GENETIC POLYMORPHISM OF CASSAVA (*Manihot esculenta* Crantz) LANDRACES COMMONLY GROWN BY RUVUMA REGION FARMERS USING SIMPLE SEQUENCE REPEAT (SSR) MARKERS

BY

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DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CROP SCIENCE OF SOKOINE UNIVERSITY OF AGRICULTURE.

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ABSTRACT

The study aimed at investigating the morphological and genetic relationships of cassava (*Manihot esculenta* Crantz) landraces grown in Ruvuma Region in Tanzania. Thirty nine cassava landraces were collected from farmers' fields in August, 2006. Purposive sampling was used to select study villages. The cluster analysis and similarity matrix was computed using morphological traits and Simple Sequence Repeat (SSR) markers based on Jaccard's coefficient following the unweighted pair group method with arithmetic averages (UPGMA) method using Sequential and Hierarchical Numeric option (SAHN) program of NTSY-pc. A set of 13 SSR primers out of 20 that were screened generated 75 (84.3%) polymorphic bands out of 89 amplified DNA bands. Morphological characterization, SSR allele diversity and association mapping were performed using 22 morphological characters and 13 selected SSR primers. The jaccard's coefficient for morphological traits and SSR markers varied from 0.53-0.91 and 0.33-0.88, respectively. The result obtained showed that use of morphological traits and SSR markers readily distinguished the 39 cassava landraces at 0.91 and 0.88 similarity coefficients, respectively. The results, summarized on both dendrograms, showed narrow genetic diversity among the cassava landraces. Further, the dendrogram showed inter and intra-cluster variation among the landraces used providing scope for its improvement through hybridization and selection. In both methods used (i.e. morphological and SSR techniques) no cassava duplicate was identified, the most morphologically closely related cassava landraces were *Sindani* and *Ndingiwaka* with similarity coefficient of 0.91. Similarly the most genetically closely related

cassava landraces were *Mama* and *Mahuhu* that had jaccard coefficient similarity of 0.88. The result from the two methods used in study indicated that only four landraces (i.e. *Pesangani, Chilabula, Mwaya* and *Kasindano*) were found to be unrelated both morphologically and genetically. Such findings indicate that these four cassava landraces could be included in National Cassava Improvement Programs (NCIP) in Tanzania.

DECLARATION

I, LIBERATUS DOMINICK, do hereby declare to Sokoine University of Agriculture that the work presented here is my own, and has not been submitted for a higher degree in any other training institute.

..... 23/09/2008

Liberatus Dominick — Date

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The declaration confirmed

..... 23/09/2008

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LIST OF ACRONYMS

µl:	microliter
AFLP:	amplified fragment length polymorphism
APS:	ammonium persulfate
ARI:	Agricultural Research Institute
bp:	base pair
cal:	calories
CBB:	cassava bacterial bright
CBSD:	cassava brown streak disease
CGM:	cassava green mites
CIAT:	International Centre for Tropical Agriculture
CMD:	cassava mosaic disease
COSCA:	collaborative study of cassava in Africa
CS:	cluster
DALDO :	district agricultural and livestock development officer
dATP:	2' deoxyadenosine 5' triphosphate
dCTP:	2' deoxycytidine 5' triphosphate
dGTP:	2' deoxyguanosine 5' triphosphate
DNA:	deoxyribonucleic acid
dNTP:	deoxyribonucleoside triphosphate
dTTP:	2' deoxythymidine 5' triphosphate
EDTA:	ethylenediamine tetraacetic acid
FAO:	Food and Agriculture Organization
g:	gram
GD:	genetic distance

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SSRs:	simple sequence repeats
Taq:	*thermus aquaticus*
TBE:	tris-borate EDTA
TE:	tris- EDTA
TEMED:	N, N, N', N'- tetramethylethylenediamine
Tris:	tris(hydroxymethyl)methylamine
UPGMA:	unweighted pair group method with arithmetic averages algorithm

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Cassava (*Manihot esculenta* Crantz) a root crop that belong to family *Euphorbiaceae,* and is the major source of carbohydrate food for millions of people in tropics. Cassava is produced mostly by smallholder farmers on marginal lands in the humid and subhumid tropics. It is efficient in carbohydrate production, adapted to wide range of environments, and tolerant to drought and acidic soils. An estimated 70 million people obtain more than 500 000cal per day from cassava; more than 500 million people in humid and subhumid tropics consume 100 000cal per day. In tropical Africa, cassava is cultivated mainly for its storage root, which is the most important source of calories in the diet. In other cases the storage root is used for making chips and flour. Both in developed and developing countries the crop is utilized for industrial purposes, in preparing starch flakes, and pearls, and in livestock industry, where it forms a component in animal feeds (Kawano, 2003; Hahn, 1989; Cock and Reyes, 1985).

The crop has gained economic importance by transformation from a rural subsistence staple to a cash crop. This situation has led to rapid increase in production and marketing of the crop and prompted it being given second priority in nation ranking of crops in Tanzania (Kapinga *et al.,* 2001; Nweke *et al.,* 2001). Cassava is reportedly the fourth major contributor to a dietary source of calories, superseded only by rice, maize and sugar within the tropics (Cock and Reyes, 1985).

An important feature of the crop is its adaptability to wide range of ecosystems. Some of the key characteristics are its ability to grow and produce well in impoverished soils, withstand attack by insect pests such as cassava mealybug (*Phenacoccus manihoti* Mat.-Fer) and cassava green mite (*Mononychellus tanajoa* Bondar). The crop is also tolerant to drought conditions, even though it thrives on fertile, sandy-clay soils (Cock and Reyes, 1985; FAO, 2000).

In Africa, with increasing population growth and declining soil fertility, cassava is one of the crops that can still be successfully grown under low soil fertility conditions. In Southern India and Java, with population increase, cassava production increased on low fertility lands, which were unsuitable for rice cultivation (Cock and Reyes, 1985). The facts along with the wide uses of the crop and its high flexibility with respect to the timing of planting and harvesting, cassava plays an essential role for food security, especially in those regions prone to drought and with poor soils, its production figures reflect its paramount importance (Hillocks *et al.*, 2001).

1.1.1 Cassava Production in Tanzania

Tanzania is among the top 10 largest cassava producers in the world (Table 1). Between 2001 and 2005 the country produced seven million metric tons of cassava fresh roots per year on an estimated 670 000ha of land (FAO, 2005). More than 84% of the total production was consumed as human food, about 15% as waste and the remaining was for livestock feed (Mtunda *et al.*, 2002).

Major cassava producing areas in Tanzania include: the coastal strip along the Indian Ocean (i.e. Tanga, Coast, Dar es Salaam, Lindi, and Mtwara), around Lake Victoria,

Lake Tanganyika and along the shores of Lake Nyasa. About 48% of the total production is produced in the coastal area, 23.7% of cassava comes from the Lake Zone, and 13.7% from Lake Nyasa areas and the remaining 14.6% being produced elsewhere. In the areas mentioned above, cassava is regarded as the first or second staple food (Mtunda *et al.,* 2002). In Tanzania marker assisted-selection (MAS) project seeks to improve local varieties for disease and pest resistance and provide a proof of concept for MAS paradigm in cassava breeding, but more importantly it is expected to transfer useful cassava variability from the crop's centre of diversity of cassava to Africa (Kulembeka, H. personal communication, 2008).

Table 1: Major cassava-producing countries worldwide

Country	Production (tons of fresh roots
Nigeria	41 565 000
Brazil	25 872 000
Indonesia	19 459 000
Thailand	16 938 000
Democratic Republic of Congo	14 974 000
Mozambique	11 458 000
Ghana	9 567 000
Tanzania	7 000 000
India	6 976 000
Uganda	5 756 000
Paraguay	4 785 000
China	15 700

Source: FAO (2005)

1.1.2 Production Constraints in Tanzania

In Tanzania, the estimated average cassava root yield is 10.45 tons per hectare (FAO, 2005) which is below the potential yield of the crop. The low yield is caused by a number of factors including biological, environmental and socio-economic.

1.1.2.1 Biological

Alongside its adaptability to wide range of climatic conditions, cassava faces serious challenges as a vegetatively propagated crop that is prone to genetic erosion as well as pest and virus infestations. The most important diseases affecting cassava production in Tanzania include cassava mosaic virus disease (CMVD), cassava brown streak virus disease (CBSD) and cassava bacterial blight (CBB). These diseases have been identified as the major biotic constraints to cassava production in Tanzania. Cassava mosaic virus causes an estimate of 50% root yield reductions (Hillocks *et al.*, 2001; Theiberge, 1985). The causal agents of CMD is *Geminiviruses* of the family *Geminiviridae* transmitted by the whitefly (*Bamisia tabaci* Gennadius). While *Ipomovirus* of the family *potyviridae* is the causal agents of CBSD and *Xanthomonas campestris pv manihoti* is the causative agent of CBB (Akparobi *et al.*, 1998). Whilst Cassava green mite (*Mononychellus tanaja* Bondar) and cassava mealybug (*phenococus manihot)* is the major arthropods pests (Yaninek, 1994). CBSD has been recorded to be endemic in all East African coastal cassava growing areas; its symptoms include foliar chlorosis and, sometime stem lesions. The disease also affects the tuberous roots which develop a yellow/brown, dry, corky necrosis within the starch bearing tissues, sometimes accompanied by pitting and distortion that is visible externally (Hillocks, 1997). Root necrosis accounts for the quantitative and qualitative reduction in total yield through the presence of necrotic lesions or discoloration of the root, rendering them unpalatable and non-marketable. Preliminary investigation in Tanzania showed an average yield loss of 34% due to CBSD (SARRNET, 1994).

Current research on cassava crop is to come up with landraces that produce root that can be harvested within 8-12 months after planting. According to collaborative study of cassava in Africa (COSCA) report of 1996, the late bulking of cassava is perceived to be one of the most important food security problems for subsistence farmers who sometime need to harvest earlier.

1.1.2.2 Environmental

In Tanzania cassava is grown under a wide range of agro-ecological conditions and in very diverse cropping systems (Masumba, 2006). The variations in temperature and rainfall favour the prevalence of viral diseases. For example CBSD which has been recorded as endemic in all East Africa coastal cassava growing areas, but restricted to low and medium altitudes below 1000m asl (Nichols,1950; Mtunda *et al.*, 2002).

Cassava grows in a wide range of soils but the crop is not well adapted to heavy soil, particularly soils with cracking clays such vertisols, which is not only suitable for growing cassava but also make harvesting extremely difficult. Gravelly or rocky soils are also not recommended for cassava production (Howeler, 1985). Similarly drought is also a growth limiting factor of the crop.

1.1.2.3 Socio-economic

Cassava farmers in marginal area often lack adequate postharvest facilities and essential infrastructure such as roads, means of communication and markets. These postharvest and market constrains hamper the development of cassava trade in tropics significantly, and often lead to the situation that any surplus beyond the immediate home consumption become waste. The market potential of cassava

remains largely underexploited in the tropics including Tanzania. The rapid postharvest deterioration of fresh cassava, as well as labour bottleneck in processing, are the major constraints of cassava commercialization (FAO, 2006). Additionally cassava is considered as food for impoverished households.

1.1.3 Cassava breeding

Plant breeding has one of the highest rates of return among the investments in agricultural research. It has been reported that the remarkable increase in the productivity of many crops during the twentieth century was due to genetic gains achieved trough crop breeding. Cassava has also benefited from technological inputs in the area of breeding (Kawano, 2003). New varieties in Africa, Asia, Latin America and the Caribbean satisfy the needs of farmers, processors, and consumers, bringing millions of dollars in additional income to small farmers. New technologies in the area of tissue culture, genetic transformation and molecular biology have also made positive contributions (DeVries and Toenniessen, 2001; Puonti-Kaerlas *et al.*, 1997).

1.1.3.1 Cassava breeding in Tanzania

Currently, the effort in cassava improvement in Tanzania are aimed at producing cultivars that are early maturing, resistant to insect pests and diseases, high yielding, low level of toxins and good quality root. In Tanzania cassava research is carried out under National Root and Tuber Crops Research Program. In the past the work was initiated at Amani (the East African Agriculture Research Institute) located in Muheza District in Tanga Region (Kapinga *et al.*, 2001). The institute was responsible for the improvement of the introduced landraces and did further

distribution of the planting materials. Some of the developed landraces include hybrids 46106/27, derivative of *Mahihot glaziovii*, and 5543/156, a cross made in 1955 between a first backcross derivative of *M. melanobasis* and fourth backcross of *M. glaziovii* as described by Jennings (2003), cited by Masumba (2006). In 1970s cassava improvement activities were transferred to Muguga, in Kenya under the East African Community. Later in 1974 the program was transferred to Ukiruguru research station in Mwanza, Tanzania (Kapinga *et al.*, 2001).

Recently, in Tanzania the effort has been embarked on the use of molecular markers in cassava improvement. The earliest genetic markers reported in Tanzania were based on the use of simple sequence repeat marker (SSR) and random amplified polymorphic deoxyribonucleic acid (RAPD) (Fregene *et al.*, 2003; Herzberg *et al.*, 2004).

1.2 Problem statement and justification

Based on importance of cassava worldwide, efforts are being made to develop genotypes with high resistance to diseases, insectpests and stable yield across different agro-ecologies. Success in these efforts depends on the availability of genetic variability. Some amounts of variation necessary for genetic improvement programme of cassava cultivars already exist within the several germplasm collections from different parts of origin and Africa (Beeching *et al.*, 1993).

In Tanzania, several studies on cassava molecular characterization using SSR markers have been conducted to fingerprint and study the genetic diversity. This is very important in cultivar identification for selection of parental materials in

breeding programs (Masumba, 2006; Fregene *et al.*, 2003 and Herzberg *et al.*, 2004). However, these studies based only in Southern zone (Mtwara and Lindi regions), Lake zone (Mwanza, Mara, Kagera, Tabora, Shinyanga, and Kigoma regions) and East zone which includes Morogoro, Coast and Dar-es-salaam regions, excluding Ruvuma Region which contribute significantly in cassava production in Tanzania as described in section 1.1.1 of this dissertation. It is vital for cassava breeding programs to have sufficient diversity available to allow for production of new varieties that are aimed towards the improvement of cassava productivity and able to withstand damage from biotic and abiotic factors. In this respect, efforts have also been made to predict the prospects of developing superior genotypes from a cross by the measuring genetic distance (GD) between the parents.

Historically, cassava around the Lake Nyasa was brought by farmers from different parts including nearby countries such Malawi and Democratic Republic of Congo. This evidence indicates that the area around Lake Nyasa is endowed by many cassava genotypes which have not been genotyped, and not included in cassava germplasm. This study aimed at determining the morphological and genetic diversity of cassava landraces in study area which will provide information to cassava breeders to maximize utilization of cassava landraces in the study areas. The use of SSR techniques, therefore, will provide quick evaluation of genetic diversity and hold promise for understanding the complexity of cultivar relationships as well as selection of desirable genotypes for crop improvement. Defining the genetic entities is crucial in breeding to identify and/or develop new cassava genotypes to be used as targets for efforts in producing high yielding landraces that meet the demands of farmers and consumers.

Several molecular markers have been used in cassava characterization. This includes Random amplified polymorphic DNA (RAPDs) (Tonukari *et al.*, 1997; Herzberg *et al.*, 2004) Amplified fragment length polymorphism (AFLP) and simple sequence repeats (SSR) (Moyib *et al.*, 2007; Raghu *et al.*, 2007; Masumba, 2006; Herzberg *et al.*, 2004 and Fregene *et al.*, 2003). In this study however, SSR marker was used because of their high level of polymorphism, high informative, highly available and cost effective, which make them particularly useful for genetic diversity studies.

1.3 Overall objective

The study aimed at conducting morphological and genetic characterization of cassava landraces commonly grown by farmers in Ruvuma Region using simple sequence repeats (SSRs) markers.

1.3.1 Specific objectives were to

i. Assess morphological and genetic characteristics of cassava landraces grown by farmers in the study area
ii. Determine the genetic relationships among the cassava landraces grown by farmers.
iii. Identify potential cassava landraces for incorporation in national cassava improvement programs.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Cassava: origin and distribution

Cassava, which is a shrub reaching 1-4m height, is commonly known as *tapioca* in India, *manioc*, *mandioca*, *yuca* in Southern America, *kaspe* in Indonesia and *muhogo(*plural *mihogo)* in kiswahili in East Africa. The crop belongs to the dicotyledon family *Euphorbiaceous*, subfamily *Crotonoideae* and tribe *Manihotae*. The *Manihot* genus is reported to have about 100 species, among which the only commercially cultivated one is *Manihot esculenta* Crantz (Alves, 2002). According to Olsen and Schaal (2001) and Theiberge (1985) the savannah of Colombia, Venezuela, Guatemala and Mexico had earlier been proposed as likely places of origin due to large number of landraces.

Wild accessions of cassava species grow over much of the American neotropics in Brazil, Bolivia, Peru, Venezuela and Guyana. Three are recognized, these are *M. esculenta* which is the domesticated subspecies and includes all cultivar known in agricultural production. The wild *M. esculenta peruviana* is most found in Eastern Peru and Western Brazil. The wild *M. esculenta flabellifolia* has a wider distribution that began in central Brazil all the way to Venezuela Amazon (Allem, 1994).

The exact origin of cassava is not known, but apparently it was first domesticated somewhere in Southern Brazil. Therefore, by the time the first Europeans reached the New World, the crop was already cultivated in all of neotropical America (Cock and Reyes, 1985). On arrival of the conquistadors from the old world, the crop was observed throughout the lowland tropics of the Americas and the Caribbean. The

introduction of cassava into Asia is not well documented. The Portuguese may have taken cassava to Gao in India in the early 18th century. At about the same time cassava was taken from Mexico to Indonesia and the Philippines. There is some evidence that it was then taken from Indonesia to Mauritius in mid 18th century. Later toward the end of 18th century and in the first half of the 19th century cassava was firmly established in South and South East Asia as both a staple in some areas and as a source of starch for export in others (Olsen and Schaal, 2001).

2.1.1. Cassava climate and soil requirements

2.1.1.1 Climate

Cassava is produced between 30° north and south latitudes, and near the equator up to an altitude of about 2 300masl. Because of the crop's tolerance to drought and low soil fertility, it is generally produced in marginal areas with poor soils and/or high risk of drought. The crop grow best in areas with a mean temperature of 25-29°C, and a soil temperature of about 30°C. The temperature below 10°C the plants stop growing. While the crop grows best in areas with an annual well distributed rainfall of 1000-1500 mm and tolerate semi-arid conditions with rainfall as low as 500mm, and may have a competitive advantage over other crops under those conditions (Onwueme and Sinha, 1991).

2.1.1.2 Soil requirements

Cassava can grow on a wide range of soils, but is best adapted to well- drained, light-texture, deep soils of intermediate fertility. Under high fertility conditions top growth may be stimulated at the expense of root growth. Optimum soil pH is

between 4.5 and 6.5. The crop does not grow well in heavy poor drained soils (such vertisol), gravelly or saline soils or in soils with a hardpan (Howeler, 1985).

2.1.2 Cassava introduction and distribution in Africa

With the development of trade between Africa and Brazil by the Portuguese sailors in the second half of the 16th century, through the western and eastern coast of Africa, cassava was introduced to the Congo River basin in the 16th century. In the 18th century the crop was introduced into Madagascar and the East Coast of Africa, from where it moved inland (Cock and Reyes, 1985). According to Jennings 1976 as cited by Ng and Ng (2002), the crop reached the East Africa from West Africa by Congolese farmers and through the eastern coast by Portuguese who settled in Zanzibar and Kilwa in Tanzania and Mombasa in Kenya.

2.1.3 Cassava introduction and distribution in Tanzania

In Tanzania cassava reached the Lake Tanganyika from West Africa by the Congolese farmers, from there cassava move inland Tanganyika through farmer to farmer diffusion (Carter *et al.*, 1992). Also spread of cassava in Tanzania was from the eastern coast through the trade route made by Portuguese sailors as described in section 2.1.2. Further spread was reinforced by the colonial administrators who encouraged farmers to grow cassava and increased cultivation in the late 19th and 20th century. Again its ability to survive in harsh conditions and viability of the cuttings facilitate the natural spread of the crop (Masumba, 2006).

Currently, in Tanzania cassava is grown almost in every area below 2000masl. These include areas around mountain Meru in Arusha, Kilimanjaro, Mbeya, Kagera and

central Tanzania regions, where until the mid 1970s cassava crop was regarded as less important crop.

The International Institute of Tropical Agriculture (IITA) has developed varieties resistance to cassava brown streak disease (CBSD) in collaboration with the National agriculture research in Tanzania. The challenge is now to replace the susceptible varieties with the newly released varieties. Currently in Zanzibar four varieties which are resistant to CBSD had been released by the Ministry of Agriculture Food Security and co-operatives these includes Kibaha (KBH) 517, KBH 482, KBH 477 and KBH 494 (IITA, 2007). Also some cassava landraces which have been tasted in different locations in southern zone had showed resistance to CMD and CBSD, these include Nachinyaya, Kiroba, Kitumbua and Naliendele 94 (Hillocks, 2001).

2.2 Morphological characteristics of cassava

According to International Plant Genetic Resource Institute (IPGRI) and Cornell University (2003) define the morphological characteristics as the observable and measurable characteristics of individual. These characteristics can easily be influenced by environments. These measures have the advantage of being readily available, do not require sophisticate equipment and are the most direct measure of phenotype. However, morphological determinations need to be taken by an expert in the species, they are subject to changes due to environmental factors and may vary at different developmental stages.

The morphological characteristics of cassava are highly variable, which indicate high degree of interspecific hybridization. Cassava landraces are usually characterized on

the basis of morphological and agronomic descriptors (Alves, 2002) but sometime the crop utility. Based on IPGRI as cited by Alves (2002) there are 75 cassava descriptors of which 54 are morphological and 21 are agronomic. Morphological descriptors (i.e., lobe shape, root pulp colour, stem external colour) have a higher heritability than agronomic character (such as root length, number of root per plant and root yield). Among morphological descriptors, the following are considered as the minimum or basic descriptors that should be taken in mind during identifying a cultivar. These are apical leaf colour, apical leaf pubescence, central lobe shape, petiole colour, stem cortex colour, stem external colour, root peduncle presence, root external colour, root cortex colour, root pulp colour, root epidermis texture and flowering characteristics (Allem, 2002).

2.2.1 Cassava root

Roots are the main storage organs in cassava. In plants propagated from true seeds a typical primary tap root system is developed, similar to dicotyledon species. Anatomically, cassava root is not a tuberous root, but a true root, which cannot be used for vegetative propagation. The mature cassava storage root has three distinct tissues: bark (periderm), peel or cortex and parenchyma (edible portion of root). Cassava root can be used in morphological characterization by looking its variability for example the external colour can be, white, cream, yellow, light brown or dark brown; cortex colour can be white, cream, yellow, pink or purple; parenchyma can be white, cream yellow or pink; epidermis texture can be smooth or rugose, root peduncle can be sessile, peduculate or both; root constriction can be none, little,

medium or many; root shape can be conical, conical-cylindrical, cylindrical or irregular as described by Fukuda and Guevara (1998) cited by Alves (2002).

2.2.2 Cassava stem

The mature stem is woody, cylindrical and formed by alternating nodes and internodes. On the nodes of the oldest parts of the stem, there are protuberances, which are the scars left by the plant's first leaves. A plant grown from stem cuttings can produce as many primary stems as there are viable buds on the cutting. In some cultivars with strong apical dominance, only one stem develops. The cassava plant has sympodial branching, the main stem(s) divide dichotomously, trichotomously or tetrachotomously. Produce secondary branches that produce other successive branching, these branching which are induced by flowering have been called 'reproductive branching' (Connor *et al.*, 1981).

2.2.3 Cassava leaves

Cassava leaf is formed by lamina and petiole. The leaf is lobed with palmated veins. There is generally uneven number of lobes ranging from three to nine. Cassava leaves can be used in morphological characterization by determining their variability, for example the apical leaf colour, apical pubescence, shape of central lobe, petiole colour, mature leaf colour and number of lobes (Irikura *et al.*, 1979).

2.2.4 Cassava flowers

Cassava is monoecious with a small number of pistillate flowers and a larger number of staminate flowers borne on the same inflorescence. Flowering is frequent and regular in some cultivars, while in others it is rare or non-existent. Cassava flower

are borne on terminal panicles, with the axis of the branch being continuous with that of the panicle inflorescence. The female flowers occur closer to the base, they have five petals and an ovary. The male flowers occur near the tip, they have 10 stamens and open after the female flowers have bloomed (Bueno, 1985). However, according to Cock *et al.* (1979) this characteristic is not among the basic constituent of the plant that is required for cassava yield.

2.2.5 Cassava fruits

After pollination and subsequent fertilization, the ovary develops into the young fruit, which takes 70 to 90 days to mature. The mature cassava fruit is a globular capsule (diameter 1 to 1.5cm), with six narrow longitudinal wings. The woody endocarp contains three locules, each with one seed. Practically however, seed set is normally less than three. When the fruit is dry, the endocarp splits explosively to release the seeds (Bueno, 1985). Farmers usually do not propagate cassava by the use of seed because produces thin and low number of roots. However, under natural conditions as well as plant breeding programs, seed propagation is common.

2.3 Cassava genetic characteristics

According to IPGRI and Cornell University (2003) define genetic diversity as the variation of genes within species, that is, the heritable variation within and between population of organisms. All variation exists in the sequence of the four base pairs (i.e., Adenine, Guanine, Cytosine and Thymine/Uracil) that compose the DNA molecule and constitute the genetic code. The genetic variation is the result of selection, mutation, migration, genetic drift and/or recombination, all this phenomena cause changes in gene and allele frequencies, leading to the evolution of populations.

Other kinds of genetic diversity can also be identified at all levels of organization in the nucleus, including the amount of DNA per cell, chromosome number and DNA structure.

2.3.1 Techniques of determining genetic diversity

Genetic markers have become fundamental tools for understanding the inheritance and diversity of natural variation. The earliest markers in cassava were morphological which based on shape and root colour, later on, eight morphological markers were discovered located on leaves and roots (Ocampo *et al.*, 1992). The second generation of markers were biochemical, such as isozymes, and they provided a useful tool for genetic 'fingerprinting' and the study of genetic diversity in cassava (Lefevre and Charrier, 1993a). Isozymes have been applied to characterizing relationships among accessions of African cassava germplasm and fingerprinting of the international cassava collection held at International Centre for Tropical Agriculture (CIAT) (Ocampo *et al.*, 1992).

The most prominent molecular marker systems include; minisatellites (Jeffreys *et al.*, 1985), restriction fragment length polymorphism (RFLP) (Botstein *et al.*, 1980), random amplified polymorphic DNAs (RAPDs) (Williams *et al.*, 1990), microsatellites, also known as simple sequence repeat markers (SSRs) (Litt and Lutty, 1989a,) and amplified fragment length polymorphisms (AFLPs) (Vos *et al.*, 1995).

Molecular characterization based mainly on deoxyribonucleic acid (DNA) molecular markers, has been very useful in evaluating the germplasm genetic diversity (Marcio

et al., 2004; Wang *et al.*, 2003; Beeching *et al.*, 1993). Latin America, which is a centre of origin for cassava, has been reported by Porto *et al.* (1991) to have generally a wide genetic diversity of the crop. Fregene *et al.* (2003) identified genetic variation and differentiation of cassava from both primary and secondary centre of cassava diversity using simple sequence repeats (SSR). These include some African countries i.e., Tanzania and Nigeria and Southern America countries i.e.,Brazil, Peru, Columbia, Venezuela, Guatemala, Mexico and Argentina. The result showed that cassava genetic diversity between the two centers of diversity is comparable. Such observations of cassava crop indicate to have a wide diversity in tropical countries such as Tanzania.

Karp *et al.* (1997) reported that, the estimation of the diversity of plant genetic resources has become more simple and reliable due to rapid advance of laboratory methods to perform genetic analysis. The most common applied techniques include isozyme polymorphism, restriction fragment length polymorphism (RFLP), and numerous marker assays based on polymerase chain reaction (PCR) such as random amplified polymorphic deoxyribonucleic acid (RAPD), simple sequence repeats (SSRs) and amplified fragment length polymorphism (AFLP). Fregene *et al.* (2003) reported that, there are possibilities of analyzing the genetic diversity of the cassava landraces using SSR marker system to identify the genetic identity and so identify duplicates. The method involves first isolation of DNA then amplification by PCR reactions. Fregene *et al.* (2000) reported that the use of RFLP markers was possible only to research centres that can afford the technology, thereby limiting the use of this technology to the research centres, which can not afford the technology. In attempt to make markers technology widely available in cassava, an effort was

embarked upon to place on the cassava map simple sequence repeat (SSR) markers. Markers that are (PCR)-based and highly polymorphic and best meet the criteria required for the transfer of the markers technology to research facilities in developing countries.

2.3.2 Simple sequence repeats (SSR) markers

Simple Sequence Repeats (SSR) markers are found in all eukaryotic genomes. They are short tandem repeat usually consists of 1-6 base pair (bp) of nucleotides. They were first referred to as microsatellites by Litt and Lutty (1989) and later as simple sequence repeats (SSR) by Jacob *et al.* (1991). Conserved region flanking the repeats are suitable designing PCR Primer pairs to be used for amplifying the intervening repeat loci. These loci are highly variable on account of number of repeat units found for each locus in any given population (Morgante and Oliveri, 1993).

The high level of heterozygosity, codominant and PCR-based nature of these repeat loci have made SSR the molecular markers of choice for genetic mapping and diversity studies (Gupta *et al.*, 1996; Wang *et al.*, 1994). Microsatellites are hypervariable and therefore able to distinguish among closely related plant cultivars (Davila *et al.*, 1998). The SSR markers had become powerful ideal tools for genotype assignment, marker assisted breeding, genetic mapping, and diversity assessment (Gupta and Varshney, 2000). Comparative studies in crop plants have shown that microsatellite markers are more variable than most other molecular markers. Recent studies have shown that SSR are conserved in related species, and may allow for the analysis of different species by the same microsatellite loci. The

SSR loci are randomly dispersed in the genome and suitable for wide comparisons (Morgante and Oliveri, 1983). Furthermore, SSR polymorphism can be detected easily and robustly by polymerase chain reaction (PCR) and DNA sequence.

According to Chavariaga-Aguirre *et al.* (1999) SSR markers were used to evaluate the genetic diversity of the core collection. About 600 accessions of the cassava world germplasm bank at the International Centre for Tropical Agriculture (CIAT) were studied. The results showed high levels of heterozygosity (up to 0.88) of the markers, revealed putative duplicates and indicated the unequal representation of cassava diversity, by country, in the core collection.

CHAPTER THREE

3.0 MATERIAL AND METHODS

3.1 Cassava plant materials

3.1.1 Collection of cassava landraces

Cassava stems used in this study were collected from farmers' fields in 17 villages in Ruvuma region as shown in Fig. 1:. In each district (Fig. 1) the District Agricultural and Livestock Development Officer (DALDO) assigned Agricultural extension office to survey the villages where cassava was commonly grown. In each village, the cassava farmers were randomly selected using the simple random sampling method. Sampling was restricted in the areas indicated in Figure 1, because the remaining areas such as Tunduru plain agro-ecological zone had already been worked on by other researchers such as Masumba (2006) and Fregene *et al.* (2003). Different cassava landraces were identified according to their morphological characteristics as well as the local names given to the landrace by farmers. The agro-ecological name and altitude (masl) were identified during sample collection as indicated in Table 2. Most of the samples were collected from the western part of the Region where cassava is widely cultivated. A total of 39 cassava landraces (Table 2) were collected, labeled, transported, planted and maintained at Agricultural Research Institute (ARI) Kibaha in the Coast Region.

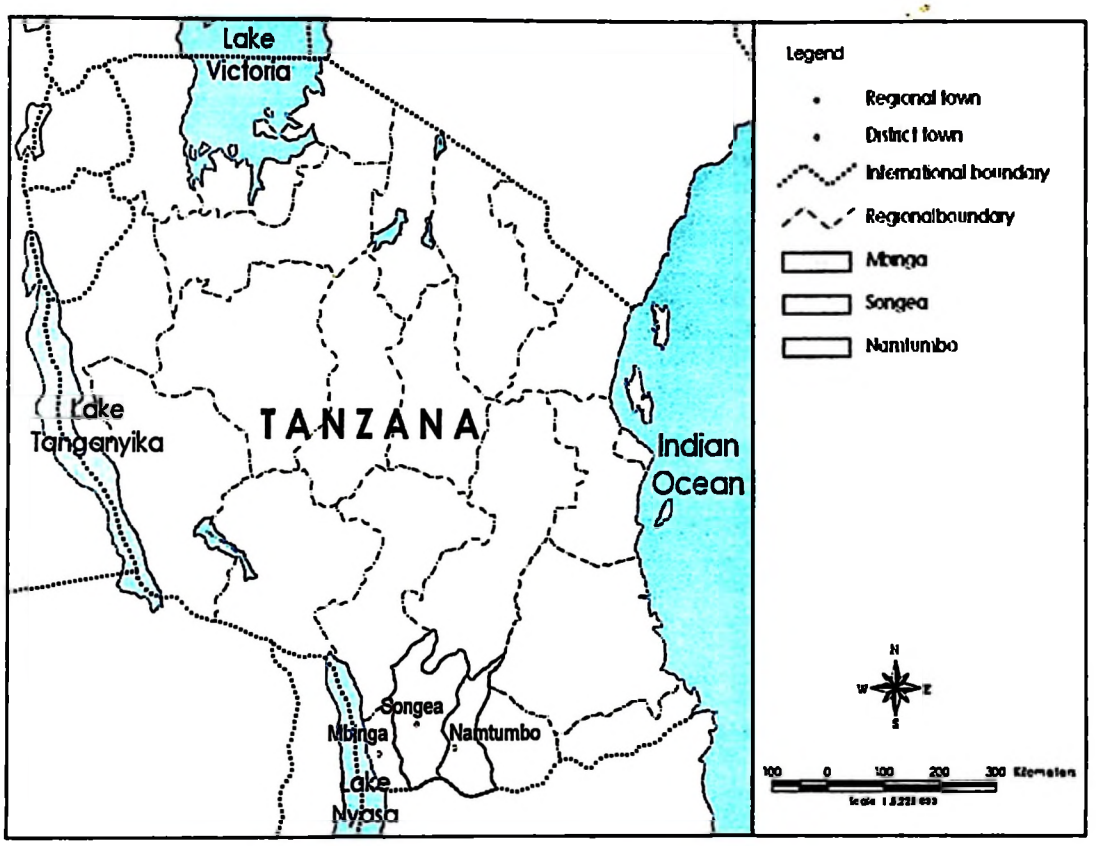

Figure 1. Map of Tanzania showing districts where cassava planting materials were collected

3.1.2 Establishment of plant materials in screen house

The growth media was made from forest soils (chemical characteristics not determined) and sterilized in barrel at 90°C. Two kilogram (kg) of sterilized soil was put in plastic pot (2litres) as a growth media. A stem of each cassava landrace was cut into three portions (cuttings), each cutting was 30cm long taken from the middle of the stem with approximately six nodes. The cuttings with three nodes were buried into the soil and the remaining nodes were left above the ground. Planting was done on 20 August, 2006 and kept in the screen-house at ARI-Kibaha. As there were no disease and pest incidence, crop maintenance was only by irrigation as no other control measures were undertaken. Three months after planting, four cassava young leaves per genotype were sampled and stored at -20 °C in deep freezer at Mikocheni laboratory for deoxyribonucleic acid (DNA) extraction.

Table 2: List of 39 cassava landraces collected from different villages in Ruvuma Region

Lab. No.	Landrace name	Village name	Agro-ecological zone
1	Pesangani	Ndengele	Lowland Nyasa
2	Kifuu cha nazi	Peramiho	Medium altitude(Songea urban)
3	Mkita	Uzena-mbinga	Mbinga Highland altitude
4	Sindani	Kikola	Lowland (Nyasa)
5	Kaendaenda	Chiulu	Lowland Nyasa
6	Kalimbe	Chiulu	Lowland Nyasa
7	Mbundamule	Saluti	Medium altitude Namtumbo
8	Kigoma	Kilosa	Lowland Nyasa
9	Meda	Kilosa	Lowland Nyasa
10	Gomani	Magagula	Medium altitude Songea rural
11	Mwaya	Mbambabay	Lowland Nyasa
12	Kanyela	Kipera	Mbinga Highland
13	Mwarabu	Ndengele	Lowland Nyasa
14	Eliza-cheupe	Mbambabay	Lowland Nyasa
15	Eliza	Mtipwili	Lowland Nyasa
16	Beatrice	Mtipwili	Lowland Nyasa
17	Beatrice-mkeka	Kilosa	Lowland Nyasa
18	Tupuka	Nyoni	Mbinga–Highland
19	Mtwara	Liuli	Lowland Nyasa
20	Mswaluka	Saluti	Medium altitude Namtumbo
21	Mtutuma	Uzena	Mbinga Highland
22	Waridi	Nyoni	Mbinga–Highland
23	Mkangawandu	Mikalanga	Mbing –Highland
24	Lepulepu	Kipera	Mbinga–Highland
25	Mama	Kipera	Mbinga–Highland
26	Mahuhu	Mkuka	Mbinga Highland
27	Mandunda	Mkuka	Mbinga Highland
28	Faraja	Mkuka	Mbinga Highland
29	Kasindano	Uzena	Mbinga Highland
30	Gomani-cheupe	Liuli	Lowland Nyasa
31	Mkawe	Kikola	Lowland Nyasa
32	Chilambula	Ndengele	Lowland Nyasa
33	Ndingiwaka	Chiulu	Lowland Nyasa
34	Unknown No. 1	Mbambabay	Lowland Nyasa
35	Unknown No. 2	Kilosa	Lowland Nyasa
36	Unknown No.3	Kilosa	Lowland Nyasa
37	Kaniki	Mkwaya	Mbinga Highland
38	Kandinginya	Chiulu	Lowland Nyasa
39	Nakalai	Mkwaya	Lowland Nyasa

Lowland Nyasa 560-800m asl

Medium altitude 900-1200m asl

Mbinga Highlands 1200-1800masl as described by de Pauw (1984)

3.2 Assessment of cassava morphological characteristics under field and screen-house conditions.

Morphological characteristics were obtained under two different conditions i.e., under field and screen house conditions. Under farmers' fields both above and below ground characteristics were recorded from all 39 landraces used in the study. These included root characteristics such outer skin colour, inner skin pulp colour, root shape, root constriction, root peduncle and the taste of root as described by Mahungu and Kanju (1997) and shown in Appendix i. Root taste was assessed by using individual farmers who owned the landraces from where the sample was collected. Above ground characteristics includes leaf shape, lobe shape, lamina colour, mature stem colour, immature stem colour and petiole colour. Farmers could not reveal exact age of each plant stand in their fields at the time of sampling. Under screen-house conditions the basic constituents of cassava plants that were: nodal units consisting of leaf blades, petioles, pubescence and internodes were observed at three months after planting.

3.3 Assessment of genetic variation of cassava landraces

3.3.1 Preparations

Fresh leaf tissue from single plants of each landrace was harvested and placed in a cool box the temperature was about-20 °C ready to be transported to ARI-Mikocheni for DNA extraction. Seventy percent (70%) ethanol was used as disinfectant to wash hands before harvesting. Motor and pestle were cleaned using 70% ethanol and rinsed with sterilized water.

3.3.2 Deoxyribonucleic acid (DNA) extraction

Total genomic DNA extraction was done as described by Dellaporta *et al.* (1983). However, some modifications to fit the prevailing conditions were done as follows: 15ml of Dellaporta extraction buffer (100ml of 1M Tris-HCL at pH 8.0; 100ml of 0.5M EDTA at pH 8.0; was used in DNA extraction. The resulting solution was incubated in -80 °C refrigerator over night for the purpose of extracting large quantity of DNA as a replacement for using 20 minutes which is stipulated by standard protocol. Additional step of phenol protocol was used with the following ratio Phenol: chloroform: isoamyl (25:24:1) for the purpose of purifying and increase the integrity of extracted DNA as shown in Appendix vii.

3.3.3 Deoxyribonucleic (DNA) quantification

According to Herzberg *et al.* (2004) 20-25ng of DNA concentration is required for cassava amplification. DNA Quantification was done with the aim of getting the right quantity (25ng) which was used in PCR amplification. The following standards were used to compare with DNA sample 100ng, 50ng and 25ng. The DNA sample of nine landraces was diluted with tris-ethyldimethlytetra acetate (TE) buffer in different concentration (i.e. 1:50, 1:25, and 1:10 dilutions). Both the standards (lambda) and DNA sample was electrophoresed on one gel tank. The band intensity of the DNA sample was compared with the standard bands. The dilution of 1:25 which is equivalent to 25ng of DNA was used throughout the experiment.

3.3.4 Primers screening and DNA amplification

The total of 20 primers were tested to amplify the isolated DNA (Appendix iii) and their composition has been established by Mba *et al.* (2001). For PCR amplifications, a 25 µl reaction mixture was used and it contained 16.6 distilled water, 3µl of genomic DNA, 0.4µl of primers (forward and reverse), 0.2µl of taq polymerase (Promega Madison WI USA), 2.0µl of 10X PCR buffer (Promega Madison WI USA), 0.8µl of $MgCl_2$ and 1.6µl of dNTP (deoxyribonucleoside triphosphate)(Appendix iv). Amplifications were performed in DNA amplification thermocycler (GeneAmp® PCR SYSTEM 9700, version 3.08). The apparatus was programmed to execute the following conditions: 95 °C for 5minutes (1 cycle), 94 °C for 30 seconds (denaturation), 55°C for 1minute (annealing), 72 °C for 1minute extension temperature) for 35 cycles. A final extension of 72 °C for 5 minutes was included as illustrated in Appendix v.

The primer screening results revealed 13 arbitrary oligonucleotides derived from commercial company (MWG-Biotech-AG). These primers were able to prime PCR amplification of all cassava genomic DNA. Seven out of 20 primers screened were unable to amplify the DNA samples. These include SSRY 100, SSRY 106, SSRY 64, SSRY 108, SSRY 96, SSRY 89 and SSRY 70. Primer pairs were initially screened for polymorphism in a set of four landraces (i.e., *Mkita, Mwaya, Kanyela* and *Mwarabu*). The results indicate that four primers selected (i.e. SSRY82, SSRY182, SSRY135 and SSRY69) produce clear and visible bands as described in Appendix vii.

To reduce the possibility of cross contamination in the amplification reactions, a master reaction mixture was routinely prepared and two controls were used. One consists of reaction mixture excluding DNA template and the second consists of reaction mixture and double distilled water excluding DNA template. The amplifications were performed several times and only reproducible products were taken into account for further data analysis. The amplification products were separated in 1.8% agarose gels in 1X TAE buffer and detected by staining with ethidium bromide (0.5µl) for two hours at 80V according to Sambrook *et al.* (1989).

3.3.5 Polyacrylamide gel (PAGE) analysis

Polyacrylamide gel analysis was done as described by Marcio *et al.* (2004). Again here reagent optimizations were made to fit the prevailing conditions as follows. The PCR products (Amplicons) were separated on 6% polyacrylamide sequencing gel (BIO-RAD sequencing sequi-Gen® GT), with the following composition: 12.44ml of Tris-Borate EDTA (10X TBE buffer), 17.72ml of Acrylamide solution, 18.66ml Bisacrylamide solution, 0.1g of ammonium persulfute (APS) and 125µl of N, N, N', N'-tetramethylethylenediamine (TEMED) run in 1 x TBE (pH 8.8) at 1000V, 90mA and 100W for three hours (Appendix vi). The amplified DNA was denatured at 95 °C for five minutes and 4µl of DNA was loaded per well after addition of sequencing loading buffer (formaldehyde), the gel was scanned using computer scanner, and the bands were scored with the aid of lighting box.

3.3.6 Silver staining

The Procedure used for silver staining was that of Sanguinetti *et al.* (1994). However, some modifications were done as follows: After removing the gel from

ear glass, the gel was soaked into 10% acetic acid to fix the gel; slightly shaking was applied for 30 minutes. Then the gel was soaked into double distilled water for 10 minutes to wash the acetic acid. The gel was again soaked into silver nitrate solution (0.1g/l of silver nitrate plus 1.5ml of formaldehyde) for 45 minutes to stain the bands, after staining; the gel was rinsed in clear single distilled water for 5 seconds. Then the gel was transferred into chilled developing solution (30g/l of sodium carbonate, 1.5ml of formaldehyde plus 8µl of sodium thiosulfate) and continued shaking until clear bands were visible. When all bands were visible, stop solution was applied (7.5% acetic acid) to stop further development of bands, which might have caused dark background to the glass. The gel was again transferred into distilled water to wash the remaining acetic acid.

3.4. Data collection and analysis

3.4.1 Morphological data

Morphological data were obtained from farmers' field during sample collections and in the screen house. Sixteen morphological characteristics recommended by IITA were analyzed according to Mahungu and Kanju (1997) as shown in Appendix i.

The morphological characteristics with their class numbers within parentheses used were: colour of unexpanded apical leaves (3), pubescence (2), leaf shape (2), petiole colour (3), colour of lamina (4) colour of mature leaf veins (5), shape of the leaf central lobe (5), immature stem colour (5), mature stem colour (4), root taste (2), root arrangement (5), outer skin colour (3), inner skin colour (3), storage root pulp colour (2), neck length (4), root shape (5).

All the traits were recorded according to the description list (Appendix i). Morphological data was transformed into binary data and the transformation was done as follows; for the traits which had 2 classes only like the shape of the leaves (1=narrow, 2= broad). These were scored in the normal binary matrix i.e. (1) was scored for narrow and (0) for broad. For traits with more than 2 classes such as colour and shape traits, the scoring considered the whole range of diversity of that trait, such as mature stem colour having four classes 1= silvery green, 2=light brown/orange, 3= dark brown and 4= reddish –red. In this case for a silvery green plant, (1) was scored for silvery green category and (0) for the rest of the three options. (Masumba, 2006).

3.4.2 Morphological data analysis

Before analysis the categorical variables were transformed into binary variables and standardized to 0 and 1 at complete disjunctive table (0 = absent and 1= present). Hence a two-way matrix (39 x 57 classes) was created in Microsoft excel program-2003 version.

3.4.3 Genetic diversity data analysis

Cassava genetic diversity data were obtained by scoring shared bands between pairs of landraces. Only the strongest bands were considered because light bands may have been stutter bands that resulted from slippage of the Tag polymerase during PCR (Wu and Tanksley, 1993). The binary code (1) designated the presence and (0) absence of a band at a specific location for each landrace. Missing values (e.g. resulting from a failed PCR reaction or faint (uncertain bands) were not considered. Both coded data of morphological and genetic diversity were computed and arranged

in similarity matrix on the basis of Jaccard's coefficient as described by Tonukari *at el.* (1997). The number of alleles and percentage of polymorphism were calculated for each locus and species.

The coefficient was calculated as: $JC_{ij}=a/(a+b+c)$

Where:

"a" is the number of fragments present in both landrace "i" and "j",

"b" the number of fragments present in "i" absent in "j"

"c" the number of fragments presents in "j" and absent in "i"

The cluster analysis based on the unweighted pair group method with arithmetic averages (UPGMA) was used by selecting Sequential and Hierarchical Numeric option (SAHN). The clustering was used to construct a dendrogram that revealed the relationship among landraces collected by selecting the tree plot option in the numerical taxonomy and multivariate analysis system software package (NTSYS-pc version 2.10*i*) as described by Rohlfs (2000). Furthermore, a three-dimensional scatter plot was generated on the basis of a principal component analysis using variance and covariance matrix for further investigating the relationship among landraces. The polymorphic information content (PIC) values that revealed the ability of each primer to distinguish the 39 cassava landraces were calculated for each of the 13 primers used. The formula used for calculation of the polymorphic information content is.

$PIC= 1-\sum n (Pi)^2$

Where n=the total number of alleles present in a primer

P= is the proportion of number of alleles present in genotypes

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1. Above-ground morphological characteristics

Although flowers and fruits are very important in the cassava breeding programs (Jennings and Iglesias, 2002), these reproductive characters were not sampled as cassava landraces in the study area take long time to mature (i.e. 1 ½ -2 ½ years from planting). Hence these characteristics were not used for morphological characterization. Again cassava flowering is highly influence by the environment, seed set and germination are low and it harbors high levels of heterogeneity and heterozygosity. Further, cassava has a relatively long generation time (Muhana and Mtunda, 2002). Moreover, these characters are not important in cassava yield and selection by farmers (Alves, 2002).

In this study different colours were recorded from different parts based on different growth stages (Appendix ii). The colours assessed were from petioles, young shoots, immature stems, lamina and mature stems as indicated in Plates 1, 2 and 3. For petioles, three colours were observed as indicated in Plate 1. Plate 1(a) indicates green purple petiole with light green lamina colour while Plate 1(b) shows greenish colouration with green lamina colour and lastly Plate 1(c) shows the purple petiole with green lamina colour. From the total sample used (n= 39) 18 (46%) were found to have green purple petiole colouration while 12 (31%) were found to be purple and lastly nine (23%) landraces were with green colour.

In all landraces assessed 19 (49%) were found to have light green lamina colouration, 14 (36%) landraces were greenish while five (13%) landraces were with green purple

colouration and lastly one (2%) landrace was with purple colouration. These results are similar to those documented by Onwueme (1982), who observed that in very young leaves, the lamina may be purplish or grey, but as it matures the leaf colour changes to light green or greenish colouration.

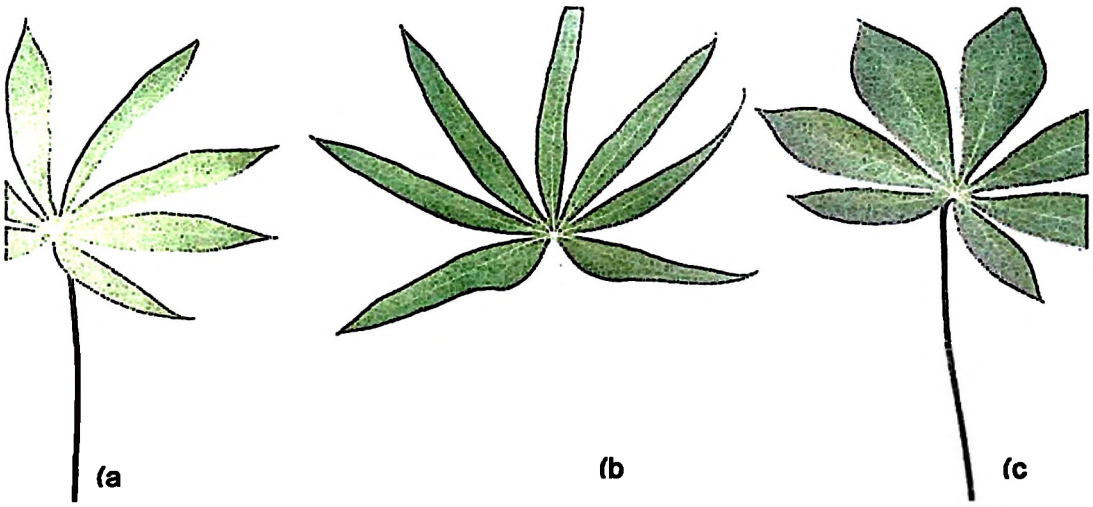

Plate 1: Petiole and leaf shapes (a) purple petiole with broad leaf (b) green petiole with narrow leaf (c) green purple petiole with broad leaf

As indicated in Plate 2, young shoot of cassava was also among morphological characteristic which was assessed during the study. Plate 2(a) shows the greenish while Plate 2(b) shows purple and lastly Plate 2(c) indicates green purple colouration. For the all landraces collected 16 (41%) landraces were found to be green purple while 13 (33%) landraces were purple and 10 (26%) landraces were with green colour.

Plate 2: **Cassava shoots showing different colouration (a) green (b) purple (c) green purple**

Twenty eight (72%) landraces assessed had green immature stem colour while nine (23%) landraces were of green purple and two (5%) landraces were with purple colouration. Silvery greenish colouration was found in 24 (62%) landraces, while six (15%) landraces were light brown and five (13%) landraces were of dark brown lastly reddish colour were found in four (10%) landraces. The finding shows that most of landraces assessed are relatively similar especially lamina colouration. However, as observed in this study the use of colour in morphological characterization of cassava sometimes may bring confusion because of their resemblance, different varieties may be classified/grouped together while they are quite different. This result concurs with Masumba (2006) who also did the cassava morphological characterization of cassava in Eastern and Western agro-ecological zones of Tanzania and Raghu *et al.* (2007) who did cassava morphological characterization in India. Other studies have indicated that sometime the colour of

cassava different parts is also influenced by the environmental changes as reported by Bayley (1983).

4.1.1 Leaf shape

Thirty one (80%) of the total landraces had broad leaves and only eight (20%) of the collected samples had narrow leaves. Additionally cassava plant used in the study area had different central leaf lobe shapes as shown in Plate 3 and Table 3. Nineteen (49%) landraces out of 39 had lanceolate lobe shape while 10 (25%) landraces had pandurate lobe shape in addition six (16%) landraces had oblanceolate while two (5%) landraces had elliptic and the remaining two (5%) landraces had linear lobe shape. Furthermore, the result shows that 25 (65%) landraces collected had hairy structure at young stage of growth while 14 (35%) landraces had no hair structures as indicated in Table 3.

In this study, cassava leaf shapes were strongly used in morphological characterization because they are not influenced by environment and they show typical variation of cultivar, rather than using colour of different parts of the plants which may sometime bring perplexity in identifying cultivars. Similarly Raghu *et al.* (2007) concluded that the use of leaf shape provided useful information regarding the variation of cassava landraces. Different types of shapes identified during the study are shown in Plates 3(a), 3(b), 3(c), 3(d) and 3(e) i.e., elliptic, oblanceolate, pandurate, lanceolate and linear respectively.

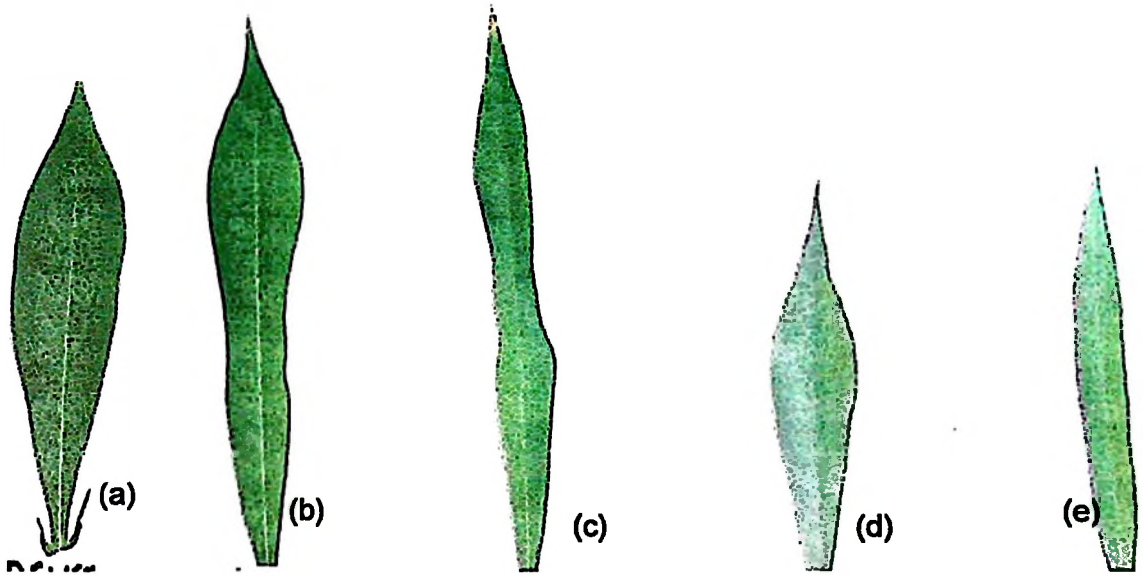

Plate3: **Cassava central lobe shapes: (a) elliptic (b) oblanceolate (c) pandurate (d) lanceolate (e) linear**

Table 3: Cassava leaf shape and pubescence

Landrace Name	Lobe shape	Leaf shape	Pubescence
Pesangani	Lanceolate	Narrow	Absent
Kifuu Cha Nazi	Pandurate	Narrow	Present
Mkita	Lanceolate	Broad	Absent
Sindani	Lanceolate	Broad	Present
Kaendaenda	Lanceolate	Broad	Absent
Kalimbe	Lanceolate	Broad	Present
Mbundamule	Lanceolate	Broad	Absent
Kigoma	Lanceolate	Broad	Absent
Meda	Pandurate	Broad	Present
Gomani	Lanceolate	Broad	Present
Mwaya	Pandurate	Broad	Absent
Kanyela	Lanceolate	Broad	Absent
Mwarabu	Lanceolate	Broad	Present
Eliza-Cheupe	Pandurate	Broad	Absent
Eliza	Pandurate	Narrow	Present
Beatrice	Oblanceolate	Narrow	Absent
Beatrice-Mkeka	Pandurate	Narrow	Present
Tupuka	Oblanceolate	Broad	Present
Mtwara	Pandurate	Narrow	Absent
Mswaluka	Oblanceolate	Broad	Absent
Mtutuma	Pandurate	Broad	Absent
Waridi	Lanceolate	Broad	Absent
Mkangawandu	Oblanceolate	Broad	Absent
Lepulepu	Lanceolate	Broad	Absent
Mama	Oblanceolate	Broad	Present
Mahuhu	Lanceolate	Broad	Present
Mandunda	Elliptic	Broad	Absent
Faraja	Lanceolate	Broad	Present
Kasindano	Lanceolate	Broad	Present
Gomani-Cheup	Lanceolate	Broad	Present
Mkawe	Elliptic	Broad	Present
Chilambula	Pandurate	Broad	Present
Ndingiwaka	Lanceolate	Broad	Absent
Unknown N0. 1	Linear	Broad	Present
Unknown No. 2	Lanceolate	Broad	Absent
Unknown No. 3	Lanceolate	Broad	Absent
Kaniki	Oblanceolate	Broad	Present
Kandinginya	Lanceolate	Broad	Present
Nakalai	Oblanceolate	Broad	Present

Lanceolate = 49%, pandurate = 25% oblanceolate = 16%, elliptic = 5% and linear = 5%; Broad leaves = 80%, narrow leaves = 20%; presence of pubescence = 65%, absence of pubescence =35%

4.2 Root characteristics

4.2.1 Root shape and colour

The root characteristics that were recorded are as shown in Plate 4 and Table 4. As shown in Plate 4 outer skin root colouration was mostly light brown. The roots were found to be either smooth (Plate 4(a)) or constricted (Plates 4(b) and (c)) while the pulp colour was mostly white/cream as shown in Plate 4(c). From the literature as described by Oti and Ene (1992) such characteristics are mostly affected by soil factors such as soil moisture and sometime soil texture. Other root characteristics which were assessed during field study included root taste, root outer skin colour, root inner skin colour, pulp colour and root shape as indicated in Plate 4.

From the total sample used 18 (46%) landraces were found to be light brown while 12 (31%) were white/cream and lastly nine (13%) landraces were with dark brown outer skin colouration. Thirty (77%) landraces were found to be white/cream while five (13%) landraces were found to be pink and four (10%) landraces were with yellow inner skin colouration. According to Oti and Ene (1992) white/cream coloured landraces usually contain high cyanide (HCN) contents. Such results give clear picture that most of landraces collected (77%) from the study area were bitter due to white/cream colouration of the inner skin. Twenty six (67%) landraces found to be white/cream pulp colouration, while 13 (33%) landraces were yellow in colour as indicated in Table 4.

(a) (b) (c) (d)

Plate 4: **Cassava different root characteristics (a) smooth shape (b) root constriction (c) white pulp colour (d) root constriction with dark brown colour**

4.3.2 Cassava root taste

According to farmers' tastes assessed during the field data collection, 77% of the cassava landraces sampled were found to be of bitter taste, while 23% were found to be sweet (Table 4). These results support the observation made earlier on effect of skin coloration as discussed in section 4.3.1 of this study. Again such characteristic could be influence by soil as described by Oti and Ene (1992) and Howeller (1985).

Table 4: Root characteristics assessed during the study

Landrace Name	Outer colour	Inner colour	Root shape	Root arrangement	Root taste[a]
Pesangani	dark brown	white/ cream	fusiform	Horizontal	bitter
KifuuCha Nazi	dark brown	white/cream	fusiform	Horizontal	sweet
Mkita	dark brown	white/cream	fusiform	Horizontal	bitter
Sindani	light brown	white/cream	irrgular	tending vertical	bitter
Kaendaenda	light brown	white/cream	fusiform	tending vertical	sweet
Kalimbe	light brown	white/cream	cylindrical	tending vertical	sweet
Mbundamule	white/cream	white/cream	fusiform	tending horizontal	bitter
Kigoma	white/cream	pink	irregular	Horizontal	sweet
Meda	white/cream	white/cream	cylindrical	tending horizontal	bitter
Gomani	light brown	white/cream	fusoform	tending horizontal	bitter
Mwaya	white/cream	white/cream	fusiform	tending horizontal	sweet
Kanyela	dark brown	white/cream	cylindrical	tending horizontal	sweet
Mwarabu	dark brown	pink	conical	Horizontal	sweet
Eliza-Cheupe	white/cream	white/cream	conical	Horizontal	bitter
Eliza	white/cream	white/cream	cylindrical	Horizontal	bitter
Beatrice	light brown	yellow	fusiform	tending horizontal	bitter
Beatrice-keka	white/cream	white/cream	conical	Horizontal	bitter
Tupuka	light brown	white/cream	fusiform	tending vertical	bitter
Mtwara	dark brown	white/cream	conical	tending horizontal	bitter
Mswaluka	light brown	yellow	fusiform	tending vertical	sweet
Mtutuma	light brown	yellow	cylindrical	Horizontal	bitter
Waridi	white cream	white/cream	fusiform	tending horizontal	bitter
Mkangawandu	dark brown	white/cream	irregular	Horizontal	bitter
Lepulepu	light brown	pink	cylindrical	Horizontal	bitter
Mama	light brown	white/cream	irregular	Horizontal	bitter
Mahuhu	white/cream	pink	fusiform	tending horizontal	bitter
Mandunda	light brown	yellow	conical	tending vertical	bitter
Faraja	light brown	pink	conical	tending horizontal	sweet
Kasindano	white/cream	white/cream	conical	tending horizontal	sweet
Gomani-Cheup	light brown	white/cream	conical	tending vertical	bitter
Mkawe	light brown	white/cream	conical	tending horizontal	biter
Chilambula	light brown	white/cream	fusiform	tending vertical	bitter
Ndingiwaka	light brown	white/cream	conical	tending vertical	bitter
Unknown.No 1	dark brown	white/cream	cylindrical	tending horizontal	bitter
Unknown.No.2	dark brown	white/cream	irregular	tending horizontal	bitter
Unknown No.3	light brown	white/cream	irregular	tending vertical	bitter
Kaniki	light brown	white/cream	fusiform	tending horizontal	bitter
Kandinginya	white/cream	white/cream	fusiform	tending horizontal	bitter
Nakalai	white/cream	white/cream	irregular	tending vertical	bitter

[a] **farmers taste during field data collection**

Outer skin colour: Light brown (46%), Dark brown 23%), White/cream 31%). **Inner skin colour:** White/cream (77%), Pink 13), Yellow (10%), **Root taste**: Bitter (77%), Sweet (23%), **Root shape**: fusiform (39%), cylindrical (23%), irregular (18%) Conical (20%), **Root arrangement:** Tending vertical (28%), Tending horizontal (41%), Horizontal (31).

4.3 Morphological relationships of cassava landraces

4.3.1 Morphological dendrogram composition

The results obtained after morphological data transformation into binary variables as indicated in section 3.4.1 of this study are shown in the phenetic dendrogram (Fig. 2). Phenetic dendrogram revealed four clusters at 0.53- 0.91 jaccard similarity level, indicating all landraces were morphologically closely related at that range. Jaccard's coefficient similarity showed that 0.53 was for most unrelated landraces while 0.91 for closer related landraces.

Further as indicated in Fig 2:, these results show that cluster one (SC1) comprised six landraces that were collected from Mbinga lowland agro-ecological zone and one landrace collected from Namtumbo-medium agro-ecological zone resulting into a total of seven landraces. These landraces include *Pesangani, Kigoma, Mkita, Kanyela, Mwarabu, Mkawe* and *Mbundamule.* As indicated in Appendix ii theses landraces were similar in morphological characteristics that had green petiole colour, broad leaves and lanceolate lobe shape except landrace *Mkawe* which had elliptic lobe shape. These cassava landraces had their similarity level ranging between 0.74-0.85 (Table 5).

Cluster two (CS2) was the largest group with 28 landraces (72%) of the total landraces collected (Fig.2). All these landraces were collected from Mbinga lowland and highland agro-ecological zone except *Mswaluko* which was collected from Namtumbo -medium agro-ecological zone.

Cluster three (CS 3) encompassed of three landraces (i.e. *Mkangawandu, Unknown No.1, Unknown No.2*) while cluster four (CS 4) had *Kifuu cha nazi*. These landraces were all found to be bitter in taste, dark brown outer skin and white/cream inner skin colour plus irregular root shape. *Mkangawandu* was easily identified by farmers because of its height that was around 4.9m and its bitterness. According to village extension officer this landrace *(Mkangawandu)* has even caused neural disease (locally known as *Konzo*) to 22 people at *Kipera* village in Mbinga District. As reported by Kawano (2003) working at the International Institute of Tropical Agriculture (IITA) this disease is very serious only to malnourished people those lack essential sulfur-rich amino acids that allow for cyanide detoxification in their bodies.

Cassava landraces referred to as *Unknown No.1, Unknown No.2* and *Kifuu cha nazi* were more interesting because of their reddish stem colour, deep purple petiole colour and purple colour of young shoot. However, the landraces *Kifuu cha nazi* collected from medium agro-ecological zone differed considerably from all the others, having only 0.53 similarities with *Lepulepu* and *Unknown no. 3*. Then again cluster 3 (*Mkangawandu, Unknown no.1* and *Unknown no.2*) differ considerably morphologically with all other clusters and their genetic relationship with other landraces are presented in Table 5. The majority of the landraces in cluster 2 are closer morphologically related, however, in the cluster 2 the landraces are sub-divided into 5 sub-cluster, these results show that even if the landraces are closer morphologically related there is degree of variation between them. Even though the similarity among vegetatively propagated crop is not uncommon as pointed out by

other researchers who worked on sweet potato and cassava (Zhang *et al.*, 2001; Tonukari *et al.*, 1997).

Jaccard coefficient similarity level as shown in Table 5 indicate that majority of the landraces ranged between 0.74-0.88, suggesting closer relation within and between clusters. The similarity between landrace *Lepulepu* and landrace *Kifuu cha nazi* was 0.53 while the similarity between landrace *Unkonown* No.1 and landraces *Mbundamule, Mwaya,* and *Kasindano* was 0.53. The results presented on the phenetic dendrogram indicate only two landraces (*Sindani* and *Ndingiwaka*) were found to be 0.91 morphologically closer related.

Morphological traits are useful tool for preliminary evaluation, because they provide a fast and useful approach for assessing the extent of diversity. The estimation of descriptive statistics of 22 different morphological traits studied in this work however, revealed narrow morphological diversity among 39 landraces collected. Moreover no duplicate were identified indicating each genotype stand in its own. This result provides scope for improvement through hybridization and selection. The results also concur with the report made by Tonukari *et al.* (1997) who studied the genetic diversity of cassava using RAPD marker who also identified low genetic diversity among cassava accession collected from Benin Republic. However, this finding was in disagreement with studies reported by Raghu *et al.* (2007) and Calvalho *et al.* (2001) identified the high degree of genetic variability among cassava landrace in India and Brazil respectively. The reason for this inconsistency is due to fact that Asia and Latin America were confirmed to be the origin of cassava (Alves, 2002) hence there greater variation among cassava landraces compared to Africa.

The morphological traits that showed maximum variations among 39 landraces after conducting descriptive statistics using Statistical Package For Social Science (SPSS) includes tuber attachment on parent cuttings, tuber inner skin colour, central leaf lobe shape , root shape, tuber outer skin colour and pulp colour, the rest of the traits showed resemblance to each other. These results are in agreement with study done by Raghu *et al.* (2007) in morphological characterization of cassava done in India.

In cluster analysis the similarity matrix was computed using morphological markers and SSR markers based on Jaccard's coefficient following the UPGMA method using Sequential and Hierarchical Numeric option (SAHN) program of NTSYS-pc version 2.02*i*. The jaccard's coefficient for morphological data and SSR data varied from 0.53-0.91 and 0.33-0.88, respectively (Fig. 2 and 3). This indicated that the morphological diversity of the collected landraces is lower than that observed in SSR techniques. Raghu *et al.* (2007) also reported the same in India. This Might be due to high polymorphic information content (PIC) showed by SSR marker used in this study (Table 6). This might also stem from the fact that some of the landraces had similar morphological traits for example lamina colour which is green to all landraces. Again Moyib *et al.* (2007) reported high polymorphic information content of SSR markers used to identify relationships among cassava landraces in Nigeria. Based on morphological data, 39 cassava landraces were found to be grouped into 4 main clusters at 0.70 similarity. This similarity is in agreement with the finding reported by Moyib *et al.* (2007). The clusters obtained varied in size from 1-28, cluster two was found to be comprised 28 landraces while cluster one contains seven landraces. The landraces *Mkangawandu, Unknown No.1* and *Unknown No.2* (CS3) having unique characters like bitter taste, broad leaves, purple young leaf, purple

petiole colour, erect bush type and tallness character could have made these landraces to fall into one cluster. *Kifuu cha nazi* (CS4), which is having unique characters like reddish stem colour, deep purple petiole colour, purple colour of young shoot, spreading canopy habit and lack of root peduncle which is present to few landraces, that could have been the reason for forming a different cluster. Similarly Tonukuri *et al.* (1997) and Herzberg *et al.* (2004) reported low degree of genetic distance in Republic of Benin and Tanzania respectively. However, Fregene *et al.* (2000) analyzed the genetic relationship between African and Latin America accessions using morphological traits and AFLP markers and found high genetic diversity among accessions, the reason might be due to different techniques used.

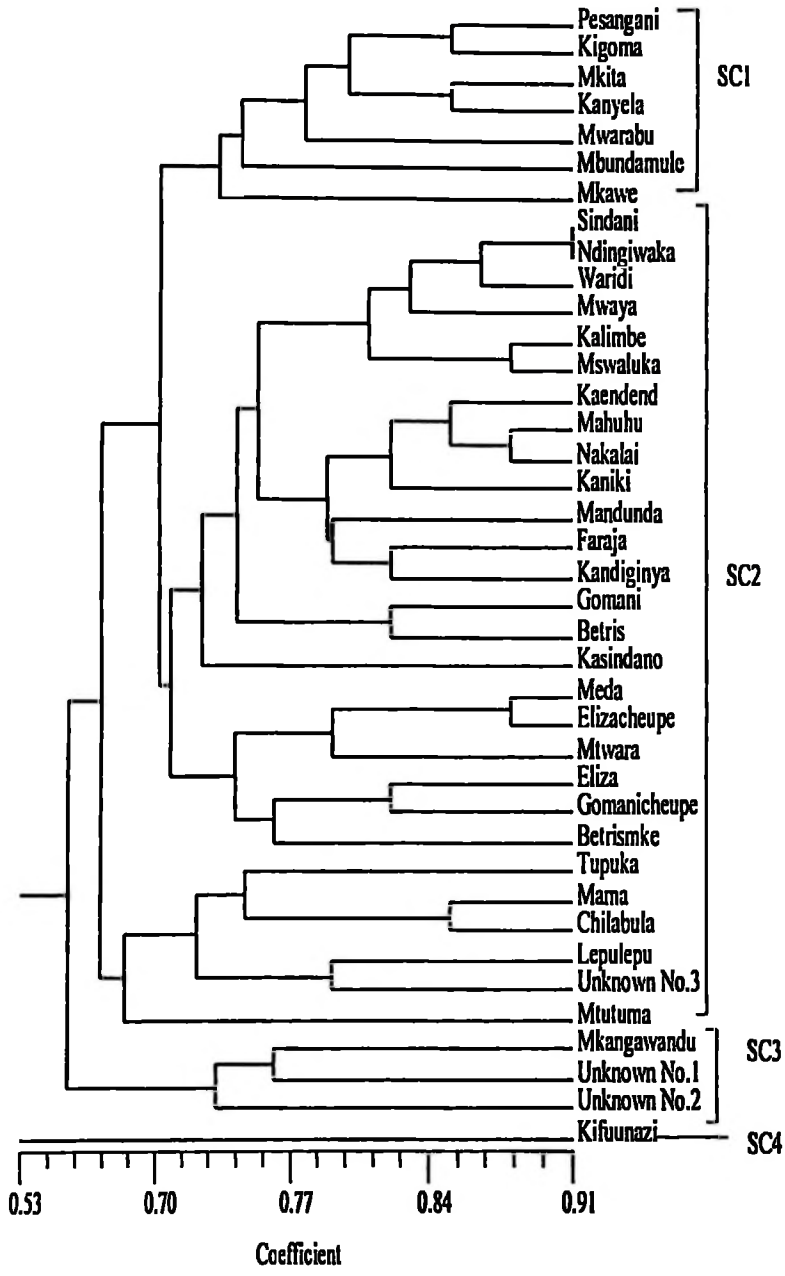

Figure 2: **Dendrogram based on morphological variation of 39 cassava landraces used**

CS1= cluster one, CS2= cluster two, CS3= cluster three and CS4= cluster 4

Table 5: Morphological relationship of cassava landraces

Cultivar	Pes	Kif	Mki	Sin	Kae	Kal	Mbu	Kig	Med	Gom	Mwa	Kany	Mwa	Eli-c	Eli	Bet	BetM	Tup	Mtw	Msw	Mtu	War	Mkan	Lepu	Mam	Mah	Man	Far	Kasi	Gom-c	Mka	Chil	Ndin	Unk1	Unk2	Unk3	Kand	Nak	Kan
Pes	1																																						
Kif	0.76	1																																					
Mki	0.82	0.75	1																																				
Sin	0.74	0.59	0.82	1																																			
Kae	0.68	0.56	0.74	0.82	1																																		
Kal	0.71	0.65	0.76	0.82	0.79	1																																	
Mbu	0.76	0.65	0.76	0.76	0.71	0.79	1																																
Kig	0.85	0.68	0.85	0.76	0.68	0.68	0.74	1																															
Med	0.71	0.68	0.74	0.76	0.68	0.68	0.62	0.68	1																														
Gom	0.76	0.71	0.76	0.76	0.82	0.76	0.74	0.74	0.68	1																													
Mwa	0.71	0.59	0.74	0.79	0.76	0.74	0.65	0.71	0.79	0.74	1																												
Kan	0.74	0.62	0.85	0.85	0.74	0.74	0.71	0.79	0.82	0.76	0.79	1																											
Mwa	0.74	0.59	0.79	0.74	0.76	0.76	0.76	0.79	0.68	0.74	0.68	0.79	1																										
Eliz	0.68	0.65	0.71	0.71	0.76	0.62	0.59	0.65	0.88	0.74	0.74	0.76	0.68	1																									
Eli-C	0.71	0.62	0.76	0.79	0.79	0.74	0.74	0.68	0.74	0.71	0.76	0.71	0.68	0.76	1																								
Bet	0.71	0.71	0.74	0.71	0.74	0.71	0.59	0.65	0.71	0.82	0.68	0.68	0.65	0.76	0.65	1																							
BetM	0.76	0.74	0.74	0.68	0.65	0.62	0.68	0.68	0.76	0.68	0.74	0.68	0.68	0.74	0.76	0.68	1																						
Tup	0.71	0.59	0.68	0.74	0.74	0.74	0.74	0.65	0.62	0.71	0.68	0.62	0.68	0.59	0.74	0.68	0.68	1																					
Mtw	0.68	0.65	0.68	0.71	0.68	0.56	0.62	0.68	0.79	0.59	0.59	0.68	0.68	0.79	0.76	0.65	0.71	0.68	1																				
Msw	0.71	0.62	0.76	0.88	0.82	0.88	0.76	0.71	0.76	0.76	0.79	0.79	0.74	0.71	0.74	0.76	0.62	0.74	0.62	1																			
Mtu	0.59	0.59	0.68	0.68	0.68	0.59	0.65	0.65	0.68	0.65	0.65	0.76	0.76	0.71	0.71	0.59	0.65	0.62	0.71	0.65	1																		
War	0.71	0.65	0.76	0.88	0.82	0.79	0.68	0.71	0.82	0.79	0.85	0.79	0.74	0.82	0.79	0.76	0.71	0.68	0.68	0.88	0.68	1																	
Mka	0.71	0.65	0.74	0.74	0.65	0.62	0.65	0.68	0.59	0.71	0.74	0.68	0.62	0.59	0.68	0.68	0.62	0.71	0.62	0.68	0.65	0.65	1																
Lep	0.65	0.53	0.71	0.71	0.74	0.68	0.68	0.68	0.62	0.65	0.74	0.71	0.76	0.65	0.74	0.62	0.74	0.71	0.68	0.62	0.76	0.68	0.71	1															
Mam	0.65	0.65	0.65	0.68	0.71	0.65	0.71	0.68	0.59	0.71	0.62	0.59	0.74	0.59	0.62	0.74	0.68	0.76	0.68	0.74	0.65	0.65	0.74	0.71	1														
Mah	0.62	0.62	0.68	0.79	0.85	0.74	0.62	0.68	0.74	0.79	0.76	0.71	0.65	0.74	0.74	0.71	0.71	0.68	0.62	0.76	0.59	0.85	0.59	0.68	0.65	1													
Man	0.68	0.65	0.74	0.71	0.82	0.68	0.68	0.76	0.71	0.76	0.74	0.74	0.76	0.68	0.71	0.62	0.74	0.71	0.68	0.71	0.68	0.71	0.59	0.68	0.68	0.79	1												
Far	0.59	0.65	0.65	0.71	0.79	0.74	0.68	0.71	0.71	0.79	0.68	0.71	0.68	0.71	0.68	0.65	0.68	0.62	0.65	0.76	0.62	0.74	0.53	0.59	0.68	0.88	0.82	1											
Kas	0.62	0.65	0.62	0.74	0.71	0.71	0.62	0.68	0.65	0.74	0.65	0.62	0.59	0.65	0.71	0.68	0.71	0.68	0.65	0.65	0.59	0.74	0.62	0.65	0.68	0.82	0.65	0.76	1										
GomC	0.68	0.62	0.79	0.82	0.76	0.76	0.76	0.74	0.76	0.71	0.68	0.76	0.79	0.71	0.82	0.65	0.76	0.76	0.76	0.76	0.68	0.74	0.65	0.65	0.74	0.71	0.79	0.74	0.74	1									
Mka	0.74	0.65	0.76	0.68	0.76	0.71	0.68	0.76	0.71	0.71	0.68	0.71	0.76	0.71	0.74	0.71	0.71	0.59	0.71	0.71	0.68	0.71	0.62	0.68	0.65	0.65	0.74	0.71	0.68	0.74	1								
Chil	0.62	0.68	0.71	0.62	0.65	0.62	0.71	0.65	0.65	0.65	0.59	0.62	0.76	0.65	0.65	0.62	0.68	0.74	0.74	0.65	0.74	0.62	0.65	0.71	0.85	0.59	0.68	0.68	0.59	0.74	0.65	1							
Ndi	0.71	0.56	0.79	0.91	0.76	0.76	0.74	0.79	0.76	0.76	0.85	0.82	0.74	0.71	0.76	0.71	0.74	0.74	0.68	0.82	0.68	0.85	0.74	0.76	0.71	0.76	0.74	0.71	0.76	0.79	0.68	0.65	1						
Unk1	0.56	0.56	0.59	0.68	0.68	0.56	0.53	0.62	0.59	0.68	0.74	0.65	0.53	0.62	0.59	0.71	0.59	0.59	0.59	0.65	0.62	0.62	0.76	0.68	0.71	0.68	0.65	0.65	0.62	0.53	0.62	0.59	0.68	1					
Unk2	0.62	0.68	0.71	0.82	0.71	0.74	0.68	0.65	0.71	0.68	0.71	0.74	0.62	0.59	0.68	0.62	0.65	0.65	0.65	0.76	0.56	0.71	0.76	0.65	0.74	0.74	0.68	0.74	0.71	0.76	0.65	0.68	0.76	0.71	1				
Unk 3	0.65	0.53	0.74	0.76	0.71	0.76	0.74	0.71	0.62	0.65	0.68	0.74	0.79	0.62	0.68	0.65	0.62	0.76	0.65	0.74	0.68	0.68	0.68	0.79	0.74	0.59	0.68	0.59	0.59	0.79	0.62	0.74	0.79	0.62	0.71	1			
Kand	0.65	0.59	0.65	0.76	0.79	0.71	0.71	0.65	0.74	0.79	0.68	0.71	0.71	0.71	0.74	0.68	0.71	0.76	0.76	0.76	0.71	0.74	0.68	0.65	0.76	0.76	0.76	0.82	0.79	0.79	0.74	0.74	0.76	0.68	0.71	0.62	1		
Nak	0.68	0.62	0.76	0.82	0.85	0.74	0.65	0.71	0.76	0.82	0.79	0.76	0.74	0.74	0.71	0.76	0.71	0.74	0.68	0.79	0.65	0.85	0.65	0.74	0.76	0.88	0.79	0.76	0.76	0.74	0.65	0.71	0.85	0.68	0.71	0.68	0.79	1	
Kan	0.68	0.65	0.68	0.71	0.79	0.68	0.62	0.65	0.71	0.79	0.76	0.68	0.68	0.76	0.71	0.74	0.74	0.79	0.65	0.74	0.59	0.79	0.74	0.68	0.76	0.82	0.76	0.74	0.76	0.74	0.62	0.68	0.76	0.65	0.68	0.68	0.79	0.85	1

Pes= Pesangani, Kif =Kifuu Cha Nazi, Mki=Mkita, Sin=Sindani, Kae=Kaendaenda, Kal= Kalimbe, Mbu= Mbundamule, Kig= Kigoma, Med= Meda, Gom= Gomani, Mwa= Mwaya, Kany=Kanyela, Mwar=Mwarabu,Eli-C= Eliza Cheupe, Eli=Eliza,Bet=Betriece, Betm=Betriece Mkeka, Tup= Tupuka, Mtw= Mtwara, Msw=Mswaluka, War=Waridi, Mtu=Mtutuma, Mka=Mkangawandu,Lep=Lepulepu, Mam=Mama, Mah=Mahuhu, Far= Faraja, Gomc= Gomani Cheupe, Man=Mandunda, Kasi=Kasindano, Mka=Mkawe,Chil=Chilambula Ndin=Ndingiwaka, Unk1=Unknown No.1 Unk2= Unknown No.2, Unk3= Unknown No.3 Kandi=Kandinginya, Nak=Nakalai, Kan= Kaniki.

The principle component analysis (PCA) shown Appendix viii was developed to supplement the results obtained earlier and presented in Fig 2: Although PCA was based on a different algorithm using Eigen, the grouping of cassava landraces was found to be similar to that of dendrogram developed in Fig 2:. The PCA revealed four different groups. The findings indicated that *Mahuhu* was grouped differently from the rest of the landraces while landrace *Sindani* and landrace *Ndingiwaka* were again confirmed to be very closely related.

4.4 Cassava molecular characterization and genetic relationships

4.4.1 Simple sequence repeats marker analysis

The results from *"PAGE"* and silver staining analysis revealed 13 informative oligonucleotide primers which detected polymorphisms among 39 landraces. The primers reveal a total of 89 clear and easily scorable bands with a mean of 6.8 (Table 6). Seventy five (i.e., 84.3%) of the 89 bands were polymorphic among the cassava landraces used in this study. Cassava SSR markers revealed higher levels of genetic polymorphism in this study, these results are in agreement with studies done by Raghu *et al.*(2007); Moyib *et al.* (2007); Masumba (2006) and Fregene *et al.* (2003) on the same crop. The higher level of polymorphism associated with SSR markers may be a function of the unique replication slippage mechanism responsible for generating SSR allelic diversity (Mba *et al.*, 2001). The number of allele per primer ranged from 2 to 10. The SSRY 110 had the lowest number of allele (2), while SSRY 69 had the highest number (10) as indicated in Plate 5. Similarly SSR markers generated more number of alleles reported by Moyib *et al.* (2007); Raghu *et al.* (2007) and Luiz *at al.* (2001) on cassava crop. The size of DNA bands that were produced in the *"PAGE"* analysis ranged from 100 to 3000bp, but most of the bands

were between 400 and 1000bp. These results are in agreement with those reported by Herzberg *et al.* (2004); Luiz e*t al.* (2001) and Tuonukari *et al.* (1997) who worked on cassava. The number of loci reported in this study demonstrates variability of the patterns among the 39 individual cultivars and also between the primers. However, blank lanes observed on the most of the "*PAGE*" analysis may have been due to salts contamination experienced during the work and incomplete PCR premixes or degraded genomic DNA template and not because of absent priming sites. This type of result are in agreement with those reported by other researchers such as Herzberg *et al.* (2004) who worked on cassava in German on cassava materials that had been collected from Tanzania.

The 'PAGE' analysis Plate 5 describes the number of allele obtained using SSR 69 primer. The line and letters on the right indicate the number of alleles. Alleles were numbered starting from bottom to the top. From the example the bottom allele is SSR69/1 and the highest is allele SSR69/10 with alleles 2-9 lying in between. The left numbers indicate the size of the band, in this example the weight range from 500-3000bp.

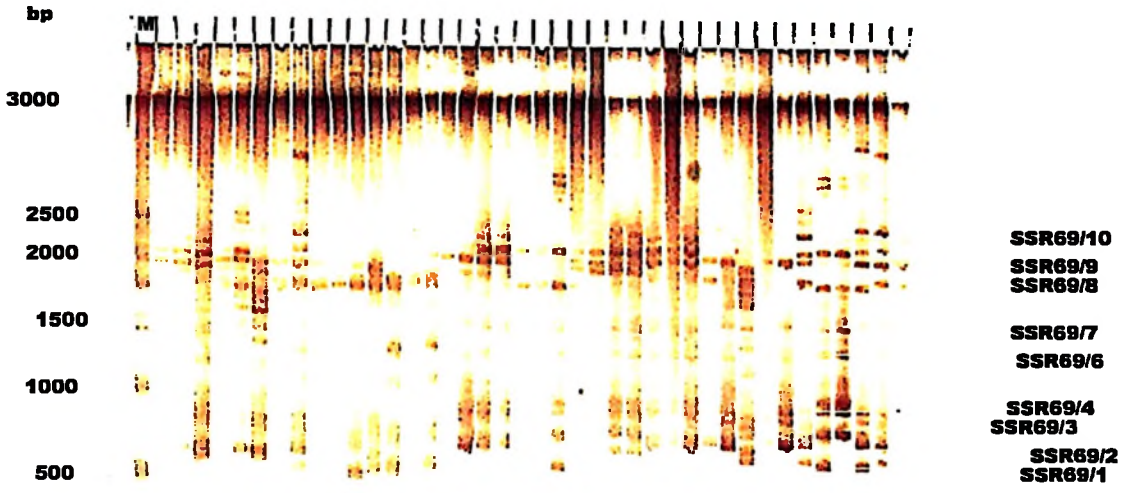

Plate 5: **SSR-based multi-allelic fingerprints in 39 cassava landraces. Line and letters on the right show the putative alleles. Alleles were numbered starting with the shortest sequence.**

Table 6: Fragment analysis of SSR primers included in genetic variation analysis of 39 cassava landraces

S/No.	SSR primer	Sequence(5'-3')	Sequence (3'-5')	Total number of loci	Polymorphic loci	PIC values
1	SSRY 59	GCAATGCAGTGAACC ATCTT	CGTTTGTCCTTTCT GATGTTC	6	6	0.74
2	SSRY 52	CCATGCAGATGTGCC ATCTTT	ATTTTCACCAACCG CAACTC	7	7	0.83
3	SSRY 69	CGATCTCAGTCGATA CCCAAG	CACTCCGTTGCAG GCATTA	10	9	0.85
4	SSRY 63	TCAGAATCATCTACCT TGGCA	AAGACAATCATTTT GTGCTCCA	9	5	0.67
5	SSRY 82	TGTGACAATTTTCAGA TAGCTTCA	CACCATCGGCATT AAACTTTG	8	4	0.69
6	SSRY 171	ACTGTGCCAAAATAG CCAAATAGT	TCATGAGTGTGGG ATGTTTTTATG	7	6	0.22
7	SSRY 177	ACCACAAACATAGGC ACGAG	CACCCAATTCACC AATTACCA	3	3	0.49
8	SSRY 102	TTGGCTGCTTTCACTA ATGC	TTGAACACGTTGA ACAACCA	4	4	0.74
9	SSRY 182	GGAATTCTTTGCTTAT GATGCC	TTCCTTTACAATTC TGGACGC	7	7	0.84
10	SSRY 110	TTGAGTGGTGAATGC GAAAG	AGTGCCACCTTGA AAGAGCA	4	2	0.65
11	SSRY 135	CCAGAAACTGAAATG CATCG	AACATGTGCGACA GTGATTG	9	8	0.85
12	SSRY 181	GGTAGATCTGGATCG AGGAGG	CAATCGAAACCGA CGATACA	9	9	0.86
13	SSRY 61	GGCTGCTTTACCTTCT ACTCAGA	CAAGAACGCCAAT ATGCTGA	6	5	0.77
		Total		89	75	9.18
		Mean		6.8	5.8	0.71

PIC= polymorphic information content

4.4.2 Genetic dendrogram composition

Landrace *Kifuu cha nazi*, which differed considerably morphologically with other genotypes in Fig. 2, shows some genetic relationship with *Gomani, Pesangani, Sindani, Waridi, Mkangawandu, Mbundamule, Meda, Mswaluka* and *Mtutuma in* cluster1 (CS 1) of Fig 3:. These landraces were collected from low and medium agro-ecological zone. The second cluster (CS2) comprised of 13 landraces from Mbinga lowland-Nyasa. These included *Mkita, Betrice, Mtwara, Betrice mkeka, Kaendaenda, Kalimbe, Kigoma, Mwarabu, Mwaya, Kanyela, Eliza cheupe, Eliza, Gomani cheupe* and *Mkawe, a*nd one landrace i.e., *Kanyela* from Mbinga highland agro-ecological zone. The third cluster (CS3) involved nine landraces from Mbinga Lowland-Nyasa and five landraces from Mbinga highland agro-ecological zone. These were *Unknown No.1, Unknown No.2, Unknown No. 3 Tupuka, Kasindano, Chilambula, Ndingiwaka, Lepulepu, Mama, Mahuhu, Mandunda,* and *Faraja.* The fourth cluster (CS4) contain three landraces from Mbinga Lowland Nyasa, these includes *Kandinginya, Nakalai* and *Kaniki.*

4.4.3 Cassava genetic relationship

Genetic diversity in cassava has been previously studied using DNA molecular primers, isozyme markers (Sariria *et al.,* 1992), RFLP (Angel *et al.,* 1992) RAPD (Tonukari *et al.,* 1997) and SSR by Moyib *et al.* (2007); Raghu *et al.* (2007) Masumba (2006) and Fregene *et al.* (2003) low to high genetic diversity has been observed in these studies. In this study low genetic diversity among the landraces collected from the study area were also identified. This might be due to the reason that the landraces were domesticated in same area with narrow genetic base for long

time or might be due to the fact that no introduction of other varieties to the area, either through farmer to farmer diffusion or deliberately by cassava researchers.

The Jaccard's coefficient of similarity based on 13 SSR markers ranged from 0.33-0.88 (Fig. 3). This range is in agreement with the study done by Moyib *et al.* (2007) in cassava who reported the similarly the same range. At 0.60 coefficient similarity, the 39 cassava landraces were clustered into four main clusters. Among the 39 landraces studied, the highest similarity index (0.88) was recorded between the landraces *Mama* and *Mahuhu* and the lowest similarity index (0.33) was recorded between landraces *Pesangani* and *Kaendanda.* The dendrogram revealed that the landraces in the first cluster (CS1) had the following genetic distances *Pesangani* and *Kifuu cha nazi* (0.83), *Sindani* and *Waridi* (0.73), *Waridi* and *Mkangawandu* (0.73), *Meda* and *Gomani* (0.67) and *Mswaluka* and *Mtutuma* had (0.81) genetic distances as indicated in Table 7. The results showed that the landraces in cluster one were closely related, but without duplication of landraces.

Again the genetic relationships of cassava landraces in cluster two were also found to be closely related with the following genetic distance *Mkita* and *Betris* (0.68), *Eliza* and *Eliza cheupe* (0.83), *Mwaya* and *Kanyela* (0.80), *Kaendaenda* and *Kalimbe* (0.76). Additionally in cluster three the majority of the landraces were found to be closely related with the similarity coefficient ranged 0.67-0.88 (Table 7). Furthermore the similarity coefficient of landraces in cluster four was high indicating closer relationships among landraces. However, there is wide genetic distance between cluster formed, for example the landraces in cluster one show wide variation with the landraces found in the rest of the clusters (i.e. *Pesangani* found in cluster

one and *Kanyela* located in cluster two had coefficient similarity of 0.35, *Pesangani* and *Kaendanda* had similarity coefficient of 0.33). These inter and intra-clusters variations of landraces provide useful information to cassava breeders in selection of related or unrelated parental materials. The present results will enable the breeder to maximize variability in their breeding programs and also to maximize utilization of landraces which sometime may produce good segregating progenies.

The dendrograms generated using morphological markers and SSR markers based on Jaccard's coefficients, showed that cluster two of both methods shared some of the landraces. These include *Betrice, Betrice mkake, Mwaya, Kalimbe, Kaendaenda, Eliza, Eliza cheupe* and *Gomani cheupe*. While cluster one and three shared only two landraces i.e. *Pesangani* and *Mbundamule* and *Unknown No.1* and *Unknown No.2*, respectively. However, the present findings indicate that four landraces (i.e., *Pesangani, Chilabula, Mwaya* and *Kasindano*) were found to unrelated morphologically and genetically.

The SSR primers have shown high level of polymorphism in many other important crops including *Sorghum bicolaor* (Agrama and Tuinstra, 2003; Dje *et al.*, 2000), Tunisia date palm (Zehdi *et al.*, 2004). Similar results obtained in this study, SSR primers showed high level of polymorphism in cassava landraces assessed. The results indicated that each of the 13 SSR primer detected polymorphism among the 39 cassava landraces.

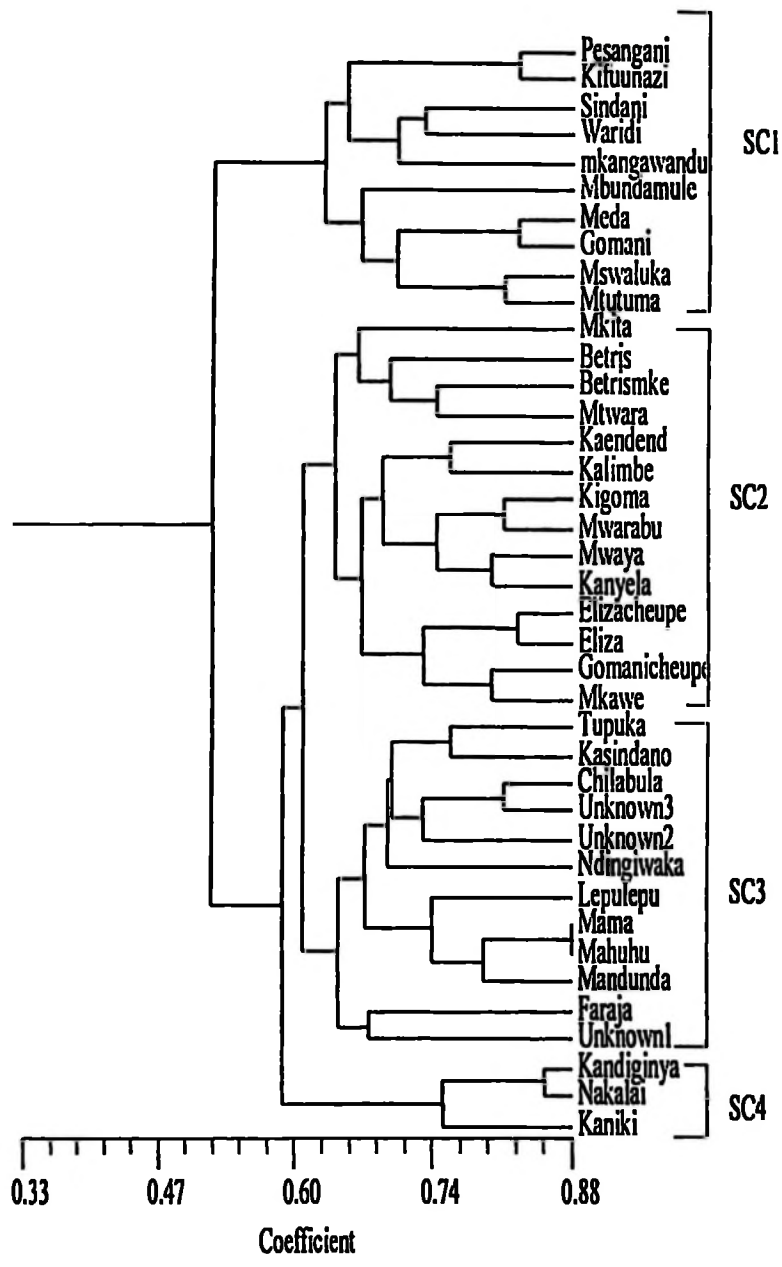

Figure 3: Phenetic dendrogram of genetic analysis computed from a similarity matrix of genetic distance based on jaccard's coefficient.
CS1= cluster one, CS2= cluster two, CS= cluster three and CS4 = cluster four

Table 7: Genetic relationship of 39 cassava landraces based on Jaccard's coefficient similarity

	Kif	Mki	Sin	Kae	Kal	Mbu	Kig	Med	Gom	Mwa	Kany	Mwar	Eli-C	Eli	Bet	Tup	Mtw	Msw	Mtu	War	Mka	Lep	Mam	Mah	Man	Far	Kas	Gom	Mka	Chil	Ndin	Unk1	Unk2	Unk3	Kand	Nak	Kan
Pes	1.00																																				
Kif	0.83	1.00																																			
Mki	0.52	0.61	1.00																																		
Sin	0.68	0.75	0.68	1.00																																	
Kae	0.55	0.59	0.65	0.71	1.00																																
Kal	0.41	0.43	0.71	0.55	0.76	1.00																															
Mbu	0.64	0.65	0.43	0.64	0.61	0.45	1.00																														
Kig	0.40	0.49	0.69	0.56	0.67	0.69	0.52	1.00																													
Med	0.67	0.63	0.56	0.69	0.67	0.56	0.68	0.57	1.00																												
Gom	0.68	0.64	0.49	0.65	0.60	0.49	0.67	0.43	0.83	1.00																											
Mwa	0.41	0.45	0.65	0.63	0.76	0.76	0.56	0.72	0.61	0.60	1.00																										
Kany	0.35	0.39	0.59	0.48	0.64	0.69	0.49	0.71	0.55	0.51	0.80	1.00																									
Mwar	0.35	0.44	0.69	0.53	0.67	0.67	0.57	0.81	0.60	0.51	0.80	0.76	1.00																								
Eli-C	0.52	0.53	0.55	0.57	0.63	0.57	0.67	0.59	0.72	0.68	0.63	0.67	0.67	1.00																							
Eli	0.43	0.47	0.64	0.53	0.69	0.72	0.55	0.73	0.57	0.51	0.75	0.81	0.79	0.83	1.00																						
Bet	0.60	0.67	0.68	0.71	0.65	0.63	0.56	0.59	0.64	0.60	0.63	0.59	0.59	0.68	0.69	1.00																					
Bet-M	0.45	0.52	0.67	0.56	0.56	0.69	0.47	0.65	0.47	0.43	0.64	0.65	0.65	0.51	0.65	0.69	1.00																				
Tup	0.39	0.43	0.68	0.47	0.55	0.63	0.43	0.67	0.48	0.49	0.68	0.64	0.75	0.57	0.67	0.65	0.75	1.00																			
Mtw	0.47	0.51	0.65	0.63	0.65	0.65	0.51	0.69	0.67	0.57	0.73	0.67	0.75	0.60	0.67	0.71	0.75	0.73	1.00																		
Msw	0.65	0.59	0.49	0.60	0.68	0.57	0.69	0.53	0.69	0.71	0.65	0.64	0.59	0.71	0.67	0.68	0.51	0.55	0.60	1.00																	
Mtu	0.68	0.61	0.49	0.65	0.60	0.47	0.64	0.45	0.67	0.76	0.57	0.59	0.51	0.71	0.61	0.63	0.43	0.49	0.55	0.81	1.00																
War	0.63	0.64	0.57	0.73	0.63	0.55	0.64	0.53	0.56	0.60	0.57	0.48	0.51	0.49	0.56	0.63	0.53	0.44	0.57	0.63	0.63	1.00															
Mka	0.63	0.61	0.52	0.68	0.52	0.49	0.61	0.48	0.59	0.65	0.49	0.43	0.43	0.52	0.48	0.65	0.53	0.49	0.55	0.57	0.65	0.73	1.00														
Lep	0.48	0.52	0.53	0.59	0.64	0.59	0.65	0.60	0.63	0.56	0.61	0.55	0.68	0.64	0.63	0.56	0.63	0.64	0.64	0.64	0.48	0.64	0.61	1.00													
Mam	0.41	0.40	0.52	0.52	0.60	0.63	0.53	0.64	0.48	0.47	0.71	0.59	0.64	0.60	0.67	0.57	0.64	0.73	0.63	0.57	0.47	0.57	0.49	0.77	1.00												
Mah	0.40	0.39	0.56	0.51	0.56	0.59	0.49	0.71	0.52	0.48	0.67	0.63	0.68	0.59	0.63	0.48	0.65	0.69	0.67	0.53	0.48	0.56	0.48	0.71	0.88	1.00											
Man	0.49	0.48	0.49	0.52	0.55	0.52	0.53	0.53	0.51	0.55	0.63	0.51	0.61	0.55	0.53	0.52	0.59	0.73	0.63	0.52	0.47	0.63	0.55	0.75	0.81	0.77	1.00										
Far	0.59	0.49	0.45	0.48	0.53	0.43	0.63	0.47	0.55	0.69	0.56	0.52	0.57	0.64	0.55	0.59	0.55	0.64	0.59	0.69	0.64	0.59	0.53	0.63	0.67	0.65	0.75	1.00									
Kasi	0.41	0.43	0.60	0.63	0.55	0.55	0.53	0.64	0.53	0.49	0.63	0.59	0.69	0.63	0.61	0.63	0.59	0.76	0.65	0.52	0.49	0.57	0.55	0.61	0.68	0.69	0.71	0.64	1.00								
Gom	0.49	0.45	0.57	0.52	0.60	0.60	0.56	0.59	0.67	0.63	0.61	0.67	0.67	0.76	0.69	0.65	0.56	0.63	0.63	0.68	0.63	0.55	0.55	0.64	0.60	0.64	0.60	0.67	0.73	1.00							
Mka	0.43	0.41	0.59	0.53	0.61	0.67	0.49	0.65	0.55	0.53	0.75	0.73	0.71	0.69	0.79	0.72	0.68	0.75	0.72	0.67	0.59	0.56	0.51	0.71	0.77	0.71	0.67	0.65	0.72	0.80	1.00						
Chil	0.33	0.40	0.57	0.49	0.52	0.65	0.43	0.64	0.48	0.44	0.71	0.69	0.67	0.55	0.67	0.63	0.72	0.76	0.73	0.55	0.47	0.52	0.49	0.61	0.71	0.72	0.73	0.56	0.71	0.68	0.80	1.00					
Ndi	0.45	0.52	0.59	0.59	0.56	0.51	0.49	0.57	0.57	0.53	0.61	0.57	0.57	0.51	0.57	0.69	0.63	0.69	0.72	0.59	0.56	0.67	0.56	0.63	0.69	0.68	0.69	0.63	0.69	0.64	0.76	0.75	1.00				
Unk1	0.53	0.52	0.53	0.59	0.59	0.56	0.60	0.49	0.60	0.61	0.61	0.55	0.57	0.59	0.57	0.67	0.63	0.67	0.61	0.59	0.59	0.64	0.64	0.68	0.64	0.60	0.75	0.68	0.67	0.59	0.71	0.67	0.68	1.00			
Unk2	0.44	0.40	0.49	0.47	0.52	0.57	0.45	0.53	0.45	0.36	0.60	0.67	0.59	0.55	0.64	0.65	0.67	0.68	0.63	0.52	0.41	0.52	0.49	0.67	0.68	0.61	0.68	0.59	0.71	0.65	0.77	0.76	0.69	0.72	1.00		
Unk3	0.36	0.45	0.65	0.52	0.60	0.65	0.43	0.64	0.45	0.39	0.60	0.56	0.64	0.44	0.56	0.55	0.72	0.73	0.65	0.44	0.36	0.49	0.52	0.64	0.63	0.64	0.68	0.51	0.63	0.55	0.64	0.81	0.67	0.64	0.71	1.00	
Kand	0.33	0.40	0.55	0.41	0.52	0.68	0.40	0.56	0.45	0.39	0.55	0.64	0.59	0.49	0.59	0.55	0.64	0.63	0.60	0.44	0.36	0.41	0.41	0.59	0.52	0.51	0.52	0.43	0.52	0.57	0.64	0.68	0.56	0.56	0.68	0.71	1.00
Nak	0.37	0.39	0.53	0.45	0.48	0.61	0.41	0.57	0.47	0.40	0.61	0.68	0.60	0.59	0.65	0.61	0.63	0.64	0.64	0.56	0.45	0.45	0.37	0.60	0.64	0.63	0.56	0.52	0.53	0.61	0.73	0.75	0.60	0.55	0.72	0.67	0.85	1.00
Kan	0.44	0.45	0.55	0.47	0.47	0.52	0.43	0.53	0.43	0.39	0.55	0.64	0.53	0.55	0.64	0.60	0.67	0.65	0.60	0.49	0.49	0.52	0.49	0.56	0.60	0.59	0.55	0.53	0.57	0.57	0.67	0.65	0.59	0.56	0.65	0.60	0.73	0.77	1.00

Pesangani, Kif =Kifuu Cha Nazi, Mki=Mkita, Sin=Sindani, Kae=Kaendaenda, Kal= Kalimbe, Mbu= Mbundamule, Kig= Kigoma, Med= Meda, Gom= Gomani, Mwa= Mwaya, Kany=Kanyela, Mwar=Mwarabu,Eli-C= Eliza Cheupe, Eli=Eliza,Bet=Betriece, Betm=Betriece Mkeka, Tup= Tupuka, Mtw= Mtwara, Msw=Mswaluka, War=Waridi, Mtu=Mtutuma, Mka=Mkangawandu,Lep=Lepulepu, Mam=Mama, Mah=Mahuhu, Far= Faraja, Gomc= Gomani Cheupe, Man=Mandunda, Kasi=Kasindano, Mka=Mkawe,Chil=Chilambula Ndin=Ndingiwaka, Unk1=Unknown No.1 Unk2= Unknown No.2, Unk3= Unknown No.3 Kand=Kandinginya, Nak=Nakalai, Kan= Kaniki.

Generally the microsatellite analysis indicated that, these landraces were genetically closely related based on their similarity coefficient (Table 7). This condition is common especially for crops such as cassava, which is vegetatively propagated, the phenomenon which is also reported by Moyib *et al.* (2007) in Nigeria, Herzberg *et al.* (2004) in German, Fregene *et al.* (2000, 2003) in Tanzania, Luiz *et al.* (2001) in Brazil, Tonukari *et al.*(1997) in Benin and Beeching *et al.* (1993) who also worked on cassava genetic characterization using SSR and RAPD marker.

The results obtained in the dendrogram were also supported by the principle component analysis as shown in Appendix ix. Although PCA is based on a different algorithm using Eigen and vector, the grouping of landraces was similar to that of dendrogram as indicated in Fig 3:. The principal component analysis (PCA) performed on the basis of the similarity matrix of 39 landraces which reveal four major clusters, and confirms the results of cluster analysis using UPGMA analysis.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Morphological characterization of cassava (*M. esculanta* Crantz) landraces revealed low morphological diversity among landraces. However, the dendrogram based on morphological traits showed inter and intra-cluster variation providing scope for cassava improvement through selection.

Despite the narrow genetic base of the cassava landraces, the SSR primers used in this study detected cassava genetic variability at the similarity coefficient of 0.33-0.88 in the four main clusters i.e. cluster 1, 2, 3 and 4.

In both methods used (i.e. morphological and SSR techniques) no cassava duplicate was identified. The most morphologically closely related cassava landraces were *Sindani* and *Ndingiwaka* with similarity coefficient of 0.91. Similarly the most genetically closely related cassava landraces were *Mama* and *Mahuhu* that had jaccard coefficient similarity of 0.88.

The present findings showed that four landraces (i.e. *Pesangani, Chilabula, Mwaya* and *Kasindano*) that were found to unrelated both morphologically and genetically could be advocated to be included in National Cassava Improvement Programs (NCIP) in the country.

5.2 Recommendations

i. Due to the fact that some important cassava morphological characteristics usually are influenced by age and sometime by the environment where the plants grows, it is advised that the use of cassava morphological characteristics particularly colour of stems and lamina in cassava characterization should not be relied upon. However, such characteristics could be used to complement the genetic characterization. It is also suggested that simple sequence repeat (SSR) markers be used whenever possible (i.e. with available manpower and infrastructure) in characterizing and identifying cassava landraces because are simple, high informative and codominant.

ii. It is also advocated from these results that *Pesangani, Chilabula, Mwaya* and *kasindano* cassava landraces that were found to be morphologically and genetically distantly related be included in breeding of cassava in National cassava improvement programs in Tanzania.

iii. As majority (77%) of cassava landraces grown by farmers were found to be bitter taste, it is therefore, suggested that further research work be conducted with the aim of establishing the chemical causes of cassava bitterness in the study area.

iv. Both morphological and genetic information of 39 landraces used in study provided usefully information to breeders and could be added to the national

germplasm for the purpose of conserving and using it in National Cassava Improvement Programs.

v. The use of biotechnology such as tissue culture techniques should be engaged with the aim of accelerating the multiplication of genetically unrelated cassava materials in order to make easier and quicken availability to researchers and farmers for use.

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APPENDICES

Appendix i: Descriptors for morphological characteristics as described by Mahungu and Kanju (1997)

A. Shoot characteristics:

i. Colour of the young shoot
   1=green
   2=purplish green
   3=purple

ii. Pubescence (presence of hairs on young shoots)
   0=nil/none
   1=positive

iii. Leaf shape
   1=narrow
   2=broad

iv. Petiole colour
   1=green
   2=green-purple
   3=purple

v. Colour of the lamina
   1=light green
   2=green
   3=dark green
   4=purple

vi. Colour of the mature leaf veins (veins on the lower surface of the first fully expanded leaf)
   1=light green
   2=dark green
   3=green-purple
   4=purple
   5=green red

vii. Shape of the leaf central lobe
   1=oblanceolate
   2=linear
   3=elliptic
   4=pandurate
   5=lanceolate

viii. Number of lobes (most common number)

ix. Immature stem colour
1=green
2=green-purple
3=purple
4=green-red
5=red

x. Mature stem colour
1=silvery green
2=light brown/orange
3=dark brown
4=reddish-red

xi. Branching height
0=no branching or generally unbranched
I=low branching (less than 1/3 of total plant)
2=medium branching (between 1/3 and 2/3 of total plant height)
3=high branching (above 2/3 of the total plant height)

xii. Number of forking branches i.e. branching levels (actual number of levels)

xiii. Number of primary stems (actual number of stems)

xiv. Number of suckers (actual number of suckers)

B. Root characteristics

i. Root taste
1=bitter
2=sweet

ii. Root arrangement/position
1=tending towards vertical
2=tending towards horizontal
3=horizontal
4=irregular
5=one sided

iii. Outer skin colour (root surface)
1=white to cream
2=light brown
3=dark brown

iv. Inner skin colour
1=white/cream
2=yellow
3=pink

v. Colour of the pulp
   1=white/cream
   2=yellow

vi. Neck length (root peduncle)
   0=absent
   1=short (less than 5 centimeter)
   2=intermediate (5-10 centimeter)
   3=long (greater 10 centimeter)

vii. Root shape
   1=conical
   2=conical/cylindrical
   3=cylindrical
   4=fusiform
   5=irregular

Appendix ii: Cassava foliar characteristics (colour)

Landrace Name	Young Shoot	Petiole	Lamina	Immature Stem	Mature Stem
Pesangani	Purplish Green	green	Light Green	Green	Silvery Green
Kifuu Cha Nazi	purple	Purple	Light Green	Green	Reddish
Mkita	Purplish Green	Green	Light Green	Green	Reddish
Sindani	Purple	Purple	Green	Green Purple	Silvery Green
Kaendaenda	Purple	Green Purple	Light Green	Green	Silvery Green
Kalimbe	Purplish Green	Green	Light Green	Green Purple	Silvery Green
Mbundamule	Purplish Green	Green	Light Green	Green Purple	Silvery Green
Kigoma	Purplish Green	Green	Light Green	Green	Silvery Green
Meda	Purplish Green	Green Purple	Light Green	Green	Dark Brown/Orange
Gomani	Green	Green Purple	Green	Green	Silvery Green
Mwaya	Purplish Green	Green Purple	Light Green	Green	Silvery Green
Kanyela	Purplish Green	Green	Light Green	Green	Dark Brown
Mwarabu	Purple	Purple	Light Green	Green Purple	Silvery Green
Eliza-Cheupe	Purplish Green	Green	Dark Green	Green	Reddish
Eliza	Green	Green Purple	Green	Green	Silvery Green
Beatrice	Green	Green	Green	Green	Light Brow/Orange
Beatrice-Mkeka	Green	Green	Light Green	Green	Silvery Green
Tupuka	Purplish Green	Dark Green	Purple	Green	Silvery Green
Mtwara	Purple	Purple	Dark Green	Green	Light Brown/Orange
Mswaluka	Purplish Green	Green Purple	Light Green	Green Purple	Silvery Green
Mtutuma	Purple	Green Purple	Light Green	Green	Dark Brown
Waridi	Green	Green Purple	Green	Green	Silvery Green
Mkangawandu	Purplish Green	Green Purple	Dark Green	Green	Reddish
Lepulepu	Purple	Purple	Dark Green	Green	Dark Brown
Mama	Purple	Purple	Light Green	Green Purple	Silvery Green

Appendix ii continue...

Landrace Name	Young Shoot	Petiole	Lamina	Immature Stem	Mature Stem
Mahuhu	Green	Green Purple	Green	Green	Silvery Green
Mandunda	Purple	Green Purple	Light Green	Green	Silvery Green
Faraja	Green	Green	Lightgreen	Green Purple	Silvery Green
Kasindano	Green	Green Purple	Green	Green	Silvery Green
Gomani-Cheupe	Purplish Green	Green	Light Green	Green	Silvery Green
Mkawe	Purple	Green	Light Green	Green	Light Brown/Yellow
Chilambula	Purple	Purple	Green	Green Purple	Reddish
Ndingiwaka	Purplish Green	Green Purple	Light Green	Green	Silvery Green
Unknown 1	Purple	Purple	Purple	Green Red	Reddish
Unknown 2	purple	Purple	Light Green	Green Purple	Reddish
Unknown 3	Purplish Green	Purple	Green	Reddish Red	Dark Brown
Kaniki	Purple	Green Purple	Green	Green	Silvery Green
Kandinginya	Purple	Green Purple	Green	Green	Silvery Green
Nakalai	Green	Green Purple	Green	Green	Silvery Green

Appendix iii: Properties of 20 primer pairs

S/No.	SSR primer	motif	Left primer	Right primer	Product size	Annel. temp	$MgCl_2$(mM)
1	SSRY 59	$(CA)_{20}$	GCAATGCAGTGAACCATCTT	CGTTTGTCCTTTCTGATGTTC	158	55	1.5
2	SSRY 52	$(GT)_{19}$	CCATGCAGATGTGCCATCTTT	ATTTTCACCAACCGCAACTC	266	55	1.5
3	SSRY 69	$(CT)_{18}ATT(AT)_2(N)7(CTTT)_2$	CGATCTCAGTCGATACCCAAG	CACTCCGTTGCAGGCATTA	239	55	1.5
4	SSRY 63	$(GA)_{16}$	TCAGAATCATCTACCTTGGCA	AAGACAATCATTTTGTGCTCCA	290	55	1.5
5	SSRY 82	$(GA)_{24}$	TGTGACAATTTTCAGATAGCTTCA	CACCATCGGCATTAAACTTTG	211	55	1.5
6	SSRY 171	$(TA)_5CATA(GTA)_8GC(GA)_{23}(GTGA)_2$	ACTGTGCCAAAATAGCCAAATAGT	TCATGAGTGTGGGATGTTTTTATG	291	55	1.5
7	SSRY 177	$(CCT)_6CT(N)_{65}(CT)_4AT(CT)_{18}$	ACCACAAACATAGGCACGAG	CACCCAATTCACCAATTACCA	268	45	1.5
8	SSRY 102	$(GT)_{11}$	TTGGCTGCTTTCACTAATGC	TTGAACACGTTGAACAACCA	179	55	1.5
9	SSRY 182	$(CA)_{17}(N)_{31}GAGG(GA)_8$	GGAATTCTTTGCTTATGATGCC	TTCCTTTACAATTCTGGACGC	256	55	1.5
10	SSRY 110	$(GT)_{12}$	TTGAGTGGTGAATGCGAAAG	AGTGCCACCTTGAAAGAGCA	247	55	1.5
11	SSRY 135	$(CT)_{16}$	CCAGAAACTGAAATGCATCG	AACATGTGCGACAGTGATTG	253	45	1.5

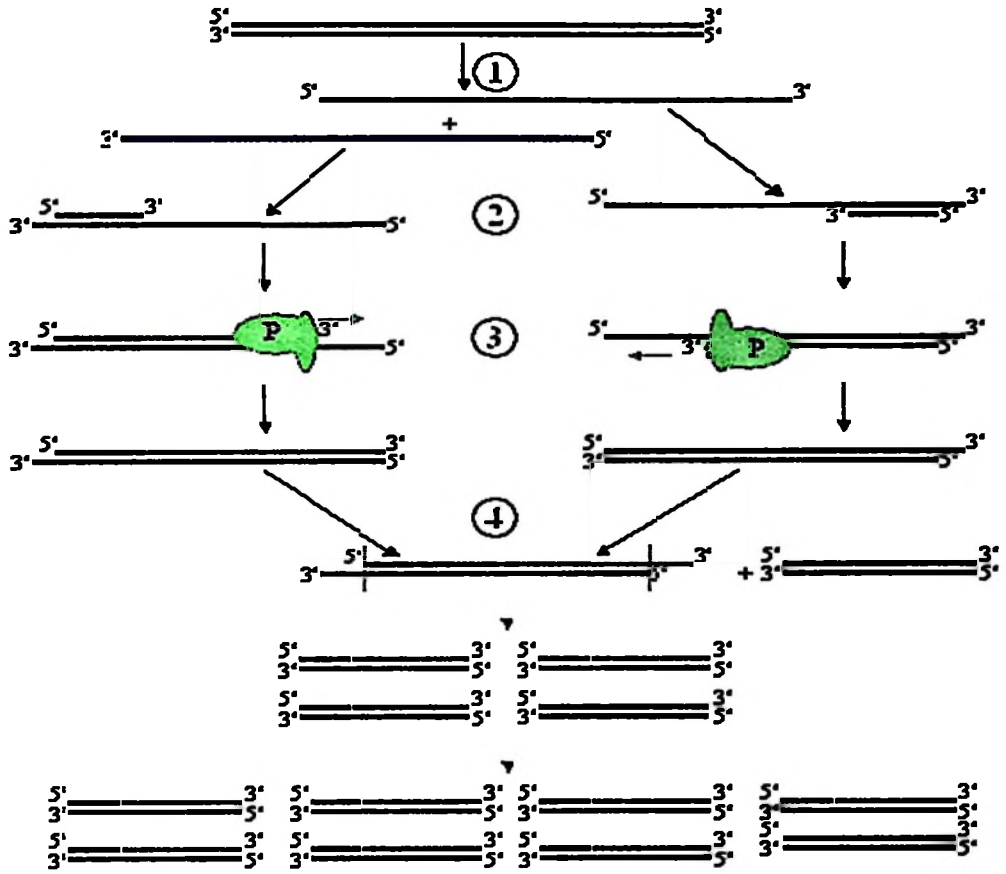
Appendix iii continue...

S/ No.	SSR primer	motif	Left primer	Right primer	Product size	Annealing temperature	$MgCl_2$(mM)
12	SSRY 181	$(GA)_{22}(G)_3C(GA)_3GGAA(GA)_4$	GGTAGATCTGGATCGAGGAGG	CAATCGAAACCGACGATACA	199	55	1.5
13	SSRY 61	Total	GGCTGCTTTACCTTCTACTCAGA	CAAGAACGCCAATATGCTGA	233	55	1.5
14	SSTY64	$(CT)_{13}CG(CT)_6$	CGACAAGTCGTATATGTAGTATTCACG	GCAGAGGTGGCTAACGAGAC	194	55	1.5
15	SSRY70	$(GT)_{18}$	CGCTATTAGAATTGCCAGCAC	CGCTTGTTGTATCCATTGGC	249	55	1.5
16	SSRY 89	$(GT)_{19}$	AGTTGAGAAAACCTTGCATGAG	GGCTGTTCGTGATCCTTATTAAC	120	55	1.5
17	SSRY108	$(CT)_{24}CCT$	ACGCTATGATGTCCAAAGGC	CATGCCACATAGTTCGTGCT	203	55	1.5
18	SSTY 100	$(CT)_{17}TT9CT)_7$	ATCCTTGCCTGACATTTTGC	TTCGCAGAGTCCAATTGTTG	210	55	1.5
19	SSRY 106	$(CT)_{24}$	GGAAACTGCTTGCACAAAGA	CAGCAAGACCATCACCAGTTT	270	55	1.5
20	SSRY 96	$(GT)_{12}$	GAGCAATCAAATTCAACAGCA	AAGCCGAAGCTTATGAAGGA	194	55	1.5

Appendix iv: PCR mixture used throughout the experiment

reagents	Reagent per sample (µl)	Reagent for 40 samples(µl)
Double distilled water	16.6	664
10x pcr buffer	2.0	80
dNTPs (5mM)	1.6	64
MgCl2 (25mM)	0.8	32
Primer reverse	0.4	16
Primer forward	0.4	16
Tag-polymerase	0.2	8
DNA template	3	

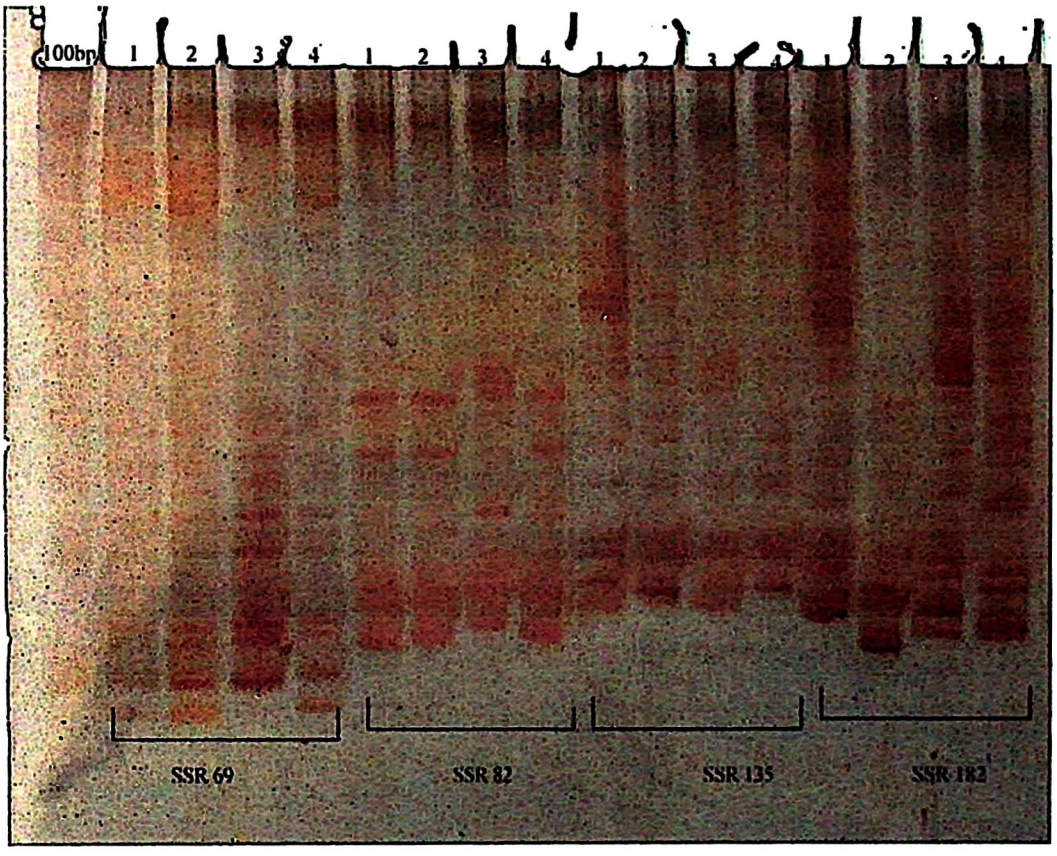
Appendix v: Schematic drawing of the PCR cycle. (1) Denaturing at 94°C. (2) Annealing at 55°C. (3) Elongation at 72°C (P=Polymerase). As described by Griffiths et al. (1996)



Appendix vi : Polyacrylamide gel solution

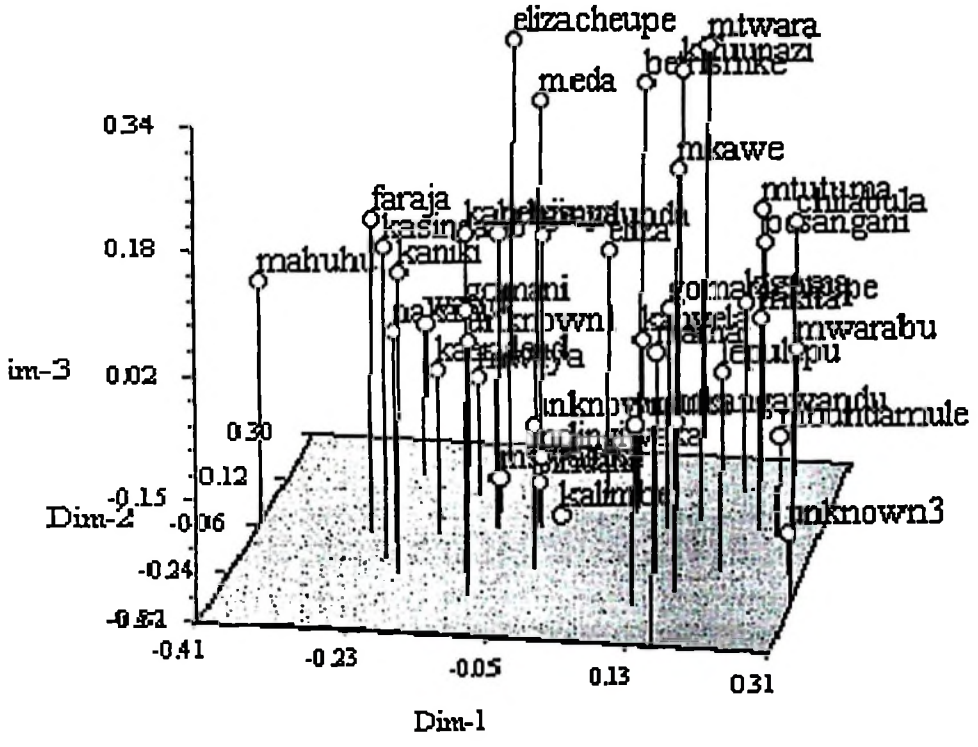
- 12.44ml of 10X TBE (Tris-Borate EDTA) buffer
- 17.72ml of Acrylamide solution
- 18.66ml Bisacrylamide solution
- 0.1g of ammonium persulfute (APS)
- 125µl of N, N, N', N'-tetramethylethylenediamine (TEMED
- 1 x TBE (pH 8.8) at 1000V, 90mA and 100W for about three hours

Appendix vii: Primer screen results



1= *Mkita* 2= *Mwaya* 3= *Kanyela* 4= *Mwarabu*).

Appendix viii: Principal component analysis (PCA) of cassava landraces used based morphological variations.



Appendix ix: Principal component analysis of 39 cassava landraces based on genetic distance calculated with 13 SSRY primers.