POPULATION STRUCTURE AND DIVERSITY OF EAST AFRICAN TARO [*Colocasia esculenta* (L.) SCHOTT]

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(I56/12254/09)

A thesis submitted in partial fulfillment of the requirements for the award of the degree of Master of Science in Biotechnology in the School of Pure and Applied Science of Kenyatta University.

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Population structure and diversity of East

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DECLARATION

DECLARATION BY CANDIDATE

I declare that I have independently prepared this work with no other than the indicated sources and support. This thesis has not been presented elsewhere for research or as an examinable paper.

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DEDICATION

This thesis is dedicated to my parents Steven M. Macharia and Lucy N. Macharia who have encouraged, supported and funded my education including my MSc studies.

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ACRONYMS AND ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
CTAB	Cetyltrimethyl Ammonium Bromide
DNA	Deoxyribonucleic Acid
EDTA	EthyleneDiamineTetraAcetic
Kb	Kilobase
PAGE	Polyacrylamide gel electrophoresis
PCA	Principle Component Analysis
PCR	Polymerase Chain Reaction
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
SSR	Simple sequence repeats
Taq	*Thermus aquaticus*
TE	Tris/EDTA
TBE	Tris/Borate/EDTA
TLB	Taro Leaf blight

ABSTRACT

Taro [*Colocasia esculenta* (L) Schott] belongs to the family Araceae. Taro is mainly produced in Africa by small holder farmers and plays an important role in the livelihood of millions of poor people in less developed countries. There is need to develop breeding programmes that target development of resistant and improved taro varieties with high yield, yield stability across broad agro-ecological sites, resistance to TLB and good eating quality. The genetic diversity of East African taro has not been determined; consequently there is no breeding programme for this vital root crop. This work establishes the genetic diversity of taro in East Africa using DNA microsatellite markers. Plant material consisted of 98 taro cultivars obtained from East Africa (Kenya, Tanzania and Uganda). DNA was extracted using the Cetyltrimethyl Ammonium Bromide method and six microsatellite primers previously shown to reveal high level of polymorphism in Polynesian taro were used to analyze 5 populations of Taro from three different regions of East Africa. Principal component analysis of SSR data indicated variation but did not show any geographical structure. Population diversity estimates was high for accessions sourced from Lake Victoria basin. Analysis of molecular variance (AMOVA) revealed most variation among individuals within population. Cluster analysis indicates that relatedness is not based on geographical proximity alone. It is envisaged that the result of this study will assist in establishing a regional collection that will be conserved and ensure a broad genetic base for available varieties and enable development of improved varieties through breeding programmes.

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CHAPTER ONE

INTRODUCTION

1.1 Background Information on Taro

Taro, *Colocasia esculenta* (L.) Schott or cocoyam is a member of the plant family Araceae. It is one of the most important food crops worldwide. The family comprises at least 100 genera and more than 1500 species (Cho *et al.*, 2007b). Taro is a traditional root crop of the tropics grown for its edible corms and leaves, and is believed to be one of the earliest cultivated root crops in the world (Plucknett, 1976). Worldwide production is on the increase, with Food and Agriculture Organization (FAO) records indicating that taro production has doubled over the past decade (FAO, 2000), and taro is now the fifth most-consumed root vegetable worldwide. Cultivated types are mostly diploid ($2n = 2x = 28$), although some triploids are also found ($2n = 3x = 42$) (Singh, 2004).

There are two botanical varieties of taro – *C. esculenta* var. *esculenta,* commonly known as dasheen, and *C. esculenta* var. *antiquorum*, commonly known as eddoe. Dasheen varieties have large central corms, with suckers and/or stolons, whereas eddoes have a relatively small central corm and a large number of smaller cormels (Mace *et al.*, 2006). In East Africa, taro is erroneously known as arrow root and most commonly by one of the local dialects (Kikuyu) name 'nduma'

(a)

(b)

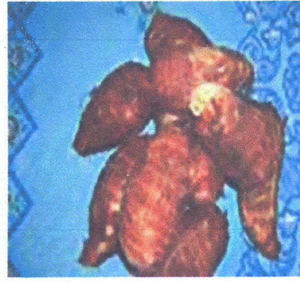
(c)

Figure 1: The taro plant and corms. (a) The taro plant. (b) Taro corm dasheen variety. (c) Taro corm eddoe variety. Dasheen has large central corm while eddoes have a small central corm with larger numbers of smaller cormels

1.1.1 Taro Distribution

Taro is postulated to have originated in southern or southeast Asia, and to have been dispersed to Oceania through the Island of New Guinea very many centuries ago. It is believed to have arrived on the east coast of Africa over 2,000 years ago from polynesia; it was taken by voyagers, first across the continent to West Africa, and later by slave ships to the Caribbean (Mbouobda *et al*., 2007). Although taro is a popular crop in West Africa, in East Africa, the extent of its production and consumption is not known, partly because it has been ignored as a legitimate crop for research and development, and is managed outside the conventional agricultural production, marketing and economic channels (Tumuhimbise, 2009).

The greatest intensity of its cultivation, and its highest percentage contribution to the diet, occurs in the Pacific Islands. However, the largest area of cultivation is in West Africa, which therefore accounts for the greatest quantity of production. In 1998, 6 million tonnes of taro/tannia were produced in the world on an area of 1.07 million hectares (the statistics combine taro and tannia).Tannia is from *Xanthosoma*

sagittifolium which is an aroid related to taro that is cultivated for its starchy corm (Chien-ying *et al.*, 2007a). The bulk of the production and area were in Africa, with Asia producing about half as much as Africa, and Oceania about one tenth as much (Onwueme, 1999).

1.1.2 Economic Importance of Taro

Taro plays an important role in the livelihood of millions of relatively poor people in less developed countries. Taro leaves and corms are used for human food in most producing countries. Taro peels and wastes are fed to domestic livestock. Efforts have been made to produce silage from the large quantities of taro tops which are left after the corms are harvested (Kuruvilla and Singh, 1981). Taro corms are highly priced in urban markets hence generates extra income to the rural farmers while its trade provides employment to many people .The crop also maintains ground cover in the fields (Tumuhimbise *et al.*, 2009). However, there is very limited local research on Taro in East Africa and its actual contribution to food security and economy is underestimated. Also, its profile on the national research and conservation agenda is low. In Uganda for example, the average taro yields in is less than 1 t ha^{-1}.For the majority of smallholder producers annually (Tumuhimbise *et al.*, 2009), compared to the African and world average of 5.9 and 6.6 t ha-1, respectively (FAO, 2008). It is possible to increase the status of taro in east Africa to the level of potato and sweet potato. This can be done by diversifying the taro germplam grown in East Africa including the Dryland (unflooded) or upland taro varieties.

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1.2 Problem Statement and Justification

Taro was introduced to East Africa through transoceanic crop transfer that took place across the Indian Ocean (Purseglove, 1972). A comprehensive analysis of the extent and distribution of the genetic variation in taro is essential for sound genetic conservation strategies. Conservation and sustainable use of genetic resources is essential to meet the demand for future food security. Successful conservation of any given gene pool is largely dependent on understanding the diversity and its distribution in a given region (Zhang *et al.*, 2000). Morphological characters in tubers are highly variable among the genotypes. This high variability is a result of natural mutations for traits like root and skin color, leaf and vine characteristics (Karuri *et al.*, 2009). However morphological variability may be as a result of environmental changes and therefore it is important to carry out a genetic diversity study using molecular markers. This study utilized 6 SSR molecular markers to analyze genetic diversity.

Recent molecular data reveal low genetic diversity in taro cultivars from Polynesia. If East African taro cultivars were founded from a narrow genetic base, the bulk of East African taro cultivars have low genetic diversity and are thus poorly adapted to cope with the threats of changing ecosystem. Low genetic diversity also implies limited alleles for manipulation in improvement programmes. In addition, taro in East Africa is mainly cultivated by small holder farmers that grow a few popular varieties mainly propagated vegetatively leading to fixation of a few plants of a particular genetic base and potentially, loss of some valuable genetic resources (Lebot and Aradhya, 1991). This study aims to quantify the level of genetic diversity and distribution of the genetic variation of taro germplasm within the East African region.

This information is crucial in formulating breeding programs aimed in improving the quality of taro germplasm in East Africa.

1.3 Null Hypothesis of the Study

Taro varieties in East Africa have no genetic diversity.

1.4 Objectives

1.4.1 General Objective

To determine genetic diversity of East African taro cultivars, using microsatellites markers.

1.4.2 Specific Objectives

i) To select appropriate markers for identification of Taro cultivars in East Africa.

ii) To determine the genetic diversity among and within Lake Victoria basin (Kenya, Uganda and Tanzania), Central Kenya and Eastern Kenya taro cultivars using microsatellites.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Colocasia esculenta* (L.) Schott (Taro)

2.1.1 Taro Taxonomy and Nomenclature

Taro (*Colocasia esculenta*) is a herbaceous plant belonging to the order *Alismatales*, family *Araceae*, subfamily *Aroideae*, tribe *Colocasiae*, genus *Colocasia* (Onwueme, 1994). In East Africa taro is erroneously known as arrow root. In Kenya it is popularly known by the local dialect word *Nduma* and *Magimbi* in Tanzania.

2.1.2 Ecology, Biology and Reproduction of Taro

Taro is a perennial root crop plant which grows to a height of 1-2m. The plant consists of a central corm (lying just below the soil surface) from which leaves grow upwards, roots grow downwards, while cormels, daughter corms and runners (stolons) grow laterally. The root system is fibrous and lies mainly in the top one meter of soil (Schnell *et al.*, 1998). Two principal botanical varieties of taro are recognized: *C. esculenta* var. *esculenta,* commonly known as dasheen, and *C. esculenta* var. *antiquorum*, commonly known as eddoe. Dasheen varieties have large central corms, with suckers and/or stolons, whereas eddoes have a relatively small central corm and a large number of smaller cormels (Mace *et al.*, 2006). Partly because of their large transpiring surfaces, taro plants have a high requirement for moisture for their production. Normally, rainfall or irrigation of 1,500-2,000mm is required for optimum yields. Taro thrives best under very wet or flooded conditions. Dry conditions result in reduced corm yields. Corms produced under dry conditions also tend to have a

dumb-bell shape; the constrictions reflect periods of reduced growth during drought. Taro does best in soil of pH 5.5-6.5 (Onwueme, 1994).

Taro is vegetatively propagated, generally from suckers, but this is difficult where cultivars have been selected for large corm size but produce very few suckers (Chien-Ying *et al.*, 2007b). *In vitro* techniques offer an alternative, reliable method for production of planting material (Pearson *et al.*, 1999).

In the dasheen types of taro, the corm is cylindrical and large. It is up to 30cm long and 15cm in diameter, and constitutes the main edible part of the plant. In eddoe types, the corm is small, globoid, and surrounded by several cormels (stem tubers) and daughter corms. The cormels and the daughter corms together constitute a significant proportion of the edible harvest in eddoe taro. Daughter corms usually give rise to subsidiary shoots even while the main plant is still growing, but cormels tend to remain dormant and will only give rise to new shoots if left in the ground after the death of the main plant. Each cormel or each daughter corm has a terminal bud at its tip, and axillary buds in the axils of the numerous scale leaves all over its body (Matthews and Eyzaguirre, 1993).

Corms, cormels and daughter corms are quite similar in their internal structure. The outmost layer is a thick brownish periderm. Within this lies the starch-filled ground parenchyma. Vascular bundles and laticifers ramify throughout the ground parenchyma. Idioblasts (cells which contain raphides or bundles of calcium oxalate crystals) also occur in the ground tissue and in nearly all other parts of the taro plant (Plucknet *et al.*, 1970). The raphides are associated with acridity or itchiness of taro, a

factor that is important in the marketing of taro corms (Roberts *et al.*, 1999). The density and woodiness of the corm increase with age.

Trimanto *et al.* (2009) found that occasionally in the field, some taro plants are observed to produce runners. These structures grow horizontally along the surface of the soil for some distance, rooting down at intervals to give rise to new erect plants. In eddoe and dasheen types of taro, the central corm represents the main stem structure of the plant. The surface of each corm is marked with rings showing the points of attachment of scale leaves or senesced leaves. Axillary buds are present at the nodal positions on the corm (Xu *et al.*, 2009). The apex of the corm represents the plant's growing point, and is usually located close to the ground level. The actively growing leaves arise in a whorl from the corm apex (Djukri, 2006). These leaves effectively constitute the only part of the plant that is visible above ground. They determine the plant's height in the field.

Each leaf is made up of an erect petiole and a large lamina. The petiole is 0.5-2m long and is flared out at its base where it attaches to the corm, so that it effectively clasps around the apex of the corm. The petiole is thickest at its base, and thinner towards its attachment to the lamina. Internally, the petiole is spongy in texture, and has numerous air spaces which presumably facilitate gaseous exchange when the plant is grown in swampy or flooded conditions (Matthews, 1990). For most taro types, the attachment of the petiole to the lamina is peltate, meaning that the petiole is attached, not at the edge of the lamina, but at some point in the middle. This peltate leaf attachment generally distinguishes taro from tannia which has a hastate leaf i.e. the petiole is attached at the edge of the lamina (TaroGen, 2000).

The lamina of taro is 20-50cm long, oblong-ovate, with the basal lobes rounded. It is entire (not serrated), glabrous, and thick (Onwueme, 1999). Three main veins radiate from the point of attachment of the petiole, one going to the apex, and one to each of the two basal lamina lobes. Some prominent veins arise from the three main veins, but the overall leaf venation is reticulate (net-veined).

2.1.3 Morphological Diversity in Taro Germplasm

Both wild type and domesticated varieties of taro differ in many ways. Wild type taro is much more acrid, has relatively small corms (swollen stems that store starch), bears long thin stolons (runners) rather than short, thick and starchy side-corms, and has leaves that are almost entirely green. Wild type plants are not genetically uniform, but they are very similar in morphology over a large geographical range (Lebot *et al.*, 2004). Domesticated forms of taro are found over a much larger geographical and environmental range, and are very diverse in morphology, eating qualities, maturation speed, and storage characteristics. There are possibly hundreds of tropical, subtropical and temperate-adapted forms of cultivated taro, but no exact figures can be given - partly because there are no fixed criteria for distinguishing cultivars (Matthews, 1991).

Out of the morphological parameters studied, a list of minute descriptors for the proper characterization and evaluation of cocoyam have been established for both the white cultivar and the red cultivar. Standardized morphological descriptors used for characterizing national germplasm collections are: growing conditions, altitude, botanical variety, growth habit, stolon formation, plant height, shape of lamina, orientation of lamina, leaf lamina margin, lamina, color, sinus, vein junction, color of

leaf petiole, variation on petiole, flowering, resistance against leaf blight, maturity, corm shape, flesh color and eating quality (Lebot *et al.*, 2004). These descriptors, besides being genotype-dependent, are probably dependent on the climatic and edaphic conditions. Indeed, although cultivated under the same microclimate, these plants conserved the characteristics acquired previously and provided vital information on the morphological diversity.

2.2 Taro Constraints

The greatest constrain to taro production is taro leaf blight disease caused by *Phytophthora colocasiae*. It was first reported in Java about a century ago, and has since spread to various parts of Asia and the Pacific (Onwueme, 1999). The disease begins as purple-brown water-soaked lesions on the leaf. A clear yellow liquid oozes from the lesions. These lesions then enlarge, join together and eventually destroy the entire lamina in 10-20 days. Free water collecting on older leaves, as well as high temperature and high humidity are conducive to onset and spread of the disease and germination of the spores (Yen, 1991). The disease can be spread from plant to plant by wind and splashing rain. Spores survive in planting material for three or more weeks. Thus, infected planting material is one common means of spreading the disease over long distances and from season to season. The disease can cause yield losses of 30-50%, and results in lowering of the quality of the reduced harvest. Also taro leaves for human consumption are rare in affected areas. Most countries where the disease has been reported are under strict quarantine isolation (Irwin *et al.*, 1998).

Various approaches have been used to try to control the taro leaf blight. Agronomic methods that have given partial success include careful choice of planting material,

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planting at high density, intercropping taro with other crops rather than growing it as a sole crop, and crop rotation. Field removal of infected leaves has also been useful, but it is extremely laborious. In Samoa, control has been achieved by an intensive spraying programme with Ridomil or Manzate, and more recently with phosphorous acid (Hollyer, 2007). Chemical control is extremely tedious, expensive, and not totally effective. An integrated control approach combining cultural and chemical methods seems to be the best at present. The ultimate solution must lie in the breeding and release of resistant cultivars. The taro breeding programme in Bubia, Papua New Guinea, has already identified several promising lines in this regard (Mace and Godwin, 2006).

In some countries, the taro leaf blight is present but causes relatively minute or economic damage. This is true of the Philippines, Thailand and Hawaii. In other cases such as Samoa and American Samoa, the disease can be devastating (Jackson *et al.*,1980; Jatala and Bridge, 1993). This situation has led to conjectures about the possibility that various strains of *Phytophthora colocasiae* may exist, and that in south-east Asia in particular, some of these strains may have evolved along with the taro crop and may be less virulent (Onwueme, 1994). This factor is in addition to differences in the genetic make-up and genetic diversity of the taro crop in each country.

The alomae virus disease is also a constraint of taro and is caused by a complex of two or more viruses acting together. The two viruses that are definitely involved are the taro large bacilliform virus (TLBV) which is transmitted by the plant hopper *Tarophagus proserpina*, and the taro small bacilliform virus (TSBV) which is

transmitted by the mealybug *Planococus citri* (Rodoni, 1995). Neither virus is transmissible by mechanical contact, nor does their host range seem limited to aroids only. The full-blown alomae disease occurs when these two viruses (and possibly others) are present. Presence of only TLBV alone results in bobone, a milder form of the disease.

Alomae virus disease first starts as a feathery mosaic on the leaves. Lamina and veins become thick. The young leaves are crinkly and do not unfold normally. The petiole is short and manifests irregular outgrowths on its surface. The entire plant is stunted and ultimately dies. The symptoms of bobone are similar, but the leaves are more stunted and the lamina is curled up and twisted. With bobone, complete death of the entire plant does not usually occur.

Severe cases of alomae virus disease can result in total crop loss, while bobone can cause up to 25% yield loss. However, in many instances, only isolated plants in taro fields seem to be affected by either disease, and in the case of bobone infected plants may recover from the symptoms. Gunua and Kokoa (1995) reported the alomae/bobone disease complex in Papua New Guinea and Solomon Islands. The disease is controlled by pulling out diseased plants in the field, and by careful selection to ensure disease-free planting material. Ultimately, control will have to rely on breeding and disseminating resistant cultivars. Some tolerant cultivars bred through recurrent selection, have been released in Solomon Islands since 1992.

While the alomae/bobone disease is mainly confined to the Pacific, the dasheen mosaic virus disease occurs world-wide (Onwueme, 1997). Most taro-producing

countries in the Asia/Pacific region have the disease. Dasheen Mosaic Virus is caused by a stylet-borne, flexuous, rod-shaped virus that is spread by aphids. It is characterized by chlorotic and feathery mosaic patterns on the leaf, distortion of leaves, and stunted plant growth. The disease is not lethal, but yield is depressed. Control is through the use of Dasheen Mosaic Virus-free planting material, field sanitation, and quarantine measures (Lebot, 2004). Other diseases and pests of taro include: i) Corm and root rots caused by the fungi *Pythium* spp and *Phytophthora,* ii) nematodes, iii) taro planthopper, *Tarophagus proserpina* which not only transmits virus diseases, but can cause wilting and death of the plant in heavy infestations, iv) aphids, v) taro hornworm which defoliates the plant and vi) armyworms or cluster caterpillars which can also do extensive damage to the leaves.

While these diseases and pests may be considered minute, these pathogens infect the leaves, destroying tissues and thereby reducing plant growth, corm size and quality (Lebot *et al.*, 2004). Other pests of the taro include the taro beetle. To control taro pests and disease, the use of insecticides has been the most commonly adopted method (Onwueme, 1999). Due to the deleterious effects of insecticides to man, environment and livestock, alternative strategies for the insect pest management and disease have been developed including breeding programmes that target development of resistant cultivars.

These programmes target the development of improved taro varieties with high yield, yield stability across broad agro-ecological sites, resistance to Taro leaf blight and high food quality. The genetic diversity contained in different varieties provides breeders with options to develop, through selection and breeding, new and more

productive crops that are resistant to pests and diseases (Lebot *et al.*, 2005). Genetic diversity in taro has been surveyed with tests for ribosomal DNA (Matsuda, 2001), chloroplast DNA (Tahara *et al.*, 1999) and mitochondrial DNA (Matthews et al., 1992). All of these studies indicate that cultivated forms of taro are very heterogeneous.

This study has used molecular genetic markers to assess the genetic differentiation of taro cultivars in East Africa and determine their phylogenetic origin. This data will aid in formulating informed programmes for future genetic improvement of taro germplasm in East Africa.

2.3 Approaches of Measuring Genetic Diversity

Genetic markers can be divided into three classes; morphological, biochemical and more recent DNA based markers.

2.3.1 Morphological markers

Morphological markers displaying Mendelian inheritance have been in use for long to characterise genetic variation. They are difficult to use in plant genetics for the following reasons: they are often recessive in nature therefore heterozygotes are not identifiable; they are generally mutations and often confer a deleterious phenotype to the organism, therefore decreasing its fitness; they may also exhibit epistatic effects (interaction between genes) or their expression may be affected by environmental conditions and may confer a phenotype that is only apparent at one stage of an organism (Geburek and Turok, 2005).

2.3.2 Biochemical markers

Biochemical markers can either be at protein level such as isozymes or at organic chemical levels such as terpenes and they examine the products of genes (Ferguson and Grabe, 1986). Protein markers were among the first group of biochemical markers exploited for genetic diversity assessment and genetic linkage map development (Hash and Bramel-Cox, 2000).

The weakness of isozyme markers is that each of the proteins that are being scored may not be expressed in the same tissue and at the same type of development. Therefore several samplings of the genetic population need to be made. Biochemical markers have not been used in assessing diversity of taro cultivars.

2. 3.3 DNA Based Markers

Molecular markers are neutral and detect variation in non-coding regions of the genomes, which are fast evolving (Powell *et al.*, 1996). These are therefore able to detect high levels of genetic variation such as single nucleotide mutation as well as insertions and deletions (Powell *et al.*, 1996). Among these molecular markers, hybridization based DNA markers such as restriction fragment length polymorphism (RFLP) and PCR based DNA markers such as random amplified polymorphic DNAs (RAPDs), Microsatellites and amplified fragment length polymorphisms (AFLP), have been used in genetic diversity studies.

2.3.3.1 Restriction Fragment Length Polymorphism

Restriction Fragment Length Polymorphism is defined as the variation(s) in the length of DNA fragments produced by a specific restriction endonuclease from genomic DNAs of two or more individuals of a species (Kahl, 2001). RFLP was first developed in the early 1980s for use in human genetic applications for determining genetic relationships (Botstein *et al.,* 1980) and was later applied to plants (IAEA, 2002). RFLPs have been used to document genetic diversity in cultivated plants and their wild relatives (Diers and Osborn, 1994; Weising *et al.,* 2005). Variations in fragment length between individuals or species can arise either when mutations alter restriction sites, or result in insertions / deletions between them (Burr *et al*., 1983).

Because heterozygous individuals are distinguishable, RFLPs are co-dominant markers. The complexity in performing RFLP analysis, coupled with the widespread use of short-lived radioisotopes, has led to its limitation for routine application in large-scale crop improvement programmes (Yamamoto *et al.,* 1999). Although highly specific, performing RFLPs is quite tedious and expensive since it requires large amounts of pure quality DNA and expertise in handling radioactivity.

2.3.3. 2 Random Amplified Polymorphic DNA (RAPD)

The development of polymerase chain reaction (PCR) for amplifying DNA led to a revolution in the applicability of molecular methods and a range of new technologies have been developed which can overcome many of the technical limitations of RFLPs. The most common version is RAPD (Random amplified polymorphic DNA) analysis, in which the amplification products are separated on agarose gels in the

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presence of ethidium bromide and visualized under ultraviolet light (William *et al.*, 1990). It's cost-effective and easy to perform approach which gives satisfactory results even with crude DNA preparations. The enormous attractions of these arbitrary priming techniques are; there is no requirement for DNA probes or sequence information for the design of specific primers, since the procedure involves no blotting or hybridizing steps, it is quick, simple and automatable and very small amounts of DNA (10 nanograms per reaction) are required.

It is absolutely critical, however, to maintain strictly constant PCR reaction in order to achieve reproducible profiles (Karp and Edwards, 1996). RAPDs have therefore been extensively used in assessing genetic relationships amongst various accessions of different plant species (Wachira *et al.*, 1995). However, it has limitations associated with dominance and the potential non-homology of apparently similar characters states and its lack of specificity and reproducibility. It has been observed that RAPD profiles are highly sensitive to variations in the concentrations of template DNA (Davin-Regli *et al.*, 1995), magnesium ions, *Thermus aquaticus* polymerase and thermal cycler used. Thus, the results obtained through RAPDs can be arbitrary. The results from a molecular study of taro genetic using randomly amplified polymorphic markers (RAPD) by Irwin *et al.* (1998), confirmed that although the cultivars in the pacific region exhibit morphological variation the genetic base is very low.

2.3.3.4 Amplified Fragment Length Polymorphism

Amplified fragment length polymorphisms, a PCR based assay for plant DNA fingerprinting, combines the specificity of restriction analysis with PCR amplification (Zabeau and Vos, 1993; Vos *et al.*, 1995). The steps involved in AFLP are restriction

digestion/ligation, pre-selective amplification, selective amplification and visualization. The technique has become an attractive tool because it generates a high number of polymorphic products (Powell *et al.*, 1996), and is reproducible across laboratories (Jones *et al.*, 1997). Amplified Fragment Length Polymorphism analysis has been used to analyze the geographical differentiation, phylogenetic relationships and to identify AFLPs linked to TLB resistant genes of Indian taro. Significant differentiation in taro cultivars was demonstrated by AFLPs. Results showed that AFLP can be used to distinguish taro cultivars by their unique and different banding patterns (Kamal *et al.*, 2008). This further supports the evidence for the genetic variation in the genome of *C. esculenta.*

2.3.3.5 Simple Sequence Repeats (SSR)/Microsatellite

Microsatellites are DNA sequences composed of a tandem repetition of a simple short sequence, occurring in the genome of many higher organisms (Rafalski *et al.*, 1993). Simple sequence repeats are widely distributed in higher plants (Morgante and Olivieri, 1993). Although they are ubiquitous (Kijas *et al.*, 1995), retrieval of SSRs has not been easy in plants because of their relatively low abundance compared with animal/human genomes. The variation in size of SSR's comes from differences in number of repeat units originating from error in copying of DNA during replication by DNA polymerase (Robinson and Haris, 1999). Providing the sequence of the DNA surrounding a microsatellite is known and suitable PCR primers can be designed, the segment of DNA incorporating the microsatellite can be amplified and its length determined by electrophoresis. Multiple allelic length variants can be identified at

most microsatellite loci (Rafalski *et al.,* 1993). Microsatellite markers are highly polymorphic, codominant loci and are abundant in most of species genomes.

However, the high number of alleles per locus causes some bias in diversity estimates due to increased heterozygosity levels. The high mutation rates also means that microsatellites suffer from homoplasty problems and may also increase within-population component of variation. Since each marker has advantages and disadvantages, the choice of a specific marker depends on project objectives, time required for analysis, availability of equipments and skilled personnel as well as financial resources (Glaubitz and Moran, 2000). Simple sequence repeats (SSR), have been employed to study taro resources; sixteen microsatellites have already been developed for *C. esculenta* (Mace and Godwin, 2002), and seven of them were used in the SSR analysis for the taro germplasm resources in the Pacific Island region (Mace *et al.* 2006). In China, 11 novel microsatellite markers were isolated and developed from taro germplasm.

Mace and Godwin (2002), using microsatellites from an enriched library of *C. esculenta* revealed polymorphism among the different accessions of *C. esculenta* from the Pacific island region and Southeast Asia. Using seven primers they had designed they were able to characterize a core collection of 81 accessions in Papua New Guinea. Mace *et al.*, (2006), characterized taro germplasm from the Pacific island region and recommended 221 accessions for inclusion in the final regional core collection based on the molecular characterization using the same primers. However, the microsatellite markers were not transferable across genera to *Xanthosoma* (Singh *et al.*, 2008).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Plant material

Plant material consisted of taro cultivars obtained from East Africa in particular the Lake Victoria basin that covers three countries: Kenya, Tanzania and Uganda. Additional samples from Central and Eastern Kenya were included in the study. The different cultivars were collected from farmers. Collections involved both principal botanical varieties of taro – *C. esculenta* var. *esculenta*, commonly known as dasheen, and *C. esculenta* var. *antiquorum*, commonly known as eddoe. Dasheen varieties have large central corms, with suckers and/or stolons, whereas eddoes have a relatively small central corm and a large number of smaller cormel (Purseglove, 1972). The planting material was collected as apical 1-2 cm of the corm with the basal 15-20 cm of the petioles attached; use of apices, as planting material is particularly advantageous because it does not entail the utilization of much material that is otherwise edible. Moreover, apices establish very quickly and result in vigorous plants. The apices attached to part of the corms were planted in pots in the green house and appropriately labeled. The plants were watered every day to ensure growth, after a month young shoots had already formed.

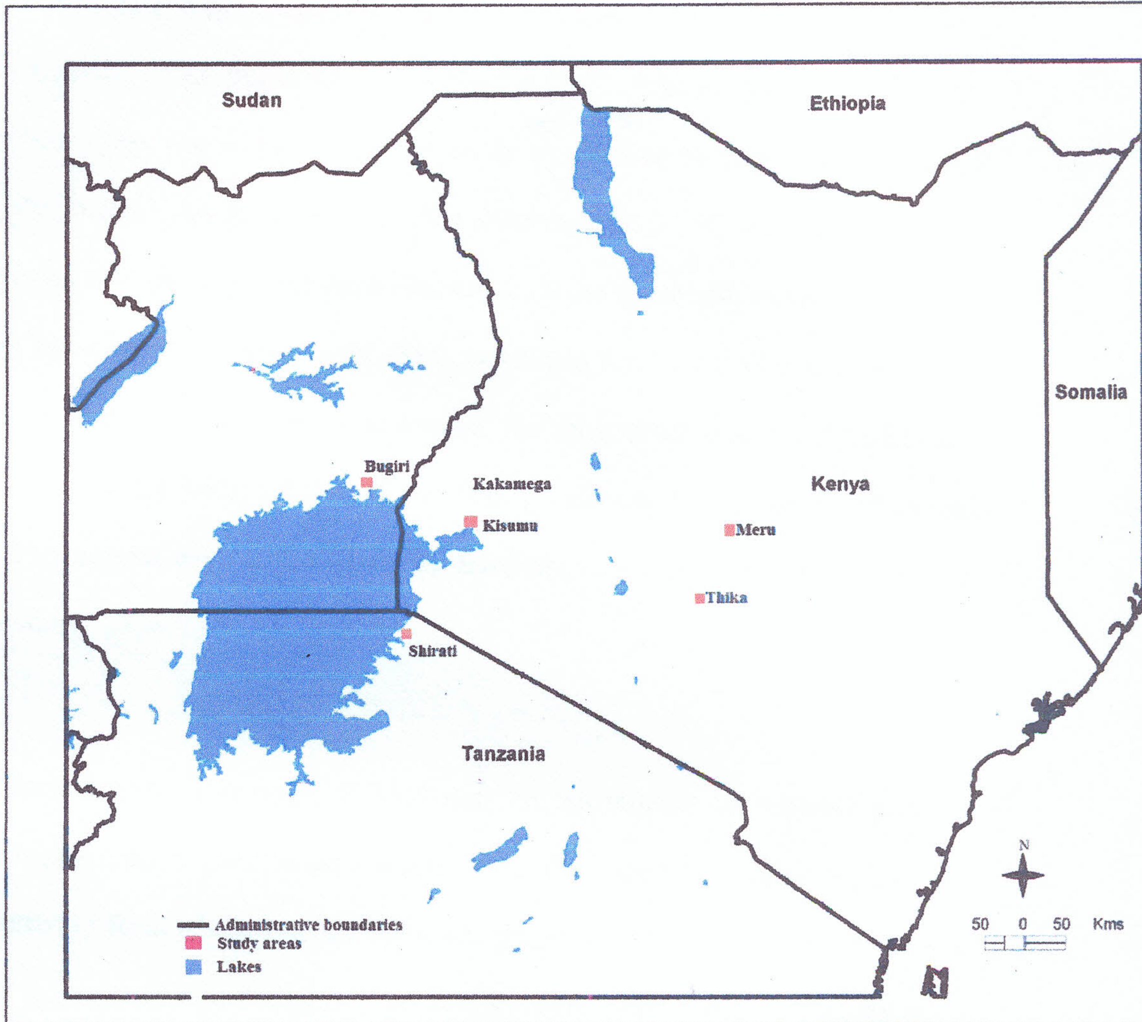

Figure 1: Study sites where plant materials for the study were collected in East Africa.

3.2 DNA Isolation

DNA was extracted according to the CTAB protocol modified and optimised by Sharma *et al*. (2008). 500 mg of fresh leaf material was collected and washed in distilled water and rinsed with 80% ethanol. The surface sterilised leaves were weighed and placed in 1.5 ml tube; a stainless steel ball was placed inside each tube (to aid in lyophilisation of frozen leaves) and locked.

The tubes were placed in liquid nitrogen and vortexed until the tissue was converted to fine powder. CTAB buffer was pre-warmed at 60 °C. In each tube, 1.5 ml of CTAB extraction buffer was added and mixed gently by swirling to homogenize the tissue with the buffer. The samples were then incubated at 60 °C for 1hr with gentle continuous rocking. The tubes were removed from the water bath and allowed to cool for 10 minutes. The samples were then centrifuged for 10 minutes at 3500 rpm at room temperature. Two layers were formed; the supernatant was transferred to new labelled tubes and 500µl of chloroform: Isoamyl alcohol (24:1) was added to each tube. The samples were mixed gently by continuous rocking for 10 minutes at room temperature.

The samples were centrifuged at 3500 rpm for ten minutes to generate a yellow aqueous phase and a green organic phase tubes. The supernatant aqueous phase was transferred to fresh labelled tubes. DNA was precipitated by adding equal volume of chilled isopropanol into the tubes. The samples were incubated at -20° C for 1hr in order to form a precipitate. The samples were centrifuged at 3500 rpm for ten minutes. The supernatant was drained taking much care not to spill the pellet. The tube was inverted on clean soft tissue for 1hr; to dry the pellet. The pellet was re-suspended in 50µl of distilled water, and then 5µl of sodium acetate solution (NaoAc) and 550 ml of absolute ethanol were added. The mixture was incubated at -20° C for 1hr. The mixture was then centrifuged at 3500rpm for 8 minutes and the supernatant drained. The tube was inverted on soft tissue for 1hr to dry the pellet. The DNA pellet was re-suspended in 50µl of double distilled water and left overnight at room temperature. The DNA was then stored at 4° C awaiting further analysis.

3.2.1 Determination of DNA Quantity

DNA samples were electrophoresed in a 1% agarose gel in 1× TBE buffer (0.045 M Tris-Borate and 0.001 M EDTA) at 100 V for 1 hour. Each DNA sample (5µl) was mixed with 6 X gel loading dye (0.25% xylene cyanol and 30% glycerol) prior to loading. The quantity of DNA in the sample was then estimated by comparing florescent yield of the sample with standard cut Lambda DNA run alongside the DNA samples.

The spectrophotometer method was used for checking the purity of DNA extracted, The Optical Density (OD) was taken at 260nm and 280nm and the ratio OD 260: OD 280 calculated. This ratio provided an estimate of purity of nucleic acid. Pure preparations of DNA have OD 260: OD 280 ratios of 1.8 (Sambrook *et al.*, 1989: Hoisington *et al.*, 1994).

3.3 Microsatellite-PCR Reaction

Six SSR primer previously shown to reveal high level of polymorphism in Polynesian taro cultivars (Mace and Godwin, 2002) were used as shown on Table 1 . The PCR reaction mixture was similar for each primer with varying cycling conditions. The PCR mixture (10µl) contained 1µl of template DNA, 0.05µl forward primer and 0.05µl reverse primer, 0.05µl of each dNTP, 1µl of reaction buffer that contained magnesium chloride and 0.25ul *Taq* DNA polymerase. The PCR regime included; initial denaturation (94° C for 5minutes), 35 cycles each consisting of 30s denaturation (94° C), 1 minute at annealing temperature ranging from 55° C to 59° C

(Table 4.1) and 2 minutes elongation (72°C). Finally an extension period of 10 minutes was included.

For detection via electrophoresis only samples amplified with primer Xuqtem110 were analyzed using Polyacrylamide gel electrophoresis since its products were small and could not be resolved using agarose gel electrophoresis. Native PAGE (10% acrylamide/bisacrylamide (29:1), solution for a minigel was prepared as follows. For each 10ml of mixture 3.45ml of distilled water was mixed with 4ml of 40% acrylamide-bisacrylamide mix (29:1). Buffer (2.6 ml of 1×TBE) was added followed by 100µl of 10% ammonium persulfate and finally 10µl of TEMED. The acrylamide solution was poured between the plates of the minigel apparatus and a comb was put in between the plates taking care not to form any bubbles. The gel was allowed to dry for one hour after which the plates were placed in the vertical gel tank. The comb was removed to reveal the wells and 2µl aliquot of product was mixed with 1µl of loading dye and 1µl of SYBR green (a nucleic acid stain) was be loaded in each well. Buffer (1×TBE) was added and the system closed after which it was run at 70V for 1hr. The gel was removed carefully from the plates and dried at room temperature for 10minutes and then visualized under UV light.

All other products that were amplified using primers Xuqtem73, Xuqtem 55, Xuqtem88, Xuqtem97, and Xuqtem91 were resolved using 2 % agarose gel. Agarose gel was prepared using TAE buffer. The samples were premixed with 1µl of SYBR green and a µl of loading dye. The samples were run at 100V for 2 hours and the gel visualized under UV light.

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Table 1: Primers used in the study

SSR primer	Repeat type	Primer sequence (5→□′)	*Tm* (°C)	Resulting size in (bp)	No. of alleles per locus
Xqutem110	(TGA)6(TGGA)4	FWD: 2 AGCCACGACACTCAACTATC RVS: 2 GCCCAGTATATCTTGCATCTCC	57.0	250-400	3
Xqutem73	(CT)15	FWD: 3 ATGCCAATGGAGGATGGCAG RVS: 3 CGTCTAGCTTAGGACAACATGC	55.0	100-500	7
Xqutem55	(CAC)5	FWD: 4 CTTTTGTGACATTTGTGGAGC RVS: 4 CAATAATGGTGGTGGAAGTGG	57.0	300-550	5
Xqutem88	(CAT)9	FWD: 5 CACACATACCCACATACACG RVS: 5 CCAGGCTCTAATGATGATGATG	59.0	100-600	7
Xqutem97	(CA)8	FWD: 6 GTAATCTATTCAACCCCCCTTC RVS: 6 TCAACCTTCTCCATCAGTCC	56.0	200-500	6
Xqutem91	(TG)6(GA)4	FWD: 7 GTCCAGTGTAGAGAAAAACCAG RVS: 7 CACAACCAAACATACGGAAAC	55.0	250-500	4

3.4 Data analysis

3.4.1 Nei's Estimates and Cluster Analysis

Banding patterns observed at particular locus were observed as a presence/absence matrix. The bands were scored in a binary form which were configured as an input file and analyzed with POPGENE 1.31(Yeh and Yang, 1999). The index proposed by

Nei and Li (1979) was used to calculate genetic similarities (S_{ij}) between cultivars(i) and (j) as;

$$S_{ij=}2N/ (N_i+N_j)$$

Where Nj = the number of bands (alleles) in common between cultivars $_i$ and $_j$, N_i and N_j are the number of alleles for cultivars $_{j,\text{ and } I}$ respectively. The similarities were used to derive genetic diversity trees by average linkage cluster analysis. Differentiation among group samples (i.e. from the different taro growing areas) was estimated using average linkage.

3.4.2 Principal Component Analysis

A principal component analysis (PCA) was carried out using GenAlEx 6.3 (Peakall and Smouse, 2009) software according to variance/covariance method to determine the one variable that can be used to assess the differences among taro varieties. PCA explores common patterns of variation among groups and subgroups of accessions based on their variance/covariance structure and reduces the observed variation to a few (usually 2 or 3) linear combinations of the original variables (Ortiz, 1999).

3.4.3 Analysis of Molecular Variance (AMOVA)

Based on individual product profiles, a Euclidean distance matrix was generated and analyzed with the ARLEQUIN ver.3.11 software package (Excoffier *et al.*, 2005). Genetic variation was subsequently partitioned within and between populations according to an analysis of molecular variance and significance values assigned to

variance components based on random permutation (99 times) of individuals assuming no genetic structure.

CHAPTER FOUR

RESULTS

4.1. DNA Extraction

High quality DNA was obtained using a modified CTAB protocol. The quantity of DNA samples was estimated to be 20ng/μl using ethidium bromide staining and a series of DNA standards of known concentration before use in SSR profile analysis.

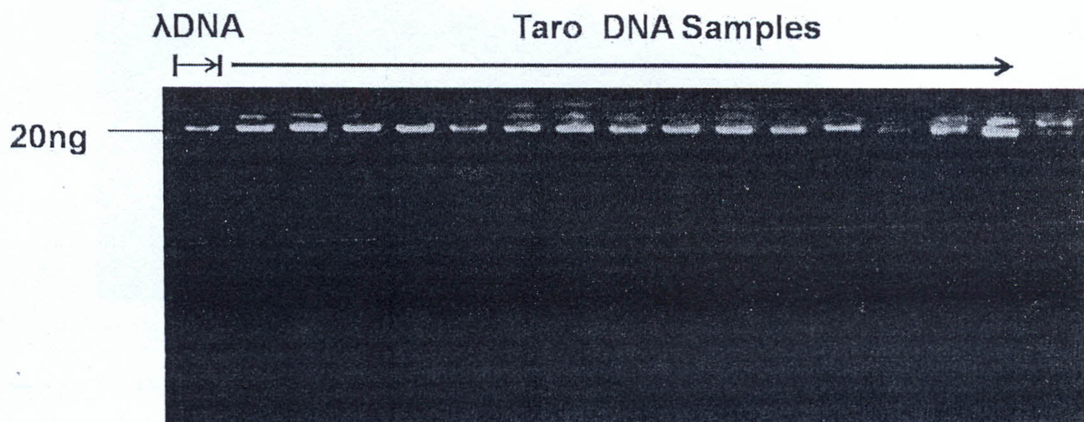

Figure 3:1% Agarose gel showing quality and quantity of Taro DNA extracted using the CTAB method

4.2 Analysis of SSR data

Six primers developed by Mace and Godwin (2002) were used and showed different levels of polymorphism. The six primers used differed in the ability to identify unique multiband phenotypes among the 98 accessions. The number of individual bands generated by each primer was checked visually and scored as presence and absence matrix. In total 31 alleles were amplified from six SSR primers across 98 accessions of which 85% were polymorphic. No region specific markers were amplified. Xuqtem 88 and Xuqtem 73 had the highest number of alleles; 7 alleles while Xuqtem 110

amplified only two alleles. Alleles were between 1500bp and 100bp.Varieties from Central region exhibited a maximum of 4 alleles regardless of the primer used. Varieties from Lake Victoria basin and Eastern Kenya had exhibited all the possible alleles. Primer Xuqtem 110 and Xuqtem 91 exhibited polymorphism across all the populations.

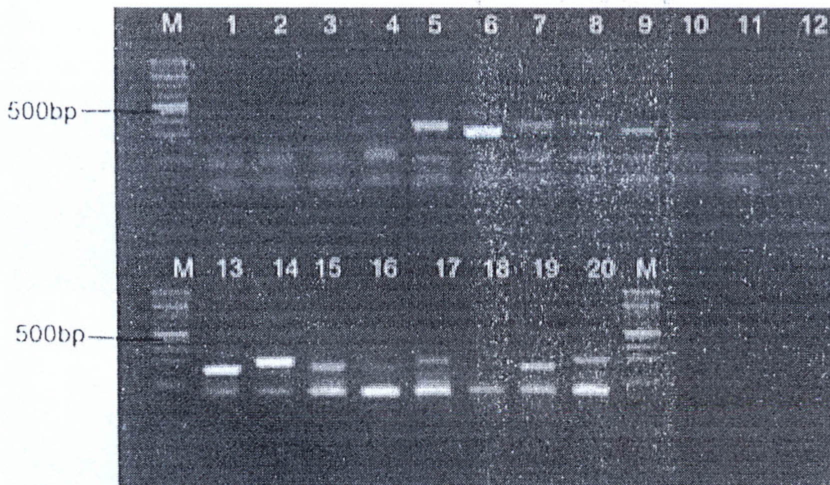

Figure 2: Detection of polymorphism using SSR primers (Xqutem 110) M is 1Kb marker. Samples 1-12) represent random samples amplified from the Lake Victoria basin while 13 -20 represents random samples from Eastern Kenya.

4.5 Marker Analysis

All markers used in this study were informative with Xqutem 91 having the highest heterrozygosity of 0.4 across all the populations. There were no private alleles among all the populations in figure 5.

Table 2: A table showing band Frequency, Estimated Allele Frequency (p & q), Samples Size, No. Alleles, No. Effective Alleles, Information Index, Expected and Unbiased Expected Heterozygosity.

Pop	Locus	Band Freq.	p	q	N	Na	Ne	I	He	uHe
(girigasha)Central	1	0.500	0.293	0.707	10.000	2.000	1.707	0.605	0.414	0.436
	2	0.000	0.000	1.000	10.000	0.000	1.000	0.000	0.100	0.000
	3	0.700	0.452	0.548	10.000	2.000	1.982	0.689	0.495	0.522
	4	0.000	0.000	1.000	10.000	0.000	1.000	0.000	0.300	0.000
	5	0.000	0.000	1.000	10.000	0.000	1.000	0.000	0.213	0.000
	6	0.000	0.000	1.000	10.000	0.000	1.000	0.000	0.110	0.000
Eastern Kenya	1	0.783	0.534	0.466	23.000	2.000	1.991	0.691	0.498	0.509
	2	0.217	0.115	0.885	23.000	2.000	1.256	0.358	0.204	0.209
	3	0.435	0.248	0.752	23.000	2.000	1.595	0.560	0.373	0.381
	4	0.000	0.000	1.000	23.000	0.000	1.000	0.000	0.000	0.000
	5	0.174	0.091	0.909	23.000	2.000	1.198	0.305	0.166	0.169
	6	0.783	0.534	0.466	23.000	2.000	1.991	0.691	0.498	0.509
kigoi(Central Kenya)	1	0.200	0.106	0.894	10.000	2.000	1.233	0.337	0.189	0.199
	2	0.100	0.051	0.949	10.000	2.000	1.108	0.202	0.097	0.102
	3	0.600	0.368	0.632	10.000	2.000	1.869	0.658	0.465	0.489
	4	0.000	0.000	1.000	10.000	0.000	1.000	0.000	0.050	0.000
	5	0.000	0.000	1.000	10.000	0.000	1.000	0.000	0.070	0.000
	6	0.400	0.225	0.775	10.000	2.000	1.537	0.534	0.349	0.368
tannia (lake region)	1	0.727	0.478	0.522	22.000	2.000	1.996	0.692	0.499	0.511
	2	0.636	0.397	0.603	22.000	2.000	1.919	0.672	0.479	0.490
	3	0.364	0.202	0.798	22.000	2.000	1.476	0.504	0.323	0.330
	4	0.136	0.071	0.929	22.000	2.000	1.151	0.255	0.131	0.134
	5	0.409	0.231	0.769	22.000	2.000	1.552	0.541	0.356	0.364
	6	0.455	0.261	0.739	22.000	2.000	1.629	0.575	0.386	0.395
taro(lake region)	1	0.727	0.478	0.522	33.000	2.000	1.996	0.692	0.499	0.507
	2	0.667	0.423	0.577	33.000	2.000	1.953	0.681	0.488	0.496
	3	0.636	0.397	0.603	33.000	2.000	1.919	0.672	0.479	0.486
	4	0.333	0.184	0.816	33.000	2.000	1.428	0.477	0.300	0.304
	5	0.061	0.031	0.969	33.000	2.000	1.063	0.137	0.060	0.061
	6	0.697	0.450	0.550	33.000	2.000	1.980	0.688	0.495	0.503

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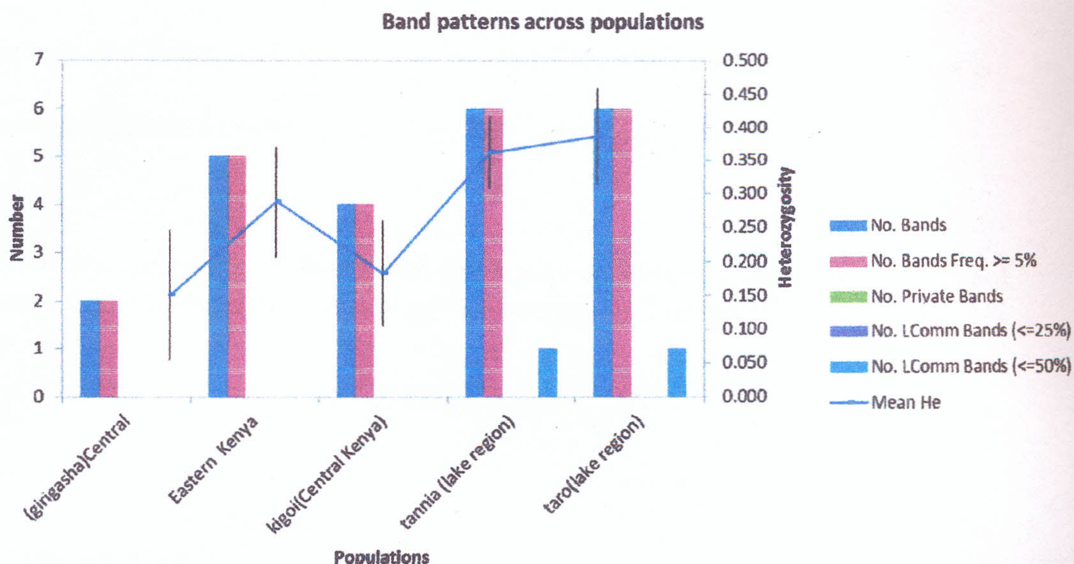

4.3 Population genetic structuring

4.3.1 Mean diversity Estimates

Five populations namely, Lake Victoria basin (taro), Lake Victoria basin (tannia), Central Kenya(Taro-girigasha),Central Kenya (Taro-Kigoi) and Eastern Kenya(Taro). were analyzed The gene diversity values (H) ranged from 0.2783 for Lake Victoria basin (Taro) and 0.1863 for Eastern Kenya as shown in table 4.2. The highest proportion of percentage polymorphic loci was found in Taro from Lake Victoria basin while the lowest was found in Central Kenyan taro (Kigoi variety). Girigasha variety from Central Kenya had 64 % of polymorphic loci while taro from Eastern Kenya had 58.06% polymorphic loci (Table 3). A general trend between the highest % polymorphic loci and highest gene diversity was observed. The standard error for all the population was generally high from between ± 0.1859 in taro from private alleles across the entire collection. Accesions of Taro from Lake Victoria basin were the most distinguishable based on the SSR data set.

alleles across the entire collection. Accesions of Taro from Lake Victoria basin were the most distinguishable based on the SSR data set.

Table 3: Mean diversity estimates (H) for 5 populations of C.esculenta generated from &SSR markers. (The percentage polymorphic loci and sample size (N) is shown).

Ecotypes	N	*H*	I	% Polymorphic Loci
Lake Victoria basin (Taro)	33	0.2783	0.4871	90.32%
Lake Victoria basin (Tannia)	22	0.2478	0.4672	80.65%
Central Kenya (Taro-Girigasha)	10	0.1774	0.3590	64.52%
Central Kenya (Taro- Kigoi)	10	0.1261	0.2476	48.39%
Eastern Kenya (Taro)	23	0.1863	0.3406	58.06%

4.3.2 Analysis of Molecular Variance (AMOVA)

AMOVA of SSR data (Table 4.3) indicated that 79% of overall genetic variation could be ascribed to differences between samples within varieties. 6% variation occurred among regions while 14% occurred among populations.

Table 4 : Analysis of molecular variance (AMOVA) for SSR among C.esculenta sampled from Kenya, Uganda and Tanzania.

Degrees of freedom (d.f) and significance (P) of the variance components are shown

Source of variation	df	Ss	Ms	Est.var	percentage	P-value
All population						
Among regions	2	61.083	30.541	0.357	6	0.01
Among population	4	39.088	19.544	0.824	14	0.01
Within population	93	422.676	4.545	4.545	79	0.01
Total	97	522.847		5.726	100	

4.3.3 Principal Component analysis (PCA)

A plot of the first two principal components of analysis of SSR variation represented an overall variation of 32.36% in the first axis and 19.14% in the second axis (Fig 4.4). Labeling individuals by their ecotype of origin is informative. There was no distinguishable pattern of clustering of accessions from a certain region. Taro-Kigoi samples from central Kenya revealed little variation in the first axis. Taro-Girigasha from Central Kenya was also in the first axis and cluster together with Taro kigoi. Tannia and Taro from Lake Victoria basin showed high variation across the two axes, this was also true for the Eastern Kenya Taro.

Table 5: Eigen value (EV), per cent variability and accumulative variability explained by first 12 principle components (PC 1–8) based on characterization of 99 taro accessions

Axis	Eigen Value	Inertia %
1	0.05896	12.84
2	0.04254	9.27
3	0.03374	7.35
4	0.02748	5.99
5	0.02418	5.27

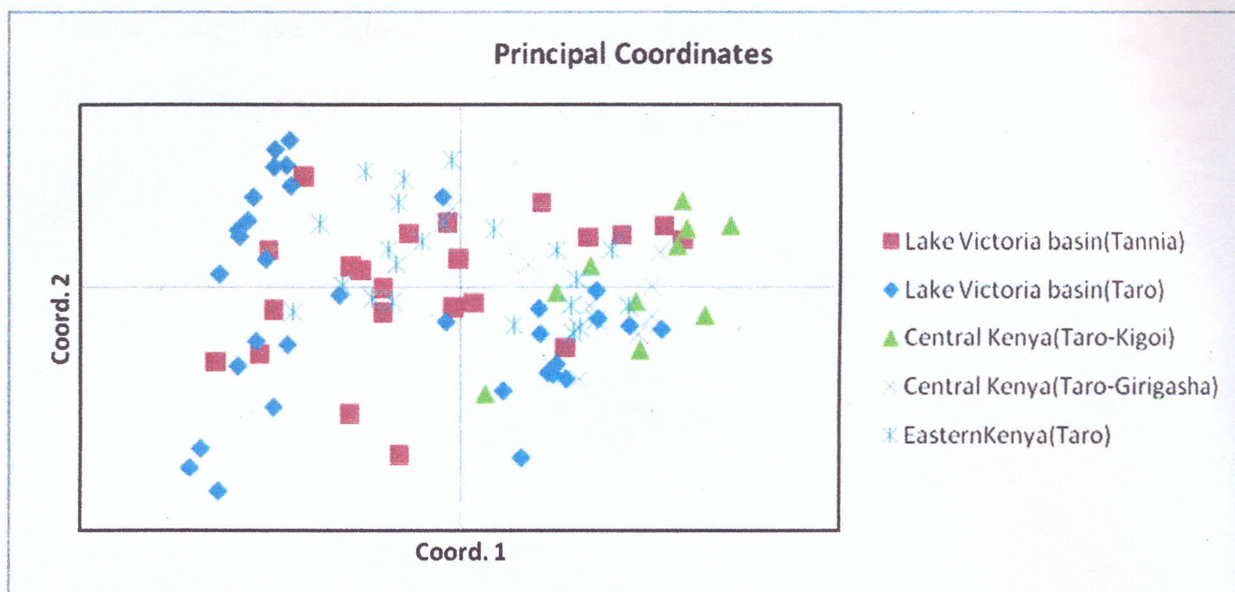

Figure 6:Distribution of 98 accessions on the first and second coordinates of PCA performed with six markers. The accessions are represented according to their geographical region

4.4 Cluster analysis

Phenogram based on Nei's genetic distance (Table 6) showed four major clusters (Figure 4).Only one cluster was not population based while the rest clustered on basis of population. The estimates of genetic distance between populations showed a range of 0.0766 and 0.270. The samples clustered to a larger degree on basis of population rather than geographical location. Taro and Tannia, which are populations from the Lake Victoria basin do not cluster together. Instead, Tannia clusters Eastern Taro and the two areas are geographically distant. A large difference was observed between Kigoi and girigasha as they do not cluster together yet they are from the same geographical region. Kigoi was separate from all other populations. The greatest distance between populations was between Taro from Lake Victoria basin and Taro-girigasha from central Kenya (0.2733).The smallest genetic distance was between

Tannia from Lake Victoria basin and Taro from Eastern Kenya (0.0371). These results correlate with PCA results (Figure 6).

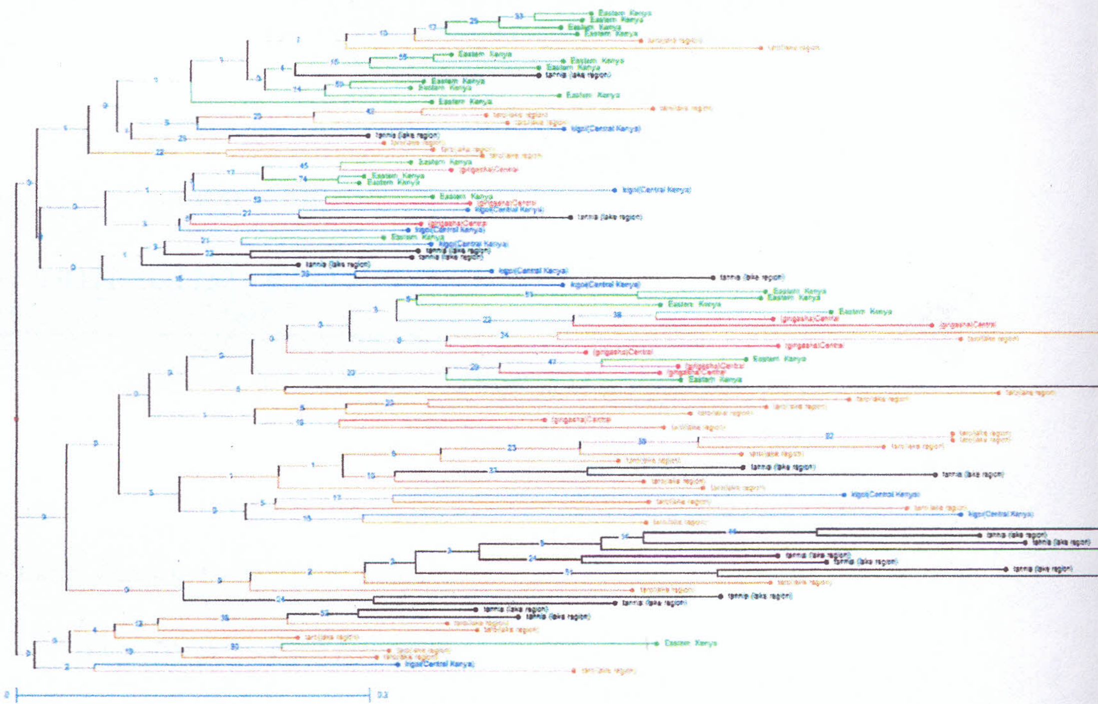

Figure 7: Phenogram based on Nei and Li (1978) genetic distance for 5 populations (99 accessions) of C.esculenta from Kenya, Uganda and Tanzania generated using 6 SSR markers

Table 6 : Matrix of unbiased genetic distance according to Nei (1978) among 5 populations of C.esculenta based on 6 SSR markers

Pop ID	1	2	3	4	5
1					
2	0.0766				
3	0.2733	0.1931			
4	0.1597	0.128	0.1265		
5	0.0876	0.0371	0.1753	0.0894	

Key

Population 1- Lake Victoria basin (Taro)

Population 2- Lake Victoria basin (Taro-tannia)

Population 3- Central Kenya (Taro-girigasha)

Population 4- Central Kenya (Taro-Kigoi)

Population 5- Eastern Kenya (Taro)

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

An understanding of the extent and distribution of genetic variation within and among taro populations is essential for determining appropriate genetic management strategies for the species. The amount of molecular marker information has considerable impact on the results of studies of germplasm genetic relationships in crop (Cheng-lai *et al*, 2010). In this study,a total of 31 alleles were amplified with an average of 6.1 alleles per locus, this agrees with results obtained by Mace *et al.* (2006) who used similar primers to rationalize taro germplasm in the Pacific; in their study a total of 38 allelles were amplified in 515 accesions of taro. No allele was found to be specific for any population.

Different primers revealed different allelic sizes; for example, Xqutem 88 and Xqutem 73 had the highest number of alleles having 7 alleles while Xqutem 110 amplified only two alleles. All the alleles amplified were between 1500bp and 100bp but only the expected size alleles were scored, the larger alleles though polymorphic were treated as non-specific. There was a difference in the expected allelic sizes between some accessions; for example all accessions from central Kenya did not have 250 bp amplified by Xqutem 73 as in other populations. Allelic size variation of the loci is common in most plant species for various reasons. Xie *et al.* (2006) while studying SSR allelic variation in almond concluded that the significant allelic size variation observed in almond was exclusively due to difference in the structures of repeat motifs which involved interruptions or occurrence of new repeat patterns in addition to varying number of AG/CT repeats. Study of allelic diversity is important

in breeding programmes as was reported by Rousell *et al.*, (2005) from a study of allelic diversity changes in wheat varieties released from the year 1840 to 2000. It was observed that total number of alleles was stable up to the 1960s, from which time it regularly decreased.

Variation in genes is necessary to allow organisms to adapt to ever-changing environments. Average genetic diversity (H) for all the taro populations assessed was 0.20318 with the highest being Taro and Tannia from Lake Victoria basin populations (H= 0.2783) and (H=0.2478) respectively. This is low compared to genetic diversity of sweet potato in Kenya whose average was 0.75 with the lowest being 0.21 (Karuri *et al.*, 2009). In the genetic diversity of cassava in the great lakes region value of H was above 0.5 which was considered sufficient for a conservation program (Pariyo *et al.*, 2009; Tumwegamire *et al.*, 2011). The highest genetic diversity was in Lake Victoria basin, this could be attributed to the fact that the Lake Victoria basin covers three countries namely: Kenya, Uganda and Tanzania. The three countries are separated by a lake, this feature is a probable reason as the individual countries material has been selected and bred separately and there is no germplasm exchange among farmers in the three countries. This further implies that cultivars in each country are isolated and hence leading to a genetic drift where each population evolved on its own. This is in agreement with Epperson (1992) who found that isolated populations evolve separately as they adapt to new ecological habitat leading to changes in allele frequencies hence the genetic differentiation.

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Taro from central Kenya harbored low genetic diversity; girigasha (H=0.1774) and Kigoi (H=0.1261). These two populations consist of two different varieties which explain the difference in the value of H. None of the accessions from Kigoi overlap with cultivars from Girigasha, this is clearly shown by the PCA and cluster analysis. The low genetic diversity in the Central region is attributed to clonal propagation indicating the use of planting material from a common source. It is worth noting that clonal plants have lower genetic diversity than non-clonal plants (Harper, 1977).This phenomenon was reported by Farhado *et al.* (2002) and Gichuki *et al.* (2003), who all studied genetic diversity in the clonally propagated sweet potato. Despite these findings, Pujol *et al.* (2005) while studying genetic diversity of cassava emphasizes the incorporation of volunteer seedlings, of predominantly vegetatively propagated crops, by traditional farmers as an important mechanism for increasing genetic variability and a potential avenue for avoiding genetic erosion. Diversity in clonally propagated crops is attributed to accumulation of random mutations resulting via stem cuttings and adventitious buds arising from storage (Karuri et al., 2009). Low levels of genetic diversity are detrimental to populations as they lead to inbreeding depression. However, they can be of interest in evolutionary studies as they may indicate ongoing evolution and speciation (Shepherd, 1999). The results signify the importance of assessing populations for variability for conservation purposes.

Results from AMOVA analysis showed low genetic variation among populations but high genetic variation within population. This agrees well with Kreike *et al.* (2004) and Mace *et al.* (2006).This may be attributed to the limited number of taro accessions introduced to populations.

This study was not for determination of regional core development and therefore the accessions collected from the three countries were grouped into populations across the regions, namely: Lake Victoria basin (which covers Kenya, Uganda and Tanzania) Central Kenya and Eastern Kenya. In general no population was found to be unique and some varieties had duplicates in the collection. Some accessions that had been identified (Chien-ying *et al.*, 2007a) as different morphologically were actually similar genetically; this is especially true for taro and tannia in the Lake Victoria basin, these results are in agreement with Labra *et al.* (2001) who found that grapevines that looked different morphologically actually shared 80 percent of SSR alleles. It can be concluded that taro and tannia cultivars probably do have a common ancestor. However, they do not share many morphological characteristics.

From the principle component analysis, a few accessions from the Lake Victoria basin were distinct and formed one cluster displaying divergence from other regions. Varieties from Eastern region have a higher similarity to the tannia varieties from the Lake Victoria basin as compared to varieties from central region implying that planting material must have been sourced from the same area.

The cluster analysis of the entire data set revealed that most accessions in a population did not differ significantly. This is in accord with Elameen *et al.* (2008) who found that genotypes collected in a given region often displayed the same variation as that observed over the entire sampled area. The genetic distances between populations were large with the highest being between Taro from Lake Victoria basin and Taro-girigasha from central Kenya (0.2733). The smallest genetic distance was between

Tannia from Lake Victoria basin and Taro from Eastern Kenya (0.0371).This large genetic distances might be due to restrictive breeding activities of taro.

Many clones of taro do not flower naturally (Djukri, 2006). In other clones, natural flowering is sporadic and not predictable enough for breeding (Ivancic and Lebot, 2000). Gichuki *et al.* (2003) obtained small genetic distances (0.01 and 0.05) in sweet potato populations attributed to high inbreeding activities between the populations studied. This indicates that originally taro cultivars in East Africa could have been introduced from a single clone. However P71, P59, P59, P44, P51 and P47 are very distinct from all other accessions and cluster at a genetic distance between 0.05 and 0.08. This difference could be attributed to ploidy levels; diploid and triploid cultivars have no close relationships (Kreike *et al.*, 2004). Kreike *et al.* (2004) found that triploid cultivars had very high genetic diversity and this is as a result of polyploidisation that happened a long time ago. Kuruvilla and Singh (1981); Irwin *et al.* (1998) determined in there study that the botanical variety *C. esculenta var. esculenta* has a dasheen-type corm and is said to be diploid, while the *C. esculenta var. antiquorum* has an eddoe-type corm and is said to be triploid. However a study conducted by Kreike et al.(2004) analyzed ploidy level using flow cytometry (that could effectively discriminate triploid and diploid) but they could not find any relation between triploidy and an eddoe-type corm shape. The genetic distance between the populations girigasha and Kigoi is large which is unexpected since they are from the same geographical region this is attributed to the fact that the two populations do not share a large proportion of alleles.

The clustering of Tannia with Eastern Kenya taro was not expected because taro and tannia are in a different genus. The microsatellites used in this study are not transferable across genera (Mace *et al.*, 2002). In this study accessions designated as tannia revealed amplifications. This could be attributed to the fact that *Xanthosoma sagittifolium* (tannia) is closely related to *C.esculenta var antiquorum* (taro-eddoe) and therefore misidentified. Morphological misidentification is not uncommon in sample collection as in the case of misidentified accessions of the genus *Kummerowia* which is closely to the genus *Lespedeza* (Wang *et al.*, 2009). Another explanation could be that the microsatellite markers used are actually transferable across genus. This is not a unique phenomenon in SSR markers, which are very specific at species level. Yamamoto *et al.* (2002) used SSR primers used in genetic diversity of apple and applied them in a study of pear. SSR alleles in pear were amplified and compared with SSR allele of apple genome. All of the tested SSR primers derived from apple produced discrete amplified fragments in all pear accessions. Nucleotide repeats were detected in the amplified bands by Southern blotting and sequence analysis. The difference in fragment sizes was due to differences in repeat numbers. Therefore the amplification of some tannia cultivars by microsatellites developed from taro is possible and it could as well be misidentification during plant material collection.

Overall results indicated that accessions did not cluster as dasheens or eddoes and neither did they cluster according to geographical region and cultivars known by the popular names did not always cluster together. This indicates diversity within the variety or misidentification. A similar diversity study carried out in South east Asia and the pacific showed that different accessions clustered according to countries and revealed presence of two major gene pools which has a major implication in

conservation of germplasm (kreike *et al.*, 2004). A similar genetic diversity study of taro in Papua New Guinea was used to establish a first nation core collection of any species based on molecular markers (Singh, 2007).

This study has contributed critical knowledge about the distribution of genetic variation within and among the taro germplasm collections of the East African regions. There is a narrow genetic base among and within taro cultivars in the East African taro and only the accessions from Lake Victoria basin analyzed have sufficient genetic diversity that can be used to enhance breeding and conservation. This agrees with a study conducted by Elameen *et al.* (2008) on sweet poatato varieties who found that only a few of the populations studied had sufficient diversity. Lack of genetic diversity implies that the crop may be susceptible to pest and diseases as there is not enough material to evaluate superior varieties and thus leading to enormous loss of crop. This was the case of Samoan Archipelago in 1993, seriously damaging the crop. The severity of this epidemic was mainly due to extensive plantings of the same susceptible taro cultivar. A USDA program, Agricultural Development in the American Pacific (ADAP), financed from 1994 to 1996 the collection, evaluation, and distribution of leaf blight resistant taro to American Samoa. Taro production quickly recovered, but almost 10 years later, the number of different varieties remains (TaroGen, 2007).Genetic improvement of East African taro is needed to increase resistance to adverse environmental conditions (i.e. high salt and soil pH, low rain fall) and pests in order to increase plant vigor and yield. This study will further help advocate for use of molecular markers for the successful development of a core collection of taro germplasm for the East African region.

5.2 Conclusion

- This study provided insights into the genetic composition of the taro crop in East African regions with Lake Victoria basin hosting the greatest diversity and Central Kenya having the lowest diversity.
- Partitioning of genetic variation of the populations indicated that the species is characterized by high within population genetic diversity.
- The study has also demonstrated the usefulness of SSR markers in genetic diversity analysis of clonally propagated crop.
- SSR results showed that the sampled populations of *C.esculenta* are not significantly different.
- Despite concerns of genetic erosion due to clonal propagation the accessions obtained from the same variety harbored some genetic differences. Partitioning of genetic variation of the populations indicated that the species is characterized by high within population genetic diversity.

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5.3 Recommendations

1. Priority of conservation and collection of germplasm should be given to population with higher genetic diversity as it has more alleles that are found in other populations.
2. The results of this study have major implications for future breeding programmes. For the successful breeding of novel taro varieties with new combinations of desired characters, a high genetic diversity between the parents is desirable.
3. Collection of accessions to be used in validating a core collection should involve a wider geographical area in order to capture maximum diversity from the three countries.
4. Wild taro is highly diverse and comprises important material for long term breeding purposes and should therefore be incorporated in the core collection.
5. Apart from the 6 microsatellites used in this study, more should also be included in a further diversity study of taro.

REFERENCES

Botstein, B., White, R., Skolnick, M., & Davis, R. (1980). Construction of a genetic Linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics, 32*, 314-331.

Burr, B., Evola, S., Burr, F., & Beckmann, J. (1983). The application of restriction fragment length polymorphisms to plant breeding. : Genetic Engineering Principles andMethods. *Plenum, 5*, 45-59.

Cheng-lai, W., Sheng-fu, L., Bing-xue, D., Qian-qian, Z., & Chun-qing, Z. (2010). Determination of the Number of SSR Alleles Necessary for the Analysis of Genetic Relationships between Maize Inbred Lines. *Agricultural Sciences in China, 9*(12), 1713-1725.

Chien-ying, K., Ji-ping, K., & Mcdonald, R. (2007). In vitro micro propagation of white dasheen (*Colocasia esculenta*). *African Journal of Biotechnology, 7*, 41-43.

Cho, J. J., Yamakawa, R. A., & Hol, J. (2007). Hawaiian kalo, past and future. Davin-Regli. A., Charrel, N., Bollet, C., & de Mico , P. (1995). Variations in DNA concentrations significantly select the reproducibility of RAPD fingerprint patterns. *Research Microbiology, 146*, 561-568.

Diers, B., & Osborn, T. (1994). Genetic diversity of oilseed Brassica napus germplasm based on restriction fragment length polymorphisms. *Theoretical Applied Genetics, 88*, 662-668.

Djukri, K. (2006). The plant characters and corm production of taro as catch Crop under the young rubber stands. *Biodiversitas, 7*(3), 256-259.

Eckert, C. G. (2002). The loss of sex in clonal plants. *Evolutionary Ecology, 15*, 501-520.

Elameen, A., Siri, F., Arild, L., Odd, A., Rognli, L. S., Susan, M. (2008). Analysis of genetic diversity in a sweet potato (Ipomoea batatas L.) germplasm collection from Tanzania as revealed by AFLP. *Genetic Resources Crop Evolution 50, 55*, 397–408.

Epperson, B. (1992). Spatial structure of genetic variation within populations of forest trees. *New Forests, 6*, 257–278.

Excofferier, L., Smouse, P., & Quattro, M. (2005). Analysis of molecular variance inferred from metric distances among DNA haplotypes : applications to human mitochondria DNA restriction data. *Genetics, 131*, 479-491.

Fajardo, D. D., La Bonte, D. R., & Jarret, R. L. (2002). Identifying and selecting genetic diversity in Papua New Guinea sweet Ipomea batatas

(L.)Lam.Germplasm collected as botanical seed. *Genetic Resource Crop Evolution, 49*, 463-470.

FAO. (2008). FAOSTATbase. *http://www.fao.org, (Accessed 20 January 2009).*

FAO. (2000). FAO statistical database: agricultural production of primary crops. *Available from http://apps.fao.org/default.htm, (Accessed July 2009).*

Ferguson, J., & Grebe, D. (1986). Identification of cultivars of perennial rye grass by SDSPAGE of seed proteins. *Crop Science, 26*, 170-176.

Geburek, T., & Turok, J. (2005). Conservation and Management of Forest Genetic Resource In Europe. *Arbora Publisher, 3, 30-35.*

Gichuki, S. T., Berenyi, M., Zhang, D. P., Hermann, M., Schmidt, J., Glossl, J., et al. (2003). Genetic diversity in sweet potato (Ipomoea batatas (L.) in relationship to geographic sources as assessed with RAPD markers. *Genetic Resource Crop Evolution 50*, 429–437.

Gunua, A., & Kokoa, P. (1995). Taro research in PNG - An overview. *Paper presented at South Pacific Commission Taro Seminar II, Lae, 1995.*

Harper, J. L. (1977). Population biology of plants. *Academic Press, London.*
Hash, C., & Bramel-Cox, P. (2000). Survey of Molecular markers. *Applications of Molecular Markers in Plant Breeding.*

IAEA. (2002). Mutant Germplasm charachterization using Molecular Markers. *A manual prepared by the joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture.Training Course series No.19, .International Atomic Agency , Viena 2002.*

Irwin, S. V., Kaufusi, P., Banks, K., de la Pena, R., & Cho, J. J. (1998). Molecular characterization of taro Colocasia esculenta using RAPD markers. *Euphytica 99*, 183-189.

Ivancic, A., Lebot, V., Roupsard, O., Garcia., & Okpul, T. (2004). Thermogenic flowering of taro(*Colocasiaesculenta,Araceae*).*Canadian Journal of Botany, 82.*

Jackson, G. V. H., Gollifer, D. K., & Newhook, F. J. (1980). Studies on the taro leaf blight fungus Phytophthora colocasiae in Solomon Islands: Control by fungicides and spacing. *Applied Biology, 96*(16), 1-10.

Jatala, P., & Bridge, J. (1993). Nematode parasites of root and tuber crops. *Nematodes in Subtropical and Tropical Agriculture*, 137-180.

Jones, C. J., Edwards, K. J., Castaglione, S., Winfield, M. O., Sala, F., van deWiel, C., et al. (1997). Reproducibility testing of RAPD,

AFLP and SSR markers in plants by a network of European laboratories. *Molecular Breeding, 3*, 381–390.

Kahl, G. (2001). The Dictionary of Gene Technology. *Wiley-VCH,Weinheim.*

Kamal, S., Ajay, K. M., & Raj, S. M. (2008). Analysis of AFLP variation of taro population and markers associated with leaf blight resistance gene.1(3). *Academic Journal of Plant Sciences 1*, 42-48.

Kan, H., Xing, F. H., Wei, D. K., & Yi, D. (2009). Characterization of 11 new microsatellite loci in taro (*Colocasia esculenta*). *Molecular Ecology Resources 9*, 582–584.

Karp, A., & Edwards, J. (1996). DNA Markers: a global overview . In: Caetano-anolle`s G and PM Gresshorf eds. *Protocol, Applications and Overviews WilerVCH, New York*, 75-85.

Karuri, H. W., Ateka, E. M., Amata, R., Nyende, A. B., Muigai, A. W. T., Mwasame, E., et al. (2010). Evaluating diversity among Kenyan sweet potato genotypes using morphological and SSR markers. *International Journal of Agriculture and Biology, 12*, 33–38

Kay, D. E. (1973). Crop and product digest 2. Root Crops. *Tropical Products Institute, London*, 245 pp.

Kijas J.M.H., Fowler, J. C. S., & Thomas, M. R. (1995). n evaluation of sequence tagged microsatellite site markers for genetic analysis with citrus and related species. *Genome 38*, 349-355.

Kreike, C. M., Van Eck, H. J., & Lebot, V. (2004). Genetic diversity of taro, *Colocasia esculenta* (L.) Schott, in Southeast Asia and the Pacific. *Theoretical and Applied Genetics, 109*, 761–768.

Kuruvilla, K. M., & Singh, A. (1981). Karyotypic and electrophoretic studies on taro and its origins. *Euphytica, 30*, 405–412.

Labra, M., Carreno-Sanchez, E., Bardini, M., Basso, B., Sala, F., & Scienza, A. (2001). Extraction and purification of DNA from grapevine leaves. *Vitis, 40*, 101-102.

Lebot, V., & Aradhya, K. M. (1999). Isozyme variation in taro (*Colocasia esculenta* (L)schott) in Asia and Oceania. *Euphytica, 56*, 55-56.

Lebot, V., Ivancic.K., & Abraham, K. (2005). The Geographical distribution of allelic diversity,a practical means of preserving and using minutesor root crop genetic resources. *Exploring Agriculture, 41*, 475-489.

Lebot, V., Prana, M. S., Kreike, N., Heck, H., Pardales, J., & Okpul, T. (2004). Characterisation of taro (*Colocasia esculenta* (L) schott) genetic

resources in South East Asia and Oceania *Genetic Resources and Crop Evolution, 51*, 381-392.

Mace, E. S., & Godwin, I. D. (2002). Development and characterization of polymorphic microsatellite markers in taro, *Colocasia esculenta* (L.) Schott. *Genome, 45*, 823-832.

Mace, E. S., & Godwin, I. D. (2002). Development and characterization of polymorphic microsatellite markers in taro,*Colocasia esculenta*(L) schott. *Genome, 45*, 823-832.

Mace, E. S., Mathur, P. N., Izquierdo, L., Hunter, D., Taylor, M. B., Singh, D., et al. (2006). Rationalization of taro germplasm collections in the pacific island region using simple sequence (SS) markers. *Plant Genetic Resources, 4*, 210-220.

Matsuda, M. (2001). Taro in Japan and its dispersal in East and Southeast Asia. *Vegeculture in East¬ern Asia and Oceania, 5*, 117-134

Matthews, P. J. (1990). The origins, dispersal and domestication of taro. *PhD Thesis, Australian National University.*

Matthews, P. J. (1991). A possible tropical wildtype taro:colocasia esculenta var aquatilis. *Indo-Pacific Prehistory Bulletin, 11*, 69-81.

Matthews, P. J., & Eyzaguirre, P. B. (1993). The Global Diversity of Taro: Ethnobotany and Conservation. Rome: IPGRI; Osaka. *National Museum of Ethnology (in press).*

Matthews, P. J., Matsushita, Y., & Hirai, M. (1992). Ribosomal and mitochondrial DNA variation in Japanese taro, *Colocasia esculenta* (L.) Schott. *Japanese Journal of Breeding, 42*, 825-833.

Mbouobda, H. D., Boudjeko, T., Djocgoue, P. F., Tsafack, T. J. J., & Omokolo, D. N. (2007). Morphological characterization and agronomic evaluation of cocoa yam Germplasm in Cameroon. *Journal of Biological Sciences, 7*, 27-33.

Morgante, M., & Olivieri, A. M. (1993). PCR-amplified microsatellites as markers in plant genetics. *Plant Journal, 3*, 175–182.

Nei, M., & Li, W. H. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. *National Academic Science USA, 76*(5269-5273).

Onwueme, I. C. (1994). Tropical root and other tuber crops production,perspectives and future prospects. *FAO Plant Production &Protection*, 126-228.

Onwueme, I. C. (1999). Taro cultivation in Asia and the pacific. *RAP publication:Food and Agriculture Organization of the United Nations Regional Office for Asia and Pacific, Bangkok, Thailand,* 16.

Ortiz, R. (1999). Statistical analysis of DNA marker characterization of germplasm.In (Jonathan,H.C. and Tenkousonou A.(eds) DNA marker assisted improvement of the staple crops of Sub-Saharan Africa. *Proceedings of the Work shopon DNA markers at IITA held by crop improvement division,IITA,Ibadan,Nigeria*, 21-22.

Pariyo, A., Baguma, Y. K., Kawuki, R. S., Omongo, C. A., Alicai, T., Edema, R., et al. (2009). Genetic diversity of cassava landraces in the great lakes region of Africa assessed using SSR markers. *Centro Internacional de Agricultura Tropical (CIAT), Cali, CO. 1.*

Peakall, R., & Smouse, P. (2009). GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes, 6*, 288-295.

Pearson, M. N., Chand, H., & Lovell, P. H. (1999). Rapid vegetative multiplication in *Colocasia esculenta* (L) schott(taro). *Plant Cell,Tissue and Organ culture, 55*, 223-226.

Plucknett, D. L. (1976). Edible aroids: *Alocasia, Colocasia, Cyrtosperma, Xanthosoma*. In: Simmonds NW (ed.). *Evolution of Crop Plants, London: Longman*, 10–12.

Plucknett, D. L., de la Pen˜a, R. S., & Obrero, F. (1970). Taro (*Colocasia esculenta*). *Field Crop Abstracts, 23*(413–426).

Powell, W., Morgante, M., Andre, C., Hanafey, M., Vogel, J., Tingey, S., et al. (1996). The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Molecular Breeding 2*, 225–238.

Purseglove, J. K. (1972). Tropical Crops. Monocotyledons I. *Longman, London.*

Rafalski, J. (1993). Randomly amplified polymorphic DNA (RAPD) analysis. *DNA markers: Protocols, Applications and Overviews. Wiley-Liss Inc, New York*, 75-84.

Ramon, S. (1980). Development of new taro varieties through breeding. *HITAHR*, 5547-5552.

Robert, E., Paull, C., Tang, K., Gross, & Gail, U. (1999). The nature of the taro acridity factor. *Postharvest Biology and Technology, 16*(1), 71-78.

Robinson, J. P., & Harris, S. A. (1999). Amplified Fragment Length Polymorphisms and Microsatellites: A phylogenetic perspective.In:Which DNA Marker. *Final Compedium of the Research Project*

Development, Optimization for Molecular Tools for Assessment of Biodiversity in Forest Trees in Europen Union.

Rodoni, B. (1995). Alomae disease of taro. *Australian Centre for International Agricultural Resources, 15.*

Roussel, V., Leisova, L., Exbrayat, F., Stehno, Z., & Balfourier, F. (2005). SSR allelic diversity changes in 480 European bread wheat varieties released from 1840 to 2000. *Theoretical and Application Genetics, 111*(1), 162-170.

Sambrook, J., Fritsch, E. F., & Maniatis, T. (1989). Molecular Cloning:A laboratory manual. *Cold Spring Harbor Laboratory Pres,Cold Spring Harbor ,N.Y.*

Schnell, R. J., Goenaga, R., & Olano, C. T. (1998). Genetic similarities among cocoa yam cultivars based on Randomly Amplified Polymorphic DNA(RAPD) analysis. *Scientia Horticulturae, 80*, 267-276.

Sharma, K., Mishra, A. K., & Misra, R. S. (2008). Identification and characterization of differentially expressed genes in the resistance reaction in taro infected with *P. colocasiae*. *Molecular Biology Reporter, 93.*

Shepherd, I. (1999). The genic view of the process of speciation. *Evolutionary. Biology, 14*, 851–865.

Singh, D., Mace, E. S., Godwin, I. D., Mathur, P. N., Okpul, T., Taylor, M., et al. (2007). Assessment and rationalization of genetic diversity of Papua New Guinea taro (*Colocasia esculenta*) using SSR DNA fingerprinting. *Genetic Resources and Crop Evolution, 55*(6), 811-822.

Singh, D., Mace, E. S., Godwin, I. D., Matthur, P. N., OkpuL, T., Taylor, M., et al. (2008). Assesment and rationalization of genetic diversity of PNG taro(*Colocasia esculenta*) using SSR DNA fingerprinting. *Genetic Resource Evolution., 55*, 811-825.

Tahara, M., Nguyen, V. X., & Yoshino, H. (1999). Isozyme analyses of Asian diploid and triploid taro, *Colocasia esculenta* (L.) Schott. *Aroideana, 22*, 72-78.

TaroGen. (2000). Taro Genetic Resources: Conservation and Utilisation Project.Annual Report 1999/2000. *Secretariat of the Pacific Community, Noumea, New Caledonia.*

TaroGen. (2000). AusAID/SPC Taro Genetic Resources: Conservation and Utilisation Project.

Annual Report 1999/2000. Secretariat of the Pacific Community, Noumea, New Caledonia.

Zabeau, M. (1995). AFLP: a new technique for DNA fingerprinting. *Nucleic Acid Research, 23*, 4407-4414.

Zabeau, M., & Vos, P. (1993). Selective Restriction Fragment Amplification: A general method for DNA fingerprints. *Nucleic Acid Research, 22*, 4233-4235

Zhang, D. P., Cervantes, J. C., Huaman, Z., Carey, E. E., & Ghislain, M. (2000). Assesing genetic diversity of Sweet potato[(Ipomea batatas(L) Lam.] Cultivars from Tropical America using AFLP. *Genetic Resources Crop Evolution 47*, 659-655.

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