Genetic diversity analysis and nutritional assessment of cocoyam genotypes in Malawi

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DECLARATION

"I declare that the dissertation hereby submitted by me for the degree Magister Scientiae Agriculturae at the University of the Free State is my own independent work and has not previously been submitted by me to another University/Faculty.

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Obed John Mwenye Date

DEDICATION

This piece of work is dedicated to my parents John and Ruth Mwenye and my fiancée Trintus Njete whom I met in the course of my studies. You all deserve to enjoy the fruits of your understanding, patience and love.

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LIST OF ABBREVIATIONS

°C	Degree Celsius
°N	North latitude
°S	South latitude
µl	Microlitre
µm	Micrometre
µM	Micromolar
AFLP	Amplified fragment length polymorphism
AMOVA	Analysis of molecular variance
ANOVA	Analysis of variance
ARET	Agriculture Research and Extension Trust
ATP	Adenosine 5′-triphosphate
BC	Before Christ
BSA	Bovine albumin serum
Ca	Calcium
cm	Centimetre
cmol/kg	centimole per kilogram
Coy	Cocoyam
Cu	Copper
CV	Coefficient of variation
DARS	Department of Agriculture Research Services
DNA	Deoxyribonucleic acid
dNTPs	2′-Deoxynucleoside 5′-triphosphate
DRC	Democratic Republic of Congo
DTT	Dithiothreitol
EDTA	Ethylene-diaminetetraacetate
EPA	Extension Planning Area
FAO	Food and Agriculture Organisation
FAOSTAT	Food Agriculture and Organisation Statistics
Fe	Iron

g	Gram
g	Relative centrifugal force
GPX	Guaiacol peroxidise
ha	Hectare
HDM	High dry matter
HNO	Nitric acid
IBPGR	International Board for Plant Genetic Resources
IITA/SARRNET	International Institute of Tropical Agriculture/Southern Africa Root Crops Research Network
IPGRI	International Plant Genetic Resources Institute
K	Potassium
Kcal	kilocalories
KCl	Potassium chloride
Kg	Kilogram
LEISA	Low External Input and Sustainable Agriculture
LSD	Least significant difference
M	Molar
m	Metre
MAS	Marker-assisted selection
Masl	Metres above sea level
Mg	Magnesium
$MgCl_2$	Magnesium chloride
ml	Millilitre
mm	Millimetre
mM	Millimolar
Mn	Manganese
MoAFS	Ministry of Agriculture and Food Security
mtDNA	Mitochondrial DNA
n	Chromosome number
Na	Sodium
NaCl	Sodium chloride

NCSS	Number Cruncher Statistical System
NH_4NO_3	Ammonium vanadate
ng	Nanogram
NILs	Near-isogenic line(s)
nm	Nanometre
NTSYS	Numerical taxonomy and multivariate analysis system
P	Phosphorous
PC	Principal component
PCA	Principal component analysis
PCoA	Principal coordinate analysis
PCR	Polymerase chain reaction
pH	Power of hydrogen
pmol	Picomole
ppm	Parts per million
PVP	Polyvinylpyrrolidone
QTL	Quantitative trait loci
RAPD	Random amplified polymorphic DNA
RCBD	Randomised complete block design
RFLP	Restriction fragment length polymorphism
SADC/ICART	Southern African Development Community/Implementation and Coordination of Agricultural Research and Training in the SADC Region
SDS	Sodium dodecyl sulphate
SE	Standard error
SNP	Single nucleotide polymorphism
SOD	Superoxide dismutase
SPC	South Pacific Community
SSA	Sub-Saharan Africa
SSCP	Single strand conformation polymorphism
SSR	Simple sequence repeat
STS	Sequence tagged site

Subsp	Subspecies
Taq	*Thermus aquaticus*
TBE	Tris – Boric acid-EDTA
TE	Tris-HCl/EDTA
Tris-HCl	Tris(hydroxymethyl) aminomethane hydrochrolide
U	Unit
UPGMA	Unweighted pair group method of arithmetic averages
USA	United States of America
UV	Ultra-violet
var	Variety
V	Volts
v/v	Volume per volume
VNTR	Variable number of tandem repeats
W	Watt
w/v	Weight per volume
WHO	World Health Organisation
Zn	Zinc

CHAPTER 1

General introduction

1.1 Motivation and objectives

Cocoyam (*Colocasia esculenta* (L.) Schott and *Xanthosoma sagittifolium* (L.) Schott) is a stem tuber crop that is widely cultivated in tropical and subtropical regions of the world (Okonkwo, 1993). The two most cultivated species worldwide are *C. esculenta* and *X. sagittifolium*. There are seven species of *Colocasia* (*taro*) that originated from Asia and about 40 species of *Xanthosoma* (*tannia*) that originated from the American continent (Purseglove, 1972). The main economic parts in both species are the corms and cormels, as well as the leaves. The corms and cormels are usually boiled, baked, roasted or fried and consumed in conjunction with other foods like fish and coconut preparations. The leaves are usually boiled or prepared in various ways mixed with other condiments like spinach (Onwueme, 1999; Janseens, 2001).

According to Onwueme (1999), cocoyam is an important crop in many parts of the world, mainly for smallholder farmers. The crop plays a major role in the lives of many as a food security crop and has rich economic and socio-cultural connotations. It is a cash crop and foreign exchange earner, as well as an important component in the rural development of many areas and individuals.

As an ancient crop, cocoyam maintains considerable socio-cultural importance for the people. The adulation and prestige attached to cocoyam is only equalled by yam in certain communities. It is considered as a prestige crop and crop of choice for loyalty, thanks-giving, traditional feasting and fulfilment of obligations (Onwueme, 1999). The crop features prominently in the folklore and old traditions of many cultures in west Africa, Oceania and south east Asia. Various parts of the cocoyam plant are used as traditional medicine. In fact, to highlight its importance in other countries, Samoa and Tonga, for example, each have a

depiction of cocoyam (*taro*) as a main feature on one of their currencies (Onwueme, 1999; Caillon *et al*., 2006).

According to FAO (2005) the total world production of cocoyam in 2005 was 10 million tonnes. Africa as a continent is the major producer of cocoyam, followed by Asia, with about half of the African production and Oceania with just a tenth of the total African production. The Oceania region surpasses any other region in terms of production, utilisation and dependence on cocoyam for food (Onwueme, 1999).

Cocoyam is mainly produced and consumed on a subsistence basis. However, a considerable amount is produced as a cash crop. Surpluses from subsistence production are sold, thereby playing a role in poverty alleviation of smallholder farmers who are the main growers of the crop. Countries like Fiji, Tonga, Cook Islands, Tuvalu, Thailand and Samoa were (up until 1993 before leaf blight disease destroyed most cocoyam plantations) the major exporters of the crop, mainly to New Zealand and Australia and earned the much desirable foreign exchange for their economies (Onwueme, 1999; Janseens, 2001).

In major cocoyam producing countries, the industry provides meaningful employment to a large number of people, mostly in rural areas. Exportation facilities for cleaning, sorting, packaging and shipping of cocoyam provide additional avenues for poverty alleviation through employment generation in the rural areas. In Hawaii, processed forms of cocoyam (*taro*) are produced in rural cottage industries. Therefore the role of cocoyam in rural development is even further enhanced (Onwueme, 1999).

Cocoyam products have shown considerable potential in different industries. In livestock production, cocoyam leaves have proven to be useful as replacement for different soybean flour mixes and the flour has shown potential as raw material for the production of lager beer. Recent studies have revealed that cocoyam starch is fine and contains small granules, a property required in many industries (Onwuka and Enech, 1996; Perez *et al*., 2005; Rodriguez *et al*., 2006).

Like many other crop plants, cocoyam production is affected by both biotic and abiotic stresses. Pests and diseases such as leaf blight disease and cocoyam beetle (*Papuana* species)

reduce cocoyam yield to a greater extent. Other constraints of cocoyam production include: weeds, laboriousness of the production system and scarcity of labour, scarcity of planting material and improved varieties, post-harvest handling and marketing and limited research and extension services (Onwueme, 1999; Janseens, 2001). This was corroborated by Serem *et al*. (2008) and they furthermore observed that land scarcity was the major factor limiting cocoyam production in various areas around Lake Victoria in Kenya, Uganda and Tanzania. This was attributed to the fact that cocoyam cultivation is restricted to wetlands, which was already a limited resource in the region and other factors that influence the production of the crop (Serem *et al*., 2008). Research and development agencies therefore need to develop appropriate cocoyam production technologies to mitigate existing constraints.

Malawi utilises and depends mainly on root and tuber crops. Cassava, sweet potato and potato are the main root and tuber crops. Other minor traditional root and tuber crops like yam, cocoyam, Livingstone and/or African potato (*Plectranthus esculentus,* N.E. Br.) also play an important role in the traditional setting (Sandifolo, 2003). In Malawi, like in most developing countries, root and tuber crops such as cassava, sweet potato, yam and cocoyam act as principal sources of food, nutrition and cash income especially to most food insecure households. These crops, cocoyam included, have a greater ability to produce more edible energy per hectare per day compared to other commodities and produce satisfactory under adverse conditions where other crops may fail (Onwueme, 1978; Malawi Government Report, 1996; Moyo *et al.*, 1999; Sandifolo, 2003). This suggests that the potential of such crops like cocoyam, for food security, income generation and nutritional enhancement in the households are grossly underutilised. Cocoyam, despite possessing such rare attributes such as yielding 30-60 ton/ha, being rich in minerals and vitamins and possessing small starch grains, remains an indispensable, yet neglected, food crop especially for predominantly malnourished rural households (Ekwe *et al*., 2009).

According to Burlingame *et al*. (2009) there exists a need for increased efforts and resources to analyse, compile and disseminate data on the nutrient composition of wild, underutilised and under-appreciated food biodiversity. The availability of this data at genetic resource level assists countries to promote local species and varieties and to value and maintain the ecosystems that produce them.

On the other hand, Onwueme (1999) pointed out that the major challenge of cocoyam production is in fact the loss of a large pool of cocoyam germplasm which is mostly held in farmers' fields and in the wild. These losses pose a threat to cocoyam germplasm conservation, whose very existence determines the future of the crop. To guard such genetic erosion in cocoyam, there exists a need for detailed collection and conservation of the crop in all growing and production areas. These genetic resources would serve as a rich source for diversity for the crop's improvement as well as protection against further genetic erosion.

Preservation and use of major root crop genetic resources (cassava, potato and sweet potato) has greatly benefited from international funds allocated to economically important species. Many developing countries, however, experience difficulty in sustaining conservation and genetic improvement of lesser root crops, mostly aroids (cocoyam and yams) (Lebot *et al*., 2005). Hence, these minor root and tuber crops are being lost due to lack of knowledge on the importance of such traditional crops and lack of research and conservation measures. Most of these crops are being conserved by the elder generations and/or are being left to grow on their own (Malawi Government Report, 1996).

Detailed conservation and characterisation of cocoyam would not only help in conserving the national heritage in minor root and tuber crops but also generate information on their worthiness in terms of nutritional and potential industrial utilisation and on the genetic diversity that exists. The process has already started through the collection and characterisation of yams (Malawi Government Report, 1996; Luhanga, 2005; Msowoya, 2005).

In the case of cocoyam, there exists a need for improved understanding of the genetics and chemistry in order to enhance efficiency and effectiveness of improvement programmes (Smith and Duvick, 1989). There are several genotypes/accessions of cocoyam in Malawi and limited or no work has been undertaken to collect, characterise and assess the genetic yield potential for the local cocoyam genotypes/accessions. It is therefore important that cocoyam varieties from farmers' local genotypes/accessions be collected and studied.

Beeching *et al*. (1993) stated that a prerequisite for any genetic improvement programme is the knowledge of the extent of genetic variation present between accessions and genetic distance between all closely related species with which hybrids could be produced. This can be achieved through the characterisation of the germplasm using either morphological, biochemical and/or DNA markers.

This study therefore was aimed at assessing the morphological and genetic diversity of cocoyam genotypes of Malawi using ethno-botany, morphological and amplified fragment length polymorphism (AFLP) markers. The study also determined the mineral composition of the local (Malawi) cocoyam genotypes.

1.2 References

Beeching, J., Garada, M., Nairot, M., Haysom H.R., Hughes M.A., and Charrier, A. (1993). An assessment of genetic diversity within collections of cassava (*Manihot esculenta* Crantz) germplasm using molecular markers. Annals of Botany 72: 515-520.

Burlingame, B., Charrondiere, R., and Mouille, B. (2009). Food composition is fundamental to the cross-cutting initiative on biodiversity for food and nutrition. Journal of Food Composition and Analysis, in press.

Caillon, S., Quero-Garcia, J., Lescure, J.P., and Lebot, V. (2006). Nature of *taro* (*Colocasia esculenta* (L.)) genetic diversity prevalent in a Pacific Ocean island, Vanua lava, Vanuatu. Genetic Resources and Crop Evolution 53: 1273-1289.

Ekwe, K., Nwosu, K., Ekwe, C., and Nwachukwu, I. (2009). Examining the underexploited values of cocoyams (*Colocasia* and *Xanthosoma* species) for enhanced household food security, nutrition and economy in Nigeria. In: Jaenicke, H., Ganry, J., Zeledon-Hoeschle, I., and Kahane, R. (Eds.). Proceedings of the international symposium on underutilized plants for food security, income and sustainable development. Acta Horticulturae 806, ISHS, pp. 71-78.

FAO. (2005). http://www.fao.org/es/ess/top/commodity.html (2008/06/02).

Janseens, M. (2001). Crop production in tropical Africa. Bonn, Germany: Institut fur obst- und germusebau, abt. Tropicscher pflanzenbau, Rheinische Friedrich-Wilhelms-Universitat Bonn, auf dem Hugel 6, D-53121 Bonn, Germany. Directorate general for international co-operation, Karmelietenstraat 15, B-1000 Brussels, Belgium pp. 221-228.

Lebot, V., Ivancic, A., and Abraham, K. (2005). The geographical distribution of allelic diversity, a practical means of preserving and using minor root crop genetic resources. Experimental Agriculture 41: 475-489.

Luhanga, R. (2005). Molecular characterisation and genetic diversity of wild and cultivated yams (*Dioscorea species*) in Malawi. Zomba: University of Malawi, MSc Thesis.

Malawi Government Report. (1996). Country report to the FAO international technical conference on plant genetic resources. Leipzig: FAO, pp. 9-28.

Moyo, C.C., Mahungu, M., Soko, R., and Sandifolo, V. (1999). Progress in developing cassava varieties in Malawi. In: Akaroda, M. and Teri, J. (Eds.). Food security and crop diversification in SADC countries: The role of cassava and sweet potato. Proceedings of the scientific workshop of the Southern Africa Root Rrops Research Network (SARRNET), pp. 143-150.

Msowoya, W. (2005). Morphological characterisation and genetic diversity of wild and cultivated yams (*Dioscorea* species) in Malawi. Zomba: University of Malawi, MSc Thesis.

Okonkwo, C. (1993). *Taro, Colocasia* spp. In: Bergh, G. (Eds.) Genetic improvement of vegetable crops. Oxford: Pergamon, pp. 709-715.

Onwueme, I. (1978). Tropical tuber crops*:* Yams, cassava, sweet potato and cocoyams. John Wiley and Sons, pp. 199-225.

Onwueme, I. (1999). *Taro* cultivation in Asia and Pacific: RAP Publication 1999/16. Bangokok, Thailand: Food and Agriculture Organisation of the United Nations regional office for Asia and the Pacific.

Onwuka, N., and Enech, C. (1996). The cocoyam, *Xanthosoma sagittifolium* as a potential raw material source for beer brewing. Plant Foods for Human Nutrition 49: 283-293.

Perez, E., Schultz, F.S., and Delahaye, E.P. (2005). Characterisation of some properties of starches isolated from *Xanthosoma sagittifolium* (*tannia*) and *Colocasia esculenta* (*taro*). Carbohydrate Polymers 60: 139-145.

Purseglove, J. (1972). Tropical crops: Monocotyledons 1. London: Longman, pp. 66-74.

Rodriguez L., Lopez D.J., Preston T.R., and Peters K., 2006. New cocoyam (*Xanthosoma sagittifolium*) as partial replacement for soyabean meal in sugar cane diets for growing pigs. Livestock Research for rural development 18. Retrieved from http://www.lrrd.org/lrrd. (2009/09/10).

Sandifolo, V. (2003). Estimation of crop losses due to different causes in root and tuber crops: The case of Malawi. Proceedings of the Expert Consultation on Root Crop Statistics-Volume II: Invited Papers. Harare, Zimbabwe: Food and Agriculture Organisation of The United Nations, Statistics division, Regional Office for Africa. FAO, Rome, pp. 41-47.

Serem, A.K., Palapala, V., Talwana, H., Nandi, J., Ndabikunze, B., and Korir, M. (2008). Socio-economic constraints to sustainable cocoyam production in the Lake Victoria crescent. African Journal of Environmental Science and Technology 2: 305-308.

Smith, J., and Duvick, D. (1989). Germplasm collections and private breeder. In: Brown, A., Marshal, D., Frankel, O., and Williams, J. (Eds.). The use of plant genetic resources. Cambridge: Cambridge University press, pp. 16-31.

CHAPTER 2

Literature review

2.1 Introduction

In the past, mankind depended on a much wider range of plant species for food, fibre, health security and other needs. The advent of the Green Revolution has, however, changed peoples' needs throughout the world and only a limited number of crops are being utilised to meet the needs of staple diets and few major non-food crops are being used to meet other associated needs (Williams and Haq, 2002). Thousands of plant species and many more varieties fall into a category defined as underutilised or neglected crops. These crops are marginalised by both agricultural and nutritional research (Global forum for underutilised species, 2009). This over reliance on just a few crops is risky to the world populace. Crops may fail, wars and strife wreak havoc on harvests and commodity prices oscillate. Recently, with the problems of climate change, crop production has been destabilised. The ever increasing global population also continues to push the Green Revolution to its limits (Ramana and Hodgkin, 2002). However, bringing these 'underutilised' crops (species with underexploited potential for contributing to food security, nutrition, health income generation and environmental services) out of the shadows into the mainstream would not only help to spread the risk but also claim the marginal land (National Academy of Science, 1975; Ramana and Hodgkin, 2002; Williams and Haq, 2002; Global forum for underutilised species, 2009).

Cocoyam, like many other indigenous tropical root crops, has been largely neglected. Despite its adaptability, acceptance and commercial food value, cocoyam has received little attention by researchers (National Academy of Science, 1975; Ekwe *et al*., 2009). Cocoyam usually thrives in infertile or difficult terrains that are not well suited for large-scale commercial agriculture of most conventional staple crops. Since the poor are often the main inhabitants of such areas, underutilised crops like cocoyam give them alternative sources of income-paths out of poverty (Williams and Haq, 2002).

Cocoyam holds great potential in commercial agriculture as it produces fine granule starch desired in specialised industries (Onwueme, 1978). Its future depends on selection of high yielding, good quality genotypes and development of low cost technologies that will enhance its production (National Academy of Science, 1975). However, in order to exploit this crop better, an understanding of its genetic diversity and distribution will be essential for its conservation and use (Ramana and Hodgkin, 2002).

2.2 Taxonomical description of cocoyam

The taxonomy of cocoyam is confusing. The many common names of the crop further add to the confusion (Onyilanga *et al*., 1987). It is known by several common names in English as *tannia, tania, yautia, cocoyam, tanier*; Spanish as *yautía, malanga* (Antilles), *macal* [(Mexico, (Yucatan)], *quiscamote* (Honduras), *tiquisque* (Costa Rica), *otó* (Panama), *okumo* (Venezuela), *uncucha* (Peru), *gualuza* (Bolivia), *malangay* (Colombia); Portuguese as *taioba, mangareto, mangarito, mangarás* (Brazil) and French as *chou Caribe* (Antilles). Names in other languages include *queiquexque* (Mexico), *tannia tanier*a (Antilles), *dasheen, tannia, dalo* (Fijian), *arri, kachu* (Indian), *talla* (Malayan), *taro* (Polynesian) and *gabi* and *yautia* (Tagalog, in the Philippines) (Williams *et al*., 1982).

Cocoyam is therefore a non-specific term, applied to various edible aroids (members of the Araceae family). Various tropical aroids that produce edible tubers are also collectively referred to as '*taro*'. There is therefore some confusion as to the use of the terms cocoyam and *taro* without distinction among the genera (Janseens, 2001). In the Oceania region and many other tropical producing countries, the common name *taro* is used for all edible aroids. Nevertheless, each variety has its common name. *Colocasia esculenta* is referred to as the true cocoyam, old cocoyam or *taro* and several common names including *tannia, yatua, malanga, callalo, coco* or new cocoyam are used to refer to domesticated *Xanthosoma* species which share substantially the same uses (O'Hair, 1990; South Pacific Community (SPC), 1993; Janseens, 2001). The confusion in the naming of the crop is even greater among the different continents. According to Onyilanga *et al*. (1987), *taro, dasheen, eddoe, curcas*, true cocoyam and old cocoyam are all forms of a plant originally described as *Arum esculentum* L. but now referred to as *C. esculenta* (L) Schott or *C. antiquorum* Schott. All

types are known as *taro* in the Pacific region. However, the name cocoyam in west Africa is used for both *Colocasia* and *Xanthosoma* species. The names *dasheen* and *eddoe* exist as different forms of cocoyam in west India, but the *dasheen* of the southern United States of America (USA) is regarded as the *eddoe* of Trinidad (Onyilanga *et al*., 1987).

Cocoyam belongs to the monocotyledonous family Araceae. The family (often referred to as aroids) contain several plants which are cultivated and used for food in various parts of the world (Onwueme, 1978). The Araceae family consists of some 100 genera and more than 1500 species. Members of this family are mostly tropical and subtropical plants (Purseglove, 1972). The aroids grow mainly in moist or shady habitats. Some are terrestrial, while others are vines, creepers or climbers. Many species are also epiphytes (Ekanem and Osuji, 2006).

The major edible aroids are classified into two tribes and five genera: *Clasioideae* with the genera *Cyrtosperma* and *Amorphophallus* and *Colocasioideae* with the genera *Alocasia*, *Colocasia* and *Xanthosoma* (Purseglove, 1972). The basic chromosome number is generally n=14, except for the genera *Xanthosoma* where n=13 (Janseens, 2001). The chromosomes of cocoyam are prone to unpredictable behaviour during cell division, such that the chromosome number per cell is not uniform within the crop. Chromosome numbers of 2n=22, 26, 28, 38 and 42 have all been reported. New types or variants of cocoyam, therefore, occur quite frequently in nature or under cultivation (Onwueme, 1978; Janseens, 2001).

Cocoyam production relies on traditional genotypes/accessions mostly kept by elderly members of the growing societies. Numerous botanical varieties of the crop exist. New agricultural accessions have been developed and they generally fall into two main groups namely the *eddoe* type, with small corm and large cormels and the *dasheen* type with large corms and small cormels (Onwueme, 1978). Genotypes from different species of cocoyam are distinguished based on the flesh colour of the corms and cormels, lamina and vein colour, petiole colour, acidity of tuber and leaves, shape, acridity (due to their ethenedioic or oxalic acid content), fibre content and palatability (Williams *et al*., 1982).

Purseglove (1972) suggested that the main distinguishing feature between the two common types of cocoyam i.e. *C. esculenta* and *X. sagittifolium* is the position of the leaf attachment on the petiole. In *C. esculenta*, the attachment of the petiole to the lamina is not at the edge of the lamina, but at some point in the middle of the lamina while in *X. sagittifolium*, the petiole is attached to the indentation and extends from the base of the leaf in *X. sagittifolium* and constitutes the midrib and the plants are usually large (Janseens, 2001).

2.3 Origin, classification, history and diffusion of cocoyam

Cocoyam originated in south central Asia, probably in India or Malaysia (Onwueme, 1978). The three genera of cocoyam, *Colocasia, Alocasia* and *Cyrtosperm*, are reported to have originated in south east Asia and the Pacific islands. The Indian subcontinent is considered as the primary centre of origin for genera *Colocasia* and *Alocasia*, whereas the genus *Cyrtosperma* originated from Indonesia (Janseens, 2001).

Jianchu *et al*. (2001) described the Yunnan province in south west China, at the margins of the centres of diversity in Assam and south east Asia, as an important region for ethno-botanical and genetic diversity of cocoyam (*C. esculenta*). Further evidence is given by historical records which clearly show the importance of *taro* cultivation in the same area nearly 2000 years ago. *Qi-Ming-Yao Shu*, a book on agricultural technology written by Jia Sixi in 600 BC, described *taro* cultivation in great detail.

Cocoyam cultivation dates back some 10000 years and has been cultivated longer than wheat or barley. Cocoyam, now used as a staple in parts of Asia, the Pacific, the Caribbean and Hawaii, was prevalent in the Mediterranean long before potato made an appearance (Jianchu *et al*., 2001).

The requirement of flooded conditions for *taro* (*C. esculenta*) convinced anthropologists that cocoyam was indeed the first irrigated crop and that the ancient "rice" terraces of Asia were originally constructed for cocoyam (Plucknett, 1976). *Tannia* (*X. sagittifolium*) on the other hand, does not tolerate waterlogged conditions as much (Onwueme, 1978). During prehistoric times, cocoyam cultivation spread to the Pacific islands. Around 500 BC, *Colocasia* was introduced to Africa, through Egypt, the Nile basin and east Africa. From

west Africa, the crop spread to the west Indies and the tropical parts of America (Onwueme, 1978; Kuruvilla and Avtar, 1981; Janseens, 2001).

On the other hand, FAO (1990) suggested that *Colocasia* species spread to Egypt from India and south east Asia and thence to Europe. Subsequently, it was taken from Spain to tropical America and then to west Africa. It spread to the west Indies with the slave trade as it was used as food for slaves. *Colocasia* species are today a staple food in many islands of the south Pacific, such as Tonga, western Samoa and Papua New Guinea. Due to its tolerance to shady conditions, cocoyam is often planted under permanent plantations like banana, coconut, citrus, oil palm and especially cocoa (FAO, 1990).

Jianchu *et al*. (2001) suggested that the crop flourished in ancient times due to its hardness that suited primitive agriculture. The corms and cormels were able to survive long periods of desiccation. The crop would grow quickly and in the right climate and could produce three small or two large crops per year. Dried cocoyam became an essential for survival on long journeys that the Polynesians made from south east Asia to the Pacific islands. It was these long, open-boated voyages that assisted in diffusion and distribution of slips and cuttings of more than 100 plants, including cocoyam, breadfruit, yams and coconut palm (Jianchu *et al*., 2001). This corroborates the fact that the spread of most root and tuber crops was facilitated by their ability to thrive under varied adverse tropical conditions. The level of water tolerance varies considerably among root and tuber crops, ranging from the waterlogged conditions required for cocoyam to the drought tolerance and minimal water supplies needed for cassava once it has been established (Wilson, 1984).

On the other hand, *Xanthosoma* species are reported to have originated and been first cultivated in tropical America, especially in central America and the Caribbean. It has since spread to south east Asia, the Pacific islands and Africa. Spread of the crop to the south Pacific and Africa occurred in recent times (Onwueme, 1978; Janseens, 2001). It was spread by the Spanish and Portuguese, who also introduced it to Europe and Asia. It moved from the Caribbean, in the late 19th century to Africa, first to Sierra Leone and then to Ghana (FAO, 1990).

FAO (1990) suggested that *Xanthosoma* species are now more important than *Colocasia* species, being popular for their corms, cormels, leaves and young stems. Although *Xanthosoma* species are relatively new to the Pacific region, they have spread rapidly and widely, becoming quite important in many of the islands. It is widely cultivated in Puerto Rico, the Dominican Republic and Cuba and is important along the coastal mountains of South America, in the Amazon basin and in central America (FAO, 1990).

Today cocoyam is grown nearly in all parts of the tropics, as well as in some subtropical regions. It is being grown nearly in all latitudinal regions between 10^0N and 10^0S. *Colocasia esculenta* var. *esculenta* is widespread in its region of origin, but also in all hot and humid regions in Africa. *Colocasia esculenta* var. *antiquorum* is cultivated in Japan, China and India. *Xanthosoma sagittifolium* is today found in all the hot and humid areas of the world and is grown extensively throughout west Africa (Janseens, 2001).

There is limited information as to how cocoyam was introduced into Malawi. However, two routes seem probable. Like many other root crops, it has been cultivated in suitable parts of southern Africa for centuries and was probably introduced by the Portuguese traders. It could have also been introduced by the early settlers of Malawi who migrated from Zaire in central Africa, present day the Democratic Republic of Congo (DRC) (http://www.bioverrsityexplorer.org).

2.4 Cocoyam morphology

Cocoyam is a perennial monocotyledonous herb (Onwueme, 1978). It consists of a central corm from which cormels, roots and shoots arise (Figure 2.1, Onwueme, 1978; Williams *et al*., 1982). Cocoyam is naturally a perennial crop, but for practical purposes is harvested after 5-12 months of growth (Onwueme, 1978). Its growth and developmental cycle goes through three main periods. During the first two months, growth is slow. This first period starts with sprouting of shoots and ends when the cormels emerge. The second period is characterised by a rapid increase in shoot growth, until 6-7 months after planting. It is during this period that plants achieve their maximum leaf area, pseudo stem diameter and height. During the third period, the leaves start to wilt and the total dry weight of the above ground plant parts decreases until harvest. This is the period of major movement of photo-assimilates from

leaves to the corm and cormels. The senescence of the plant at the end of this period is used by farmers as harvest period (Castro, 2006).

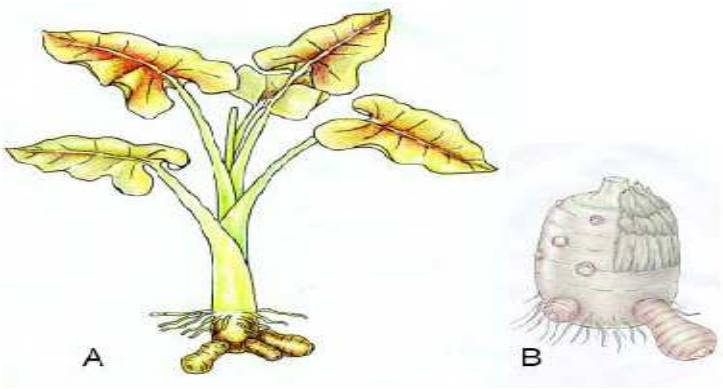

Figure 2.1 (A) Cocoyam plant, (B) Corm and cormels (Acuna, 2006).

Immediately after planting, there is a rapid increase in shoot growth until about six months after planting. There after growth of the shoot (mostly leaves) as well as the total dry weight of the shoot declines. This pattern of growth holds true for both the *C. esculenta* and *X. sagittifolium* (Onwueme, 1978).

Propagation of cocoyam is generally through vegetative means using tubers or the apical portions of large tubers harvested at maturity. Segmentation of the setts is possible as in yams. In regions where the growing season is interrupted by dry periods, *Xanthosoma* species are usually first multiplied by means of shoots and later transplanted (Janseens, 2001). In cases where shoots are used for multiplication instead of corms, yields are usually higher compared to using lateral buds. However, shoots have to be pruned to encourage growth of the corms (Williams *et al*., 1982; Janseens, 2001).

The shoot of the cocoyam plant consists mainly of the leaves which arise in a whorl from the apex of the corm. The terminal bud remains close to this apex. The leaves are the most prominent aerial organ of the plant. Plant height is determined by the height of the leaves. It ranges from 1-2 m. Each leaf consists of a long erect petiole and large lamina (Onwueme, 1978; Williams *et al*., 1982).

The leaf lamina is large, thick, entire and globrous. It is more or less rounded, except for a slight indentation at the base and pointedness at the top. The petiole may be one metre long and is thick along its entire length, but thicker at the base than near the attachment to the lamina. The base of the petiole where it is attached to the corm is flaced out so that it clasps around the apex of the corm. The petiole is solid throughout its length, but replete with large air spaces. These air spaces function as conduits for aeration of the subterranean organs when the plant is grown under swampy or flooded conditions (Onwueme, 1978; Williams *et al*., 1982; Janseens, 2001). The leaves and the shoots are normally consumed as a spinach-like vegetable (Janseens, 2001).

The main stem is an edible starch-rich, underground stem (rhizome) structure called the corm, from which offshoots, termed cormels; develop (Purseglove, 1972). The corm may be up to 30 cm long and 15 cm in diameter in *dasheen* types and is usually smaller in *eddoe* types. Within the corm lies the parenchyma, which is densely packed with stored starch. Scattered vascular bundles and a few lacifers occur in the ground parenchyma. Cells containing raphides (bundles of calcium oxalate) occur in the tissues of the corm, and to a lesser extent, in all other parts of the plant (Onwueme, 1978; Janseens, 2001).

The cormels arise from the auxiliary buds on the corms. Morphologically, they represent the lateral branches of the plant stem, while the corm represents the main stem (Onwueme, 1978). A terminal bud is present at the distal end of the cormel. Scale leaves are also present on the body of the cormel. Cormels, like the corm, are edible and less woody than the corm (Onwueme, 1978; Williams *et al*., 1982; Janseens, 2001). Secondary corms may arise from auxiliary buds of the corm of cocoyam, which give rise to suckers or daughter plants (Onwueme, 1978). In *Xanthosoma*, the corm is more spherical and the cormels are flask shaped, usually larger and more numerous than in the *Colocasia* genera (Williams *et al*., 1982).

Flowering in cocoyam is sporadic. When flowers occur, they appear shortly after planting, sometimes before any of the leaves have expanded. The inflorescence arises from the leaf axils or the centre of the cluster of unexpanded leaves. A plant may bear two or more inflorescences. The peduncle is stout and relatively short (Onwueme, 1978; Williams *et al*.,

1982). The inflorescence consists of a cylindrical spadix of flowers enclosed in a spathe. The flowers are unisexual with the female flowers located at the base of the spadix and the male flowers at the top. Sterile flowers are located in between the pistillate and staminate flowers (Purseglove, 1972).

The inflorescence of cocoyam is protogamous and pistillate flowers are normally receptive 2-4 days before pollen is shed. The spadices are seldom fertile and produce few viable seed (Castro, 2006). Flowers are fragrant and pollination is probably by insects, especially flies. Fruit and seed setting in cocoyam are even more uncommon than flowering. Many inflorescences wither without setting any seed. The fruits are clustered at the basal portion of the spadix. Each fruit is a berry about 3-5 mm in diameter. The seed is hard and contains endosperm and germinates with extreme difficulty (Onwueme, 1978; Castro, 2006).

The root system of cocoyam is fibrous and confined mostly to the top layers of the soil. The roots arise from the lower portions of the corm (Onwueme, 1978).

2.5 Environmental conditions for cocoyam growth

Cocoyam is a crop of the humid tropics. It requires a hot and relatively humid climate. It does best at average temperatures above 21°C and minimum temperatures that do not fall below 10°C. Cocoyam does not perform under frosty conditions (Onwueme, 1978; Janseens, 2001). Photosynthesis is optimal between 25-29°C (Janseens, 2001). Cocoyam generally has a high moisture requirement. Best yields are obtained where annual rainfall is above 2000 mm. Low rainfall reduces corm growth and of the two types of cocoyam, *eddoe* types tolerate drier conditions better than *dasheen* types. The *dasheen* types grow better under flooded conditions (Onwueme, 1978).

Cocoyam can be grown both under flooded (lowland) and unflooded (upland) conditions. In drier areas, it can be grown in swampy areas or under irrigation. Cocoyam usually grows best as a first crop after clearing. It requires heavy and moist soils, with good fertility and sufficient organic matter. Cocoyam prefers a soil pH of 5.5-6.5 and tolerates saline soils better than many other crops (Williams *et al*., 1982). Some cocoyam genotypes thrive better

than others in water logged conditions. However, intermittent moisture stress results in corms that have dumb-bell shapes and poor quality (Onwueme, 1978).

Yields are generally higher under flooded conditions, due to the greater ability of the plant to produce suckers, the larger leaf area and the slow rate of leaf senescence. Expanded leaves of the plant act as an extensive transpiring surface through which large quantities of water are lost. Cocoyam grown under flooded soil conditions is able to transport oxygen from aerial parts of the plant to the roots enabling roots to respire and grow normally. This makes cocoyam a valuable crop in areas where there is water logging and flooding problems (Onwueme, 1978; Janseens, 2001). However, the crop takes a long time to mature and the amount of effort expended is greater (Janseens, 2001). In Taiwan, upland-cultivated cocoyam had higher mineral content than ones grown in paddy (wetland) conditions (Huang *et al*., 2007).

2.6 Nutritional content and utilisation of cocoyam

Cocoyam is an important crop in tropical and subtropical areas because of the carbohydrates, proteins, fats, vitamins and minerals, as well as for the cash income it provides to farmers (Onokpise *et al*., 1999). It is a staple food for many people in developing countries in Africa, Asia and the Pacific. It is produced mainly in Africa (especially in Nigeria) and in Asia (mainly in China), but on the basis of per capita availability, it is most important in Oceania. In fact, it is an important food crop for more than 400 million people worldwide, especially in the tropics and subtropics (Onokpise *et al*., 1999; Onwueme, 1999).

Studies conducted on nutritional composition of the crop suggested that it is a good source of carbohydrates and minerals (Huang *et al*., 2007; Njoku and Ohia, 2007; Perez *et al*., 2007) and that leaves have quite substantial amounts of proteins and vitamins (Onwueme, 1999). It consists of 63.6-72.4% moisture, 21.1-26.2% starch and 1.75-2.57% crude protein and provides total energy in the range of 97.1-118.3 kcal/100 g fresh cocoyam. The corms have reasonably high contents of potassium (K) and magnesium (Mg) in the ranges of 2251-4143 and 118-219 mg/100 g dry matter respectively and are moderately good sources of water-soluble vitamins such as thiamine, riboflavin and ascorbic acid, compared to other tropical

roots. Essential amino acid contents are fairly good except for the sulphur containing amino acids such as tryptophan and histidine, (Huang *et al*., 2007).

Njoku and Ohia (2007) found cocoyam to be a good source of minerals [sodium (Na), potassium (K), magnesium (Mg) and calcium (Ca)] whose salts regulate the acid-base balance of the body system. However, the apical section of the corms are reported to have higher protein contents compared to the distal section which has high ash, fibre, Mg and phoshorous (P) contents (Sefa-Dedeh and Kofi-Agyir, 2004).

Cocoyam leaves are a good source of proteins, minerals and vitamins, i.e. they are rich in β-carotene and ascorbic acid (Thomas and Oyediran, 2008) and have great potential to qualify as good vegetables for hypersensitive, diabetic and obese people due to their anti-oxidant properties (Englberger *et al*., 2008; Thomas and Oyediran, 2008). Despite being a good source of carbohydrates and minerals, the corms and cormels contain anti-nutritional factors namely the trypsin inhibitor, total oxalate, soluble oxalate and calcium oxalate (Sen *et al*., 2006). Boiling of the tubers reduce the level of soluble oxalates in the cooked tissue to below detectable levels as soluble oxalates gets leached and hydrolysed in cooking solutions (Sen *et al*., 2006; Catherwood *et al*., 2007). Soluble and insoluble oxalates are also found in the young and older leaves of cocoyam (Oscarsson and Savage, 2007) and cooking furthermore significantly reduces the anti-nutritional factors and enhances the availability of crude fibre and proteins in cocoyam leaves (Oscarsson and Savage, 2007; Lewu *et al*., 2009).

According to Janseens (2001), fresh corms and cormels of cocoyam are used in the same way as potatoes. They are usually consumed after being boiled, baked, roasted or fried in oil. In parts of west Africa, the boiled corms and cormels may be pounded into a paste (fufu), similar to pounded yam. Some communities also produce flour to make pulp and Perez *et al*. (2007) found that the flour of cocoyam (*C. esculenta* and *X. sagittifolium*) produced chemical, physical and physicochemical properties which were similar to those shown by conventional flour of cereals. These studies showed considerable potential of cocoyam flours in terms of nutritional quality and good microbiological stability (Janseens, 2001; Perez *et al*., 2007).

Recently, the use of cocoyam corms, cormels and leaves as an animal feed seems to be gaining recognition. Others have tried to explore cocoyam derivatives as potential sources of raw material in various industries such as beer and starch production (Onwuka and Enech, 1996; Rodriguez *et al*., 2006; Mweta *et al*., 2008; Abdulrashid and Agwunobi, 2009). Mweta *et al*. (2008) investigated the physiochemical and functional properties of starch from cocoyam and cassava grown in Malawi to unravel their potential industrial applications. Cocoyam starch granules exhibited polygonal truncated shapes and small sizes (average of 7 μm) and gave lower values of amylose content and paste clarity with higher phosphorus content, maximum wavelength of iodine complex absorption and blue-value compared to cassava starches. It exhibited lower swelling power and solubility compared to cassava starches. There were similar enthalpy values for gelatinisation and retrogradation characteristics of cocoyam and cassava starch. However, cocoyam starch displayed higher retrogradation tendencies than cassava starches.

Onwuka and Enech (1996) investigated the potential of a widely cultivated cocoyam cultivar in Nigeria as a potential replacement of malt in lager beer production. Cocoyam was superior to barley and sorghum as a substrate because of its potentially higher carbohydrate content (71-78%) compared to barley (65%) and sorghum (70-73%).

Rodriguez *et al*. (2006) found no significant differences in feeding pigs with different mixtures of cocoyam leaves and soybean meal. Results, though preliminary, indicated the potential of fresh cocoyam leaves to replace up to half the soybean protein in diets based on sugarcane juice for growing pigs. Furthermore, Abdulrashid and Agwunobi (2009) suggested that proper processed cocoyam meal can effectively replace maize at 25% (raw sundried) and 50% (boiled and sundried) as a major source of energy in diets of broiler chicken finisher feed.

Like most root and tuber crops, cocoyam faces post-harvest losses during storage which are mainly influenced by storage conditions, the state of maturity at harvest and the morphological characteristics of the cormels. Wound pathogens are a major cause of post-harvest deterioration in cocoyam cormels and the causal pathogen of cormel decay is *Sclerotium rolfsii* Sacc (Onwueme, 1978; Agbor-egde and Rickard, 1991).

Post-harvest losses in cocoyam are reduced under conditions of low temperature (15°C) and high humidity (85%). Storage life under these conditions can be prolonged for periods of up to five to six weeks. *Xanthosoma* genotypes, however, tolerate storage under tropical ambient conditions of more or less the same periods. Post-harvest fungicide treatment is important especially for *Colocasia* species, which may register up to 60% decay in storage (Onwueme, 1978; Agbor-egde and Rickard, 1991).

Sajev *et al*. (2004) suggested that storing cocoyam at ambient storage temperatures bring about changes in both textural and rheological characteristics, compared to evaporative cooled room and refrigerated storage. This is attributed to the dehydration of the cormels and the biochemical changes of especially calcium induced hardness of pectic and related materials, which affects the rheological properties of the extracted flour.

The starchy corms and cormels are also stored in the form of dried chips and flour in order to avoid post-harvest losses, especially in developing countries (Perez *et al*., 2007). This guarantees year-round supplies of the tuber, which should encourage consumption of this flour and may contribute to new product development by regional food industries (Onwueme, 1978). The corms and cormels can also be stored underground. They are normally left in the ground after maturity and harvested when needed. This field storage for the crop compensates for the poor storability of most genotypes. Underground storage in pits is also common (Onwueme, 1978).

2.7 Genetic diversity assessment of cocoyam

Plant genetic resources are a valuable resource in agriculture, food security and forestry because they provide genetic diversity necessary for both farmers and breeders to obtain new cultivars (Laurentin, 2009). The ability to identify genetic variation is indispensable to effective management and use of genetic resources in a breeding programme (Rao, 2004), as a proper analysis of the genetic variation and relationships between accessions or genotypes is important to (a) understand the genetic variability available and its potential use in breeding programmes; (b) estimate any possible loss of genetic diversity; (c) offer evidence of the evolutionary forces shaping the genotypic diversities and (d) choose priority genotypes for conservation (Smith and Duvick, 1989).

According to Beeching *et al*. (1993) a prerequisite for any genetic improvement programme is knowledge of the extent of genetic variation present between genotypes and the genetic distance between all closely related species with which hybrids could be produced. This can be achieved through the characterisation of the germplasm using either morphological, biochemical or genetic markers. Genetic diversity analysis and characterisation allows evaluation of genetic variability, which is a fundamental element in determining breeding strategies and genetic conservation plans. As such, knowledge is even necessary before the breeding materials are exploited further (Gholiazadeh *et al*., 2008).

Smith and Duvick (1989) reported that a breeding programme that is genetically broad-based provides ideal results: steady gains under selection and the ability to respond readily to changed environments, diseases and economic trends. In contrast, a narrow-based programme would provide slow response to selection and increase the likelihood of crises triggered by outbreaks of diseases and insect pests. A lack of genetic variability across breeding programmes could exacerbate these deficiencies nationally or internationally, conceivably threatening the usefulness of available varieties and, of longer term significance, the usefulness of breeding stocks.

Cocoyam has been neglected by research and conservation efforts. There is limited information available on the amount of diversity that exists (Jianchu *et al*., 2001; Lebot *et al*., 2005). Hence, there exists a need to assess the extent of genetic diversity and how much has been conserved (Jianchu *et al*., 2001). Quero-Garcia *et al*. (2004) observed that with the lack of an accurate assessment of the genetic distance present between local genotypes, cocoyam breeders often face a difficult choice in making selections. The use of phenotypic values to assess the degree of diversity in cocoyam is challenging due to somatic fixation and often morphotypes are quite distinct even though they share the same genetic background.

Of late, alternative procedures such as biochemical and molecular genetic markers have received much attention (Lebot and Aradhya, 1991; Schnell *et al*., 1999; Caillon *et al*., 2006). Biochemical markers often make use of seed protein and enzyme electrophoresis to analyse genetic diversity as they reveal differences between seed storage proteins or enzymes encoded by different alleles at one (allozymes) or more gene loci (isozymes) (Rao, 2004).

Molecular makers, on the other hand, identify polymorphism at DNA sequence level and provide information about allelic variation at a given locus and have overcome limitations of both morphological and biochemical markers (Laurentin, 2009).

2.8. Methods of assessing genetic diversity

2.8.1 Morphological markers

Morphological traits have their basis in genetic alterations that lead to visible differences in the phenotype. Since only a small fraction of genes code for traits that are manifested in observable phenotypes, i.e. classical morphological markers, the level of information that can realistically be obtained from morphological markers is limited. For targeted studies of allelic variation at specific genes, however, analysis of morphological markers give useful information about genetic diversity analysis (Smith and Smith, 1989).

Morphological traits used in selecting breeding material are associated with large genetic distances. Therefore morphological traits used as genetic markers are often undesirable in genotypes and if used in selection must be removed in the final stages of the breeding programme. In addition, the number of morphological markers on genetic maps is generally limited. Therefore their utility is reduced due to the large genetic distances (Swiecicki and Timmer-Vuaghan, 2004).

Morphological markers are usually of limited use as they are often affected by environmental conditions or developmental growth stages. However, in some cases phenotypic markers such as leaf tip necrosis associated with *Lr*34 and *Yr*18 (wheat rust resistance genes) have been intensively used in selection of durable resistance in wheat (Feuillet and Keller, 2004).

Smith and Smith (1989) observed that the use of morphological markers alone immediately excludes analysis of those portions of the genome containing non-coding sequences, which in plants can often account for more than 90% of the complete DNA sequence.

In cocoyam, morphological traits such as colour of the flesh of the corm and cormels, lamina and vein colour and petiole colour have been used to distinguish genotypes. Size of the corm and cormels are also important (Onwueme, 1978). Mbouobda *et al.* (2007) characterised

Cameroonian cocoyam using leaf margin colour, leaf petiole colour, main vein colour, leaf sheath colour, number of cormels per plant, weight of cormels and corms per plant. These parameters represented 70.7% of the total variation. In a different study, Quero-Garcia *et al.* (2004) used more or less the same agro-morphological traits to stratify cocoyam germplasm. In addition, leaf lamina characters like margin, colour, variegation and orientation, stolon formation, corm shape, maturity period, growth habit, corm flesh colour and taste were also used.

2.8.2 Biochemical markers

Measurement and characterisation of genetic diversity have always been a primary concern in population and evolutionary genetic studies, because genetic variability provides a basis for evolutionary change. Over the years, methods for detecting and analysing genetic diversity have gradually progressed from Mendelian analyses of discrete morphological traits, to statistical characterisation of continuously varying quantitative characters, to electrophoretic assays of biochemical variants and, most recently, to molecular examinations of DNA sequence variation (Zhang *et al*., 1993).

Isoenzymes (isozymes) and allozymes are common biochemical markers that have been developed. Isoenzymes are different molecular forms in which proteins may exist with the same enzymatic specificity. This meant that different variants of the same enzymes have identical or similar functions and are present in the same individual. On the other hand, allozymes are variant proteins produced by allelic forms of the same locus (isozymes encoded by the allelic genes) (Zeidler, 2000). Due to the amino acid charge differences, allozymes can be differentiated by their relative migration speed during gel electrophoresis (Mueller and Wolfenbarger, 1999).

These biochemical markers are powerful tools for gene variability within and between populations of plants, in genetic relatedness studies, mating system estimations and genetic diversity assessments in clonally propagated plant species. Most allozymes are co-dominant and are expressed at all stages of the life cycle (Zeidler, 2000).

Biochemical markers have been used in different studies in cocoyam. They have been used in diversity analysis as well as pathogen identification and characterisation (Lebot and Aradhya, 1991; Tambong *et al*., 1999; Mitra *et al*., 2007).

Electrophoretic analysis of isozymes was used to differentiate *Phytophthora* blight susceptible cocoyam genotypes from resistant ones (Mitra *et al*., 2007). Further assessment of the differential expression of enzymes and their isozymes was done to determine their implications in *Phytophthora* blight disease resistance in cocoyam. Induction and/or increased activity of particular isoform(s) of superoxide dismutase (SOD) and guaiacol peroxidase (GPX) against infection of *Phytophthora colocasiae* Rac in the resistant genotypes and absence of such expressions in the susceptible genotype led to the conclusion of linkage of isozyme expression with blight resistance in cocoyam. This indicated the scope of using SOD and GPX as biochemical markers for disease resistance. These markers could be helpful in characterising the plants and for molecular breeding for leaf blight resistance (Sahoo *et al*., 2007).

Electrophoretic analysis of isozymes helped to differentiate cultivated cocoyam genotypes from wild types from Asia and Oceania. There was greater isozyme variation in Asia than in Oceania, with Indonesia being the area of greatest diversity. However, no correlations were found between zymotypes and morphotypes or ploidy levels (Lebot and Aradhya, 1991).

Manzano *et al*. (2001) found that isoenzyme analysis of esterases and peroxidases allowed for the characterisation of clones and confirmed that there were no duplicates in the cocoyam collection studied in Cuba, as each clone had its characteristic banding pattern in the esterase system. The study found a strong African and Japanese, as well as south east Asian and Philippine influence on the origin of the Cuban accessions.

2.8.3 Genetic markers

Genetic or molecular markers are powerful diagnostic tools used to detect DNA polymorphism both at the specific loci and whole genome level (Somers, 2004). According to Somers (2004) and Gupta and Varshney (2004), DNA markers reveal polymorphism in a DNA sequence, or the presence or absence of a particular DNA sequence at a particular site

in the genome (normally a restriction site or polymerase chain reaction (PCR) primer binding site). In most cases these polymorphisms manifest themselves in variation in the length of homologous DNA fragments and can thus be visualised and quantified by electrophoretic separation of fragments. In its simplest form, however, a DNA polymorphism may comprise no more than the substitution of a single nucleotide in a defined DNA segment. The DNA-based molecular markers have revolutionised the ability to characterise genetic variation. Significant progress has been made in the application of molecular markers to plant genetic resources' characterisation and evaluation (Gupta and Varshney, 2004).

Molecular markers are not only used in diversity and relationship analysis studies. They can also be utilised in DNA fingerprinting of germplasm and construction of molecular genetic maps of whole genomes. Identification of molecular markers that are tightly linked to genes/quantitative trait loci (QTL) controlling important traits is also possible. This help in gene tagging and is used in marker-assisted selection (MAS) in plant breeding programmes, leading to desirable gene stacking or pyramiding (Somers, 2004).

Molecular markers have several advantages over traditional phenotypic markers. They offer great scope for improving the efficiency of conventional plant breeding by carrying out selection not directly on the trait of interest but on molecular markers linked to that trait. This, of course, requires a molecular marker to be tightly linked to the trait of interest. Besides, these markers are not environmentally regulated and are, therefore, unaffected by conditions in which the plants are grown and are detectable in all stages of plant growth (Mohan *et al*., 1997).

The different marker systems that have been developed and are being applied to a range of crop plants include: restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), AFLP and simple sequence repeat (SSR) or microsatellites. In recent years newer techniques have been developed which include sequence tagged sites (STSs), single nucleotide polymorphism (SNP) and others (Rao, 2004).

Powell *et al*. (1996) observed that all marker assays have different properties: SSRs have the highest expected heterozygosity while AFLPs are characterised by a high multiplex ratio. RAPDs are intermediate in heterozygosity and multiplex ratio, while RFLPs have moderate heterozygosity and are uniquely appropriate for studying synteny. However, the most important criteria determining choice of assay should be informativeness and ease of genotyping for the specific crop system.

2.8.3.1 Application of AFLP analysis in cocoyam diversity assessment

AFLP (Vos *et al*., 1995) is a DNA fingerprinting technique which combines the merits of both the RFLP and the PCR techniques. It is widely used in many types of genetic analyses (Somers, 2004).

Vos *et al*. (1995) reported that the AFLP technique is based on the selective PCR amplification of restriction fragments from a total digest of genomic DNA. The AFLP technique involves three steps: (a) restriction of the DNA and ligation of oligonucleotide adapters, (b) selective amplification of sets of restriction fragments and (c) gel analysis of the amplified fragments. AFLP analysis does not require any sequence information of the nucleotides to visualise PCR products as it uses a limited set of genetic primers. The method can be used for DNA of any origin or complexity. The number of fragments detected in a single reaction can be tuned by selection of specific primer sets. The technique is highly reproducible. Fingerprints can be used to distinguish even between closely related organisms, including near-isogenic lines (NILs). It allows scoring of a large number of markers in a given population. It is robust and reliable because of the stringent conditions that are used for primer annealing.

According to Vos *et al*. (1995), the major advantage of the technology is in the high marker density that can be obtained. The frequency with which AFLP markers are detected depends on the sequence polymorphism between the tested DNA samples. The molecular basis of AFLP polymorphism will usually be single nucleotide polymorphism in the restriction sites or selection nucleotides adjacent to the restriction sites. In addition, deletions, insertions and rearrangements affecting the presence or size of restriction fragments will result in detectable polymorphism.

AFLP has proved to be a molecular marker of interest in cocoyam diversity studies because of its robustness. AFLP is preferred in most cocoyam studies conducted so far because it can analyse more samples per assay, it is reproducible, easy to use and does not require prior sequence information. This is of great importance in a crop like cocoyam which has not been exposed to advanced molecular research (Mueller and Wolfenbarger, 1999). However, compared to co-dominant microsatellite markers, AFLP markers suffer from their dominant nature, i.e. markers are scored as present or absent (null) and thus does not allow identification of homologous alleles. This renders the marker less useful for studies that require precise assignment of allelic states (Mueller and Wolfenbarger, 1999).

The technique was used to study the cocoyam genetic diversity in China and the Pacific Ocean islands. In all these cases the AFLP fingerprints differentiated the cocoyam morphotypes, most of which did not present significant intra-clonal variation (Jianchu *et al*., 2001; Caillon *et al*., 2006). AFLP assays were able to validate cocoyam genotypes stratified using ethno-botany and morphological data. This showed the efficiency of the AFLP assay, especially if ethno-botany is incorporated in the interpretation of data (Jianchu *et al*., 2001). AFLP markers proved to be useful for detecting duplicates and fingerprinting of cocoyam accessions in genebanks. This will be an important contribution to cocoyam breeding programmes where diversity is much sought after (Quero-Garcia *et al*., 2004).

The robustness of the AFLP assays were demonstrated when three AFLP primer combinations generated a total of 465 scorable amplification products. The 255 accessions from Vietnam, Thailand, Malaysia, Indonesia, the Philippines, Papua New Guinea and Vanuatu were grouped according to their country of origin, ploidy level (diploid or triploid) and habitat - cultivated or wild (Kreike *et al*., 2004).

Caillon *et al*. (2006) reported that the AFLP technique was reliable in identifying duplicates, somatic mutants, related genotypes and feral plants of cocoyam. They further observed that without farmers' knowledge, this powerful technique would not have been able to list and quantify the distinct but complementary diversifying patterns of cocoyam germplasm and especially to highlight the importance of sexual reproduction.

Sharma *et al*. (2008) used AFLP assays to analyse the geographical differentiation, phylogenetic relationships and to identify molecular markers linked to cocoyam leaf blight resistance genes of Indian cocoyam. The significant differentiation in Indian cocoyam genotypes and identification of AFLP markers linked to the leaf blight resistance gene clearly demonstrated that AFLP assays can distinguish cocoyam genotypes by their unique and different banding patterns. This may also provide a starting point for map-based cloning of this important gene. Jiachun *et al*. (2004) emphasised the efficiency of AFLP markers for investigating genetic relationships in one of the Araceae ornamental foliage plants usually propagated vegetatively, similar to cocoyam. About 54 cultivars of *Aglaonema* species were classified into eight clusters using AFLP markers.

Other markers have also been used in cocoyam studies. RAPD markers have been used in cocoyam diversity analysis studies in Ghana and USA (Schnell *et al*., 1999; Offei, *et al*., 2004). In a study by Schnell *et al*. (1999), RAPD markers suggested low levels of genetic variation (0.86-0.97 genetic similarity) among cocoyam genotypes from the United States of America Department of Agriculture/Agricultural Research Services (USDA/ARS) germplasm collection. Seven random primers generated 40 RAPD loci, that revealed that 11 of the 18 genotypes assessed were identical at all RAPD loci. In a different study, Offei *et al*. (2004) used 10 random primers to study the genetic diversity and structure of the experimental material in Ghana and a total of 120 different bands were detected. Levels of polymorphic fragments detected by the 10 primers ranged from 69.2-100%.

Mace and Godwin (2002) identified microsatellites from enriched genomic libraries of *taro* cocoyam, which were mostly dinucleotide or trinucleotide repeats. Singh *et al*. (2008) used 30 agro-morphological descriptors and DNA fingerprints of seven SSR primers to assess and rationalise cocoyam diversity in Papua New Guinea. The sample was efficiently reduced to 10% of the total collection, removing unnecessary duplicates.

Cocoyam holds great potential as a cash, food security and industrial crop. Although limited information exists on the amount of diversity in different cultivating regions, much of germplasm is held *in situ* by farmers maintaining the germplasm. A lot of variants of the crop exists due to among other reasons the unpredictable behaviour of the chromosome numbers. Genetic diversity and nutritional studies of the crop in different niches will help generate the information needed for an efficient conservation and improvement programme.

2.9 References

Abdulrashid, M., and Agwunobi, L.N. (2009). *Taro* cocoyam (*Colocasia esculenta*) meal as feed ingredient in poultry. Pakistan Journal of Nutrition 8: 668-673.

Acuna, P.M. (2006). Cocoyam plant drawing. In: Castro, G. (Eds.). Studies on cocoyam (*Xanthosoma* spp) in Nicaragua, with emphasis on mosaic virus. Uppsala: PhD thesis-Swedish University of Agricultural sciences, pp. 8.

Agbor-egde, T., and Rickard, J. (1991). Study on the factors affecting storage of edible aroids. Annals of Applied Biology 119: 121-130.

Beeching, J., Marmey, P., Garada, M., Nairot, M., Haysom, H., Hughes, M., and Charrier, A. (1993). An assessment of genetic diversity within collections of cassava (*Manihot esculenta* Crantz) germplasm using molecular markers. Annals of Botany 72: 515-520.

Caillon S., Quero-Garcia, J., Lescure, J.P., and Lebot, V. (2006). Nature of *taro* (*Colocasia esculenta* (L.)) genetic diversity prevalent in a Pacific Ocean island, Vanua lava, Vanuatu. Genetic Resources and Crop Evolution 53: 1273-1289.

Castro, G. (2006). Studies on cocoyam (*Xanthosoma* spp) in Nicaragua, with emphasis on dasheen mosaic virus. PhD thesis-Swedish University of Agricultural Sciences, Uppsala, pp. 7-8.

Catherwood, D., Savagea, G., Masona, S., Schefferb, J., and Douglas, J. (2007). Oxalate content of cormels of Japanese *taro* (*Colocasia esculenta* (L.) Schott) and the effect of cooking. Journal of Food Composition and Analysis 20: 147-151.

Ekanem, A., and Osuji, J. (2006). Mitotic index studies on edible cocoyam. African Journal of Biotechnology 5: 846-849.

Ekwe, K., Nwosu, K., Ekwe, C., and Nwachukwu, I. (2009). Examining the underexploited values of cocoyams (*Colocasia* and *Xanthosoma* species) for enhanced household food security, nutrition and economy in Nigeria. In: Jaenicke, H., Ganry, J., Zeledon-Hoeschle, I., and Kahane, R. (Eds.). Proceedings of the international symposium on underutilized plants for food security, income and sustainable development. Acta Horticulturae 806, ISHS, pp. 71-78.

Englberger, L., Schierle, J., Kraemer, K., Aalbersberg, W., Dolodolotawake, U., Humphries, J., Graham, R., Reid, A.P., Lorens, A., Albert, K., Levendusky, A., Johnson, E., Paul, Y., and Sengebau, F. (2008). Carotenoid and mineral content of micronesian giant swamp *taro* (*Cyrtosperma*) cultivars. Journal of Food Composition and Analysis 21: 93-106.

FAO (1990). Food and Agriculture Organisation of the United Nations. Roots, tubers, plantain and bananas in human nutrition, Food and Nutrition series, No. 24: FAO code: 86, AGRIS: SO1 ISBN 92-5-102862-1. Rome.

Feuillet, C., and Keller, B. (2004). Molecular markers for disease resistance: The example of wheat. In: Lorz, H., and Wenzel, G. (Eds.). Biotechnology of Agriculture and Forestry, Vol 55. Molecular marker systems. Springer-Verlag, Berlin Heidelberg, pp. 335-350.

Gholiazadeh, M., Mianji, G.R., and Zadeh, H.S. (2008). Potential use of molecular markers in genetic improvement of livestock. Asian Journal of Animal and Veterinary Advances 3: 120-128.

Global forum for underutilised species. (2009). http://www.underutilised-species.org/about_GFU.asp. (2009/09/05).

Gupta, P., and Varshney, K., (2004). The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. Euphytica 113: 163-185.

Huang, C.C., Chen, W.C. and Wang, C.C.R. (2007). Comparison of Taiwan paddy- and -upland cultivated *taro* (*Colocasia esculenta* L.) cultivars for nutritive values. Food Chemistry 102: 250-256.

http://www.bioversityexplorer.org/plants/araceae/colocasia_esculenta.htm. (2008/03/20).

Janseens, M. (2001). Crop production in tropical Africa. Bonn, Germany: Institut fur obst- und germusebau, abt. Tropicscher pflanzenbau, rheinische friedrich-wilhelms-Universitat Bonn, auf dem hugel 6, D-53121 Bonn, Germany. Directorate general for international co-operation, karmelietenstraat 15, B-1000 Brussels, Belgium, pp. 221-228.

Jiachun, C., Devanand, P.S., Norman, D.J., Henny, R.J., and Chih-Chen T.C. (2004). Genetic relationships of *Agloenema* species and cultivars inferred from AFLP markers. Annals of Botany 193: 157-166.

Jianchu, X., Yongping, Y., Yingdong, P., Ayad, W.G., and Eyzagnire, P. (2001). Genetic diversity in *taro* (*Colocasia esculenta* Schott, Araceae) in China: An ethnobotanical and genetic approach. Economic Botany 55: 14-31.

Kreike, C., Van Eck, H., and 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., and Avtar, S. (1981). Karyoptic and electrophoretic studies on *taro* and its origin. Euphytica 30: 405-413.

Laurentin, H. (2009). Data analysis for molecular characterisation of plant genetic resources. Genetic Resources and Crop Evolution 56: 277-292.

Lebot, V., and Aradhya, K.M. (1991). Isozyme variation in *taro* (*Colocasia esculenta* (L.) Schott) from Asia and Oceania. Euphytica 56: 55-66.

Lebot, V., Ivancic, A., and Abraham, K. (2005). The geographical distribution of allelic diversity, a practical means of preserving and using minor root crop genetic resources. Experimental Agriculture 41: 475-489.

Lewu, M., Adebola, P., and Afolayan, A. (2009). Effect of cooking on the proximate composition of the leaves of some accessions of *Colocasia esculenta* (L.) Schott in Kwazulu-Natal province in South Africa. African Journal of Biotechnology 8: 1619-1622.

Mace, E., and Godwin, I. (2002). Development and characterisation of polymorphic microsatellite markers in *taro* (*Colocasia esculenta*). Genome 45: 823-832.

Manzano, A., Nodals, A., Gutierrez, M., Mayor, F., and Alfonso, C. (2001). Morphological and isozyme variability of *taro* (*Colocasia esculenta* (L.) Schott) germplasm in Cuba. Plant Genetic Resources Newsletter 126: 31-40.

Mbouobda, H.D., Boudjeko, T., Djocgoue, P.F., Tsafack, T.J.J., and Omokolo, D.N. (2007). Morphological characterization and agronomical evaluation of cocoyam (*Xanthosoma sagittifolium* (L.) Schott) germplasm in Cameroon. Journal of Biological Sciences 7: 27-33.

Mitra, S., Sinha, B., Pal, H., and Tarafdar, J. (2007). Comparative studies on morphological characters, yield, nutritional status and isozymes activity of some elite genotypes of *taro* [*Colocasia esculenta* (L.) Schott var. *antiquorum*] grown in West Bengal. In: Chandha M.L., Kuo, G., and Gowda, C.L.L. (Eds.). Proceedings of the First International Conference on Indigenous Vegetables and Legumes. Acta Horticulturae, 752 ISHS, pp. 219-230.

Mohan, M., Nair, S., Bhagwat, A., Krisnhna, T.G., Yano M., Bhatia C.R., and Sasaski T. (1997). Genome mapping, molecular markers and marker-assisted selection in crop plants. Molecular Breeding 3: 87-107.

Mueller, U., and Wolfenbarger, L.L. (1999). AFLP and fingerprinting: Reviews. Tree 14: 389-394.

Mweta, D.E., Labuschagne, M.T., Koen, E., Benesi, I.R.M., and Saka, J.D.K. (2008). Some properties of starches from cocoyam (*Colocasia esculenta*) and cassava (*Manihot esculenta* Crantz.) grown in Malawi. African Journal of Food Science 2: 102-111.

National Academy of Science. (1975). Underxploited tropical plants with promising economic. Report of ad hoc panel of the advisory committee on technology inovations, national academy of sciences, USA. Washington D.C.

Njoku, P.C., and Ohia, C.C. (2007). Spectrophometric estimation studies of mineral nutrient in three cocoyam cultivars. Pakistan Journal of Nutrition 6: 616-619.

Offei, S., Asante, I.K., and Danquah, E.Y. (2004). Genetic structure of seventy cocoyam (*Xanthosoma sagittifolium*, (L.), Schott) accessions in Ghana based on RAPD. Hereditas 140: 123-128.

O'Hair, S.K. (**1990).** Tropical root and tuber crops. In: Janick, J. and Simon, J.E. (Eds.). Advances in new crops. Timber Press, Portland, OR, pp. 424-428.

Onokpise, O.U., Wutoh, J.G., Ndzana, X., Tambong, J.T., Meboka, M.M., Sama, A.E., Nyochembeng, L., Aguegia, A., Nzietchueng S., Wilson J.G., and Burns, M. (1999). Evaluation of macabo cocoyam germplasm in Cameroon. In: Janick, J. (Eds.). Perspectives on new crops and new uses. Alexandria, VA: ASHS Press, pp. 394-396.

Onwueme, I. (1978). The tropical tuber crops: Yams, cassava, sweet potato and cocoyams. John Wiley and Sons, pp. 199-225.

Onwueme, I. (1999). *Taro* cultivation in Asia and Pacific: RAP Publication 1999/16. Bangokok, Thailand: Food and Agriculture Organisation of the United Nations regional office for Asia and the Pacific.

Onwuka, N., and Enech, C. (1996). The cocoyam, *Xanthosoma sagittifollium*, as a potential raw material source for beer brewing. Plant Foods for Human Nutrition 49: 283-293.

Onyilanga, J., Omenyi, A., Illoh, H., and Lowe, J. (1987). *Colocasia esculenta* (L.) Schott., *Colocasia antiquorum* Schott., How many species? A preliminary investigation. Euphytica 36: 687-692.

Oscarsson, K., and Savage, G. (2007). Composition and availability of soluble and insoluble oxalates in raw and cooked *taro* (*Colocasia esculenta* var. Schott) leaves. Food Chemistry 101: 559-562.

Perez, E., Guti'errez, M., Pacheco de Delahaye, E., Tovar, J., and Lares, M. (2007). Production and characterisation of *Xanthosoma sagittifolium* and *Colocasia esculenta* flours. Journal of Food Science 72: 367-372.

Plucknett, D.L., 1976. Edible Aroids: *Alocasia, Colocasia Cyrtosperma, Xanthosoma.* In: Simmonds, N.W. (Ed.). Evolution of Crop Plants. London and New York: Longman, pp. 10-12.

Powell, W., Morgate, M., Chadre, C., Hanafey, M., Vogel, J., Tingey, S., and Rafasalki, A. (1996). The comparison of RFLP, AFLP, RAPD, and SSR (microsatellites) markers for germplasm analysis. Molecular Breeding 2: 225-238.

Purseglove, J. (1972). Tropical crops: Monocotyledons Vol 1. Essex England: Longman, pp. 66-74.

Quero-Garcia, J., Noyer, J., Perrier, X., Marchand, J.L., and Lebot, V. (2004). A germplasm stratification of *taro* (*Colocasia esculenta*) based on agro-morphological descriptors, validation by AFLP markers. Euphytica 137: 387-395.

Ramana, R.V., and Hodgkin, T. (2002). Genetic diversity and conservation and utilization of plant genetic resources. Plant Cell, Tissue and Organ Culture 68: 1-19.

Rao, K.N. (2004). Plant genetic resources: Advancing conservation and use through biotechnology. African Journal of Biotechnology 3: 136-145.

Rodriguez, L., Lopez D.J., Preston T.R., and Peters, K. (2006). New cocoyam (*Xanthosoma sagittifolium*) as partial replacement for soya bean meal in sugar cane

diets for growing pigs. Livestock Research for rural development 18(7). Retrieved from http://www.lrrd.org/lrrd. (2009/09/09).

Sahoo, M., Madhumita, D., Kole, P., Bhat, J., and Mukherjee, A. (2007). Antioxidative enzymes and isozymes analysis of *taro* genotypes and their implications in *Phytophthora* blight disease resistance. Mycopathologia 163: 241-248.

Sajev, M., Manikantan, M., Kingsly, A., Moorthy, S., and Sreekumar, J. (2004). Texture analysis of *taro* (*Colocasia esculenta* (L.) Schott) cormels during storage and cooking. Journal of Food Science 69: 315-321.

Schnell R.J., Goenaga R., and Olano C.T. (1999). Genetic similarities among cocoyam cultivars based on Randomly Amplified Polymorphic DNA (RAPD) analysis. Scientia Horticulturae 80: 267-276.

Sefa-Dedeh, S., and Kofi-Agyir, S.E. (2004). Chemical composition and the effect of processing on oxalate content of cocoyam *Xanthosoma sagittifolium* and *Colocasia esculenta* cormels. Food Chemistry 85: 479-487.

Sen, S., Bhattacharya, A.D.M., Sen, D., Das, A., and Pal, S. (2006). Nutrient and antinutrient composition of cormels of *Colocasia esculenta* var. *antiquorum*. Journal of Vegetable Science 11: 17-33.

Sharma, K., Mishra, A.K., and Misra, R. (2008). Analysis of AFLP variation of *taro* population and markers associated with leaf blight resistance gene. Academic Journal of Plant Sciences 1: 42-48.

Singh, D., Mace, E., Godwin, I., Mathur, P., Okpul, T., Taylor, M., Hunter, D., Kambuou, R., Ramanatha, Rao, V., and Jackson, G. (2008). Assessment and rationalization of genetic diversity of Papua New Guinea *taro* (*Colocasia esculenta*) using SSR DNA fingerprinting. Genetic Resources and Crop Evolution 55: 811-822.

Smith, J.S.C., and Duvick, D. (1989). Germplasm collections and private plant breeder. In: Brown, A., Marshal, D.R., Frankel, O. and Williams, J. (Eds.). The use of plant genetic resources. Cambridge University Press, IBPGR. pp. 16-31.

Smith, J.S.C., and Smith, O.S. (1989). The description and assessment of distances between inbred lines of maize. II. The utility of morphological, biochemical and genetic descriptors and a scheme for testing of distinctiveness between inbred lines. Maydica 34: 151-161.

Somers, D.J. (2004). Molecular marker systems and their evaluation for cereal genetics. In: Gupta, P.K. and Varshney, R., (Eds.). Cereal genomics. Netherlands: Kluwer Academic Press, pp. 19-34.

South Pacific Community (SPC). (1993). *Taro*: South Pacific foods leaflet 1. Suva, Fiji: Community educational material training centre.

Swiecicki, W.K., and Timmer-Vuaghan, (2004). The localisation of important traits: the example pea (*Pisum sativum* L.) In: Lorz, L. and Wenzel, G., (Eds.). Biotechnology of Agriculture and Forestry, Vol 55. Molecular marker systems. Springler-verlag, Berlin Heidelberg, pp. 155-166.

Tambong, T.J., Poppe J., and Hofte, M. (1999). Pathogenicity, electrophoretic characterization and in *planta* detection of the cocoyam root rot disease pathogen, *Pythium myriotylum*. European Journal of Plant Pathology 105: 597-607.

Thomas, A., and Oyediran, O. (2008). Nutritional importance and micronutrient potentials of two non-conventional indigenous green leafy vegetables from Nigeria. Agricultural Journal 3: 362-365.

Vos P., Hogers R., Bleeker, M., Reijans, M., Van de Lee, T., Hormes M., Friters A., Pot J., Paleman, J., Kuiper, M. and Zabeau, M., (1995). AFLP: A new technique for DNA fingerprinting. Nucleic Acids Research 23: 4407-4414.

Williams, C., Chew, W., and Rajaratnam, J. (1982). Cocoyam. In: Payne, W. (Ed.). Tropical and field crops of the wetter regions of the tropics. Longman, pp. 210-211.

Williams, J., and Haq, N. (2002). Global research on underutilised crops: An assessment of current activities and proposals for enhanced cooperation. Southampton, UK: International Centre for Underutilised Crops.

Wilson, J. (1984). Cocoyam. In: Goldsworthy, P., and Fischer, N. (Eds.). The physiology of tropical field crops. New York and London: John Willey and Sons Ltd, pp. 589-605.

Zeidler, M. (2000). Acta universitatis palackianea olomecensis Facultas Rerum Naturalium Biolgica 38: 8-6.

Zhang, Q., Saghai Maroof, M.A., and Kleinhofs, A. (1993). Comparative diversity analysis of RFLPs and isozymes within and among populations of *Hordeum vulgare* ssp. *spontaneum.* Genetics 134: 909-916.

CHAPTER 3

Ethno-botanical and morphological characterisation of cocoyam germplasm in Malawi

3.1 Introduction

Cocoyam is one of the major root and tuber crops of the tropics (O'Hair, 1990). It is widely grown as a staple food and the potential of the crop is high in the humid and sub-humid tropics (Onwueme, 1978; Okonkwo, 1993). It is a member of the Araceae family and the two most important species of the edible aroids are *C. esculenta* and *X. sagittifolium* (Purseglove, 1972). *Colocasia esculenta* is commonly known as *taro* or old cocoyam and *X. sagittifolium* as *tannia* or new cocoyam. They are generically called cocoyam in most parts of the tropics and both produce edible starchy corms and cormels (Purseglove, 1972; Janseens, 2001).

The cocoyam plant is a perennial herb but is cultivated as an annual crop. It has a thick, tuberous underground stem with simple, broad and long-petiole leaves (Okonkwo, 1993). Generative propagation in natural conditions seldom occurs due to erratic flowering and poor germination of seeds. Propagation under cultivation is therefore by vegetative means through the corms and cormels and in the wild through stolons (Purseglove, 1972; Onwueme, 1978). It is a monoeciuos and highly variable plant. Thousands of species exist and it is nearly impossible to describe all existing morphological variation and in most cases only the most important and genetically inherited traits are used as distinguishing characters (Purseglove, 1972; Kurvilla and Singh, 1981; Ivancic *et al*., 2003). Much of the visible phenotypic diversity of cocoyam cultivars is attributed to vegetative mutation and selection by growers for specific attributes. Therefore there exists great diversity in colour patterns in the corms and leaves (Mathews, 2004).

Cocoyam, though an important staple for millions of people in the world, is cultivated mainly in developing countries and rarely on large plantations, but rather on small farms with little use of technology (Chien-Chun *et al*., 2007). It has been neglected by science and most knowledge regarding its diversity lies with farmers in different ethnic communities (Jianchu

et al., 2001). Cocoyam genetic resources are generally maintained in *ex situ* collections. *In situ* collections are limited and there is a dearth of information regarding the existing level of genetic diversity and how much has been conserved in different communities (Lebot and Aradhya, 1991). Furthermore, various systems for growing and uses of cocoyam in different cocoyam growing regions have been poorly documented.

A better understanding of cocoyam genetic diversity and its distribution lies in the ability of researchers and breeders to tap the indigenous knowledge from ethnic groups. This will not only help determine the level of diversity that exists but also what to collect, conserve and use of the different species and wild relatives of cocoyam (Ramana and Hodgkin, 2002). Information on the distribution of genetic diversity and its uses by farmers, as well as the within-crop gene pool, is essential in the conservation and maintenance of genetic diversity in cultivated cocoyam (Lebot and Aradhya, 1991). Characterisation is one of the critical steps in conservation and maintenance of genetic diversity since it helps to identify different accessions and discern genetic relationships among genotypes in a germplasm collection. Traditional approaches for the measurement of diversity have relied upon the ability to resolve differences in morphological traits (Karp *et al*., 1996). These approaches have been successful in cocoyam germplasm characterisation with the aid of ethno-botany and curinary knowldge (Prana, 2000; Jianchu *et al.*, 2001; Mathews, 2004). However, they are limited in accurate identification of accessions due to a limited number of traits, especially with highly heritable traits which show less variation over most of the material studied and are often affected by environmental influences (Rao, 2004; Laurentin, 2009).

Cocoyam exhibits a wide range of agro-morphological polymorphism. Numerous variable but clonally stable traits are being used as markers for varietal identification and assessment of genetic diversity (Mathews, 2004). Prana (2000) used 19 botanical and agronomical traits to unravel the genetic variability in 335 cocoyam accessions from West Java. The study succesfuly identified *C. esculenta* var. *esculenta* as the dominant species being used as a carbohydrate source in West Java. *Colocasia gigantea* and other wildtypes were furthermore identified. Jianchu *et al*. (2001) and Mathews (2004) demonstrated how indigenous and culinary knowledge added efficiency in cocoyam germplasm characterisation and management. Mbouobda *et al*. (2007) found significant variability in Cameroonian cocoyam

germplasm using agro-morphological traits. The principal component analysis (PCA) suggested that this represented 70.7% of the total variability. Jianchu *et al.* (2001) used ethno-botany data to elucidate cocoyam genetic diversity that exists in China. Mathews (2004) used a culinary knowledge survey and molecular approaches to study the origin, domestication and dispersal of cocoyam in the Pacific. This study established that cocoyam is an ancient root crop in Asia, Africa and the Pacific which is genetically diverse. International Plant Genetic Resources Institute (IPGRI) descriptors are also being utilised in many cocoyam studies (IBPGR, 1989; IPGRI, 1999). Quero-Garcia *et al*. (2004) and Opoku-Agyeman *et al*. (2004) used cocoyam agro-morphological descriptors developed by IPGRI to characterise cocoyam germplasm in Vanuatu and Ghana, respectively.

Based on many circumstantial stories and scientific reports, the ethno-botany of the Araceae family is diverse and fascinating (Mathews, 2004). In Malawi, like China, Vietnam and many developing countries (Hue *et al*., 2001; Jianchu *et al*., 2001), most of its native germplasm is managed by smallholder farmers (Malawi Government Report, 1996). These farmers not only demonstrate elaborate knowledge of the crops they grow and conserve, but also indigenous knowledge of local classification and safety of these crops. Mkumbira *et al*. (2003) and Benesi (2005) demonstrated how extensive knowledge by Malawian farmers helped in the classification of cassava into bitter and sweet cultivars, based on folk taxonomy. Farmers hold enormous ethno-botanical and ethno-ecological knowledge of the cultivars they grow. Common descriptors used by farmers to characterise cocoyam include leaf, petiole, root and tuber characters. In Nepal, Rijal (2004) found direct and positive links between diversity of food traditions, ecological variations and cocoyam genotypes grown. The diversity was strongly linked to ecological variation, local uses and income generation for the smallholder farmers.

Knowledge of the different culinary qualities i.e. different qualities that affect preparation and consumption is important to plant breeders because it is critical for the acceptance of new cultivars by consumers. So far, no effort has been made to relate specific genes in cocoyam to specific culinary qualities or traditional uses and limited information on traditional uses of cocoyam has been documented (Mathews, 2004).

The initiation of cocoyam research in Malawi through an initial collection of germplasm calls for a detailed characterisation and proper conservation of such material. This will be successful if optimal existing indigenous knowledge is obtained from farmers to help guide the direction of conservation and establishment of a successful breeding programme.

The objectives of this study were to (a) determine the genetic diversity of cocoyam genotypes using ethno-botany and morphological markers and (b) to assess farmers' preferences and uses of cocoyam in Malawi.

3.2 Materials and methods

3.2.1 Collection of Malawian cocoyam germplasm and gathering of ethno-botany data

Cocoyam germplasm were collected from several districts in Malawi in 2007, jointly by the Department of Agricultural Research Services (DARS) and the International Institute of Tropical Agriculture/Southern Africa Root Crops Research Network (IITA/SARRNET). This collection was done in the northern and the southern regions of Malawi. In the north it was done along the lake shore districts of Nkhatabay, Rumphi, Karonga and Chitipa. In the south collection was done in the Shire highlands and the Shire valley districts of Mangochi, Machinga, Zomba, Mulanje, Thyolo and Chikwawa.

Each accession collected had detailed passport data. This included: accession code, accession name, sample status, name of farmer, ethnic group, village, traditional authority, district, extension planning area (EPA), collecting institution, collection date, taste, maturity period, target use, period that cultivar has been with the farmer and preferred characteristics of the cultivar. The location of collection in terms of latitude, longitude and altitude was captured. Collected accessions were multiplied at Chitedze Research Station in July 2007.

3.2.2 Morphological characterisation using descriptors

3.2.2.1 Plant material

Plant material consisted of 28 accessions from the collection from several districts across Malawi that was multiplied at Chitedze Research Station. Pre-sprouted corms were planted in

a randomised complete block design (RCBD), with three replicates of five plants each. The planting distance was 0.9 m between rows and 0.9 m between plants.

The Chitedze Research Station is located between latitude 13°59' S and longitude 33°38' E with an altitude of about 1146 m above sea level (masl) on the Lilongwe plain. It has a mean annual temperature of 20°C and mean annual rainfall of 892 mm [Ministry of Agriculture and Food Security (MoAFS, 2008)].

3.2.2.2 Morphological traits

The morphological (above ground) parameters were evaluated between 3-6 months after planting. Data for 30 above ground qualitative characters were collected according to the International Plant Genetic Resources Institute (IPGRI) Taro descriptor (IPGRI, 1999. Morphological data for the 28 accessions were converted into a binary matrix using the procedure of Benesi (2005). Thus traits with two categories were scored in a normal binary matrix and traits with multiple categories of description i.e. colour and shape, were coded considering the range of diversity of the trait and scored against that particular class. For example, predominant position (shape) of leaf lamina surface (PPLLS) ranged from 1=drooping, 2=horizontal, 3=cup-shaped, 4=erect-apex up and 5=erect-apex down. If an accession's predominant position (shape) of leaf lamina surface was drooping, it was scored as 1 against PPLLS1 (drooping), and 0 for PPLLS2 (horizontal), PPLLS3 (cup-shaped), PPLLS4 (erect) and PPLLS5 (erect-apex down) (Appendix 1, IPGRI, 1999).

3.2.3 Data analysis

Similarity coefficients for morphological data were calculated using the Dice coefficient (Dice, 1945) and the NTSYSpc version 2.2c computer package (Rohlf, 2000). Dendrograms were constructed using the unweighted pair group method of arithmetic averages (UPGMA) in SAHN programme parameters (Rohlf, 2000). The goodness of fit of clustering matrices was calculated using COPH and MXCOMP programmes in NTSYSpc.

3.3 Results and discussion

3.3.1 Ethno-botany: prevalence, preference and utilisation of cocoyam in Malawi

Results indicated that cocoyam is grown in both the southern and northern regions of Malawi. It is grown from as low as 87 masl and as high as 1470 masl provided there is ample moisture. It is mainly grown as a homestead-backyard crop in the southern region. In the northern region it is cultivated as an intercrop to most *dimba* (wetland) crops like banana/plantains. It is grown as a food crop in the two regions covered in this study (Table 3.1).

The crop is known by different names in the different regions. This is mainly influenced by the different ethnic groups found in the region. The northern region (lake shore) is mainly dominated by the *Tumbuka* and *Tonga* tribes that refer to cocoyam as *masimbe*. Sometimes they distinguish the local genotypes from introductions from China by the Chinese experts as *chinese*. In the southern region cocoyam is generally referred to as *koko.* The *Yao* tribe in the southern districts along the Shire highlands also refer to cocoyam as *zigumbwa* and *dumbe.* The *Lhomwe* and *Mang'anja* tribes in the southern region also refer to cocoyam as *koko*. All the local names however, do not have specific meanings other than cocoyam (Table 3.1).

Results showed that people from the two regions prefer cocoyam with high dry matter (HDM) content. Other traits that farmers preferred included taste, high yield in terms of tuber size, mealyness, resilience to bad weather, early maturity and good cooking properties (Table 3.2). In both regions people opted for tubers with HDM content (41%), followed by taste (17%), high yield (17%) and good cooking properties like boiling (17%), mealyness (8%) and suitability for making pulp (*msima*) (8%) (Table 3.2).

The preferences varied between the two regions. The northern region of Malawi is the only region which consumes root crops as their staple i.e. cassava in Nkhatabay district (Mkumbira *et al*., 2003). Hence people in the region preferred cocoyam with high yield, taste, good quality for making pulp, HDM content and mealyness. On the other hand, the southern region consumes cocoyam as a snack during breakfast. Hence they prefer traits that will not affect palatability and taste such as HDM, taste and after boiling properties. These traits are not only important to the consumers but also for breeders. Breeders need to

incorporate these traits if they are to be efficient in their breeding efforts. Breeding for cocoyam that incorporates these traits would help in diffusion of the new varieties to the end users.

Table 3.1 Passport data of the cocoyam accessions indicating exact collecting points, topography, soil type and local names

	Acc. No.[1]	Region	District	Ethnic group[2]	Altitude (masl)	Latitude	Longitude	Topography	Soil type	Local name
1	**MH/CHE/2007/Coy1**	South	Mangochi	*Yao*	491	8416962	0694616	Flood-plain	Sandy-loam	*Koko*
2	**MH/LI/2007/Coy3**	South	Mangochi	*Yao*	-	-	-	Hill/Mountain	Sandy-clay-loam	*Dumbe*
3	**MH/MDU/2007Coy5**	South	Mangochi	*Yao*	809	8410092	0779941	Undulating	-	*Koko*
4	**MHG/BA/2007/Coy6**	South	Machinga	*Yao*	-	-	-	-	-	*Koko*
5	**MHG/NTU/2007/Coy9A**	South	Machinga	*Yao*	689	8321745	0745218	Undulating	Loam	*Zigumbwa*
6	**ZA/FIKI/2007/Coy13**	South	Zomba	*Yao*	669	8310041	0741085	Undulating	Loam	*Koko*
7	**ZA/MDO/2007/Coy14**	South	Zomba	*Yao*	632	8309052	0740177	Undulating	Sandy-loam	*Koko*
8	**ZA/MA/2007/Coy16**	South	Zomba	*Yao*	642	8307949	0739696	Undulating	Loam	*Koko*
9	**MHG/CHA/2007/Coy20**	South	Machinga	*Yao*	761	8321301	0756851	Undulating	Loam	*Koko*
10	**MHG/NA/2001/Coy21**	South	Mangochi	*Yao*	644	8320916	0766748	Flood-plain	Sandy-loam	*Koko*
11	**ZA/NSA/2007/Coy21/4**	South	Zomba	*Yao*	750	8314423	0755149	Flood-plain	Sandy-loam	*Koko*
12	**MHG/NA/2007/Coy22**	South	Machinga	*Yao*	644	8320916	0766748	Plain	Sandy-loam	*Koko*
13	**MHG/MTE/2007/Coy23**	South	Machinga	*Yao*	632	8315754	0767374	Flood-plain	Sandy-loam	*Koko (Chinese)*
14	**ZA/BU/2007/Coy28**	South	Zomba	*Yao*	1091	8307476	0750996	Hill/mountain	Sandy-loam	*Koko*
15	**MHG/KWE/2007/Coy30**	South	Machinga	*Yao*	621	8315830	0742474	Undulating	-	*Koko*
16	**ZA/MU/2007/Coy31**	South	Zomba	*Chewa*	789	8297105	0756067	Flood-plain	Sandy-loam	*Koko*
17	**MJ/NA/2007/Coy34**	South	Mulanje	*Lhomwe*	662	8221091	0789589	Undulating	Loam	*Koko*
18	**TO/CHI/2007/Coy37**	South	Thyolo	*Lhomwe*	850	8214137	0732542	Undulating	Loam	*Koko*
19	**CK/MKHU/2007/Coy42**	South	Chikwawa	*Mang'anja*	87	8223070	0699476	Flood-plain	Sandy-loam	*Koko*
20	**NB/KWE/2007/Coy49**	North	Nkhata-bay	*Tonga*	514	8662516	0614353	Lake shore	Loam	*Masimbi*
21	**NB/MU/2007/Coy50**	North	Nkhata-bay	*Tonga*	492	8664064	0615054	Lake shore	Sandy-loam	*Masimbi*
22	**NB/MTE/2007/Coy51**	North	Nkhata-bay	*Tonga*	516	8691588	0623979	Lake shore	Sandy-loam	*Masimbi*
23	**NB/KA/2007/Coy53**	North	Nkhata-bay	*Tonga*	534	8723858	0631438	Flood-plain	Clay-loam	*Masimbi (Chinese)*
24	**NB/CHI/2007/Coy55**	North	Nkhata-bay	*Tonga*	1070	8740380	0627943	Undulating	Loam	*Masimbi*
25	**NB/CHI/2007/Coy56**	North	Nkhata-bay	*Tonga*	1070	8740380	0627943	Undulating	Loam	*Masimbi*
26	**NB/CHI/2007/Coy57**	North	Nkhata-bay	*Tumbuka*	-	-	-	-	-	*Masimbi*
27	**RU/KHA/2007/Coy60**	North	Rumphi	*Tumbuka*	1093	8802084	0610223	Flood-plain	Clay-loam	*Masimbi*
28	**RU/KA/2007/Coy61**	North	Rumphi	*Tumbuka*	1203	8810392	0612944	-	Loam	*Masimbi*
29	**RU/KA/2007/Coy62**	North	Rumphi	*Tumbuka*	1203	8810392	0612944	-	Loam	*Masimbi*
30	**RU/CHA/2007/Coy63**	North	Rumphi	*Tumbuka*	1332	8812708	0612085	Flood-plain	Loam	*Masimbi (Chinese)*
31	**RU/NKHA/2007/Coy64**	North	Rumphi	*Tumbuka*	1324	8812628	0612199	Undulating	Sandy-clay-loam	*Masimbi*
32	**RU/MA/2007/Coy65**	North	Rumphi	*Tumbuka*	1288	8813996	0618130	Phoka hills	Sandy-clay-loam	*Koko*
33	**CP/KA/2007/Coy66A**	North	Chitipa	*Msukwa*	1470	8931548	0558533	Undulating	Sandy-clay-loam	*Masimbi (Chinese)*
34	**RU/MWA/2007/Coy70**	North	Rumphi	*Tumbuka*	1139	8813606	0627654	Undulating	Sandy-clay-loam	*Masimbi*

[1]Accession name of the genotypes include the district, traditional leader of the area and year of collection. In the subsequent section and chapters, only the accession code e.g. Coy1, is used. Only 28 of the above accessions were further characterised, during the study.

[2]The ethnic group of the individual and/or farmer donating the accession and not necessarily the ethnicity of the region or area

masl-metre above sea level, - missing data

Table 3.2 Frequencies of desired cocoyam traits in Malawi as reported by farmers at collection points

Preferred characteristics	North		South		General	
	No	%	No	%	No	%
High dry matter	2	6	12	35	14	41
Taste	3	8	3	8	6	17
High yield (tuber size)	5	14	1	3	6	17
Good for breakfast (boiled tubers)	3	8	3	8	6	17
Mealyness	3	8	0	0	3	8
Good for *msima* (pulp)	3	8	0	0	3	8
Easy to cook (cooks fast)	1	3	0	0	1	3
Resilience to bad weather (adaptability)	2	6	0	0	2	6
Early maturing	0	0	1	3	1	3
Tuber flesh colour	1	3	0	0	1	3
Good flowers (ornamental)	1	3	0	0	1	3
Total number of respondents	-	34	-	34	-	34

Results of Table 3.3 suggest that most Malawians use cocoyam as food. About 88% of the respondents use cocoyam as food in the form of boiled tubers, 12% as *msima* (pulp) and 38% use the leaves as a vegetable. The study showed that mostly people from the northern region use cocoyam for making pulp. Both the northern and the southern region used cocoyam leaves as vegetable although more people from the south (29% versus 9%) used the leaves as vegetable. Eating cocoyam as boiled snacks is common in both regions and seems to be the main way of consuming cocoyam. Caillon *et al.* (2006) suggested that farmers in Vanuatu in the Pacific region used agronomic traits such as time required to mature, yield and taste in cultivar selection. Tasty, "strong" *taros* with HDM content are used to make *nalot*, a cocoyam pudding that is highly valued socially. Non-irritating and soft cocoyam with low HDM are reserved for another important Melanesian (Papua New Guinea and Vanuatu) meal made from grated corms known as *laplap*. Non-irritating dry, but soft cocoyam are roasted. However, Caillon *et al.* (2006) observed that over the last two generations selection criteria have changed from a preference for a dry corm that can be roasted, to a strong corm that can

be boiled. This has coincided with the introduction of cooking pots. Studies by Aregheore and Perera (2003) agreed with the idea that most people use HDM and firm texture after cooking as selection criteria for cocoyam genotypes. In Samoa, cocoyam genotypes with such characters as HDM were found to be more acceptable. Results are similar to what Rijal (2004) and Hue *et al*. (2001) found in Nepal and Vietnam, respectively. Farmers' priority traits of cocoyam included uses of plant parts, areas of adaptation and eating quality.

Table 3.3 Frequencies of common uses of cocoyam in Malawi as reported by farmers at collection points

Most common uses	North		South		General	
	No	%	No	%	No	%
Food - boiling tubers	15	44	15	44	30	88
Food - *Msima* (pulp)	4	12	0	0	4	12
- Leaves as vegetables	3	9	10	29	13	38
Source of income (sale of fresh tubers)	7	21	0	0	7	21
Livestock feed - fish	0	0	1	3	1	3
- pigs	0	0	1	3	1	3
Medicine - for ring worms	0	0	1	3	1	3
Ornamental flowers	1	3	0	0	1	3
Total number of respondents	-	34	-	34	-	34

According to this study, cocoyam is used as a source of income to most (21%) growers in the northern region (Table 3.3). Mature tubers are usually sold along the main roads. It is also used as a livestock feed in the southern region (6%). The leaves are fed to pigs and used as fish feed in fish ponds (Table 3.3). The use of cocoyam for pigs is not widespread. In Cambodia it was observed that farmers lack knowledge of how to use and process cocoyam leaves and of the benefits of cocoyam leaves for pigs (Buntha *et al*., 2008). Stems, leaves and the petioles are usually peeled and boiled before being fed to livestock, particularly pigs, to reduce the itching effect (Miller, 1992). According to Agwunobi *et al*. (2004) boiling reduces the amount of anti-nutritional factors in cocoyam cormels and that feed intake, weight gain

and feed efficiency for the diets containing boiled cocoyam were better than for non-boiled and sun-dried cocoyam cormels.

Results indicated that people from the south use the leaf sap as an ointment to treat ringworms i.e. a fungal skin infection (Table 3.3). This could be in line with Rijal (2004), Brown *et al.* (2005) and Chen *et al*. (2007) who highlighted members of the Araceae family, including the genera *Xanthosoma* and *Colocasia,* as important medicinal plants. They are a valuable source for glycosidase inhibitors that are anti-diabetic, anti-metastatic, anti-viral and immunomodulatory agents. The powder of *Homalomena aromatica* rhizomes, another member of the family Araceae, is used as an anti-inflammatory agent, a tonic for treatment of skin disease in India (Chen *et al*., 2007). In Hawaii, a preliminary study (*in vivo*) by Brown *et al.* (2005) suggested that *poi,* a paste made from *C. esculenta,* have novel tumour specific anti-cancer activities. On the other hand, cocoyam is used as a medicine for headache in Nepal (Rijal, 2004).

Cocoyam is also used as an ornamental plant in the north (Table 3.3). Most people in and around town grow it as an ornamental in their backyards. Péreira *et al.* (2005) suggested that despite the fact that some genotypes do not flower at all, most cocoyam plants have characteristics which qualify them as ornamental plants, mainly as potted plants or as parts of gardens.

3.3.2 Morphological description of 28 cocoyam accessions from Malawi

This study considered only morphological traits and not agronomical traits since morphological traits typify varieties and are used in characterisation of germplasm. Agronomical traits are used for evaluation purposes with the aim of recommending a variety for farmers. Agronomical evaluation has to be done in several environments due to genotype by environment interaction of agronomical traits and their polygenic nature (Benesi, 2005).

Results indicated similarities and differences in the above-ground morphological characteristics (Table 3.4). Of the 30 above-ground characteristics studied only one was monomorphic (Another characteristic, leaf main vain variegation (LMVV) was absent in all

accessions except for one (Coy5), all the accessions studied had open cross section of the lower part of the petiole (CSLPP)).

The cocoyam accessions showed high levels of variability for plant growth habit, in terms of plant span (PS) and plant height (PH) (Table 3.4). Most of the clones (75%) had a medium (50-100 cm) plant span, 21.4% had a wide span (>100 cm) and only 1% had a narrow plant span (<50 cm). Most of the accessions (78.6%) were medium in height (50-100 cm) whilst 17.9% of the accessions were dwarfs (<50 cm) (Table 3.4). This suggested that a large number of accessions were tall with a wide plant span and will need a wide spacing to perform well. These characteristics are important as they also determine the maturity period of cocoyam plants. Prana (2000) found a strong correlation in plant height and corm maturity. Dwarf types matured early (<6 months) and medium plants were ready between 6-9 months whilst giant/tall types took 9-12 months to mature. Accession Coy5 from the southern region exhibited a narrow plant span and dwarf height. However, this could be as a result of the agro-ecological conditions that existed during the growing season. All accessions did not flower, corroborating Onwueme (1978) that flowering seldom occurs in cocoyam. Side shoots (stolons) and suckers are important in cocoyam production as sources of planting materials. Few (10.7%) accessions produced stolons and 78.6% of the accessions produced at least 1 to 5 suckers (direct shoots) (Table 3.4).

Farmers prefer accessions with suckers for multiplication purposes. However, excessive production of stolons reduces corm production and increases production costs, hence farmers tend to select against this trait. Lebot *et al*. (2004) suggested that the presence of long stolons is a characteristic of wild genotypes and is often associated with small elongated corms, continuous growth and high concentration of calcium oxalate that causes acridity.

Table 3.4 Morphological descriptors of 28 cocoyam accessions from Malawi

Acc.No.	PS	PH	SS	SL	NOS	LBA	PPLLS	LBM	LBC	LBCV	TOV	COV	LBMC
Coy1	Medium	Medium	None	N/A	Absent	Sagittate	Cup shaped	Entire	Green	Absent	Absent	Absent	Purple
Coy3	Medium	Dwarf	None	N/A	Absent	Peltate	Erect-apex down	Entire	Yellow-green	Present	Stripe	Yellow-green	Purple
Coy5	Narrow	Dwarf	None	N/A	Absent	Sagittate	Cup shaped	Entire	Green	Present	Mottle	Yellow-green	Purple
Coy6	Medium	Medium	None	N/A	1 to 5	Sagittate	Erect-apex down	Entire	Dark-green	Absent	Absent	Absent	Purple
Coy9A	Medium	Medium	None	N/A	1 to 5	Peltate	Erect-apex down	Undulate	Yellow-green	Present	Mottle	Yellow-green	Purple
Coy13	Medium	Medium	None	N/A	1 to 5	Sagittate	Cup shaped	Undulate	Dark-green	Present	Fleck	Yellow-green	Purple
Coy14	Medium	Medium	None	N/A	Absent	Sagittate	Cup shaped	Entire	Dark-green	Absent	Absent	Absent	Purple
Coy16	Wide	Tall	6 to10	Long	1 to 5	Peltate	Erect-apex down	Undulate	Green	Absent	Absent	Absent	Purple
Coy20	Medium	Medium	None	N/A	1 to 5	Peltate	Erect-apex down	Undulate	Yellow-green	Present	Mottle	Yellow-green	Purple
Coy22	Medium	Dwarf	None	N/A	1 to 5	Sagittate	Erect-apex down	Undulate	Dark green	Absent	Absent	Absent	Purple
Coy23	Wide	Medium	1 to 5	Long	1 to 5	Peltate	Erect-apex down	Sinute	Dark-green	Present	Mottle	Dark-green	Purple
Coy28	Wide	Medium	None	N/A	1 to 5	Sagittate	Cup shaped	Undulate	Green	Present	Mottle	Yellow-green	Purple
Coy34	Medium	Medium	None	N/A	1 to 5	Sagittate	Cup shaped	Entire	Green	Absent	Absent	Absent	Purple
Coy37	Medium	Medium	None	N/A	Absent	Sagittate	Cup shaped	Entire	Dark-green	Absent	Absent	Absent	Purple
Coy42	Medium	Medium	None	N/A	1 to 5	Sagittate	Cup shaped	Entire	Dark-green	Present	Mottle	Yellow-green	Purple
Coy49	Medium	Dwarf	None	N/A	1 to 5	Sagittate	Cup shaped	Entire	Light-green	Absent	Absent	Absent	Whitish
Coy51	Medium	Medium	None	N/A	1 to 5	Sagittate	Cup shaped	Entire	Green	Present	Mottle	Dark-green	Whitish
Coy53	Medium	Medium	1 to 5	Long	1 to 5	Peltate	Erect-apex down	Undulate	Dark-green	Absent	Absent	Absent	Purple
Coy55	Medium	Medium	None	N/A	1 to 5	Peltate	Erect-apex down	Undulate	Dark-green	Present	Fleck	Yellow-green	Purple
Coy56	Medium	Dwarf	None	N/A	1 to 5	Sagittate	Cup shaped	Entire	Green	Absent	Absent	Absent	Yellow
Coy57	Medium	Medium	None	N/A	1 to 5	Sagittate	Cup shaped	Entire	Yellow-green	Present	Mottle	Yellow-green	Whitish
Coy60	Wide	Medium	None	N/A	1 to 5	Sagittate	Cup shaped	Entire	Dark-green	Present	Mottle	Yellow-green	Whitish
Coy61	Medium	Medium	None	N/A	1 to 5	Sagittate	Cup shaped	Entire	Green	Absent	Absent	Absent	Whitish
Coy62	Wide	Medium	None	N/A	1 to 5	Sagittate	Cup shaped	Entire	Green	Present	Mottle	Yellow-green	Whitish
Coy63	Medium	Medium	None	N/A	1 to 5	Sagittate	Cup shaped	Entire	Green	Absent	Absent	Absent	Purple
Coy65	Wide	Medium	None	N/A	Absent	Sagittate	Cup shaped	Entire	Green	Absent	Absent	Absent	Whitish
Coy66A	Medium	Medium	None	N/A	1 to 5	Sagittate	Cup shaped	Entire	Green	Absent	Absent	Absent	Yellow
Coy70	Medium	Medium	None	N/A	1 to 5	Sagittate	Erect-apex down	Entire	Yellow-green	Present	Mottle	Yellow-green	Whitish

Key: PS-plant span, PH-plant height, SS-side shoots (stolons), SL-stolon length, NOS-number of suckers (direct shoots), LBA-leaf base shape, PPLLS-predominant position (shape) of leaf lamina surface, LBM-leaf blade margin, LBC-leaf blade colour, LBCV-leaf blade colour variegation, TOV-type of variegation, COV-colour of variegation, LBMC-leaf blade margin colour.

Table 3.4 (Continued)

Acc. No.	PJP	PJC	SCLBT	LMVC	LMVV	VP	CPTT	CPMT	CPBT	PS
Coy1	Absent	Absent	Milky	Green	Absent	Y-pattern	Light-green	Purple	Purple	Absent
Coy3	Small	Purple	Pink	Green	Absent	Y-pattern extended to sec. Veins	Purple	Purple	Brownish	Present
Coy5	Absent	Absent	Milky	Yellow	Present	Y-pattern extended to sec. Vein	Light-green	Green	Brownish	Absent
Coy6	Absent	Absent	Milky	Green	Absent	y-pattern	Light-purple	Purple	Purple	Absent
Coy9A	Medium	Purple	Brownish	Green	Absent	Y-pattern extended to sec. Veins	Brownish	Brownish	Light-green	Absent
Coy13	Absent	Absent	Milky	Light-green	Absent	Y-pattern	Light-green	Green	Light-green	Absent
Coy14	Absent	Absent	Milky	Green	Absent	Other	Light-green	Light-green	Brownish	Absent
Coy16	Small	other	Yellow	Green	Absent	Y-pattern extended to sec. Veins	Light-green	Light-green	Light-green	Absent
Coy20	Small	Purple	Pink	Yellow-green	Absent	Y-pattern extended to sec. Veins	Light-purple	Brownish	Green	Present
Coy22	Absent	Absent	Milky	Green	Absent	Y-pattern	Light-green	Purple	Purple	Absent
Coy23	Small	Purple	Brownish	Green	Absent	Y-pattern extended to sec. Veins	Brownish	Light-green	Light-green	Absent
Coy28	Absent	Absent	Whitish	Light-green	Absent	Y-pattern extended to sec. Veins	Light-green	Purple	Brown/purple	Present
Coy34	Absent	Absent	Milky	Green	Absent	Y-pattern	Light-green	Other	Brownish	Absent
Coy37	Absent	Absent	Milky	Green	Absent	Y-pattern extended to sec. Veins	Light-green	Purple	Purple	Absent
Coy42	Absent	Absent	Milky	Light-green	Absent	Y-pattern	Light-green	Purple	Purple	Absent
Coy49	Absent	Absent	Milky	Light-green	Absent	Y-pattern	Light-green	Light-green	Light-green	Absent
Coy51	Absent	Absent	Milky	Green	Absent	Y-pattern	Light-green	Green	Light-green	Absent
Coy53	Small	Purple	Milky	Green	Absent	Y-pattern	Purple	Brownish	Green	Absent
Coy55	Small	Purple	Pink	Green	Absent	Y-pattern extended to sec. Veins	Purple	Green	Green	Absent
Coy56	Absent	Absent	Milky	Green	Absent	Y-pattern extended to sec. Veins	Light-green	Green	Green	Absent
Coy57	Absent	Absent	Milky	Green	Absent	Y-pattern	Light-green	Green	Light-green	Absent
Coy60	Absent	Absent	Milky	Green	Absent	Y-pattern	Light-green	Green	Light-green	Absent
Coy61	Absent	Absent	Milky	Green	Absent	Y-pattern	Light-green	Green	Light-green	Absent
Coy62	Absent	Absent	Milky	Light-green	Absent	Y-pattern extended to sec. Veins	Light-green	Green	Light-green	Absent
Co 63	Absent	Absent	Milky	Green	Absent	Y-pattern	Light-green	Green	Green	Absent
Coy65	Absent	Absent	Milky	Green	Absent	Y-pattern	Light-green	Green	Light-green	Absent
Coy66A	Absent	Absent	Milky	Green	Absent	Y-pattern	Light-green	Green	Light-green	Absent
Coy70	Absent	Absent	Milky	Green	Absent	Y-pattern	Light-green	Green	Light-green	Absent

Key: PJP-petiole junction pattern, PJC-petiole junction colour, SCBLT-sap colour of leaf blade tip, LMVC-leaf main vein colour, LMVV-leaf main vein variegation, VP-vein pattern, CPTT-colour of petiole top third, CPMT-colour of petiole middle third, CPBT-colour of petiole basal third, PS-petiole stripe

Table 3.4 (Continued)

Acc. No.	PSC	LA	PBRC	CSLPP	LSC	LSEC	LW
Coy	Absent	Absent	Brown	Open	Red-purple	Dark brown-continuous	Absent
Coy3	Purple	Absent	White	Open	Light-green	Other	Low
Coy5	Absent	Present	Green	Open	Red-purple	Dark brown-not continuous	Low
Coy6	Absent	Absent	White	Open	Red-purple	Dark brown-continuous	Low
Coy9A	Other	Absent	Other	Open	Light-green	Other	Low
Coy13	Absent	Absent	White	Open	Light-green	Purple	Low
Coy14	Absent	Absent	White	Open	Red-purple	Other	Low
Coy16	Absent	Absent	White	Open	Light-green	Other	Low
Coy20	Green	Absent	Green	Open	Light-green	Purple	Low
Coy22	Absent	Absent	White	Open	Light-green	Purple	Low
Coy23	Absent	Absent	Green	Open	Light-green	Dark brown-not continuous	Low
Coy28	Light-green	Absent	Green	Open	Brownish	Dark brown-continuous	Low
Coy34	Absent	Absent	White	Open	Whitish	Other	Low
Coy37	Absent	Absent	White	Open	Light-green	Other	Low
Coy42	Light-green	Absent	Green	Open	Red-purple	Dark brown-not continuous	Low
Coy49	Absent	Absent	White	Open	Light-green	Purple	Low
Coy51	Absent	Absent	White	Open	Light-green	White	Absent
Coy53	Absent	Absent	White	Open	Light-green	Dark brown-not continuous	Low
Coy55	Absent	Absent	White	Open	Light-green	Other	Low
Coy56	Absent	Absent	White	Open	Light-green	Purple	Low
Coy57	Absent	Absent	White	Open	Light-green	Dark brown-not continuous	Low
Coy60	Absent	Present	Green	Open	Light-green	Dark brown-not continuous	Absent
Coy61	Absent	Present	White	Open	Light-green	Dark brown-not continuous	Low
Coy62	Absent	Absent	Green	Open	Light-green	White	Low
Coy63	Absent	Absent	White	Open	Light-green	Purple	Low
Coy65	Absent	Absent	White	Open	Light-green	Dark brown-not continuous	Absent
Coy66A	Absent	Present	White	Open	Light-green	Other	Low
Coy70	Absent	Absent	White	Open	Light-green	Purple	Low

Key: PSC-petiole stripe colour, LA-leaf lamina appendage PBRC-petiole base ring colour, CSLPP-cross-section of lower part of petiole, LSC-leaf sheath colour, LSEC-leaf sheath edge colour, LW-leaf waxiness.

The key to distinguish the two most common edible Araceae members, namely *Colocasia* and *Xanthosoma*, is based on the predominant leaf base shape (Purseglove, 1972). *Colocasia* species has peltate leaves while *Xanthosoma* species has sagittate or hastate leaves with pointed top tips (not rounded). Based on the predominant leaf base shape, 25% of the clones had peltate and 75% sagittate leaves as displayed in Figure 3.1 (Table 3.4). This suggests that Malawi has both *Colocasia* and *Xanthosoma* species being conserved and cultivated by farmers. Danquah *et al*. (2006), in characterising a mutant population of cocoyam (*X. sagittifolium*), found that the plants had a non-peltate petiole attachment. All the *Xanthosoma* plants had sagittate leaf shapes.

The predominant leaf lamina position (PPLLS) was cup-shaped (64.3%), whilst 35.7% of the accessions exhibited leaves with lamina-apex facing down. The leaf blade colour (LBC) ranged from green (42.9%) to dark-green (35.7%). Other accessions showed yellow-green (17.9%) and light-green (3.6%) leaves. Results suggest that most (67.9%) of the leaves of the accessions had an entire margin (LBM) whilst few were undulate (28.6%) and sinute (3.6%) (Table 3.4). There was a close relationship between the predominant leaf base shape (LBS) and predominant position of leaf lamina surface (PPLLS). All accessions with a peltate leaf base shape had an erect-apex down leaf lamina surface position as opposed to almost all accessions (90.5%) with sagittate leaves which exhibited a cup-shaped leaf lamina surface position (Table 3.4). The peltate leaf base shaped accessions (25%) had a small petiole junction pattern which was absent in the sagittate leaf base shaped accessions (75%) (Table 3.4 and Figure 3.1). The petiole junction pattern in the peltate leaves varied from light-green to purple. These traits seemed to be closely related and can be effectively used for cultivar identification.

A **B**

Figure 3.1 (A) Cocoyam (*C. esculenta*) plant exhibiting peltate leaf shape with an erect-apex facing down position, (B) Cocoyam (*X. sagittifolium*) plant exhibiting sagittate leaf shape with a predominant cup-shaped position.

Leaf blade colour (LBC) of the 28 accessions was basically green. However, it varied from light-green, green to dark-green with some plants showing yellow-green leaves. There was a high level of variation for leaf blade colour variegation. Fifty percent of the accessions showed leaf blade colour variegation (TOV) that varied from stripes (3.6%), to mottling (39.3%) to flecks (7.1%). The colour of variegation (COV) was mostly yellow-green (42.9%) and dark-green (7.1%) with the rest of the accessions showing no colour variegation on the leaf blade. Results further suggested that a number of accessions had a purplish leaf blade margin colour (LBMC) (64.3%) whilst the other accessions exhibited white (28.6%) and yellow (7.1%) leaf blade margin colour (Table 3.4).

The accessions' sap colour of leaf blade tip (SCBLT) ranged from milky-white (75%), white i.e. transparent (3.6%), pink (10.7%) as well as brown (7.1% (Table 3.4). It was observed that most of the accessions with a sagittate leaf base shape had milky-white sap and the peltate leaf base shaped accessions white (transparent), pink and brown coloured sap. The vein pattern (VP) of the leaves varied from y-pattern (57.1%) to y-pattern extending to

secondary veins (39.3%) whilst accession Coy14 was not very distinct. There was a relationship between vein pattern and sap colour of leaf blade tip. All accessions which produced coloured sap had a vein pattern of y-with extension to secondary veins except for accessions Coy37, Coy56 and Coy62 which had milky-white sap. Accessions with a vein pattern of y-extending to secondary veins had peltate leaves suggesting that they belong to the genus *Colocasia*. This corroborates reports that suggest that the two main genera of cocoyam are distinct in the vein pattern of their leaves (Onwueme, 1978).

The accessions showed low levels of variability on leaf waxiness, leaf sheath edge colour and petiole stripe colour. All accessions had an open cross-section at the lower part of petiole and the main vein colour was basically green. However, with the heterogeneous nature of cocoyam (Ivancic and Lebot, 2000), it was difficult to score some characters as was the case in a study by Quero-Garcia *et al.* (2004).

These results corroborate studies by Prana (2000) where high levels of variation was found in morphological characters, especially in the petiole, such that they could be used as diagnostic characters to distinguish different *taro* genotypes. In China and Taiwan ethno-botanical data not only provided clues on domestication and genetic evolution of cocoyam, but also gave a solid indication of genetic diversity (Hue *et al*., 2001; Jianchu *et al*., 2001). Like in China, morphological characteristics observed in the field and indigenous knowledge of people about uses, folk names, selection and management provided a good indication and measure of the available cocoyam genetic diversity in Nepal (Rijal, 2004).

3.3.3 Clustering of the 28 characterised cocoyam accessions from Malawi

The 28 cocoyam accessions from Malawi were characterised using 30 morphological characteristics. The data was coded into a binary matrix (section 3.3.2.2) and used to create a dendrogram. The dendrogram was constructed using the Dice similarity coefficient and the UPGMA clustering method. The accessions separated into two main clusters, I and II, mainly corresponding to the two species based on leaf base shape i.e. sagittate and peltate leaf base shapes and region of collection (Figure 3.2). Sagittate leaf base shaped accessions clustered in main cluster I while the peltate leaf base shaped accessions clustered in main cluster II.

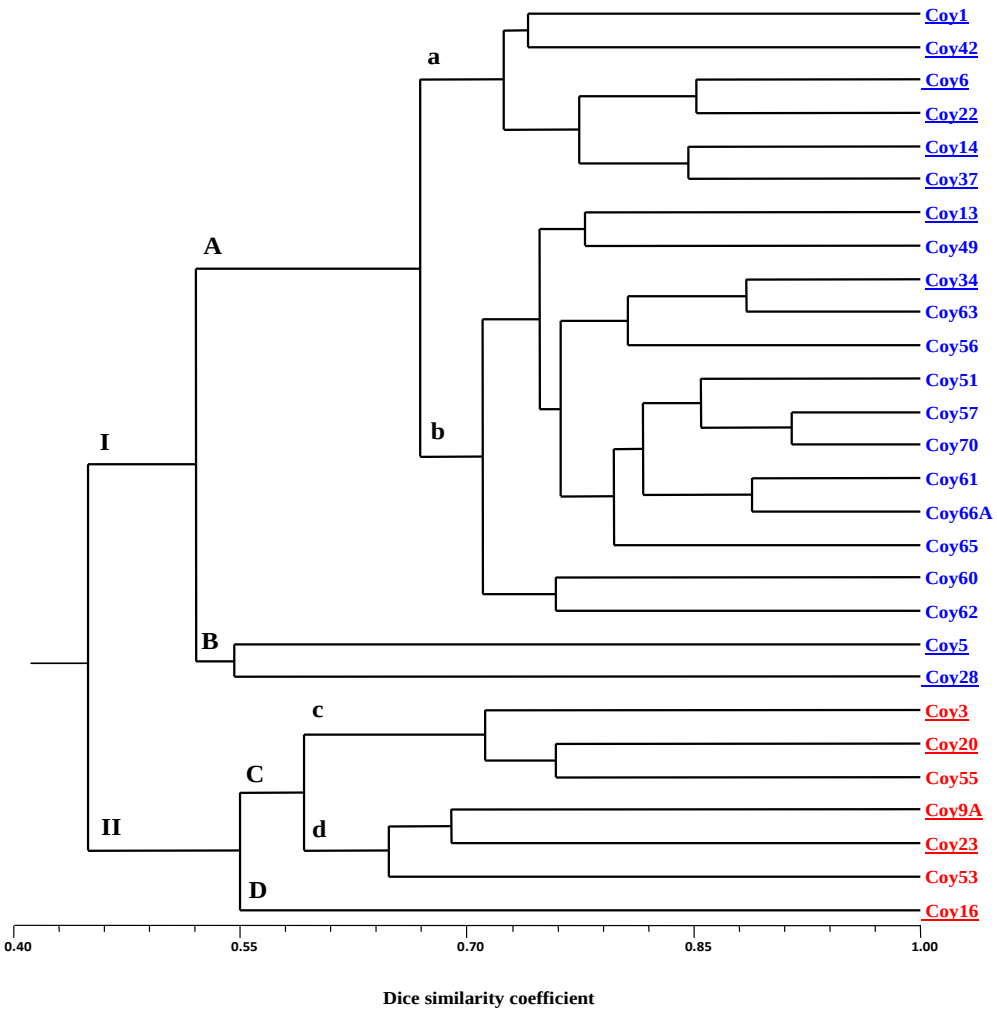

Figure 3.2 Clustering of 28 cocoyam accessions from Malawi based on 30 morphological characters and UPGMA clustering using the Dice similarity coefficient. Red represents accessions with peltate leaf base shapes, blue, accessions with sagittate leaf base shapes, while accessions collected from the south are underlined and the rest are from the north.

There were two sub-clusters A and B, in main cluster I Within cluster I (sagittate), accessions from the southern and northern regions mostly clustered separately (Figure 3.2). Most (19) of the sagittate leaf base shaped accessions clustered in cluster A and only two accessions (Coy5 and Coy28) in sub-cluster B. Cluster A further subdivided into two sub-clusters, a and b. Most accessions from the south clustered in sub-cluster a, whilst all accessions from the north clustered in sub-cluster b, with two accessions from the south (Coy13 and Coy34). Two of the accessions from the south (Coy5 and Coy28) clustered separately in sub-cluster B and were characterised by yellow-green mottled leaves. These two accessions were the most dissimilar within the main cluster I, i.e. 55% similar, whilst accessions Coy57 and Coy70, both from the north, were the most similar accessions in both clusters I and II, i.e. 92% similar (Figure 3.2).

The main cluster II (peltate) subdivided into two sub-clusters, C and D. Sub-cluster C contained six accessions and was sub-divided into two sub-clusters, c and d. Sub-cluster c contained two accessions from the north and the rest from the south. Sub-cluster d contained a single accession, Coy16 from the south clustering on its own. Two accessions identified by farmers as *Chinese*, Coy23 (south) and Coy53 (north) clustered together in sub-cluster C. The most similar accessions in cluster II were Coy20 and Coy55 (75%) and the most dissimilar accession was Coy16, which was only 55% similar to the rest of the accessions in cluster II (Figure 3.2). Accessions of cluster I (sagittate) were 45% similar to accessions of cluster II (peltate). Accessions within cluster I were more closely related compared to those in cluster II. However, accessions Coy5 and Coy28 which clustered outside the main cluster I, contributed much to the variation in this cluster. Accessions in Cluster II showed high levels of variation.

Clustering methods will always cluster data whether or not there are really clusters in the data, it was therefore important to run some check for the existence of clusters i.e. how well the cluster analysis represents the original matrix of distances among genotypes/accessions. The matrix correlation based on the goodness of fit of clustering to data matrixes (Table 4.3) was therefore calculated using COPH and MXCOMP programmes. The r value gives a measure of goodness of fit for a cluster analysis and 0.87220 in the present study suggested a good fit.

Table 3.5 Pair-wise genetic similarity coefficient matrix for 28 cocoyam genotypes calculated from morphological characters

	Coy1	Coy3	Coy5	Coy6	Coy9A	Coy13	Coy14	Coy16	Coy20	Coy22	Coy23	Coy28	Coy34	Coy37	Coy42	Coy49	Coy51	Coy53
Coy1	1.000																	
Coy3	0.407	1.000																
Coy5	0.556	0.414	1.000															
Coy6	0.741	0.519	0.444	1.000														
Coy9A	0.407	0.567	0.379	0.482	1.000													
Coy13	0.593	0.448	0.517	0.704	0.557	1.000												
Coy14	0.741	0.518	0.593	0.778	0.407	0.704	1.000											
Coy16	0.333	0.482	0.259	0.444	0.519	0.482	0.444	1.000										
Coy20	0.296	0.700	0.310	0.482	0.667	0.552	0.370	0.481	1.000									
Coy22	0.667	0.556	0.444	0.852	0.519	0.778	0.704	0.556	0.482	1.000								
Coy23	0.259	0.448	0.310	0.407	0.690	0.414	0.370	0.630	0.517	0.407	1.000							
Coy28	0.510	0.351	0.545	0.431	0.421	0.582	0.431	0.385	0.500	0.471	0.393	1.000						
Coy34	0.731	0.436	0.518	0.769	0.400	0.704	0.808	0.491	0.364	0.731	0.327	0.482	1.000					
Coy37	0.769	0.545	0.518	0.808	0.436	0.704	0.846	0.491	0.400	0.808	0.400	0.482	0.769	1.000				
Coy42	0.741	0.400	0.655	0.778	0.500	0.759	0.704	0.296	0.467	0.704	0.448	0.667	0.655	0.691	1.000			
Coy49	0.593	0.444	0.481	0.667	0.407	0.778	0.704	0.482	0.407	0.741	0.333	0.431	0.731	0.692	0.667	1.000		
Coy51	0.704	0.414	0.517	0.667	0.483	0.759	0.667	0.444	0.379	0.667	0.414	0.473	0.741	0.667	0.665	0.778	1.000	
Coy53	0.407	0.518	0.222	0.593	0.630	0.556	0.482	0.593	0.593	0.630	0.667	0.275	0.528	0.528	0.482	0.444	0.444	1.000
Coy55	0.370	0.724	0.345	0.593	0.690	0.690	0.518	0.593	0.759	0.630	0.586	0.407	0.482	0.556	0.483	0.444	0.517	0.741
Coy56	0.604	0.518	0.556	0.642	0.444	0.667	0.679	0.528	0.444	0.717	0.333	0.440	0.745	0.745	0.556	0.755	0.741	0.491
Coy57	0.630	0.483	0.586	0.667	0.621	0.793	0.667	0.407	0.444	0.667	0.448	0.509	0.704	0.704	0.759	0.778	0.862	0.519
Coy60	0.556	0.310	0.586	0.519	0.483	0.655	0.519	0.333	0.345	0.519	0.483	0.509	0.519	0.519	0.690	0.593	0.759	0.407
Coy61	0.630	0.370	0.556	0.630	0.482	0.704	0.630	0.444	0.333	0.630	0.370	0.431	0.731	0.654	0.630	0.741	0.815	0.518
Coy62	0.556	0.414	0.655	0.556	0.512	0.759	0.593	0.482	0.519	0.556	0.483	0.691	0.630	0.630	0.724	0.741	0.793	0.333
Coy63	0.741	0.481	0.556	0.778	0.482	0.815	0.667	0.519	0.482	0.778	0.370	0.520	0.885	0.808	0.593	0.778	0.852	0.444
Coy65	0.741	0.370	0.556	0.593	0.407	0.667	0.778	0.444	0.259	0.593	0.370	0.431	0.692	0.692	0.704	0.704	0.852	0.593
Coy66A	0.667	0.407	0.556	0.704	0.444	0.778	0.704	0.482	0.370	0.704	0.333	0.471	0.808	0.731	0.630	0.778	0.852	0.482
Coy70	0.582	0.542	0.508	0.727	0.610	0.780	0.655	0.509	0.508	0.727	0.441	0.464	0.691	0.655	0.678	0.764	0.847	0.848

Table 3.5 (Continued)

	Coy55	Coy56	Coy57	Coy60	Coy61	Coy62	Coy63	Coy65	Coy66A	Coy70
Coy55	1.000									
Coy56	0.593	1.000								
Coy57	0.552	0.704	1.000							
Coy60	0.448	0.556	0.793	1.000						
Coy61	0.482	0.755	0.852	0.815	1.000					
Coy62	0.512	0.704	0.793	0.759	0.704	1.000				
Coy63	0.630	0.868	0.815	0.630	0.815	0.741	1.000			
Coy65	0.407	0.679	0.815	0.778	0.815	0.741	0.778	1.000		
Coy66A	0.519	0.830	0.815	0.704	0.889	0.741	0.889	0.778	1.000	
Coy70	0.610	0.691	0.915	0.712	0.764	0.780	0.800	0.727	0.800	1.000

r = 0.87220

Accessions separated based on species as reported by Purseglove (1972) and Onwueme (1978) who stated that sagittate leaf base-shaped cocoyam plants belong to the species *Xanthosoma* and peltate leaf base-shaped plants to *Colocasia*. Accessions were successfully clustered into the two major species of the Araceae family based on the 28 polymorphic morphological characters. The high levels of variation between the two species are due to the extreme heterogeneous nature of the plant which, according to Morton (1972) and Ivancic and Lebot (2000) may be due to large variation in chromosome structure and number, such that the morphological differences among and within the two species are large. This variation may be associated with mutations and intensive selection by isolated human communities in diverse environments, followed by continuous vegetative propagation which resulted in the phenotypic diversity observed. The dendrogram showed that accessions from the same region (location) clustered together, suggesting movement of germplasm within the regions as farmers' source of planting material is solely famer to farmer exchange. The fact that the Chinese introductions grouped together corroborates farmers' knowledge on the introduction and domestication of cocoyam in Malawi. Using the 28 polymorphic morphological characteristics all accessions were distinguished from each other. Results furthermore suggested that much of the germplasm fall within *Xanthosoma* species. This corroborated Onwueme (1978) and Janseens (2001), who suggested that *Xanthosoma* species have become much more important world wide compared to *Colocasia* species world wide.

3.4 Conclusions and recommendations

This study has shown that there exists a great amount of cocoyam diversity in Malawi. Most of the cocoyam genotypes found in Malawi belonged to the *Xanthosoma* species compared to the *Colocasia* species. Farmers maintaining this diversity also hold rich ethno-botanical and ecological knowledge of the cocoyam genotypes that they are conserving. Farmers' preferences regarding cocoyam genotypes include uses of plant parts, areas of adaptation and eating quality. Farmers in Malawi use cocoyam mainly as a food crop and to a lesser extent as feed for livestock, an ornamental plant and as a medicinal plant. These preferences and uses of cocoyam vary between the two regions of Malawi. Preferences and uses also tend to shape the way genotypes are disseminated from farmer to farmer, hence the diversity. Researchers need to incorporate this indigenous knowledge if they are to disseminate new

cocoyam genotypes. It is recommended that a thorough collection be done in all regions of Malawi to conserve all the cocoyam germplasm being held by farmers as it is a heritage of the nation. This would also help to determine the amount of the cocoyam diversity of the country after proper characterisation.

3.5 References

Agwunobi, L., Angwukam, P., Cora, O., and Isika, M. (2004). Study on the use of *Colocasia esculenta* (*taro* cocoyam) in the diets of weaned pigs. Tropical Animal Health and Production 34: 241-247.

Aregheore, E., and Perera, D. (2003). Dry matter, nutrient composition and palatability/acridity of eight exotic cultivars of cocoyams-*taro* (*Colocasia esculenta*) in Samoa. Plant Foods for Human Nutrition 58: 1-8.

Benesi, I. (2005). Characterisation of Malawian cassava germplasm for diversity, starch extraction and its native and modified properties. PhD thesis in Plant Breeding Department of Plant Sciences (Plant Breeding), Bloemfontein, University of the Free state, South Africa, pp.199.

Brown, A., Reitzenstein, J., Liu, J., and Jadus, M. (2005). The anti-cancer effects of *poi* (*Colocasia esculenta*) on colonic adenocarcinoma cells *in vitro*. Phytotherapy Research 19: 767-771 .

Buntha, P., Borin, K., Preston, T., and Ogle, B. (2008). Survey of *taro* varieties and their use in selected areas of Cambodia. Livestock Research for Rural Development: 20 (supplement). http://www.lrrd.org/lrrd. (2009/10/09).

Caillon S., Quero-Garcia, J., Lescure, J.P., and Lebot, V. (2006). Nature of *taro* (*Colocasia esculenta* (L)) genetic diversity prevalent in a Pacific Ocean island, Vanua lava, Vanuatu. Genetic Resources and Crop Evolution 53: 1273-1289.

Chen, J., Henny, R., and Liao, F. (2007). Aroids are important medicinal plants. In: Yadav, A., (Ed.). Proceedings of the International Symposium on Medicinal and Nutraceutical Plants. Acta Horticulturae. 756, ISHS, pp. 347-354.

Chien-Chun, H., Woan-Ching, C., and Chiun, C.R.W. (2007). Comparison of Taiwan paddy-and upland-cultivated *taro* (*Colocasia esculenta* L.) cultivars for nutritive values. Food Chemistry 102: 250-256.

Danquah, A., Offei, S.K., Blay, E.T., Asare, E., and Danquah, E.Y. (2006). Characterisation of mutant population of cocoyam (*Xanthosoma sagitifolium* L. Schott). International Journal of Botany 2: 128-132.

Dice, L. (1945). Measures of amount of ecologic association between species. Ecology 26: 297-302.

Hue, N.N., Trinh, L.N., Ha, N.P., Sthapit, B., and Jarvis, D. (2001). *Taro* cultivar diversity in three ecosites of North vietnam. In: Tuan, D.H., Hue, N.N., Sthapit, B.R., and Jarvis, D.I., (Eds.). Onfarm management of agricultural biodiversity in Vietnam. Hanoi, Vietnam: IPGRI, pp. 58-62.

IBPGR. (1989). Descriptor for *Xanthosoma*. International Board for Plant Genetic Resource, Rome.

IPGRI. (1999). Descriptor for *taro* (*Colocasia esculenta*). International Plant Genetic Resources Institute, Rome .

Ivancic, A., and Lebot, V. (2000). The genetics and breeding of *taro*. Montpellier, France: Series Reperes.

Ivancic, A., Quero-Garcia., A., and Lebot, V. (2003). Development of visual tools for selecting qualitative corm characteristics of *taro* (*Colocasia esculenta* (L) Schott). Australian Journal of Agricultural Research 54: 581-587.

Janseens, M. (2001). Crop production in tropical Africa. Bonn, Germany: Institut fur obst- und germusebau, abt. Tropicscher pflanzenbau, rheinische friedrich-wilhelms-Universitat Bonn, auf dem hugel 6, D-53121 Bonn, Germany. Directorate general for international co-operation, karmelietenstraat 15, B-1000 Brussels, Belgium, pp. 221-228.

Jianchu, X., Yongping, Y., Yingdong, P., Ayad, W.G., and Eyzagnire, P. (2001). Genetic diversity in *taro* (*Colocasia esculenta* Schott, Araceae) in China: An ethnobotanical and genetic approach. Economic Botany 55: 14-31.

Karp, A., Serberg, O., and Buiatti, M. (1996). Molecular techniques in the assessment of botanical diversity. Annals of Botany 78: 43-149.

Kurvilla, K., and Singh, A. (1981). Karyoptic and electrophoretic studies on *taro* and its origin. Euphytica 30: 405-413.

Laurentin, H. (2009). Data analysis for molecular characterisation of plant genetic resources. Genetic Resources and Crop Evolution 56: 277-292.

Lebot, V., and Aradhya, K. (1991). Isozyme variation in *taro* (*Colocasia esculenta* (L) Schott) from Asia and Oceania. Euphytica 56: 55-66.

Lebot, V., Prana, M., Kreike, N., van Heck, V., Pardales, J., Okpul, T., Gendua, T., Thongjien, M., Hue, H., Viet, N., and Yap, T.C. (2004). Characterisation of *taro* (*Colocasia esculenta* (L.) Schott) genetic resources in Southeast Asia and Oceania. Genetic Resources and Crop Evolution 51: 381-392.

Malawi Government Report. (1996). Country Report to the FAO International Technical Conference on Plant Genetic Resources. Leipzing: FAO.

Mathews, P. (2004). Genetic diversity in *taro* and the preservation of culinary knowledge. Ethnobotany Research and Applications 2: 55-71.

Mbouobda, H.D., Boudjeko, T., Djocgoue, P.F., Tsafack, T.J.J., and Omokolo, D.N. (2007). Morphological characterization and agronomical evaluation of cocoyam (*Xanthosoma sagittifolium* (L.) Schott) germplasm in Cameroon. Journal of Biological Sciences 7: 27-33.

Miller, C.D. (1992). Food values of breadfruit, taro leaves, coconut and sugar cane. Bernice P. Bishop Museum. Bulletin 64. Honolulu, Hawaii.

Ministry of Agriculture and Food Security (MoAFS). (2008). Chitedze Agricultural Research Station guide. Lilongwe, Malawi.

Mkumbira, J., Chiwona-Karltun, L., Largecrantz, U., Mahungu, N., Saka, J., Mhone, A., Bokanga, M., Brimer, L., Gullberg, U., and Rosling, H. (2003). Classification of cassava into 'bitter' and 'cool' in Malawi: From farmers' perception to characterisation by molecular markers. Euphytica 132: 7-22.

Morton, J. (1972). Cocoyams (*Xanthosoma caracu*, *X. atrovirens* and *X. nigrum*): Ancient root-and-leaf vegetables, gaining in economic importance. Florida State Sorticultura Society: 85-94.

O'Hair, S.K. (**1990).** Tropical root and tuber crops. In: Janick, J., and Simon, J.E. (Eds.). Advances in new crops. Timber Press, Portland, OR, pp. 424-428.

Okonkwo, C. (1993). *Taro, Colocasia* spp. In: Bergh, G. (Ed.). Genetic Improvement of Vegetable Crops. Oxford: Pergamon, pp. 709-715.

Onwueme, I. (1978). The tropical tuber crops: Yams, cassava, sweet potato and cocoyams. John Wiley and Sons, pp. 199-225.

Opoku-Agyeman, M., Bennett-Lartey, S., and Markwei, C. (2004). Agro-morphological and sensory characterization of cocoyam (*Xanthosoma sagittifolium* (L) (Schott) germplasm in Ghana. Ghana Journal of Agricultural Sciences 37: 23-31.

Péreira, F., Puiatti, M., and Finger, F. (2005). Ornamental potential of *taro* (*Colocasia esculenta* (L.) Schott) accessions. In: Tombolato, A., and Dias-Tagliacozzo, G. (Eds.). Proceedings of the Vth International Symposium on New Flor. Crops. Acta Horticulturae. 683, ISHS pp. 307-312.

Prana, M. (2000). Morphological and agronomical traits variation of *taro* (*C. esculenta* (L) Schott) from West Java. Annalels Bogorienses 7: 1-10.

Purseglove, J. (1972). Tropical crops: Monocotyledons 1. London: Longman, pp. 66-74.

Quero-Garcia, J., Noyer, J., Perrier, X., Marchand, J.L., and Lebot, V. (2004). A germplasm stratification of *taro* (*Colocasia esculenta*) based on agro-morphological descriptors, validation by AFLP markers. Euphytica 137: 387-395.

Ramana, R.V. and Hodgkin, T. (2002). Genetic diversity and conservation and utilization of plant genetic resources. Plant Cell, Tissue and Organ Culture 68: 1-19.

Rao, K.N. (2004). Plant genetic resources: Advancing conservation and use through biotechnology. African Journal of Biotechnology 3: 136-145.

Rijal, D. (2004). Revealing hidden knowledge on *taro* (*Colocasia* and *Xanthosoma*) in Nepal. In: Sthapit, B., Upadhyay, M., Shrestha, P., and Jarvis, D., (Eds.). On-farm conservation of agricultural biodiversity in Nepal: Volume I. Assessing the amount and distribution of genetic diversity on-farm. Nagarkot, Nepal, pp. 148-160.

Rohlf, F. (2000). NTSYS-pc: Numerical taxonomy and multivariate analysis system. Applied Biostatistics. New York: Exerter Publishing Ltd.

CHAPTER 4

Genetic diversity of Malawian cocoyam germplasm as revealed by AFLP markers

4.1 Introduction

Cocoyam is significant in world agriculture for food, nutrition and the role it plays in subsistence economies, as well as crop diversification (FAO, 1990; Onwueme, 1999; Caillon *et al*., 2004). The advent of the Green Revolution saw a desperate fight for survival from hunger and starvation, hence the introduction of quick growing, high energy sustaining starch crops as staples in farming and food systems of the world (Ekwe *et al*., 2009). This left cocoyam and similar crops as un-enterprising commodities, overshadowed by the "new crops" which have gained more credence as staple foods (Ekwe *et al*., 2009; Global Forum for Underutilised Species, 2009). The consequence is that cocoyam has become grossly marginalised while its rich values remain underexploited for enhancing household food security and economic empowerment (Ekwe *et al*., 2009).

Cocoyam now faces genetic erosion due to changes in the cropping pattern, introduction of improved varieties and changes in the ecosystem. Therefore an assessment of genetic diversity prevalent in local germplasm needs immediate attention (Lakhanpaul *et al*., 2003). A better understanding of genetic diversity, i.e. variation present in all species, their genetic material and the ecosystems, as well as its distribution, is essential for germplasm conservation and use. Ramana and Hodgkin (2002) suggested that central to any effective conservation programme is an understanding of genetic diversity in the species of concern. This has not been the case in most conservation efforts, either *in situ* or *ex situ*, which have proceeded with little information on the genetic diversity being conserved. Therefore there exists an urgent need to remedy the situation (Ramana and Hodgkin, 2002).

Several techniques are used to identify genetic variation between and within species. These include traditional methods of assessing diversity by measuring variation in phenotypic traits (both qualitative and quantitative) (Karp *et al*., 1996), biochemical methods based on protein

and enzyme electrophoresis (Brown, 1978) and DNA-based techniques that identify polymorphisms due to differences in DNA sequences (Karp *et al*., 1996; Rao, 2004). DNA-based markers have become methods of choice in genetic diversity studies, as they analyse variation at DNA level. This excludes all environmental influences and time specificity, since analysis can be performed at any growth stage using any plant part and requires only small amounts of material (Mueller and Wolfenbarger, 1999; Rao, 2004). DNA-based markers that are being used in diversity studies in crop plants including cocoyam are RFLP, RAPD, AFLP, SSRs or microsatellites, STSs and SNPs (Karp *et al*., 1996) as well as variable number of tandem repeats (VNTR) and single strand conformation polymorphisms (SSCP). Other markers such as specific mitochondrial DNA (mtDNA) and Y chromosomes are used in identification of maternal and paternal lineages (Erhardt and Weimann, 2007).

In Asia, Ochiai *et al*. (2001) differentiated cocoyam (*taro*) cultivars based on geographical and phylogenetic relationships. RAPD and isozyme analyses established evolutionary and genetic relationships among Asian cocoyam. In a separate study, Lakhanpaul *et al.* (2003) used RAPD marker analysis to assess the genetic diversity as well as genetic basis of morphotypic classification in Indian *taro*. Three out of 13 random decamer primers analysed showed 100% polymorphism. However, the clustering pattern did not show any strict relationship with regard to geographical distribution, morphotype classification and genotypic diversity. RAPD markers were used to assess the genetic diversity in cocoyam germplasm in the USA and Ghana (Schnell *et al*., 1999; Blay *et al*., 2004). On the other hand, SSR markers proved powerful in genetic diversity studies of cocoyam by identifying duplicates in the Pacific region germplasm (Mace and Godwin, 2002), as well as rationalising and reducing cocoyam diversity previously revealed by agro-morphological descriptors (Mace *et al*., 2006; Singh *et al*., 2008).

AFLP markers (Vos *et al*., 1995), developed for assessing genetic variability and constructing genetic maps in many species, are effective in plant cultivar identification (Arnau *et al*., 2001). AFLP markers were used to validate cocoyam germplasm stratified using agro-morphological descriptors in Vanuata (Quero-Garcia *et al.*, 2004) and to reveal genetic diversity of cocoyam (*taro*) in south east Asia and the Pacific region (Kreike *et al*., 2004). AFLP analysis grouped cultivars according to their country of origin, ploidy level

(diploid or triploid) and habitat - cultivated or wild. AFLP markers were used to analyse the geographical differentiation and phylogenetic relationships and to identify markers linked to cocoyam leaf blight resistance genes of Indian *taro* (Sharma *et al*., 2008).

Cocoyam is usually asexually propagated except for breeding purposes (Wilson, 1990). Sexual propagation, though rarely present, occurs due to the erratic flowering nature of the plant (Onwueme, 1978). Lasso (2008) suggested that it is improbable for most asexually reproducing species to transfer exact replicas of the maternal genome to their offspring over longer periods of time. Genetic changes slip in, due to genetic or epigenetic mutations, endo-symbiotic activities or yet unknown mechanisms. Genetic variations in clonal plants such as cocoyam therefore often occur from somatic embryogenesis which promotes somatic mutations or somaclonal variation (Larkin and Scowcroft, 1981; Santelices *et al*., 1995). Thousands of years of asexual propagation are thought to have fixed somatic mutations in cocoyam such that morphotypes and/or clones are quite distinct even when they share the same genetic background (Quero-Garcia *et al*., 2004).

There is limited information as to the prevalence and extent of intra-clonal variations and its effect on genetic diversity of cocoyam. There is no clear measure with which one can identify a threshold of genetic dissimilarity distance below which cocoyam plants can be considered to represent a single or different clone. Studies have shown that clones, i.e. individuals that grow and propagate by self-replication of genetically identical units, do not always present identical genetic fingerprints (Lasso, 2008). Often clonal fingerprints are less than 100% similar due to differences between ramets (all units forming a clone) and replicate runs of the same template DNA. To this extent, AFLP markers have been successfully used in identifying variation within clones (Douhovnikoff and Dodd, 2003). The highly polymorphic AFLP fragments maximise the sensitivity of the analysis in order to separate clones as well as sibs. To provide an objective means of identifying clones, several authors suggested different threshold Jaccard similarity indices to provide a lower limit of fingerprint similarities of different clonal species. In two separate studies Arens *et al*. (1998) and Douhovnikoff and Dodd (2003) set their minimum threshold similarity for the identification of clones at 0.98, based on DNA extractions of the same clone. On the other hand, Winfield *et al.* (1998) detected similarities between duplicate leaf samples of *Populus nigra* subsp.

betulifolia trees ranging from 0.96 to 1.0 and predicted that trees having a similarity index of 0.95 were close to clonal scoring range. Lasso (2008) observed that most studies indicated that individuals having less than 2-4% differences in their fingerprint profiles have been classified as part of the same clone. These threshold values (2-4%) have been found to be consistent across studies, such that they are almost being used universally.

Hence, this study determined the genetic diversity of cocoyam genotypes of Malawi. Specifically the study aimed at assessing (a) the genetic diversity among Malawian cocoyam accessions and (b) the intra-clonal genetic diversity within accessions using AFLP markers.

4.2 Materials and methods

4.2.1 Plant material

Plant materials were obtained from the cocoyam germplasm collection at the Malawi Plant Genetic Resources Centre of Malawi at Chitedze Research Station, Lilongwe, Malawi. Twenty-eight cocoyam accessions, randomly sampled from the collection, were used for AFLP analysis (Table 4.1). Three plants randomly sampled from each cocoyam accession were analysed to assess the intra-clonal variation that exists within the Malawian cocoyam accessions. The analysis involved three individual samples (Coy5, Coy6 and Coy20) and a bulk sample of each of the three sampled accessions.

4.2.2 Genomic DNA isolation

The modified Dellaporta *et al*. (1983) DNA minipreparation method was used to extract total genomic DNA from cocoyam plant samples. Fresh young leaves were collected from the gene-bank and kept on ice. The leaf samples were freeze-dried in a FreezeMobile II freeze-drier. Approximately 250 µl of the freeze-dried leaf material was ground to a fine powder using the TissueLyser (Qiagen, Retsch) homogeniser. A volume of 800 µl extraction buffer (100 mM Tris(hydroxymethyl) aminomethane hydrochrolide (Tris-HCl), 50 mM Ethylene-diaminetetraacetate (EDTA) and 500 mM NaCl and 1% (w/v) of polyvinylpyrrolidone (PVP) and 0.2% β-mercaptoethanol) was added to approximately 250 µl lyophilised leaf material of the individual samples and incubated at 65°C for one hour. After an hour, 2% sodium

dodecyl sulphate (SDS) was added and the samples were further incubated for 15 min at 65°C with intermittent shaking.

Table 4.1 List of 28 accessions used in AFLP analysis

Accession No.	Region	District	Leaf base shape
MH/LI/2007/Coy3	South	Mangochi	Peltate
MH/NDU/2007/Coy5	South	Mangochi	Sagittate
MHG/BA/2007/Coy6	South	Machinga	Sagittate
MHG/NTU/2007/Coy9A	South	Machinga	Peltate
ZA/FIKI/2007/Coy13	South	Zomba	Sagittate
ZA/NDO/2007/Coy14	South	Zomba	Sagittate
ZA/MA/2007/Coy16	South	Zomba	Peltate
MHG/CHA/2007Coy20	South	Machinga	Peltate
ZA/NSA/2007/Coy21/4	South	Zomba	Unknown
MHG/NA/2007/Coy22	South	Machinga	Sagittate
MHG/NTE/2007/Coy23	South	Machinga	Peltate
ZA/BU/2007Coy28	South	Zomba	Sagittate
MHG/KWE/2007/Coy30	South	Machinga	Unknown
ZA/MU/2007/Coy31	South	Zomba	Unknown
TO/NJO/2007/Coy38	South	Thyolo	Unknown
CK/MKHU/2007/Coy42	South	Chikwawa	Unknown
NB/KA/2007/Coy49	North	Nkhata-bay	Unknown
NB/MU/2007/Coy50	North	Nkhata-bay	Unknown
NB/MTE/2007/Coy51	North	Nkhata-bay	Sagittate
NB/KA/2007/Coy54	North	Nkhata-bay	Unknown
NB/CHI/2007/Coy55	North	Nkhata-bay	Peltate
NB/CHI/2007/Coy56	North	Nkhata-bay	Sagittate
NB/CHI/2007/Coy57	North	Nkhata-bay	Sagittate
RU/KHA/2007/Coy60	North	Rumphi	Sagittate
RU/KA/2007/Coy62	North	Rumphi	Sagittate
RU/CHA/2007/Coy63	North	Rumphi	Sagittate
RU/NKHA/2007/Coy64	North	Rumphi	Unknown
RU/MWA/2007/Coy70	North	Rumphi	Sagittate

The samples were cooled for 2 min after which 5 M Potassium acetate was added to the mixture and incubated on ice for 20 min. Phases were separated by centrifugation at 12000 g for 10 min. The DNA was precipitated from the aqueous phase with 0.66 volumes ice-cold isopropanol and incubated at -80°C for one hour. The resultant mixture was centrifuged at

12000 g for 10 min. The supernatant was discarded and the DNA pellets air-dried for another hour. The pellets were resuspended in 500 µl Tris-EDTA (TE) buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA, pH 8.0) and incubated for 15 min at 65°C. DNA was precipitated with 500 µl ice-cold isopropanol and incubated at -80°C for 1 hour. The resultant mixture was centrifuged at 12000 g for 10 min and pellets were air-dried for one hour to remove excess isopropanol. The DNA was resuspended in 200 µl TE buffer (pH 8.0) to which 0.2 mg/ml RNase was added and incubated at 37°C for two hours. Samples were stored at 4°C for further use.

4.2.3 DNA concentration and purity determination

DNA concentration and quality were estimated using an ultra-violet (UV) spectrophotometer by measuring absorbencies at 260 nm and 280 nm and by electrophoresis in 0.8% agarose gel (w/v) in 1x UNTAN running buffer (40 mM Tris-HCl, 2 mM EDTA, pH adjusted to 7.4 with acetic acid) for 60 min at 80 V. DNA was visualised using ethidium bromide. The genomic DNA was diluted to 200 ng/µl, depending on the concentration of each sample.

4.2.4 AFLP analysis

AFLP analysis was performed according to Vos *et al*. (1995) as modified by Herselman (2003). DNA was digested using *Eco*RI (rare 6-base cutter) and *Mse*I (frequent 4-base cutter). Primer combinations are given in Table 4.2.

4.2.4.1 Restriction enzyme digestion and ligation reactions

Genomic DNA (1 µl) was digested with 4 U of *Mse*I and 1x *Mse*I-buffer [50mM NaCl; 10 mM Tris-HCl, pH 7.9; 10 mM $MgCl_2$ and 0.1 mM dithiothreitol (DTT)] for 5 hours at 37°C and followed by an overnight digestion at 37°C using 5 U *Eco*RI and NaCl to a final concentration of 100 mM. Digestions were carried out in a final volume of 50 µl. Adapter ligation were done by addition of 10 µl ligation-mix [1x ligase buffer (66 mM Tris-HCl, pH 7.6; 6.6 mM $MgCl_2$; 10 mM DTT and 66 µM ATP); 0.4 mM adenosine 5′-triphosphate (ATP) 50 pmol *Mse*I-adapter, 5 pmol *Eco*RI adapter and 1 U T4 DNA ligase] to the 50 µl restriction reaction and incubated at 16°C overnight.

4.2.4.2 Pre-amplification reactions

Pre-amplification reactions were done using 5 µl template DNA from the restriction/ligation mixture using 30 ng of each of the pre-amplification primers (*Eco*RI- and *Mse*I-primer+0, Table 4.1), 1x Promega *Taq* polymerase buffer (10 mM Tris-HCl; pH 9.0; 50 mM KCl and 0.1% (v/v) Triton X-100), 2 mM $MgCl_2$, 200 µM of each 2′- deoxynucleoside 5′-triphosphate (dNTP) and 1 U *Taq* DNA polymerase (Promega, Madison, WI, USA). PCR was done at 5 min as 94 °C followed by 30 cycles of 30 sec at 94°C, 60 sec at 56°C, and 60 sec at 72°C and a final elongation of 10 min at 72 °C. Quality and quantity of pre-amplification reactions were determined by electrophoresis in 1.5% (w/v) agarose gels. The pre-amplified DNA was diluted accordingly prior to selective amplifications (1:10 to 1:20).

4.2.4.3 Selective amplification reactions

Selective amplifications carried were out using diluted pre-amplification products. The selective amplification reactions were carried out in a total volume of 20 µl containing 5 µl diluted pre-amplified DNA, 1x Promega *Taq* DNA polymerase buffer, 2 mM $MgCl_2$, 200 µM of each dNTP, 100 µg/ml bovine serum albumin (BSA), 30 ng *Mse*I primer+3, 30 ng *Eco*RI primer+3 and 0.75 U Promega *Taq* polymerase. The PCR programme used was as follows: one cycle of denaturation at 94°C for 5 min followed by one cycle of 30 sec at 94°C, 30 sec at 65°C and 60 sec at 72°C. The annealing temperature was lowered by 1°C per cycle during the next eight cycles after which 25 cycles were performed at 94°C for 30 sec, 56°C for 30 sec and 72°C for 60 sec followed by one last elongation of 10 min at 72°C. *Eco*RI and *Mse*I primers were coded beginning with E and M respectively (Table 4.2).

AFLP analysis on the three individuals and their bulked samples for intra-clonal assessment was done using four primer combinations (E-AGG/M-CAT; E-ACT/M-CAT; E-ACT/M-CTA and E-ACC/M-CTA).

4.2.4.4 Gel electrophoresis

PCR products were mixed with 20 µl formamide dye [98% (v/v) de-ionised formamide; 10 mM EDTA, pH 8.0; 0.05% (w/v) bromophenol blue and 0.05% (w/v) xylene cynol] and denatured by incubation for 5 min at 95°C.

Table 4.2 Adapter, primer pair combinations (*Eco*RI and *Mse*I) and primer sequences used in AFLP analysis

Enzyme	Type	Sequence (5'-3')
*Eco*RI	Adapter-F	CTCGTAGACTGCGTACC
	Adapter-R	AATTGGTACGCAGTCTAC
*Mse*I	Adapter-F	GACGATGAGTCCTGAG
	Adapter-R	TACTCAGGACTCAT
*Eco*RI	Primer + 0	GACTGCGTACCAATTC
	Primer + 3	GACTGCGTACCAATTCNNN
		NNN = AAC, ACA, ACC, ACT, AGG
*Mse*I	Primer + 0	GATGAGTCCTGAGTAA
	Primer + 3	GATGAGTCCTGAGTAANNN
		NNN = CAC, CAT, CTA, CTT

Mixtures were immediately placed on ice prior to loading. PCR products were separated using 5% (w/v) denaturing polyacrylamide gels [19:1 acrylamide: bis-acrylamide; 7 M urea and 1x TBE buffer (89 mM Tris-HCl; 89 mM boric acid and 2.0 mM EDTA)]. Electrophoresis was performed at constant power of 80 W for approximately two hours.

The silver staining process for DNA visualisation of the denaturing acrylamide gels was done using the Silver Sequence^{TM} DNA Sequencing System of Promega. Gels were fixed in 10% (v/v) acetic acid for 30 min, and rinsed three times in de-ionized water, first for 10 min and 5 min each the last two washes. Gels were stained in a solution of 0.1% (w/v) silver nitrate and 0.056% (v/v) formaldehyde for 30 min and rinsed in de-ionized water for 10 sec before being immersed in a cold (4-10°C) developer [3% (w/v) sodium carbonate; 0.056% (v/v) formaldehyde and 2 µg/ml sodium thiosulphate]. The gels were shaken manually in the developer until the DNA fragments became visible. The 10% acetic acid was used to stop the developing process and shaking continued for a further 2-3 min. The gel was rinsed in de-

ionised water again and left to dry at room temperature overnight. A photograph of the gel was taken by exposing the photographic paper (Ilford multigrade IV RC de luxe) directly under the gel to dim light for 20 sec.

4.2.5 Data analysis

DNA fingerprint analysis was done by scoring fragments into a binary matrix as present (1) or absent (0). Similarity coefficients were calculated using Dice similarity coefficients (Dice, 1945; Nei and Li, 1979) using the NTSYSpc version 2.11c computer package (Rohlf, 2000). Dendrograms were constructed using UPGMA clustering in SAHN programme parameters (Rohlf, 2000). The goodness of fit of clustering data matrices was calculated using COPH and MXCOMP programmes, in NTSYSpc.

4.3. Results and discussion

4.3.1 Assessment of intra-clonal diversity within selected cocoyam accessions using AFLP analysis

The dendrogram (Figure 4. 1) indicated that there were no genetic variation within the different individuals from the same accessions and the bulked sample except for accession Coy20. The three individual samples and the bulked sample of accessions Coy5 and Coy6 were 100% similar. The three individuals and the bulked sample of Coy20 all grouped together. However, only one of the three individual samples of Coy20 was 100% similar to the bulked sample. The other two individuals (Coy20-1 and Coy20-11) were 96% and 98% similar, respectively to the bulked sample (Coy20-bulk). This suggested a 2-4% genetic variation within accession Coy20. The genetic variation within accession Coy20s' individual samples and the bulk was within the similarity threshold index levels for clones presumed to be similar in intra-clonal diversity studies (2-4%) (Lasso, 2008).

According to Rozenfeld *et al*. (2007) the genetic structure and diversity of clonal populations present a challenge in diversity studies. It needs a comparison of the extent of genetic variability among individuals within and among populations. Therefore calls for a thorough assessment of the population using appropriate molecular markers and statistically representative sample of the individuals. The genetic dissimilarity observed from the few

accessions tested in the present study was within the minimum threshold levels set and observed in similar clonal diversity studies (Arens *et al*., 1998; Douhovnikoff and Dodd, 2003; Lasso, 2008).

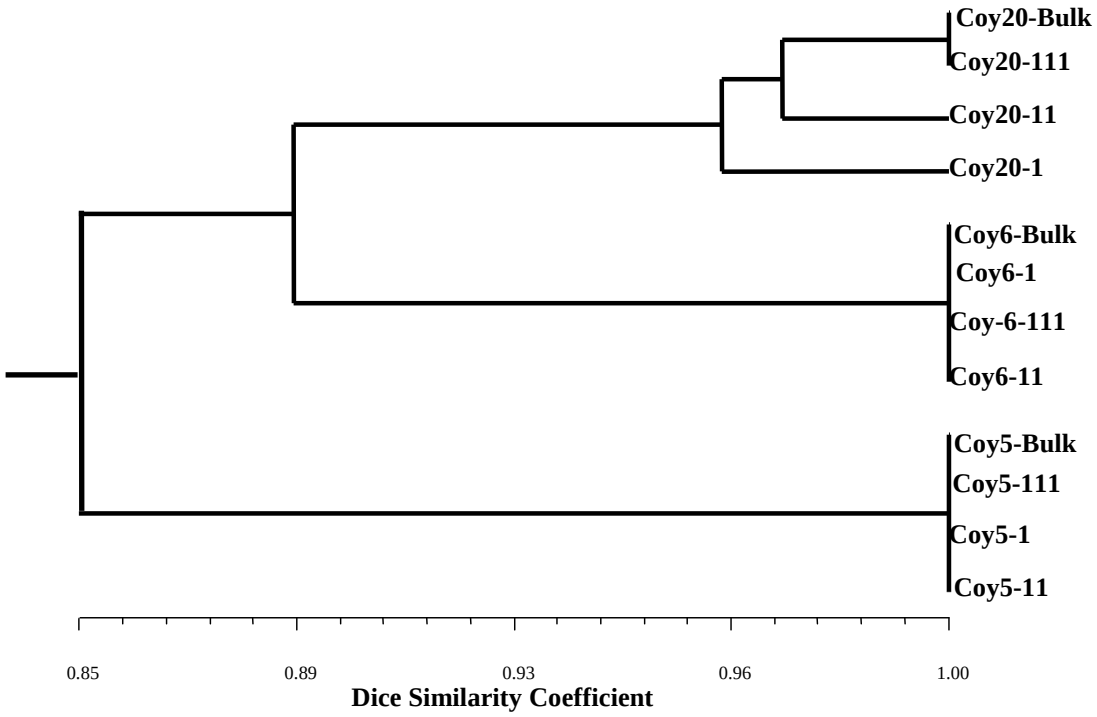

Figure 4.1 Dendrogram showing the intra-clonal similarities within three randomly selected cocoyam accessions. Dendrogram was created based on AFLP analysis and UPGMA cluster analysis using the Dice similarity coefficient.

Although a detailed study is required to elucidate if the variation is representative of the Malawian cocoyam populations, the present study suggested limited diversity within accessions. The diversity observed within Coy20 could be attributed to clonal growth, selfing and somatic mutations as suggested by Rozenfeld *et al.* (2007) as well as differences between ramets (all units forming a clone) and replicate runs of the same template DNA (Lasso,

2008). Out-crossing and migration which are dominant processes in genetic diversity in clonal populations appear to be marginal here as all accessions exhibited non-flowering behaviour (Chapter 3). Based on the low levels of genetic diversity within clones, AFLP analysis on the entire set of 28 accessions was performed using a bulk sample (of three individuals) of each accession. By bulking DNA from three individuals per accession, a representative sample for each accession was obtained.

4.3.2 Assessment of genetic diversity among the cocoyam accessions using AFLP analysis

Eight AFLP primer combinations were randomly selected and tested on the 28 cocoyam accessions. The eight selected primer combinations produced a total of 241 scorable fragments. A total of 223 fragments were polymorphic with an average of 30 polymorphic fragments per primer pair combination, representing 93% polymorphism. Primer combinations E-AAC/M-CTA, E-ACC/M-CTT, E-ACC/M-CTA, E-ACA/M-CTA and E-ACT/M-CTA produced the highest number of amplified fragments. Primer combination E-ACT/M-CAC produced the lowest number of amplified fragments. High levels of polymorphism were observed for all primer combinations used ranging from 83% (E-ACT/M-CTA) to 100% (E-ACT/M-CTA) (Table 4.3).

The dendrogram constructed based on AFLP markers revealed two main clusters I and II (Figure 4.2). Main cluster I contained 11 accessions, mostly from the southern region. All but one (Coy55) of the peltate leaf base shaped accessions grouped together in main cluster I, i.e. accessions Coy3 and Coy9A as well as Coy16, Coy20 and Coy23. Cluster I subdivided into two clusters, A and B. Cluster A contained ten of the accessions in this group. Only one accession, Coy63 from the northern region clustered on its own in cluster B.

Low levels of variation were observed among the first nine accessions within cluster I (85.3% similarity). The other two accessions within cluster I, Coy16 and Coy63, clustered separately from other accessions and were the most dissimilar in this cluster with a Dice genetic similarity coefficient of 79.3% and 68.8% in relation to the other accessions in this group, respectively. Accessions Coy3 and Coy9A were the most similar accessions in the cluster with a Dice similarity coefficient of 96.1%.

Table 4.3 Information generated using eight AFLP primer combinations

Primer combinations	Total scorable fragments	Polymorphic fragments	% Polymorphism
E-AAC/M-CTA	40	37	92.5
E-ACC/M-CTT	40	36	90.0
E-ACT/M-CAC	12	10	83.3
E-ACC/M-CTA	35	34	97.1
E-ACT/M-CTA	32	31	96.9
E-AAC/M-CTT	29	28	96.6
E-ACA/M-CTT	19	19	100.0
E-ACA/M-CTA	34	30	88.2
Total	241	223	93.0
Average	30	28	93.0

The second main cluster II contained 17 accessions from both the southern and northern regions. All accessions with known leaf base shapes in cluster II, except for Coy55, exhibited sagittate base shaped leaves. The most closely related accessions in cluster II were accessions Coy55 and Coy56, with a Dice genetic similarity of 97.4%. The cluster further sub-divided into clusters C and D. Cluster C contained nine accessions, mostly from the south. Cluster C further sub-divided into sub-clusters a and b. Sub-cluster a contained two accessions Coy5 and Coy70, from the south and north respectively. Sub-cluster b contained seven accessions from the south. However, cluster D contained eight accessions all of which were collected from the north, most of which exhibited sagittate base shaped leaves. Only accession Coy55 in cluster II exhibited peltate base shaped leaves. Cluster II represented low levels of diversity, with all accessions in this cluster being 88% similar.

High levels of genetic diversity were detected between the two main clusters i.e. 38.1%. However, the genetic relationship among accessions within each main cluster (I and II) was close. The accessions in main cluster I were 83.3% similar, excluding accessions Coy16 and Coy63 which clustered separately from the rest of the accessions while accessions in cluster II were 87.7% similar.

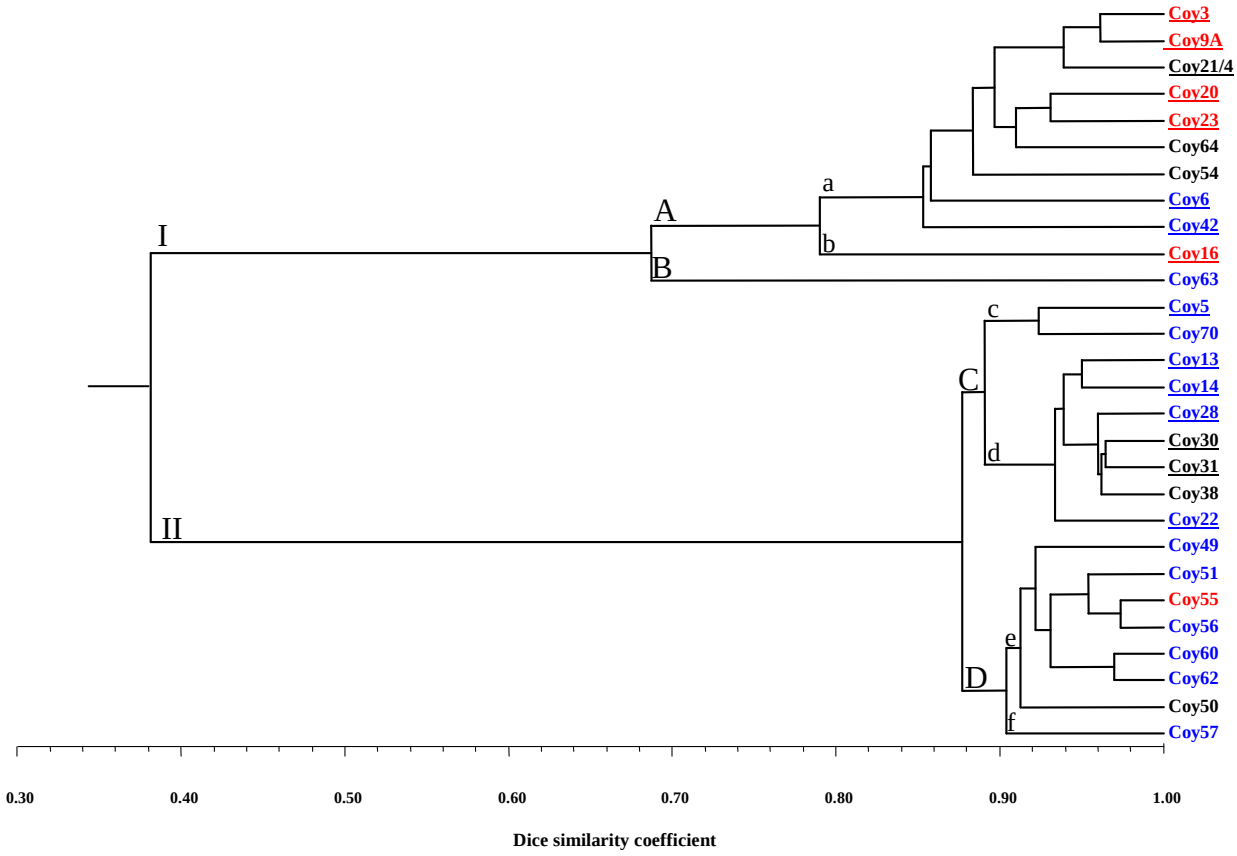

Figure 4.2 Clustering of 28 cocoyam accessions based on AFLP analysis and UPGMA clustering using the Dice similarity coefficient. Red represents accessions with peltate leaf base shapes while blue represents accessions with sagittate leaf base shapes. Accessions collected from the south are underlined, and the rest are from the north. The leaf base shapes of the accessions in black are unknown.

AFLP markers proved a valuable tool for cultivar identification, especially in such a collection with a narrow genetic base. Although some genotypes were genetically similar, all accessions could be uniquely distinguished from each other. Results agreed with that of earlier studies by Quero-Garcia *et al*. (2004). In practical terms, however, crosses between genetically closely related genotypes such as those identified by the AFLP analysis, will have to be avoided.

The correlation matrix based on the goodness of fit of clustering to data matrixes (Table 4.4) was calculated using COPH and MXCOMP programme. The r value gives a measure of goodness of fit for a cluster analysis. The r-value of 0.98097 in the present study suggested a very good fit.

Results indicated that accessions mostly clustered according to species i.e. *Colocasia* and *Xanthosoma*. Cluster I contained mostly peltate leaf based accessions while cluster II contained sagittate leaf base shaped accessions. According to Purseglove (1972) and Onwueme (1978), *Colocasia* species is characterised by peltate leaf base shape while *Xanthosoma* is non-peltate i.e. sagittate. The high levels of variation between the two clusters further suggested the presence of the two different species of cocoyam, differentiated based on AFLP fingerprints. Variations within clusters suggest continuous gradation within the cocoyam species which has been suggested by several authors, such that further classification into finer groupings is complicated (Murton, 1972; Onwueme, 1978). This contrasts a study by Sharma *et al*. (2008) where AFLP markers unveiled high levels of variation within *C. esculenta*. Apart from *X. sagittifolium* and *C. esculenta* which exist in numerous forms, other species of *Colocasia* and *Xanthosoma* also exists. These include *X. atrovirens*, *X. caracu* and *X. violaceum* whilst *C. esculenta* var. *antiquorum* and *C. esculenta* var. *esculenta* have been characterised as distinct varieties (Murton, 1972). Kreike *et al*. (2004) observed very distinctive AFLP fingerprint patterns for *C. esculenta*. The closely related species like *C. gigantea*, *Alocasia* spp., *X. sagittifolium, X. violacaum, X. brasiliense, X. robustum* and *Amorphophallus campanulatus* did not show any resemblance to the *C. esculenta*. AFLP banding patterns and therefore were excluded from the genetic diversity analysis of *taro* (*C. esculenta*) in south east Asia and the Pacific.

Table 4.4 Pair-wise genetic similarity coefficient matrix for 28 cocoyam genotypes calculated from AFLP analysis data

	Coy3	Coy5	Coy6	Coy9A	Coy13	Coy14	Coy16	Coy20	Coy21/4	Coy22	Coy23	Coy28	Coy30	Coy31	Coy38	Coy42	Coy49	Coy50
Coy3	1.000																	
Coy5	0.272	1.000																
Coy6	0.870	0.391	1.000															
Coy9A	0.961	0.244	0.898	1.000														
Coy13	0.288	0.939	0.417	0.260	1.000													
Coy14	0.319	0.897	0.430	0.283	0.950	1.000												
Coy16	0.778	0.354	0.727	0.810	0.349	0.368	1.000											
Coy20	0.896	0.391	0.859	0.891	0.403	0.434	0.825	1.000										
Coy21/4	0.929	0.310	0.853	0.949	0.333	0.349	0.807	0.924	1.000									
Coy22	0.251	0.932	0.440	0.271	0.944	0.926	0.377	0.383	0.275	1.000								
Coy23	0.887	0.349	0.841	0.868	0.372	0.421	0.798	0.931	0.917	0.374	1.000							
Coy28	0.281	0.918	0.419	0.277	0.949	0.937	0.343	0.394	0.313	0.943	0.368	1.000						
Coy30	0.277	0.920	0.420	0.288	0.947	0.942	0.372	0.404	0.326	0.935	0.368	0.956	1.000					
Coy31	0.294	0.917	0.420	0.276	0.933	0.950	0.366	0.411	0.342	0.928	0.397	0.964	0.965	1.000				
Coy38	0.308	0.905	0.440	0.305	0.259	0.923	0.386	0.431	0.348	0.924	0.403	0.961	0.961	0.962	1.000			
Coy42	0.854	0.256	0.847	0.874	0.885	0.278	0.770	0.830	0.855	0.273	0.852	0.272	0.282	0.308	0.323	1.000		
Coy49	0.382	0.868	0.481	0.361	0.844	0.888	0.450	0.495	0.414	0.859	0.466	0.901	0.896	0.918	0.902	0.402	1.000	
Coy50	0.321	0.836	0.417	0.324	0.880	0.842	0.376	0.403	0.351	0.861	0.378	0.875	0.892	0.872	0.876	0.332	0.900	1.000
Coy51	0.298	0.896	0.439	0.304	0.321	0.872	0.385	0.440	0.352	0.895	0.400	0.901	0.910	0.897	0.908	0.338	0.917	0.927
Coy54	0.861	0.295	0.842	0.931	0.879	0.371	0.795	0.862	0.878	0.356	0.876	0.343	0.360	0.382	0.374	0.865	0.416	0.383
Coy55	0.304	0.893	0.426	0.320	0.885	0.863	0.350	0.398	0.323	0.892	0.338	0.902	0.912	0.890	0.902	0.326	0.912	0.937
Coy56	0.287	0.905	0.412	0.299	0.824	0.869	0.364	0.413	0.324	0.897	0.365	0.897	0.914	0.865	0.905	0.303	0.921	0.924
Coy57	0.324	0.862	0.481	0.349	0.858	0.831	0.368	0.446	0.324	0.870	0.393	0.878	0.853	0.865	0.870	0.369	0.884	0.880
Coy60	0.356	0.854	0.482	0.354	0.824	0.843	0.404	0.462	0.386	0.848	0.425	0.874	0.873	0.865	0.870	0.365	0.925	0.884
Coy62	0.362	0.862	0.484	0.368	0.858	0.848	0.414	0.479	0.402	0.857	0.440	0.871	0.884	0.869	0.880	0.371	0.934	0.904
Coy63	0.681	0.489	0.792	0.740	0.469	0.461	0.679	0.688	0.667	0.547	0.636	0.496	0.486	0.484	0.514	0.659	0.536	0.462
Coy64	0.886	0.377	0.843	0.902	0.402	0.428	0.803	0.898	0.900	0.368	0.923	0.391	0.399	0.404	0.417	0.850	0.484	0.450
Coy70	0.350	0.924	0.439	0.307	0.877	0.851	0.407	0.464	0.359	0.870	0.416	0.870	0.857	0.860	0.865	0.322	0.878	0.840

Table4.4 (Continued)

	Coy51	Coy54	Coy55	Coy56	Coy57	Coy60	Coy62	Coy63	Coy64	Coy70
Coy51	1.000									
Coy54	0.378	1.000								
Coy55	0.952	0.400	1.000							
Coy56	0.957	0.353	0.974	1.000						
Coy57	0.922	0.391	0.914	0.937	1.000					
Coy60	0.918	0.371	0.918	0.934	0.898	1.000				
Coy62	0.936	0.363	0.928	0.951	0.896	0.970	1.000			
Coy63	0.504	0.667	0.526	0.498	0.541	0.538	0.546	1.000		
Coy64	0.434	0.896	0.395	0.400	0.407	0.446	0.457	0.661	1.000	
Coy70	0.884	0.346	0.875	0.912	0.876	0.863	0.888	0.509	0.428	1.000

r = 0.98097

In this study, there was no to limited prior knowledge of the germplasm but clear banding patterns were observed among accessions, suggesting the presence of two different species as seen in the dendrogram (Figure 4.2). However, other accessions exhibited unfamiliar bands and clustered out of the main two clusters, which suggested the possible presence of the other species or subspecies.

As has been observed in other clonally propagated crops, genetic clusters revealed by AFLP markers are marked with similar morphotypes and similar genotypes being distributed closely. This is attributed to the absence of active sexual reproduction and constant movement of clonal material across the growing regions (Crouch *et al*., 2000). The dendrogram (Figure 4.2) suggested a clear genetic differentiation in accordance to spatial patterns. Most accessions from the south grouped together in cluster I and sub-cluster C of the main cluster II. On the other hand, accessions with sagittate leaf base shapes collected from the northern region clustered together in sub-cluster c. The constant movement of clonal material within each region may explain the fact that most of the morphotypes and genotypes of Malawi are similar within each region. However, the presence of genotypes from different regions in the same cluster suggested that there is limited isolation between the two regions. The between cluster heterogeneity was much larger than the within cluster heterogeneity. This contradicts results observed in Vanuatu where AFLP markers were used to validate a stratification of *taro* germplasm (Quero-Garcia *et al*., 2004), which could be due to the fact that only a single known species was investigated as opposed to the present study.

4.4 Conclusions and recommendations

The AFLP technique was successful in uniquely identifying all accessions as well as unveiling the presence and level of intra-clonal variation within and between accessions. The intra-clonal variation was within the minimum threshold to assume that the members belonged to the same clone. Hence any member of such accessions exhibiting intra-clonal variation could be used to represent other members without affecting the position of the accession in population diversity. The dendrogram suggested the presence of the two main cocoyam species *Colocasia* and *Xanthosoma* and possible subspecies of the two species. The AFLP technique indicated high levels of variation between the two cocoyam species and low

levels of variation among accessions in each of the two species identified. The genetic diversity of cocoyam in Malawi showed differentiation patterns according to geographical level as several genotypes from the same location clustered together. The results of the AFLP analysis need to be corroborated with agro-morphological characterisation to elucidate the pattern of the clustering of the genotypes and how the diversity is stratified across the country. It is also recommended that a mitotic index study be conducted to see if the accessions cluster according to their ploidy levels.

4.5 References

Arens, P., Coops, H., Janseen, J., and Vosman, B. (1998). Molecular genetic analysis of black poplar (*Populus nigra* L.) along Dutch rivers. Molecular Ecology 7: 11-18.

Arnau, G., Lallemand, J., and Bourgoin, M. (2001). Are AFLP markers the best alternative for cultivar identification? In: Doré, C., Dosba, F., and Baril C. (Eds.). Proceedings of the International Symposium on Molecular Markers. Acta Horticulturae 546, ISHS, pp. 301-306.

Blay, E., Offei, S., and Danquah, E. (2004). Genetic diversity in cocoyam as revealed by random amplified polymorphic DNA (RAPD) markers. In: Genetic improvement of under-utilised and neglected crops in low income food deficit countries through irradiation and related techniques. Proceedings of a final research coordination meeting organised by the joint FAO/IAEA division of nuclear techniques in food and agriculture. Pretoria, South africa: IAEA, pp. 131-142.

Brown, A. (1978). Isozymes, plant population genetic structure and genetic conservation. Theoretical and Applied Genetics 52: 145-157.

Caillon, S., Quero-Garcia, J., and Guarino, L. (2004). *Taro* in Vanuatu: towards a dynamic conservation strategy. LEISA Magazine, March, pp: 18-20.

Crouch, H., Crouch, J., Madsen, S., D.R., V., and Ortiz, R. (2000). Comparative analysis of phenotypic and genotypic diversity. Theoretical and Applied Genetics 101: 1056-1065.

Dellaporta, S., Wood, J., and Hicks, J.R. (1983). A plant DNA minipreparation: version II. Plant Molecular Biology Report 1: 19-21.

Dice, L. (1945). Measures of amount of ecological association between species. Ecology 26: 297-302.

Douhovnikoff, V., and Dodd, R. (2003). Intra-clonal variation and a similarity threshold for identification of clones: application to *Salix exigua* using AFLP molecular markers. Theoretical and Applied Genetics 106: 1307-1315.

Ekwe, K., Nwosu, K., Ekwe, C., and Nwachukwu, I. (2009). Examining the underexploited values of cocoyams (*Colocasia* and *Xanthosoma* species) for enhanced household food security, nutrition and economy in Nigeria. In: Jaenicke, H., Ganry, J., Zeledon-Hoeschle, I., and Kahane, R. (Eds.). Proceedings of the underutilized plants for food security, income and sustainable development. Acta Horticulturae 806, ISHS, pp. 71-78.

Erhardt, G., and Weimann, C. (2007). Use of molecular markers for evaluation of genetic diversity and in animal production. Archivos Latinoamericanos de Produccion Animmal 15 (Supplement 1): 63-66.

FAO (1990). Roots, tubers, plantain and bananas in human nutrition. Rome: Food and Agriculture Organisation of the United Nations.

Global Forum for Underutilised Species (2009). http://www.underutilised-species.org. (2009/09/05).

Herselman, L. (2003). Genetic variation among Southern African cultivated peanut (*Arachis hypogea* L.) genotypes as revealed by AFLP analysis. Euphytica 133: 319-327.

Karp, A., Serberg, O., and Buiatti, M. (1996). Molecular techniques in the assessment of botanical diversity. Annals of Botany 78: 143-149.

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

Lakhanpaul, S., Velayudhan, K., and Bhat, K. (2003). Analysis of genetic diversity in Indian *taro* (*Colocasia esculenta* (L.) Schott) using random amplified polymorphic DNA (RAPD) markers. Genetic Resources and Crop Evolution 50: 603-609.

Larkin, P., and Scowcroft, W. (1981). Somaclonal variation - a novel source of variability from cell cultures for plant improvement. Theoretical and Applied Genetics 60: 197-214.

Lasso, E. (2008). The importance of setting the right genetic distance threshold for identification of clones using amplified fragment length polymorphism: a case study with five species in the tropical plant genus *Piper*. Molecular Ecology Resources: Technical advances 8: 74-82.

Mace, E., and Godwin, I. (2002). Development and characterisation of polymorphic microsatellite markers in *taro* (*Colocasia esculenta*). Genome 45: 823-832.

Mace, E., Murthur, P., Iziquierdo, L., Hunter, D., Taylor, M., Singh, D., DeLacy, I.H., Jackson, G.V.H., and Godwin, I.D. (2006). Rationalization of *taro* germplasm collections in the Pacific Island region using simple sequence repeat (SSR) markers. Plant Genetic Resources 4: 210-22.

Mueller, U., and Wolfenbarger, L.L. (1999). AFLP and fingerprinting: Reviews. Tree 14: 389-394.

Murton, J. (1972). Cocoyams (*Xanthosoma caracu*, *X. atrovirens* and *X. nigrum*): Ancient root-and-leaf vegetables, gaining in economic importance. Florida State Horticultura Society: 85-94.

Nei, M., and Li, W. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences USA 76: 5269-5273.

Ochiai, T., Nguyen, V., Tahara, M., and Yoshino, H. (2001). Geographical differentiation of Asian *taro, Colacasia esculenta* (L.) Schott, detected by RAPD and isozyme analyses. Euphytica 122: 219-234.

Onwueme, I. (1978). Tropical tuber crops*:* Yams, cassava, sweet potato and cocoyams. John Wiley and Sons, pp. 199-225.

Onwueme, I. (1999). *Taro* cultivation in Asia and the Pacific. FAO: Regional Office for Asia and the Pacific.

Purseglove, J. (1972). Tropical crops: Monocotyledons Vol 1. Essex England: Longman. pp. 66-74.

Quero-Garcia, J., Noyer, J., Perrier, X., Marchand, J., and Lebot, V. (2004). A germplasm stratification of *taro* (*Colocasia esculenta*) based on agro-morphological descriptors, validation by AFLP markers. Euphytica 137: 387-395.

Ramana, R.V., and Hodgkin, T. (2002). Genetic diversity and conservation and utilization of plant genetic resources. Plant Cell, Tissue and Organ Culture 68: 1-19.

Rao, K.N. (2004). Plant genetic resources: Advancing conservation and use through biotechnology. African Journal of Biotechnology 3: 136-145.

Rohlf, F. (2000). NTSYS-pc: Numerical taxonomy and multivariate analysis system. Applied Biostatistics. New York: Exerter Publishing Ltd.

Rozenfeld, A., Arnaud-Hound, S., Hernamdez-Garcia, E., Eguiluz, V., Matias, M., Serrao, E., and Duarte, C.M. (2007). Spectrum of genetic diversity and networks of clonal organisms. Journal of the Royal Society Interface 4: 1093-1103.

Santelices, B., Aedo, D., and Varela, D. (1995). Causes and implications of intra-clonal variation in *Gracilaria chilensis* (*Rhodophyta*). Journal of Applied Phycology 7: 283-290.

Schnell, R.J., Goenaga, R., and Olano, C.T. (1999). Genetic similarities among cocoyam cultivars based on Randomly Amplified Polymorphic DNA (RAPD) analysis. Scientia Horticulturae 80: 267-276.

Sharma, K., Mishra, A.K., and Misra, R.S. (2008). Analysis of AFLP variation of *taro* population and markers associated with leaf blight resistance gene. Academic Journal of Plant Sciences 1: 42-48.

Singh, D., Mace, E., Godwin, I., Mathur, P., Okpul, T., Taylor, M., Hunter, D., Kambuou, R., Ramanatha Rao, V., and Jackson, G. (2008). Assessment and rationalization of genetic diversity of Papua New Guinea *taro* (*Colocasia esculenta*) using SSR DNA fingerprinting. Genetic Resources and Crop Evolution 55: 811-822.

Vos P., Hogers R., Bleeker, M., Reijans, M., Van de Lee, T., Hormes M., Friters A., Pot J., Paleman, J., Kuiper, M., and Zabeau, M. (1995). AFLP: A new technique for DNA fingerprinting. Nucleic Acids Research 23: 4407-4414.

Wilson, J. (1990). Agro facts, crops: *Taro* breeding 3/89. Apia, Western Samoa: Institute for Research, Extension and Training in Agriculture (IRETA) publications.

Winfield, M., Amold, G., Cooper, F., Le Ray, M., White, J., Karp, A., and Edwards, K.J. (1998). A study of genetic diversity in *Populus nigra* subsp *betulifolia* in the upper severn area of the UK using AFLP markers. Molecular Ecology 7: 3-10.

CHAPTER 5

Comparison of genetic diversity among Malawian cocoyam germplasm using morphological characters and AFLP analysis

5.1 Introduction

Conservation and sustainable use of plant genetic resources are essential to meet the demand for future food security (Rao, 2004). Plant genetic resources are an important component of biodiversity and provide the basic genetic variability, for breeding that allows new and improved cultivars to be developed (Powell *et al*., 1995). Numerous germplasm collections have been established and it is important to know the extent of diversity that exist as well as identify species that are present (Ferguson, 2007). Information on genetic diversity and relationships within and among crop species and their wild relatives is essential for the efficient utilisation of plant genetic resource collections (Mohammadi and Prasanna, 2003; Ferguson, 2007).

A number of methods are available for the analysis of genetic diversity in germplasm accessions, breeding lines and populations. These methods include agro-morphological (Karp *et al*., 1996), biochemical (Brown, 1978) and molecular (DNA-based) markers (Rao, 2004). IPGRI descriptor lists are often used for evaluation of morphological characteristics, supplemented by cytogenetic studies of accessions which are useful in the establishment of chromosome number and genome composition. On the other hand, biochemical and molecular methods describe accessions using biochemical and molecular markers. These are readily detectable sequences of DNA or proteins whose inheritance can be monitored (Biodiversity International, Rural Development Administration, 2009). There are several methods that can be employed in biochemical and molecular characterisation, which differ from each other in terms of ease of analysis, reproducibility and level of polymorphism, number and genome distribution of loci (Rao, 2004; Biodiversity International, Rural Development Administration, 2009).

However, in order to elucidate clear genetic relationships within and between populations and/or individuals, agro-morphological data have to be correlated with molecular data. According to Mohammadi and Prasanna (2003), for a reasonably accurate and unbiased estimate of genetic diversity, adequate attention has to be devoted to (a) sampling strategies, (b) utilisation of various data sets on the basis of understanding of their strengths and constraints, (c) choice of genetic distance measure(s), clustering procedures and other multivariate methods in analyses of data and (d) objective determination of genetic relationships.

Determining true genetic (similarity or dissimilarity) relationships between individuals is therefore an important and decisive point for clustering and analysing diversity within and among populations, as different procedures (indices) yield conflicting outcomes. Several approaches are used to study genetic diversity within and among populations or groups of individuals (Kosman and Leornado, 2005). Assessing genetic diversity within a group implies quantifying genetic variability in a group and comparing individuals belonging to the same group using similarity coefficients such as Jaccard, Dice and simple matching. On the other hand, classification and ordination methods are used to visualise relationship patterns of individuals and/or groups. Comparing different groups of individuals involves different statistics like Wright's F statistics and Nei's parameters (Laurentin, 2009).

Cluster and ordination methods type individuals of a population into groups in a hierarchical structure, such that similar individuals belong to the same group and dissimilar individuals in different groups. UPGMA is the most frequently used clustering method (Laurentin, 2009) and principal coordinate analysis (PCoA) (Gower, 1966) and principal component analysis (PCA) are the most used ordination methods in assessment of plant diversity. Cluster analysis and ordination techniques' complementarity in interpretation of the diversity among individuals is often observed. Similarities in the analysis pattern of both techniques is used as a measure of the technique's empirical accuracy (Drossou *et al*., 2004**;** Laurentin, 2009).

Overall, diversity in assessment of plant genetic resources may be partitioned into within and between groups of the populations under investigation. This is achieved by analysis of molecular variance (AMOVA). AMOVA identifies molecular variation within and between

population groups, based on squared distances for comparing pair-to-pair all the band patterns at different hierarchical levels (Excoffier *et al.,* 1992). AMOVA eliminates the normality assumption used in the conventional analysis of variance (ANOVA) to test the significance of the variance components with the use of permutations. Sources of variation are partitioned into among groups and within groups of individuals and error (Laurentin, 2009).

Cocoyam, a member of the Araceae family (Onwueme, 1978), has been cultivated for generations (Mathews, 2004). Genotypes have been selected by subsistence farmers to conform to local agro-ecological conditions and social requirements. Local importance has declined with the availability of productive shorter season root crops. Demand for cash crops and shortage of land due to population pressure has led to the narrowing of its diversity (Singh *et al.,* 2008). The serious repercussion of the loss of cocoyam genetic diversity has led to the collection, evaluation and conservation of cocoyam genetic resources in different regions, Malawi inclusive.

Estimates of genetic diversity in cocoyam have been obtained by analysing phenotypic characters (Quero-Garcia *et al*., 2004; Danquah *et al*., 2006; Murakami *et al.,* 2006; Mbouobda *et al*., 2007), isozyme or storage proteins (Lebot and Aradhya, 1991; Tambong *et al.,* 1999; Manzano *et al*., 2001), DNA sequences (Mace and Godwin, 2002; Kreike *et al.*, 2004) and a combination of two or all techniques (Lebot *et al.*, 2004; Quero-Garcia *et al*., 2004; Singh *et al.,* 2008). However, phenotypic descriptors alone do not always allow the quantification of the genotypic difference or similarity between cultivars as do genetic distances based on DNA polymorphism (Lefebvre *et al.,* 2001). As observed by Okpul *et al.* (2005), phenotypic variation did not exactly reflect genotypic variation at molecular level in cocoyam. Singh *et al.* (2008) suggested that information derived from agro-morphological characterisation should therefore be treated with caution. However, the use of predominant and stable agro-morphological traits can provide basic information and stratification prior to thorough molecular characterisation. According to Soller and Beckmann (1983) use of molecular markers as an additional tool for germplasm characterisation and description add efficiency to the whole process.

This study was aimed at determining the genetic diversity in cocoyam accessions from Malawi using morphological characters, AFLP analysis and a combination of the two methods. Specifically the study compared the efficiency of using these methods in determining cocoyam genetic diversity.

5.2 Materials and methods

5.2.1 Plant materials

Plant materials and experimental design for morphological characteristics and AFLP analysis techniques are given in Sections 3.2.2.1 and 4.2.1 respectively.

5.2.2 Morphological data collection

Data collection procedures for morphological characteristics of the cocoyam accessions are as described in Section 3.2.2.2.

5.2.3 DNA extraction

Total genomic DNA from cocoyam samples was extracted according to the modified Dellaporta *et al*. (1983) DNA minipreparation method as described in Section 4.2.2.

5.2.4 AFLP analysis

AFLP analysis was performed according to Vos *et al*. (1995) as modified by Herselman (2003). DNA was digested using *Eco*RI (rare 6-base cutter) and *Mse*I (frequent 4-base cutter) as described in Section 4.2.4. Primer combinations are given in Table 4.2.

5.2.5 Genetic similarities and clustering analysis

Morphological data for the accessions were converted into a binary matrix using the procedure of Benesi (2005) in Section 3.2.2.2. DNA fingerprint analysis was done by scoring fragments into a binary matrix as present (1) or absent (0). Dendrograms for morphological, AFLP analysis and a combination of the two analyses were constructed using the Dice similarity coefficient (Dice, 1945; Nei and Li, 1979) and UPGMA clustering in SAHN programme parameters using the NTSYSpc version 2.2c computer package (Rohlf, 2000). For combined analysis, only accessions for which both morphological and AFLP data were

available (21 in total), were used. PCA bi-plots were prepared using the Dice dissimilarity coefficient and PCA algorithms using DARwin 5.0.155 software (Perrier *et al*., 2003; Perrier and Jacquemand-Collet, 2006). AMOVA analysis was done using the software ARLEQUIN 3.11 (Excoffier *et al*., 2005). For morphological data, the total variance among accessions was partitioned into variance among groups and within groups. The groups were defined on the basis of leaf base shape, either peltate or sagittate (representing the two species *Colocasia* and *Xanthosoma*, respectively). For AFLP and combined data, the groups were based on the two clusters obtained during the construction of the dendrograms, because some accessions used during AFLP analysis did not have complete morphological data, especially leaf base shapes were unknown for some accessions. The significance for partitioning of the genetic components was tested using 16000 permutations. Although an AMOVA is usually done using genetic data only, in the present study, AMOVA was also done using the morphological data and combined morphological and AFLP data. This was done since the morphological data was also in binary matrix (0 and 1) and because the study wanted to elucidate the partitioning of variance within and between the two assumed species, *Colocasia* and *Xanthosoma*, based on morphological and AFLP data. Similar AMOVA analysis (including morphological and AFLP data) were done by Donini *et al*. (2000) to assess the diversity in United Kingdom winter wheat.

5.3 Results and discussion

5.3.1 Clustering of the cocoyam accessions based on combined morphological and AFLP data

The dendrogram in Figure 5.1 was drawn with combined data from morphological characters and AFLP analysis of 21 accessions that could be characterised using both morphological and AFLP data. Results suggested that accessions grouped based on species. Two groups were identified, one group belonging to *Colocasia* and the other to *Xanthosoma* species, based on the type of leaf base shapes (attachment to the petiole) i.e. peltate (*Colocasia*) and sagittate (*Xanthosoma*) (Purseglove, 1972; Onwueme, 1978). Cluster I contained all but one (Coy55) peltate leaf base shaped accessions (in red). On the other hand, cluster II contained predominantly sagittate leaf shaped accessions.

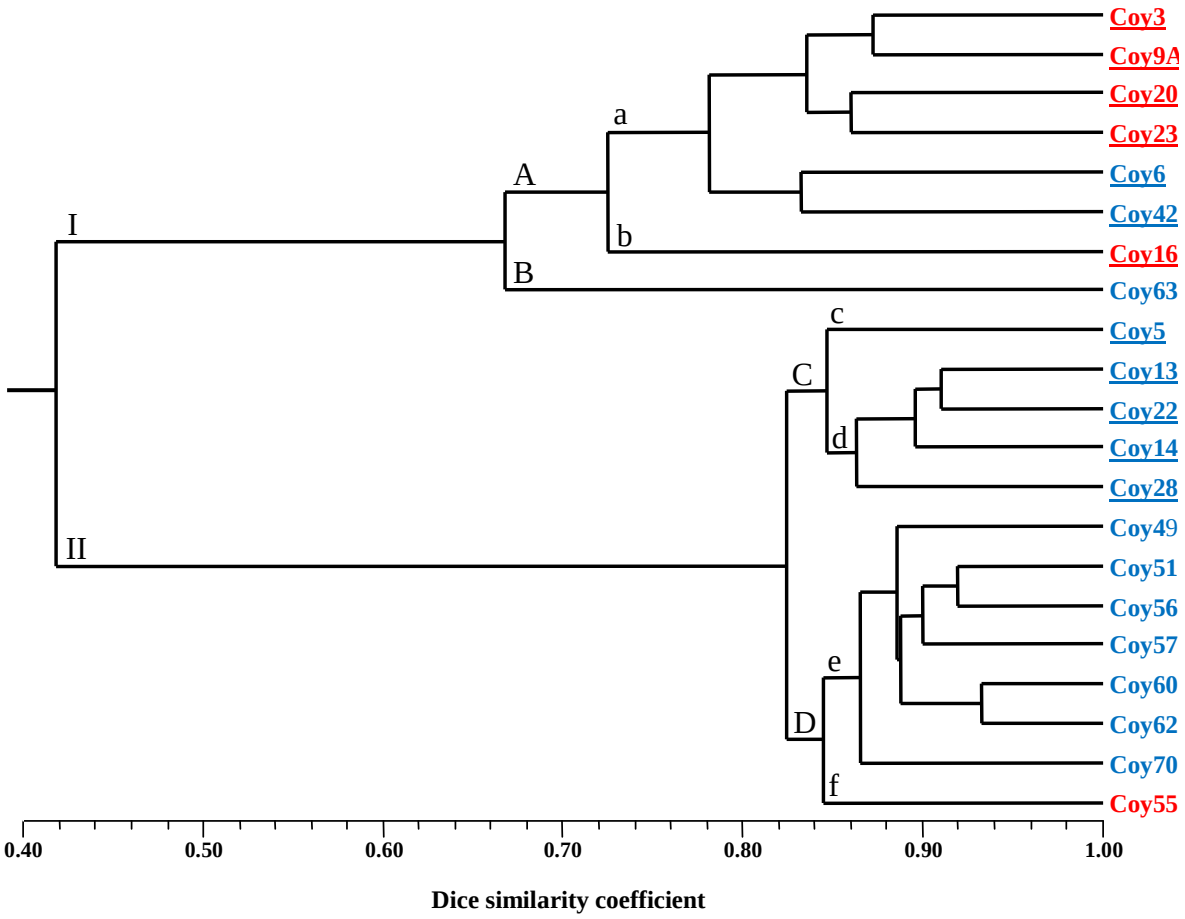

Figure 5.1 **Clustering of 21 cocoyam accessions from Malawi based on combination of 30 morphological characters and AFLP analysis (seven primer combinations). Red-peltate leaf base shaped, blue-sagittate leaf base shaped, underlined-accessions collected from the south and the rest from the north. Dendrogram was constructed using the Dice similarity coefficient and UPMGA clustering.**

However, accession Coy55 with peltate leaf shapes grouped in sub-cluster D with the sagittate leaf shaped accessions. Three sagittate leaf base shaped accessions (Coy6, Coy42 and Coy63) grouped with the peltate leaf base accessions in cluster I.

Results (Figure 5.1) further suggested that differentiation of accessions was based on region of collection. Cluster I mainly contained accessions from the southern region (underlined). Accession Coy63 from the northern region, was exceptional in this cluster in that it clustered separately from the other accessions and happened to be the most dissimilar accession of the group (66% similarity). Main cluster II contained accessions from both the southern and northern regions of Malawi. However, the two populations clearly separated. Sub-cluster C contained accessions from the southern region only. Sub-cluster D, on the other hand, only contained accessions collected from the northern region.

In comparison with dendrograms constructed based on morphological characters (Figure 3.2) and AFLP analysis (Figure 4.2) differentiation of the accessions based on combined data was more similar to the AFLP dendrogram than the morphological data dendrogram. This could be as a result of the number of data points present in the AFLP analysis (241) as opposed to the morphological characters (156). Variation between the two population groups (*Colocasia* and *Xanthosoma* species) was more pronounced in AFLP analysis (38.1% similar) as opposed to morphological characterisation (45%) and combined analysis (42% similar). Variation within each main group was lower using AFLP and combined analysis compared to morphological characterisation. Using AFLP analysis, accessions with sagittate leaf base shapes were 88% similar and using combined analysis 82%, while using morphological characterisation similarity was 45%. Similarity within accessions with peltate leaf base shapes was 69% using AFLP analysis, 66% for combined data and 48% using morphological data.

For all analyses (morphological, AFLP and combined data), accession Coy16 showed the highest level of variation within the peltate leaf shaped accessions that clustered together in main cluster I. Accession Coy55, that clustered separately from the other peltate leaf shaped accessions using AFLP and combined data, was the only accession with peltate leaf shape that was collected from the northern region.

The matrix correlation based on the goodness of fit of clustering to data matrixes (Table 5.1) was calculated using the COPH and MXCOMP programme. The r value gives a measure of goodness of fit for a cluster analysis. The r-value of 0.96636 found suggested a very good fit. This is intermediate between the r-value for AFLP analysis (r=0.98097) and morphological characterisation (r=0.872220).

All three analyses suggested the presence of the two distinct species i.e. *Colocasia* and *Xanthosoma*, if species definition is based on the leaf base shape (petiole attachment) characteristics. Variation between accessions was furthermore based on the region of collection. The southern region accessions tended to group separately from ones from the northern region for all three analyses.

Variation was higher within the *Colocasia* species i.e. peltate accessions than the *Xanthosoma* i.e. sagittate accessions. Accessions Coy5 and Coy28 clustered separately from the rest of the accessions using morphological characters while accession Coy63 clustered separately using AFLP and combined data analysis. Breeders and conservationists interested in maintaining high levels of genetic diversity should consider using Coy16 (representative of peltate leaf base shaped accessions) and Coy5, Coy28 and Coy63 (representative of sagittate leaf base shaped accessions) in their breeding and conservation efforts.

Table 5.1 Pair-wise genetic similarity coefficient matrix for 21 cocoyam genotypes calculated using combined morphological and AFLP data

	Coy3	Coy5	Coy6	Coy9A	Coy13	Coy14	Coy16	Coy20	Coy22	Coy23	Coy28	Coy42	Coy49	Coy51	Coy55	Coy56	Coy57	Coy60
Coy3	1.000																	
Coy5	0.299	1.000																
Coy6	0.811	0.400	1.000															
Coy9A	0.872	0.273	0.817	1.000														
Coy13	0.319	0.858	0.467	0.323	1.000													
Coy14	0.353	0.845	0.487	0.307	0.908	1.000												
Coy16	0.722	0.337	0.678	0.748	0.373	0.382	1.000											
Coy20	0.860	0.376	0.799	0.844	0.430	0.424	0.765	1.000										
Coy22	0.312	0.835	0.518	0.325	0.910	0.884	0.414	0.401	1.000									
Coy23	0.806	0.384	0.770	0.830	0.379	0.412	0.768	0.860	0.359	1.000								
Coy28	0.293	0.849	0.420	0.305	0.881	0.855	0.350	0.410	0.854	0.373	1.000							
Coy42	0.752	0.345	0.833	0.778	0.371	0.363	0.669	0.754	0.374	0.769	0.354	1.000						
Coy49	0.393	0.804	0.510	0.369	0.867	0.859	0.455	0.481	0.838	0.444	0.826	0.453	1.000					
Coy51	0.320	0.825	0.478	0.341	0.857	0.838	0.396	0.429	0.850	0.403	0.830	0.408	0.894	1.000				
Coy55	0.389	0.780	0.456	0.403	0.840	0.800	0.399	0.467	0.835	0.386	0.804	0.363	0.829	0.864	1.000			
Coy56	0.328	0.843	0.450	0.327	0.856	0.838	0.393	0.419	0.862	0.360	0.818	0.355	0.894	0.919	0.901	1.000		
Coy57	0.358	0.804	0.516	0.411	0.818	0.800	0.376	0.447	0.830	0.404	0.804	0.464	0.865	0.910	0.314	0.981	1.000	
Coy60	0.347	0.804	0.488	0.380	0.817	0.789	0.389	0.441	0.784	0.436	0.809	0.434	0.871	0.889	0.823	0.868	0.876	1.000
Coy62	0.371	0.824	0.496	0.399	0.840	0.806	0.426	0.485	0.800	0.447	0.838	0.444	0.903	0.910	0.848	0.909	0.875	0.933
Coy63	0.643	0.502	0.790	0.687	0.536	0.519	0.647	0.651	0.596	0.588	0.497	0.670	0.579	0.570	0.547	0.566	0.598	0.555
Coy70	0.387	0.844	0.489	0.372	0.858	0.817	0.422	0.472	0.841	0.420	0.794	0.403	0.859	0.877	0.820	0.872	0.884	00.834

Table 5.1 (Continued)

	Coy62	Coy63	Coy70
Coy62	1.000		
Coy63	0.581	1.000	
Coy70	0.868	0.566	1.000

r = 96636

5.3.2 Principal component analysis (PCA) for cocoyam accessions from Malawi

The PCA is one of the ordination methods that are used to visualise genetic relationships within populations. According to Laurentin (2009) PCA does not group individuals according to a hierarchical structure but rather represents the relationship based on presence or absence of markers, in a low-dimension space. With this relationship, ordination of the individuals according to similarity/dissimilarity may be easily visualised. The PCA was computed to visualise the relationship among the cocoyam accessions analysed using morphological characters (Figure 5.2A), AFLP analysis (Figure 5.2B) and a combination of both (Figure 5.2C).

Results from PCA analysis using morphological trait data showed two distinct groups based on the leaf base shapes i.e. peltate and sagittate shapes (Figure 5.2A). All sagittate leaf base shaped accessions were on the left side (in blue) and peltate (in red) on the right side of the PCA bi-plot. Results further suggested that peltate leaf base shaped leaves were not as closely related as the sagittate leaf base shaped group. Two distinct groups were visible within the peltate leaf base shaped accessions, with Coy9A, Coy20 and Coy23 forming a separate group from Coy3, Coy16, Coy53 and Coy55.

Figure 5.2B shows the relationship of the accessions in a PCA bi-plot based on AFLP analysis. Results suggested a closer relationship between accessions within the two distinct groups. Accessions with sagittate leaf base shapes appear to be more closely related as opposed to those with peltate shapes. Accession Coy55, which had a peltate leaf base shape, clustered together with sagittate leaf base shaped accessions while Coy6 and Coy42 (sagittate) grouped together with the peltate leaf base shaped accessions. Accession Coy63 clustered separately from both the peltate and sagittate leaf base shaped accessions. Accessions in black were not morphologically characterised, hence their leaf base shapes are unknown.

Figure 5.2C represents the relationship of the cocoyam accessions based on a combination of morphological characters and AFLP analysis.

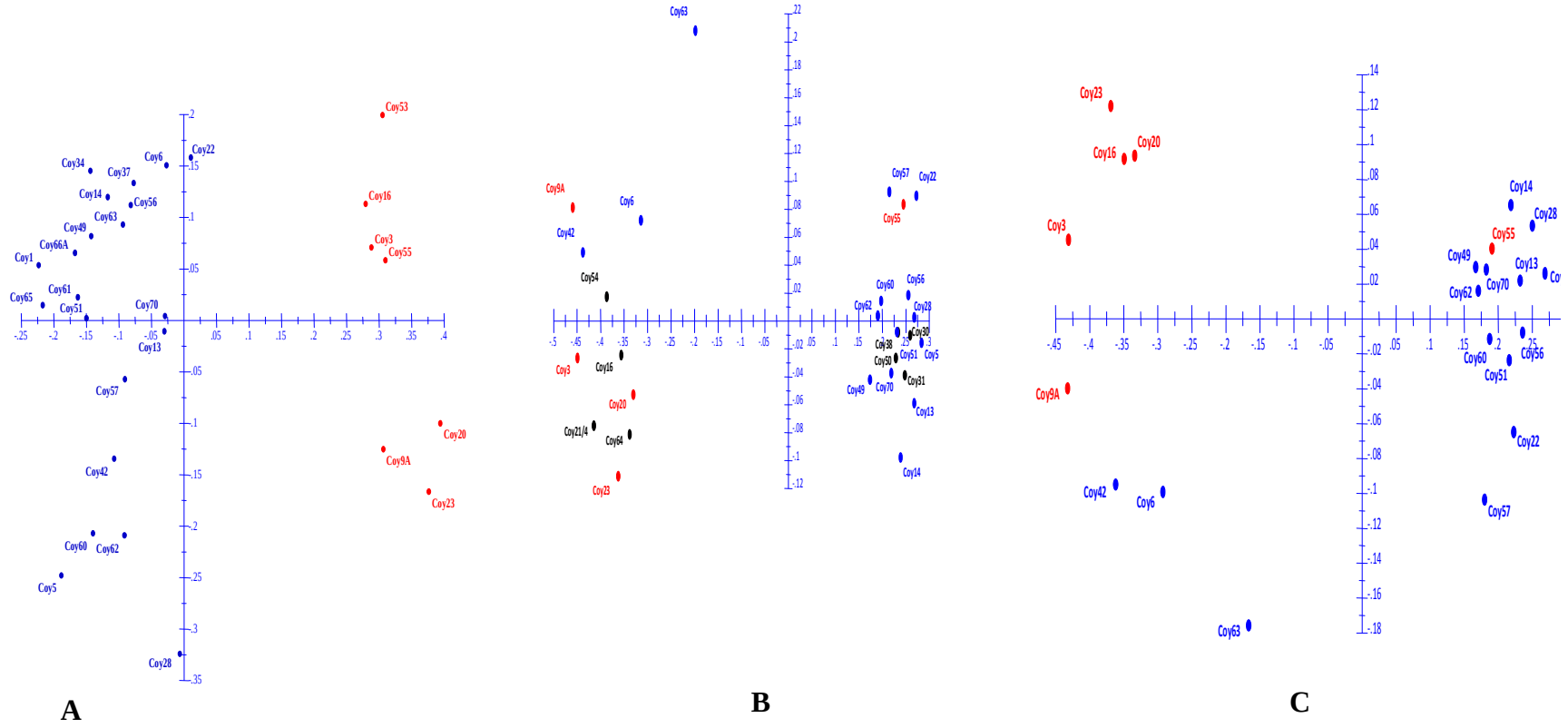

Figure 5.2 PCA of the 21 cocoyam accessions from Malawi (A) based on morphological traits analysis (B) based on AFLP analysis and (C) based on combination of morphological trait and AFLP analysis. Red represents accessions with peltate base shaped leaves, blue accessions with sagittate base shaped leaves while the leaf base shape of accessions in black was unknown.

Results suggested a closer relationship within the sagittate base shaped accessions than within the peltate leaf based accessions. The two groups of accessions were distinct from each other. As for AFLP (Figure 5.2B), accession Coy55 clustered among the sagittate leaf shaped accessions, despite being characterised as peltate, while three accessions characterised as sagittate (Coy6, Coy42 and Coy63) grouped closer to the peltate group than the sagittate group. The combined PCA also suggested bigger differentiation between the two populations, representing the *Colocasia* and *Xanthosoma* species.

The three PCA biplots showed similar relationships among and within the cocoyam accessions and similarity to the hierarchical clustering of the data. In all PCAs there was a clear distinction among the two morphologically differentiated populations, *Colocasia* and *Xanthosoma* species based on peltate and sagittate leaf base shapes. The PCAs suggested low levels of variation among the sagittate accessions. The PCA based on AFLP analysis was similar to PCA based on combined analysis, both showed two distinct groups. In both PCAs accession Coy63 clustered separately from the rest of the peltate accessions.

5.3.3 Analysis of molecular variance (AMOVA) among and within cocoyam accessions from Malawi

The total variance among the cocoyam accessions was partitioned into variance among populations and within populations. Populations for AMOVA analysis using morphological data were defined on the basis of morphological classification into *Colocasia* and *Xanthosoma* species i.e. peltate and sagittate leaf base shapes. Since not all accessions used for AFLP analysis were characterised using morphological characters, populations for AMOVA analysis based on AFLP and combined data could not be defined based on leaf shapes. However, the partioning of accessions into main groups during AFLP and combined cluster analysis were used to define two main groups for AMOVA. The significance of the partitioning of the genetic variance components was tested using 16000 permutations.

Results in Table 5.2 suggests highly significant differences among and within populations based on morphological characters ($P<0.000$). Results showed that most (64.3%) of the variation was within the population i.e. within the peltate and sagittate population groups

compared to among populations (35.7%). The F_{ST} value shows the degree of genetic variation among the population and is relatively high, at 0.35725.

Table 5.2 Analysis of molecular variance among and within cocoyam accessions from Malawi based on morphological data

Source of variation	Sum of squares	Variance components	Percentage variation
Among cocoyam populations	69.230	5.70370	35.72529 (P<0.000)
Within cocoyam populations	244.846	10.26173	64.27471 (P<0.000)
Total	314.075	15.96543	

Table 5.3 shows the AMOVA results for the accessions based on AFLP data (genetic data). Results indicated that the variance components were highly significant (P<0.000) among and within the cocoyam populations (Table 5.3). Results further suggested that the highest differentiation was among (79.2%) populations compared to within populations (20.8%). The F_{ST} value of 0.79209 is high, suggesting high levels of variation among the two populations i.e. group I (probably peltate) and group II (probably sagittate) of the AFLP dendrogram (Figure 4.2). Results indicated that almost 80% of the variation revealed by AFLP analysis could be attributed to the two main clusters (probably peltate and sagittate) and that AFLP analysis revealed low levels of variation between accessions of the same group (probably low levels of variation within each of the two species, *Colocasia* and *Xanthosoma*, but high levels of variation between the two species).

Table 5.3 Analysis of molecular variance among and within cocoyam accessions from Malawi based on AFLP data

Source of variation	Sum of squares	Variance components	Percentage variation
Among cocoyam populations	828.333	64.73673	79.20962 (P<0.000)
Within cocoyam populations	415.980	16.99164	20.79038 (P<0.000)
Total	1244.314	81.72836	

AMOVA results using combined morphological and AFLP data indicated highly significant differences among and within the populations (P<0.000). The analysis showed that 67.4% of the variation lied among the two population groups and 32.6% within the population groups (group I and group II, Figure 5.1). The F_{ST} value of 0.67395 is high, further suggesting high differentiation among the two population groups i.e. probably the two species, *Colocasia* and *Xanthosoma* species. The variation among populations was lower compared to using only AFLP data, but higher when using morphological data.

Table 5.4 Analysis of molecular variance among and within cocoyam accessions from Malawi based on combined morphological and AFLP data

Source of variation	Sum of squares	Variance components	Percentage variation
Among cocoyam populations	624.802	63.19634	67.39526 (P<0.000)
Within cocoyam populations	539.517	30.57337	32.60474 (P<0.000)
Total	1164.320	93.76971	

5.3.4 Analysis of genetic diversity among Malawian cocoyam accessions

The major output after characterisation of plant genetic resources is quantification of the genetic diversity and to know the genetic relationship within and/or among groups of the accessions (Laurentin, 2009). In the present study, cluster analysis and ordination methods have been used to visualise and quantify the intra- and inter-group relationships of the cocoyam accessions from Malawi. Two clear population groups were identified by all three dendrograms (morphological, AFLP and combined analysis). To display the relationships among the cocoyam accessions in terms of their position relative to coordinate axes, principal component analyses were performed. The PCA bi-plots clearly separated the morphologically similar accessions into two groups i.e. *Colocasia* (peltate) and *Xanthosoma* (sagittate). In agreement to what Lefebvre *et al*. (2001) found, grouping of accessions in the PCA bi-plots were similar to the clustering of the cocoyam accessions in the dendrograms. Within similar groups low levels of variation were observed. As suggested by Laurentin (2009) the PCA bi-plots gave a better visualisation of the cocoyam accessions' relationship

than the UPGMA clustering. Relationships of accessions (Coy5, Coy28 and Coy63) which appeared to be dissimilar to other accessions in their clusters in dendrograms could be crearly observed.

Results of the AMOVA further alluded to the presence of the two species within the germplasm collection as differentiation among the population groups observed was high. The three AMOVAs confirmed the presence of the two major species of cocoyam in the collection as was observed with the UPGMA clustering and the PCA bi-plots of the accessions. However, all three AMOVAs indicated low levels of variation within the two population groups i.e. *Colocasia* and *Xanthosoma* species. The high F_{ST} values observed among the two distinct populations further supported the variation of the accessions based on species. The AMOVA demonstrated how efficient the two methods used were in identifying the two species. In a different study by Stedje and Bukenya-Ziraba (2003) an AMOVA analysis showed that variation among species and groups was less than 10%, whereas the variation within species and groups was more than 90%. This was attributed to the fact that the groups which were morphologically distinct were only sub-species of Solanaceae family (*Solanum anguivi* Lam and *S. aethiopicum* L.) and could not be distinctively separated using RAPD as was the case with two separate species dealt with in the present study.

The present study corroborated the results reported by Lebot *et al*. (2004) who detected low variation within cocoyam *taro* (*C. esculenta*) germplasm of south east Asia and Oceania using both morphological and AFLP analysis. In a similar study Quero-Garcia *et al.* (2004) also found low genetic variation within cocoyam species. However, the AFLP fingerprints used did not identify any duplicates in the sample.

Assessment of genetic variability between accessions is of interest in practical applications such as conservation of genetic resources and broadening the genetic basis of genotypes for breeding purposes. In plant genetic resource conservation, it is useful to know whether individuals that are phenotypically similar display similar gene combinations. For breeding purposes, information on genetic distances between different genotypes helps to predict their ability to combine (Charcosset *et al*., 1998; Lefebvre *et al*., 2001). The low levels of variation within the two species observed morphologically and validated by AFLP analysis pose a

challenge in selection of parents for making improvements to the germplasm. This is similar to what Lebot and Arhadya (1991) observed that one of the major challenges in cocoyam breeding is the narrow genetic base of the crop such that it is necessary to further explore the available genetic diversity within the species. Combination of morphological characters and molecular analysis has indicated that accessions with contrasting morphological characteristics usually should not cluster together. However, in rare cases they could cluster together, which according to Lebot (1992), is a clear manifestation of the clonally propagated nature of cocoyam *taro* and "sport" type mutations which do not have any underlying genetic basis. In such instances Lebot (1992) suggested that the level of similarity based on molecular analyses is considered a better indicator of genetic similarity than morphological characterisation. Similarly the accession Coy55, characterised morphologically as *Colocasia* species but grouping with *Xanthosoma* species using AFLP analysis, could be regarded as the latter. This however, needs further investigation both on morphological and genetic level.

5.4 Conclusions and recommendations

Genetic diversity of cocoyam accessions from Malawi has revealed that the germplasm consists of two species of the Araceae family i.e. *Colocasia* and *Xanthosoma*. The morphological, AFLP and combined analyses suggested the presence of two different species within the studied germplasm. The study has furthermore revealed low levels of genetic variation within the different cocoyam species. The study has shown the efficiency of combining morphological characters and molecular analysis in genetic diversity assessment studies. Results based on AFLP analysis were more similar to the combined analysis than morphological traits alone. This showed the efficiency of the AFLP technique in diversity analysis of cocoyam. There was consistency in the relationship of the cocoyam accessions using the different cluster and ordination methods as well as the AMOVA. The dendrograms, PCA bi-plots and AMOVA showed high levels of variation between the two species and low levels within the species. The data analysis also revealed that the genetic diversity of cocoyam accessions in Malawi was related to the different regions of collection. Accessions from the southern regions separated from the ones from the northern region. This study, though informative, only covered a subset of the cocoyam germplasm in Malawi due to time limitation, but acted as an initial investigation into cocoyam germplasm conservation and

breeding. A broader spectrum investigation into the genetic diversity of the crop is therefore recommended to cover all regions.

5.5 References

Benesi, I. (2005). Characterisation of Malawian cassava germplasm for diversity, starch extraction and its native and modified properties. PhD thesis in Plant Breeding, University of the Free State, South Africa. pp 199.

Biodiversity International, Rural Development Administration. (2009). A training module for the international course on plant genetic resources and genebank management. Suwon, Republic of Korea (2009/09/7-18). Biodiversity International, Rural Development Administration, pp. 27-28

Brown, A. (1978). Isozymes, plant population genetic structure and genetic conservation. Theoretical and Applied Genetics 52: 145-157.

Charcosset, A., Bonnseau, B., Touchebeuf, O., Burstin, J., Dubreuil, P., Barriere, Y., Gallis, A., and Denis, J.B. (1998). Prediction of maize hybrid silage performance using marker data: comparison of several models for specific combining ability. Crop Sciences 38: 38-44.

Danquah, A., Offei, S.K., Blay, E.T., Asare, E., and Danquah, E.Y. (2006). Characterisation of mutant population of cocoyam (*Xanthosoma sagitifolium* L. Schott). International Journal of Botany 2: 128-132.

Dellaporta, S., Wood, J., and Hicks, J.R. (1983). A plant DNA minipreparation: version II. Plant Molecular Biology Report 1: 19-21.

Dice, L. (1945). Measures of amount of ecologic association between species. Ecology 26: 297-302.

Donini, P., Law, J., Koebner, R., Reeves, J., and Cooke, R. (2000). Temporal trends in the diversity of UK wheat. Theoretical and Applied Genetics 100: 912-917.

Drossou, A., Katsiosis, A., Leggert, J., Loukas, M., and Tsakas, S. (2004). Genome and species relationships in genus *Avena* based on RAPD and AFLP molecular markers. Theoretical and Applied Genetics 109: 48-54.

Excoffier, L., Smouse, P., and Quattro, J. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131: 179-191.

Excoffier, L., Laval, G., and Schneider, S. (2005). Arlequin (version 3.11): An integrated software package for population genetics data analysis. Evolutionary Bioinformatics 1: 47-50.

Ferguson, R. (2007). The need for characterisation and evaluation of germplasm: Kiwi fruit as an example. Euphytica 154: 371-382.

Gower, J. (1966). Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika 53: 325-338.

Herselman, L. (2003). Genetic variation among Southern African cultivated peanut (*Arachis hypogea* L.) genotypes as revealed by AFLP analysis. Euphytica 133: 319-327.

Karp, A., Serberg, O., and Buiatti, M. (1996). Molecular techniques in the assessment of botanical diversity. Annals of Botany 78: 143-149.

Kosman, E., and Leonardo, K. (2005). Similarity coefficients for molecular markers in studies of genetic relationships between individuals for haploid, diploid and polyploid species. Molecular Ecology 14: 415-424.

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

Laurentin, H. (2009). Data analysis for molecular characterisation of plant genetic resources. Genetic Resources and Crop Evolution 56: 277-292.

Lebot, V. (1992). Genetic vulnerability of Oceania's traditional crops. Experimental Agriculture 29: 309-323.

Lebot, V., and Aradhya, K.M. (1991). Isozyme variation in *taro* (*Colocasia esculenta* (L) Schott) from Asia and Oceania. Euphytica 56: 55-66.

Lebot, V., Prana, M.S., Kreike, N., Van Eck, H., Pardales, J., Okpul, T., Gendua, T., Thongjiem, M., Hue, H., Viet, N., and Yap, T.C. (2004). Characterisation of *taro* (*Colocasia esculenta* (L.) Schott) genetic resources in Southeast Asia and Oceania. Genetic Resources and Crop Evolution 51: 381-192.

Lefebvre, V., Goffinet, B., Chauvet, J., Caromel, B., Signoret, P., Brand, R., and Palliox, A. (2001). Evaluation of genetic distances between pepper inbred lines for cultivar protection purposes: comparison of AFLP, RAPD and phenotypic data. Theoretical and Applied Genetics 102: 741-750.

Mace, E., and Godwin, I. (2002). Development and characterisation of polymorphic microsatellite markers in *taro* (*Colocasia esculenta*). Genome 45: 823-832.

Manzano, A., Nodals, A., Gutierrez, M., Mayor, F., and Alfonso, C. (2001). Morphological and isozyme variability of *taro* (*Colocasia esculenta* (L.) Schott) germplasm in Cuba. Plant Genetic Resources Newsletter 126: 31-40.

Mathews, P. (2004). Genetic diversity in *taro* and the preservation of culinary knowledge. Ethnobotany Research and Applications 2: 55-71.

Mbouobda, H.D., Boudjeko, T., Djocgoue, P.F., Tsafack, T.J.J., and Omokolo, D.N. (2007). Morphological characterization and agronomical evaluation of cocoyam (*Xanthosoma sagittifolium* (L.) Schott) germplasm in Cameroon. Journal of Biological Sciences 7: 27-33.

Mohammadi, S.A., and Prasanna, B.M. (2003). Analysis of genetic diversity in crop plants-salient statistical tools and considerations. Crop Science, Review and Interpretation 43: 1235-1249.

Murakami, K., Takashi, K., and Ogawa, K. (2006). Morphological variation of corms in plants regenerated from calluses of *taro* (*Colocasia esculenta* Schott). Proceedings of the Vth International Symposium on In Vitro Culture and Horticuluture Breeding. Fári, M.G., Holb, I., and Bisztray, Gy.D. (Eds.). Acta Horticlturae. 725, ISHS, pp. 401-405.

Nei, M., and Li, W. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences USA 76: 5269-5273.

Okpul, T., Mace, E., Godwin, I., Singh, D., and Wagih, M. (2005). Evaluation of variability among breeding lines and cultivars of *taro* (*Colocasia esculenta*) in Papua New Guinea using ISSR fingerprinting and agro-morphological characterisation. Plant Genetic Resources Newsletter 143: 8-16.

Onwueme, I. (1978). Tropical tuber crops*:* Yams, cassava, sweet potato and cocoyams. John Wiley and Sons, pp. 199-225.

Perrier, X., and Jacquemond-Collet, J. (2006). DARwin software. http://darwin.cirad.fr/darwin.

Perrier, X., Flori, A., and Bonnot, F. (2003). Data analysis methods. In: Hamon, P., Perrier, X., and Graszmann, J. (Eds.). Genetic diversity of cultivated tropical plants. Montipellier: Enfielol, Science Publishers, pp. 76.

Powell, W., Orozco-Castillo, C., Chalmers, K., Provan, J., and Waugh, R. (1995). Polymerase chain reaction-based assays for the characterisation of plant genetic resources. Electrophoresis 16: 1726-1730.

Purseglove, J. (1972). Tropical crops: Monocotyledons 1. London: Longman, pp. 66-74.

Quero-Garcia, J., Noyer, J., Perrier, X., Marchand, J.L., and Lebot, V. (2004). A germplasm stratification of *taro* (*Colocasia esculenta*) based on agro-morphological descriptors, validation by AFLP markers. Euphytica 137: 387-395.

Rao, K.N. (2004). Plant genetic resources: Advancing conservation and use through biotechnology. African Journal of Biotechnology 3: 136-145.

Rohlf, F. (2000). NTSYS-pc: Numerical taxonomy and multivariate analysis system. Applied Biostatistics. New York: Exerter Publishing Ltd.

Singh, D., Mace, E., Godwin, I., Mathur, P., Okpul, T., Taylor, M., Hunter, D., Kambuou, R., Ramanatha Rao, V., and Jackson, G. (2008). Assessment and rationalization of genetic diversity of Papua New Guinea *taro* (*Colocasia esculenta*) using SSR DNA fingerprinting. Genetic Resources and Crop Evolution 55: 811-822.

Soller, M., and Beckmann, J. (1983). Genetic polymorphism in varietal identification and genetic improvement. Theoretical and Applied Genetics 67: 27-33.

Stedje, B., and Bukenya-Ziraba, R. (2003). RAPD variation in *Solanum anguivi Lam.* and *S. aethiopicum* L. (Solanaceae) in Uganda. Euphytica 131: 293-297.

Tambong, T.J., Poppe, J., and Hofte, M. (1999). Pathogenicity, electrophoretic characterization and in *planta* detection of the cocoyam root rot disease pathogen, *Pythium myriotylum*. European Journal of Plant Pathology 105: 597-607.

Vos P., Hogers R., Bleeker, M., Reijans, M., Van de Lee, T., Hormes M., Friters A., Pot J., Paleman, J., Kuiper, M., and Zabeau, M. (1995). AFLP: A new technique for DNA fingerprinting. Nucleic Acids Research 23: 4407-4414.

CHAPTER 6

Mineral composition of Malawian cocoyam genotypes

6.1 Introduction

Cocoyam contributes significantly to the human diet in parts of the Pacific region, Latin America, Africa and Asia (FAO, 2001). It is ranked the fifth most consumed root and tuber crop in the world after potato, cassava, sweet potato and yam (FAOSTAT, 2005). Its starchy corms and cormels are used as a subsistence staple as they provide a cheap source of carbohydrates in many parts of Africa, south east Asia, the Pacific islands, Hawaii, Philippines, West Indies and parts of South America. Cocoyam serves as a source of income for many families in the tropics and subtropics (Tambong *et al*., 1997; Sajeev *et al*., 2004). Young leaves, petioles and stems of cocoyam are used as a leafy vegetable (Okonkwo, 1993; Janseens, 2001). In addition to its importance in the diet, cocoyam is closely integrated into the social and cultural life of most of the cultivating communities (Caillon *et al*., 2004).

The main nutrient provided by cocoyam as with many other root and tuber crops is the dietary energy supplied by carbohydrates (O'Hair, 1990). According to Onwueme (1978) cocoyam tubers contain an average of 20-25% carbohydrates (fresh weight), mostly starch which consists predominantly of amylopectin as well as 17-18% amylose. The starch granules of cocoyam are small (4-7 µm) and hydrolyses easily comparative to other root and tuber crops (Onwueme, 1978; Mweta *et al*., 2008) and as such it is readily digestible (Onwueme, 1978; Okonkwo, 1993). For this reason, cocoyam starch is utilised in the preparation of speciality foods for individuals that require carbohydrate as a source of energy that will not stress metabolic processes i.e. peptic ulcer patients, patients with pancreatic disease, chronic liver problems and inflammatory bowel as well as gall bladder disease and infant meals (Sefa-Dedeh and Kofir-Agyir, 2004; Emmanuel-Ikpeme *et al*., 2007). The protein fraction of cocoyam tubers is low (1-3%) and like most root and tuber crop proteins, sulphur containing-amino acids are limiting. The proteins in cocoyam are limited to histidine, lysine, isoleucine, tryptophan and methionine (FAO, 1990; Onwueme, 1999).

The corms and cormels of *X. sagittifolium* are superior to *C. esculenta* in terms of energy and proteins (Bradbury and Holloway, 1988), but less digestible due to slightly bigger starch granules (Janseens, 2001). Sefa-Dedeh and Kofir-Agyir (2004) observed variations in chemical composition of the different sections of cocoyam cormels (distal, middle and apical). The apical section contained high protein content and starch was the highest at the middle section while the distal section contained high levels of ash, fibre and minerals, disagreeing with what Onwueme (1978) suggested that the variation is from the distal section to the growing apex (geotropic).

Cocoyam corms and cormels are good sources of the essential mineral nutrients that contribute to growth as well as health maintenance and general well being (South Pacific Community (SPC), 1993). The major mineral nutrient in cocoyam is K (FAO, 1990) and it is also rich in Fe, Zn and Ca which are essential for building blood and bones (Bradbury and Holloway, 1988; Englberger *et al*., 2008). In their study, Wills *et al*. (1983) observed variable mineral nutrient levels between different cultivars of cocoyam in Papua New Guinea. The cormels contained K (250-480 mg/100 g), Mg (19-37 mg/100 g), Ca (11-45 mg/100 g), Zn (0.2-6.3 mg/100 g), Fe (0.6-1.8 mg/100 g) and Na (0-3 mg /100 g). In a different study Agbor-Egbe and Rickard (1990) observed variation in mineral composition of the two genera of cocoyam, with *C. esculenta* corms showing higher mineral levels than *X. sagittifolium*. *Xanthosoma sagittifolium* corms contained, on fresh weight basis, Ca (0.5-4.0 g/kg), P (2.2-4.7 g/kg), K (15.1-39.1 g/kg), Mg (1.0-2.1 g/kg), Fe (12.9-26.9 mg/kg), Na (38.2-147.7 mg/kg), Mn (7.2-11.3 mg/kg), Zn (13.4-16.2 mg/kg), and Cu (8.3-13.9). On the other hand *C. esculenta* var *antiquorum* corms contained Ca (0.2-8.0 g/kg), P (2.0-3.7 g/kg), K (13.3-21.2 g/kg), Mg (0.8-1.3 g/kg), Fe (13.4-18.5 mg/kg), Na (53.4-173.7 mg/kg), Mn (9.7-14.8 mg/kg), Zn (14.8-19.2 mg/kg) and Cu (2.9-17.6 mg/kg). Njoku and Ohia (2007) suggested that cocoyam is a good source of Na, K, Mg and Ca whose salts regulate the acid-base balance of the body. Wide variations observed in the mineral composition values between sections of the cormels as well as among different cultivars of cocoyam have been attributed to differences in genetic background as well as climate, soil, season and agronomic factors (FAO, 1990).

Studies by Englberger *et al*. (2003; 2008) suggested that cocoyam tubers contained good levels of provitamin A carotenoids. A wide range of provitamin A carotenoid levels were found in cocoyam cultivars (especially in the giant swamp *taro*) and coloured cultivars showed high levels of α- and β-carotene and essential minerals like Zn and Ca.

The tender leaves, petioles as well as the stems of cocoyam which are eaten as a leafy vegetable (Okonkwo, 1993; Janseens, 2001), are reported to be a good source of proteins, vitamin A, C, B_1 (riboflavin) and B_2 (thiamin) (Aregheore and Perera, 2003) as well as essential dietary mineral nutrients especially K, Ca, P, Mg, Zn and Fe (Ejoh *et al*., 1996; Barminas *et al*., 1998). According to Janseens (2001) cocoyam leaves contain 20% proteins (on dry matter basis) and all essential amino acids except for methionine and cystine as is the case with most leafy vegetables (Gerloff *et al*., 1965; Ejoh *et al*., 1996). In a separate study, Thomas and Oyediran (2008) found that cocoyam leaves were rich in β-carotene, ascorbic acid and micronutrients of nutritional importance because of their anti-oxidant properties, but low in Na, carbohydrates and energy content. In a more recent study in South Africa, Lewu *et al*. (2009) found high levels of crude protein in cocoyam leaves. The study also suggested that cooking improved the availability of protein, fibre as well as lipid contents of the cocoyam leaves. This was attributed to the breakdown in tannin during cooking which form complexes with proteins, thereby, inhibiting its availability. This suggested that cocoyam leaves may be a good source of plant protein for marginal resource communities and a good compliment to its tubers which are low in proteins (Lewu *et al*., 2009).

The effective use of cocoyam is, however, hampered with the acrid factors found in tubers (skin or peels and flesh) (Catherwood *et al*., 2007) and leaves (Oscarsson and Savage, 2007), which causes a sharp irritation and burning of the throat and mouth when uncooked material is ingested (Emmanuel-Ikpeme *et al*., 2007) and this is caused by the presence of calcium oxalate raphides (Janseens, 2001). Traditional processing methods of anaerobic fermentation in underground pits for several weeks, removal of the thick layer of skin of the corms and cormels, drying, soaking in water, prolonged cooking or baking and ethanol extraction have been reported to reduce the acridity factor significantly (Onwueme, 1978; Sefa-Dedeh and Kofir-Agyir, 2004; Catherwood *et al*., 2007).

The importance of taking adequate essential micro-nutrients has been well documented (FAO/WHO, 2000; Graham *et al*., 2001; Welch, 2002). In countries like Malawi, where most people's diets are low in animal proteins, i.e. with a meat consumption per capita of 5.1 against 13.0 kg/person/year of sub-Saharan Africa (SSA) (FAOSTAT, 2004), the risk of micro-nutrient deficiency is high. Cocoyam nutritional composition studies suggested that it contains a range of important macro- and micro-nutrients (Agbor-Egbe and Rickard, 1990; Sen *et al*., 2006; Njoku and Ohia, 2007; Englberger *et al*., 2008). However, unlike the minerals, the protein-calorie contribution of the corms and cormels of cocoyam have been well documented (National Academy of Science, 1975; Wills *et al.*, 1983; Aregheore and Perera, 2003; Sen *et al*., 2006).

According to Burlingame *et al*. (2009) recent research has provided data to confirm the micro-nutrient superiority of some lesser known crops like cocoyam and their wild varieties over other more extensively utilised crops. However, the existence of many cocoyam genotypes and/or cultivars with distinct botanical characteristics suggests the presence of variation in nutritional composition due to differences in habitat, growth conditions and genetic background. Therefore, in order to develop criteria to improve these cultivars through selection or breeding, nutritional composition of corms and cormels need to be determined and compared together with yields traits (Sen *et al*., 2006). Data on nutritional composition would help to re-assess the value of neglected varieties and encourage their sustainable use as well as coming up with a detailed database of micro-nutrient rich plant species that would help in planning nutritional intervention programmes as well as save the loss of micronutrient rich plant species and their edible wild relatives (Grivetti and Ogle, 2000). Burlingame *et al*. (2009) further suggested that nutritional composition data are expected to have a major impact on micro-nutrient intake estimations, which become complex due to micro-nutrient composition variation that exists in many cultivars.

This study assessed Malawi cocoyam germplasm with respect to micro-nutrient content in order to identify germplasm that could be used to develop lines that would help combat micro-nutrient deficiencies among the marginal resource farmers. Specifically the study assessed (a) the mineral levels (K, P, Ca, Mg, Mn, Na, Fe and Zn) and (b) their variation among the different cocoyam accessions from the Malawian cocoyam germplasm.

6.2 Materials and methods

6.2.1 Trial site

The trial was planted at Chitedze Research Station. Soil samples were collected from trial sites and analysed for pH, organic matter, organic carbon and available minerals at the Agricultural Research and Extension Trust (ARET) soil laboratory. Samples were taken from top- and sub-soil.

6.2.2 Collection and preparation of test samples

Cocoyam samples used were collected from the germplasm bank (collected for genetic diversity studies) at Chitedze Research Station, Lilongwe Malawi. Forty-five samples were collected representing 15 accessions each replicated three times and were sampled based on maturity of the accessions (corms). The corms were thoroughly washed with water and the outer skins peeled off using a kitchen knife. The fleshy part of the cormels were grated, air-dried for 72 hours and ground manually into a fine powder using a laboratory metallic motor. The powder of each sample was stored in transparent air-tight plastic bottles as stock samples until required for analyses.

6.2.3 Preparation of sample solutions and reading of the minerals

Exactly 2 g of each of the cocoyam samples was weighed in pre-heated and cooled crucibles and ashed in a furnace at 550°C for three hours. Samples were removed from the furnace and allowed to cool. Samples were digested with 1-2 ml of HNO (55%) and left to evaporate in a hot sand-bath. Samples were further ashed at 550°C for 30 minutes. Upon cooling, the ashed samples were digested again with 10 ml of 1:2 HNO and desiccated for a few minutes. About 50 ml of distilled water was used to rinse the digested samples into 100 ml flasks and the flasks were filled up to the marks with distilled water and read (Fe, Zn, Mn, Na, K, Ca and Mg) on an atomic absorption spectrophotometer. Phosphate was determined by adding the ammonium vanadate (NH_4VO_3) colour reagent and read on a thermo-spectromic meter (Hesse, 1971).

6.2.4 Data analysis

Mineral composition data for the cocoyam accessions were subjected to ANOVA using Agrobase (2000). Correlation coefficients among the different cocoyam minerals were estimated using the Pearson`s product moment method of correlation analysis. In order to ascertain the accession with the best minerals attributes, PCA, a data reduction technique, was performed. The goal of this analysis was to construct linear combinations of the original variables (Fe, Zn, Mn, Na, Ca, K, Mg and P) that accounted for as much of the total variation as possible. In order to reduce the influence of outliers and scale differences during PCA, data were standardised as follows: The mean observation for each genotype was standardised by subtracting the mean value of the variable and subsequently dividing with its respective standard deviation (Bekele, 2005; Sen *et al*., 2006). This results in standardised values for each variable with an average of zero and standard deviation of one or less. These standardised values were used to perform PCA. During PCA, the 15 cocoyam accessions were represented by rows and the eight mineral nutrients by columns. A scatter plot of factor scores was drawn with PC1 as the X-axis and PC2 as the Y-axis to observe the relative position of the cocoyam accessions as a result of component loadings of those characters. PCA, correlation analyses and the scatter plot were performed using the Number Cruncher Statistical System, NCSS 2000 (Hintze, 1998).

6.3 Results and discussion

There were highly significant ($p \leq 0.001$) differences among the Malawian cocoyam accessions studied with regard to mineral composition (Table 6.1). The average micro-nutrient composition for the accessions were 50.93 mg/kg, 21.76 mg/kg, 13.49 mg/kg, 192.0 mg/kg, 207.42 mg/kg, 15078.62 mg/kg, 725.04 mg/kg and 1204.9 respectively for Fe, Zn, Mn, Na, Ca, K, Mg and P. Results suggested that K is the major mineral present in the cocoyam accessions studied followed by P, Mg and Ca, corroborating earlier studies (Enomfon and Umoh, 2004; Njoku and Ohia, 2007). The results further suggested higher levels of the trace elements (Fe, Zn and Mn) in the cocoyam accessions than previously reported in other studies (Wills *et al*., 1983; Njoku and Ohia, 2007).

Table 6.1 Mineral composition (mg per kg) of cocoyam accessions from Malawi

Accession	Fe	Zn	Mn	Na	Ca	K	Mg	P
Coy10	43.72	16.17	5.67	170.2	81.0	11900	662	1206.0
Coy5	50.28	17.33	6.17	272.5	76.7	14241	650	1156.3
Coy27	42.67	17.50	6.00	330.0	70.9	21877	715	1358.7
Coy42	68.88	19.17	6.33	343.9	85.5	8523	605	1146.2
Coy34A	153.57	39.33	31.00	30.5	206.8	7633	760	1234.2
Coy24	45.95	17.17	6.33	230.0	136.1	14917	553	1129.2
Coy39	35.77	16.83	7.00	322.0	128.3	10717	595	1144.7
Coy34B	53.11	18.00	11.33	59.5	143.4	22506	635	1169.0
Coy53	45.94	28.67	42.50	22.8	588.3	8367	1283	868.5
Coy33	40.82	20.83	7.59	113.6	358.0	18517	730	1240.5
Coy14	30.79	12.17	5.17	275.7	131.5	15106	626	1117.5
Coy23	41.87	37.51	42.55	29.0	638.1	13293	1316	1256.0
Coy12	39.33	22.84	8.25	260.3	133.9	16223	553	1401.3
Coy45	37.16	25.46	6.25	150.3	198.8	27389	659	1297.2
Coy75	34.06	17.47	10.25	270.3	133.9	14973	534	1348.7
Mean	50.9	21.8	13.5	192.0	207.4	15078.6	725.0	1204.9
CV%	12.33	11.57	3.63	18.73	4.62	9.84	10.28	6.12
SE (±)	5.13	2.06	1.77	29.37	7.82	1210.94	60.87	60.23
$LSD_{0.05}$	10.51	4.21	16.07	60.17	13.30	2480.51	124.69	123.38
Sign	**	**	**	**	**	**	**	**

LSD-least significant difference, CV-coefficient of variation, SE-standard error, ** significance at $p \leq 0.0001$.

Cocoyam accessions Coy45, Coy34B and Coy27 showed high levels of K, while accessions Coy12, Coy75 and Coy27 showed high levels of P and Mg was high in accessions Coy23, Coy53 and Coy34A. Accessions that showed high levels of trace elements i.e. Fe and Zn were Coy34A, Coy42, Coy23 and Coy53. The mean values of the essential minerals Fe, Ca and Zn suggested that the mineral levels present in these accessions were above that of other major root and tuber crops (Wanasundera and Ravindran, 1994; Ravindran *et al*., 1995; Charles *et al*., 2005). On the other hand cultivars Coy10, Coy5 and Coy33 showed low mineral levels. This mineral variation among cultivars suggests a wide range of diversity in the cocoyam accessions in terms of mineral levels and offers potential genetic material to

improve the micro-nutrient levels in cocoyam accessions through breeding (Burlingame *et al*., 2009).

Variation in mineral composition among the cocoyam accessions is probably due to differences in the genetic potential of each cultivar to obtain nutrients from the soil (Onwueme, 1978; Guchhait *et al*., 2008) since different cocoyam genotypes have different nutrient-use efficiencies (Goenaga and Chardon, 1995). In their study, Lebot *et al*. (2004) found high levels of variability in south east Asia and Oceania *taro* germplasm with regard to chemical composition i.e. minerals, lipids, proteins, amylose, HDM, glucose, fructose and saccharose. They suggested that cultivar selection would be efficient for their improvement since these traits are genetically controlled.

Cocoyam is characterised by a long growing period and high fertiliser requirements, especially in N, P, K and Ca (Kabeerathumma *et al*., 1985). According to Wang *et al*., (2008) availability of N, P, K and S fertilisers increase yield as well as nutritional quality of root and tuber crops. Phosphorous stimulates root development and enhances N uptake and K plays an important part in the transportation of starch and sugars from above ground plant parts to tubers whilst the amount of available Ca and S in the soil solution is directly linked to the available Ca and S in the tubers. The high mineral levels obtained in the current study may be as a result of the available and exchangeable minerals from both the top- and sub-soils, especially P and K which are important for root development (Table 6.2) (Miyasaka *et al*, 2001; Li *et al*., 2005). The variation in minerals composition could also be attributed to genetic differences between the accessions in absorbing the soil ions and anions in an acidic soil. The soils at Chitedze Research Stations are sandy clay loam (MoAFS, 2008). Table 5.2 indicates that the soils were acidic and values of soil organic C, total N, available P, exchangeable Ca, K and Mg were high for both top- and sub-soils. This could be attributed to residual fertilisers from previous cropping seasons.

The correlation matrix revealed positive and highly significant ($p \leq 0.01$) correlation between Mg and Mn ($r^2 = 0.904$), Mg and Ca ($r^2 = 0.927$) as well as Ca and Mn ($r^2 = 0.861$) and negative significant correlation between Mg and Na ($r^2 = -0.589$), Ca and Na ($r^2 = -0.5780$)

and Mn and Na (r^2= 0.630) (Table 6.3). No significant correlations were observed between K, P, Fe and Zn with the rest of the minerals.

Table 6.2 Pre-planting soil chemical properties at trial site

Depth	pH	OC	OM	N	Total	P	Na	K	Ca	Mg
	$CaCl_2$	(%)	(%)	(%)	**N** (%)	ppm	Meq %	Meq %	Meq %	Meq %
Top	4.57	1.45	2.94	0.15	0.13	21.70	0.35	0.27	3.12	0.83
Sub	4.60	1.45	2.94	0.15	0.10	20.74	0.31	0.27	3.60	0.80
Top	4.59	1.60	3.25	0.15	0.14	17.20	0.30	0.34	3.76	0.90
Sub	4.85	1.14	2.31	0.12	0.10	18.30	0.23	0.26	4.28	1.00

Top-soil 0-20 cm, Sub-soil 20-40 cm, OC-organic carbon, OM-organic matter, ppm-parts per million, Meq-milliequivalent

Table 6.3 Pearson's correlation matrix for the eight minerals of the tested cocoyam accessions

	Zn	Mn	Na	Ca	K	Mg	P
Fe	0.194	0.349	-0.336	-0.031	-0.425	0.045	-0.027
Zn		0.297	-0.343	0.325	0.361	0.274	0.167
Mn			-0.630*****	0.861******	-0.428	0.904******	-0.362
Na				-0.580*****	0.054	-0.589*****	0.312
Ca					-0.193	0.927******	-0.354
K						-0.269	0.481
Mg							-0.416

* $P \leq 0.05$, ** $P \leq 0.01$

These results are in contrast to findings by Sen *et al.* (2006) where they found significant positive correlations in Mg with both P and K as well as Ca to P and K. This could be attributed to differences in the genotype nutrient-use efficiency and differences in environment. The presence of significant negative correlation among different minerals suggest a major challenge to breeders to enhance specific minerals in these genotypes

without taking into consideration the associated effect on other minerals (Burlingame *et al*., 2009).

The PCA grouped the eight mineral nutrients into eight components, which accounted for 100% of the variability existing among the cocoyam accessions. Table 6.4 indicates that the first six principal components (PC) explained 98.66% of the total variation. The first three eigenvectors (the only ones with eigenvalues greater than one) accounted for a cumulative value of 83.34% of the entire variability among the tested cocoyam accessions.

Table 6.4 Eigenvectors, eigenvalues, individual and cumulative percentage of variation explained by the first three principal components (PC) for the eight mineral nutrients of the cocoyam accessions studied

Variables	**Eigenvectors**		
	PC1	**PC2**	**PC3**
Fe	-0.16	-0.17	-0.82
Zn	-0.17	0.59	-0.28
Mn	-0.49	0.00	-0.05
Na	0.38	-0.17	0.17
Ca	-0.46	0.15	0.27
K	0.21	0.63	0.17
Mg	-0.48	0.07	0.23
P	0.27	0.41	-0.26
Eigenvalues	3.77	1.67	1.24
Individual percentage variation explained	47.09	20.81	15.43
Cumulative percent variation explained	47.09	67.91	83.34

The first PC, which explained 47.09% of the total variation among the accessions, was mainly attributed to variation in Mn, Mg, Ca, Na, P and K. Likewise, 20.81% of the total variability among the genotypes accounted for the second PC originated from variation in K, Zn and P. The third PC, which explained 15.43% of the total variation were due to variation in Fe only. Two of the mineral nutrients, K and P contributed significantly to variation in two

of the three significant PCs, making them relatively more important. This agrees with earlier studies where K was portrayed as the major mineral nutrient component and that cocoyam is a good source of Na, Mg, Ca and P (FAO, 1990; Enomfon and Umoh, 2004; Njoku and Ohia, 2007) as well as having significant levels of Fe, Cu and Zn, especially in purple and yellow or pink-fleshed cultivars (Englberger *et al*., 2003; 2008). However, this study did not assess the variation in micronutrients in relation to corm flesh colour.

During PCA the accession with the most desirable component score appears in the upper right quadrant of the graph and as it has the highest PC1 and PC2 values. The scattergram (Figure 6.1) suggests that accession Coy45 is the best cultivar as it appears in the upper quadrant of the scattergram.

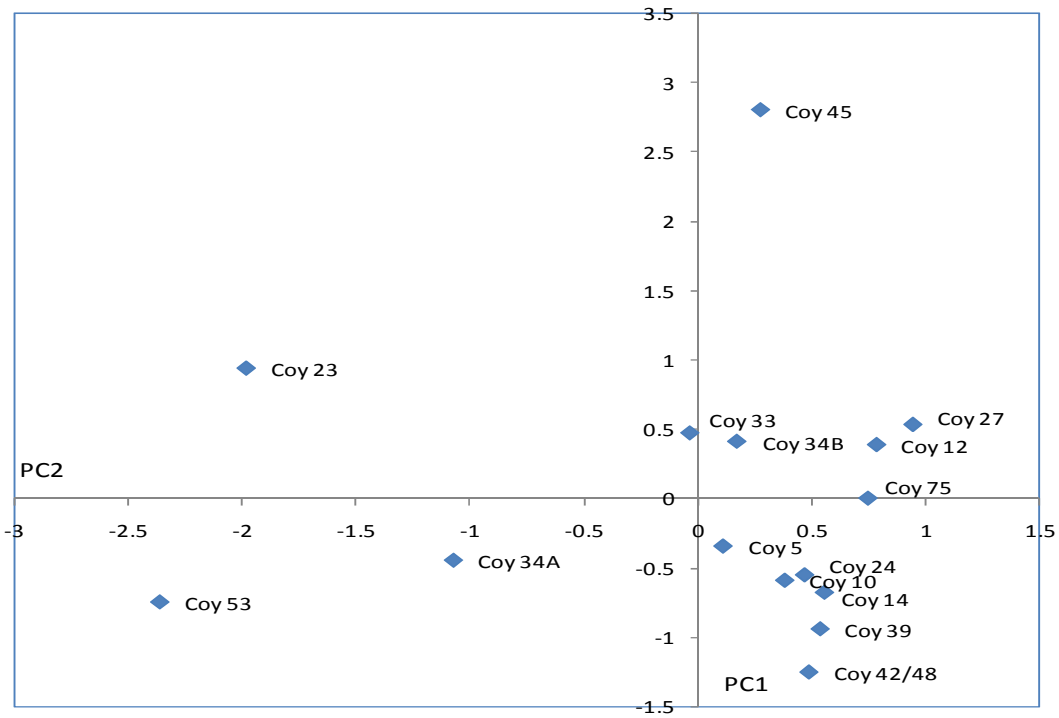

Figure 6.1 Scattergram showing the relative positions of cocoyam accessions due to their mineral composition.

This accession had the highest K level which is the main mineral element present in the cocoyam. Accessions Coy27, Coy12 and Coy34B appear in the right quadrant of the graph and have above average levels of the minerals K and P which are the major mineral elements.

It also had appreciable levels of Mn, Mg, Na, Ca, Zn and Fe. Based on these results, accessions Coy45, Coy27, Coy12 and Coy34B represent promising genetic material from which improved lines may be developed.

6.4 Conclusions and recommendations

Cocoyam can serve as a cheap dietary source of essential minerals required by humans. The crop is capable of absorbing a wide range of minerals with relevance to human health. The results presented in this study revealed that cocoyam accessions from Malawi are high in K, P and Mg. Accessions are also rich in essential minerals Ca, Fe and Zn. There was a wide variation in mineral composition among accessions. Accessions Coy45, Coy27, Coy12 and Coy34B were identified as genotypes with good mineral composition and potential for becoming parental lines to enrich the germplasm. The cocoyam accessions' K, P, Mg, Fe, Zn and Mn levels are well above the adult recommended dietary allowances and minimum requirements (FAO/WHO, 2000). Taking Ca and Fe rich cocoyam corms would be good for women and growing children who need a lot of Ca and Fe in their diet. Calcium helps to make strong bones and teeth and Fe helps keep the blood healthy. Zn helps the body in protection against infection, builds the blood and protects against vitamin A deficiency. However, due to time limitations the study did not assess the macro-nutrients (carbohydrates, lipids and proteins) and anti-nutrient factors present in the accessions to reveal a full nutritional and anti-nutritional composition as well as the genotype by environment effect. It is therefore recommended that time permitting, this study should be repeated on the macro-nutrients and in several environments (locations and seasons) to compliment the micro-nutrient data presented in this study. A complete information package on the nutritional and anti-nutritional composition of the local cocoyam germplasm would help to guide policy makers, nutritionists and research in incorporating the crop into the diversification programme undertaken by the Malawi government in order to curb drought recurrence due to over reliance on predominantly cereal-based diets.

6.5 References

Agbor-Egbe, T., and Rickard, J. (1990). Evaluation of the chemical composition of fresh and stored edible aroids. Journal of Science, Food and Agriculture 53: 487-495.

Agrobase. (2000). Agrobase 20 for Windows. Copyright 1990-1998, 1999 by Agromix Software, Inc. Winnipeg, Manitoba, Canada.

Aregheore, E., and Perera, D. (2003). Dry matter, nutrient composition and palatability/acridity of eight exotic cultivars of cocoyams-*taro* (*Colocasia esculenta*) in Samoa. Plant Foods for Human Nutrition 58: 1-8.

Barminas, J., Charles, M., and Emmanuel, D. (1998). Mineral composition of non-conventional leafy vegetables. Plant Foods for Human Nutrition 53: 29-36.

Bekele, Y.D. (2005). Assessment of cup quality, morphological, biochemical and molecular diversity of *Coffea arabica* L. genotypes of Ethiopia. PhD thesis in Plant Breeding, University of the Free State, South Africa, pp. 197.

Bradbury, J., and Holloway, W. (1988). Chemistry of Tropical root crops: Significance for nutrient and agriculture in the Pacific. ACIAR. Monograph No. 6: 201.

Burlingame, B., Charrondiere, R., and Mouille, B. (2009). Food composition is fundamental to the cross-cutting initiative on biodiversity for food and nutrition. Journal of Food Composition and Analysis, in press.

Caillon, S., Quero-Garcia, J., and Guarino, L. (2004). *Taro* in Vanuatu: towards a dynamic conservation strategy. LEISA Magazine, pp. 18-20.

Catherwood, D., Savagea, G., Masona, S., Schefferb, J., and Douglasc, J. (2007). Oxalate content of cormels of Japanese *taro* (*Colocasia esculenta* (L.) Schott) and the effect of cooking. Journal of Food Composition and Analysis 20: 147-151.

Charles, A., Sriroth, K., and Huang, T. (2005). Proximate composition, mineral contents, hydrogen cyanide and phytic acid of five cassava genotypes. Food Chemistry 92: 615-620.

Ejoh, A., Mbiapo, T., and Fokou, E. (1996). Nutrient composition of the leaves and flowers of *Colocasia esculenta* and the fruits of *Solanum melongena*. Plant Foods for Human Nutrition 49: 107-112.

Emmanuel-Ikpeme, C., Eneji, C., and Essiet, U. (2007). Storage stability and sensory evaluation of taro chips in fried in palm oil, palm olein oil, groungnut oil, soybean oil and their blends. Pakistan Journal of Nutrition 6: 570-575.

Englberger, L., Schierle, J., Kraemer, K., Aalbersberg, W., Dolodolotawake, U., Humphries, J., Graham, R., Reid, A.P., Lorens, A., Albert, K., Levendusky, A., Johnson, E., Paul, Y., and Sengebau, F. (2008). Carotenoid and mineral content of micronesian giant swamp *taro* (*Cyrtosperma*) cultivars. Journal of Food Composition and Analysis 21: 93-106.

Englberger, L., Schierle, J., Marksa, G., and Fitzgerald, M. (2003). Micronesian banana, *taro* and other foods: newly recognized sources of provitamin A and other carotenoids. Journal of Food Composition and Analysis 16: 3-19.

Enomfon, J., and Umoh, I. (2004). Effects of heat and tracycline treatment on the food quality and acridity factors in cocoyam (*Xanthosoma sagittifolium* (L) Schott). Pakistan Journal of Nutrition 3: 240-243.

FAO (1990). Food and Agriculture Organisation of the United Nations. Roots, tubers, plantain and bananas in human nutrition, Food and Nutrition series, No. 24: FAO code: 86, AGRIS: SO1 ISBN 92-5-102862-1. Rome.

FAO (2001). Production Year Book: 55. Rome: Food and Agriculture of the United Nations.

FAOSTAT (2004). Food and Agricultural Organisation of the United Nations, FAOSTAT on-line statstical service, Rome. http://apps.fao.org. (2009/10/08).

FAOSTAT (2005). Food and Agricultural Organisation of the United Nations, FAOSTAT statistical database, Rome. Agricultural production of primary crops. http://apps.fao.org. (2008/06/02).

FAO/WHO (2000). Preliminary report on recommended nutrient intakes. Joint FAO/WHO Export consultation on Human Vitamin and mineral requirements, 21-30 September 1998. Bangkok, Thailand: Food and Agricultural Organisation of the United Nations and World Health Organisation, Rome. pp. 1-16.

Gerloff, E., Lima, I., and Stohmann, M. (1965). Leaf protein as food stuffs: Amino acid composition of leaf protein concentrate. Journal of Agriculture and Food Chemistry 13: 139-142.

Goenaga, R., and Chardon, U. (1995). Growth, yield and nutrient uptake of *taro* grown under upland conditions. Journal of Plant Nutrition 18: 1037-1048.

Graham, R., Welch, R., and Bouis, H. (2001). Addressing micronutrient malnutrition through enhancing the nutritional quality of staple foods: principles, perspectives and knowledge gaps. Advances in Agronomy 70: 77-142.

Grivetti, L., and Ogle, B. (2000). Value of traditional foods in meeting macro- and micronutrient needs: the wild plant connection. Nutritional Research Reviews 13: 31-46.

Guchhait, S., Bhattacharya, A., Pal, S.M., Chattopadhyay, A., and Das, A.K. (2008). Quality evaluation of cormels of new germplasm of *taro*. International Journal of Vegetable Science 14: 304-321.

Hesse, P. (1971). A textbook of soil chemical analysis. London: John Murray Ltd.

Hintze, J. (1998). NCSS-2000 Statistical System for Windows. Number Cruncher Statistical Systems. Kaysville, UT.

Janseens, M. (2001). Crop production in tropical Africa. Bonn, Germany: Institut fur obst- und germusebau, abt. Tropicscher pflanzenbau, rheinische friedrich-wilhelms-Universitat Bonn, auf dem hugel 6, D-53121 Bonn, Germany. Directorate General for International Co-operation, karmelietenstraat 15, B-1000 Brussels, Belgium, pp. 221-228.

Kabeerathumma, S., Mohankumar, B., and Nair, P. (1985). Nutrient uptake by *taro* (*Colocasia esculenta),* 2. Uptake pattern of secondary and micronutrients at different stages of growth. Journal of Root Crops 11: 51-56.

Lebot, V., Prana, M., Kreike, N., van Heck, V., Pardales, J., Okpul, T., Gendua, T., Thongjien, M., Hue, H., Viet, N., and Yap, T.C. (2004). Characterisation of *taro* (*Colocasia esculenta* (L.) Schott) genetic resources in Southeast Asia and Oceania. Genetic Resources and Crop Evolution 51: 381-392.

Lewu, M., Adebola, P., and Afolayan, A. (2009). Effect of cooking on the proximate composition of the leaves of some accessions of *Colocasia esculenta* (L.) Schott in Kwazulu-Natal province of South Africa. African Journal of Biotechnology 8: 1619-1622.

Li, M., Liu, R., Christie, P., and Li, X. (2005). Influence of three arbuscular mycorrhizal fungi and phosphorus on growth and nutrient status of *taro*. Communications in Soil Science and Plant Analysis 36: 2383-2396.

Ministry of Agriculture and Food Security (MoAFS). (2008). Chitedze Agricultural Research Station Guide. Lilongwe, Malawi.

Miyasaka, S., Hollyer, J., and Kodani, R. (2001). Mulch and compost effects on yield and corm rots of *taro*. Field Crops Research 71: 101-112.

Mweta, D.E., Labuschagne, M.T., Koen, E., Benesi, I.R.M., and Saka, J.D.K. (2008). Some properties of starches from cocoyam (*Colocasia esculenta*) and cassava (*Manihot esculenta* Crantz.) grown in Malawi. African Journal of Food Science 2: 102-111.

National Academy of Science. (1975). Underxploited tropical plants with promising economic potential. Report of Ad hoc Panel of the Advisory Committee on Technology Inovations, National Academy of Sciences USA. Washington D.C.

Njoku, P.C., and Ohia, C.C. (2007). Spectrophometric estimation studies of mineral nutrient in three cocoyam cultivars. Pakistan Journal of Nutrition 6: 616-619.

Okonkwo, C. (1993). *Taro, Colocasia* spp. In: Bergh, G. (Ed.) Genetic improvement of vegetable crops. Oxford: Pergamon, pp. 709-715.

O'Hair, S.K. (**1990).** Tropical root and tuber crops. In: Janick, J. and Simon, J.E. (Eds.), Advances in new crops. Timber Press, Portland, OR, pp. 424-428.

Onwueme, I. (1978). The tropical tuber crops: Yams, cassava, sweet potato and cocoyams. John Wiley & Sons, pp. 199-225.

Onwueme, I. (1999). *Taro* cultivation in Asia and Pacific: RAP Publication 1999/16. Bangkok, Thailand: Food and Agriculture Organisation of the United Nations regional office for Asia and the Pacific.

Oscarsson, K., and Savage, G. (2007). Composition and availability of soluble and insoluble oxalates in raw and cooked *taro* (*Colocasia esculenta* var. Schott) leaves. Food Chemistry 101: 559-562.

Ravindran, V., Ravindran, G., Sivakanesan, R., and Rajaguru, S. (1995). Biochemicamal and nutritional assessment of tubers from 16 cultivars of sweet potato (*Ipomoea batatas* L.). Journal of Food Chemistry 43: 2646-2651.

Sajeev, M., Manikantan, M., Kingsly, A., Moorthy, S., and Sreekumar, J. (2004). Texture analysis of *taro* (*Colocasia esculenta* L. Schott) cormels during storage and cooking. Journal of Food Science: 69: 315-321.

Sefa-Dedeh, S., and Kofir-Agyir, S. (2004). Chemical composition and the effect of processing on oxalate content of cocoyam *Xanthosoma sagittifolium* and *Colocasia esculenta* cormels. Food Chemistry 85: 479-487.

Sen, S., Bhattacharya, A.M., Sen, H., Das, A.K., and Pal, S. (2006). Nutrient and antinutrient composition of cormels of *Colocasia esculenta* var. *antiquorum*. Journal of Vegetable Science 11: 17-33.

South Pacific Community, (SPC). (1993). *Taro*: South Pacific foods leaflet 1. Suva, Fiji: Community educational material training centre.

Tambong, J., Ndzana, X., Wutoh, J., and Dadson, R. (1997). Variability and germplasm loss in the Cameroon national collection of cocoyam (*Xanthosoma sagittifolium* Schott (L.)). Plant Genetic Resources Newsletter 112: 49-54.

Thomas, A., and Oyediran, O. (2008). Nutritional importance and micronutrient potentials of two non-conventional indigenous green leafy vegetables from Nigeria. Agricultural Journal 3: 362-365.

Wanasundera, J., and Ravindran, G. (1994). Nutritional assessment of yam (*Dioscorea alata*) tubers. Plant Foods for Human Nutrition 46: 33-39.

Wang, Z.-H., Li, S.-X., and Malhi, S. (2008). Effects of fertilization and other agronomic measures on nutritional quality of crops. Journal of the Science of Food and Agriculture 7: 7-23.

Welch, R. (2002). The impact of mineral nutrients in food crops on global human health. Plant and Soil 247: 83-90.

Wills, R., Lim, J., Greenfield, H., and Bayliss-Smith, T. (1983). Nutrient composition of *taro* (*Colocasia esculenta*) cultivars from the Papua New Guinea highlands. Journal of Science of Food and Agriculture 34: 1137-1142.

CHAPTER 7

General conclusions and recommendations

Plant genetic resources are an important component in breeding. Knowledge of genetic variation present within cultivars and/or closely related species is a prerequisite to a sustainable plant genetic resource conservation and improvement programme. Limited or no information exists on the levels and patterns of genetic diversity, as well as utilisation of cocoyam in Malawi. Hence, cocoyam accessions collected from local farmers in Malawi were characterised using ethno-botany, morphological characters and AFLP analysis. Mineral composition of a selection of cocoyam accessions was determined to identify suitable cultivars that would help combat micro-nutrient deficiencies in the country.

Information on prevalence, farmer's preferences and utilisation of cocoyam cultivars in Malawi was gathered during germplasm collection. Results indicated that cocoyam is grown in both regions of the country covered in this study i.e. northern and southern Malawi. The crop is cultivated in both low and high altitude areas provided water is not limiting. Although cocoyam is known by different names based on ethnicity of a particular cultivating area, it is generally referred to as *koko* throughout the country. Other names include *masimbi* in the north (*Tumbuka* and *Nkhonde* tribe) and *zigumbwa* and *dumbe* in the south among the *Yao*.

The study showed that farmers in Malawi hold rich ethno-botanical and ecological knowledge of cocoyam accessions they are conserving. Farmer's preferences regarding cocoyam cultivars included uses of plant parts, adaptation and eating quality. Specifically farmers preferred high yielding cultivars in terms of size of tubers, HDM content, mealyness, taste, shorter maturity period, good cooking properties (making pulp and fast cooking) as well as resilience to detrimental weather conditions and good ornamental characters. Preferences tended to vary between the two regions. In Malawi, cocoyam is mainly grown as a food crop and a source of income, but also as an ornamental plant. It is usually consumed as a snack in the form of boiled tubers and to a lesser extent tubers are dried and ground for

making pulp (msima). Tender leaves are used as a leafy vegetable and livestock feed (fish and pigs). The leaves are also used as a traditional medicine for ringworms.

Characterisation of cocoyam using morphological traits indicated that farmers in Malawi are maintaining high levels of cocoyam diversity. Of the 30 traits used to characterise the germplasm, only one was monomorphic (cross-section of lower the part of the petiole) and one character (main vein colour) was monomorphic in all but showed difference in one accession. Traits such as predominant leaf base shape, predominant leaf lamina position, petiole junction pattern and colour, sap colour of leaf blade tip and vein pattern of the leaves were highly polymorphic and contributed significantly to the total variation of the accessions. These morphological characters were highly variable and could be used to uniquely identify cultivars in a breeding programme. The results furthermore suggested that most of the genotypes in Malawi belong to the *Xanthosoma* species rather than to the *Colocasia* species.

The dendrogram constructed using morphological characters separated the accessions according to species based on leaf base shapes (petiole attachment) i.e. peltate leaf base accessions (*Colocasia*) and sagittate (*Xanthosoma*). Accessions Coy5 and Coy28 were the most dissimilar among the *Xanthosoma* species (sagittate group). Accession Coy16 was the most dissimilar among the *Colocasia* species (peltate) group. The accessions were further differentiated within the main clusters based on region of collection. Accessions from the northern region clustered separately from the ones from the southern region. High levels of variation were identified between the two cocoyam species (45% similar). Variation within the species was moderate. However, it was higher within the *Colocasia* species (peltate) compared to the *Xanthosoma* species (sagittate).

The AFLP technique successfully illustrated the genetic variation within (intra-clonal) and among accessions. The observed intra-clonal variation (2-4%) was within the minimum acceptable dissimilarity threshold for genotypes to belong to the same clone. The dendrogram constructed using AFLP fingerprints confirmed the presence of the two species identified using morphological characters. Accessions that were morphologically characterised as *Colocasia* (peltate) and *Xanthosoma* (sagittate) grouped separately, with the exception of four accessions.

AFLP analysis revealed high levels of diversity between the two species. However, within each of the two species the level of genetic variation was low. The genetic diversity of the cocoyam accessions also exhibited a differentiation based on geographical level. Accessions Coy16 and Coy63 were the most dissimilar in the *Colocasia* (sagittate) species. On the other hand, accessions Coy5, Coy70 and Coy57 clustered separately from the other accessions of the *Xanthosoma* (sagittate) species. Accession Coy55, morphologically identified as *Colocasia* (peltate), clustered among the sagittate group and conversely accessions Coy6, Coy42 and Coy63 clustered among the peltate group.

Combined morphological traits and AFLP data analyses further confirmed the results suggested by dendrograms constructed using morphological and AFLP data. The dendrogram constructed using combined morphological characters and AFLP data was more similar to the one constructed using AFLP data than using morphological data. For all three analyses, accessions separated into two main groups according to the two species based on leaf base shape of accessions. High levels of variation were observed between the two species. Variation within accessions was higher in the *Colocasia* compared to *Xanthosoma* species. Variation between accessions was furthermore mainly based on collection region since accessions from the south tended to group separately from ones from the north.

The three PCA bi-plots (morphological characters, AFLP data and combined morphological and AFLP data) showed similar relationships among and within cocoyam accessions and similarity to the hierarchical clustering of the data. A clear distinction was visible among the two morphologically differentiated populations (*Colocasia* and *Xanthosoma* species). Accessions Coy6, Coy42 and Coy63 separated from the rest of the *Colocasia* (peltate) species while accession Coy55 clustered among the *Xanthosoma* (sagittate) species.

The AMOVAs partitioned the total variance among cocoyam accessions into variance among and within populations, basically defined on morphological characterisation into the two main species of cocoyam *Colocasia* (peltate) and *Xanthosoma* (sagittate). Results indicated that most of the variation lied between the two populations compared to within populations. This agreed with the hierarchical clustering and PCA bi-plots results which showed high

levels of variation between the two populations compared to the variations within the populations (*Colocasia* and *Xanthosoma* species).

Cocoyam accessions showed high levels of diversity in terms of mineral composition. Results confirmed that K is the major mineral component of the tubers. The tubers also exhibited high levels of essential minerals (Ca, Fe and Zn). Incorporating cocoyam into the diet of resource poor farmers would help reduce the micro-nutrient deficiency prevalent in the country. Accessions Coy12, Coy27, Coy34B and Coy45 showed high levels of minerals and could be utilised as parents to incorporate these traits into new cultivars.

Although the accessions exhibited high morphological variation, the AFLP technique revealed low levels of genetic variation within the germplasm. The narrow genetic base poses a major challenge to conservationists and breeders as cocoyam germplasm is vulnerable to future disease epidemics and insect damage. The high levels of morphological variation in the germplasm is due to the high rate of vegetative propagation after somatic mutations and famers' selections that fix different morphotypes having more or less the same genetic base. The AFLP technique which detects polymorphism in the entire genome reduced the high levels of variation observed morphologically, (morphological traits are mainly expressed by a limited number of genes). The study has therefore shown that the two methods can best elucidate the level and pattern of genetic diversity if used complementary.

Results are similar to what others found in major cocoyam growing regions of south east Asia and the Oceania region. The narrow genetic base and high variability and level of mineral composition has been observed in most growing regions and different species of cocoyam.

It is therefore recommended that farmers' ethno-botanical knowledge be preserved and incorporated into the future improvement programmes of the crop. Farmers' preferences, if incorporated into a breeding programme, tend to shape and enhance the adoption of newly developed cultivars. Apart from a few accessions (Coy5, Coy16, Coy28, Coy55 and Coy63) that showed high levels of variation within each of the two species, it is recommended that

genotypes from other regions be utilised to broaden the existing narrow genetic base available for crosses.

The information revealed by this study will help improve efficiency in cocoyam conservation and future improvement programmes. The low levels of genetic variation among the accessions should help conservationists and breeders design better conservation efforts of the crop in case of disease epidemics and pest outbreaks. Breeders will have options of parents for making crosses, as the genetic distances among the genotypes has been uncovered. The most distinct morphological traits identified in this study will be used by the breeders and conservationists in cultivar characterisation and identification required by variety release committee. Farmers' will have improved cultivars with all the desired traits as breeders have knowledge of the farmers' preferences and desired traits. This will in turn ease adoption of released cocoyam cultivars. The study should also help nutritionists, technologists and extension agents, both public and private, to help them advocate and market the crop and its products to resource poor farmers and potential industries.

The study only covered a subset (southern and northern regions) of the existing germplasm in the country. In future a broad spectrum collection and study on genetic diversity (agro-morphological, molecular and nutritional) should be conducted in the country to confirm the extent of the genetic diversity prevalent in Malawi. Future studies should also consider use of a combination both morphological characters and AFLP analysis to uncover the pattern and extent of genetic diversity in the country. A cytogenetic study would also help to identify any possible association between the pattern of diversity and the ploidy nature of the crop.

SUMMARY

Cocoyam (*C. esculenta* (L.) Schott and *X. sagittifolium* (L.) Schott) belongs to the family Araceae. Cocoyam has the potential to contribute significantly to world agriculture in terms of food, nutrition and crop diversification. Despite this the crop remains neglected in terms of research focus. Limited or no information exists on the prevalence, preference and utilisation as well as the pattern and level of diversity that exists in cocoyam germplasm found in Malawi. This study determined the level and pattern of genetic diversity of cocoyam from Malawi using ethno-botany, morphological characters and AFLP markers. Mineral composition of selected genotypes was also determined. The ethno-botanical survey showed that farmers in Malawi maintain a large amount of cocoyam germplasm. These farmers have rich ethno-botanical and ecological knowledge of the cocoyam cultivars they conserve. Farmer's preferences regarding cocoyam cultivars included uses of plant parts, adaptation and eating quality. Morphological characters showed high levels of variation among accessions. Two main species of cocoyam, *Colocasia* and *Xanthosoma* were identified based on leaf base shapes (petiole attachment). AFLP markers showed low levels of genetic diversity between accessions as opposed to the high morphological diversity. A combined (morphological characters and AFLP data) analysis using UPGMA clustering, PCA and AMOVA further alluded to the presence of the two cocoyam species within the germplasm. High levels of variation were detected between the two species and low levels of variation were observed within each of the two species (*Colocasia* and *Xanthosoma*). The PCA exhibited a better representation of the genetic diversity pattern than the hierarchical clustering. Accessions showed high levels of mineral composition. Potassium was identified as the major mineral component. Accessions also exhibited high levels of essential minerals (Ca, Fe and Zn). Incorporating cocoyam into the diet of the resource poor farmers could help in the fight of the most prevalent micro-nutrient deficiencies. A study on morphological and genetic diversity as well full nutritional assessment of the tubers and leaves of cocoyam from the whole country is recommended. In order to broaden the narrow genetic base observed importation of foreign material is recommended.

OPSOMMING

Amadumbie (*C. esculenta* (L.) Schott en *X. sagittifolium* (L.) Schott) behoort aan die Araceae familie. Amadumbie het die potensiaal om betekenisvol tot wêreldlandbou in terme van voedsel, voedingswaarde en gewasdiversifikasie by te dra. Ten spyte hiervan word die gewas nog steeds in terme van navorsing afgeskeep. Beperkte of geen inligting is oor die voorkoms, voorkeure en gebruik van die gewas sowel as die patroon en vlak van diversiteit wat voorkom in kiemplasma van Malawi beskikbaar. In hierdie studie is die patroon en vlak van genetiese diversiteit in amadumbie kiemplasma van Malawi met behulp van etno-botanie, morfologiese eienskappe en AFLP merkers bepaal. Minerale inhoud van geselekteerde genotipes is ook bepaal. Die etno-botaniese opname het getoon dat boere in Malawi ‘n groot hoeveelheid genotipes onderhou. Die boere wat die kiemplasma aanplant het ‘n ryk etno-botaniese en ekologiese kennis van die genotipes wat hulle bewaar. Die boere se voorkeur in terme van amadumbie cultivars sluit in die potensiële gebruik van plantdele, aanpassing en eetbaarheid. Morfologiese eienskappe het hoë vlakke van variasie tussen genotipes getoon. Die twee hoof spesies van amadumbie, *Colocasia* en *Xanthosoma*, is op grond van blaarbasis vorms (petiool aanhegting) geïdentifiseer. AFLP merkers het lae vlakke van diversiteit tussen genotipes getoon, wat teenstrydig was met die hoë morfologiese diversiteit. Gekombineerde analises (morfologiese eienskappe en AFLP data) met UPGMA groepering, PCA en AMOVA het die teenwoordigheid van twee amadumbie spesies in die kiemplasma bevestig. Hoë vlakke van variasie tussen die spesies en lae vlakke binne die twee spesies (*Colocasia* en *Xanthosoma*) is waargeneem. Die PCA het ‘n beter verteenwoordiging van die genetiese diversiteitspatrone as hierargale groepering gegee. Genotipes het hoë mineraalinhoudvlakke getoon. Kalium was die belangrikste minerale komponent. Genotipes het ook hoë vlakke van noodsaaklike minerale (Ca, Fe en Zn) gehad. Die insluiting van amadumbie in die dieet van hulpbron-arm boere kan tot die stryd teen mikro-element tekorte bydra. ‘n Volledige studie van morfologiese en genetiese diversiteit sowel as voedingswaarde analise van die knolle en blare van amadumbie van die hele land word aanbeveel. Die invoer van kiemplasma word aanbeveel, om die smal genetiese basis van die gewas te verbreed.

APPENDIX 1

Morphological descriptor used for cocoyam characterisation

No	Description	Categories	Code
1.	Maximum horizontal distance reached by leaves	1-Narrow (<50 cm) 2- Medium (50-100 cm) 3-Wide (>100 cm)	7.1.1
2.	Maximum vertical distance reached by leaves, relative to ground level	1-Dwarf (<50 cm) 2-Medium (50-100 cm) 3-Tall (>100 cm)	7.1.2
3.	Number of stolons (side shoots)	0- None 1-1 to 5 2- 6 to 10 3-11 to 20 4- >20	7.1.3
4.	Measure of the longest stolon	1-Short (<15 cm) 2-Long (≥15 cm)	7.1.3.1
5.	Number of suckers (direct shoot)	0- None 1-1 to 5 2- 6 to 10 3-11 to 20 4- >20	7.1.4
6.	Leaf base shape (with regard to the petiole attachment)	1-Peltate 99-Other e.g. sagittate, hastate specify	7.2.1
7.	Predominant position (shape) of leaf lamina surface	1- Drooping 2-Horizontal 3-Cup-shaped 4-Erect - apex up 5-Erect - apex down 99-Other (specify)	7.2.2
8.	Leaf blade margin	1-Entire 2-Undulate 3-Sinuate 99-Other (specify)	7.2.3
9.	Leaf blade colour	1-Whitish 2-Yellow or yellow green 3-Green 4-Dark green 5-Pink 6- Red 7-Purple 8-Blackish (violet-blue) 99-Other (specify	7.2.4
10.	Leaf blade colour variegation	0- Absent 1-Present	7.2.4.1
11.	Type of variegation	1-Fleck 2-Mottle 3-Stripe	7.2.4.2
12.	Colour of variegation	1-Whitish 2-Yellow 3-Orange	7.2.4.3

		4-Green 5-Pink 6-Red 7-Purple 99-Other (specify)	
13.	Leaf blade margin colour (observed on the upper side of blade)	1-Whitish 2-Yellow 3-Orange 4-Green 5-Pink 6-Red 7-Purple 99. Other (specify)	7.2.5
14.	Leaf lamina appendages	0-Absent 1-Present	7.2.6
15.	Petiole junction pattern- Area of spots at vein junction on upper surface of leaf	0-Absent 1-Small 2-Medium 3-Large	7.2.8
16.	Petiole junction colour (observed on the upper side)	0-Absent 1-Yellow 2-Green 3-Red 4-Purple 99-Other (specify)	7.2.9
17.	Sap colour of leaf blade tip	1-Whitish (transparent) 2-Yellow 3-Pink 4-Red 5-Dark red 6-Brownish 99-Other (specify)	7.2.10
18.	Leaf main vein colour (observed the upper side of leaf blade, beyond junction)	1-Whitish 2-Yellow 3-Orange 4-Green 5-Pink 6-Red 7-Brownish 8-Purple 99-Other (specify	7.2.11
19.	Leaf main vein variegation (observed the upper side of leaf blade)	0-Absent 1-Present	7.2.11.1
20.	Vein pattern (shape of pigmentation on veins on leaf lower surface)	1-V pattern (in a ‘V’ space) 2-I pattern (in an ‘I’ shape) 3-Y pattern (in a ‘Y’ shape) 4-Y pattern and extending to secondary veins 99-Other (specify)	7.2.12
21.	Petiole colour-colour of top third	1-Whitish 2-Yellow 3-Orange 4-Light green	7.2.14.1

		4-Green 6-Red 7-Brown 8-Purple 99-Other (e.g. 'bronze', black specify)	7.2.14.2
22.	Petiole colour-colour of middle third	1-Whitish 2-Yellow 3-Orange 4-Light green 4-Green 6-Red 7-Brown 8-Purple 99-Other (e.g. 'bronze', black specify)	
23.	Petiole colour-colour of bottom third	1-Whitish 2-Yellow 3-Orange 4-Light green 4-Green 6-Red 7-Brown 8-Purple 99-Other (e.g. 'bronze', black specify)	7.2.14.3
24.	Petiole stripe	0-Absent 1-Present	7.2.15
25.	Petiole stripe colour	1-Whitish 2-Yellow 3-Orange 4-Light green 4-Green 6-Red 7-Brown 8-Purple 99-Other (e.g. 'bronze', black specify)	7.2.15.1 7.2.16
26.	Petiole basal-ring colour	1-White 2-Green (yellow green) 3-Pink 4-Red 5-Purple 99-Other (specify)	
27.	Cross-section of lower part of petiole (observed on healthy and fully developed leaves of the same age	1-Open 2-Closed	7.2.17
28.	Leaf sheath colour	1-Whitish 2-Yellow 3-Light green 4-Red purple 5-Brownish 99-Other (specify)	7.2.19
29.	Leaf sheath edge colour	1-Dark brown (continuous) 2-Dark brown(not continuous)	7.2.19.1

30.	Leaf waxiness	99-Other (specify) 0-Absent 3-Low 5- Medium 7-High	7.2.20