accessions of cassava into the different clusters based on their quantitative traits

Cluster number	Number of Accessions	Name of accessions	Region of Collection
I	16	Acw10, Acw157, Acw23, Acw37, Acw5, Jk90005	Western
		B89031	Eastern
		Mc90022, Jk90033	Central
		Eop9803	G. Accra
		Kav90022, Kav90007, Kav90010	Volta
		Kaa90067, Kaa90062, Kaa90060	Ashanti
II	15	Acw107, Acw125, Acw29, Jk90021	Western
		Mc90010*	Central
		Kav90005, Kav90013	Volta
		B89003, B89013, Kaa90063, Kae90050, Mc90005, Kae90034, Kae90046	Eastern
		Eop9804	G. Accra
III	7	Acw11, Acw2, Acw86, Jk90006, Jk90022	Western
		Mc90031, Mc90036	Central
IV	29	Acw111, Acw126, Acw16, Acw24, Acw71, Acw75, Acw82, Acw9, Acw91, Jk90001, Jk90009, Jk90026, Jka90013	Western
		B89029, Btl97006	Eastern
		Acc497, Jk90031, Mc90015, Mc90019, Mc90021, Mc90028, Mc90030, Mc90033	Central
		So96002	Upper East
		Kav90006, Kav90008, Kav90028	Volta
Dmgk02, Dmgk05	Northern		
V	22	Acw19, Acw36, Acw6, Acw62, Jk90008, Jk90012, Jk90013, Jk90016, Jka90008	Western
		Mc90012, Mc90034,	Central
		Kaa90087, Kaa90830, Mc90006	Ashanti
		Dmgk12, Dmgk11	Northern
		Kae90033, Mc90004, B89015, B89037, B89032	Eastern
		Kav90002	G. Accra
VI	11	Acw61, Jka90016*	Western
		B89001	Eastern
		Mc90013, Mc90020, Mc90029, Mca90010	Central
		Eop9801, Eop9802, Eop9805, Kav90004	G. Accra

Table 4.8: Means of cluster of cassava accessions associated according to quantitative agronomic traits in cassava

Agronomic character	Cluster number					
	I	II	III	IV	V	VI
Plant establishment at 4 weeks	79.50	80.90	68.80	53.30	70.60	83.00
Number of leaf lobes	7.00	7.00	7.00	6.65	6.73	6.73
Plant stand at harvest	78.50	82.50	70.30	51.90	69.30	83.00
Storage root length (cm)	4.67	5.80	5.71	4.14	5.09	5.45
Storage root diameter (cm)	5.25	6.00	5.29	4.64	5.32	5.91
Hydrogen cyanide potential	4.52	4.72	4.96	4.44	4.34	4.36
Percentage foliage retention	32.30	38.80	36.40	29.60	33.90	36.70
Nodes to first branching	3.50	103.30	118.20	6.80	66.10	27.10
Height at branching (cm)	7.78	145.50	166.60	10.10	103.00	53.40
Plant height at harvest (cm)	253.70	251.70	256.50	167.50	207.80	238.00
Number of fresh tubers per plant	4.61	7.06	3.95	2.00	3.60	6.69
Number of fresh tubers per plot	36.90	56.50	31.60	16.00	28.80	53.50
Fresh tuber yield (tons per ha)	12.90	29.40	12.50	4.80	13.60	24.20
Harvest index	0.06	0.15	0.07	0.15	0.11	0.06

Figure. 4.2 shows a three-dimensional presentation of the accessions in nine clusters. The various colours and symbols represent specific clusters. Cluster 1 with five accessions formed a more dispersed cluster. The other clusters formed fairly tight and distinct clusters.

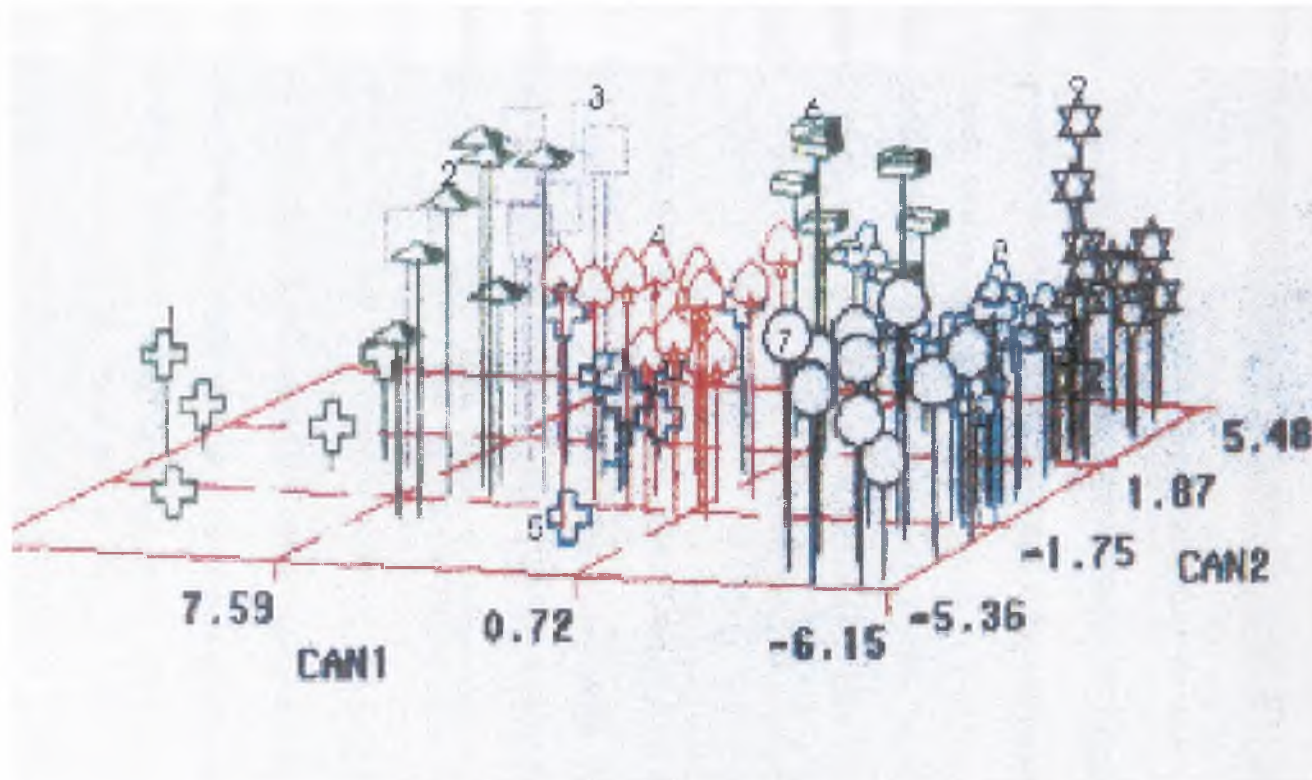


Fig. 4.7. First three new variables from canonical discriminant analysis of 100 accessions of cassava on quantitative traits.

4.3.2 Morphological Characterisation Using Qualitative Traits

Table 4.9 shows the relative contribution of each of the different qualitative characters in determining total variation among the accessions. The first 10 new variables accounted for 75% of the total variation measured. The first new variable contributed for 17% of the total variation. The variation was greatly explained by three traits, first fully expanded leaf colour, storage root surface colour and storage root surface texture. The first two characters showed large negative influence whereas the third showed positive influence. The second new variable accounted for 12% of the total variation. The length of stipules and petiole, root form and position showed high positive influence on the variation. The length of stipules and storage roots showed high negative influence on the sixth new variable. Mealiness showed positive influence on the sixth new variable. The sixth new variable only accounted for 5% of the variation, hence the large positive effect from the length of stipules and storage root form second new variable was not cancelled out, with the high negative influence on the sixth new variable.

The 100 accessions were grouped into six clusters (Table 4.10). Cluster II was the smallest group with 6 accessions representing 50% of the regions where the accessions were obtained. The largest group was cluster III with 32 accessions from six of the eight regions. The accessions from Western, Volta and Central were found in all the six groups. Moreover, the five accessions from the northern part of Ghana were found in four different clusters.

The cluster means for the qualitative characters studied are presented in (Table 4.11). There were considerable differences in first fully expanded leaf colour, anthocyanin pigmentation, storage root form, position of root, colour of outer surface and storage root texture. These characters had higher effect on distinguishing between clusters than the other characters.

Table 4.9: Eigen values and loading from principal component analysis of qualitative Agronomic traits in 100 accessions of cassava.

Character	Principal component axes (loading of components)									
	1*	2	3	4	5	6	7	8	9	10
First fully expanded leaf colour	-0.36	0.17	0.04	0.02	0.02	0.04	0.28	-0.01	-0.01	-0.08
Pubescence on young leaf	0.27	0.10	-0.06	-0.34	0.25	0.10	0.31	0.00	-0.07	0.24
Length of stipules	-0.04	0.30	0.02	0.07	0.15	-0.50	0.19	-0.21	0.01	0.02
Petiole length	0.08	0.35	0.21	-0.03	-0.15	-0.14	0.07	0.05	-0.03	-0.23
Petiole colour	0.08	-0.11	0.55	0.02	-0.08	0.11	0.22	0.08	0.13	0.11
Anthocyanin pigmentation	0.02	-0.09	0.55	0.01	0.10	-0.13	0.23	0.18	0.05	0.12
Stem colour	0.12	0.12	0.09	0.04	0.13	-0.08	-0.36	0.46	-0.36	0.46
Prominence of scars	-0.28	-0.03	0.11	-0.04	0.29	0.00	-0.06	-0.09	0.05	0.31
Presence or absence of flowers	-0.16	0.22	-0.23	0.12	-0.12	0.11	0.17	0.13	0.19	0.29
Storage root peduncle length	0.06	0.22	0.22	-0.16	-0.09	0.26	0.07	0.30	0.06	0.03
Storage root form	0.05	0.32	-0.04	0.21	0.06	-0.36	-0.06	0.07	-0.16	0.22
Constriction on storage roots	-0.09	0.25	0.05	0.12	0.38	0.12	0.22	0.24	0.08	0.26
Position of roots	0.06	0.35	0.15	0.05	-0.15	0.32	-0.20	0.14	0.16	-0.24
Storage root surface colour	0.35	0.24	-0.06	0.07	-0.09	-0.10	-0.02	-0.07	-0.01	0.08
Storage root surface texture	-0.36	0.20	-0.06	0.06	-0.11	-0.07	-0.09	-0.13	-0.01	0.17
Ease of root periderm removal	0.27	-0.07	-0.08	0.34	0.10	0.13	0.03	0.26	0.07	0.11
Colour of outer surface	0.22	-0.24	-0.08	0.21	-0.24	0.09	0.11	0.15	-0.03	0.15
Mealiness	0.08	-0.04	0.23	0.32	0.16	0.31	0.06	-0.33	-0.08	0.07
Taste	0.04	0.17	0.13	0.31	0.14	0.11	0.27	0.33	0.49	0.24
Eigen value	3.52	2.84	1.86	1.51	1.10	0.96	0.89	0.81	0.75	0.71
% variability	16.70	12.10	9.70	8.00	6.20	5.30	4.80	4.20	3.80	3.70
Cumulative variability	16.70	28.80	38.50	46.50	52.70	58.00	62.80	67.00	70.80	74.50

* Refers to coefficient for first, second, third, fourth.....tenth principal component equations

Table 4.10: Distribution of 100 accessions of cassava into different clusters based on their qualitative traits

Cluster Number	Number of Accessions	Name of accessions	Region of collection
I	11	Jk90001, Acw126, Acw24, Acw37, Acw71, Acw9 Acc497, Mc90028 B89029 Kav90008 Dmgk02	Western Central Eastern Volta Northern
II	6	Acw86, Acw10, Jk90022 Eop9803 Mc90036 Kav90006	Western G. Accra Central Volta
III	32	Acw107, Acw11, Acw111, Acw125, Acw29, Acw5, Acw6, Acw75, <i>Jk90008*</i> Jk90005, Jk90009, Jk90012, Jk90026, <i>Jka90008*</i> B89001, B89003, B89013, B89037, Bti97006, Mc90005, Kae90034 <i>Mc90010*</i> , Mc90012, Mc90013, Mc90020, Mc90021, Mc90030, <i>Mca90010*</i> Kaa90087 Kav90028 Eop9802	Western Eastern Central Ashanti Volta G. Accra
IV	19	<i>Acw91*</i> , Acw36, <i>Jka90016*</i> , <i>Jk90016*</i> , Acw61, Acw62, Acw82 Dmgk05, Dmgk11 Jk90031, Mc90022 Kae90050, B89031 Kav90007, Kav90013, Kav90005 Kav90004, Eop9805, Eop9804	Western Northern Central Eastern Volta G. Accra
V	17	Jk90033, Mc90019, Mc90031, Mc90034 B89015, Kaa90063, <i>Kae90033*</i> , Kae90046 Acw16, Acw19, Acw23, Jk90021 Kav90010 Dmgk12 <i>Eop9801*</i> , Kaa90067, Kaa90830	Central Eastern Western Volta Northern G. Accra Ashanti
VI	15	Acw157, Acw2, Jk90006, <i>Jk90013*</i> , <i>Jka90013*</i> Kav90022 Kav90002 Kaa90060, <i>Kaa90062*</i> , Mc90006 So96002 Mc90015, Mc90033, <i>Mc90029*</i> B89032	Western Volta G. Accra Ashanti Upper East Central Eastern

Table 4.11: Means of clusters of cassava accessions associated according to Qualitative agronomic traits in cassava.

Agronomic character	Cluster mean					
	I	II	III	IV	V	VI
First fully expanded leaf colour	5.50	4.33	6.16	5.95	3.70	3.63
Pubescence on young leaf	2.73	5.33	1.81	1.45	3.68	1.20
Length of stipules	4.64	5.67	5.31	5.10	4.82	4.73
Petiole length	3.82	5.67	5.03	4.58	4.82	4.53
Petiole colour	6.82	7.33	8.80	4.03	7.23	8.47
Anthocyanin pigmentation	1.43	1.75	2.76	0.31	1.50	2.47
Stem colour	2.91	2.75	2.92	2.97	2.88	2.90
Prominence of scars	5.09	3.50	4.61	4.21	3.65	3.60
Presence or absence of flowers	0.41	0.17	0.64	0.74	0.41	0.10
Storage root form	1.45	2.08	1.83	2.00	1.88	1.63
Constriction on storage roots	0.64	0.58	0.58	0.68	0.56	0.40
Position of roots	1.27	1.67	1.78	1.66	1.79	1.50
Storage root surface colour	2.00	3.00	2.09	2.03	2.94	2.50
Storage root surface texture	4.68	6.33	4.54	4.37	6.29	5.37
Ease of root periderm removal	3.64	3.67	3.62	3.53	4.59	4.37
Colour of outer surface	1.54	1.08	1.47	1.24	2.12	2.20
Mealy	3.04	2.62	3.29	2.80	3.28	3.13
Taste	3.50	3.25	3.50	3.55	3.56	3.43

Figure 4.3 is a three dimensional presentation of cluster grouping into nine groups as per canonical discriminant analysis. The first three new variables were used as the x, y, z axes. Colour and symbols represent the various groups. The closely packed accession depicts less intra-cluster distances, hence close relatedness. The other groups showed dispersed accessions. Figure 4.4 is a three dimensional presentation of cluster grouping of cassava accessions based on qualitative and quantitative morphological traits. The first three new variables were used as the x,y,z axes. Nine clusters were obtained from canonical discriminant analysis. The accessions in each cluster are in appendix II.

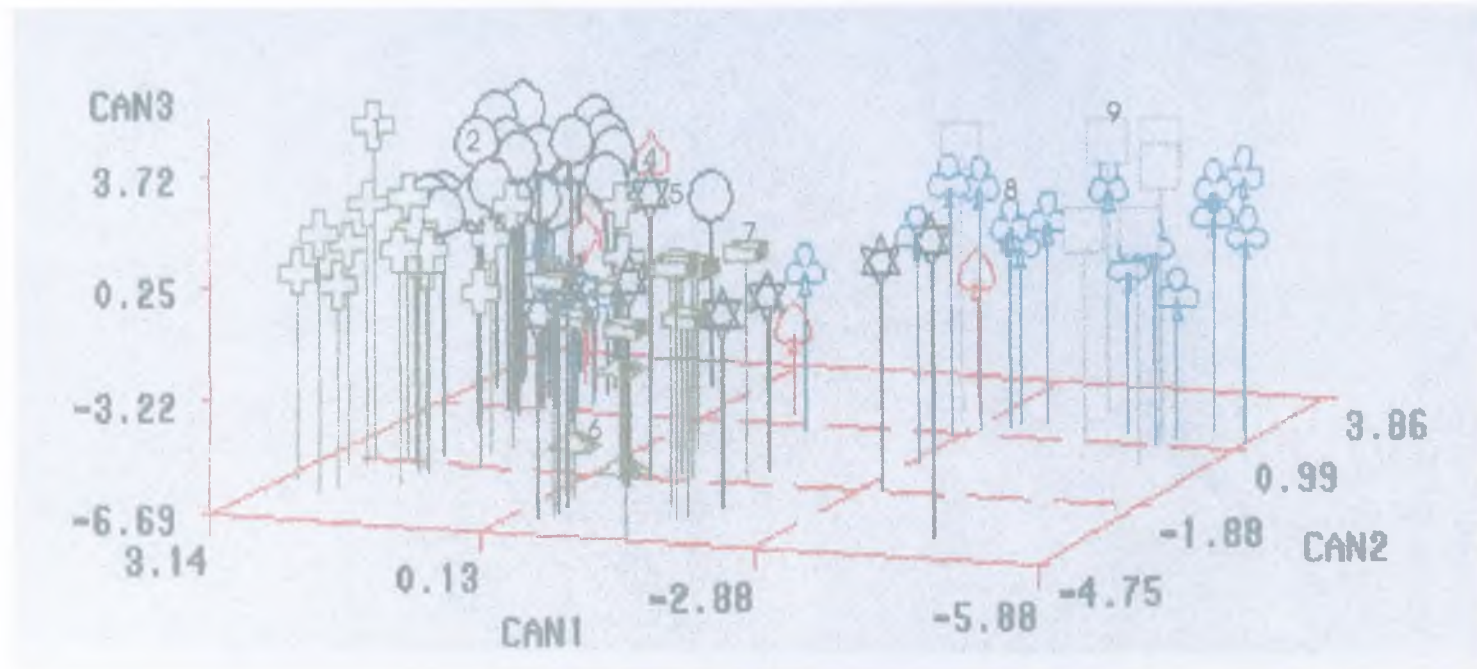


Fig 4.8 First three axes from canonical discriminant analysis of 100 accessions of cassava on qualitative traits.

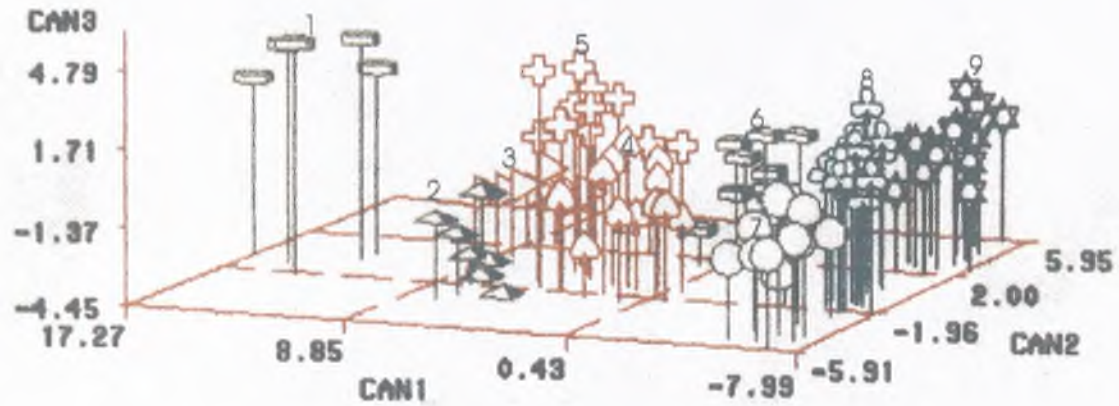


Fig. 4.9 First three axes from canonical discriminant analysis of 100 accessions of cassava on qualitative and quantitative traits.

4.4 Molecular Characterization

Eighty random oligonucleotide primers (Operon Technologies set) A,O,P,Q,R,S,J,K and E sets evaluated for the ability to prime PCR amplification of cassava genomic DNA. In a preliminary test, genomic DNA of two of the accessions, Jk90033 and Mc90021 were used as templates. Fifty-eight of the primers did not amplify the two DNA templates or resulted in only limited amplification that gave faint bands or smears. The remaining twenty-two primers were able to amplify the genomic DNA, giving scorable, reproducible RAPD amplification patterns with individual bands that stained intensely.

Ten out of the twenty-two primers were used for the study. Table 4.14 shows the sequence of the ten primers used and the results of the RAPDs. The ten primers detected polymorphism among the ninety-six cassava accessions used. Figures 4.5 and 4.6 show the banding patterns for primer OPJ20 and OPR9. In all, the ten primers used revealed a total of 63 clear and easily scorable bands with 59% polymorphic loci. The number of bands per primer ranged between 5 and 9.

Table 4.12. Ten oligonucleotide primers used for the RADP analysis of the cassava accessions showing the amplified and polymorphic fragments

Serial no	Operon code	Nucleotide Sequence	Total no. of RAPD loci	No of Polymorphic	No. of Monomorphic
1	OPJ-14	CACCCGGATG	8	4	4
2	OPJ-20	AAGCGGCCTC	9	5	4
3	OPJ-5	CTCCATGGGG	5	1	4
4	OPK-1	CATTCGAGCC	5	4	1
5	OPK-11	AATGCCCCAG	5	5	0
6	OPK-16	GAGCGTCGAA	5	4	1
7	OPQ-18	AGGCTGGGTG	8	3	5
8	OPR-2	CACAGCTGCC	6	2	4
9	OPR-9	TGAGCACGAG	6	4	2
10	OPS-18	CTGGCGAACT	6	5	1
			63	37 (59%)	26 (41%)

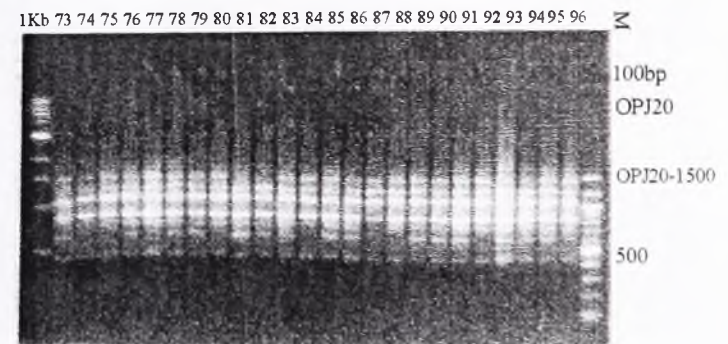
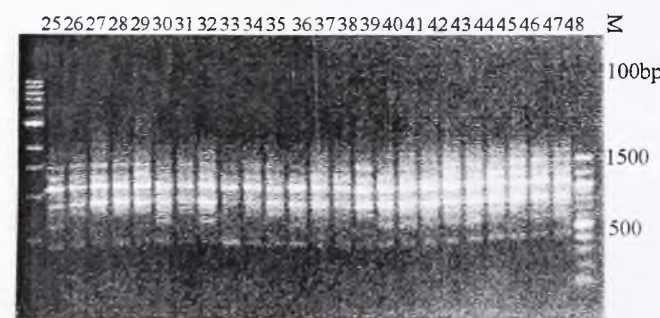
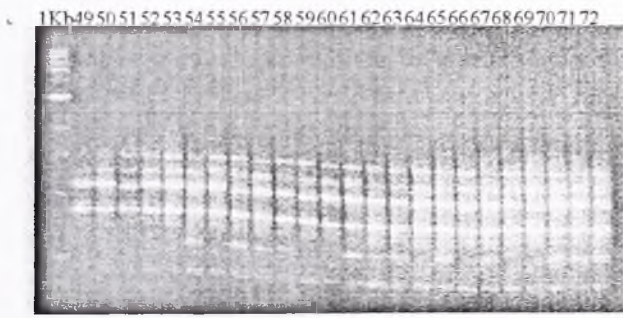
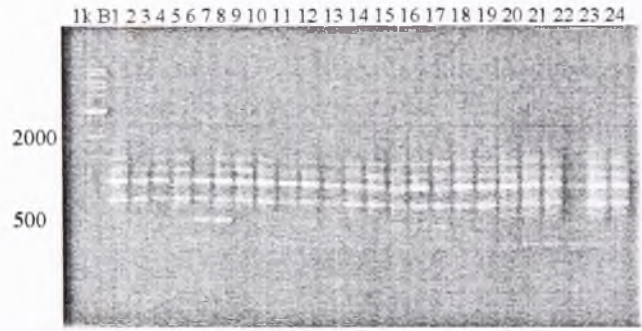


Fig. 4.10 Amplification products from RAPD analysis using primer OPJ20

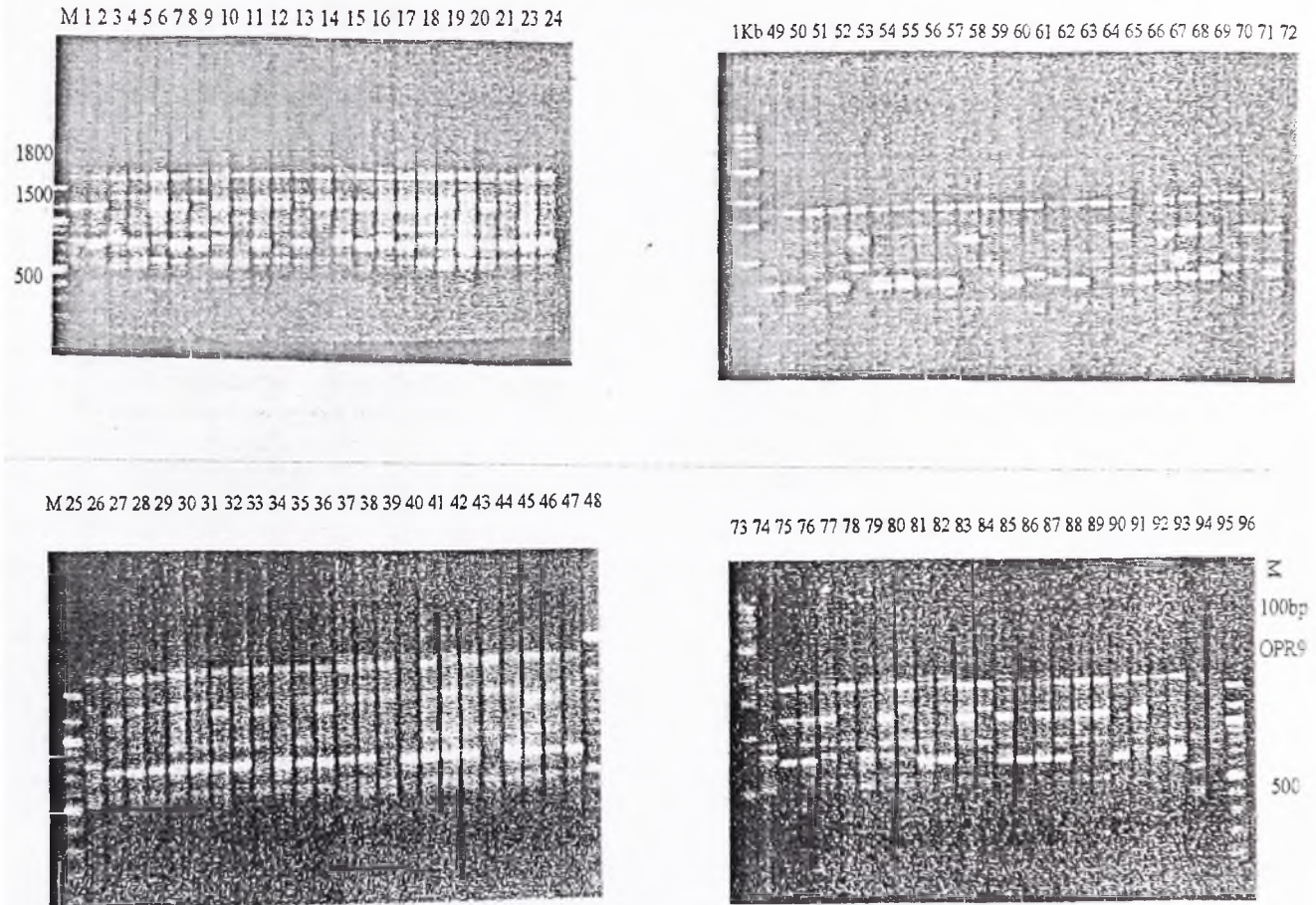


Fig. 4.11 Amplification products from RAPD analysis using primer OPR9

The accession Jk90033 (Nsawam) showed the highest polymorphic bands, Acw71 (Adroble) showed the least. The size of DNA bands that were produced in the PCR reaction ranged from 300 to 2000 (base pairs), but most of the bands were between 400 to 1500 (base pairs).

Figure 4.12 shows a dendrogram from the UPGMA analysis from the 96 cassava accessions. The accessions formed eight groups at the 60% Jaccard similarity coefficient level. There were no identical RAPD patterns (100% Jaccard similarity or zero dissimilarity coefficient). Jaccard similarity coefficient ranged from 87% for the most closely related accessions to 8% for those most distantly related. The distribution of accessions in relation to regions of collection and cluster groups is presented in Figure 4.13. The eight regions of collection are a representation of the agro-ecological zones. There was no definite relationship between the groups and the regions of collection.

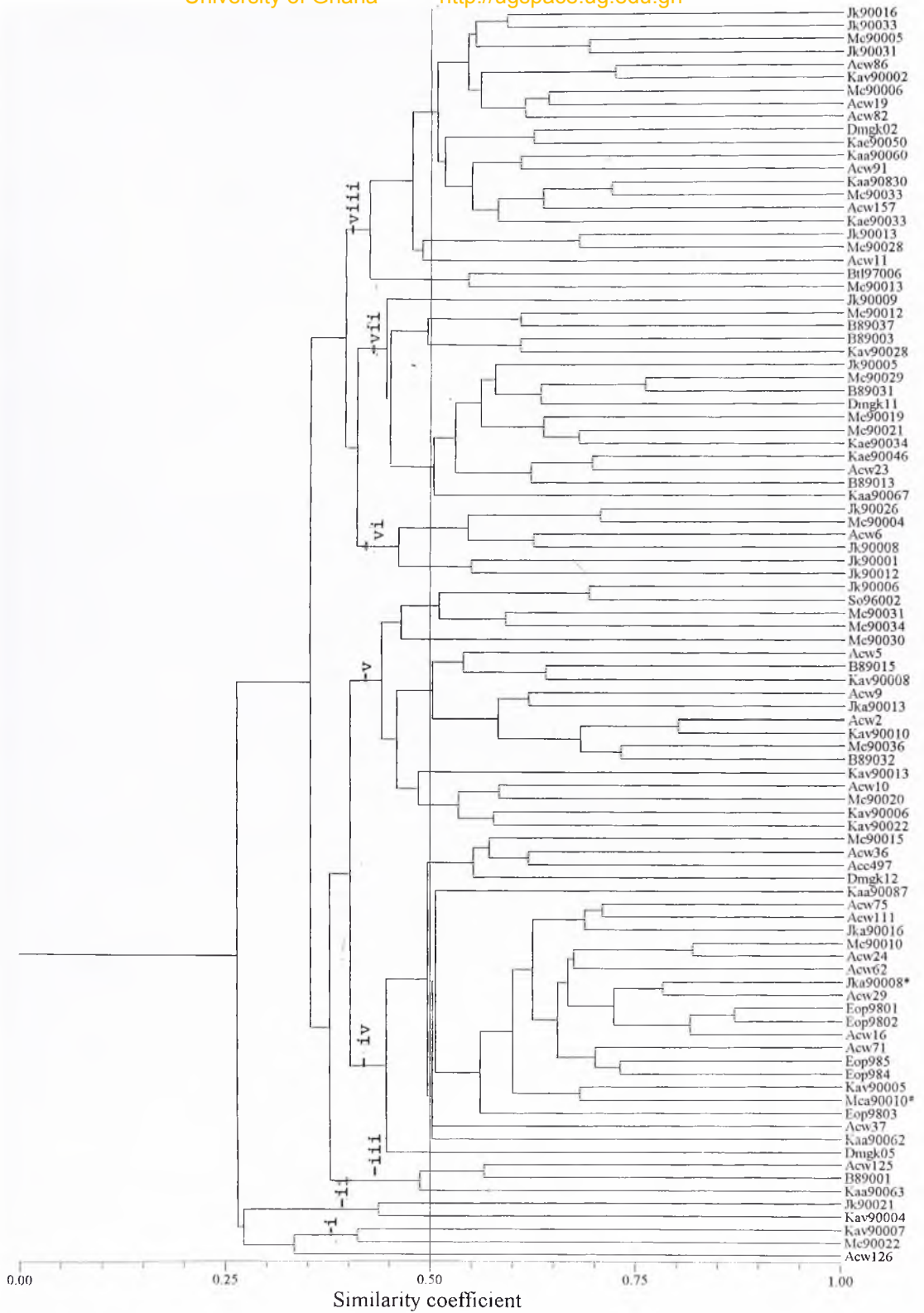


Figure 4.12 Dendrogram generated by UPGMA cluster analysis of pairwise distance data for 96 accessions of cassava using RAPDs.

A core collection of seventeen accessions could be selected based on morphological genetic variation in the accessions. Cluster groups and genetic distances are a better guide for this selection. The accessions were selected for conservation based on the polymorphism revealed by the molecular markers (RAPDs) used.

Table 4.13 List of core collection of cassava accessions for conservation

Clone	Name	Cluster	Meaning
Kav90007	Katawia	8	Umbrella/Canopy
Acw157	Ankrah	1	farmer's name
Eop9803	Katawire	5	Skin for covering
Eop9801	Yebesi	5	The god's plant (early maturing)
Acw5	Besrebema	4	Too good to part with
Kae90050	Bankyesono	1	Cassava as big as elephant
Dmgk11	Agric	2	Improved/exotic variety
Acw82	Adzo Congo	3	Farmer's name, and source
Kav90002	Ankrah	4	Farmer's name
Kaa90063	Akosua Tumtum	6	Dark traits
Kav90004	Trailasko	7	Locomotive smoke
Mc90034	Steer bekum driver	4	Plant branch like the steer of a car
Dmgk05	Bian bisa	5	Pays off debt
Kaa90062	Bankye bodee	5	Cassava with plantain colour
Kae90046	Bankye tumtum	2	Dark Traits
Jk90005	Akwada pe	2	Children like it
Acw19	Awona Congo	1	Ewe Congo

CHAPTER FIVE

5.0 DISCUSSION

5.1 Morphological variation and heritability estimates

Yield and colour characters gave the clearest indicators of intra-specific and inter-specific variation. In this respect accessions with similar quantitative characters appeared grouped in the three dimensional cluster and the derived dendrogram. However, qualitative descriptors showed little intra-specific variation. Luis *et al.*(1998), observed a similar trend on qualitative descriptors in the study on passionflora. The efficiency of the use of qualitative descriptors was very low. The subjectivity of such characters could be a factor for this observation.

The accessions from eight out of the ten regions of Ghana were distributed in six different clusters using the principal component axes. Both the qualitative and quantitative markers distributed accessions into cluster groupings with no unique cluster relationship with the regions. The eight regions covered the different agro-ecological zones in Ghana. The spatial distribution patterns of the accessions could be attributed to the ecological distribution. The genetic affinity observed between the accessions from different regions could however be due to the selection and exchange of cassava between farmers from different regions.

Cassava is a vegetative crop, and may have been selected by farmers based on characters that best fit their food culture. This has generated a large number of traditional vernacular (Mignouna *et al.*, 1998) names given to the same varieties according to ethnic groups. Accessions with common local names and duplicates from source were found in the same cluster. Principal component analysis clustering based on qualitative traits showed that cluster three had accession, Acw111 and Mc90013 (Bankye Santum), Acw75 and Acw5 (Besrebema), Jk90008 and Jka90008 (Manoa), Mc90010 and Mca90010 (Kweku Sam) being duplicates

sharing common local names. The accessions Acw91, Jk90016, Jka90016 all had a common local name (Steer bekum driver). The local name describes the plant architect, four level branching type that make the foliage resemble the steer of a car. Cluster five had accessions Eop8901 and Kae90033 (Bosom nsia) an early maturing type with common local name. A duplicate from source Jk90013 and Jka90013 (Abish) were in cluster six. Accession Acw157 and Kav90022 (Ankrah), a popular local variety was also found in the group.

Clustering based on quantitative traits also showed accessions with common local names in the same cluster. Accession Acw157 and Kav90022 were put in the same cluster as observed with the clustering based on qualitative traits. Duplicates from source such as (Manoa), also followed a similar trend. However, duplicates from source (Abish) and (Steer) in cluster V were separated into cluster IV and VI respectively. Common local names such as (Tuaka, Bianbasi, Ohyewkaw) (debt payer) in different dialects were found in cluster IV. Accession Acw24 and Acw75 (Besrebema-“too good to give out”) were in the same cluster with Acw111, Mc90019 (Bankye Santum-“potato-like cassava”). This poliferation of common local names has been discussed by other workers, to be the result of the distribution mode of cassava among farmers (Mignouna *et al.*, 1998). Accessions that are morphologically similar will be difficult to identify using only morphological characters.

The large ratios of genotypic to phenotypic variances (broad sense heritability) for length of stipules, plant height at harvest, number of fresh tuber and fresh tuber yield, indicate that, these characters are highly heritable and that differences between accessions are real. Genotypic influence on petiole colour and length of stipules has been established (IITA, 1989). Farmers use the length of stipules and petiole colour to select for healthy accessions with good cooking qualities.

Fresh tuber yield is a genetically controlled character, hence cassava improvement programmes have selected genotypes with high stable yields across locations. A study of the genotype by environment interaction and optimum resource allocation for yield and components of yields (Dixon and Nukenine, 2000) have selected genotypes with stable yields across agroecological zones. Morphological characterisation has been used effectively for the evaluation of cassava genotypes for tolerance for shade (Skreekumari *et al.*, 1988). Other useful morphological data-based studies have detected three morphological markers in cassava, namely leaf lobe shape, root surface colour and male sterility (CIAT 1993). In this study the root surface colour was highly heritable and showed positive correlation with the number of fresh tuber yield.

The morphological markers used for quantitative and qualitative traits as showed in the clusters I and IV accounted for maximum inter-cluster distance. These accessions would be expected to produce large variability in segregating generations (Sindhu, 1985). Most parents are usually believed to come from distant and distinct geographical regions. However, this may not be the situation in all cases. The direction of selection practiced in the different geographical locations may determine genetic divergence. Directional selection for desired economic traits could alter the genetic variation of the accessions.

In Ghana, farmers at different locations and with different farming systems may select for culinary qualities, plant architecture, plant height, petiole colour and yield. This suggests that parents for hybridization should be considered based on the magnitude of genetic divergence and not on geographical distances or differences. Correlation analysis revealed traits with joint variation. There was a significant correlation between the severity of CBB and ACMV in the accessions. Percentage plant establishment at four weeks after planting and plant height at harvest, showed a high positive correlation.

Percentage foliage retention and yield characters were positively correlated. Several experiments have confirmed the correlation between leaf area index (LAI) and yield in cassava (Doku, 1965).

There was a positive correlation between hydrogen cyanide content and the storage root diameter and length. Since in cassava, foliage and storage roots develop simultaneously, assimilates supply is thus divided between foliage and storage roots leading to an intense competition. Yet if too little assimilate goes into leaf growth, photosynthetic process is reduced and consequently yield will be low. There was a linear relationship between total dry weight and the dry weight of the storage roots, suggesting that the rate of root growth kept pace with the rate of crop growth (Osiru *et al.*, 1995). The correlation between the yield traits and percentage foliage retention in this study confirms this trend.

5.2 Genetic variation by RAPD Markers

The PCR-RAPD amplification analysis of the present study produced 63 loci, with 37 polymorphic loci (59%). Jaccard similarity coefficient ranged from 87% to 8% for the most distant accessions. Accession Eop9802 (Yebesi) and Eop9801 (Bosomnsia) both collected from Pokuase in the Greater Accra region were the most related showing 87% Jaccard similarity coefficient. The most distant accessions, with 8% similarity were Kav90004 (Trailasko) from the Volta region and Eop9803 (Katawire) from the Greater Accra region, of cluster II and IV respectively. The wide range of similarity revealed a large amount of polymorphism.

The 59 % polymorphic loci observed in this study was relatively higher than the 32% of the 78 bands realised in 28 cassava cultivars used in a similar study in the Republic of Togo (Tonukari *et al.*, 1997). The large size of the accessions from a wide range of geographical areas,



is very essential for genetic distance estimation (Nei,1978). Marmey *et al.*(1994), identified *Manihot* species and 19 cassava, collected from many countries using RAPDs. Laminski *et al.*, 1997 studied genetic variation in elite cassava cultivars in South Africa.

In this study, a combination of extensive regional collection of accessions, and the large sample size used made it possible to access a large number of loci. A large number of loci were analysed, which resulted in good estimates of the genetic variation in the accessions.

The accessions with common names, such as 'Ankrah', 'Steerbekum driver' and 'Bankye boodee' were four each in the collection. However, except for 'Bankye boodee' the set of accessions with common local names, were distributed in different clusters. The distribution of the accessions into the groups had no unique geographical relationship. Five of the accessions from the northern part of Ghana were put in separate clusters.

A core collection of seventeen accessions could be selected based on morphological genetic variation in the accessions. These will be kept for future breeding work.

CHAPTER SIX

6.0 CONCLUSION

Qualitative morphological variability in the accessions was detected mainly by petiole colour, fully expanded leaf colour and distribution of anthocyanin pigmentation. The fully expanded leaf colour gave the widest variability.

There was considerable variability in plant establishment, plant stand and height at harvest among the accessions. The plant height at first branching, number of nodes to first branching and leaf lobes also distinguished the accessions. Yield traits measured on storage root length, storage root diameter, weight and number of fresh tuber per plant, harvest index and percentage foliage retention showed significant variation in the accessions. The HCN content and severity scores of ACMV and CBB, also showed significant variability among the accessions.

Variability among accessions for plant establishment at four weeks after planting, number of leaf lobes, storage root length, plant height at harvest, number of fresh tuber and weight of fresh tuber were largely due to genetic effects.

Principal component analysis (PCA) using quantitative traits showed that number and weight of fresh tuber yield, number of leaf lobes and plant height at harvest, plant stand at harvest, number of nodes to first branching height at branching, storage root diameter and harvest index contributed immensely to observed variability among accessions. Qualitative traits used for PCA showed first fully expanded leaf colour, storage root surface colour and texture, length of stipules, and petiole length, storage root form and storage root position contributed greatly to the variation among the cassava accessions.

Genetic variability assessed by RAPD markers generated eight distinct clusters. In comparing the characterisation based on morphological and molecular markers, it was clear that one method complemented the other. RAPD markers showed more variability in the accessions than the morphological markers. There was no definite relationship between geographic diversity and genetic diversity. Seventeen accessions were selected and kept as a core collection based on RAPD and morphological markers.

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Appendix I**LIST OF REAGENTS**

Reagent	Manufacturer
Agarose	Sigma
Isopropanol	Sigma
Mineral oil	Sigma
CTAB	Sigma
Chloroform	Sigma
Phenol	Sigma
Ethanol	Sigma
PVP	Sigma
Taq Polymerase	Promega
DNTPs	Promega
Beta - Mercaptoethanol	Sigma
Ethidium bromide	Sigma
1-Kb ladder	Promega
Tris-EDTA Buffer	Fisher scientific
Primers	Operon
Isoamylalcohol	Sigma
Glacial Acetic acid	Sigma
DNA Ladder	Sigma

Appendix II

Table of accessions and their clusters as per canonical discriminant analysis using morphological qualitative and quantitative traits (Fig. 4.9)

Accession	Local Name	Cluster
Acw62	Nda-Aya	1
Jk90001	Appiah	1
Mc90015	Adjoa Beesiwa	1
Acw86	Kwesi Tutu	1
Acc497	Ohyewkaw	1
Mc90019	Santum	2
So96002	Banke	2
Dmgk11	Agric	2
Eop9803	Katawire	2
Eop9802	Yebesi	2
B89013	B89013	2
Kae90034	Agege	2
B98003	B98003	3
Mc90004	Tuaka	3
Acw11	Duafra	3
Kav90005	Aduse	3
Kav90013	Sokla	3
Eop9805	Afisiafi	4
Kav90004	Trailasko	4
Eop9804	Quarantine	4
Kae90050	Bankye Sono	4
Mc90021	Ketekewusie	4
Jka90013	Abish	4
Kav90008	Tuaka	4
Kav90002	Akpanya	4
Mc90036	Paafio	4
Mc90029	Bankye Bodee	4
Acw157	Ankrah	4
Acw19	Awona Congo	4
Kaa90060	Asona	4
Eop9801	Bosom Nsia	5
Kav90010	Kpenyivi	5
Acw2	Congo	5
B89032	B89032	5
B89015	B89015	5
Kae90033	Bosom Nsia	5
Mc90005	Ankrah Tuaka	5
Kaa90067	Betea	5
Kav90022	Ankrah	5
Jk90033	Nsawam	5

Dmgk12	Kplaso	5
Kaa90830	Kaa90830	6
Kav90006	Ankrah	6
Acw10	Tabu	6
Acw16	Agric Taboo	6
Mc90033	Afua Beyaw	6
Kaa90062	Bankye Bodee	6
Acw23	Nimme	6
Mc90006	Adimadin	6
Mc90034	Steer Bekum Driver	7
Kaa90063	Akosua Tuntum	7
Kae90046	Bankye Tuntum	7
Mc90031	Saakwa	7
Jk90021	Esi Panyin	7
Acw29	Neme	7
Jk90008	Manoa	7
Jk90022	Etibire	7
Acw107	Essabayem	7
Jk90006	Kofi Asem	7
Mc90030	Gari Bankye	7
Kaa90087	Basare Bankye	7
Jk90012	Aduane Asa	8
Jk90026	Okwadwofo	8
Acw5	Besrebema	8
B89031	B89031	8
Mc90020	Saakwanya	8
B89001	B89001	8
Acw61	Adakola	8
Mca90010	Kwaku Sam	8
Mc90010	Kwaku Sam	8
Mc90013	Bankye Santum	8
Jka90008	Manoa	8
B89029	B89029	8
Acw75	Besrebema	8
Acw37	Adwofuflule	8
Jk90005	Akwada pe	8
Acw111	Bankye Santum	8
Jk90009	Kasa fanti	8
Btl97006	Btl97006	8
Acw24	Besrebema	8
Acw9	Aduobile	8
Dmgk05	Bian Basi	8
Acw71	Adroble	8
Acw82	Adzo Congo	8
Mc90022	Gyanoa	9
Kav90007	Katawia	9

Dmgk02	Made in dozen	9
Acw125	Esi Panyin	9
B89037	B89037	9
Acw126	Kwesi Itu	9
Acw6	Kwesi Itu	9
Mc90012	Awona Bankye	9
Mc90028	Bankye Mpapro	9
Jk90013	Abish	9
Kav90028	Sakpa	9
Acw91	Steer Bekum Driver	9
Jka90016	Steer Bekum Driver	9
Acw36	Anounla Bede	9
Jk90031	Half Assin	9
Jk90016	Steer Bekum Driver	9

39	B89037	0.33	0.34	0.31	0.39	0.52	0.39	0.46	0.44	0.37	0.47	0.41	0.39	0.48	0.43	0.44	0.36	0.39	0.50	0.41	0.84	0.30	0.38	0.42	0.40	0.38	0.40	0.46	0.35	0.61	0.36	0.39	0.48	0.40	0.45	
40	Mc90021	0.48	0.40	0.41	0.45	0.52	0.46	0.38	0.31	0.43	0.52	0.41	0.40	0.33	0.43	0.40	0.37	0.35	0.39	0.38	0.42	0.38	0.43	0.32	0.36	0.29	0.46	0.63	0.31	0.60	0.37	0.46	0.43	0.41	0.61	
41	Kae90034	0.54	0.35	0.36	0.56	0.52	0.40	0.42	0.45	0.43	0.47	0.37	0.35	0.33	0.48	0.45	0.37	0.45	0.39	0.42	0.38	0.27	0.39	0.32	0.36	0.29	0.36	0.63	0.36	0.54	0.28	0.40	0.54	0.46	0.50	
42	Mc90029	0.63	0.39	0.39	0.59	0.55	0.48	0.45	0.43	0.37	0.45	0.45	0.39	0.41	0.57	0.54	0.41	0.48	0.48	0.36	0.45	0.30	0.33	0.46	0.34	0.23	0.39	0.50	0.35	0.46	0.36	0.33	0.52	0.39	0.53	
43	B89031	0.47	0.39	0.35	0.34	0.50	0.43	0.45	0.43	0.32	0.45	0.40	0.39	0.41	0.47	0.48	0.41	0.43	0.48	0.36	0.45	0.34	0.33	0.46	0.44	0.28	0.56	0.55	0.35	0.58	0.41	0.38	0.64	0.44	0.59	
44	Kae90046	0.43	0.39	0.35	0.39	0.46	0.44	0.32	0.30	0.42	0.38	0.46	0.39	0.28	0.54	0.44	0.36	0.39	0.33	0.32	0.37	0.30	0.38	0.42	0.25	0.32	0.46	0.46	0.41	0.37	0.42	0.33	0.37	0.40	0.40	
45	Dmgk11	0.46	0.26	0.35	0.22	0.37	0.44	0.28	0.30	0.37	0.42	0.36	0.30	0.32	0.43	0.44	0.36	0.44	0.38	0.32	0.32	0.35	0.29	0.36	0.30	0.22	0.40	0.37	0.41	0.37	0.42	0.39	0.42	0.35	0.62	
46	Acw5	0.38	0.26	0.27	0.26	0.32	0.30	0.32	0.22	0.23	0.22	0.31	0.26	0.19	0.33	0.34	0.21	0.26	0.33	0.24	0.28	0.25	0.29	0.42	0.21	0.27	0.35	0.37	0.29	0.37	0.31	0.28	0.37	0.40	0.40	
47	Acw23	0.43	0.35	0.41	0.40	0.52	0.45	0.38	0.40	0.38	0.42	0.37	0.35	0.33	0.59	0.50	0.42	0.45	0.44	0.38	0.42	0.36	0.39	0.54	0.36	0.33	0.46	0.52	0.42	0.43	0.42	0.35	0.54	0.52	0.50	
48	B89013	0.30	0.27	0.28	0.41	0.43	0.36	0.25	0.27	0.50	0.39	0.32	0.36	0.29	0.50	0.41	0.27	0.31	0.30	0.33	0.36	0.50	0.43	0.36	0.28	0.42	0.60	0.36	0.50	0.38	0.35	0.44	0.42	0.46		
49	Kav90004	0.20	0.21	0.23	0.16	0.15	0.26	0.19	0.21	0.29	0.21	0.27	0.21	0.23	0.15	0.21	0.14	0.16	0.18	0.11	0.15	0.19	0.25	0.20	0.25	0.44	0.25	0.24	0.31	0.29	0.33	0.29	0.35	0.32	0.28	
50	Kav90007	0.21	0.18	0.20	0.27	0.21	0.27	0.21	0.18	0.24	0.19	0.19	0.14	0.15	0.13	0.14	0.12	0.10	0.15	0.13	0.17	0.07	0.21	0.17	0.16	0.35	0.21	0.30	0.19	0.24	0.17	0.18	0.19	0.21	0.20	
51	Acw125	0.28	0.29	0.18	0.24	0.27	0.29	0.31	0.29	0.17	0.21	0.21	0.38	0.31	0.28	0.24	0.19	0.38	0.27	0.36	0.31	0.19	0.32	0.35	0.28	0.36	0.28	0.31	0.17	0.28	0.29	0.21	0.31	0.33	0.22	
52	Mc90022	0.42	0.27	0.20	0.32	0.30	0.32	0.35	0.38	0.29	0.27	0.28	0.18	0.19	0.26	0.27	0.22	0.27	0.25	0.25	0.25	0.12	0.21	0.27	0.21	0.29	0.21	0.30	0.32	0.24	0.22	0.18	0.35	0.26	0.29	
53	Kae90063	0.30	0.31	0.21	0.36	0.43	0.31	0.33	0.36	0.33	0.30	0.37	0.41	0.29	0.34	0.31	0.27	0.36	0.35	0.43	0.38	0.36	0.39	0.38	0.48	0.39	0.42	0.48	0.30	0.38	0.38	0.29	0.38	0.48	0.41	
54	Kav90028	0.38	0.39	0.45	0.44	0.37	0.39	0.52	0.56	0.42	0.47	0.41	0.34	0.42	0.43	0.50	0.42	0.50	0.44	0.32	0.41	0.21	0.38	0.31	0.30	0.38	0.46	0.46	0.48	0.48	0.36	0.61	0.46	0.35		
55	Acw126	0.29	0.17	0.23	0.30	0.23	0.21	0.28	0.30	0.27	0.21	0.17	0.21	0.27	0.20	0.21	0.15	0.21	0.28	0.19	0.19	0.11	0.24	0.20	0.24	0.26	0.19	0.42	0.17	0.32	0.15	0.27	0.38	0.29	0.31	
56	B89015	0.42	0.30	0.31	0.43	0.45	0.39	0.36	0.34	0.41	0.37	0.35	0.34	0.28	0.47	0.48	0.27	0.43	0.33	0.45	0.41	0.34	0.47	0.36	0.30	0.37	0.30	0.50	0.30	0.37	0.27	0.33	0.41	0.50	0.44	
57	Kav90013	0.35	0.21	0.29	0.37	0.30	0.28	0.30	0.32	0.34	0.31	0.29	0.24	0.26	0.35	0.28	0.24	0.32	0.23	0.19	0.30	0.28	0.35	0.29	0.19	0.40	0.23	0.43	0.32	0.34	0.24	0.26	0.30	0.32	0.33	
58	Acw10	0.38	0.34	0.20	0.44	0.32	0.30	0.37	0.30	0.37	0.33	0.31	0.39	0.32	0.38	0.39	0.26	0.34	0.29	0.32	0.32	0.35	0.43	0.36	0.30	0.32	0.25	0.46	0.41	0.42	0.31	0.23	0.42	0.30	0.40	
59	B89001	0.36	0.45	0.29	0.40	0.42	0.40	0.33	0.31	0.33	0.34	0.32	0.45	0.38	0.43	0.40	0.32	0.40	0.34	0.42	0.38	0.31	0.39	0.42	0.31	0.33	0.31	0.47	0.26	0.29	0.37	0.25	0.33	0.36	0.36	
60	Kav90008	0.41	0.29	0.30	0.33	0.31	0.29	0.35	0.29	0.31	0.32	0.34	0.29	0.23	0.28	0.33	0.21	0.33	0.32	0.31	0.27	0.33	0.41	0.30	0.29	0.31	0.29	0.45	0.23	0.31	0.25	0.38	0.36	0.38	0.43	
61	Mc90031	0.32	0.33	0.34	0.43	0.45	0.33	0.35	0.33	0.36	0.36	0.30	0.38	0.31	0.37	0.29	0.21	0.38	0.32	0.35	0.35	0.38	0.46	0.25	0.33	0.31	0.29	0.45	0.23	0.23	0.35	0.32	0.31	0.44	0.39	
62	Mc90034	0.36	0.32	0.25	0.54	0.56	0.42	0.34	0.32	0.35	0.35	0.24	0.32	0.25	0.46	0.37	0.23	0.42	0.36	0.39	0.34	0.32	0.36	0.28	0.32	0.29	0.22	0.63	0.32	0.30	0.28	0.36	0.40	0.50	0.43	
63	Kav90006	0.45	0.41	0.29	0.52	0.54	0.37	0.43	0.41	0.30	0.35	0.33	0.41	0.34	0.50	0.46	0.38	0.46	0.46	0.48	0.43	0.32	0.35	0.44	0.32	0.25	0.32	0.48	0.38	0.30	0.29	0.21	0.44	0.42	0.33	
64	Mc90020	0.35	0.37	0.26	0.46	0.39	0.41	0.39	0.32	0.30	0.28	0.29	0.41	0.26	0.35	0.32	0.24	0.37	0.27	0.26	0.28	0.28	0.35	0.29	0.28	0.40	0.32	0.54	0.43	0.44	0.44	0.36	0.44	0.37	0.42	
65	Kav90022	0.48	0.40	0.36	0.45	0.47	0.45	0.47	0.45	0.48	0.47	0.58	0.50	0.43	0.43	0.45	0.37	0.56	0.44	0.47	0.52	0.41	0.43	0.42	0.36	0.44	0.36	0.47	0.55	0.38	0.54	0.40	0.43	0.41	0.41	
66	Acw9	0.48	0.29	0.26	0.44	0.41	0.44	0.41	0.33	0.36	0.37	0.30	0.33	0.26	0.42	0.38	0.24	0.38	0.32	0.31	0.27	0.39	0.42	0.35	0.39	0.30	0.28	0.46	0.33	0.31	0.29	0.26	0.42	0.45	0.44	
67	Jka90013	0.46	0.29	0.34	0.48	0.40	0.38	0.50	0.48	0.41	0.41	0.34	0.33	0.31	0.41	0.43	0.35	0.48	0.37	0.40	0.40	0.33	0.37	0.35	0.33	0.26	0.29	0.40	0.39	0.36	0.30	0.27	0.41	0.50	0.34	
68	Acw2	0.45	0.38	0.31	0.42	0.39	0.42	0.39	0.33	0.35	0.40	0.30	0.42	0.35	0.50	0.47	0.34	0.47	0.37	0.39	0.39	0.43	0.41	0.44	0.44	0.33	0.31	0.33	0.48	0.38	0.40	0.44	0.37	0.45	0.48	0.52
69	Mc90030	0.46	0.33	0.34	0.48	0.45	0.54	0.50	0.43	0.41	0.45	0.39	0.29	0.31	0.46	0.43	0.40	0.54	0.42	0.35	0.29	0.32	0.30	0.33	0.26	0.38	0.50	0.39	0.41	0.30	0.32	0.41	0.44	0.54		
70	Kav90010	0.57	0.43	0.31	0.48	0.45	0.54	0.45	0.34	0.41	0.45	0.35	0.39	0.32	0.57	0.48	0.38	0.48	0.33	0.41	0.41	0.39	0.42	0.41	0.30	0.23	0.34	0.45	0.35	0.32	0.41	0.29	0.37	0.39	0.53	
71	Mc90036	0.48	0.41	0.37	0.36	0.38	0.41	0.47	0.41	0.34	0.39	0.42	0.36	0.34	0.44	0.45	0.38	0.45	0.40	0.34	0.38	0.32	0.31	0.43	0.32	0.26	0.41	0.42	0.37	0.34	0.43	0.31	0.48	0.41	0.45	
72	B89032	0.43	0.35	0.29	0.35	0.38	0.45	0.38	0.31	0.38	0.38	0.41	0.35	0.33	0.48	0.50	0.37	0.40	0.34	0.38	0.42	0.31	0.30	0.42	0.31	0.33	0.46	0.47	0.42	0.43	0.42	0.35	0.43	0.41	0.50	
73	Dmgk12	0.29	0.30	0.31	0.30	0.32	0.30	0.37	0.26	0.23	0.26	0.19	0.39	0.32	0.38	0.34	0.21	0.26	0.29	0.28	0.32	0.30	0.38	0.55	0.35	0.38	0.30	0.37	0.24	0.32	0.31	0.23	0.48	0.40	0.27	
74	Kae90087	0.26	0.27	0.24	0.41	0.43	0.31	0.33	0.23	0.33	0.30	0.19	0.31	0.24	0.39	0.27	0.27	0.27	0.25	0.38	0.33	0.31	0.39	0.43	0.26	0.28	0.17	0.43	0.15	0.29	0.18	0.19	0.33	0.36	0.32	
75	Acw37	0.19	0.35	0.29	0.40	0.38	0.24	0.33	0.27	0.29	0.27	0.24	0.31	0.29	0.34	0.35	0.28	0.24	0.34	0.29	0.29	0.27	0.43	0.37	0.38	0.29	0.31	0.52	0.13	0.33	0.19	0.25	0.43	0.36	0.32	
76	Acw36	0.30	0.31	0.24	0.41	0.38	0.31	0.43	0.31	0.29	0.30	0.19	0.38	0.																						

79	Jka90016	0.27	0.32	0.29	0.37	0.34	0.32	0.39	0.32	0.34	0.35	0.25	0.28	0.30	0.31	0.32	0.38	0.28	0.31	0.34	0.34	0.28	0.31	0.44	0.37	0.35	0.37	0.48	0.22	0.44	0.29	0.38	0.50	0.37	0.42
80	Mc90010	0.27	0.24	0.29	0.32	0.30	0.28	0.30	0.24	0.30	0.31	0.21	0.28	0.22	0.31	0.32	0.33	0.28	0.27	0.34	0.30	0.37	0.35	0.44	0.42	0.35	0.48	0.43	0.27	0.39	0.33	0.36	0.39	0.48	0.42
81	Kav90005	0.28	0.21	0.34	0.38	0.35	0.33	0.31	0.29	0.41	0.32	0.30	0.29	0.27	0.37	0.38	0.35	0.29	0.32	0.31	0.27	0.29	0.37	0.35	0.39	0.31	0.33	0.45	0.28	0.36	0.30	0.32	0.36	0.50	0.39
82	Jka90008*	0.35	0.28	0.38	0.41	0.39	0.37	0.34	0.28	0.39	0.39	0.29	0.32	0.26	0.40	0.41	0.38	0.32	0.36	0.39	0.34	0.37	0.40	0.44	0.37	0.30	0.42	0.48	0.32	0.39	0.38	0.42	0.39	0.48	0.47
83	Kaa90062	0.19	0.29	0.22	0.38	0.36	0.24	0.31	0.16	0.26	0.28	0.21	0.29	0.17	0.32	0.29	0.19	0.20	0.27	0.27	0.38	0.33	0.37	0.35	0.33	0.38	0.28	0.52	0.22	0.48	0.24	0.26	0.36	0.28	0.34
84	Mca90010*	0.18	0.26	0.27	0.30	0.28	0.26	0.28	0.18	0.23	0.22	0.19	0.30	0.23	0.25	0.30	0.21	0.22	0.33	0.28	0.24	0.21	0.29	0.31	0.35	0.27	0.30	0.41	0.19	0.37	0.28	0.28	0.37	0.40	0.31
85	Eop9801	0.31	0.32	0.29	0.32	0.34	0.32	0.39	0.28	0.30	0.35	0.25	0.32	0.28	0.31	0.32	0.33	0.28	0.31	0.34	0.34	0.32	0.31	0.50	0.32	0.40	0.37	0.39	0.32	0.39	0.38	0.38	0.44	0.37	0.38
86	Eop9802	0.32	0.33	0.38	0.33	0.35	0.33	0.44	0.33	0.35	0.40	0.34	0.33	0.31	0.32	0.38	0.34	0.33	0.41	0.35	0.39	0.29	0.38	0.44	0.29	0.36	0.38	0.39	0.33	0.40	0.39	0.37	0.45	0.38	0.38
87	Eop9803	0.21	0.30	0.27	0.34	0.41	0.26	0.28	0.18	0.28	0.29	0.23	0.30	0.19	0.33	0.30	0.26	0.22	0.33	0.32	0.28	0.30	0.38	0.42	0.30	0.22	0.30	0.37	0.19	0.23	0.28	0.19	0.28	0.30	0.27
88	Acw111	0.27	0.28	0.33	0.32	0.30	0.28	0.34	0.24	0.30	0.31	0.29	0.37	0.30	0.35	0.41	0.33	0.28	0.36	0.34	0.34	0.28	0.40	0.50	0.37	0.35	0.42	0.43	0.27	0.44	0.29	0.26	0.44	0.37	0.29
89	Acw16	0.36	0.29	0.38	0.38	0.39	0.42	0.35	0.29	0.35	0.40	0.30	0.29	0.24	0.38	0.38	0.34	0.33	0.32	0.35	0.35	0.29	0.32	0.39	0.29	0.36	0.38	0.44	0.38	0.35	0.44	0.42	0.40	0.38	0.42
90	Acw29	0.34	0.31	0.41	0.40	0.47	0.40	0.38	0.31	0.33	0.42	0.28	0.27	0.25	0.43	0.40	0.37	0.31	0.34	0.38	0.42	0.36	0.34	0.48	0.31	0.33	0.38	0.47	0.31	0.33	0.37	0.40	0.43	0.41	0.45
91	Acc497	0.30	0.36	0.37	0.41	0.43	0.36	0.43	0.31	0.38	0.39	0.32	0.48	0.38	0.50	0.46	0.38	0.31	0.40	0.33	0.43	0.36	0.44	0.57	0.36	0.39	0.48	0.43	0.36	0.44	0.38	0.29	0.44	0.48	0.28
92	Acw71	0.27	0.24	0.29	0.37	0.39	0.37	0.30	0.24	0.26	0.31	0.18	0.24	0.22	0.35	0.32	0.29	0.32	0.31	0.39	0.34	0.23	0.27	0.38	0.32	0.30	0.28	0.43	0.27	0.26	0.29	0.31	0.34	0.42	0.33
93	Eop685	0.26	0.24	0.29	0.31	0.38	0.31	0.38	0.31	0.29	0.34	0.24	0.27	0.29	0.39	0.35	0.32	0.35	0.34	0.38	0.42	0.31	0.30	0.48	0.38	0.29	0.36	0.42	0.26	0.33	0.28	0.30	0.43	0.41	0.36
94	Eop984	0.44	0.36	0.45	0.50	0.52	0.50	0.52	0.45	0.48	0.52	0.38	0.41	0.43	0.53	0.50	0.43	0.45	0.50	0.47	0.52	0.32	0.44	0.54	0.41	0.34	0.41	0.57	0.37	0.43	0.38	0.41	0.54	0.52	0.45
95	Acw24	0.35	0.24	0.33	0.41	0.39	0.37	0.34	0.28	0.39	0.35	0.25	0.32	0.26	0.40	0.37	0.29	0.28	0.31	0.34	0.34	0.37	0.45	0.50	0.42	0.40	0.37	0.48	0.32	0.39	0.33	0.36	0.39	0.54	0.42
96	Acw62	0.39	0.35	0.29	0.56	0.52	0.40	0.38	0.27	0.43	0.38	0.28	0.35	0.21	0.39	0.31	0.28	0.31	0.30	0.47	0.42	0.31	0.39	0.37	0.36	0.33	0.31	0.63	0.31	0.43	0.32	0.35	0.38	0.41	0.45

		35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68		
35	Acw6	1.00																																			
36	Kaa90067	0.34	1.00																																		
37	Mc90018	0.40	0.50	1.00																																	
38	Jk90008	0.63	0.50	0.41	1.00																																
39	B89037	0.44	0.44	0.40	0.50	1.00																															
40	Mc90021	0.39	0.45	0.67	0.45	0.44	1.00																														
41	Kaa90034	0.39	0.56	0.61	0.40	0.39	0.68	1.00																													
42	Mc90029	0.33	0.54	0.59	0.54	0.48	0.85	0.65	1.00																												
43	B89031	0.33	0.54	0.59	0.48	0.54	0.65	0.54	0.76	1.00																											
44	Kaa90048	0.29	0.50	0.56	0.44	0.38	0.50	0.44	0.60	0.48	1.00																										
45	Dmgk11	0.24	0.50	0.45	0.39	0.38	0.56	0.44	0.67	0.60	0.57	1.00																									
46	Acw5	0.38	0.39	0.50	0.44	0.29	0.39	0.39	0.54	0.43	0.50	0.44	1.00																								
47	Acw23	0.39	0.56	0.61	0.50	0.44	0.50	0.56	0.72	0.59	0.70	0.56	0.56	1.00																							
48	B89013	0.40	0.41	0.46	0.41	0.46	0.58	0.52	0.50	0.50	0.59	0.52	0.40	0.65	1.00																						
49	Kav90004	0.30	0.21	0.23	0.28	0.13	0.16	0.12	0.11	0.20	0.24	0.13	0.18	0.21	0.19	1.00																					
50	Kav90007	0.20	0.22	0.24	0.22	0.20	0.18	0.18	0.13	0.13	0.25	0.15	0.20	0.22	0.21	0.33	1.00																				
51	Acw125	0.32	0.44	0.30	0.33	0.27	0.20	0.24	0.23	0.28	0.22	0.18	0.18	0.29	0.23	0.15	0.29	1.00																			
52	Mc90022	0.25	0.32	0.33	0.32	0.25	0.18	0.32	0.31	0.28	0.30	0.25	0.25	0.32	0.21	0.25	0.41	0.35	1.00																		
53	Kaa90063	0.35	0.48	0.41	0.41	0.35	0.36	0.36	0.30	0.39	0.35	0.25	0.25	0.41	0.42	0.25	0.21	0.45	0.32	1.00																	

94	Eop984	0.40	0.45	0.50	0.45	0.45	0.50	0.50	0.53	0.53	0.50	0.45	0.45	0.61	0.52	0.19	0.33	0.39	0.33	0.41	0.45	0.46	0.48	0.47	0.35	0.45	0.48	0.43	0.48	0.52	0.42	0.55	0.50	0.54	0.57													
95	Acw24	0.36	0.41	0.42	0.41	0.31	0.41	0.41	0.40	0.40	0.46	0.36	0.52	0.58	0.54	0.27	0.39	0.35	0.33	0.42	0.31	0.42	0.66	0.48	0.41	0.41	0.50	0.44	0.44	0.43	0.48	0.41	0.59	0.50	0.59													
96	Acw62	0.39	0.40	0.41	0.40	0.34	0.45	0.45	0.34	0.34	0.39	0.30	0.39	0.40	0.46	0.16	0.32	0.38	0.32	0.46	0.26	0.40	0.54	0.41	0.44	0.45	0.48	0.46	0.54	0.46	0.52	0.50	0.50	0.48	0.57													
		69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96																			
69	Mc90030	1.00																																														
70	Kav90010	0.52	1.00																																													
71	Mc90036	0.48	0.70	1.00																																												
72	B89032	0.48	0.65	0.73	1.00																																											
73	Dmgk12	0.23	0.38	0.56	0.44	1.00																																										
74	Kaa90087	0.29	0.39	0.37	0.31	0.52	1.00																																									
75	Acw37	0.29	0.34	0.41	0.35	0.50	0.48	1.00																																								
76	Acw36	0.33	0.50	0.46	0.46	0.59	0.55	0.58	1.00																																							
77	Acw75	0.33	0.43	0.55	0.56	0.56	0.52	0.62	0.65	1.00																																						
78	Dmgk05	0.24	0.26	0.32	0.36	0.40	0.48	0.41	0.36	0.46	1.00																																					
79	Jka90016	0.34	0.40	0.47	0.46	0.46	0.54	0.64	0.61	0.71	0.42	1.00																																				
80	Mc90010	0.34	0.50	0.57	0.58	0.58	0.48	0.58	0.61	0.64	0.42	0.74	1.00																																			
81	Kav90005	0.36	0.46	0.48	0.48	0.42	0.57	0.48	0.50	0.67	0.57	0.56	0.63	1.00																																		
82	Jka90008*	0.39	0.56	0.52	0.62	0.46	0.54	0.46	0.54	0.64	0.54	0.60	0.74	0.77	1.00																																	
83	Kaa90062	0.26	0.28	0.30	0.38	0.43	0.45	0.50	0.45	0.50	0.39	0.52	0.52	0.48	0.52	1.00																																
84	Mca90010*	0.28	0.33	0.45	0.44	0.50	0.40	0.50	0.46	0.63	0.52	0.52	0.58	0.68	0.58	0.57	1.00																															
85	Eop9801	0.28	0.45	0.52	0.52	0.52	0.48	0.46	0.61	0.64	0.42	0.74	0.74	0.56	0.67	0.52	0.58	1.00																														
86	Eop9802	0.31	0.45	0.57	0.52	0.46	0.43	0.47	0.54	0.69	0.48	0.65	0.65	0.62	0.72	0.52	0.64	0.67	1.00																													
87	Eop9803	0.23	0.38	0.35	0.34	0.38	0.46	0.56	0.52	0.50	0.40	0.46	0.52	0.54	0.58	0.50	0.57	0.65	0.64	1.00																												
88	Acw111	0.30	0.35	0.52	0.58	0.58	0.46	0.52	0.54	0.71	0.54	0.67	0.67	0.63	0.60	0.59	0.65	0.67	0.65	0.52	1.00																											
89	Acw16	0.35	0.50	0.52	0.52	0.41	0.43	0.42	0.48	0.63	0.48	0.59	0.65	0.68	0.79	0.46	0.58	0.79	0.84	0.64	0.59	1.00																										
90	Acw29	0.33	0.43	0.45	0.45	0.50	0.58	0.50	0.58	0.62	0.46	0.64	0.64	0.60	0.78	0.57	0.44	0.71	0.69	0.63	0.58	0.76	1.00																									
91	Acc497	0.33	0.39	0.41	0.52	0.52	0.36	0.52	0.62	0.52	0.42	0.48	0.54	0.50	0.48	0.45	0.46	0.54	0.54	0.52	0.61	0.48	0.46	1.00																								
92	Acw71	0.34	0.40	0.47	0.48	0.46	0.54	0.41	0.54	0.58	0.42	0.54	0.60	0.63	0.60	0.40	0.58	0.67	0.59	0.58	0.60	0.72	0.64	0.42	1.00																							
93	Eop985	0.38	0.39	0.55	0.56	0.56	0.52	0.50	0.65	0.68	0.46	0.64	0.71	0.54	0.58	0.50	0.56	0.71	0.69	0.56	0.64	0.63	0.68	0.52	0.71	1.00																						
94	Eop984	0.54	0.53	0.60	0.61	0.50	0.52	0.50	0.64	0.67	0.46	0.63	0.63	0.65	0.69	0.39	0.50	0.63	0.68	0.50	0.63	0.68	0.67	0.64	0.69	0.73	1.00																					
95	Acw24	0.39	0.56	0.52	0.58	0.58	0.54	0.52	0.68	0.58	0.42	0.60	0.82	0.70	0.74	0.46	0.52	0.67	0.59	0.52	0.60	0.65	0.64	0.61	0.67	0.64	0.76	1.00																				
96	Acw62	0.38	0.54	0.45	0.50	0.39	0.58	0.45	0.52	0.56	0.46	0.58	0.64	0.67	0.71	0.57	0.56	0.64	0.63	0.56	0.52	0.69	0.62	0.46	0.64	0.56	0.67	0.71	1.00																			