Morphological and Molecular Characterization, Assessment of Nutritional Composition and Micropropagation of Cocoyam (*Xanthosoma sagittifolium* (L.) Schott) from Ethiopia

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June, 2018

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A Dissertation Submitted to
the Department of Microbial, Cellular and Molecular Biology, Addis Ababa University, in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Biology (Applied Genetics)

By
Eyasu Wada Wachamo

Addis Ababa University
Addis Ababa, Ethiopia
June, 2018

Declaration

I, the undersigned, declare that this Dissertation is my original work and its composition has never been submitted elsewhere for any other award. All sources of materials used for the Dissertation have been duly acknowledged.

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ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

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A Thesis Presented to the School of Graduate Studies of the Addis Ababa University in Partial Fulfillment of the Requirements for the PhD in Biology (Applied Genetics)

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Acknowledgments

First and foremost, I am highly indebted to express my deepest and genuine appreciation to my supervisors: Dr. Tileye Feyissa, Dr. Kassahun Tesfaye and Prof. Zemede Asfaw for their excellent guiding, follow-up and support. Next, I would like to owe my special thanks to Dr. Kifle Dagne and Asfaw Kifle (PhD prospective graduate) for their special pieces of support. I would also like to thank Prof. Daniel Potter from Plant Science Department, University of California-Davis (UCDavis), USA and Prof. Dr. Birgit Gemeinholzer and Prof. Dr. Volker Wessmann from Justus Liebig University Giessen (JLU), Germany for inviting me to their Molecular Biology Laboratories as a visiting scholar. I extend my deepest acknowledgments to different institutions that have provided financial support: Department of Microbial, Cellular and Molecular Biology and Graduate Program Office of Addis Ababa University (SIDA project), Wolaita Sodo University and The German Academic Exchange Service (DAAD). I am thankful to Areka Agricultural Research Center, Department of Plant Sciences of UCDavis and Systematic Botany Group of JLU for making the laboratories accessible for this study. I am grateful to the Ethiopian Institute of Biodiversity for giving me permits to export *X. sagittifolium* leaf samples to UCDavis and JLU for molecular analyses. My unreserved thanks go to farmers for providing information of the crop. I have many thanks to driver Mr. Beyene Dilbore and field and laboratory technical assistances: Mr. Abeje Yilma, Mr. Wolde Pilto, Mr. Sied Mohammed, Mrs. Eyerusalem Natinael, Ms. Gloria Diaz-Britz, Mrs. Hela Krufzick, Mrs. Sabine Mutz and Mr. Peter Seum for providing unreserved technical help. Many other persons also stretched their hands for help during this research work. Although I cannot mention all of their names, Prof. Hoysign Won, Mr. Genene Gezahegn, Mr. Girma, Mrs. Tigist Markos, Mrs. Christena Muller, Mr. Teshome Matusala and Mr. Micheal Mears can be mentioned as their representatives. Finally, my deepest thanks go to my wife Meseret Gano, my children Fitsum Eyasu and Keleul Eyasu, and my nieces Suna Esayas and Konjit Minota for their patience and encouragement at all times of this study period.

May God Bless You All!

Dedication

This Dissertaion is dedicated to my mother Asase Anebo and my late father Wada Wachamo for giving me the foundation of learning that they never enjoyed themselves. The efforts that they made for my education are in my mind. I have been able to appreciate the value of learning.

Table of Contents

page

Table of contents continued

Table of Contents continued

Table of Contents continued

List of Tables

Page

List of Figures

page

List of Appendices

Page

List of Acronyms and Abbreviations

AFLP	Amplified Fragment Length Polymorphism
AMOVA	Analysis of molecular variance
AOAC	Association of Official Analytical Chemists
BAP	6-benzyl aminopurine
FAM	6-Carboxyfluorescein
FAO	Food and Agricultural Organization
Fst	Genetic differentiation among populations
GCV	Genotypic coefficient of variation
GLM	General linear model
Gst	Genetic differentiation among populations
h^2_b	Broad sense heritability
He	Expected heterozygosity/Nei's gene diversity
HEX	Hexachloro-fluorescein
Ho	Observed heterozygosity
I	Shanon information index
IBA	Indol-3-butyric acid
IBPGR	International Board for Plant Genetic Resource
ISSR	Inter simple sequence repeats
Kn	Kinetin
MSE	Mean square of error
MSG	Mean squares for genotypes
NAA	α-naphthalene acetic acid
Na	Number of alleles
Ne	Number of effective allele
NJ	Neighbor joining
PCA	Principal component analysis
PCoA	Principal coordinate analysis
PCV	phenotypic coefficient of variation
RAPD	Randomly amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
SNP	Single nucleotide polymorphism
SSR	Simple sequence repeats

Abstract

Morphological and Molecular Characterization, Assessment of Nutritional Composition and Micropropagation of Cocoyam (*Xanthosoma sagittifolium* (L.) Schott) from Ethiopia

Eyasu Wada Wachamo, PhD Dissertation
Addis Ababa University, June 2018

Cocoyam *(Xanthosoma sagittifolium* (L.) Schott) is a tuberous root crop in the Araceae family. It is an exotic crop to Ethiopia that was introduced fairly recently but has spread widely and already become part of the agricultural and food systems of the people, wherein tuber and root crops play an important role as sources of food. However, cocoyam has not been given research attention commensurate to its importance as it is a neglected crop by research and development community. This study was conducted to characterize cocoyam diversity at morphological and molecular levels through documenting farmers' knowledge, perceptions and management practices; determining nutritional composition; and developing a micropropagation protocol for this neglected crop. The present ethnobotanical survey results showed that the crop is given different local names by farmers and that it is locally used for food, fodder, medicine and other purposes. Furthermore, the results showed that the uses of cocoyam as a food crop and fodder are the most preferred traits as perceived by the farmers while hardness, sour taste, unpleasant smell and low market demand were the major constraits for cocoyam production. Green- and purple-colored cocoyam plants were observed during our survey. The field study helped to distinguish two classes of qualitative traits for petiole color, lamina orientation, color of veins on leaf surfaces, position of cormel apex and shape of cormels. Analysis of variance (ANOVA) revealed significant variation in 11(84.6%) of the 13 studied quantitative traits. Principal Component Analysis (PCA) reduced the 13 quantitative traits to 3 Principal components (PCs) with the eigen values >1, which explained 69.2% of the observed variations. In the genetic diversity analysis using 11 SSR markers, a total of 36 alleles were detected (mean 3.273). High SSR marker diversity was detected within populations (average Ho = 0.503 and He = 0.443) and when all collections were considered as single population (Ho: 0.508, He: 0.566). Supporting these results, genetic diversity analysis using AFLP markers revealed high Nei's gene diversity (He) within populations (He = 0.349) and at the entire collection level (He = 0.389). SSR markers revealed strong genetic differentiation among populations and between green and purple cocoyam morphotypes by Fst values 0.196 and 0.463, respectively. However, unlike SSR markers, AFLP marker-based analysis showed low genetic differentiation among populations (Gst = 0.072) as well as between green and purple cocoyam morphotypes (Gst = 0.024). The nutritional composition analysis showed that both the green- and purple-cocoyam morphotypes can provide nutrient-rich products, albeit slight differences in the quantities of proximate, minerals and antinutritional factors. A micropropagation protocol was successfully developed in which the green- and purple-cocoyam shoot tip explants were best initiated on Murashige and Skoog (MS) medium containing 2.0 mg/l BAP, best multiplied on MS medium containing 2.5 mg/l BAP and 0.5 mg/l NAA and the best IBA concentration for rooting was MS medium supplemented with 2.0 mg/l IBA. Overall, a lot of useful indigenous knowledge exists within the farming communities in the rural areas, but cocoyam is poorly studied and underutilized crop in spite of its nutritional value & its potential as food crop. The findings of this study are very important to enhance the future use of cocoyam in the country. Collaborative research intervention involving the development of varieties, making available high quality planting material for farmers & promoting value chains and market opportunities are valuable for sustainable use of the exiting diversity & to safeguard the potential end users of cocoyam in the country.

Keywords: AFLP markers, genetic diversity, indigenous knowledge, *in vitro* propagation, morphological trait, neglected crop, SSR markers

Chapter One
General Introduction

1.1 Background

Edible aroids play a significant role in the livelihood of millions of relatively poor people in the developing countries (Sarma *et al*., 2016). They are potential crops for food security, income generation and nutritional enhancement at household level. They have a greater ability to produce more energy per hectare per day and produce satisfactorily under adverse conditions where other crops fail (Onwueme, 1999). Cocoyam (*Xanthosoma sagittifolium* (L.) Schott) is one of the edible aroids in the Araceae family. It is the 6th most important root and tuber crop in the world, after potato (*Solanum tuberosum* L.), cassava (*Manihot esculenta* Crantz), sweet potato (*Ipomoea batatas* (L.) (Lam.), yam (*Dioscorea* spp.) and taro (*Colocasia esculenta* (L.) Schott) (Bown, 2000).

Cocoyam is native to the northern part of South America (Giacometti and Leon, 1994). It is widely naturalized in the Caribbean, Africa, Asia and Islands in the Pacific Ocean (Ponce, 2010). Nowadays, Africa is the major producer with the West and Central parts of the continent (Nigeria, Ghana, Cameroon) contributing over 60% of the total production (Onyeka, 2014; Owusu-darko *et al*., 2014). It was introduced into Eastern Africa through Western Africa initially coming from tropical America (Maundu *et al*., 2009). Thus, it has been known to be exotic to Ethiopia but there is no clear information on when and how it was introduced into the country. However, cocoyam has widely spread and is now part of the local food system of the people, wherein the root and tuber crops play an important role in food security.

It grows even in poor soils and under dry conditions that are too difficult for cultivation of other tuberous root crops at the Malo area of Gamo-Gofa zone (Fujimoto, 2009). It was

ranked second next to ensete (*Ensete verticosum*) (Welw.) Cheesman) among the top 10 most preferred plants around Bonga city in Kefa based on use values (Zemede Asfaw, 2001a). Amsalu Nebiyu and Tesfaye Awas (2006) alluded the presence of a considerable amount of cocoyam gene pool in south and southwest Ethiopia in farmers' fields and homegardens.

Many developing countries experience great difficulty in sustaining the conservation and genetic improvement of neglected and underutilized root and tuber crops, mostly aroids (Lebot *et al*., 2005). Most of the neglected and underutilized crops are being conserved by the elders or left to grow on their own (Matthews, 2002). Cocoyam is one of the neglected and underutilized root and tuber crops (Doungous *et al*., 2015). A large pool of germplasm is being lost due to lack of knowledge on its importance. These losses pose a threat to germplasm conservation which determines the future use of genetic resources (Onwueme, 1999).

A proper analysis of the genetic variation and relationships between accessions is important to understand the genetic variability and its potential use; estimate any possible loss of genetic diversity; offer evidence of the evolutionary forces shaping the genetic diversity and help to choose priority genotypes for conservation (Smith and Duvick, 1989). A prerequisite for any genetic improvement programme is the knowledge of the extent of genetic variation present among accessions and genetic distance among all closely related species with which hybrids could be produced (Beeching *et al*., 1993). This can be achieved through assessment of genetic diversity using genetic markers. Methods for detecting and analyzing genetic diversity have gradually progressed from Mendelian analyses of discrete morphological traits, to statistical characterization of continuously varying quantitative characters, to

electrophoretic assays of biochemical variants and to molecular examinations of DNA variation (Zhang *et al*., 1993).

Cocoyam is valuable because most parts of the plant are edible (Lebot, 2009; Vanker and Slaats, 2013). Cocoyam is mainly used as food and the plant parts are also used as fodder/feed and medicine, including its use as antipoisonous agents against tarantula, scorpion and snake bites (Boakye *et al*., 2018). The usefulness of food composition data at the level of the genetic resource (i.e. taxonomic level below species) is becoming increasingly acknowledged. Research has been providing data to confirm the nutritional superiority of some neglected and underutilized crops and their wild relatives over other more extensively utilized crops (Burlingame *et al*., 2009). Data on nutritional composition would help to assess the value of neglected and underutilized varieties and encourage their sustainable use as well as coming up with a database of nutrient rich plant species that will help in planning nutritional intervention programmes (Grivetti and Ogle, 2000).

Cocoyam is mostly produced through vegetative multiplication which is constrained by many obstacles including the dormancy period in which the initial materials for conventional propagation undergo for approximately five weeks. The conventional method for propagating cocoyam is not adequate to meet the demand because the propagules (materials used as seeds) of cocoyam such as the cormels or their fragments are used for food (Wilson, 1984). Cocoyam is usually preserved under field condition which is risky since diseases or natural catastrophes can cause the loss of genetic resources. *In vitro* storage of plants under minimal growth conditions is a suitable alternative to rely on field collections (Caula *et al.*, 2008).

1.2 Statement of the problem

Cocoyam can play a considerable role in addressing food security as its tubers and leaves serve as food source. However, research on aroid in general and cocoyam in particular is rare. Cocoyam is a neglected and underutilized crop. The use potential, genetic diversity, nutritional composition, *in vitro* propagation capacities and the likes of cocoyam grown in Ethiopia have not been fully examined. There was no known research that was carried out to document the farmers' knowledge and perceptions of cocoyam. The farmers' indigenous knowledge of the agromorphological traits, uses and management of cocoyam exists largely within the farming communities in the rural areas. The Ethiopian farmers who cultivate cocoyam are the main owners of knowledge about the crop. There was a need to compile data on farmers' knowledge and perceptions of agromorphological traits, uses and management of cocoyam for effective usage and conservation of the crop. Information on genetic diversity and nutritional composition of cocoyam is scarce in Ethiopia. It is important to assess the genetic diversity and population genetic structure, and nutritional composition of Ethiopian cocoyam and to compile and disseminate the results. Cocoyam is propagated vegetatively from corms, cormels and their fragments which are not adequate to meet the demands for seed material. Leaving cocoyam propagules under field conditions for an extended period poses risk from diseases, pests and natural catastrophes to the genetic resources. Tissue culture techniques are reliable methods for production of planting materials with the highest rates of multiplication. Currently, there is lack of tissue culture protocol for cocoyam genotypes in Ethiopia. There was a need to develop an efficient micropropagation protocol that can contribute to promote faster and massive production of cocoyam.

1.3 Research questions, hypotheses and objectives

1.3.1 Research questions

This study addressed the following broad research questions:

1. What is the knowledge held by Ethiopian cocoyam cultivators on the agromorphology, use and management of the crop?
2. What is the extent of genetic diversity among cocoyam accessions growing in Ethiopia?
3. What amount of proximate, minerals and antinutritional factors are found in cocoyam growing in Ethiopia?
4. Can efficient *in vitro* propagation protocol be developed for Ethiopian cocoyam?

1.3.2 Hypotheses

The following null hypotheses have been tested.

1. Ethiopian cocoyam farmers have no experiential/customary knowledge on cocoyam crop cultivated in their areas.
2. There is no genetic diversity within and among cocoyam accessions growing in Ethiopia.
3. Cocoyam growing in Ethiopia do not have acceptable proximate and mineral contents and antinutritional factors.
4. Cocoyam growing in Ethiopia cannot be propagated via *in vitro* culture.

1.3.3 Objectives of the study

General objective

The general objective of this study was to characterize cocoyam accessions collected from various locations in southern and southwestern Ethiopia at morphological and molecular levels through documenting farmers' knowledge and perceptions of the crop, assessing nutritional composition and developing a micropropagation protocol for cocoyam from Ethiopia.

Specific objectives

The specific objectives of this study were to:

- document farmers' knowledge and perceptions of the constraints, trait preferences, uses and management practices of cocoyam to guide production, variety design and development and commercialisation of the crop in Ethiopia;
- characterize cocoyam accessions based on morphological traits and select farmer-preferred and high performing genotypes;
- examine the genetic diversity and differentiation of cocoyam accessions using SSR markers to complement morphological data;
- assess the genetic diversity and population structure of cocoyam accessions from Ethiopia using AFLP markers to complement data collected based on SSR markers and morphological traits;
- determine proximate, minerals and antinutritional factors of green- and purple cocoyams growing in Ethiopia;
- establish micro-propagation technique using shoot tip explants derived from green- and purple- cocoyams grown in Ethiopia and to recommend optimum composition of plant growth regulators.

1.4 Organization of the dissertation

This dissertation is organized as follows:

Chapter One presents the general introduction of the dissertation which includes the background, statement of the problem and objectives of the study. Chapter Two presents the literature review on cocoyam origin, distribution, taxonomy, botany, importance and use values, growth condition and cultivation, breeding and tissue culture and genetic diversity studies. Chapter Three presents farmers' knowledge, perceptions and management practices of cocoyam based on the results of the semi structure interview conducted, together with the analyses and the discussion made. This was published in the African Journal of Agricultural Research 12 (35): 2681-2691(2017). Chapter Four presents the work on the genetic diversity, differentiation and population structure of cocoyam from Ethiopia based on morphological traits and molecular markers (SSR and AFLP), of which the AFLP molecular markers-based study part is submitted for possible publication. Chapters Five and Six, respectively, present the nutritional compositions and micropropagation experiment conducted to develop protocol for massive and faster production of green and purple cocoyam morphotypes growing in Ethiopia. These chapters are prepared as manuscripts. Chapter Seven is the part that presents the synthesis bringing together all the findings from the various aspect followed by conclusion and recommendations.

Chapter Two

Literature Review

2.1 Origin and distribution of cocoyam

Cocoyam (*Xanthosoma sagittifolium* (L.) Schott) is likely to have been domesticated and cultivated in the northern part of South America from very ancient times (Giacometti and Leon, 1994). Since recent times, it has been widely spread throughout the tropical world. The main areas of distribution of the crop include the Caribbean (Cuba, Dominican Republic, Puerto Rico, West Indies), USA (Florida, Hawaii), West Africa (Ghana, Nigeria, Cameroon, Togo) and tropical Asia (Indonesia, Malaysia, the South Pacific Islands) (Ponce, 2010). It was introduced to Central and West Africa between the 16th and 17th centuries and has become a subsistence crop in West African countries, which are the major cocoyam producers of the world (Bown, 2000). It was introduced into East Africa through Western Africa and is popular in Tanzania and common in Uganda (Maundu *et al*., 2009). In Ethiopia, it is largely unknown or considered synonymously with *Colocasia esculenta* (L.) Schott), which is locally known as taro or GODERE (Simone, 1992). There is no clear information on how and when cocoyam was introduced into the country. It was not mentioned in the Flora of Ethiopia wherein the tribes of the Araceae are described (Reidl, 1997). The species was found during an ethnobotanical survey of homegrdens and annotated as a new record for the Ethiopian Flora (Zemede Asfaw, 2001a). However, the crop was missed out in volume eight of the Ethiopian Flora, which is supposed to have a checklist of all the plants (wild and cultivated) found in Ethiopia (Hedberg *et al*., 2009).

2.2 Taxonomic description of cocoyam

The genus *Xanthosoma* belongs to the Colocasioideae subfamily, tribe Caladieae of the family Araceae. The basic chromosome number for the *Xanthosoma sagittifolium* is n=13

(Mayo *et al*., 1997). Based on the color of underground stem (corm), the color and shape of off shoots (cormels) and the color of petiole and leaf, some authors allocate the cultivated species of the genus *Xanthosoma* into *X. atrovirens* K. Koch & C.D. Bouche, *X. violaceum* Schott (*X. nigrum*, Stellfeld), *X. caracu* K. Koch & C.D. Bouche and *X. sagittifolium* (L.) Schott and *X. mafaffa* (L.) Schott (Wilson, 1984; Manner, 2011). Others allocate the cultivated species of the genus only into two main species (*X. sagittifolium* (L.) Schott and *X. violaceum* Schott) (Salazar *et al.*, 1985; Monge *et al.*, 1987; Bown, 2000, Weaver, 2000; Sarma *et al*., 2016). However, these species share some characterstics and viable fruit formation have been reported (Oghenekome *et al*., 1992; Onokpise, 1992; Tambong *et al.*, 1992), suggesting that the two may be varieties of the same species.

In general, the division of the genus *Xanthosoma* into species has been difficult (Saborio, 2007). There is much confusion, discrepancies and uncertainties regarding the taxonomy of *Xanthosoma* at the species level (Giacometti and Leon, 1994). Various names have been used synonymously (http://www.theplantlist.org; Govaerts *et al*., 2002; Lim, 2015), the same plant being given more than one species name (O'Hair and Maynard, 2003). Thus, the name of *Xanthosoma sagittifolium* (L.) Schott has usually been given to the most cultivated members of this genus (Giacometti and Leon 1994; Govaerts *et al*., 2002). In the course of this study, we refer to the accepted species status of *X. sagittifolium* in the Araceae family (Govaerts *et al*., 2002) and in The Plant List (http://www.theplantlist.org), which does not tackle taxonomy in further detail.

2.3 Etymology and common names

The etymology of the genus *Xanthosoma* comes from the Greek Xanthos (yellow) and soma, somatos (body) refers to the yellow color of the stem tissue present in several species (Mayo *et al*., 1997). Many common names have been listed for the genus *Xanthosoma* in different

publications (Morton, 1972; Giacometti and Leon, 1994; Lim, 2015). In English, for example, *Xanthosoma* species are known as cocoyam, new cocoyam, tannia, yautia, American taro, arrowleaf, elephant's ear, malanga (Lebot, 2009; Quero-Garcia *et al*., 2010; Lim, 2015). *Xanthosoma sagittifolium* (L.) Schott) and (*C. esculenta* (L.) Schott) frequently share the common terms cocoyam and taro (Shewry, 2003; Manner, 2011).

2.4 Botanical description of cocoyam

Cocoyam is an herbaceous monocotyledonous crop up to 2 m tall with long petioles (Ramawat and Merillon, 2014). There are several leaves with large sagittate or hastate (non-peltate) blades arising directly from the corm (Mayo *et al*., 1997). The leaves have a marginal vein and two large basal lobes. Cocoyam has unisexual flowers, with the female flowers located at the base of a spadix and the male flowers at the top. Between the pistillate and the staminate flowers, sterile flowers are located. The inflorescence is protogynous, pistillate flowers are normally receptive 2 to 4 days before pollen shed (Kay, 1987). Cocoyam can be distinguished morphologically from two other related aroids, mainly taro (*C. esculenta* (L.) Schott) and giant taro (*Alocasia acuminata* Schott), by the place where the petiole meets the leaf and the angle between the petiole and leaf blade. In cocoyam, the petiole attachment is at the margin of the leaf between the lobes. In taro, the petiole attachment is peltate or around the middle of the leaf (Manner, 2011). In cocoyam, mature leaves are angled about 30º off the petiole but for giant taro, the leaf blade and the petiole are in the same plane (Mayo *et al*., 1997).

2.5 Importance and use values of cocoyam

Cocoyam and taro are widely consumed, very ancient aroids included in more than 400 million people's diets. They play very important roles in the livelihood of rural farmers who often use the crops as alternative source of their daily calories (Onyeka, 2014). Cocoyam

has become much more important worldwide (Mwenye, 2009). In many tropical areas, it has overtaken taro as the main edible aroid and has been replacing taro because of its better yield (Bown, 2000). In Pacific Island, cocoyam is often grown because of the higher yield, larger tuber size and taste and hence it is increasingly being adopted by Island peoples (Manner, 2011). Cocoyam is valued as a superior species compared with taro because of its flavor and texture in most Latin America countries (Giacometti and Leon, 1994). Around Bonga city in Kefa, Ethiopia, cocoyam was ranked among the most preferred plants based on the use values, cultural significance and other reasons (Zemede Asfaw, 2001a). Cocoyam is valuable because most parts of the plant can be used for food (Lebot, 2009). It is mainly cultivated for its starchy cormels, but young leaves can be used as a green vegetable and an important source of proteins and vitamins. The central or primary tube (the corm) is acrid and not used for human consumption in most edible species of cocoyam (Lebot, 2009; Manner, 2011), rather mostly used to feed animals. Cocoyam is also used for traditional medicine (Manner, 2011).

2.6 Nutritional profile of cocoyam

Cocoyam is postulated to have superior nutritional value over other major root and tuber staples of West Africa, especially in terms of their protein digestibility and mineral composition (Calcium, Phosphorous and Magnesium) (Lim, 2016).The nutritional composition of cocoyam per 100g edible portion was reported by Kay (1987) as energy 556 kJ/100 g, moisture 70–77 %, protein 1.3–3.7 %, fat 0.2–0.4 %, carbohydrate 17–26 %, fibre 0.6–1.9 %, starch 17–34.5 %, ash 0.6–1.3 %, Ca 20 mg, Fe 1 mg, thiamine 1.1 mg, riboflavin 0.03 mg, niacin 0.0005 mg and ascorbic acid 6–10 mg. The mean values of the proximate composition of cocoyam, red flesh corms and white flesh corms from Ghana were reported by Sefa-Dedeh (2004), respectively, as moisture 57.63–77.41% g and 54.46–71.97%; protein 3.94–4.09% and 4.92–5.50%; fat 0.43–0.74% and 0.28–0.58%; ash 2.68–3.93% and

1.98–3.29%, fibre 1.16–1.77% 1.11–1.72%. The protein, fat, ash and fibre contents decreased in the cooked samples (Akpan and Umoh, 2004). The mineral contents of raw cocoyam corm based on analyses conducted in Nigeria were reported as per 100 g edible portion: Na 66 mg, K 525 mg, Mg 46.6 mg, C 18.64 mg, Fe 0.42 mg, P 70.4 gm Zn 0.4 mg and Mn 0.63 mg. Both white- and red-fleshed corms provide good sources of Na, K, Mg and Ca (Owuamanam *et al*., 2010). Mineral elements analysis showed general decreases in the cooked samples (Akpan and Umoh, 2004). Akpan and Umoh (2004) found no significant differences between the oven-dried and solar-dried samples. However, drum drying reduced the oxalate levels by approximately 50% to average levels and a general reduction in the level of antinutrients was observed after heat treatments. There was an improvement in food quality with respect to the antinutrients but with decreased values of the desired nutrients. However, there was also a general reduction in the undesirable properties of the cocoyam such as the acridity factors caused by crystal of oxalate when the cocoyam corms were cooked (Akpan and Umoh, 2004).

2.7 Growth condition and cultivation of cocoyam

Cocoyam was domesticated in the New World and was under cultivation in tropical America since the Pre-Columbian times (Purseglove, 1972). It is adapted to grow in great variety of substrates and habitats ranging from full sun to deep shaded areas beneath the canopy of natural forests (Manner, 2011). It grows in a wide range of soils except in hard clays or pure sands. It tolerates drier soils, but does not tolerate waterlogged soils (Kay, 1987; Bown, 2000). It produces the best yield on moist, mulched and well-drained soil with pH of 5.5-6.5 (Jackson, 2008). Although cocoyam is a lowland crop, it grows well in upland situations with well distributed rain fall (Manner, 2011). It can be grown under a mean annual temperature of 24°C with variations ranging from 13 to 29°C and in areas where the annual rainfall is 1,000-2,000 mm (Kay, 1987). Yellowing of older leaves indicates maturity.

Harvest usually occurs during the dry season after 9-12 months, but cormels can be harvested after 6-7 months of growth. In water stressed conditions, leaves may die but corms can continue to grow as a perennial crop (Jackson, 2008). Corms can remain viable underground and survive through unfavorable environmental conditions (Castro, 2006).

Cocoyam is propagated vegetatively from corm sets, headsets or cormels (Jackson, 2008). The portion of the corm or cormels is placed in the furrows and covered with a 5 to 7 cm layer of soil. Root formation takes place immediately after planting followed by rapid growth of the shoot (Castro, 2006). Corm set produces a quicker and higher yielding crop while headsets are the lowest yielding (Kay, 1987). To facilitate the growth, the soil needs to be well ploughed and furrowed. Plantation is just before the start of the wet season. However, in areas where rainfall is 2,000-3,000 mm and if an irrigation system is available, planting can be made throughout the year (Wilson, 1984).

2.8 Cocoyam breeding and tissue culture

Flowers are rare in most cocoyam varieties and non-existent in some. When flowers occur in some varieties, they are protogynous (Jackson, 2008), the pistillated flowers are normally receptive 2 to 4 days before pollen is shed (Wilson, 1984). This makes the use of classical breeding methods difficult, but treatments were successfully applied to induce flowering with artificial pollination techniques. Induction of flowering, hand pollination and production of seed and seedlings has become possible at experimental settings (Wilson, 1979; Goenaga and Hepperly, 1990). A spray of gibberellic acid on the tissue culture derived cocoyam plants has provided a high number of inflorescences as well as pollen quantity (Onokpise, 1992). Cocoyam genotypes were combined through artificial crossings but no new improved varieties were reported (Castro, 2006).

In Ethiopia, propagation of cocoyam is entirely convenstional from tuber fragments, which was constrained by scarcity of planting materials (Thehifet Solomon, 2010). Preserving cocoyam under field condition is risky since diseases or natural catastrophes can cause the loss of genetic resources (Caula *et al.,* 2008). Tissue culture technique will open up many possibilities for sustainable production and improvement of many crops. The technique is important in achieving rapid clonal multiplication, recovery of pathogen free plants and germplasm conservation (Larkins and Scowcroft, 1981). It has given the possibility to generate new genotypes through somaclonal variation and a variety of morphological changes were observed in callus derived cocoyam plants (Gupta, 1985). The flowering of tissue culture derived cocoyam occurred 20-30 days earlier than reported for non-tissue culture derived plants (Onokpise, 1992). Induction of tetraploid was successfully achieved by incorporating colchicine in tissue culture medium (Esnard *et al.*, 1993). Cormels from meristem derived cocoyam grew faster and the tuberization started earlier in comparison with conventionally propagated cocoyam (Castro, 2006). *In vitro* protocol was investigated for callus induction and regeneration of cocoyam, offering an opportunity for culturing virus free propagules and undertaking genetic engineering research (Paul and Bari, 2007). Temporary immersion system was reported to be an alternative micropropagation technique to obtain high quality planting material in cocoyam (Pino *et al*., 2012; Niemenak *et al*., 2013).

2.9 Assessment of genetic diversity

Plant genetic resources comprise a diversity of genetic material contained in traditional varieties, modern cultivars and crop wild relatives and other wild species. Genetic diversity provides options to develop new and more productive crops that are resistant to pests and diseases and adaptive to changing environments (Rao, 2004). Measurement and characterization of genetic diversity have always been a primary concern in population

genetic studies (Zhang *et al*., 1993). Understanding the genetic variation within and among populations is crucial for the effective conservation, management and efficient utilization of plant genetic resources. An adequate knowledge of existing genetic diversity in plant population is of fundamental interest for basic science and applied aspects. The ability to identify the genetic diversity is indispensable to effective management and utilization of genetic resources (Mondini *et al.*, 2009). Thus, genetic resource conservation activities require characterization of the germplasm (Karp *et al*., 1997), which is performed using genetic markers (Mondini *et al.*, 2009).

The genetic markers are broadly categorized into three major types: morphological markers, biochemical markers (or isozyme markers) and DNA or molecular markers (Collard *et al*., 2005).

2.9.1 Morphological markers

Genetic diversity is assessed by measuring variation in qualitative morphological traits such as texture, color, growth habit or quantitative traits like yield potential, height, size, weight, etc. The morphological traits have their basis in genetic alterations that lead to visible differences in the phenotype (Rao, 2004). The morphological characterization does not require expensive technology. This approach has, however, certain limitations: large tracts of land are often required for the experiments; highly heritable traits often show little variation; the genetic information provided by morphological characters is often limited and expression of quantitative traits is subjected to strong environmental influence because only a small fraction of genes code for traits is manifested in observable phenotypes (Karp *et al*., 1997; Mondini *et al.*, 2009). The use of morphological markers alone, therefore, 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. Thus, using morphological traits alone is often undesirable although cannot be replaced by any of the biochemical or molecular techniques (Smith and Smith, 1989). The results of biochemical or molecular studies should be considered as complementary to morphological characterization (Karp *et al*., 1997).

2.9.2 Biochemical Markers

Biochemical markers reveal differences between seed storage proteins or enzymes encoded by different alleles at one gene locus (allozymes) or more gene loci (isozymes) (Rao, 2004). Allozymes are variant proteins produced by allelic forms of the same locus. On the other hand, isozymes are different molecular forms in which proteins may exist with the same enzymatic specificity (Markert and Moller, 1959). The more general term for allozymes is isozymes, and refers to any variant form of an enzyme, whereas allozyme implies a genetic basis for the variant form. Isozymes can be differentiated by their relative migration speed during gel electrophoresis due to the amino acid charge differences (White *et al*., 2007).

The major advantages of biochemical markers consist in assessing co-dominance, ease of use and it is a fast method which requires small amounts of biological material. They allow large numbers of samples to be analyzed. The technique is comparatively inexpensive yet powerful method of measuring allele frequencies for specific genes within and among populations, in genetic relatedness studies, mating system estimations and genetic diversity assessments (Mondini *et al*., 2009). The major disadvantages of biochemical markers are the limited number and are influenced by environmental factors or developmental stage (Winter and Kahl, 1995). There are only few isozyme systems per species with corresponding markers; the enzymatic loci represent only a small and not random part of the genome (the expressed part). Therefore, the observed variability may not be representative of the entire genome. They are unable to detect low levels of variations. Comparisons of

samples from different species, loci and laboratories are problematic since they are affected by extraction methodology, plant tissue and developmental stage and environmental factors (Mondini *et al*., 2009).

2.9.3 Molecular Markers

Molecular markers are the most widely used type of genetic markers. Their analyses comprise a large variety of DNA markers which arise from different classes of DNA mutations such as substitution, insertions, deletions and errors in replication of tandemly repeated DNA (Paterson, 1996). The molecular markers are selectively neutral because they are usually located in non-coding regions of DNA. They are unlimited in number and are not affected by environmental factors and/or the developmental stage of the plant. They are detectable in all stages of plant growth (Winter and Kahl, 1995).

Molecular markers have numerous applications in plant genetics such as assessing the level of genetic diversity within germplasm and cultivar identity. They offer great opportunity 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 (Mohan *et al*., 1997). They are powerful diagnostic tools used to detect DNA polymorphism both at the specific loci and whole genome level (Laurentin, 2009).

All types of molecular marker assays have different properties. An ideal molecular marker should possess the following features: (1) be polymorphic and evenly distributed throughout the genome; (2) provide adequate resolution of genetic differences; (3) generate multiple, independent and reliable markers; (4) be simple, quick and inexpensive; (5) need small amounts of tissue and DNA samples; (6) have linkage to distinct phenotypes and (7) require no prior information about the genome of an organism. Nevertheless, no molecular marker presents all the listed advantages. Thus, the most important criteria to determine the type of

molecular marker should be informativeness and ease of genotyping for the specific crop system (Powell *et al*., 1996; Agarwal *et al*., 2008).

2.10 Molecular marker techniques

The basic molecular markers techniques have been broadly categorized into DNA-DNA hybridization-based techniques and polymerase chain reaction (PCR) based techniques. The property of complementary base pairing allowed for DNA-DNA hybridization-based techniques to be developed whereby small pieces of DNA could be used as probes to reveal polymorphisms in the sequences homologous to the probe. PCR technique enables the production of a large amount of a specific DNA sequence without cloning, starting with just a few molecules of the target sequence (White *et al*., 2007).

i. DNA-DNA hybridization-based technique

Restriction fragment length polymorphism (RFLP) is first class DNA-DNA hybridization-based markers and used for detecting variation at the DNA sequence level (Botstein *et al*., 1980). In RFLP technique, DNA polymorphism is detected by hybridizing a chemically labeled DNA probe to a Southern blot of DNA digested by restriction endonucleases, resulting in differential DNA fragment profile. The differential profile is generated due to nucleotide substitutions or DNA rearrangements like insertion or deletion (Agarwal *et al*., 2008). The RFLP markers are highly polymorphic, codominantly inherited and highly reproducible; exist throughout the genome, high heritability and locus specific. The RFLP based method provides an opportunity to simultaneously screening of numerous samples. However, the technique is time-consuming, involves expensive reagents, requires large quantities of high quality DNA and prior sequence information for probe generation. These limitations led to the conceptualization of a new set of less technically complex methods known as PCR based techniques (Mondini *et al*., 2009).

ii. The PCR based techniques

Random Amplified Polymorphic DNA (**RAPD):** The RAPD technique uses a short single primer (usually 10 bases) to amplify anonymous stretches of DNA under specific PCR condition. RAPD provides a more arbitrary sample of the genome and can detect unlimited number of loci. The number of amplified fragments depends on the distribution and number of annealing sites throughout the genome (Marwal *et al*., 2014). There is no specific target DNA, so each particular primer adheres to the template DNA randomly. As a result, the nature of the obtained products is unknown. RAPD analysis shows the difference in the pattern of bands amplified from genetically distinct individuals behaves as Mendelian genetic markers. The most limiting properties of RAPD molecular marker technique are low reproducibility, dominance inheritance and homology. Although these problems were raised, RAPDs have been widely used for studies on taxon identification, hybridization and population genetic structure studies (Semagn *et al*., 2006).

Amplified Fragment Length Polymorphism (AFLP): The AFLP is DNA fingerprinting technique that combines the power of the RFLP with the flexibility of PCR based technique by ligating primer recognition sequences (adaptors) to the restricted DNA (Lynch and Walsh, 1998). This technique involves the following steps: (1) restriction of the DNA and ligation of oligonucleotide adapters; (2) Pre-selective amplification and selective amplification of sets of restriction fragments and (3) gel analysis of the amplified fragments.

The AFLP technique is a powerful DNA fingerprinting technique applicable to any organism without the need of prior sequence information (Vuylsteke *et al*., 2007). It has a capacity to simultaneously screen representative DNA regions distributed throughout the genome (Semagn *et al*., 2006). The technique is highly reproducible and sensitive for detecting polymorphism. The molecular basis of AFLP polymorphism can be a 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 can result in detectable polymorphism (Paun and Schonswetter, 2012). The major advantage of the technology is in the high marker density which allows scoring of a large number of markers in a given population. The frequency with which AFLP markers are detected depends on the sequence polymorphism between the tested DNA samples (Somers, 2004). It has been employed for a variety of applications such as to assess genetic diversity within species or among closely related species, to infer population level phylogenies and biogeographic pattern, to generate genetic maps and to determine relatedness among cultivars (Somers, 2004; Paun and Schonswetter, 2012).

Inter Simple Sequence Repeats (ISSR): The ISSR technique is a PCR based multilocus marker system that employs oligonucleotide primers homologous to microsatellites. PCR product obtained only if simple sequence repeats (SSRs) are found in opposite orientation within a PCR amplification distance, with flanking sequence matching the oligonucleotides (Tomar *et al*., 2010). It is semi-arbitrary markers amplified by PCR in the presence of one primer. It does not require genome sequence information (Meyer, 1993). It is a fast, inexpensive genotyping technique based on variation in the regions between SSRs. It is highly polymorphic but dominant marker. The method has a wide range of uses including genetic fingerprinting, gene tagging, clonal variation detection, cultivar identification, phylogenetic analysis and assessment of hybridization (Abdel-Mawgood, 2012). Like RAPD markers, ISSR markers are quick and easy to handle, but they seem to have the reproducibility of SSR markers because of the longer length of primers (Bornet and Branchard, 2001).

Simple Sequence Repeats (SSR): The SSRs also called microsatellites are short tandem repeats, their length being 1 to 10 bp, most typically, 2-3 bp. The number of repeated units varying widely among organisms as high as 40 copies of the repeated unit (Lopez-Flores and Garrido-Ramos, 2012). When DNA is being replicated, errors occur in the process and extra sets of these repeated sequences are added to the strand. Over time, these repeated sequences vary in length between one cultivar to another. The DNA sequence flanking SSRs are known to be conserved within species and sometimes in the higher taxa. The DNA sequence flanking SSRs have been used to design suitable primers for amplification of SSR loci. Differences in length of the PCR product from different genotypes reveal the SSR polymorphism. The length difference attributed to the variation in the number of repeat units of a particular locus possibly caused by slippage during DNA replication (Tomar *et al*., 2010).

SSR markers are the most widely applied class of molecular markers used in genetic studies with applications in many fields of genetics including genetic conservation, population genetics, molecular breeding and paternity testing. This range of applications is due to the fact that they are co-dominant and multi-allelic, highly reproducible and have high resolution (Oliveira *et al*., 2006).

Single nucleotide polymorphism (SNP): The SNPs are the most abundant class of DNA markers. They are single base pair positions in genomic DNA at which different sequence alternatives (alleles) exist in normal individuals in some populations, wherein the least frequent allele has an abundance of at least 1% or greater (Brookes, 1999). SNPs are less mutable as compared to other markers, particularly microsatellites. The low rates of recurrent mutation make them evolutionarily stable. SNPs are a novel class of DNA markers that recently became highly preferred in genomic studies. They occur more frequently than

any other type of marker and are very near to or even within the gene of interest. They have tremendous applications and prospects in crop genetics. They are excellent markers for studying complex genetic traits and for understanding the genomic evolution. They can be used in association studies, tagging of economic important genes, genotyping, diversity analysis and evaluation among the species (Jehan and Lakhanpaul, 2006). The molecular marker techniques differ from each other with respect to important features (Table 2.1).

Table 2.1 Comparison of different characteristics of molecular markers

Characteristics	Molecular marker					
	RFLP	**RAPD**	**AFLP**	**ISSR**	**SSR**	**SNP**
Degree of polymorphism	High	medium	medium	medium	medium	high
Locus specific	Yes	no	no	no	no	yes
Dominance (D)/ co-dominance (C)	C	D	D	D	C	C
Reproducibility	high	low	high	high	high	high
Abundance	high	high	high	high	medium	high
Sequence information required	yes	no	no	no	yes	yes
Quantity of DNA required	high	low	medium	low	low	Low
Amenable to automation	no	yes	yes	yes	yes	yes
Technical requirement	high	low	medium	low	medium	medium
Cost per assay	high	low	medium	low	medium	medium

RFLP - restriction fragment length polymorphism; RAPD - random amplified polymorphic DNA; AFLP - amplified fragment length polymorphism, ISSR - inter simple sequence repeats; SSR - simple sequence repeat; SNP - single nucleotide polymorphism (compiled from Oliveira *et al*., 2006; Agarwal *et al*., 2008; Henry, 2008 and Mondini *et al*., 2009)

2.11 State of knowledge on cocoyam genetic diversity

Morphological, biochemical and molecular based genetic markers have been used for genetic diversity analysis of cocoyam accessions. Historical and morphological evidences were used to clarify the diagnostic features of cocoyam species growing in Nigeria (Mbouobda *et al*., 2007). The multiple component analyses of morphological parameters were used to distinguish taro and cocoyam genotypes in Malawi (Mwenye, 2009). Morphological parameters which were used to estimate genetic parameters for yield and its components in cocoyam genotypes from Bangladesh showed the presence of significant differences with wide ranges of variation among the genotypes (Paul and Bari, 2012). The

authors stated that the genotypic variances for most of the characters were remarkably higher than their corresponding environmental variances. In Ethiopia, the genetic diversity of cocoyam collections was studied using multivariate analysis at Jimma Agricultural Research Center based on 16 quantitative traits (Solomon Fantaw *et al.*, 2014a). The authors stated that the existing diversity among the cocoyam genotypes can give an opportunity for genetic improvement for desirable characters.

Xanthosoma species from different locations in Indonesia were characterized using the morphological parameters and isozyme markers. The results showed that the correlation between morphological data and data from esterase and glutamate were very good (0.97 and 0.94), but there were no obvious differences among the samples from different locations of the country (Nurmiyati *et al*., 2009).

RAPD analysis of cocoyam has revealed that very little genetic variation exists within the USDA-ARS collections in Florida (Schnell *et al*., 1999). But in Ghana, 70 cocoyam accessions analyzed with RAPD markers showed significantly higher polymorphisms (Offei *et al.*, 2004). The AFLP analysis was carried out to assess the status of species of *Caladium* versus *Xanthosoma* showed that AFLP can distinguish between the different species by their unique and different banding patterns (Loh *et al*., 2000). RAPD analyses, - (CA) 8RY-microsatellite repeat unit, chloroplast- (*trn*R/Q 01; *trn*L 03/04) and mitochondrial-specific (*NAD* 4.2/4.3; *rps*14/*COB*) primer pairs revealed 4 species of *Xanthosoma* among the cocoyam accessions from Jamaica (Brown and Asemota, 2009). SSR markers identified by Cathebras *et al*. (2014) gave variable degrees of heterozygosity, observed at levels ranging from 0.00 to 0.97. A set of six chosen long terminal repeat (LTR) primers yielded 92% polymorphic bands across 20 cocoyam accessions (Doungous *et al*., 2015).

Chapter Three

Farmers' Knowledge and Perception of the Constraints, Trait Preferences, Uses and Management Practices of Cocoyam (*Xanthosoma sagittifolium* (L.) Schott) in Ethiopia

Abstract

Cocoyam (*Xanthosoma sagittifolium* (L.) Schott) is one of the tuberous root crops in the monocotyledonous family known as Araceae and has been grown in Ethiopia for a good number of years. Less research attention is given to cocoyam as it is one of the neglected and underutilized crops in Ethiopia. There was no concrete ethnobotanical research that has been carried out to document indigenous knowledge held by Ethiopian farmers cultivating and using cocoyam. In this study, a survey was conducted to assess the state of cocoyam in Ethiopia based on farmers' knowledge and perceptions. Purposive sampling technique was used to select 50 farmers from five zones. During our survey, two morphotypes (green colored and purple colored) were observed. Different local names were given to farmer-recognized types of the crop. The most commonly encountered local names were KENI ZHANG, CUBI ZHANG, SUDAN KIDO and SAMUNA BOINA. The naming systems were, in most cases, followed by the local name given to taro (*Colocasia esculenta* (L.) Schott), as seen in the cases of ZHANG and BOINA. The local term GODERE or taro was also used for both species. Cocoyam is locally used for food (100%), fodder (60%) and other purposes such as medicine and organic fertilizer. Farmers use local methods in the preparation of cormels for food and medicine. Corms were the preferred planting materials for Ethiopian farmers. The farmers' preference to the crop was related mainly to serving as emergency food and as fodder whereas hard texture, low market demand, sour taste and unpleasant smell of cocoyam were traits disliked by farmers. In this study, useful comprehesive knowledge about cocoyam in Ethiopia was documented and this helped to sharply focus on the morphological and molecular traits, the nutritional compositions and the micropropagation aspects. The quality and productivity of cocoyam need to be improved based on farmer preferred attributes to ensure dissemination of the useful aspects and enhance its sustainable production in Ethiopia.

Keywords: Cocoyam, ethnobotany, field survey, indigenous knowledge

3.1 Introduction

Cocoyam (*Xanthosoma sagittifolium* (L.) Schott) is an herbaceous tuberous root crop that belongs to the monocotyledons in the Araceae family. Cocoyam is likely to have been domesticated in the northern part of South America where it was cultivated from very ancient times (Giacometti and Leon, 1994). It is widely cultivated in tropical America, Africa, Asia, Caribbean and other parts of the tropics mainly by small-scale farmers (Bown, 2000). It is unknown to many people in east Africa (Raemaekers, 2001). In Ethiopia, cocoyam is largely known synonymously with taro (*Colocasia esculenta* (L.) Schott), which is known to have been grown in Ethiopia since immemorial times (Simone, 1992). Cocoyam was not mentioned in the Flora of Ethiopia wherein the tribes of the Araceae are described (Reidl, 1997). Later in 2001, it was listed among the crop species cultivated in Ethiopian homegradens and annotated as a new record for the Ethiopian Flora (Zemede Asfaw, 2001a). But other reports mentioned that cocoyam accessions had been introduced into Ethiopia early in 1980s (Fujimoto, 2009). Surprisingly, however, the crop was again missed out in volume eight of the Ethiopian Flora (Hedberg *et al*., 2009), which is supposed to have a checklist of all the plants (wild and cultivated) found in Ethiopia.

Cocoyam has spread widely and has become an important part of the agricultural and food systems of indigenous communities in southern, southwestern and western parts of Ethiopia, where root and tuber crops are part of the local food systems of the people. It was ranked by farmers second among the top 10 most preferred plants around Bonga city, in Kefa, based on the use values, adaptability, cultural significance and other reasons (Zemede Asfaw, 2001b). It grows even in poor soils and under dry conditions that are too difficult for cultivation of other tuberous root crops. It diffused mainly into the lowest settlements (below 1000 m.a.s.l) (Fujimoto, 2009).

Despite its increasing importance, limited research efforts have been directed to cocoyam. The preservation and use of cocoyam is far less unlike other root crop genetic resources (Matthews, 2002). The loss of germplasm has become the major challenge to its production and poses a threat to germplasm conservation (Onwueme, 1999). Most of the research on neglected and underutilized crops are being conserved by the elders and/or are being left to grow on their own. Hence, these are being lost due to lack of knowledge on their importance (Matthews, 2002). Ethnobotanical study can be important in genetic resource conservation and application in crop improvement. Early advances in ethnobotany provided us with utilitarian benefit of plants and on that basis, plants were classified. Today, such documentation is essential for the conservation of earth's vast biological resources (Osawaru and Ogwu, 2015). Knowledge on different qualities that affect the use, preparation and consumption is important to plant breeders because it is critical for the acceptance of new cultivars by consumers (Matthews, 2002).

Research on cocoyam has been scarce in Ethiopia except a few attempts initiated at agricultural research centers to collect and maintain its germplasm. Ethiopian farmers hold enormous indigenous knowledge of the crop that they cultivate. They are the main owners of knowledge about the uses, cultivation practices and management of the crop. No concrete work to date has looked at the cocoyam grown in the country in commensurate with its importance to smallholder farmers. The indigenous knowledge of farmers on cocoyam has remained largely within the domain of farmers' knowledge in the rural areas. The main aim of this study was, therefore, to collect the knowledge that is available with the farmers on cocoyam to retrieve the knowledge held by the farming communities who cared to manage and use this neglected and underutilized crop species.

3.2 Materials and methods

3.2.1 Description of the study area

The Federal Democratic Republic of Ethiopia is composed of regional states. The regions are organized into zones which are cluster of woredas. Kebeles are the smallest administrative units within the Woredas. The study area covered the cocoyam belt between latitudes 06°20.301' N and 07°25.213' N and longitudes 035°29.829' E and 037°47.173' E. Farms were located at altitudes from 1132 to 2319 meters above sea level (Fig. 3.1; Table 3.1).

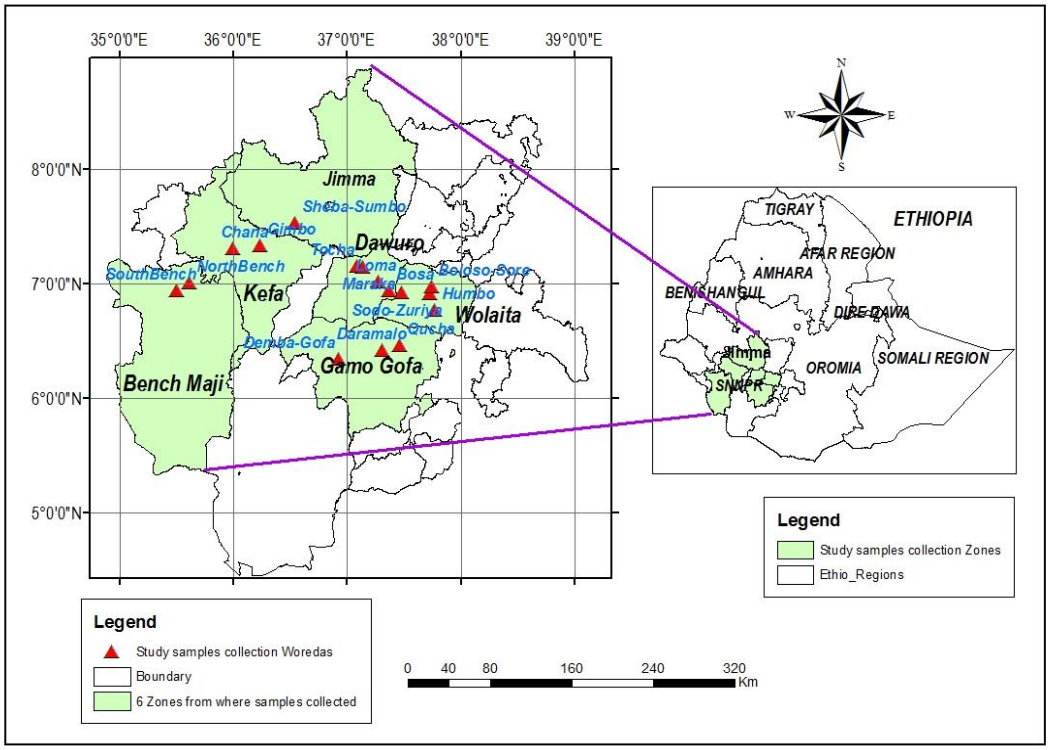

Fig. 3.1 Map of Ethiopia showing the location of the study area. Map was prepared with ArcGIS Desktop (ArcGIS Desktop 10.2.2., Esri)

3.2.2 Sampling frame and informant selection

Farmers' knowledge and perceptions of cocoyam was collected from 10 Woredas of five zones (Table 3.1) involving 50 informants, following the steps in purposive sampling technique outlined by Tongco (2007). Purposive sampling technique is a type of non-probability sampling that is most effective when one needs to describe a phenomenon and to generate data with knowledgeable informants (Tongco, 2007). To gather data from

knowledgeable and reliable informants, two Woredas in which cocoyam grows at a larger extent were purposefully selected from each of five zones by consulting zonal agricultural offices. Five farmer-experts who have rich experience in growing cocoyam were purposefully selected from each woreda with the assistance of leaders of the farmers' associations and plant experts of the farmers' associations.

Table 3.1 Study areas and number of respondents replied to interview guide

Zone	Woreda	No of respondents		Altitude range (masl)
		M	F	
Bench-Maji	South-Bench	3	2	1312-1594
	North-Bench	3	2	1288-2070
Kefa	Chena	4	1	1800-2136
	Gimbo	3	2	1405-1745
Dawuro	Tocha	4	1	1245-1535
	Loma	4	1	1910-2319
Wolaita	Kindo-Koysha	2	3	1132-1315
	Humbo	3	2	1746-1925
Gamo-Gofa	Qucha	4	1	1204-1692
	Demba-Gofa	4	1	1189-1816
Total		**34 (68%)**	**16 (32%)**	1132-2319

masl-meters above sea level

3.2.3 Ethical consideration

Before collecting farmers' perception of agromorphological traits, how they manage and use cocoyam and collection of plant materials, informants were informed about the purpose of the research and its benefits clearly underlining the fact that the results will be used for academic purposes and that no commercial interest will be attached to it. When the farmers assertively said that this research is useful and agree to provide the required information on their own will, they were interviewed.

3.2.4 Data collection and analysis

The selected farmers were interviewed (see plate below) using semi-structured interview guide (Appendix 1). As indicated by Albuquerque *et al.* (2014) in semi-structured interview, the questions are partially established by the researcher but largely flexible and more

attention is to be paid to issues that arise during the interview. Semi-structured interviews give interviewees the possibility to express their point of view. It was determined to be the most appropriate in order to gather information for qualitative research, creating the opportunity for interviewees to discuss topics that may have been dismissed and might be of importance to them. The selected farmers were encouraged to express information in the way they knew and perceived cocoyam through experience. Farmers were told to be free to tell all what they know about cocoyam on their own accord using their native languages although they were being interrupted abruptly when they deviate from the main topic of the interview. To collect information on precise themes, the following factors were mainly considered while recording the data (1) The cocoyam farming experience of farmers; (2) the introduction time of cocoyam to the farmers' locality and its origin in garden; (3) the local name given to cocoyam and its meaning; (4) whether Ethiopian farmers distinguish cocoyam from taro and if they do, how they do it; (5) the general distribution of cocoyam in the study area; (6) local uses of cocoyam; (7) the size of land allotted for cocoyam cultivation; (8) farmers' preferred and/or disliked traits of cocoyam; (9) the farmers' planting materials of cocoyam and the cultivation methods; (10) the time course of planting and harvesting and (11) methods that farmers have adopted for conservation of cocoyam. The resulting data were entered into excel spreadsheet and descriptive statistical analysis was made. The resulting values expressed as percentage.

Plate: Interview being carried out with farmers (Photo by Eyasu Wada, 2014)

3.3 Results

3.3.1 Age of farmers and their experiences in cocoyam cultivation

The age of the farmers who responded to the interview ranged from 20 to 83 years. The respondents had lived in their respective area at least for 15 years. A total of 26%, 30%, 36% and 8% of the respondents had grown the crop for 10 or less years, 11-20 years, 21-30 years and for more than 30 years, respectively. Seventy-four percent of the respondents had been involved in cocoyam farming activities for more than 10 years (Table 3.2).

Table 3.2 Age of respondents and number of years of cocoyam cultivation

Zone	Variables								
	Age (year)					Farming experience (year)			
	20-30	31-40	41-50	51-60	≥61	1-10	11-20	21-30	≥31
Bench-Maji	4	6	-	-	-	3	4	3	-
Kefa	1	5	2	2	-	2	4	3	1
Dawuro	-	5	2	1	2	2	2	6	-
Wolaita	-	3	3	1	3	4	3	2	1
Gamo-Gofa	2	2	2	1	3	2	2	4	2
Total	7 (14) *	21(42)	9 (18)	5 (10)	8(16)	13(26)	15 (30)	18 (36)	4 (8)

*numbers in parenthesis indicate the percentage values

3.3.2 Distribution and cultivation of cocoyam in the study area

During this study, only the green colored cocoyam was observed in Bench-Maji and Kefa zones while both green- and purple- colored cocoyams (Fig. 3.2) were observed in Dawuro, Wolaita and Gamo-Gofa zones. In these zones, purple cocoyam was seen more frequently than the green cocoyam. According to the farmers, cocoyam cultivation has been increasing in their localities due to its re-emerging ability from under buried corms.

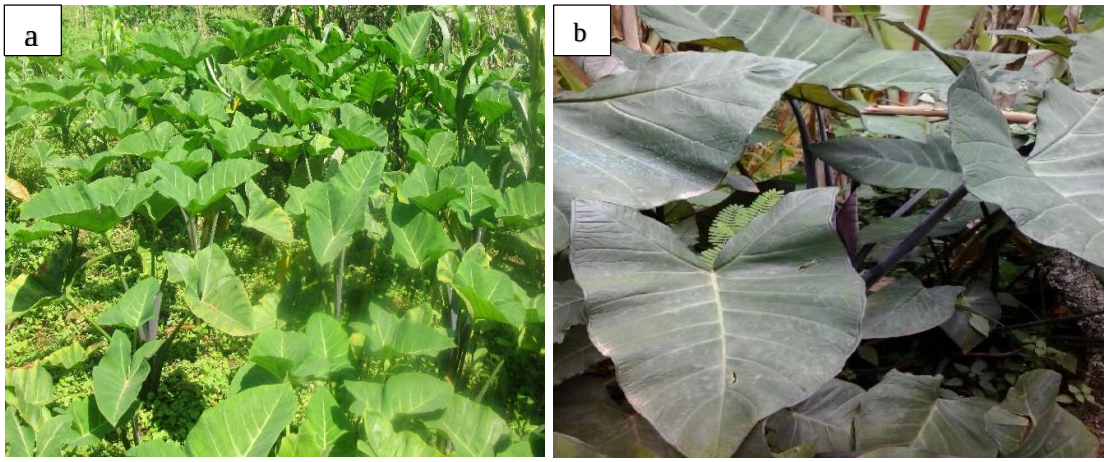

Fig. 3.2 Cocoyam (*Xanthosoma sagittifolium*) plants from Ethiopia: green cocoyam, CUBI ZHANG, from Bench-Maji Zone, South-Bench Woreda (a) and purple cocoyam, SAMUNA BOINA from Wolaita Zone, Humbo Woreda (b) (Photo by Eyasu Wada, 2014).

In all the areas, cocoyam is cultivated in the form of smallholders. Farmers mainly rely on rainfall for cocoyam cultivation. Most farmers (94%) cultivate cocoyam at homegarden patches as a backyard garden crop that grows closely associated with the houses. Cocoyam is also found in the natural ecosystem and around road sides as well as in the shade of other plants or as an ornamental plant in the urban centers (Fig. 3.3).

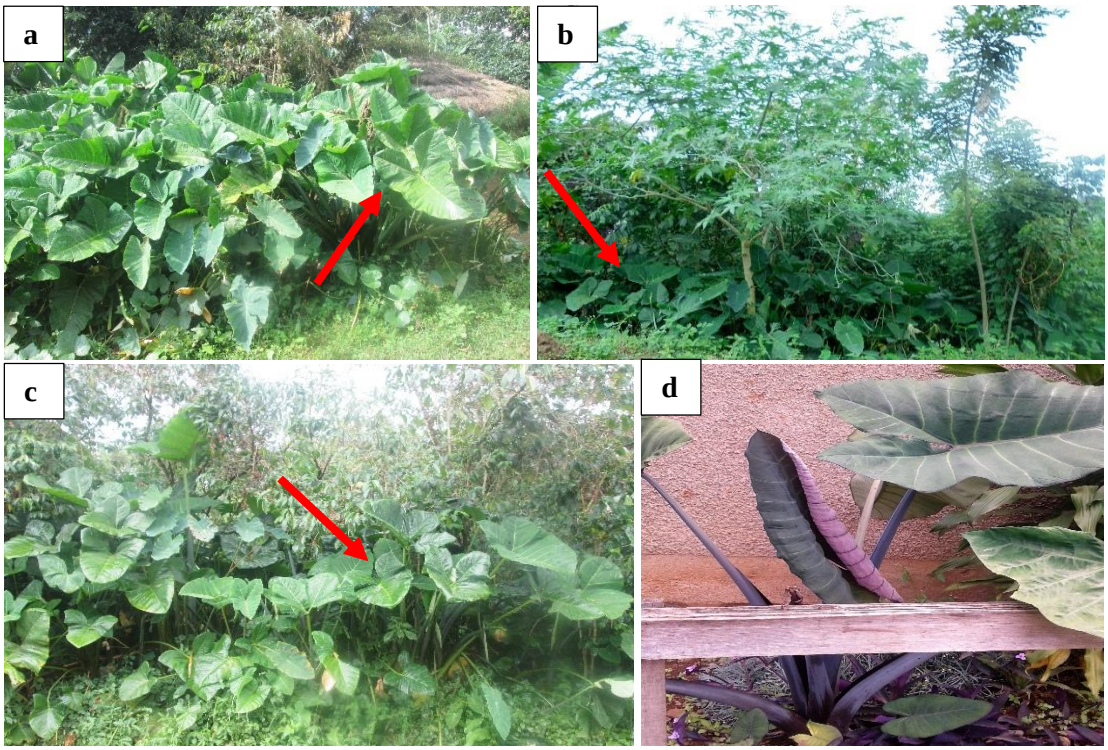

Fig. 3.3 Cocoyam plants: at homegarden (a), in natural ecosystems (b) in the shade of coffee plants (c) and as ornamental in the urban centers (photo by Eyasu Wada, 2014).

3.3.3 Local names of cocoyam and meanings

In different ethno-linguistic communities living in the surveyed areas of Ethiopia, different local names are used for *X. sagittifolium.* The naming systems are, in most cases, followed by the local name given to taro (*C. esculenta*). Most respondents (70%) consider cocoyam as a variety of taro. They distinguish it from taro mainly by leaf pigmentation and size, shape of cormels and size of corms.

The local names KENI ZHANG and CUBI ZHANG were used for cocoyam in the Bench-Maji Zone. The majority (80%) of the respondents from Bench-Maji zone relate the terms KENI or CUBI as the crop was introduced from Kenya or from Cuba, respectively. The term ZHANG is used for taro in *Bench* language. The terms GOCHELI KIDO and SUDAN KIDO were used to refer to cocoyam by the farmers of Kefa zone. According to the respondents, the term SUDAN is given to cocoyam to indicate that the crop was introduced into Kefa zone from Sudan.

The local names TEPIYA BOINA, SAMUNA BOINA, GUDETA andAGARFAwere used for cocoyam in the Dawuro zone. BOINA is a local term used for taro. Farmers use the term TEPIYA BOINAfor the green cocoyam and consider that the green cocoyam had been introduced into their areas from Tepi area of Bench-Maji Zone. The term SAMUNA meaning soap in *Dawuroto* language was given to purple cocoyam due to its cormel which has the smell of soap when cooked. In Dawuro area, prefix ZO'O meaning red was used for purple cocoyam to distinguish it from the green cocoyam.

The local names SAMUNA BOINA, DAWURO BOINA, FARANJA BOINA, TONNEKA and BADADIYAwere used for cocoyam in Wolaita zone. The meaning of the term SAMUNA is similar to that given in Dawuro zone. The term DAWURO BOINAis used for cocoyam in Kindo-Koysha Woreda of Wolaita zone, which is bounded by Dawuro zone indicating that cocoyam was introduced into Kindo-Koysha Woreda of Wolaita zone from Dawuro zone. Farmers' use the term

FARANJA BOINA, which is to mean '*foreign taro'* for cocoyam to indicate that it is an introduced crop. According to respondents the term TONNEKA is used for purple cocoyam because it has a sour nature when eaten. The term BADADIYAis used for green cocoyam to indicate its giant size. SAMUNA BOINA, TONNEKA and BADADIYAare local terms given to cocoyam in Gamo-Gofa zone. The meanings of these terms are similar to those explained in Dawuro and Wolaita zones as the languages spokem in these zones belong to *Omotic* language family.

3.3.4 Cocoyam introduction into the study areas and tuber sources for the first time garden cultivation

The majority (84%) of the farmers did not remember the time when cocoyam was introduced into their areas. Farmers in South-Bench Woreda of the Bench-Maji Zone remembered that the crop was introduced into their areas in mid 1970s by Cubans, who came to Ethiopia to build micro dams after the 1974/1975 major drought. Some farmers of Kefa Zone recall that cocoyam was introduced into their areas two to three years before the fall of the Derg regime (previous governance) in 1991. According to some respondents from Tocha and Loma Woredas of Dawuro Zone, cocoyam was introduced during the settlement program (1986). According to 34 (68%) farmers, cocoyam tubers for garden cultivation comes from market, nearby area, neighbourhood, family and relatives but 16 (32%) did not remember the origin of cocoyam in their gardens for the first time (Table 3.3).

Table 3.3 Source of cocoyam clones used for the first time planting in gardens (numbers in the table indicate the number of respondents)

Study area		**Source of cocoyam clones used for the first time palnting**				
Zone	Woreda	Market	Nearby zone	Neighbor	Family and relative	Not remember
Bench-Maji	South-Bench	-	-	2	1	2
	North-Bench	-	-	2	1	2
Kefa	Chena	-	-	2	1	2
	Gimbo	-	-	2	2	1
Dawuro	Tocha	-	2	-	-	3
	Loma	-	1	1	-	3
Wolaita	Kindo-Koysha	2	2	1	-	-
	Humbo	1	-	1	-	1
Gamo-Gofa	Qucha	-	-	2	2	1
	Demba-Gofa	1	-	2	1	1
Total		**4 (8 %)**	**5 (10%)**	**15 (30%)**	**10 (20%)**	**16 (32%)**
				34 (68%)		

3.3.5 The farmers' planting material and cropping system

Fifty percent of farmers use corm and headsets for cocoyam propagation. The remaining half use corm and cormels as planting material. Farmers prefer using corms and headsets for cocoyam propagation because cormels are used for consumption while the corms have no food value. In the study areas, cocoyam is cultivated in a mono and mixed cropping system. Thirty percent, 30% and 40% of the respondents cultivate cocoyam by mono, mixed and both cropping systems, respectively. When cocoyam grows in mixed cropping system, it grows mainly mixed with taro (*Colocasia esculenta* (L.) Schott), enset (*Ensete ventricosum* (Welw.) Cheesman), banana (*Musa* spp.) or coffee (*Coffea arabica* L.). Farmers prefer to cultivate cocoyam mixed with coffee (Fig 3.3c) because cocoyam is shade tolerant and its leaves serve as organic fertilizer for coffee when detached from the plant.

3.3.6 Land preparation, planting and harvesting of cocoyam

The time of land preparation and planting time of cocoyam varies in different zones. In Bench-Maji zone, the land is prepared in May and planting is mainly from June to July. In Dawuro, Wolaita and Gamo-Gofa zones, land preparation is from late November to January

and planting takes place from February to March at the onset of the rainy season. According to the respondents, piecemeal way of harvesting (harvesting cormels leaving the mother plant in the place as a perennial crop) take place begging from 7 months after plantation. The crop can be harvested 12 months after plantation. During harvesting, farmers use local methods, digging around the plant and applying force to uproot the crop.

3.3.7 The local uses of cocoyam

Respondents (100%) use cocoyam for food although there is difference in the use of cocoyam for food, the part of cocoyam used for food and the mode of preparation from one zone to another. Six and 3 respondents from Bench-Maji and Kefa zones, respectively, responded that leaves of cocoyam serve as organic fertilizer for coffee when leaves are detached from the plant (Fig. 3.4). Farmers of Dawuro, Wolaita and Gamo-Gofa zones, where purple cocoyam was observed, responded that the purple cocoyam has less food value when other crops are available. According to farmers of these zones, purple cocoyam serves only as emergency food and mainly eaten by those people who are at low economic status and are food deficient. The purple cocoyam is classified by the respondents to be a non-preferred food crop because of its sour taste and unpleasant smell.

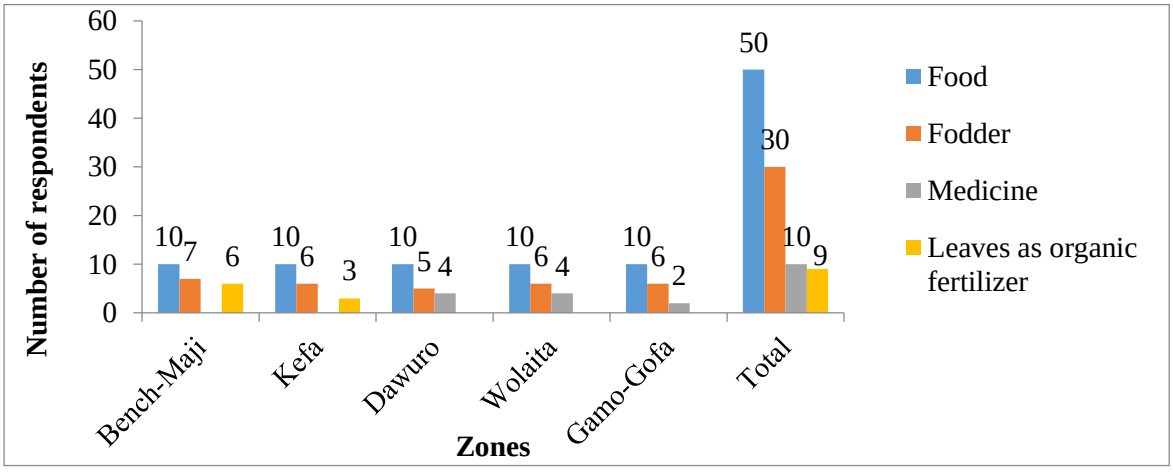

Fig. 3.4 Local uses of cocoyam in the study zones based on the interview of 10 key informants per zone. The number of respondents is shown by vertical line as well as on the top of bars

Mode of preparation when used for food: In all of the surveyed areas, cocoyam cormles are eaten after cooking. The cocoyam shoots (young leaves) are used for food by preparing as cabbage (cooked leafy vegetable) and/or mixing with cabbage in Bench-Maji Zone. Respondents from Dawuro zone indicated that only those people who are traditionally considered belonging to the lower social stratum eat the leaves of cocoyam as cooked leafy vegetable.

Cocoyam as a medicinal plant: A total of 10 farmers from Dawuro, Wolaita and Gamo-Gofa zones responded that purple cocoyam is considered to have medicinal value (Fig. 3.4). According to these farmers, purple cocoyam is used to treat WULAWUSHIYA (related with hepatitis), BARQA (postpatrum deperssion) and GERGEDA (related with rheumatoid arthritis). WULAWUSHIYA is a general term for yellow eye, for disease that affects liver or disease that burns urinary tract and symptomized by the presence of blood in urine. BARQA is a type of mood disorder associated with childbirth. To relieve from the disorder, a woman who gave birth use the purple cocoyam cormels after a course of preparation. According to respondents the meal is prepared by cooking using pot, peeled, grinded, mixed with butter and spices such as garlic (*Allium sativum* L.), black cumin seeds (*Nigella sativa* L.) and onion (*Allium cepa* L.). The respondents mentioned that the leaf of purple cocoyam is used to treat GERIGEDA which is generalized pain in joints. Farmers who use purple cocoyam to treat GERIGEDA mentioned that the pain feeling areas of the body are rubbed with young cocoyam leaves.

3.3.8 Farmers' perceptions of cocoyam characters and uses

Cocoyam was preferred by all farmers as it is serving as food crop (Table 3.4). Cocoyam was also a preferred crop by respondents due to its leaf edibility in addition to cormels and piecemeal way of harvesting (possibility of harvesting cormels by leaving mother plant in place), short cooking time and possibility of cormels to be roasted on hot stone. However,

farmers dislike cocoyam due to its sour taste and unpleasant smell (purple cocoyam), inedibility of the corm and low market demand (Table 3.4).

Table 3.4 Characters/uses of cocoyam and farmers' preference

Preferred characters/uses	No of respondent from each Zone					Total respondents	
	Bench-Maji	Kefa	Dawuro	Wolaita	Gamo-Gofa	No	%
Serve as food	10	10	10	10	10	50	100
Serve as fodder	7	6	5	4	6	28	56
Leaves serve as fertilizer	9	6	2	2	4	23	46
Short cooking time	10	8	-	-	-	18	36
Piecemeal (harvesting cormels, leaving the plant in place)	9	4	4	-	-	17	34
Young leaves edible	10	-	-	-	-	10	20
Medicinal	-	-	4	4	2	10	20
High yield	4	3	-	-	2	9	18
Disliked characters/uses							
Sour taste, unpleasant smell and continuously eating could be irritable	6	5	8	10	8	37	74
Corm inedibility	10	10	4	5	5	39	78
Hard texture to eat	6	5	6	8	7	32	64
Not appetizing	2	1	4	-	-	7	14
Low market demand	10	10	10	10	10	50	100

3.4 Discussion

In Ethiopia, different local names are used for *X. sagittifolium.* The meanings of these names were linked either with the area of collection or the crop's particular trait such as the growth condition. Similar study conducted in the Edo State, Nigeria, indicated that the local people distinguish cocoyam local types by area of collection (Osawaru and Ogwu, 2015). Various local names have also been used for *X. sagittifolium* worldwide (Giacometti and Leon, 1994; Mayo *et al*., 1997; Raemaekers, 2001; Lebot, 2009; Quero-Garcia *et al*., 2010). In this study, it was found that most of local names were followed by local names given to taro (*C. esculenta*). The Ethiopian farmers consider cocoyam as a variety of taro. According to Maundu *et al*. (2009), *Xanthosoma* and *Colocasia* frequently share the local names taro and cocoyam.

We found that the term taro or GODERE (Amharic term) is used for both aroids (*Colocasia* and *Xanthosoma*). However, the local farmers distinguish the two crops and give them different local names. In most literature, cocoyam (*X. sagittifolium*) is discussed jointly with the taro (*C. esculenta*) (Onwueme, 1999). Morton (1972) noticed that the familiarity of *Xanthosoma* had been burdened by highly localized vernacular names and hence she proposed the general adoption of the euphonious and appetizing term, cocoyam, as a collective trade name for *Xanthosoma* species. However, the term cocoyam has been used not only for *Xanthosoma* rather it has been used for both *Xanthosoma* and *Colocasia* (Lebot, 2009; Owusu-Darko *et al*., 2014, Osawaru and Ogwu, 2015). In many parts of Asia and Pacific, the term tannia which is a modification or qualification of the term taro has been used for *Xanthosoma*. Onwueme (1999) wrote in her book *Cocoyam Cultivation in Asia* and *Pacific*, taro (*C. esculenta*) should not be confused with the related aroid *Xanthosoma* species.

Cocoyam was introduced into the surveyed areas later than taro, as recalled by farmers who grow both crops. Amsalu Nebiyu *et al*. (2008) reported that cocoyam accessions which were collected from Ethiopia and introduced from abroad since 1978 were maintained at Jimma Agricultural Research Center, southwest Ethiopia. Another report mentioned that cocoyam entered the Malo area of Gamo-Gofa Zone in southwestern Ethiopia in the 1980s (Fujimoto, 2009). The majority (84%) of farmers did not remember the time when cocoyam was introduced into their areas despite the assertion by some farmers from Benchi-Maji and Kefa zones that cocoyam was introduced into their areas in 1970s. Cocoyam was widely distributed in the surveyed areas, growing mainly in the homegarden patches. Thus, the circumstantial evidences force us to believe that this crop might have much longer history in Ethiopia. During this study, farmers responded that cocoyam cultivation status has been increasing in their areas since they have known the crop. Cocoyam has expanded fast into new areas in western Africa since its introduction in the 16th or 17th century and its

importance is increasing since then (Maundu *et al*., 2009). A survey in Ethiopia indicated that cocoyam grows even in poor soils and under dry conditions (Fujimoto, 2009). Due to the related factors such as better yield, more robust and drought tolerance, cocoyam has become an important food for over 400 million people and has become the main edible aroid in many tropical areas (Giacometti and Leon, 1994; Matthews, 2002; Lebot 2009; Maundu *et al*., 2009). Farmers mentioned that cocoyam cultivation is increasing in their areas due to its re-emerging ability from under buried corm whenever it gets rain. They adopted harvesting cormels leaving the plant in place as perennial crop for germplasm conservation. It was noticed that the traditional seed exchange systems are the major way of seed supply in the surveyed areas.

In the study areas, cocoyam is cultivated in a mono and mixed cropping system. Farmers who cultivate cocoyam in mixed cropping system indicated that cocoyam is shade tolerant and it could serve as organic fertilizer when the leaves fall off. The farmers' response is in line with the research reports. Lebot (2009) pointed out that cocoyam tolerates a certain level of shade. Mazhar (2000) noted that mixed cropping of cocoyam with other crops is crucial to improve soil fertility.

During this study, the respondents mentioned that cocoyam cormels are used for human consumption after cooking or roasting while the corm is not used for human consumption. In concordant with farmers' response, it was mentioned in publications that domestication history of cocoyam was based on processes such as roasting and cooking tubers. The usable parts in cocoyam are the subterranean tuberous off shoots known as cormels and the main corm is usually acrid and is not eaten (Giacometti and Leon, 1994) or it is only eaten when no other food is available, during and after cyclones in some Pacific Islands (Lebot, 2009). This indicates the traditional way of preparation of cocoyam for food has transferred to

Ethiopia with the crop. According to farmers, the cormels of the purple cocoyam can be eaten only at the time of food emergency. Farmers in Dawuro, Wolaita and Gamo-Gofa Zones responded that purple cocoyam provides medicinal values. According to Nzietchueng (1988), some of the species of genus *Xanthosoma* such as *X. auriculatum, X. helleborifolium, X. mexicanum, X. pentaphyllum* and *X. robustum* are used as medicinal plants. Since there is dearth of information on the taxonomy of species, the purple cocoyam may be related to either of these species which are used as medicinal plant or the purple cocoyam growing in the surveyed areas may be related to *X. sagittifolium* variety growing in Pacific Islands, which are only eaten when there is shortage of other food crops (Lebot, 2009).

Farmers from Bench-Maji zone responded to the interview explained that the young leaves of cocoyam are eaten after cooking in addition to cormels. Previously Amsalu Nebiyu *et al*. (2008) reported that young leaves of the cocoyam are eaten in southwestern Ethiopia. In the literature, it was mentioned that young leaves of some cocoyam cultivars can be used as a vegetable and can be an important source of proteins and vitamins (Giacometti and Leon, 1994; Lebot, 2009). Cocoyam was originally introduced to Africa for their cormels, but their leaves are also used as a vegetable (Maundu *et al*., 2009). Traits of cocoyam that farmers prefer include serving as emergency food, fodder, young leaves edibility (Benchi-Maji Zone). Most of the farmers' preferred traits of cocoyam were traits that were also preferred by Nigerian farmers (Osawaru and Ogwu, 2015). In another way around, in Ethiopia, cocoyam traits such as sour taste, unpleasant smell, irritability with continuously eating, corm inedibility, hard texture to eat, non-appetizing nature have been hindering its potential to be a major crop as perceived by farmers. The farmers need to get these disliked traits improved. This study has shown that the farmers have useful knowledge on agromorphological traits and uses of cocoyam.

Chapter Four

Morphological Traits Based Genetic Diversity in Cocoyam (*Xanthosoma sagittifolium* (L.) Schott) from Ethiopia

Abstract

Cocoyam *(Xanthosoma sagittifolium* (L.) Schott) has been cultivated since fairly recent years in Ethiopia. Its genetic diversity has not been fully studied to date. A field study was conducted to assess the genetic diversity among cocoyam accessions from Ethiopia based on morphological traits. A total of 100 cocoyam accessions (65 green- and 35 purple-colored) were evaluated using a 10*10 simple lattice square design. Sixteen qualitative and 13 quantitative traits were studied. Two classes of qualitative traits were observed for petiole color, lamina orientation, color of the leaf surfaces, color of veins on leaf surface, position of cormel apex and shape of cormels. Analysis of variance (ANOVA) revealed significant variation in 11 (84.6%) of the 13 studied quantitative traits such as plant height, petiole length, lamina length, lamina width, circumference of pseudostem, number of cormles/plant, cormel diameter, cormel fresh weight/plant, corm length, corm diameter and corm fresh weight/plant. The estimates of genetic variance components and coefficient of variations showed that the δ^2p and PCV were higher than the δ^2g and GCV all of the 13 studied quantitative traits. However, the difference between PCV and GCV was small for 9 (69.2%) of the studied traits and there was high h^2_b% estimate for 6 (46.2%) of studied quantitative traits such as lamina length, lamina width, circumference of pseudo-stem, number of cormels/plant, corm diameter and corm fresh weight per plant, suggesting the environmental effect on these traits observed to be low. PCA reduced the original 13 characters in the experiment to 3 PCs, with the eigen values >1 which explained 69.2% of the observed variations among accessions, with PC1 has 40.02% of the total variation, PC2 and PC3, respectively, contributed, 16.10% and 12.9% of the total variation. The traits that loaded highly for PC1 were lamina length, lamina width, circumference of pseudo-stem and corm diameter, for PC2 were plant height, number of cormels/plant, corm length and corm fresh weight per plant and for PC3 were petiole length, number of cormels per plant, cormel length and cormel fresh weight per plant. Score plot has effective separated 35 purple cocoyam morphotypes from 65 green cocoyam morphotypes. Cluster analysis grouped the accessions into four clusters, irrespective of the collection sites. The study identified qualitative and quantitative traits that will help researchers in recommending best traits for morphological identification of cocoyam germplasms. The accessions should be tested in more than one environment to select elite genotypes for future utilization of cocoyam in Ethiopia.

Keywords: Cocoyam, genetic diversity, morphological traits

4.1 Introduction

Collecting, characterizing and evaluating the genetic diversity of crop plants is very important to identify genetically diverse accessions for efficient and effective usage, conservation and improvement. Morphological traits are conventional tools to analyze the genetic diversity by measuring the variation in qualitative morphological traits such as texture, color, growth habit and/or quantitative morphological traits like yield potential, height, size, weight, stress tolerance, etc. (Sinha and Kumaravadivel, 2016). The use of morphological traits to estimate genetic diversity is the most common approach to estimate the relationship between accessions. It is also an essential step for effective utilization of germplasms because it offers a useful approach for assessing the extent of diversity (Koorneef and Stam, 2001). Determination of the relevant morphological traits to describe the genetic diversity is very important in the cases of limited financial and human resource so that the least relevant traits can be eliminated (Lima *et al*., 2012). International Board for Plant Genetic Resource (IBPGR, 1989) prepared standard format that encourages the collection of data on accessions, characterization and preliminary evaluation of *Xanthosoma*. The morphological descriptors provide information underlying the conclusions on the genetic variability of cocoyam accessions.

Cocoyam (*Xanthosoma sagittifolium* (L.) Schott) is a perennial crop grown in the humid tropics and subtropics, but for practical purposes it is harvested after 9-12 months after plantation (Bown, 2000) although the time from planting to harvest can vary with genotypes and method of cultivation (Castro, 2006). In different countries, genetic diversity studies of cocoyam have been conducted using morphological traits such as plant height, the leaf lamina characters, corm shape, maturity period, growth habit, corm flesh color and taste, etc. (Mbouobda *et al*., 2007; Mwenye, 2009; Nurmiyati *et al*., 2009; Solomon Fantaw *et al*., 2014a, b). Amsalu Nebiyu and Tesfaye Awas (2006) stated that there is considerable amount

of cocoyam gene pool in south and southwest Ethiopia in farmers' fields and home-gardens. However, it is a neglected crop by research and development community. Its genetic diversity has not been fully researched in Ethiopia. The germplasms in the farmers' field are the major hope for use, conservation and improvement of cocoyam. Thus, there is a need to characterize the available cocoyam accessions for genetic diversity. A better understanding of genetic diversity will facilitate its usage, conservation and improvement.

To assess the genetic diversity of crop plants based on morphological traits, researches have used statistical methods such as basic statistical parameters (Opoku-Agyeman *et al*., 2004; Mandal *et al*., 2013) and multivariate analysis (Okpul *et al*., 2004; Tewodros Mulualem *et al*., 2013; Solomon Fantaw *et al*., 2014a). In this study, the genetic diversity of 100 cocoyam accessions collected from Ethiopia have been assessed based on morphological traits by using basic statistical parameters and multivariate analyses (principal components and cluster analysis).

4.2 Materials and methods

4.2.1 Germplasm collection

One hundred cocoyam cormels were collected from 16 woreda (Table 4.1). The individual samples (accessions) were collected from sites at least 5 km apart unless they were clearly distinguished morphologically by leaf/petiole color difference (green- and purple-colored).

4.2.2 Description of the experimental site

The field evaluation was carried out at Areka Agricultural Research Center, which is located in the Southren Nations Nationalities and Peoples Region in Wolaita zone, 303 km southwest of Addis Ababa, Ethiopia, at an altitude of 1750-1830 (masl), 07°19'N latitude and 37°08'E longitude. The site is situated in the warm sub-humid lowlands (SH_2) major agroecology, which is tepid to cool-sub-humid mid highlands. The average annual rain fall

of the study area was 1520 mm, which occurs in two seasons in the year. The first short rain season is *Belg*, which is from February to May and the second main rainy season *Mehir* which occurs from June to October. The average maximum and minimum temperature of Areka area are 25.4 ºC and 13.4ºC, respectively.

Table 4.1 List of cocoyam accessions with accession code, collection sites (Zone, Woreda and Kebele), coordinate (latitude and longitude), altitude and color of accessions

Accession Code*	Zone	Woreda	Kebele	Latitude (ºN)	Longitude (ºE)	Altitude (masl)	Color of accession	No of accessions
BS/2014/Xs1	Bench-Maji	South-Bench	Kokin	7.078	35.687	1594	Green	
BS/2014/Xs2	Bench-Maji	South-Bench	Debrework-01	6.891	35.703	1581	Green	
BS/2014/Xs3	Bench-Maji	South-Bench	Jenehu	7.023	35.651	1403	Green	
BS/2014/Xs4	Bench-Maji	South-Bench	Kite	7.040	35.606	1345	Green	
BS/2014/Xs5	Bench-Maji	South-Bench	Kite	7.134	35.542	1312	Green	
BS/2014/ Xs5	Bench-Maji	South-Bench	Kite	6.939	35.714	1349	Green	
BS/2014/Xs7	Bench-Maji	South-Bench	Kite	6.939	35.714	1349	Green	
BN/2014/ Xs8	Bench-Maji	North-Bench	Fanika	7.129	35.571	1297	Green	14
BN /2014/Xs9	Bench-Maji	North-Bench	Fanika	7.129	35.520	1288	Green	
BN/2014/Xs10	Bench-Maji	North-Bench	Woshiken	7.184	35.790	1404	Green	
BN/2014/Xs11	Bench-Maji	North-Bench	Woshiken	7.350	35.791	1404	Green	
BN/2014/Xs12	Bench-Maji	North-Bench	Temenja-Yazh	7.210	35.791	1481	Green	
BN/2014/Xs13	Bench-Maji	North-Bench	Temenja-Yazh	7.136	35.782	2070	Green	
BN/2014/Xs14	Bench-Maji	North-Bench	Wacha	7.137	35.781	2070	Green	
KC/2014/Xs15	Kefa	Chana	Wacha	7.373	35.919	2136	Green	
KC/2014/Xs16	Kefa	Chana	Daha	7.455	36.200	1993	Green	
KC/2014/Xs17	Kefa	Chana	Daha	7.439	36.261	1913	Green	
KC/2014/Xs18	Kefa	Chana	Woreta	7.437	36.006	1810	Green	
KC/2014/Xs19	Kefa	Chana	Woreta	7.544	36.194	1800	Green	
KG/2014/Xs20	Kefa	Gimbo	Jakaraba	7.531	36.518	1620	Green	
KG/2014/Xs21	Kefa	Gimbo	Ufudo	7.607	36.224	1745	Green	12
KG/2014/Xs22	Kefa	Gimbo	Ufudo	7.593	36.268	1715	Green	
KG/2014/Xs23	Kefa	Gimbo	Shomba-Kichibe	7.652	36.487	1445	Green	
KG/2014/Xs24	Kefa	Gimbo	Shomba-Kichibe	7.476	36.518	1405	Green	
JS/2014/Xs25**	Jimma	Sheba-Sumbo	Kesh	7.586	36.675	1350	Green	
JS/2014/Xs26**	Jimma	Sheba-Sumbo	Jebiye	7.719	36.768	1030	Green	
DT/2014/Xs27	Dawuro	Tocha	Gorika-Doma	7.272	37.079	1535	Green	
DT/2014/Xs28	Dawuro	Tocha	Gorika-Doma	7.272	37.080	1538	Green	
DT/2014/Xs29	Dawuro	Tocha	Wara-Wori	7.268	37.165	1357	Green	
DT/2014/Xs30	Dawuro	Tocha	Wara-Wori	7.354	37.071	1497	Green	
DT/2014/Xs31	Dawuro	Tocha	Gorika-Dama	7.289	37.186	1520	Green	
DT/2014/Xs32	Dawuro	Tocha	Gorika-Dama	7.333	37.016	1418	Green	
DT/2014/Xs33	Dawuro	Tocha	Warma-Galcha	7.246	37.259	1498	Green	
DM/2014/Xs34	Dawuro	Maraka	Shaba	7.351	37.326	1245	Green	
DM/2014/Xs35	Dawuro	Maraka	Shina-Gaburi	7.294	37.312	1290	Green	
DM/2014/Xs36	Dawuro	Maraka	Tercha-02	7.323	37.296	1327	Green	
DL/2014/Xs37	Dawuro	Loma	Gasa-Chare	7.188	37.522	2129	Green	
DL/2014/Xs38	Dawuro	Loma	Gasa-Chare	7.124	37.533	2106	Green	
DL/2014/Xs39	Dawuro	Loma	Gasa-Chare	7.124	37.533	2106	Purple	28
DL/2014/Xs40	Dawuro	Loma	Gasa-Chare	7.074	37.438	2278	Green	
DL/2014/Xs41	Dawuro	Loma	Tulama	7.054	37.345	2319	Green	
DL/2014/Xs42	Dawuro	Loma	Tulama	7.037	37.338	2299	Green	
DL/2014/Xs43	Dawuro	Loma	Tulama	7.085	37.364	2253	Purple	
DL/2014/Xs44	Dawuro	Loma	Elaa-Bacho	7.160	37.426	1910	Green	
DL/2014/Xs45	Dawuro	Loma	Elaa-Bacho	7.170	37.426	1910	Purple	

Table 4.1 continued

DB/2014/Xs46	Dawuro	Bosa-Gena	Lala-Ambe	7.038	37.435	1523	Purple
DB/2014/Xs47	Dawuro	Bosa-Gena	Lala-Ambe	7.029	37.435	1523	Green
DB/2014/Xs48	Dawuro	Bosa-Gena	Deneba	7.028	37.461	1221	Green
DB/2014/Xs49	Dawuro	Bosa-Gena	Deneba	7.029	37.462	1221	Purple
DB/2014/Xs50	Dawuro	Bosa-Gena	Zima	7.113	37.494	1222	Green
DB/2014/Xs51	Dawuro	Bosa-Gena	Zima	7.113	37.494	1222	Purple
DB/2014/Xs52	Dawuro	Bosa-Gena	Sere-Beta	7.267	37.428	1733	Green
DB/2014/Xs53	Dawuro	Bosa-Gena	Sere-Beta	7.014	37.425	1816	Purple
DB/2014/Xs54	Dawuro	Bosa-Gena	Sere-Beta	7.269	37.425	1236	Green
DB/2014/Xs55	Wolaita	Kindo-Koysha	Bayana	6.943	37.664	1132	Purple
WK/2014/Xs56	Wolaita	Kindo-Koysha	Fagena-Mata	7.005	37.503	1160	Purple
WK/2014/Xs57	Wolaita	Kindo-Koysha	Fagena-Mata	7.002	37.505	1158	Green
WK/2014/Xs58	Wolaita	Kindo-Koysha	Fagena-Mata	7.077	37.712	1156	Purple
WK/2014/Xs59	Wolaita	Kindo-Koysha	Fagena-Mata	7.078	37.714	1156	Green
WK/2014/Xs60	Wolaita	Kindo-Koysha	Bale-01	7.115	37.660	1315	Purple
WK/2014/Xs61	Wolaita	Kindo-Koysha	Bale-02	7.115	37.660	1315	Green
WH/2014/Xs62	Wolaita	Humbo	Gututo-Larena	6.803	37.785	1849	Purple
WH/2014/Xs63	Wolaita	Humbo	Gututo-Larena	7.015	38.027	1852	Green
WH/2014/Xs64	Wolaita	Humbo	Gututo-Larena	6.803	37.785	1849	Purple
WH/2014/Xs65	Wolaita	Humbo	Gututo-Larena	6.858	37.942	1785	Green
WH/2014/Xs66	Wolaita	Humbo	Gututo-Larena	6.858	37.942	1785	Purple
WH/2014/Xs67	Wolaita	Humbo	Bosa-Wanche	6.799	37.831	1755	Purple
WH/2014/Xs68	Wolaita	Humbo	Bosa-Wanche	6.826	37.831	1746	Purple
WH/2014/Xs69	Wolaita	Humbo	Demba-Koyisha	6.768	37.767	1925	Purple
WS/2014/Xs70	Wolaita	Sodo-Zuriya	Humbo-Larena	7.004	37.939	1836	Purple
WS/2014/Xs71	Wolaita	Sodo-Zuriya	Wareza-Esho	7.085	37.782	1890	Purple
WS/2014/Xs72	Wolaita	Sodo-Zuriya	Wareza-Esho	7.085	37.782	1890	Green
WS/2014/Xs73	Wolaita	Sodo-Zuriya	Gurumo-Woyde	7.051	37.891	1960	Green
WS/2014/Xs74	Wolaita	Sodo-Zuriya	Wareza-Lasho	7.085	37.931	1969	Green
WS/2014/Xs75	Wolaita	Sodo-Zuriya	Wareza-Lasho	7.085	37.931	1969	Purple
WS/2014/Xs76	Wolaita	Sodo-Zuriya	Damota-Waja	7.134	37.883	1940	Purple
WS/2014/Xs77	Wolaita	Sodo-Zuriya	Damota-Waja	7.134	37.883	1940	Green
WB/2014/Xs78	Wolaita	Boloso-Sore	Dola	6.187	37.798	1796	Purple
WB/2014/Xs79	Wolaita	Boloso-Sore	Dola	6.268	37.923	1827	Purple
WB/2014/Xs80	Wolaita	Boloso-Sore	Gurumo-Koysha	7.087	37.976	1914	Green
WB/2014/Xs81	Wolaita	Boloso-Sore	Gunnuno-01	7.146	37.875	2052	Purple
WB/2014/Xs82	Wolaita	Boloso-Sore	Gunnuno-02	7.119	37.892	2042	Purple
GQ/2014/Xs83	Gamo-Gofa	Qucha	Dana Sefera II	6.697	37.678	1692	Green
GQ/2014/Xs84	Gamo-Gofa	Qucha	Dana Sefera II	6.697	37.678	1692	Purple
GQ/2014/Xs85	Gamo-Gofa	Qucha	Basa	6.578	37.675	1390	Green
GQ/2014/Xs86	Gamo-Gofa	Qucha	Basa	6.578	37.675	1390	Purple
GQ/2014/Xs87	Gamo-Gofa	Qucha	Basa	6.578	37.672	1393	Purple
GQ/2014/Xs88	Gamo-Gofa	Qucha	Selamber-03	6.583	37.461	1413	Green
GQ/2014/Xs89	Gamo-Gofa	Qucha	Selamber-04	6.583	37.461	1413	Purple
GQ/2014/Xs90	Gamo-Gofa	Qucha	Selamber-01	6.639	37.704	1364	Green
GQ/2014/Xs91	Gamo-Gofa	Qucha	Selamber-02	6.639	37.704	1364	Purple
GQ/2014/Xs92	Gamo-Gofa	Qucha	Morka	6.706	37.667	1325	Purple
GQ/2014/Xs93	Gamo-Gofa	Qucha	Morka	6.448	37.375	1314	Purple
GQ/2014/Xs94	Gamo-Gofa	Qucha	Morka	6.448	37.375	1314	Green
GD/2014/Xs95	Gamo-Gofa	Daramalo	Dita	6.676	37.508	1204	Purple
GD/2014/Xs96	Gamo-Gofa	Demba-Gofa	Dorga	6.458	36.983	1192	Purple
GD/2014/Xs97	Gamo-Gofa	Demba-Gofa	Dorga	6.464	37.073	1816	Green
GD/2014/Xs98	Gamo-Gofa	Demba-Gofa	Dorga	6.417	37.056	1189	Green
GD/2014/Xs99	Gamo-Gofa	Demba-Gofa	Boreda	6.486	36.947	1306	Purple
GD/2014/Xs100	Gamo-Gofa	Demba-Gofa	Boreda	6.486	36.947	1306	Green

28

18

*In the accession code the first two letters stand for the zone and woreda followed by the year of collection, Xs for species name and serial number of accessions; **accessions collected from Sheba-Sumbo woreda of Jimma zone, which is close to Kefa zone. Thus, these accessions were considered with Kefa population for genetic diversity study

4.2.3 Experimental design and crop management

In 2015/16 cropping season, 100 cocoyam accessions were planted in February 16, 2015 for establishment purpose. Similar sized cormels were harvested at the end of 12th months after plantation. In 2016/17 cropping season, the 100 cocoyam accessions were planted using a 10*10 simple lattice square design in well-drained, loose soil on flat ground in February 16, 2016. Five cormels of an accession were planted in a single row plot of 3 m, spaced 0.6 m between plants and 0.75 m between rows. Weeding was conducted as required to keep plots weed free.

4.2.4 Morphological traits and data collection

Twenty-nine morphological traits were selected from International Board for Plant Genetic Resources Descriptors for *Xanthosoma* (IBPGR, 1989) (Appendix 2). The qualitative data were scored for qualitative traits such as plant growth habit, petiole attachment, petiole color (upper 2/3rd), petiole color (lower 1/3rd), color of edge of petiole, lamina orientation, leaf marigin color, leaf shape, color of upper leaf surface, color of lower leaf surface, position of cormel apex, shape of cormels, color of cormel apex and fresh cormel color at the 7th month after date of plantation, from the middle individual clone by cross-checking other clones when needed. The Munsell Plant Tissue Color Chart (Wild and Voigt, 1977) was used to discriminate colors. The quantitative data were recorded from the middle three plants of each replication, leaving the two plants grown as boarder plants. The aboveground quantitative traits such as overall plant height (cm), petiole length (cm), petiole sheath length (cm), circumference of pseudo-stem (cm), lamina length and lamina width were measured at the end of 7th months after plantation. The underground quantitative traits such as the number of cormels/plant, cormel length (cm), cormel diameter (cm), cormel fresh weight/plant (kg), corm length (cm), corm diameter (cm) and corm fresh weight/plant (kg) were recorded at the end of 11th months after date of plantation.

4.2.5 Data analysis

Frequency distribution of qualitative traits and basic statistical parameters based on quantitative morphological traits were calculated using SPSS version 23 (IBM SPSS, 2015). Analysis of variance was computed by PROC GLM procedure of SAS 9.3 (SAS, 2011) because the relative efficiency of the lattice design compared with CRBD was low (less than 105%). Total genotypic and phenotypic variances were calculated following Singh and Chaudhary (1979) by using the expectations as: Genotypic variance (δ^2g) = (MSG-MSE)/r, where MSG - mean squares for accessions, MSE-mean square of error and r is replication. Phenotypic variance (δ^2p) = δ^2g + δ^2e, where δ^2g - genotypic variance component, δ^2e Foreinenvironmental variance, which was MSE. The genotypic and phenotypic coefficient of variations were estimated by the method as suggested by Singh and Chaudhary (1979) using the formulas: Genotypic coefficient of variation (GCV%) = $\sqrt{\delta 2g}/\bar{x}$ *100 and phenotypic coefficient of variation (PCV%) = $\sqrt{\delta 2p}/\bar{x}$ *100, where $\bar{x}$ is the mean value of the particular trait of interest. Heritability in broad sense (h^2_b%) was estimated as a ratio of genotypic variance to phenotypic variance (Falconer, 1981), i.e., heritability in broad sense (h^2_b %) = δ^2g/δ^2p*100; where δ^2g genotypic variance and δ^2p phenotypic variance. Genetic advance and genetic advance as % of mean were estimated by the formula described by Johnson *et al*. (1955) as follows: Genetic advance (GA) = δ^2g/δ^2p*k*δp, where δ^2g is genotypic variance, δ^2p is phenotypic variance, δp is standard deviation of phenotypic variance and k is the selection differential at a particular selection intensity, i.e., 2.06, suggested by Falconer (1981) at 5% selection intensity. Genetic advance as percentage of mean (GA %) = GA/$\bar{x}$ *100, where $\bar{x}$ is the mean of a trait. The quantitative traits were also subjected to multivariate analysis such as principal component analysis (PCA) and cluster analysis. The PCs based on correlation matrix was calculated using Minitab 17.1 (Minitab, 2013) and the PCs with Eigen values >1.0 were selected to define the morphological trait variation among accessions. Cluster analysis was conducted by employing average linkage clustering strategy of the observation. Variables were standardized to a common scale by subtracting the means and

dividing by the standard deviation. The number of cluster was determined by following the steps recommended by Minitab 17.1 (Minitab, 2013). The correlation between clusters was computed using generalized Euclidean distance and the dendrogram showing the Euclidean distance between clusters was constructed by plotting the results of cluster analysis using Minitab 17.1 (Minitab, 2013).

4.3 Results

4.3.1 Qualitative traits

Of 16 qualitative traits, 9 discriminated 100 cocoyam accessions into two-character states but 7 characters did not discriminate the accessions.

Table 4.2 Frequency distribution of 16 qualitative traits of cocoyam

No	Plant character	Character state	%*
1	Plant growth habit	Acaulescent	100
2	Petiole attachment	Non-peltate	10 0
3	Petiole color (upper 2/3rd)	Green Purple	65 35
4	Petiole color (lower 1/3rd)	Green streaked with purple Purple	65 35
5	Color of edge of petiole sheath	The same as the rest of petiole and sheath Purple	35 65
6	Lamina orientation	One plane - apex down (Droopy) 3-dimentional (cup-shaped)	35 65
7	Leaf shape	Sagitate basal lobes	100
8	Leaf margin color	Purple edge	100
9	Color of upper leaf surface	Medium green Dark green	65 35
10	Color of lower leaf surface	Light green Purplish green	65 35
11	Color of veins on upper leaf surface	Lighter green than lamina	100
12	Color of veins on lower leaf surface	Same as color as lamina Purple	65 35
13	Position of color apex	Underground Above ground and under ground	65 35
14	Shape of cormels	Globose Ovate	65 35
15	Color of cormel apex	Red	100
16	Internal color of cormels	White streaked with purple	100

*100% indicates the character common to both morphotypes, 65% character specific to green morphotypes and 35% character specific to purple morphotypes

Two classes of petiole color (upper 2/3rd), petiole color (lower 1/3rd), color of edge of petiole sheath, lamina orientation, color of upper leaf surface, color of lower leaf surface, color of veins on lower leaf surface, position of color apex and shape of cormels existed in the accessions. Qualitative traits such as plant growth habit, petiole attachment, leaf shape, leaf margin color, color of veins on upper leaf surface, color of cormel apex and fresh cormel color did not discriminate the accessions included in this study (Table 4.2 and Fig. 4.1).

Fig. 4.1 Qualitative morphological traits of cocoyam: (a) Acaulescent plant growth habit (non-peltate petiole attachment indicated); (b) Petiole color -upper 2/3rd (green/purple); (c) Petiole color - lower 1/3rd (green streak/purple); (d) Color of edge of petiole sheath; (e) Lamina orientation (cup shaped/droopy); (f) Color of upper leaf surface (medium green/dark green; (g) Color of lower leaf surface and veins on lower leaf surface; (h) Shape of cormels (globose/ovate); (i) color of cormel apex (red) and internal color of cormels (white streaked with purple).

4.3.2 Descriptive statistical parameters and variance

Descriptive statistics such as mean, standard deviation (SD), minimum and maximum values and coefficient of variation (CV%) are summarized in Table 4.3. Plant height (cm) ranged from 57.58 to 84.83 (mean: 71.49), petiole length (cm) ranged from 42.75 to 67.83 (mean: 55.22), petiole sheath length (cm) ranged from 25.50 to 43.92 (Mean: 31.24), lamina length (cm) ranged from 29.69 to 45.25 (mean: 37.77), lamina width ranged from 17.28 to 25.47 (mean: 22.02), the circumference of pseudo-stem (cm) ranged from 21.42 to 32.42 (Mean: 28.83). The mean values of underground quantitative traits such as the number of cormels/plant, cormel length (cm), cormel diameter (cm), cormel fresh weight/plant (kg), corm length (cm), corm diameter (cm) and corm fresh weight/plant (kg), respectively, were 10.05, 9.05, 3.26, 1.28, 11.94, 5.70 and 1.18. Maximum SD (5.63) corresponded to plant height and minimum SD (0.31) corresponded to cormel fresh weight/plant (kg). The coefficient of variation (CV%) varied from 7.65% for lamina width to 29.10% for corm fresh weight/plant. Analysis of variance (ANOVA) revealed significant variation in 11(84.6%) of the 13 studied quantitative traits (Table 4.4).

Table 4.3 Basic statistics of 13 quantitative traits of cocoyam

Quantitative traits	Mean	SD	Minimum	Maximum	CV%
Plant height (cm)	71.49	5.63	57.58	84.83	7.87
Petiole length (cm)	55.22	4.82	42.75	67.83	8.72
Petiole sheath length (cm)	31.24	3.46	25.50	43.92	11.08
Lamina length (cm)	37.77	3.20	29.69	45.25	8.48
Lamina width (cm)	22.02	1.68	17.28	25.47	7.65
Circumference of pseudo-stem (cm)	28.83	2.78	21.42	34.42	9.66
Number of cormels/plant	10.05	1.81	5.92	15.33	17.98
Cormel length (cm)	9.05	1.09	6.58	11.83	12.00
Cormel diameter (cm)	3.26	0.46	2.25	4.21	13.97
Cormel fresh weight/plant (kg)	1.28	0.31	0.68	2.42	23.79
Corm length (cm)	11.94	2.11	3.49	16.92	17.71
Corm diameter (cm)	5.70	0.77	3.49	7.73	13.52
Corm fresh weight/plant(kg)	1.18	0.34	0.53	2.30	29.10

Table 4.4 Summary of mean squares of 13 quantitative traits of cocoyam

Quantitative traits	Mean squares		
	Rep (df =1)	Accessions (df =99)	Error (df =81)
Plant height (cm)	100.30	82.46*	54.46
Petiole length (cm)	294.64	49.57*	31.66
Petiole sheath length (cm)	333.62	28.16	21.48
Lamina length (cm)	20.09	16.73***	8.10
Lamina width (cm)	0.74	4.96***	2.41
Circumference of pseudo-stem (cm)	128.27	14.02***	5.72
Number of cormels/plant	5.01	7.40**	3.91
Cormel length (cm)	0.02	2.17	2.30
Cormel diameter (cm)	1.12	0.29**	0.17
Cormel fresh weight/plant (kg)	1.71	0.23**	0.13
Corm length (cm)	102.84	7.65**	4.38
Corm diameter (cm)	2.95	1.06***	0.45
Corm fresh weight/plant (kg)	1.98	0.24***	0.12

df - degree of freedom, *, ** and ******* significant at p= 0.05, 0.01 and 0.001, respectively

4.3.3 Genotypic and phenotypic variances, coefficients of variations and heritability

For each of the evaluated quantitative traits, genetic parameters including genotypic and phenotypic variance component and their coefficients of variation, broad sense heritability, genetic advance and genetic advance as % of mean are summarized in Table 4.5. Across the traits studied, $\delta^2 g$ ranged from -0.07 for cormel length to 14.00 for plant height. The lowest $\delta^2 p$ (0.18) was for corm and cormel fresh weights/plant (kg) and the highest $\delta^2 p$ (68.46) was for plant height. The GCV values were ranged from the below 0% for cormel length to 20.34% for corm fresh weight/plant (kg). PCV ranged from 8.72% for lamina width to 35.59% for corm fresh weight/plant. The heritability estimates ranged from below 0% (-2.91) for cormel length to 42.05% for circumference of pseudo-stem. Genetic advance as % of mean ranged from -0.99% for cormel length to 24.44% for corm fresh weight/plant (kg).

Table 4.5 Genetic parameters of 13 quantitative traits of cocoyam

Quantitative traits	$\delta^2 g$	$\delta^2 p$	GCV (%)	PCV (%)	h^2_b (%)	GA	GA as % of mean
Plant height (cm)	14.00	68.46	5.24	11.59	20.45	3.48	4.88
Petiole length (cm)	8.95	40.61	5.42	11.54	22.04	2.89	5.24
Petiole sheath length (cm)	3.34	24.82	5.86	15.94	13.46	1.38	4.42
Lamina length (cm)	4.32	12.42	5.51	9.32	34.76	2.52	6.67
Lamina width (cm)	1.28	3.69	5.13	8.72	34.60	1.37	6.21
Circumference of pseudo-stem (cm)	4.15	9.87	7.07	10.89	42.05	2.72	9.43
Number of cormels/plant	1.75	5.66	13.13	23.68	30.92	1.52	15.08
Cormel length (cm)	-0.07	2.24	-	16.57	-2.91	-0.09	-0.99
Cormel diameter (cm)	0.06	0.23	7.36	14.72	26.09	0.26	7.91
Cormel fresh weight/plant (kg)	0.05	0.18	17.19	32.81	27.78	0.24	18.78
Corm length (cm)	1.80	6.02	10.72	20.52	27.18	1.37	11.49
Corm diameter (cm)	0.31	0.76	9.82	15.26	40.40	0.72	12.70
Corm fresh weight/plant (kg)	0.06	0.18	20.34	35.59	33.33	0.29	24.44

$\delta^2 g$ - genotypic variance; $\delta^2 p$ - phenotypic variance; GCV - genotypic coefficient of variation; PCV - phenotypic coefficient of variation; h^2_b broad sense heritability; GA - genetic advance

4.3.4 Principal components and clustering of accessions

The PCA was conducted to determine traits that most strongly contribute to the total variation. The analysis reduced the original 13 characters in the experiment to 3 PCs, with the Eigen values >1 which explained 69.2% of the observed variations among the accessions (Table 4.6). PC1 accounted for 40.2% of the total variation. The morphological traits that loaded highly for PC1 were lamina length (0.38), lamina width (0.34), circumference of pseudo-stem (0.35) and corm diameter (0.35). PC2 accounted for 16.1% of the total variation and the traits with the greatest weight on this component were plant height (-0.50), number of cormels/plant (-0.35), corm length (0.34) and corm fresh weight per plant (0.34). PC3 contributed 12.9 % of the total variation and mainly related to petiole length (0.36), number of cormels/plant (-0.49), cormel length (-0.37) and cormel fresh weight/plant (0.50). Score plot of first component (horizontal axis) and second component (vertical axis) was drawn to study grouping of accessions. The first component was effective in separating purple cocoyam morphotypes from green cocoyam morphotypes in which most of purple cocoyam morphotypes were clustered together (Fig. 4.2).

Table 4.6 Eigen value, proportion of variability and the first 3 PCs of cocoyam

Quantitative traits	PC1	PC2	PC3
Eigen value	5.22	2.09	1.67
Proportion of variance (%)	40.2	16.1	12.9
Cumulative variance (%)	40.2	56.3	69.2
Plant height (cm)	0.22	**-0.50**	0.25
Petiole length (cm)	0.31	-0.24	**0.36**
Petiole sheath length (cm)	0.29	-0.28	0.33
Lamina Length (cm)	**0.38**	0.07	0.03
Lamina width (cm)	**0.34**	-0.03	-0.03
Circumference of pseudo-stem (cm)	**0.35**	0.05	0.11
Number of cormels/plant	0.09	**-0.35**	**-0.49**
Cormel length (cm)	0.08	-0.32	**-0.37**
Cormel diameter (cm)	0.27	0.27	-0.18
Cormel fresh weight/plant (kg)	0.20	-0.20	**-0.50**
Corm length (cm)	0.28	**0.34**	0.01
Corm diameter (cm)	**0.35**	0.20	-0.20
Corm fresh weight/plant (kg)	0.27	**0.34**	-0.07

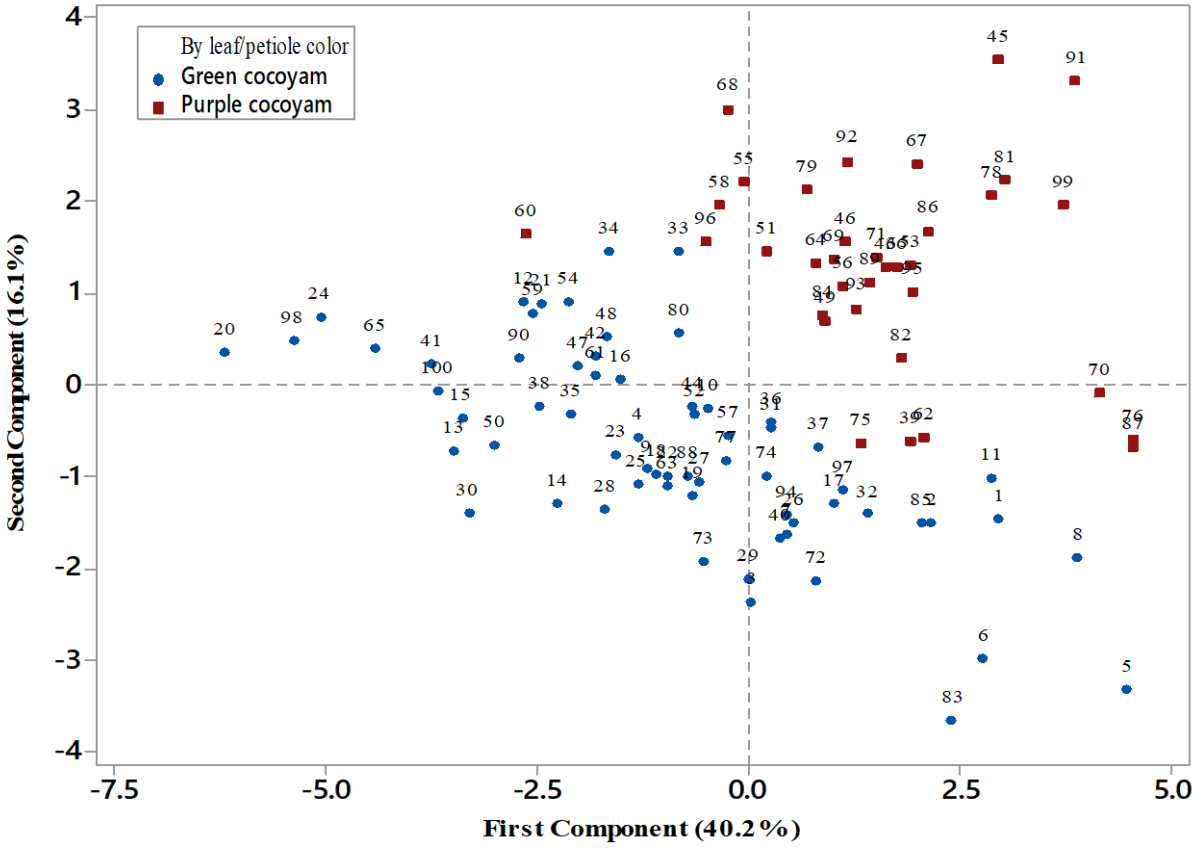

Fig. 4.2 Score plot of 100 cocoyam accessions based on 13 quantitative traits.
The numbes stand for the serial numbers of the accession code as defined in Table 4.1. The accessions 1-14, 15-26, 27-54, 55-82 and 83-100 were from Bench-maji, Kefa, Dawuro, Wolaita and Gamo-Gofa zones, respectively.

The cluster analysis based on the mean values of 13 quantitative traits grouped the 100 cocoyam accessions into 4 clusters (Fig. 4.3). The clustering means of accessions based on 13 quantitative traits is presented in Table 4.7. Cluster four, C-IV (blue), was the largest cluster composed of 41 (41%) of accessions and the least compacted cluster than all other clusters, having the largest within cluster sum of squares. Accessions in this cluster were characterized by the highest mean value for number of cormels per plant (Table 4.1.7). Cluster three, C-III (purple), was the second larger cluster contained 27 (27%) of the total accessions. Of the 27 accessions grouped in this cluster, 25 (92.3%) were purple cocoyam morphotypes. Cluster two, C-II (red), included the smallest number of accessions (11%) than all other clusters and it is a more compact cluster, which has the smallest within cluster sum of squares. Accessions in this cluster were characterized by the highest mean values for plant height, petiole length, lamina length, lamina width, circumference of pseudo-stem and corm length. Cluster one, C-I (green), contained 21 (21%) genotypes, which were characterized by the lowest mean values for most of the traits studied (Table 4.7). The cluster analysis showed that most of the accessions collected from different zones, woredas or kebeles were clustered together.

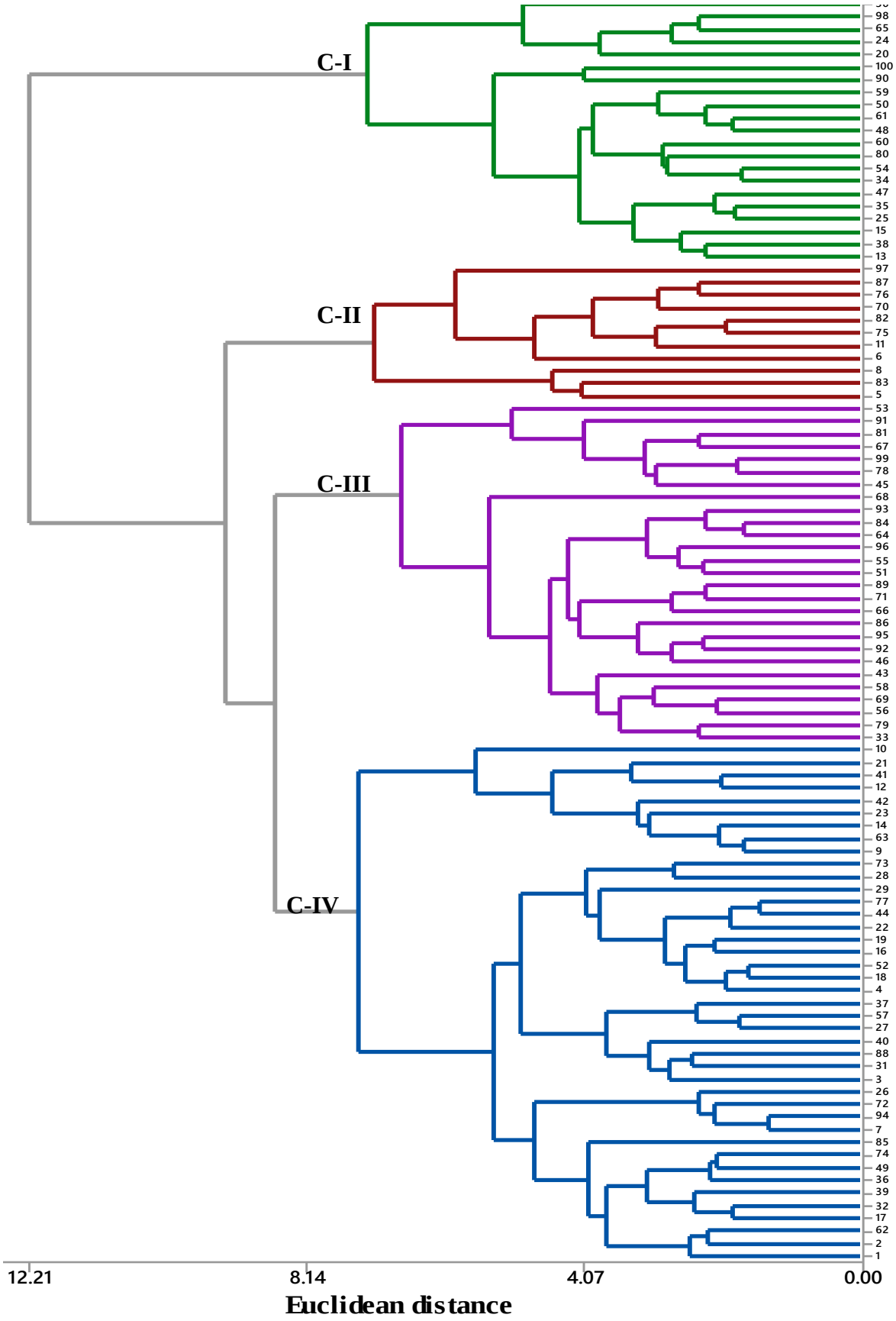

Fig. 4.3 Cluster analysis showing the relationship among 100 cocoyam accessions based on 13 quantitative traits. The numbers stand for the serial numbers of the accession code as defined in Table 4.1. The accessions 1-14, 15-26, 27-54, 55-82 and 83-100 were from Bench-maji, Kefa, Dawuro, Wolaita and Gamo-Gofa zones, respectively.

Table 4.7 Cluster means of 13 quantitative traits of cocoyam

Traits	Means values of cluster			
	C-I	C-II	C-III	C-IV
Plant height (cm)	67.26	80.00	67.60	73.93
Petiole length (cm)	50.16	63.16	55.15	55.51
Petiole sheath length(cm)	38.44	37.86	30.80	31.21
Lamina length (cm)	34.50	41.13	39.71	37.28
Lamina width (cm)	20.33	23.89	22.63	22.14
Circumference pseudo-stem (cm)	26.00	31.69	30.42	28.48
Number of cormels per plant	9.19	10.32	9.28	10.93
Cormel length (cm)	9.03	9.61	8.60	9.21
Cormel diameter (cm)	2.91	3.59	3.68	3.08
Cormel fresh weight per plant (kg)	1.02	1.43	1.31	1.35
Corm length (cm)	10.27	12.30	14.08	11.28
Corm diameter (cm)	5.00	6.19	6.31	5.55
Corm fresh weight per plant (kg)	0.92	1.29	1.51	1.07
Within cluster sum of squares	167.63	117.39	192.94	304.46

4.4 Discussion

In this study, out of 16 qualitative traits, 9 discriminated cocoyam accessions included in this study into two groups while 7 qualitative traits did not show differences among accessions. Mbouobda *et al*. (2007) reported a list of five qualitative descriptors while evaluating cocoyam in Cameroon. Opoku-Agyeman *et al*. (2004) reported 15 qualitative descriptors while evaluating cocoyam germplasms in Ghana. Among non-discriminatory qualitative traits, leaf margin color, color of cormel apex and internal color of cormels, however, showed polymorphism among cocoyam accessions from Ghana (Opoku-Agyeman *et al*., 2004). Inability of seven traits to discriminate our collections may be due to cocoyam accessions included in this study might be less diverse for the traits than the cocoyam accessions characterized by Opoku-Agyeman *et al*. (2004) in Ghana. Thirty-five (35%) of the accessions were purple colored (in generic term), having purple petiole, dark green color of upper leaf surface and purplish green color of lower leaf surface. Sixty- five (65%) of the accessions were green, having green petiole color (upper 2/3rd), green streaked with purple petiole color (lower 1/3rd), green color of leaf surface. Green- and purple-colored cocoyam

plants were observed in the farmers' field collected for this study. Farmers identify the green and purple cocoyam by different local names (Chapter 3). Diversity in leaf color is considered to be most important as it is frequently used to characterize plant germplasms and to distinguish them visually (Mandal *et al*., 2013).

Basic statistical parameters based on quantitative morphological traits showed high differences between the maximum and minimum values for plant height (57.58 cm, 84.83 cm), petiole length (42.75 cm, 67.83 cm) and petiole sheath length (19.17 cm, 48.67 cm). CV% value for 11 (84.6%) of the studied traits were <20%. According to Mohammadi and Talebi (2015) CV% <20% is considered to be good, indicating the accuracy of conducted experiments. In this study, 84.6% of the quantitative traits showed significant variation among the accessions, supporting the study of Solomon Fantaw *et al*. (2014b) that reported significant variation for all of the 16 studied quantitative traits. Villavicencio *et al*. (2016) reported that the morphological traits such as lamina width, lamina length, petiole length, fresh weight of cormels per plant and corm size manifested significant variation among the cocoyam accessions. All of these traits also showed significant variation in this study. The underground traits, except cormel length, showed significant variation. Solomon Fantaw *et al*. (2014a) reported that cocoyam accessions from Ethiopia were significantly discriminated by underground traits. The analysis of 50 taro accessions from Nagaland revealed maximum contribution of underground traits such as corm girth, corm yield per plant and number of cormels towards genetic divergence (Mezhii *et al*., 2015).

The estimates of genetic variance components and coefficient of variations showed that the δ^2p and PCV were higher than δ^2g and GCV, respectively, suggesting the presence of environmental influence on the traits studied. However, the differences between the PCV and the GCV were low for 9 (69.3%) of the 13 studied traits, implying that the environmental effect on these traits observed to be low. The PCV and GCV values greater than 20% are

regarded as high, values between 10-20% are regarded as medium and less than 10% are classified as low (Deshmukh *et al*., 1986). In our assessment, the GCV was high for only corm fresh weight per plant and the PCV were low for lamina length and lamina width.

The broad sense heritability (h^2_b) could be classified as high (>30%), medium (10-30%) and low (<10%) (Bhateria *et al*., 2006). In our assessment, the h^2_b estimates were high for 6 (46.2%) of studied quantitative traits such as lamina length, lamina width, circumference of pseudo-stem, number of cormels/plant, corm diameter and corm fresh weight per plant, suggesting the environmental effect on these traits observed to be low. Due to higher h^2_b estimates, greater benefits from clonal selection might be expected for these traits. The h^2_b and GA% were negative for cormel length. Normally, heritability values range from 0-1 but this is not always the case since the experimental error could lead to estimates outside the stated range (Conner and Hartl, 2004). Negative h^2_b for cormel length with negative GA% may indicate that cormel length is mostly influenced by environment, suggesting that limited benefit might be expected from selection of cormel length for cocoyam improvement.

PCA is often conducted to build a new set of orthogonal coordinate axes and to find out the relative significance of classification traits (Shinwari *et al*., 2014). There are no dealings to discover the worth of a coefficient but that is eigen vector (Düzyaman, 2005). The overall observations for the 3 PCs, with the eigen values >1 for 13 quantitative traits explained 69.2% of the observed variations among 100 cocoyam accessions. This indicated that the traits within these 3 PCs exhibited great influence on the phenotype of the accessions and can be used for selection among accessions. Solomon Fantaw *et al*. (2014a) reported 4 PCs which explained 70.5% of the total variation present among 64 cocoyam accessions at Jimma, Ethiopia.

The quantitative morphological traits which had loaded relatively higher to the 3 PCs were plant height, petiole length, lamina length, lamina width, circumference of pseudo-stem, number of cormels per plant, cormel length, cormel fresh weight/ plant, corm diameter and corm fresh weight per plant. These quantitative traits and 9 qualitative traits that categorized the cocoyam accessions into two groups will help the researchers in recommending best traits for morphological identification of cocoyam germplasms. Top coefficients for traits designate the relatedness of traits to the PC axes (Sneath and Sokal, 1973). Score plot effectively separated purple cocoyam morphotypes from green cocoyam morphotypes. Supporting the PC analysis, cluster analysis based on Euclidean distance had grouped most of the purple cocoyam morphotypes in distinct cluster (cluster III). This may suggest the existence of genetic difference between the two groups of cocoyam morphotypes.

Most of the accessions collected from different zones, districts or kebeles were clustered together, suggesting that cocoyam accessions might have been transported across localities. Similar study conducted by Solomon Fantaw *et al*. (2014a) showed that most of the cocoyam accessions from different districts and villages of Ethiopia were clustered together. In Ghana, 70 cocoyam accessions collected from different geographical regions clustered together (Offei *et al*., 2004). Similarly, Opoku-Agyeman (2004) reported that 78 cocoyam accessions from 7 regions of Ghana were clustered into eight different groups, irrespective of the collection sites. The study identified morphological traits for morphological identification of cocoyam accessions. The accessions should be tested in more than one environment to select elite genotypes for future utilization of cocoyam in Ethiopia.

Chapter Five

Assessment of Genetic Diversity and Differentiation of Cocoyam (*Xanthosoma sagittifolium* (L.) Schott) from Ethiopia Based on SSR Markers

Abstract

Cocoyam (*Xanthosoma sagittifolium* (L.) Schott) is originated in the tropical America and nowadays it is distributed throughout the tropical world. It is cultivated in southern, southwest and western parts of Ethiopia, for its food and feed values. There is no information available on molecular diversity of cocoyam growing in the country. This study was carried out to examine the genetic diversity and differentiation of 100 cocoyam accessions (65 green colored and 35 purple colored) using 11 polymorphic microsatellite loci. A total of 36 alleles were detected with an average of 3.273 alleles per locus with range between 2 and 5 alleles. The average Ho value across populations was 0. 503. The Ho values of green morphotypes, purple morphotypes and when all collections were considered as single population were 0.466, 0.577 and 0.508, respectively. The average He values across populations was 0.443. The He values of green morphotypes, purple morphotypes and when all collection were considered as single were 0.291, 0. 345 and 0.566, respectively. These high values of He imply that our collections contained genetically diverse cocoyam accessions with high levels of heterozygosity. The greater Ho than He for most of the loci (54.5%) may suggest high rates of vegetative propagation. Based on Nei's F-Statistics for overall loci showed strong differentiation among populations (mean Fst = 0.196). Very strong differentiation was observed between green and purple cocoyam morphotypes (mean Fst = 0.463). The analysis of molecular variance showed that the variation among population explained 14% while the variation from among individuals within populations and within individuals, respectively, explained 18% and 68% of the total variation. Dendrogram based on Nei's standard genetic distance grouped the green morphotypes together while the purple cocoyam morphotypes occupied separate position within the dendrogram. Two clusters, which were detected by using model-based Bayesian clustering, were fully associated with two cocoyam morphotypes. A high level of genetic diversity within population, within morphotypes as well as at entire collection level implies genetically diverse cocoyam accessions probably with multiple lineage have been growing in Ethiopia, and which should be considered as a good opportunity for future use and conservation of cocoyam in the country.

Keywords: Cocoyam, genetic diversity, SSR markers

5. 1 Introduction

Cocoyam (*Xanthosoma sagittifolium* (L.) Schott) is one of tuberous root crops. It was ranked as 6th among root and tuber crops in cultivation and production (Bown, 2000). It originated and first domesticated in tropical America. Nowadays, it is widely cultivated in many tropical countries for its edible tubers and leaves (Ramawat and Merillon, 2014). It has high acceptance and serve as food security crop, mainly for small scale farmers (Osawaru and Ogwu, 2015).

Cocoyam is exotic crop to Ethiopia although it is not clear when and how it was introduced into the country. The existence of cocoyam gene pool in south, southwest and western parts of the country was reported by different authors (Zemede Asfaw, 2001a, b; Amsalu Nebiyu and Tesfaye Awas, 2006; Amsalu Nebiyu *et al*., 2008; Fujimoto, 2009; Solomon Fantaw *et al*., 2014a, b; Feleke Woldeyes *et al*., 2016), but not received thoughtful research effort.

The knowledge of genetic diversity result from analysis and characterization of accessions allows evaluation of genetic variability, which is a fundamental element to determine conservation plans and breeding strategies (Mwenye, 2009). Morphological markers-based characterization of cocoyam accessions from Ethiopia was undertaken by Solomon Fantaw *et al*. (2014a) and Chapter 4 of this study. The genetic information provided by morphological traits is, however, often limited and expression of quantitative traits is subjected to strong environmental influence (Karp *et al*., 1997; Mondini *et al.*, 2009). 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 genome.

Molecular markers have several advantages over morphological markers (Kordrostami and Rahimi, 2002) although they cannot completely replace morphological traits-based

characterization. Thus, complementary to morphological characterization, the use of molecular markers-based characterization has been increasing important as it suggests more specific results (Karp *et al*., 1997). Molecular markers do not affect the phenotype of the trait of interest because they are located only near or linked to genes controlling the trait, but they can be used as signs or tags when they are located in loci, where they control specific traits within the organism (Ishikawa *et al*., 1989). They could reveal polymorphism in a DNA sequence or the presence or absence of a particular DNA sequence at a particular site in the genome.

Molecular marker-based techniques such as random amplified polymorphic DNA (RAPD) (Schnell *et al*., 1999; Offei *et al.*, 2004), amplified fragment length polymorphisms (AFLPs) (Loh *et al*., 2000), chloroplast and mitochondrial-specific primers (Brown and Asemota, 2009), simple sequence repeats (SSRs) (Cathebras *et al*., 2014), retrotransposon based molecular markers (Doungous *et al*., 2015) and inter simple sequence repeats (ISSR) (Sepúlveda-Nieto *et al*., 2017) have been used to characterize cocoyam accessions from different countries. Genetic diversity of cocoyam accessions from Ethiopia has not been reported using molecular markers. In order to enhance effective usage and conservation of this neglected and underutilized crop in the country, it is important to study its genetic diversity in detail. In this study, therefore, the genetic diversity of cocoyam in Ethiopia was assessed by using SSR markers to generate data that will be beneficial in usage and conservation of cocoyam in the country.

5.2 Materials and methods

5.2.1 Plant materials

One hundred cocoyam tubers were collected from 5 zones (Bench-Maji, Kefa, Dawuro, Wolaita and Gamo-Gofa). Twelve to twenty-eight accessions were sampled from each zone. The individual accessions were collected from at least 5 km apart unless they were clearly distinguished by leaf and petiole color difference (65 green- and 35 purple-colored) (Table 4.1). The collected accessions were planted at a common garden at Areka Agricultural Research Center, 303 km southwest of Addis Ababa, Ethiopia. Young leaf samples of 100 cocoyam accessions were collected by using silica gel (Bio lab). In the assumption that accessions might have been shared within zones more frequently than among zones, those cocoyam accessions collected from the same zone were considered as one population for genetic diversity analysis.

5.2.2 DNA extraction

Total genomic DNA was extracted by using DNeasy plant min kit (QIAGEN), briefly as follows: The dried leaf sample (~20 mg) was grinded using liquid nitrogen. Cell lysate buffer (400 µl) and RNase A (4 µl) were added and mixed by vortexing. The mixture was incubated for 30 minutes in dry heat shock by continuously mixing at 300 rpm. After adding neutralization buffer (130 µl), the sample was incubated on ice for 10 minutes and centrifuged for 5 minutes at 14,000 rpm. The lysate was pipetted into QIAGEN shredder spin column and centrifuged for 2 minutes at 14,000 rpm. The flow-through was transferred to a new tube without disturbing the pellet. Then, washing buffer (1.5 * volume of the flow through) was added, mixed by pipetting and transferred to a DNeasy mini spin column and centrifuged for 1 minute at 8,000 rpm. The flow-through was discarded, and the spin column was placed into a new 2 ml collection tube. For final washing, another washing buffer (500 µl) was added and centrifuged for 1 minute at 8,000 rpm. The spin column was removed

carefully and transferred to a new 1.5 ml microcentrifuge tube. Finally, elution buffer (100 µl) was added and incubated for 5 minutes at room temperature and then centrifuged for 1 minute at 8,000 rpm. The genomic DNA was checked by 2% agarose gel and measured by Nanodrop 2000 (Thermo Fisher).

5.2.3 SSR primers and PCR amplification

Seventeen microsatellite primer pairs identified by Cathebras *et al*. (2014) for *X. sagittifolium* were tested with 5 randomly selected samples. Twelve primer pairs, which gave an intense band on an agarose gel were selected for subsequent analysis (Table 5.1). The forward primer of each of the 12 pairs was labeled either with 6-FAM (6-Carboxyfluorescein) or Hexachloro-fluorescein (HEX). The markers were paired into 6 PCR sets with each set containing a 6-FAM labeled primer pair and a HEX labeled primer pair. These labeled primers were used to test 15 samples in a pre-analysis. PCR was performed in a total volume of 20 µl containing 2µl of 10x PCR buffer (15 mM $MgCl_2$ included), 0.4 µl dNTP mix (10 mM each), 0.5 µl (16 pmol/µl) of each of the forward and reverse SSR primers, 2 µl genomic DNA (20 ng/µl), 0.2 µl Taq polymerase and 14.4 µl ddH_2O. Amplification was performed in ABI 2720 Thermocyler. After initial denaturation at 95°C for 2 minutes, 30 cycles of denaturation at 94°C for 30 seconds, annealing at 52°C for 45 seconds, extension at 72°C for 1 minute followed with a final extension of 72°C for 10 minutes were used.

After verification of PCR amplification through 1% agarose gel electrophoresis, capillary electrophoresis was run on the same basic principles. The aliquots of PCR product (1 µl/well) were mixed with formamide (12 µl/well) and GeneScan™ 400HD ROX™ Size Standard (0.4 µl/well) in a 96 plate. The contents were denatured using a Boekel dry bath, which was heated to 95°C for 5 minutes and then the samples were quenched at -20°C for 5

minutes and then electrophoresed on an ABI 3130XL Genetic Analyzer (Applied Biosytems). The number and sizes of electrophoresed DNA fragments were identified using GeneMapper version 4.0 (Currie-Fraser *et al*., 2010), which calculates allele sizes at each microsatellite locus compared to a size standard (HD400 ROX). Data were collected, evaluated and edited by using GeneMapper and exported to a Microsoft Excel spreadsheet.

Table 5.1 Primers sequences used for amplification of microsatellite markers

Duplex	Locus name	Repeat motif	Primer sequence (5 ′-3 ′)	Primer direction
A	mXsCIR05	(CA)8 (CACA)3	6-FAM-CGCATTATTAACGAATATC	Forward
			TCATCTATGGCTATCACCT	Reverse
	mXsCIR07	(TG)7 (AG)19	HEX -GGACTGGGAGTCTGAGTAG	Forward
			CCTTTCCCCTCACTATAAA	Reverse
B	mXsCIR10	(AG)22	6-FAM-ATGTCTGTAGTGGCCTAGT	Forward
			AATTAAGTTGGGTGGTAGAT	Reverse
	mXsCIR22	(AG)22	HEX-CGTGAGAAACACCTGAATTA	Forward
			AATTTGCTCTGTCATTGTG	Reverse
C	mXsCIR11	(TG)10 (GA)16	6-FAM-AATTCTTAGCAGCATTGTTA	Forward
			CATTCGTATCAACTTCCTTT	Reverse
	mXsCIR24	(AG)23	HEX- AATTTGAAGTGAAACGATCA	Forward
			TCCTGTCATCAGAATTGTA	Reverse
D	mXsCIR12	(TC)17 (TTC)7	6-FAM-TACATTTCCATTGCCATC	Forward
		(TCCC)3 (TTCTTG)3	AAATTAAAGAGGGAGACAG	Reverse
	mXsCIR27	(AG)15 (GAA)6	HEX-TGCATGAATTGAAGAAAT	Forward
			AACAAAGAGTCTCACCACAT	Reverse
E	mXsCIR19	(AC)8 (AC)24 (AC)8	6-FAM -AACTTGTGTATCCTACATCC	Forward
			GCGTGGTTTATGTGTATCTT	Reverse
	mXsCIR21	(AG)30	HEX-CTTAACCTTGTCAGCCTCT	Forward
			GAGCGGTATAACAATTCATC	Reverse
F	mXsCIR16	(AG)15	6-FAM-CTTATTGATGCCGAGAATAC	Forward
			TTCCTCACAATATGTTCTCAT	Reverse
	mXsCIR28	(GA)9	HEX- ACAGAAGTTGACATGGAGAG	Forward
			AATGTTAAAGAGCAAAAGGA	Reverse

5.2.4 Data analysis

The number of alleles (Na), the number of effective allele (Ne) (Kimura and Crow, 1964), Shanon information index (I) (Lewontin, 1972), observed (Ho) and expected (He) heterozygosity within individual populations, within green morphotype, within purple morphotype and at entire collection level were generated using the program POPGENE version 1.32 (Yeh *et al*., 1999). The genetic differentiation among populations as well as between green- and purple-morphotypes were estimated locus by locus by using the software GenAlEx version 6.503 (Peakall and Smouse, 2006), in terms of Nei's F-statistics (Fst) (Nei, 1987). AMOVA was performed to evaluate the partitioning of molecular variation within and among populations by using the same program, by performing a significance tests using 999 permutations. The genetic clustering was assessed by performing PCoA. A neighbor joining tree, depicting relationships among individuals was constructed by using neighbor joining algorithm implemented in the computer program PHYLIP version 3.6 (Felsenstein, 2005) using Nei's genetic distance (Nei *et al.*, 1983) based on the frequencies generated by using MSA version 4.05 (Daniel *et al*., 2003). The genetic structure was determined by using the program STRUCTURE version 2.2.4 (Pritchard *et al*. 2000) based on Bayesian clustering approach (Evanno *et al*., 2005) to infer the most likely number of gene pools and the extent of genetic admixture among populations. The program was run 10 times for each K, starting from K=1 to K=20 with an initial length of 100,000 burn-in periods followed by 200,000 Markov Chain Monte Carlo (MCMC) repeats for K. The analysis was carried out using an admixture model with allele frequencies correlated among populations and the parameter of individual admixture alpha set to be the same values for all clusters with a uniform prior.

5.3 Results

5.3.1 Genetic diversity of cocoyam as revealed by SSR markers

Among a total of 12 SSR loci, 11 (91.7%) showed polymorphism. One locus (locus mXsCIR28), which showed monomorphic fragments in all analyzed samples, was not used for further analyses. A total of 36 alleles were detected. The Na at each locus ranged from 2 to 5 with mean of 3.273. The Ne per locus ranged from 1.412 (locus mXsCIR07 to 3.759 (locus mXsCIR22) with mean Ne of 2.516. The observed heterozygosity (Ho) ranged from 0.000 (loci mXsCIR19, mXsCIR16 and mXsCIR24) to 1.000 (loci mXsCIR22 and mXsCIR27) with mean Ho of 0.508. The expected heterozygosity (He) ranged from 0.294 (locus mXsCIR07) to 0.738 (locus mXsCIR22) with mean He of 0.566 (Table 5.2). Six SSR markers (54.5%) presented Ho values higher than the He values when all collections are considered as single population.

Table 5.2 Genetic diversity parameters shown by 11 polymorphic loci in 100 cocoyam accessions

Locus name	Na	Ne	I	Ho	He
mXsCIR05	4	3.499	1.304	0.800	0.720
mXsCIR07	2	1.412	0.468	0.355	0.294
mXsCIR19	2	1.851	0.652	0.000	0.462
mXsCIR10	4	2.810	1.087	0.905	0.648
mXsCIR21	4	2.498	1.098	0.372	0.603
mXsCIR22	4	3.759	1.354	1.000	0.738
mXsCIR11	4	2.158	0.969	0.650	0.539
mXsCIR24	2	1.821	0.643	0.000	0.453
mXsCIR12	3	2.368	0.972	0.505	0.581
mXsCIR27	5	3.689	1.364	1.000	0.733
mXsCIR16	2	1.807	0.639	0.000	0.449
Mean	**3.273**	**2.516**	**0.959**	**0.508**	**0.566**
St Dev	**1.103**	**0.823**	**0.318**	**0.395**	**0.142**

Na - number of different alleles, Ne - effective number of alleles, I - Shannon information index, Ho - observed heterozygosity and He - expected heterozygosity

5.3.2 Genetic diversity within populations and within morphotypes

The observed number of alleles (Na), effective number of alleles (Ne), observed heterozygosity (Ho) and expected heterozygosity (He) and Shannon information index (I) across populations are presented in Table 5.3. The average Na for individual populations varied from 1.727 (Bench-Maji, Kefa) to 3.091 (Gamo-Gofa) and the average Ne for individual populations varied from 1.560 (Kefa) to 2.660 (Gamo-Gofa), with mean Na = 2.455 and Ne = 2.091, cross-populations. The Ho values ranged from 0.446 (Bench-Maji) to 0.540 (Gamo-Gofa). The He values ranged from 0.267 (Bench-Maji) to 0.608 (Gamo-Gofa). The Shannon diversity index ranged from 0.391 (Bench-Maji) to 0.990 (Gamo-Gofa). The genetic diversity analysis conducted by grouping green and purple morphotypes separately showed that green morphotype had lower mean Na =1.909, Ne = 1.594, Ho = 0.466, He = 0.291 and I = 0.439 than purple cocoyam morphotype, which had mean Na = 2.273, Ne = 2.516, Ho = 0.508, He = 0.3447 and I = 0.5263 (Table 5.3).

Table 5.3 Genetic diversity within populations (A) and within morphotypes (B) using 11 microsatellite loci

Population	**N**	**Na**	**Ne**	**Ho**	**He**	**I**
A						
Bench-Maji	14	1.727	1.631	0.446	0.267	0.391
Kefa	12	1.727	1.560	0.515	0.289	0.399
Dawuro	28	2.818	2.093	0.492	0.476	0.787
Wolaita	28	2.909	2.513	0.520	0.574	0.935
Gamo-Gofa	18	3.091	2.660	0.540	0.608	0.990
Average across populations	**20**	**2.455**	**2.091**	**0.503**	**0.443**	**0.700**
B						
Green cocoyam	65	1.909	1.594	0.466	0.291	0.439
Purple cocoyam	35	2.272	1.770	0.577	0.345	0.526
Single population	100	3.273	2.516	0.508	0.566	0.959

N - number of accessions; Na - number of different alleles; Ne - effective number of alleles; Ho - observed heterozygosity; He - expected heterozygosity; I - Shannon information index

5.3.3 Genetic differentiation

Paired comparisons based on locus by locus analysis for 11 polymorphic microsatellite loci among populations revealed that Fst ranged from 0.080 at locus mXsCIR10 to 0.418 at locus mXsCIR21. Most of the loci have indicated strong genetic differentiation among populations as well as between green- and purple-morphotypes. The Fst values between green- and purple-morphotypes ranged from 0.208 at locus mXsCIR21 to 1.000 at loci mXsCIR19, mXsCIR24, i.e., at these two loci, green- and purple-morphotypes showed complete differentiation. This shows that alleles at these loci were private either to green or to purple morphotype. Average F-statistics over all loci showed that genetic differentiation among five populations and morphotypes were strong and very strong as revealed by mean Fst values of 0.196 and 0.463, respectively (Table 5.4).

Table 5.4 F-statistics for 11 polymorphic loci across populations and between morphotypes

Locus name	Five cocoyam populations			Green and purple morphotypes		
	Fis	Fit	Fst	Fis	Fit	Fst
mXsCIR05	-0.358	-0.177	0.134	-0.621	-0.040	0.363
mXsCIR07	-0.377	-0.176	0.146	-1.000	-0.333	0.333
mXsCIR19	1.000	1.000	0.343	N/A	1.000	1.000
mXsCIR10	-0.597	-0.469	0.080	-0.770	-0.334	0.247
mXsCIR21	0.048	0.446	0.418	0.043	0.242	0.208
mXsCIR22	-0.570	-0.415	0.099	-0.982	-0.333	0.327
mXsCIR11	-0.405	-0.209	0.140	-0.561	-0.105	0.292
mXsCIR24	1.000	1.000	0.362	N/A	1.000	1.000
mXsCIR12	-0.081	0.101	0.169	-0.714	0.047	0.444
mXsCIR27	-0.586	-0.410	0.111	-0.969	-0.326	0.326
mXsCIR16	1.000	1.000	0.339	1.000	1.000	0.943
Mean F statistics over all loci	**-0.171**	**0.0790**	**0.196**	**-0.664**	**0.105**	**0.463**

Fis - Fixation index among individuals within population or color groups, Fit- fixation index among individuals within total data set, Fst- genetic differentiation among populations or between green and purple morphotypes

The partitioning of molecular variance (AMOVA) showed that the highest genetic variability (68%) existed within individuals and the lowest variability (14%) existed among populations (Table 5.5).

Table 5.5 Summary of AMOVA for five cocoyam populations based on SSR markers

Source of variation	df	Sum of squares	Estimated variance	% of variation	Fst	P value
Among Pops	4	94.046	0.510	14	0.143	0.001
Among Indivs	95	349.994	0.637	18		
Within Indivs	100	241.000	2.410	68		
Total	199	685.040	3.558			

5.3.4 Cluster analysis and population structure

The PCoA indicated that the first coordinate fully separated green morphotype from purple morphotype. Cocoyam accessions collected from Bench-Maji and Kefa zones were separated by the first coordinate while those from Dawuro, Wolaita and Gamo-Gofa Zones were not separated by both coordinates (Fig. 5.1). When the collection data is referred, only green morphotype were observed and collected from Bench-Maji and Kefa zones while both green and purple morphotypes were collected from the other zones (Table 4.1). Individual genotype clustering is presented in the neighbor joining (NJ) tree based on Nei's genetic distance. The clustering fully separated green morphotype from the purple morphotype (Fig. 5.2), supporting PCoA.

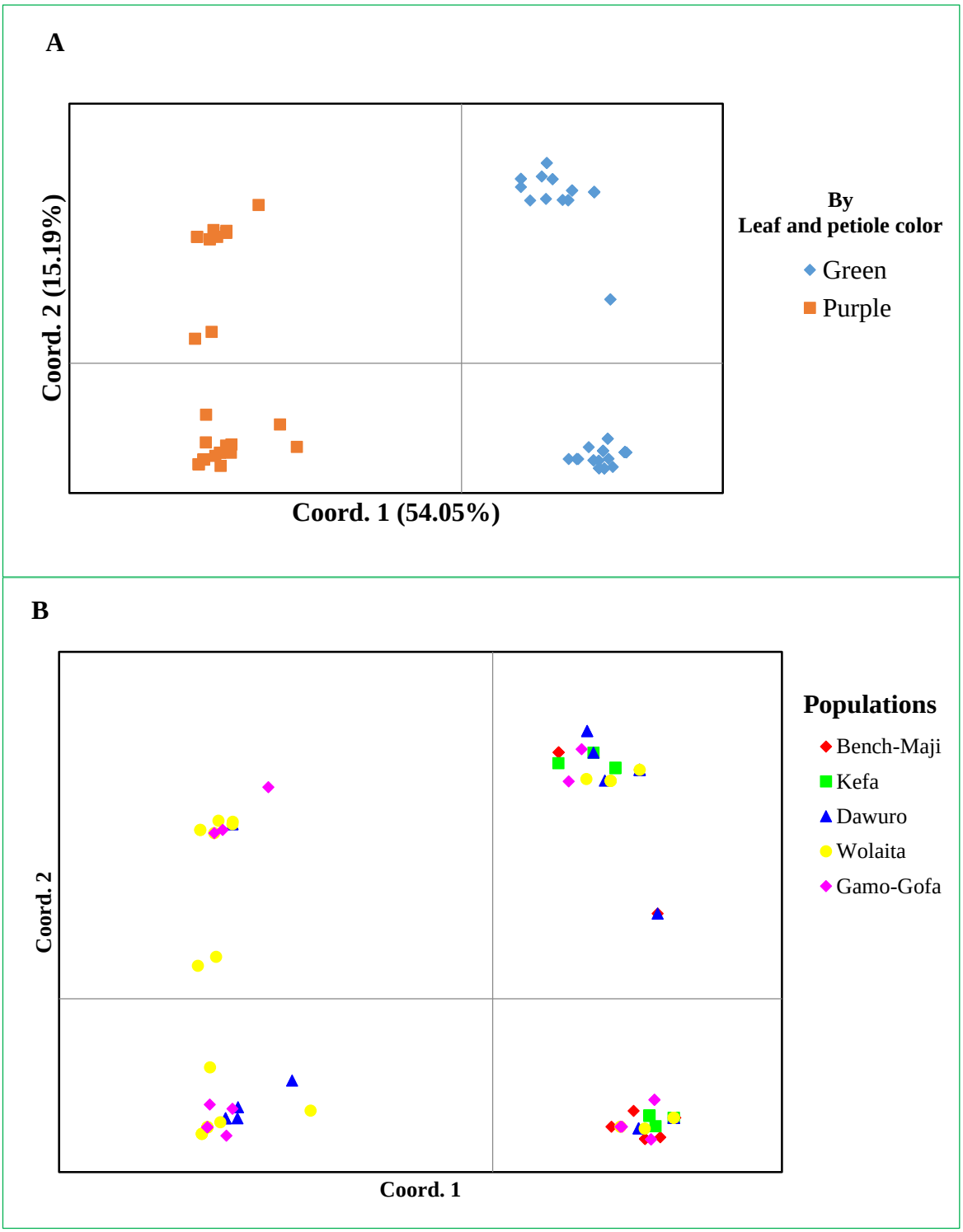

Fig. 5.1 A two-dimensional plot of the Principal Coordinate Analysis (PCoA) of 100 cocoyam accessions based on SSR data based on leaf/petiole color (A) and populations (B)

Fig. 5.2 Neighbor Joinings tree showing the relationships among 100 cocoyam accessions.

The tree was based on Nei's genetic distance (Nei *et al.*, 1983) using 11 microsatellite markers. Green- and purple-cocoyam morphotypes, represented by green and purple colors. Xs and the numbers, respectively stand for the species name and serial numbers of the accession code as defined in Table 4.1. The accessions Xs001-Xs014, Xs015-Xs026, Xs027-Xs054, Xs055-Xs082 Wolaita and Xs083 - Xs100 were from Bench-maji, Kefa, Dawuro, Wolaita and Gamo-Gofa zones, respectively.

To measure genetic structure of population and degree of admixture, the STRUCTURE algorithm was applied. The highest log-likelihood score was obtained for K=2 (Fig. 5.3a). Population structure detected by using the STRUCTURE software confirmed the results of PCoA and the neighbor joining (NJ), clustering green-colored cocoyam accessions from all of 5 populations in one group (Fig. 5.3b). The clusters identified for K=2 were 100% associated with the green and purple morphotypes, i.e., 65 green morphotype were assigned to one cluster and 35 purple morphotype were assigned to another cluster (Fig. 5.3c), where the genetic admixture was insignificant.

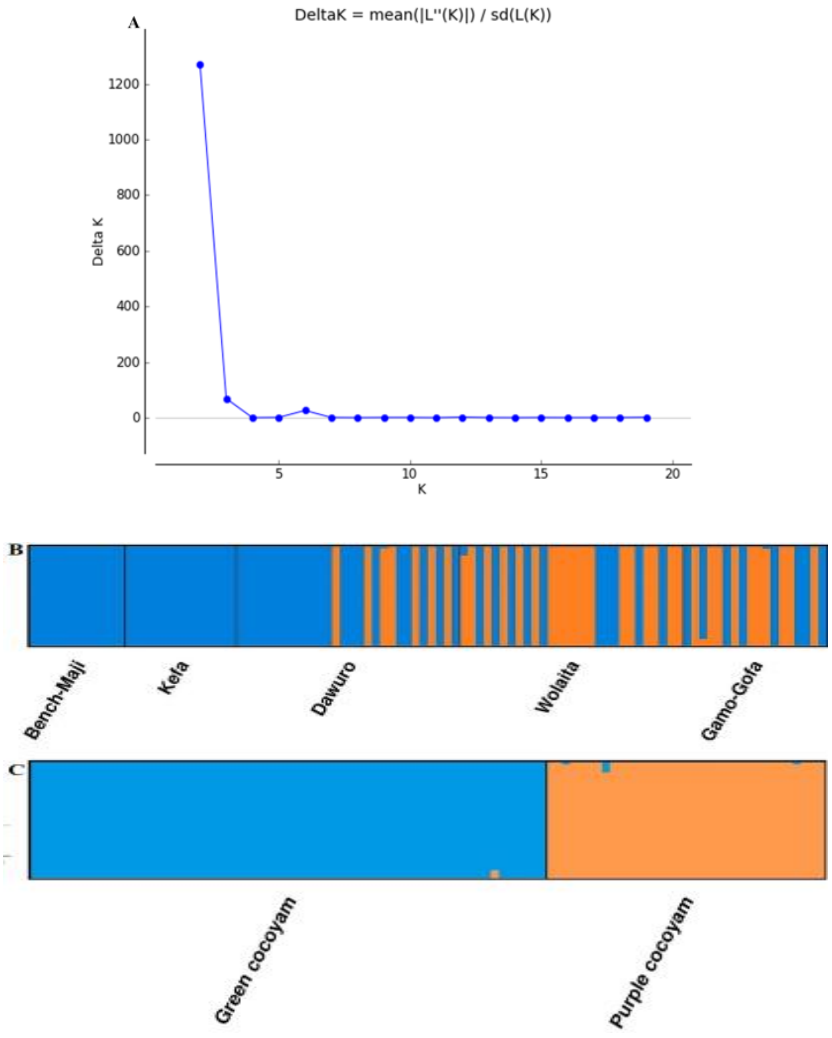

Fig. 5.3 Bayesian model-based clustering STRUCTURE analysis as inferred at K = 2 based on SSR data. The relationship between ΔK and K showing the highest peak at K = 2 (A); Each individual accession is epresented by a vertical line divided into colored segments (B). Membership of each individual within green- and purple cocoyam (C). Among the 20 runs carried out for each K, graphical representation of the highest ΔK at K=2 estimate was shown.

5.4 Discussion

The results of this study revealed that the majority (91.7%) of the SSR loci were polymorphic within 100 cocoyam accessions. In related study, Doungous *et al*. (2015) reported 88% to 97% polymorphic loci across 20 cocoyam accessions from Cameroon using retrotransposon molecular markers. The values of genetic diversity indices were high within populations, within green morphotype, within purple morphotype and at entire collection level. For example, He values across populations, within green morphotype and within purple morphotype were 0.443, 0.291, 0.345, respectively, all of which were high compared to that of *Ambrosina bassii* L. (Araceae, Ambrosineae), where mean He was 0.263 (Geraci *et al*., 2009) and *Anthurium crenatum* (L.) Kunth (Araceae) (He = 0.167) (Acosta-Mercado *et al*., 2002). The mean He (0.566) detected when all collections were considered as single population was also greater than the average He (0.456) detected in taro (*C. esculenta*) accessions by using microsatellite loci (Wansha *et al*., 2011). The high He values detected in this study imply that our collections contained genetically diverse cocoyam accessions. The average observed heterozygosity across populations as well as within green morphotype and within purple morphotype were higher than the respective average expected heterozygosity values. Six SSR markers (54.5%) presented Ho values higher than He values when all collections were considered as single population. The result was in agreement with the report of Cathebras *et al*. (2014), suggesting high rates of vegetative propagation in cocoyam. According to Bisognin (2011), vegetative propagation enables to fix high levels of heterozygosity. The progenies of vegetatively propagated crops are clones, which are genotypically similar to the parental genotypes (Simmond, 1962).

The mean values of Fst between green- and purple- cocoyam morphotype showed very strong differentiation demonstrated by Fst value of 0.463. According to Wright (1965), the genetic differentiation is low, moderate, strong and very strong when Fst value is between

0-0.05, 0.05-0.15, 0.15-0.25 and >0.25, respectively. The mean value of Fis was negative and also negative for most of the loci, implying that there is less effect of outcrossing among individuals within populations. This is may be due to rarely flowering nature of cocoyam. The analysis of molecular variance (AMOVA) showed that the variation among populations explained 14% while the variation from among individuals within populations and within individuals explained 18% and 68% of the total variation, respectively. This indicates that highest percentages of variation which can be used to improve cocoyam for traits of interest were found within individuals.

PCoA, NJ tree based on Nei's genetic distance and population genetic structure analyses resulted in clusters that were associated with green and purple morphotype, which can easily be differentiated by their leaf color (green or purple). This indicates that the significant fraction of the SSR markers between morphologically distinct cocoyams. Over all, there is high genetic diversity within populations, within green morphotype, within purple morphotype as well as when all collections were considered as single population. Very strong genetic differentiation was detected between green and purple morphotypes. This indicates that the vegetative propagation from stem underground structures (Kay, 1987), may resulted in a fixed SSR markers genetic structure and cocoyam grown in Ethiopia may have remained genetically unchanged with high heterozygosity. A high level of genetic diversity implies geneticaly diverse cocoyam accessions probably with multiple lineage have been growing in Ethiopia, and which should be considered as a good opportunity for future use and conservation of cocoyam in the country.

Chapter Six

AFLP Fingerprinting for Assessment of Genetic Diversity and Differentiation of Cocoyam (*Xanthosoma sagittifolium* (L.) Schott

Abstract

Cocoyam (*Xanthosoma sagittifolium* (L.) Schott) is an exotic crop to Ethiopia. Less research attention was given to cocoyam as it is a neglected and underutilized crop. To estimate the genetic diversity and population structure, 78 cocoyam accessions (54 green colored and 24 purple colored) were analyzed using AFLP markers. Three pairs of AFLP primers resulted in 478 scorable bands and 99.2% of polymorphic loci. The mean Nei's gene diversity (He) across populations was 0.349 the green morphotype showing 0.385 whereas purple morphotype showing 0.351. Considering all collections as a single population, the expected heterozygosity (He) was 0.389. Low level of genetic differentiation was detected among populations by the Nei's Gst = 0.072 as well as between green and purple morphotypes by the Nei's Gst = 0.024. ANOVA indicated 4% and 3% of genetic variation existed among populations and between green and purple morphotype, respectively. These low genetic differentiation results were further confirmed by PCoA, NJ clustering and Bayesian Model based STRUCTURE. Taking together, this AFLP markers-based study results showed a high level of genetic diversity within population, within green morphotype, within purple morphotype as well as when all collections were considered as single population, implying that genetically diverse cocoyams have been growing in Ethiopia. Low level genetic differentiation detected among populations indicates that there might have been movement of germplasm across the locations, possibly by farmer-to-farmer planting materials exchange. Cocoyam management and conservation strategies should concentrate on maintaining the existing high diversity within each population for sustainable utilization of the crop in Ethiopia.

Keywords: AFLP, Cocoyam, genetic diversity, genetic differentiation, *X. sagittifolium*

6.1 Introduction

The genetic diversity of cultivated plant species is the result of evolution and human intervention. Hereby, multiple factors shape the genetic structures of cultivated plants (Oyama *et al*., 2006), including genetic drift, migration, mutation and selection (Wright 1978). The ultimate goal for the conservation of genetic resources is to guarantee sustainability of genetic diversity (Kingston *et al*., 2004; Magbagbeola *et al*., 2010; Dansi *et al*., 2012). Thus, it is important to examine the genetic diversity of crop plants for conservation and for inferences in plant breeding programs (Lopes *et al*., 2014; Larranaga *et al*., 2017).

Cocoyam, tania, tannia, arrowleaf, elephant's ear, malanga, marron, taye or tayove are some of the globally used vernacular names to define a group of cultivated plants in tropical and subtropical regions of the world that belong to the genus *Xanthosoma* (Araceae). The name *X. sagittifolium* (L.) Schott has usually been given to the most cultivated members, either for their ornamental use or for food source (tubers and leaves), of this genus (Giacometti and Leon 1994; Govaerts *et al*., 2002). Various taxonomic names have also been used synonymously (Lim, 2015; http://www.theplantlist.org) as *X. sagittifolium* is one of the neglected and understudied cultivated plant species (Doungous *et al*., 2015). There are discrepancies and uncertainties regarding the taxonomy even at the species level (Castro 2006; Giacometti and Leon 1994). In the course of this study, we refer to the accepted species status of *X. sagittifolium* in the Araceae Family (Govaerts *et al*., 2002) and in The Plant List (http://www.theplantlist.org).

Cocoyam is one of the oldest cultivated aroid in the world. There are several adaptations that allow the species to survive and spread. It grows in a variety of substrates and habitats. It tolerates drier, but not waterlogged soils (Kay, 1987; Bown, 2000) and grows in full sun

or deeply shaded areas under the canopy of natural forests (Manner 2011). Although cocoyam is a lowland plant, it also grows in the highlands, e.g. in humid, tropical climate up to 1500 m.a.s.l (Manner, 2011).

Cocoyam is a perennial plant, but for practical purposes, most often cultivated as an annual crop. Harvest usually occurs during the dry season, 9-12 months after plantation (Lebot, 2009). At the end of the growing season and in water stressed conditions, leaves may die and shoots may wither completely (Onwueme and Charles, 1994; Jackson, 2008; Ramawat and Merillon, 2014). Cocoyam accessions seldom flower. When flowering occurs, the flowers are monoecious within a compound inflorescence. The female flowers are at the base of the spadix and the male flowers are above. Sterile flowers are located between the pistillate and staminate flowers. The inflorescence is protogynous; the stigma is normally receptive two to four days before pollen shed (Kay, 1987). Thus, cocoyam rarely set sexual seeds and hence it is mainly propagated vegetatively from corm sets, headsets or cormels (Jackson, 2008).

Cocoyam is believed to have originated from northern South America, spread to the Caribbean and Mesoamerica, and was subsequently introduced elsewhere into Africa, Asia and the Pacific. It has become a subsistence crop in West African countries (Ghana, Nigeria, Cameroon), which are by now the major cocoyam producers of the world (Giacometti and Leon, 1994). Cocoyam was introduced into East Africa through Western Africa and is popular in Tanzania and common in Uganda (Maundu *et al*., 2009). It is exotic crop to Ethiopia, but there is no clear information on how and when it was introduced into the country. The species is not mentioned in the Flora of Ethiopia (Reidl, 1997; Hedberg *et al*., 2009). According to Amsalu Nebiyu and Tesfaye Awas (2006) there are a considerable amount of cocoyam gene pool in south and southwest Ethiopia in farmers' fields and home-

gardens. Cocoyam has become an important part of agricultural and food system of the indigenous communities in the country (Amsalu Nebiyu *et al*., 2008; Fujimoto, 2009; Feleke Woldeyes *et al*., 2016).

Genetic diversity of cocoyam has been investigated by using Randomly Amplified Polymorphic DNA (RAPD) in Florida (Schnell *et al*., 1999) and Ghana (Offei *et al*., 2004). Doungous *et al*. (2015) used Inter-Retrotransposon Amplified Polymorphism (IRAP) to discriminate between cocoyam and taro. Brown and Asemota (2009) and Cathebras *et al*. (2014) applied microsatellite markers for the same question. Loh *et al*. (2000) applied Amplified Fragment Length Polymorphism (AFLP) to determine the intergeneric relationships between *Caladium*, *Xanthosoma* and other closely related genera within Araceae. Different marker systems are currently available for assessmet genetic diversity among populations. According to Donald (1994), AFLP is the most efficient marker to identify individual genotypes in a highly clonal species. It has the potential to increase the throughput of marker data production in organisms by allowing scoring of a large number of characters in a given population (Vuylsteke *et al*., 2008). In this study, we have used AFLP markers in order to achieve comprehensive information on the genetic diversity and differentiation of the Ethiopian cocoyam.

6.2 Materials and methods

6.2.1 Plant materials

A total of 82 *X. sagittifolium* tubers, representing four populations were collected from Bench-Maji (14), Kefa (12), Dawuro (28) and Wolaita zones (28) of Ethiopia (Table 4.1). Tubers of individual *X. sagittifolium* samples (accessions) that could be clearly distinguished by an observable morphological trait (color difference) were recorded as green and purple morphotypes. The collected tubers were planted in a common garden and young leaf samples were collected by using silica gel (Bio lab). The dried leaf samples were stored at room

temperature until further processing. The accessions that were collected from the same zone were considered as one population, assuming that clones are more likely shared within zones than among zones.

6.2.2 DNA extraction

Total genomic DNA was extracted using QIAGEN DNeasy plant min kit according to the manufacturer's instructions. The quality of DNA was checked on a 2% agarose gel. DNA concentration was assessed by using Nanodrop 2000.

6.2.3 AFLP analysis

The AFLP assay was performed according to a modified protocol of Vos *et al*. (1995). Genomic DNA was diluted to 30 ng/µl with ddH_2O and restricted using two different restriction enzymes: a frequent cutter (*Tru*1I) and a rare cutter (*Eco*RI) (Fermentas). The reaction volume was 25 µl in total (9.8 µl ddH_2O, 5 µl 10x Buffer Tango 2x Fermentas, 0.10 µl of 10 U/µl *Eco*RI, 0.10 µl of 10 U/µl *Tru*1I and 10 µl DNA). The mixture was incubated at 37°C for 1 h followed by at 65°C for another 1 h. Double stranded adapters were ligated to the restriction sites by adding 3 µl 10 mM ATP (Thermo Scientific), 0.5 µl 10x T4 DNA ligase buffer (Fermentas), 0.5 µl EA +/- (*Eco* +/-) (5 pmol) (MWG Biotech), 0.5 µl MA +/- (*Mse* +/-) (50 pmol) MWG-Biotech and 0.5 µl T4 DNA ligase (~2 U/µl) (Fermentas). The mixture was incubated at 22°C for 1 hr and 75°C for 10 min to inactivate the enzyme activities.

Two consecutive PCR amplifications were carried out with primers contained first one (+1) then three (+3) selective nucleotides at their 3`ends. The pre-PCR was carried out in 25 µl total reaction volume which contained 17.36 µl ddH_2O, 2.5 µl 10x dream Taq DNA polymerase buffer (20 mM $MgCl_2$ included), 2.5 µl dNTP-Mix (10 mM each), 0.5 µl 5 µM primer EpA (E01) MWG Biotech, 0.5 µl 5 µM primer MpC (M02) MWG Biotech, 0.14 µl

dream Taq DNA polymerase and 1.5 µl restriction-ligation product. After initial denaturation at 94°C for 3 minutes, 30 cycles were repeated at 94°C for 30 seconds, 56°C for 1 min and 72°C for 1 min, and a final extension at 72°C for 7 min. Then, the PCR product was run on 2% control agarose gel. The pre-PCR products were diluted 1:10 by using ddH_2O. Selective PCR amplification was carried out with three primer combinations (E32-AAC/M51-CCA, E35-ACA/M60-CTC, E40-AGC/M54-CCT) MWG Biotech. The sel-PCR reaction contained 8.53 µl ddH2O, 1.28 µl 10x dream Taq DNA polymerase buffer (20 mM $MgCl_2$ included), 0.28 µl dNTP Mix (10 mM each), 0.42 µl (1 µM) primer EpANN (MWG Biotech), 0.42 µl (5 µM) primer MpCNN (MWG Biotech), 0.07 µl dream Taq DNA polymerase and 1.8 µl pre-PCR product. The touchdown PCR program was run after adjusting the initial denaturation temperature at 94°C for 3 minutes, 30 cycles at 94°C for 30 seconds, 55°C for 1 min, 72°C for 1 min were repeated by reducing the primer annealing temperature by 0.7°C each and a final primers extension of 72°C for 7 minutes. After verification of PCR amplification through 1% agarose gel electrophoresis, sel-PCR products were separated by capillary electrophoresis (ABI Genescan/Applied Biosystems), which was done at LGC-Forensics/Cologne laboratory.

6.2.4 Data scoring and analyses

AFLP fragments (bands) were scored by using Genographer version 2.1.4 (Banks and Benham, 2008). Only distinct and well resolved AFLP bands in the size range of 40-520 bp were scored and a binary data matrix was constructed. POPGENE version 1.32 (Yeh *et al*., 1999) was used to calculate genetic diversities, which include the number of polymorphic loci (No. of PPL), the percentage of polymorphic loci (% PPL), the observed number of alleles (Na) and the effective number of alleles (Ne) (Kimura and Krow, 1964), Nei's gene diversity (He) (Nei, 1973) in individual populations and the Shannon's information index of diversity (I) (Lewontin, 1972). The coefficients of total population gene diversity (Ht), the

mean gene differentiation among populations (Gst) and within populations (Hs) were estimated using the same program in terms of Nei's F-statistics in subdivided populations. Analysis of molecular variance (AMOVA) was performed to evaluate the genetic variation within and among populations by using the software GenAlEx version 6.503 (Peakall and Smouse, 2006), performing significance tests using 999 permutations. The AMOVA technique was also used to determine the partitioning of molecular variation between and within green and purple morphotypes by using the same program. Potential correlations between genetic and geographic distances among individuals were estimated by using GenAlEx version 6.503 (Peakall and Smouse, 2006).

The genetic clustering was assessed by performing principal coordinate analysis (PCoA) by using the GenAlEx version 6.503 (Peakall and Smouse 2006). A neighbor joining tree was constructed by using neighbor joining algorithm (Saitou and Nei 1987) to examine the genetic relationships at individual level by SplitTree4 version 4.14.5 (Huson and Bryant, 2006). The genetic structure was also determined by using the program STRUCTURE version 2.2.4 (Pritchard *et al*., 2000) based on Bayesian and admixture models with a burn-in of 100,000 followed by a run-length of 200,000 Markov Chain Monte Carlo (MCMC) iterations for K. The analysis included 20 independent interactions and K-values ranging from 1 to 10 to determine the number of genetic clusters based on the criterion proposed by Evanno *et al*. (2005). Error rate of AFLP data was estimated using 12.8% of replicated samples according to Pompanon *et al*. (2005) because quantifying genotyping error rates is an essential component of an AFLP analysis.

6.3 Results

6.3.1 Genetic diversity of cocoyam as revealed by AFLP markers

From 82 cocoyam accessions belonging to four populations were, four accessions (accession codes 23, 36,49 and 73) were removed from analysis due to lab letdown. Thus, AFLP analysis was performed on 78 cocoyam accessions consist of 54 green and 24 purple morphotypes using three different sets of AFLP primer combinations. A total of 478 scorable bands were produced, of which, 474 (99.16%) were polymorphic. The amount and percentage of polymorphic loci (PPL) within populations ranged from 407 (85.15%) (Bench-Maji cocoyam population) to 461 (96.44%) (Dawuro and Wolaita cocoyam populations), with an average of 434.50 (90.90%) (Table 6.1). The highest Na (1.964) and Ne (1.649) exhibited by Dawuro cocoyam population while the lowest number of Na (1.852) and Ne (1.576) were observed in Bench-Maji cocoyam population. The green cocoyam morphotypes had slightly higher Na (1.985) and Ne (1.681) than purple cocoyam morphotypes which had Na = 1.941 and Ne = 1.628. Estimates of Nei's gene diversity in individual populations showed high values within each population with the highest diversity in Dawuro cocoyam population (He = 0.368) and the lowest diversity in Bench-Maji cocoyam population (He=0.327), with an average He = 0.349. The Nei's gene diversity estimate in green- and in purple-cocoyam morphotypes were high within each group, He = 0.385 and 0.351, respectively. Considering all collections as a single population, Nei gene diversity was also high (He = 0.389). The estimates of Shannon's information index showed values that are in similar trend with Nei's gene diversity (Table 6.1).

Table 6.1 Genetic diversity statistics based on AFLP data for cocoyam populations from Ethiopia: grouped according to the collection zones (A) and leaf and petiole color difference (B)

Population	N	No. of PPL	% PPL	Na	Ne	He	I
A							
Bench-Maji	14	407	85.15	1.852	1.576	0.327	0.480
Kefa	11	409	85.56	1.856	1.602	0.338	0.493
Dawuro	26	461	96.44	1.964	1.649	0.368	0.541
Wolaita	27	461	96.44	1.964	1.639	0.363	0.534
Average across pops	19.5	434.50	90.90	1.909	1.617	0.349	0.512
B							
Green cocoyam	54	471	98.54	1.985	1.681	0.385	0.564
Purple cocoyam	24	450	94.14	1.941	1.618	0.351	0.518
Single population	78	474	99.16	1.992	1.666	0.389	0.557

N – number of accessions, No. of PPL- number of polymorphic loci, % PPL - percentage of polymorphic loci, Na - observed number of alleles, Ne - effective number of alleles. He - Nei's gene diversity, I - Shannon's information index

6.3.2 Genetic differentiation and cluster analysis

Analysis of genetic variation among and within populations in terms of Nei's (1987) F-statistics in subdivided populations showed that the total population genetic diversity (Ht) was 0.378±0.02 and the mean genetic differentiation within populations (Hs) was 0.349±0.01. The mean genetic differentiation (Gst) among populations was 0.072. The Gst between green- and purple-cocoyam morphotypes was 0.024. The analysis of molecular variance (AMOVA) indicated little genetic differentiation among populations revealed by only 4.0% while most of the genetic differentiation (96.0%) found within populations. Only 3.0% of the total genetic variability explained the differentiation between green- and purple-cocoyam morphotypes (Table 6.2). Correlation between genetic distance and geographic distance among individuals was insignificant ($y=-0.0082x + 168.57$, $R^2 = 0.0053$, $p>0.05$).

Table 6.2 Summary of AMOVA for populations: grouped according to the collection zones **(A)** and morphotypes: grouped by leaf and petiole color difference **(B)**

Source of Variation	df	SS	Est. Var.	% of Var.	PhiPT	P value
A						
Among populations	3	432.953	3.397	4	0.040	0.001
Within populations	74	5993.80	80.988	96		
Total	77	6426.077	84.385	100		
B						
Between morphotypes	1	163.077	2.428	3	0.029	0.001
Within morphotypes	76	6263.000	82.408	97		
Total	77	6426.077	84.835	100		

df - degrees of freedom, SS - sum of squares, Est.Var. - estimated variance, % of Var. - percentage of variation

The two-dimensional plot of the PCoA represented 5.66% and 5.19% (in total 10.85%) of the detected variation revealing that most of the accessions, independent of origin or leaf color, cluster together whereas two groups consisted of two (accessions codes 34, 77) and five (accessions codes 29, 42, 50, 59, 61) green cocoyam accessions from Dawuro and Wolaita zones feature comparatively distinct patterns (Fig. 6.1). The PCoA analysis supported the genetic differentiation results, indicating that the genetic structure of cocoyam accessions included in this study was low. Clustering analysis also depicted a clear admixture of populations, regardless of the source populations and color groups (Fig. 6.2).

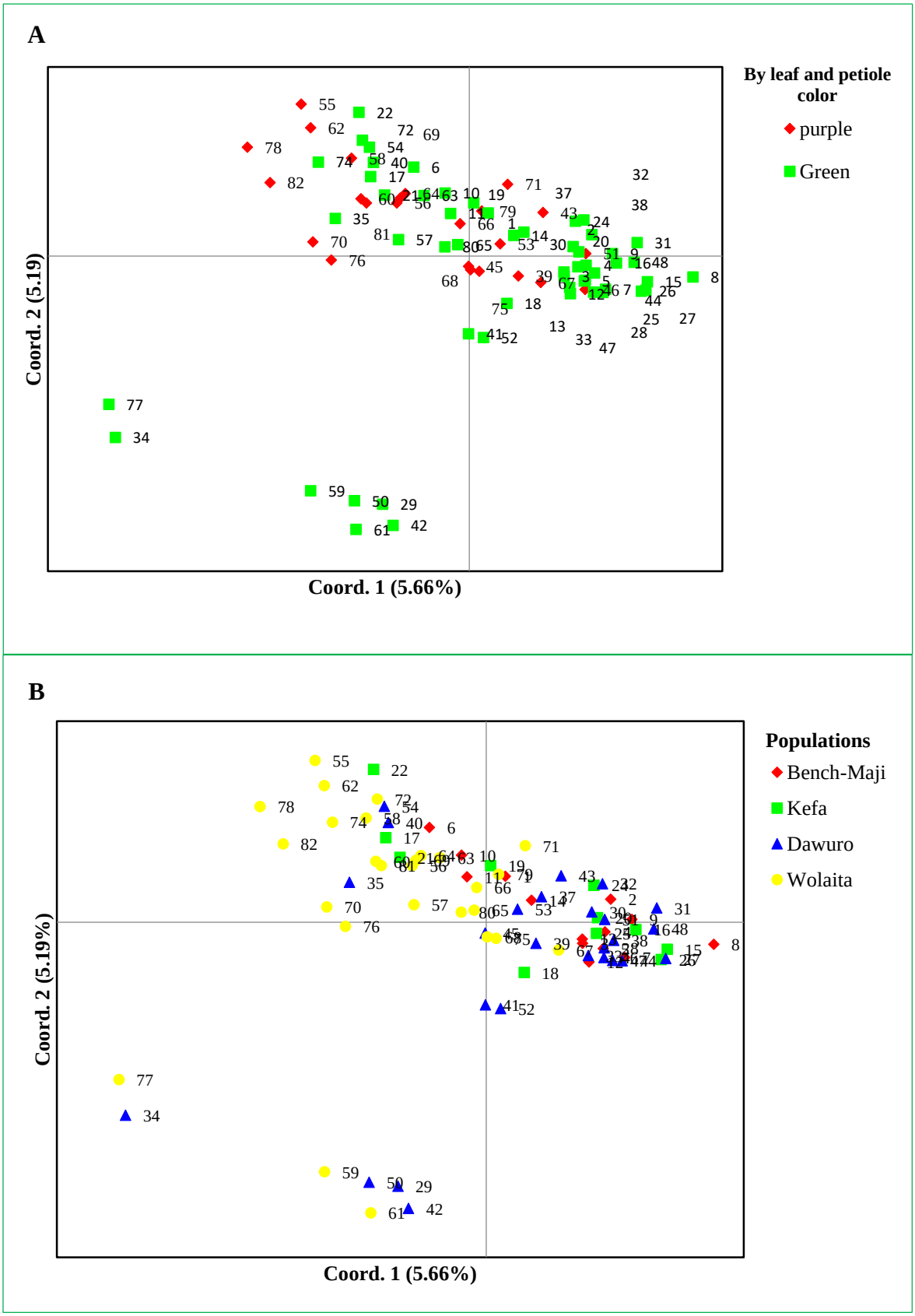

Fig. 6.1 Representation of the first two coordinates obtained from the PCoA of 78 cocoyam accessions based on AFLP data based on leaf/petiole color (A) and populations (B)

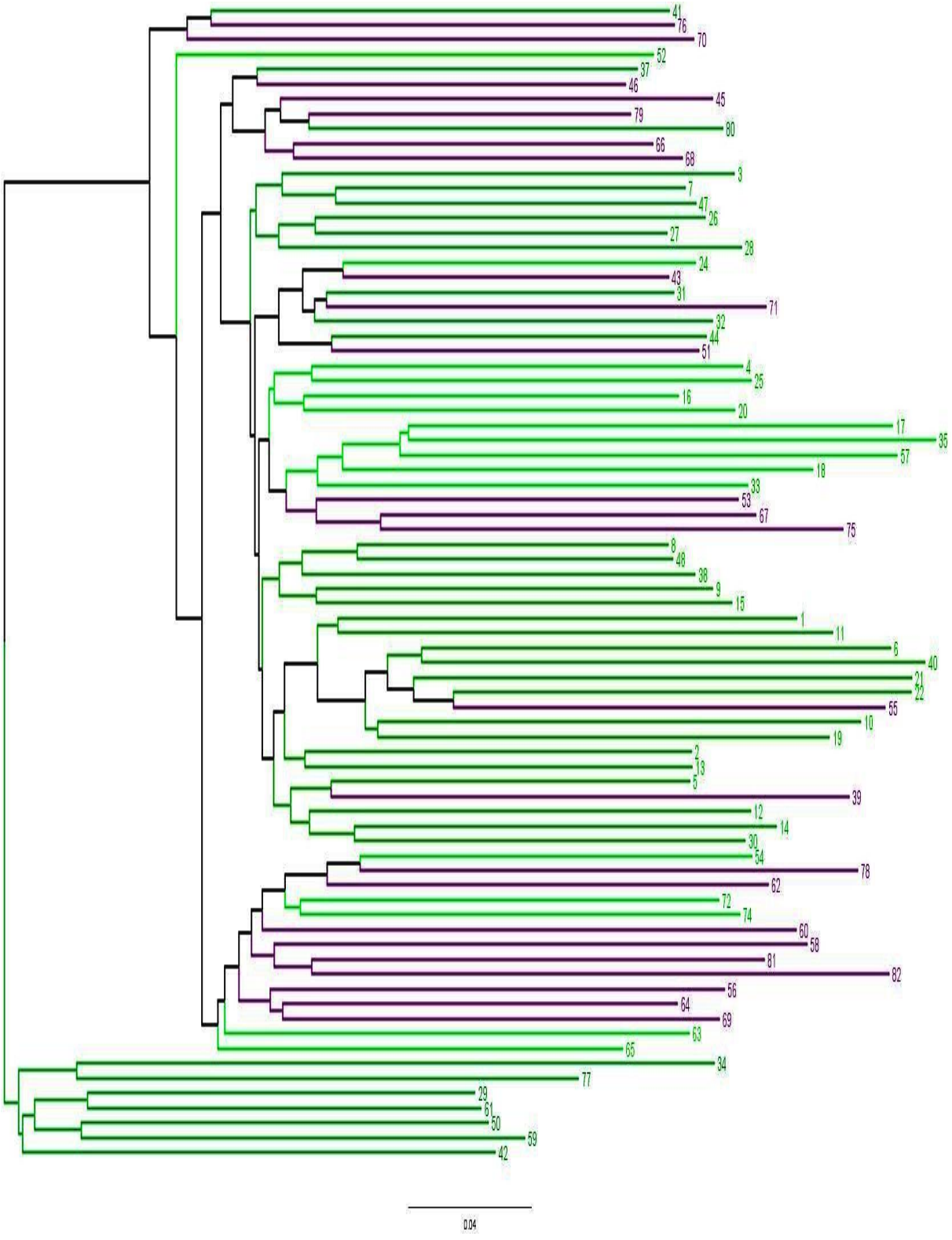

Fig. 6.2 Neighbor-Joining (NJ) tree representing clustering of cocoyam accessions generated from AFLP data. The tree was constructed by using neighbor joining algorithm implemented in the SplitTree4 version 4.14.5. The 78 accessions were clustered regardless of the source populations. Green and purple colors, respectively, indicate green- and purple-cocoyam morphotypes. The numbers stand for the serial numbers of the accession code as defined in Table 4.1. The accessions 1-4, 15-26, 27-54 and 55-82 were from Bench-maji, Kefa, Dawuro and Wolaita zones, respectively. Four accessions (23, 36, 49 and 73) were missing due to lab letdown.

The STUCTURE analysis based on the ΔK method revealed a clear maximum ΔK at K=3 in which accessions were classified into three clusters (Fig. 6.3). These results showed that the cocoyam accessions from four populations (Bench-Maji, Kefa, Dawuro and Wolaita) were clustered into three groups. This result supports the lack of association between genetic and geographic distance that was revealed by Mantle test.

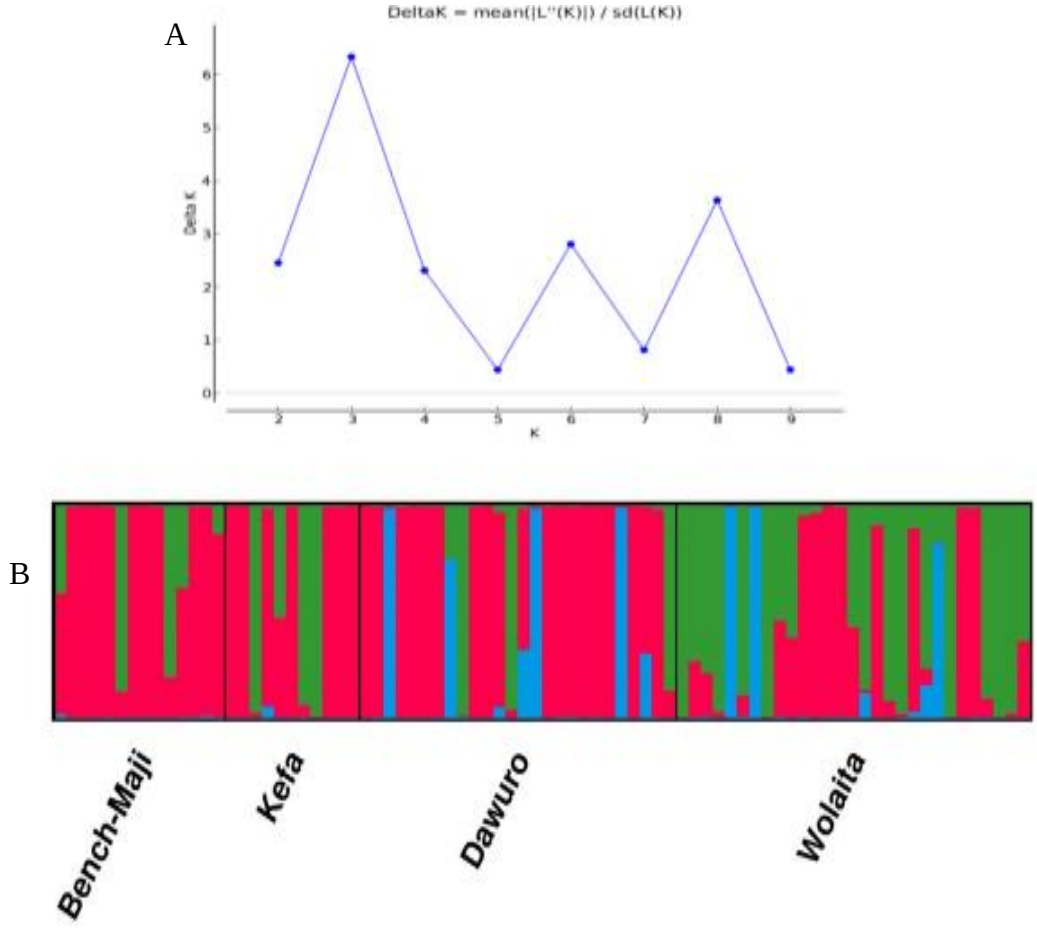

Fig. 6.3 Bayesian model-based clustering STRUCTURE analysis as inferred at K = 3 based on AFLP data. The relationship between ΔK and K showing the highest peak at K = 3; The genotype of each individual accession is represented by a vertical line divided into colored segments, the lengths of which indicate the proportions of the genome attributed to the inferred clusters (B). Among the 20 runs carried out for each K, graphical representation of the highest ΔK at K=3 estimate is shown.

6.4 Discussion

This study reports genetic diversity of Ethiopian cocoyam accessions by using AFLP markers. The three AFLP primer combinations resulted in 478 bands, of which 99.16% were polymorphic. This value is comparable to other finding by Mwenye *et al*. (2016) who detected 93% polymorphism by using eight AFLP primer combinations. Doungous *et al*. (2015) reported 92.0% polymorphic bands by using inter-retrotransposon amplified polymorphism (IRAP) markers to characterize white, red and yellow cocoyam accessions in Cameroon. Schnell *et al*. (1999) identified only 17.5% polymorphic bands by using RAPD on 18 cocoyam accessions from Florida. The analysis of eight ISSR molecular markers showed low polymorphism (4.19%) among cocoyam accessions from Brazil (Sepúlveda-Nieto *et al*., 2017). The high level of polymorphism detected in our analysis suggests that the AFLP primer combinations, used in this study, were highly discriminatory, revealing the presence of higher genetic diversity of cocoyam accessions than the previously studied accessions in Florida (Schnell *et al*., 1999) and in Brazil (Sepúlveda-Nieto *et al*., 2017).

The results of genetic diversity analysis of cocoyam detected by this study were higher than the report by Offie et al. (2004), who studied cocoyam accessions from the eastern and Volta region of Ghana by means of RAPD, where the mean Na, Ne and He were 1.99, 1.54 and 0.319, respectively. The average He value across populations, within green cocoyam morphotypes, within purple cocoyam morphotypes and when all collections were considered as single population were higher than the average He value (0.014) reported by Sepúlveda-Nieto *et al*. (2017) from Brazil by using ISSR molecular markers. The Shannon information indices (I) detected by this study were also higher than the I (0.49), reported by Doungous *et al*. (2015) who studied 20 cocoyam accessions by using IRAP markers. The genetic diversity detected in this study was also higher than the allozyme variation (mean He = 0.26) in *Ambrosina bassii* L. (Araceae) (Geraci *et al*., 2009) and *Anthurium crenatum* (L.) Kunth

(Araceae) (mean He = 0.23) (Acosta-Mercado, 2002). The high genetic diversity suggests that our collection contained genetically diverse accessions.

The detected high genetic diversity in the present study supports earlier findings based on morphological traits, which revealed the presence of diversity among cocoyam accessions in the country (Solomon Fantaw *et al*., 2014a). Since cocoyam is exotic to Ethiopia, the high genetic diversity may indicate that there might have been multiple introductions of cocoyam accessions into the country, but this needs to be investigated. Many introduced plant species had high level of diverse propagules through multiple introduction events (Rollins *et al*., 2013). The predominantly vegetative propagation of cocoyam (McKey *et al*., 2010) supports the fixation of high levels of diversity (Bisognin, 2011). The high genetic diversity detected in this study should be considered as positive opportunity for effective usage and conservation of cocoyam accessions. According to Herron and Freeman (2014), genetic diversity holds a key to the ability of species to persist through changing environments.

The results of this study rejected our assumption to find a specific pattern in cocoyam accessions by using AFLP markers due to the result revealed by the AMOVA, cluster analysis and Nei's genetic differentiation (Gst = 0.072 among populations). The most likely reason for that may be the exchange of accessions across locations. The low genetic differentiation (Gst = 0.024) between green and purple cocoyam morpthotypes furthermore indicates that the green and purple morphotypes may have hybridized in the past. This finding is in concordance to the ones reported by Mwenye *et al*. (2016) using AFLP markers in that cocoyam accessions were clustered together. Doungous et al. (2015) studied the genetic diversity of cocoyam accessions by using IRAP markers which could not discriminate between different morphotypes (white tuber flesh color and red tuber flesh color). Some sweet potato (*Ipomoea batatas* L.) accessions with different flesh colors were

assigned to the same cluster by population structure analysis, whereas some accessions with the same flesh color were assigned to different clusters based on ISSR markers (Kai *et al*., 2014).

The low levels of genetic differentiation detected among populations as well as between green and purple morphotypes by AFLP markers are not in agreement with the results from SSR markers. Some inconsistencies have been observed between the results (genetic differentiation, cluster analysis and genetic structure). Contrasting patterns are commonly found when different markers are used to detect genetic structure. The correlations of RAPD marker data with those obtained using RFLP, AFLP and SSR marker systems were lower in soybean (Powell *et al*., 1996). AFLP displayed no correspondence with RAPD and ISSR in cashew (*Anacardium occidentale* L.) (Archak *et al*., 2003). The dendrogram obtained with SSR markers was less similar to that obtained with AFLP markers in olive (*Olea europaea* L.) (Belaj *et al.,* 2003). Among the molecular markers (RAPD, RFLP, AFLP and SSR) used to estimate genetic distance of tropical maize inbred lines, AFLP and RFLP gave the most correlated results than others (Garcia *et al*., 2004). In conclusion, AFLP fingerprinting has revealed high genetic diversity but low differentiation of Ethiopian cocoyam accessions. Conservation and management plans of cocoyam in the country should concentrate on the existing high levels of genetic diversity within each population as well as when all collections were considered as single population.

Chapter Seven

Proximate, Mineral and Antinutrient Contents of Cocoyam (*Xanthosoma sagittifolium* (L.) Schott) from Ethiopia

Abstract

Cocoyam (*Xanthosoma sagittifolium* (L.) Schott) is an important food crop especially in the tropics and subtropics. Its cormels and leaves are eaten after cooking in the rural areas in Ethiopia. There is lack of information on the nutritional composition of cocoyam grown in the country. In this study, cormels of green- and purple- cocoyams were analyzed to determine proximate and mineral contents and antinutritional factors. The moisture contents (%) of green- and purple- cocoyams were 61.91 and 63.53, respectively. Crude protein (10.10%) and fiber (2.66%) contents of purple cocoyam were significantly higher than crude protein (8.48%) and fiber (2.14%) contents of green cocoyam. Fat contents (%) of the green and purple cocoyam were 0.85 and 0.22, respectively. Ash content of green cocoyam (3.25%) was significantly higher than the ash content of purple cocoyam (2.27%). The carbohydrate contents (%) and gross energy values (Kcal/100g) of green- and purple- cocoyam, respectively, were 85.36 and 378.47 and 84.76 and 380.27, showing that cocoyam grown in Ethiopia can be a good source of energy. Mineral contents (mg/100g) of green cocoyam were determined as Fe (8.20), Zn (3.07), Cu (1.04), Mg (78.77), Mn (2.48), P (120.93), Na (29.22), K (1085.70) and Ca (56.57) while purple cocoyam had Fe (9.88), Zn (3.12), Cu (1.14), Mg (82.00), Mn (3.74), P (129.87), Na (24.33), K (1223.30) and Ca (44.90). The antinutritional factors (mg/100g) of purple cocoyam (187.57 phytate and 156.1 tannin) were significantly higher than that of green cocoyam (167.76 phytate and 139.62 tannin). This study provided important information about the nutritional composition of cocoyam from Ethiopia, which can help to develop cocoyam food products and to promote production and utilization of cocoyam by encouraging its sustainable use. More detailed analyses including processing and sensory testing are suggested for further investigation.

Key Words: Ethiopia, proximate, minerals, antinutritional factors

7.1 Introduction

Humankind has used over 7000 edible plant species, at one time or another. Research, however, has concentrated on a few crops to meet the food and industrial needs. Over 50% of humankind's requirements for calories and protein are met by just three crops (maize, wheat and rice) and 95% of the world's food energy needs are provided by just about 35 crop plant species. Many plant species with a considerable importance for food security are categorized under neglected and underutilized crops. Most of these crops are particularly useful in marginal lands where they have been selected to withstand stress conditions and where they contribute to sustainable production with low inputs (GFUS, 2009). Some researchers have provided data to confirm the nutritional superiority of neglected and underutilized crops and their wild varieties over other more extensively utilized crops. Root and tuber crops are staple foods in many countries and are considered a good and inexpensive source of energy and carbohydrate in the diets (Burlingame *et al*., 2009).

Aroids are grouped with the neglected and underutilized crops which over the years have received little research attention (Adelekan, 2012) although they are important tuberous root crops playing a significant role in the livelihood of millions of relatively poor people in developing countries (Sarma *et al*., 2016). The most important food aroids are from tribes *Colocasieae* and *Caladieae*, i.e., taro (*Colocasia*) and cocoyam (*Xanthosoma*). They are often considered jointly and many developing countries depend on these aroids as a source of carbohydrates and they are important food for more than 400 million people around the world (Bown, 2000).

Cocoyam (*Xanthosoma* sagittifolium (L.) Schott has overtaken taro (*Colocasia esculenta* (L.) Schott as the main edible aroid in many tropical areas (Mayo *et al*., 1997; Matthews, 2002). Cocoyam is reported to have superior nutritional value over major root and tuber

staples of, especially in terms of their protein digestibility and mineral composition (Boakye *et al*., 2018). It is nutritionally superior to taro both in terms of proximate and mineral contents (Ndabikunze *et al.,* 2011). It plays major role in the lives of many as a food security crop, mainly for smallholder farmers. It occupies an important place in the diet of many tropical countries.

Cormels of cocoyam are boiled, baked or partly boiled and fried in oil before consumption (Kay, 1987). The corms are peeled, dried and ground to flour for pastry that can be stuffed with meat or other fillings (Lim, 2015). The young leaves can be boiled and used as vegetable similar to spinach (Mayo *et al*., 1997; Ramawat and Merillon, 2014). It was found to be superior to barley or sorghum as a substrate for brewing beer due to its high carbohydrate content (71-78 %) compared to barley (65%) and sorghum (70-73%) (Onwuka and Eneh, 1996).

In Ethiopia, cocoyam is expanding to new areas, growing even in poor soils and under dry conditions (Fujimoto, 2009; Feleke Woldeyes *et al*., 2016). The cultivation of cocoyam is increasing in the country. The cormels are eaten by cooking using pots or roasting using stones. The young leaves of green cocoyam are edible in some areas of southwestern parts of Ethiopia. Nutritional composition of roots and tubers reported to vary from place to place depending on the difference in climate, habitat, soil type, crop variety (genetic background) and other factors (FAO, 1998). Studies on varieties of cassava, sweet potato and yam showed that there are great differences in nutrient content within species, and that some varieties can provide a substantial contribution to nutritional requirements, not only for energy but also for protein and micronutrients (Burlingame *et al*., 2009). The data on the nutritional composition of cocoyam is much less than that for other root and tuber crops. There is dearth of information on nutritional composition of cocoyam grown in Ethiopia. There is a need to

analyze, compile and disseminate data on the nutritional composition of cocoyam. The main aim of this study was, therefore, to determine proximate composition (moisture, crude protein, crude fiber, crude fat, total ash, total carbohydrate and gross energy), minerals such as Iron (Fe), Zinc (Zn), Copper (Cu), Magnesium (Mg), Manganese (Mn), Phosphorus (P), Sodium (Na), Potassium (K) and Calcium (Ca), and antinutritional factors (phytate and tannin) of green- and purple- cocoyam grown in Ethiopia and to compare the difference.

7.2 Materials and methods

7.2.1 Sample collection

Fresh cormels (small, middle and large sizes) that were not attacked by pests and which were not damaged during harvesting were selected from green-and purple-cocoyam, after 9 months of plantation.

7.2.2 Preparation of cocoyam flour

Cormels of three size groups (small, medium and large) were carefully selected from green- and purple- cocoyam (one accession from each) for purpose of including the size groups. The selected samples were washed using running tap water. Then hand peeled using stainless steel knife, washed and sliced to uniform thickness (~ 5 mm). The slices were blanched in hot water (80°C) for 5 minutes followed by immediate cooling in cold water in order to inactivate enzymes that may cause browning. The slices were placed on a stainless-steel tray and dried overnight in a dry oven at 105°C. The dried cormels chips were grinded using mortar and pestle to convert into flour. Then the flour was filled in polyethylene bags, packed and kept in desiccators until analyzed for contents of proximate, mineral and antinutritional factors.

7.2.3 Determination of proximate composition

Proximate composition (total moisture content, crude protein, crude fat, crude fiber, total ash, total carbohydrate and gross energy values) of the two types of cocoyams were determined by the following methods.

Determination of moisture content: Moisture content (%) was determined in an oven drying methods at 105±5°C according to the procedure described in Association of Official Analytical Chemists (AOAC, 2005). Five grams of each fresh sample was accurately weighed in triplicate and placed in a pre-weighed aluminum dish and dried in an oven at 105±5°C till the constant weight of dry matter was obtained. The moisture content in the sample was determined as:

$$\text{Moisture (\%)} = \frac{Wt.of\ fresh\ sample - Wt.of\ dried\ sample}{Wt.of\ fresh\ sample} * 100$$

Determination of crude protein: The powdered cormel samples were analyzed for crude protein content according to the Kjeldahl's method described in the Association of Official Analytical Chemists (AOAC, 2005).

***Protein digestion*:** Five grams of the sample was weighed in an ash less filter paper and put into 250 ml digestion flask. Then 3 g of a catalytic mixture, tablet (75 g of CuSO4 and 0.7 g of K_2SO4) and 15 ml of 98% H_2SO4 were added into a digestion flask. The whole mixture was subjected to heating in a digestion chamber until transparent residue (clear light green) content was obtained. Then, it was allowed to cool. After cooling, the digest was transferred into a 100 ml volumetric flask and made up to the mark (100 ml) with distilled water and then distilled using distillation apparatus.

***Protein distillation*:** Before use, the distillation apparatus was steamed for 15 min. After which, 100 ml conical flask containing 20 ml of 40% boric acid and 2 or 3 drops of Tashiro's indicator was placed under the distillation apparatus with its out let tubes inserted into the

conical flask. The digest was washed down with distilled water followed by addition of 3-4 drops of phenolphthalein and 20 ml of 40% (w/v) NaOH solution. The distillation was continued until about 25 ml of distillate was trapped into the boric acid plus indicator solution changed from red to light grey, showing that all the ammonia liberated had been trapped. That means the digest in the condenser was steamed through until enough ammonia gas captured by the boric acid.

Titration: The solution in the receiving flask was titrated with 0.1 mM HCl to a brown color. After titration the % of nitrogen was calculated as:

Nitrogen (%) = $\frac{(Vs-VB)*mM\ HCl*0.014008}{Wt.of\ sample} * 100$, where Vs = Volume (ml) of HCl required to titrate sample; VB = Volume (ml) of acid required to titrate the blank; mM acid= Molarity of acid; W=Weight of sample (g). Then, percentage of crude protein in the sample was calculated from the % nitrogen as: % crude protein = % N x F, where, F (conversion factor) is equivalent to 6.25 (AOAC, 2005). A blank was run through along with the sample and triplicate analysis was conducted for samples.

Determination of crude fiber: Six gram of powdered sample (E) was taken into 50 ml tube and 2.5 ml of alpha-amylase was added and incubated at room temperature for 10 min. Then, 60 ml of a mixture composed of 700 ml 70% acetic acid, 100 ml 65% nitric acid and 20 g trichloroacetic acid was added. Digestion was undertaken in 250 ml flask by heating at 200°C by continuous string at 500 rpm for 30 min. Then after cooling on ice, filtrated with vacuum filtration on dry filter paper with known mass (Mf) by using distilled water until the filtrate became neutral. The residue on the filter paper was washed with 10 ml ethanol for 3 times and 10 ml acetone for 2 times to dissolve organic constituent. Then after transferring the dried residue with the filter paper into pre-weighted crucible, the residue was oven dried at 105ºC overnight to drive off moisture. The oven dried crucible containing the residue and

filter paper was cooled in a desiccator and weighted (M1). The residue and filter paper were burned first in Bunsen burner and then 550°C. The crucible containing white and grey ash (free of carbonaceous material) was cooled in a desiccator and weighted to obtain M2.

The % of crude fiber was calculated as: Crude fiber (%) = $\frac{(M1-Mf)-M2}{E} * 100$

Determination of crude fat: The crude fat in the powdered samples was determined by automated Soxhlet extraction method (AOAC, 2005). After weighting the dried flask containing sand to constant weight, 15 g of homogenized samples were measured by using filter paper of known mass and placed in extraction flask. The dried flasks (250 ml) were weighed correspondingly and filled with 150 ml of petroleum ether. The extraction thimbles were plugged tightly with cotton wool and run for 2 h. The extraction chamber continuously filled with the sample there by extracting the fat. When the optimum sensor reached, the magnetic valve was opened and the samples were washed with freshly filled solvent (petroleum ether). Finally, the solvent was recovered by collection in solvent tank. The fat was collected in filter paper. and the extract was gently evaporated to dryness. The remaining petroleum ether was removed by sonication. The extraction flask containing crude fat in the filter paper was dried in 105°C to constant weight. The % fat in the sample was calculated using the formula:

$$\text{Fat (\%)} = \frac{Wt.of\ flask\ containing\ the\ crude\ fat\ in\ filter\ paper - Wt.\ of\ flask\ plus\ filter\ paper}{Wt.of\ sample} * 100$$

Determination of total ash content: A crucible was dried at 550°C for 30 min and cooled down in a desiccator for 1 hr. The weight of crucible was measured (M1). Five grams of powdered sample was added in the dried crucible and the crucible containing sample was measured (M2). Then the sample was burned by using Bunsen burner until the steam off and then in oven at 550°C for 5 h. Ash is an inorganic residue remaining after the material has

been completely burnt. The crucible containing ash was cooled in a desiccator and then re-weighted (M3) (AOAC, 2005).

The % of ash contents in the cocoyam sample was calculated as: Ash (%) = $\frac{M3-M1}{M2-M1} * 100$.

Determination of total carbohydrate: Total carbohydrate content was calculated adding the total values of crude protein, crude fat, crude fiber and total ash contents of the sample and subtracting it from 100%.

Total carbohydrate (%) = 100 - (% crude fiber + % crude protein + % crude fat + % ash).

Determination of energy value: Gross energy value (Kcal/100g) of the samples was determined by multiplying the protein content by 4, carbohydrate content by 4 and fat content by 9 (AOAC, 2005).

Energy value = (Crude protein × 4) + (total carbohydrate × 4) + (crude fat × 9).

7.2.4 Determination of mineral content

Iron, Zinc, Copper, Magnesium, Manganese, Sodium and Potassium and Calcium were determined according to the standard method of AOAC (2005) using an Atomic Absorption Spectrophotometer (Varian SAA-20 Plus). Ashing of the samples was followed by digestion and absorption. Phosphorus was determined by AAS method of AOAC (1984).

7.2.5 Analysis of antinutritional factors

Determination of Phytate: The phytate contents of green- and purple-cocoyam were determined according to method described by Latta and Eskin (1980). Dried sample of cocoyam flour (0.1 g) was extracted with 10 ml 2.4% HCl for 1 h at room temperature and centrifuged at 3000 rpm for 30 min. The clear supernatant was used for the phytate estimation. One ml of Wade reagent (0.03% solution of FeC13.6H2O containing 0.3% sulfosalicylic acid in water) was added to 3 ml of the sample solution and the mixture was

centrifuged. The absorbance at 500 nm was measured using UV-VIS spectrophotometer. The phytate concentration was calculated from the difference between the absorbance of the control (3 ml of water + 1 m1 Wade reagent) and that of assayed sample and expressed as mg/100g.

Determination of Tannin: Tannin contents of green- and purple-cocoyam were determined using the method of Burns (1971). Cocoyam flour (0.25 g) was weighed in a screw capped test tube and 10 ml of 1% HCl in methanol was added to each test tube containing the samples. Then the tubes were put on mechanical shaker for 24 h at room temperature. After 24 h of shaking, the tubes were centrifuged at 1000 rpm for 5 minutes. One milliliter of the clear supernatant was taken and mixed with 5 ml of vanillin-HCl reagent in another test tube and this mixture was allowed to stand for 20 minutes to complete the reaction. After 20 minutes, the absorbance was read at 500 nm using spectrophotometer. The tannin concentration was calculated from the difference between the absorbance of control and that of the sample and expressed as mg/100g.

7.2.6 Statistical analysis

A comparative statistics and comparative analysis was conducted to present the difference in proximate composition (moisture, crude protein, crude fiber, crude fat, total ash, total carbohydrate and gross energy values), mineral contents (Ca, K, Na, Mg, Mn, Cu, Fe Zn and P) and antinutritional factors (phytate and tannin) in green- and purple-cocoyam samples. The analyses were performed using SPSS version 23 (IBM SPSS, 2015). Differences in means at $p < 0.05$ were considered significant.

7.3 Results

7.3.1 Proximate composition

The results of proximate analysis showed that the crude protein (10.10%), crude fiber (2.66%) and gross energy value (380.27 kcal/100g) of purple cocoyam were significantly higher than the crude protein (8.48%), crude fiber (2.14%) contents and gross energy value (378.47 kcal/100g) of the green cocoyam whereas the green cocoyam had showed significantly higher total ash content (3.25 %) than the total ash content (2.27%) of purple cocoyam. Moisture (61.19%), fat (0.85 %) and total carbohydrate (85.36%) contents of green cocoyam did not differ ($p>0.05$) from the moisture (63.53%), total carbohydrate (84.76%) and fat (0.22%) contents of purple cocoyam (Table 7.1).

Table 7.1 Proximate composition of green- and purple- cocoyams

proximate	Green cocoyam	Purple cocoyam	Significance
Moisture (%)	61.91±2.50	63.53±1.10	0.372
Crude protein (%)	8.48±0.36	10.10±0.18	*
Crude fiber (%)	2.14±0.12	2.66±0.09	**
Crude fat (%)	0.85±0.60	0.22±0.10	0.211
Total ash (%)	3.25±0.09	2.27±0.07	**
Total carbohydrate (%)	85.36±0.49	84.76±0.38	0.195
Energy value (kcal/100g)	378.47±0.71	380.27±0.43	*

Data presented are independent sample t-test. Values are means of triplicates analysis ± standard deviations. Value in level of significance are **$p < 0.01$, *$P<0.05$and mean values with $p > 0.05$ are not significantly different and their respective p-values are shown.

7.3.2 Mineral composition and antinutritional factors

Nine different minerals and two antinutritional factors were analyzed for their concentration in dry weight basis (mg/100g). The green cocoyam had Fe (8.20), Zn (3.07), Cu (1.04), Mg (78.77), Mn (2.48), P (120.93), Na (29.22), K (1085.70) and Ca (56.57) while purple cocoyam had Fe (9.88), Zn (3.12), Cu (1.14), Mg (82.00), Mn (3.74), P (129.87), Na (24.33), K (1223.30) and Ca (44.90). The result of the comparative analysis showed that Mg, Mn, P, Na, K and Ca contents of green cocoyam were significantly higher than Mg, Mn, P, Na, K

and Ca contents of purple cocoyam. The Fe, Zn and Cu contents of green cocoyam were not significantly different from the Fe, Zn and Cu contents of the purple cocoyam. Significant quantities of antinutritional factors namely: phytate and tannin were found in both cocoyam morphotypes. High antnutritional factors were determined, where the phytate (187.57) and tannin (156.11) contents (mg/100g) of purple cocoyam were significantly higher (P<0.001) than the phytate (167.76) and tannin (139.62) contents of green cocoyam (Table 7.2).

Table 7.2 Mineral contents and antinutritional factors of green- and purple-cocoyams

Minerals (mg/100g)	Green cocoyam	Purple cocoyam	Significance
Fe	8.20+0.6	9.88±1.97	0.305
Zn	3.07±0.10	3.12±0.12	0.582
Cu	1.04±0.08	1.14±0.05	0.183
Mg	78.77±0.67	82.00±1.04	*
Mn	2.48±0.19	3.74±0.06	**
P	120.93±2.07	129.87±2.06	*
Na	29.22+1.44	24.33±0.82	*
K	1085.70±32.1	1223.30±28.70	*
Ca	56.57±1.50	44.90±1.81	**
Antinutritional factor			
Phytate	167.76±2.82	187.57±0.55	***
Tannin	139.62±0.97	156.11±2.35	***

Data presented are independent sample t-test. Values are means of triplicate analysis ± standard deviations. Value in level of significance are ***p< 0.001, **p < 0.01, *p < 0.05 and mean values with p > 0.05 are not significantly different and their respective p-values are shown.

7.4 Discussion

The proximate and mineral contents and antinutritional factors (phytate and tannin) of green- and purple- cocoyam were investigated. The moisture content of the green cocoyam was 61.91% and that of the purple cocoyam was 63.53%. These values were lower than moisture content of cocoyam (70–77%) reported by Kay (1987), but the values were close to the report of Plucknett (1984) in which the mean moisture content of 37 cocoyam samples was 67.1% and within the range of moisture contents of cocoyam from Ghana (Sefa-Dedeh, 2004). The moisture contents of cocoyam reported from Tanzania and Uganda (Ndabikunze

et al., 2011) and Nigeria (Eddy *et al*., 2012) were 68.41% and 68.28%, respectively. The relative low moisture content determined in this study can be helpful for the storage of cocoyam cormels at ambient temperature.

The crude protein content of green cocoyam (8.48%) and purple cocoyam (10.10%) determined by this study were higher than mean protein contents (1.55%) of 37 samples from Tanzania and Uganda (Plucknett, 1984) and later reported by Ndabikunze *et al.* (2011) from the same countries as 4.75%. The crude protein contents were also higher than the crude protein contents of white (5.1%), yellow (5.2%) and red (5.4%) cocoyams from Cameroon (Onokpise *et al*., 1999 and the Assam State of India (2.42%) (Sarma *et al.*, 2016). Relatively, higher mean protein contents of cocoyam determined in the study may be due to cormel samples (not corms) were used for protein determination. The results were comparable with tuber proteins of some cocoyam accessions from Cameroon (up to 9.4%) (Onokpise *et al*., 1999), protein contents of Ede Uhie (8.08%) and Ede Ocha (8.74%) varieties of cocoyam cormles from Nigeria (Owuamanam *et al*., 2010) and protein content of taro varieties (10.49 to 12.13%) from Ethiopia (Melese Temesgen, 2017). The present study results were also in agreement with a review conducted by Shewry (2003) and reported that plant tubers contain protein up to 10%. According to Onwueme (1978), cocoyam (*Colocasia* and *Xanthosoma*) has relatively high protein content compared to other tuberous root crops such as cassava, yam, potato and sweet potato. According to Akpan and Umoh (2004), the protein content of cocoyam was slightly superior to taro. This study results showed that both green- and purple- cocoyams are rich in proteins, which can be considered as good opportunity since it is mainly consumed by resource poor farmers.

The crude fiber contents of green cocoyam (2.14%) and purple cocoyam (2.66%) determined in this study were lower than the minimum crude fiber content (2.80%) of two related aroids

(*Colocasia* and *Xanthosoma*) from Assam State of India (Sarma et al., 2016). The crude fiber contents were, however, higher than the range of crude fiber contents of cocoyam (0.6-1.9%) reported by Kay (1987), (0.1%) reported by Plucknett (1984) and (0.88%) reported by Akpan and Umoh (2004) and within the range of the crude fiber content (1.11-3.00%) of cocoyam reported by Sefa-Dedeh and Kofi-Agyir (2004). The purple cocoyam had significantly higher crude fiber content (2.66%) than the crude fiber content (2.14%) of green cocoyam ($p<0.01$). In contrast to this, Sarma *et al.* (2016) reported the crude fiber content of purple cocoyam (2.80%) was lower than the crude fiber content (3.50%) of dark green leaved cocoyam. The present study result showed that fiber contents in green- and purple- cocoyam grown in Ethiopia can be effective and useful source of fiber. The differences in the fibers contents could be attributed to the genotype difference.

Comparative analysis showed that the crude fat contents of green cocoyam (0.85%) was not significantly different from the fat content of purple cocoyam (0.22%) ($p>0.05$). The values were low and within the range of crude fat contents (0.2-0.4%) reported by Kay (1987) and also relatively comparable with the average crude fat contents of cocoyam reported by Akpan and Umoh (2004) (0.93%) and Ndabikunze *et al.* (2011) (0.43%), indicating cocoyam is a low-fat crop. Thus, it can be said to be preferred food crop that can contribute less to the health problems related with excess fat intake.

The total ash content of green cocoyam (3.25%) was significantly higher than the total ash content of purple cocoyam (2.27%) ($p<0.001$). The values were comparable with the total ash content of Ede ofe (3.00%) and Ede ocha (2.45%) cocoyam cultivars from Nigeria (Owuamanam *et al.*, 2010), but lower than the total ash content of white-fleshed (5%), yellow-fleshed (4.6%) and red-fleshed (4.5%) cocoyam varieties reported latter by Ihediohanma *et al*. (2014) from the same country. The values were also lower than the total

ash contents of cocoyam (3.51%) grown along the Lake Victoria Basin in Tanzania and Uganda (Ndabikunze *et al*., 2011). The differences could be the influence of the environments in which cocoyams were grown. The ash content can reflect the minerals content, which varies with the locality or soil type (Onyeike *et al*., 1995).

The total carbohydrate contents of green cocoyam (85.36%) and purple cocoyam (84.76%) were comparable with total carbohydrate content (85.65%) of released taro variety from Ethiopia (Boloso I) (Adane Tilahun *et al*., 2013), but higher than the average carbohydrate content (72.53 to 75.49 %) of different taro varieties from Ethiopia (Melese Temesgen, 2017). Gross energy values (kcal/100g) of green cocoyam (378.47) and purple cocoyam (380.27) were relatively higher than the gross energy values (kcal/100g) of Boloso I, which varied from 370 to 374 kcal/100g (Adane Tilahun *et al*., 2013). The carbohydrate contents and gross energy values indicate that cocoyam growing in Ethiopia is one of carbohydrate rich foods in supplying high energy.

The minerals such as Fe, Zn, Cu, Mn, Na and K contents (mg/100g) of green- and purple-cocoyams were higher than the Fe, Zn, Cu, Mn, Na and K content of cocoyam grown along the Lake Victoria Basin in Tanzania and Uganda (Ndabikunze *et al.*, 2011). The Mg, P and Ca (mg/100g) contents of cocoyam were lower than Mg (90.62), P (207.50) and Ca (110.17) contents of cocoyam reported by Ndabikunze *et al.* (2011). The Cu content of green cocoyam (1.04) and purple cocoyam (1.14) were higher than the Cu contents of white-fleshed cocoyam (0.52), red-fleshed cocoyam (0.78) and taro (0.78) cultivars reported by Njoku and Ohia (2007). The present study showed that both green- and purple- cocoyams are rich in minerals. According to Njoku and Ohia (2007), consumption of nutrient rich foods such as cocoyam helps the body to utilize protein, carbohydrates and other nutrients.

High phytate concentrations (mg/100g) observed in green cocoyam (167.76) and purple cocoyam (187.57). Phytate has been recognized as an anti-nutrient due to its adverse effects because it lowers the availability of many minerals such as copper, iron and zinc (Sarkiyayi and Agar, 2010). The tannin level (mg/100 g) of green- and purple- cocoyams were 139.62 and 156.11, respectively. These tannin contents were higher than the tannin content of taro-Boloso I reported by Adane Tilahun *et al*. (2013). It is known to exert negative effect on the bioavailability of proteins and minerals (Ramakrishna *et al.,* 2006). Processing methods such as boiling, fermentation and roasting can significantly reduce antinutritional factors (phytate and tannin) to low level (Ramakrishna *et al*., 2006; Adane Tilahun *et al*. 2013; Melese Temesgen, 2017; Habtamu Azene and Tesfahun Molla, 2017). These processing methods, therefore, need to be tested for the reduction of antinutritional contents of cocoyam grown in Ethiopia. These processing methods, therefore, need to be tested for the level of reduction of antinutritional factors of cocoyam grown in Ethiopia. This study provided important information about the nutritional composition of cocoyam, which can help to develop cocoyam food products and to promote production and utilization of cocoyam by encouraging its sustainable use. More detailed analyses including processing and sensory testing are suggested for further investigation.

Chapter Eight

Micropropagation of Cocoyam (*Xanthosoma sagittifolium* (L.) Schott) from Shoot Tip Explants

Abstract

Cocoyam (*Xanthosoma sagittifolium* (L.) Schott) is one of the main edible aroid in many tropical areas of the world. It contributes to the food security of households in the rural communities of south and southwestern parts of Ethiopia. Its propagation is entirely traditional from tuber fragments, which was constrained by scarcity of planting materials. This study was carried out to develop *in vitro* propagation protocol of this species using shoot tip explants. MS medium supplemented with different concentrations of plant growth regulators (BAP with Kn, BAP with NAA, and BAP with NAA and Kn) were used. The shoots were transferred to MS medium supplemented with various concentrations of auxin (IBA or NAA) for rooting. Factorial experiments were conducted in a completely randomized design with PGRs as one factor and genotype as another factor. Each treatment was replicated six times. Shoots were best initiated on MS medium supplemented with 2.0 mg/l BAP. The maximum mean number of shoots per explant, 4.56 for green cocoyam and 4.83 for purple cocoyam, were recorded on MS medium supplemented with 2.5 mg/l BAP in combination with 0.5 mg/l NAA. The maximum mean leaf number per explant, 4.67 for green cocoyam and 4.94 for purple cocoyam, were recorded on MS medium supplemented with 1.0 mg/l BAP in combination with 0.25 mg/l Kn. The longest shoots, 3.92 cm for green cocoyam and 4.36 cm for purple cocoyam, were recorded on MS medium supplemented with 5.0 mg/l BAP in combination with 1.0 mg/l Kn and 0.5 mg/l NAA. This result was, however, not significantly different from the shoot length measured on MS medium supplemented with 2.5 mg/l BAP in combination with 0.5 mg/l NAA. The highest mean root number per shoot (6.00) for green cocoyam and (5.83) for purple cocoyam were obtained on MS medium containing 2.0 mg/l IBA. The longest roots, 5.94 cm for green cocoyam and 6.44 cm for purple cocoyam, were also recorded on the same medium. Thus, maximum cocoyam shoots per explant could be obtained by initiating culture on MS medium containing 2.5 mg/l BAP in combination with 0.5 mg/l NAA and inducing roots on MS medium containing 2.0 mg/l IBA. This protocol can be used for propagation of large number of disease free planting materials within short period of time.

Keywords: Explant, *in vitro* propagation, shoot tip, *X. sagittifolium*

8.1 Introduction

Cocoyam (*Xanthosoma sagittifolium*) is one of the main edible aroid in many tropical areas of the world. The usable parts are the cormles and young leaves (Giacometti and Leon, 1994). It is contributing to the food security of households in the rural communities of south and southwestern parts of Ethiopia. However, its cultivation is constrained by scarcity of planting materials because the seeds of cocoyam such as cormels or their fragments are used for food. Preserving cocoyam under field condition is risky since diseases or natural catastrophes can cause the loss of genetic resources. *In vitro* propagation technique is a possible solution to the major problems of vegetative propagated plants associated with pathogen dissemination and subsequent loss of vigor and productivity (Paul and Bari, 2007), health and quality (Ko *et al*., 2008; Vilchez *et al*., 2009) and planting material scarcity (Tsehifet Solomon *et al.,* 2010). It is an efficient method to propagate good quality materials that can substantially improve production rate (Caula *et al.*, 2008).

Micropropagation involves using smaller propagules and a substantially faster multiplication rate than the conventional field multiplication methods (Sama *et al*., 2012). Its objective is to propagate plants as clones. Plant cells, tissues or organs can be cloned, i.e., produced in large numbers where all the individuals have the same genetic constitution as the stock parent although irregularities sometimes occur, resulting in somaclonal variation (George *et al*., 2008).

Production of a large number of plants of selected germplasms is the main prerequisite for establishment of plantations, for *ex-situ* conservation and improvement of plants (Siddique and Anis, 2009). Multiplication of many clonally propagated species in a short time is among many advantages of micropropagation systems (Panell, 1984). There are reports that have generated information to use for *in vitro* propagation of cocoyam through refining culture

media and plant growth regulator (PGR) compositions. Shoots were induced from cormel axillary bud meristems on MS media supplemented with different combinations of auxins (IAA, 2,4-D and NAA) and cytokinins (BAP and Kn) (Paul and Bari, 2007). Cocoyam was propagated *in vitro* from shoot tip through the use of BAP and Thidiazuron (TDZ) (Sama *et al*., 2012) and in temporary immersion bioreactor using sucrose as a determinant factor (Niemenak *et al*., 2013).

The ideal concentration and combination of PGRs required for micropropagation differ from species to species, genotype to genotype and explant source (Obidiegwu, 2015). According to Gomes *et al*. (2010), the composition of PGRs need to be established accurately to achieve the effective rates of multiplication. A study has been carried out to compare the conventional and *in vitro* propagation methods in order to increase planting material of cocoyam in Ethiopia (Tsehifet Solomon *et al.,* 2010). The *in vitro* propagation protocol was, however, not adequately developed for routine micropropagation. The situation justifies looking for efficient micropropagation protocol in order to achieve higher benefits in the production of cocoyam in Ethiopia. Shoot tip culture is a useful and an expanding alternative to obtain large numbers of propagules rapidly. This study was aimed to develop in vitro propagation protocol for cocoyam using shoot tip explants.

8.2 Materials and methods

8.2.1 Preparation of donor plant and stock solutions

Donor plant preparation: Cormels of green and purple cocoyam genotypes were planted in polyethylene pots containing sterile forest soil and allowed to sprout in greenhouse at average temperature of 25°C under natural photoperiod.

MS stock preparation: Murashinge and Skoog (MS) (1962) medium with its full macro and micro nutrients, vitamins compositions, sucrose and agar were used as the basic

components of the medium. All components of MS stock solution were prepared by weighing and dissolving the powder in double distilled water.

Plant growth regulators stock preparation: The stock solutions of PGRs (BAP, NAA, Kn and IBA: Indol-3-butyric acid) were prepared by weighing and dissolving the powder in double distilled water at a concentration of 1.0 mg/ml by dissolving in 3-4 drops of 1N NaOH for auxins and 1N HCl for cytokinins.

8.2.2 Culture medium preparation

Culture medium was prepared by taking proper amount of MS stock solutions and 3% (w/v) sucrose. PGR was added as treatment and pH was adjusted to 5.8 using 1N NaOH or 1N HCl. Finally, 0.6% (w/v) agar was added and dissolved by micro-oven. The medium was sterilized by autoclaving at a temperature of 121°C with a pressure 105 Kpa for 15 min. For shoot initiation, shoot multiplication and rooting, 40 ml of the medium was poured into baby jar culture vessel.

8.2.3 Surface sterilization of explant

After six weeks of planting in greenhouse, sprouts were collected. The roots were removed and the tuber was scrubbed with a brush, 0.5% sodium hypochlorite and dish soap under tap water. The lower portion of the corm was cut off, leaving 3 cm and the leaf stalk was trimmed to 2.0 to 3.0 cm. The explant was gently scrubbed with toothbrush using double distilled water, rinsed with double distilled water followed by washing in 70% ethanol for five min and washed three to four times with double distilled water. Then, the explant was placed in a clean beaker containing a solution of 1.5% sodium hypochlorite made with double distilled water and two drops of Tween-20 and the beaker was placed on a magnetic stir bar plate and stir gently for 45 min. The solution was poured off, the explant was rinsed with double distilled water and taken into the laminar flow hood. The outer leaves were

removed until inner cleaner section appears and the explant was trimmed to 1.0 cm, rinsed three to four times with sterile double distilled water, 70% ethanol for 30 seconds, 1% sodium hypochlorite for 1 min and sterile double distilled for three to four times.

8.2.4 Culture condition

Green- and purple-cocoyams were equally treated while all exogenous factors were held constant. Each culture vessel and its cap were flamed prior to closing and sealed with a strip of parafilm. The vessels were clearly labelled with the medium code, date of inoculation, name of cocoyam (Green or Purple). Three explants per culture vessel and six replications per treatment were used for shoot initiation, shoot multiplication and root induction experiments. Cultures were incubated and monitored in growth room of 12 h photoperiod under light intensity of 40 µmole m^{-2} s^{-1} provided by cool white fluorescent lamps at a temperature of 25±2°C.

8.2.5 Shoot initiation

Shoot tip (1.0 cm) comprising the apical meristem with some leaf primordia and some corm tissue at the base was used for shoot initiation (Fig. 8.1). The explants were cultured on MS medium supplemented with different concentrations of BAP (0.0, 0.5, 2.0, 3.0, 4.0 and 5.0 mg/l). The number of initiated shoots was recorded beginning from fourth day of culture and expressed as percentage of shoot initiation. The number of shoots per explant, number of leaves per explant and shoot length were recorded after two weeks of culture initiation to select the best shoot initiation medium.

Fig. 8.1 Shoot tip explant for shoot initiation: During surface sterilization (A); ready for culture (B)

8.2.6 Shoot multiplication

To screen for an optimal shoot multiplication medium, the initiated explants were cultured on 20 different PGRs concentrations including PGRs free MS medium as control. Five different combination of BAP with Kn, nine different combination of BAP with NAA and five different combinations of BAP with NAA and Kn were used (Table 8.1). The number of shoots per explant, number of leaves per explant and shoot length were recorded after three subsequent cultures at two-week interval.

Table 8.1 Compositions of plant growth regulators for shoot multiplication (mg/l)

BAP + NAA		**BAP + Kn**		**BAP + NAA + Kn**		
0	0	0	0	0	0	0
0.5	0.25	0.5	0.10	0.5	0.25	1.0
1.0	0.25	1.0	0.25	2.5	0.25	1.0
1.5	0.25	1.5	0.50	5.0	0.25	1.0
2.5	0.25	2.5	0.75	2.5	0.5	1.0
5.0	0.25	5.0	1.0	5.0	0.5	1.0
1.0	0.5					
1.5	0.5					
2.5	0.5					
5.0	0.5					

8.2.7 Rooting and acclimatization

Before conducting the rooting experiment, *in vitro* derived shoots were transferred onto a fresh PGRs free MS medium for two weeks in order to avoid any carry over effect of PGRs. Then, the shoots were cultured on MS medium supplemented with different concentrations of IBA (0.25, 0.5, 1.0, 2.0 and 3.0 mg/l) or NAA (0.25, 0.5, 1.0, 2.0 and 3.0 mg/l) for root induction. PGRs free MS medium was used as a control. The percentage of root forming shoots, number of roots per shoot and root length were recorded after three weeks of culture.

After being in rooting medium for three weeks, the rooted shoots were removed from the culture vessel and the roots were washed in double distilled water. Sixty plantlets (30 plantlets

of each of green and purple cocoyams) were planted in forest soil, coffee husk and sand in the ratio of 2:1:1, respectively. All plantlets were covered with a plastic cloche for three days before being left open in greenhouse. The number of survived plants was recorded after two weeks of acclimatization.

8.2.8 Experimental design and data analysis

The experiments were laid in a completely randomized factorial design with PGRs as one factor and genotypes (green- and purple-cocoyams) as another factor. Statistical analysis was carried out using Minitab 17.1 (2013). Treatment separation was performed using Tukey mean comparison test at a probability level of $p < 0.05$.

8.3 Results

8.3.1 Shoot initiation

The shoot tip explants responded to different BAP concentrations and turned green beginning from four days of culture (Fig. 8.2). All explants (100%) induced shoots on MS medium supplemented with 2.0 mg/l BAP. The lowest percentage shoot induction (84.19%) and (83.56%) was observed on PGRs free MS medium for green- and purple- cocoyam, respectively. The number of shoots per explant produced on PGRs free MS medium and the media containing different concentrations of BAP were not significantly different. However, among five different concentration of BAP used for shoot initiation, relatively higher shoot and leaf numbers per explant and shoot length were produced on the MS medium containing 2.0 mg/l BAP. The maximum mean number of shoots per explant (2.44 ± 0.47 and 2.50 ± 0.25), leaves per explant (3.11 ± 0.13 and 3.41 ± 0.43) and shoot length (2.17 ± 0.15 and 2.33 ± 0.16 cm) were produced, respectively, for green-and purple-cocoyam on the medium containing 2.0 mg/l BAP (Table 8.2).

Table 8.2 Effect BAP on shoot initiation of green- and purple- cocoyams from shoot tip

Green cocoyam	BAP concentration (mg/l)					
	0.0	**1.0**	**2.0**	**3.0**	**4.0**	**5.0**
Shoot initiation (%)	84.19	84.19	100.00	94.44	94.44	89.30
Mean shoot no/explant	1.50±0.21^{a}	2.00±0.42^{a}	2.44±0.47^{a}	2.22±0.20^{a}	1.89±0.25^{a}	1.55±0.07^{a}
Mean leaf no/explant	1.56±0.18^{b}	2.06±0.17^{ab}	3.11±0.13^{a}	2.78±0.28^{a}	2.67±0.19^{a}	2.89±0.20^{a}
Mean shoot length (cm)	1.53±0.21^{a}	2.08±0.15^{a}	2.17±0.15^{a}	2.11±0.24^{a}	1.78±0.44^{a}	1.92±0.21^{a}
Purple cocoyam						
Shoot initiation (%)	83.56	89.30	100.00	94.90	94.90	89.38
Mean shoot no/explant	1.94 ±0.25^{a}	2.22±0.16^{a}	2.50±0.25^{a}	2.28±0.16^{a}	2.22±0.13^{a}	1.83±0.17^{a}
Mean leaf no/explant	1.86±0.10^{b}	2.36±0.07^{ab}	3.41±0.43^{a}	3.08±0.20^{a}	2.97±0.09^{a}	3.1±0.18^{a}
Mean shoot length (cm)	1.72±0.41^{a}	2.08±0.18^{a}	2.33±0.16^{a}	2.17±0.20^{a}	1.94±0.10^{a}	1.83±0.28^{a}

Data are given as means ± standard error of means (SE); Means that do not share a letter in superscript in the same row are significantly different at $p < 0.05$ by Tukey mean comparison

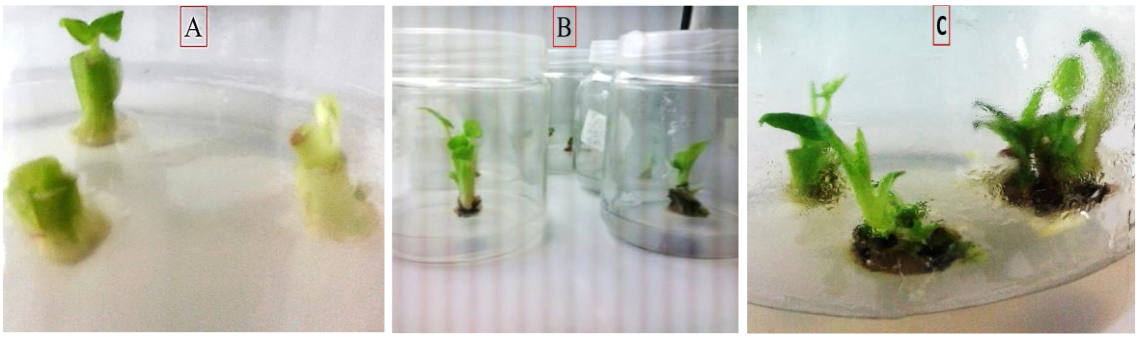

Fig. 8.2 Shoot initiation from cocoyam shoot-tip cultured on MS medium supplemented with 2.0 mg/l BAP: After 4 days of culture (A), After 7 days of culture (B) and After two weeks of culture (C)

8.3.2 Shoot Multiplication

The effects of genotypes (green- and purple-cocoyam) and PGRs on shoot multiplication of cocoyam are displayed in Table 8.3. The mean numbers of shoots and leaves per explant were not differentially influenced by genotypes. Genotypes had significant effect on the shoot length. PGRs had highly significant effect ($p<0.001$) on the number of shoots per explant, leaf numbers per explant and shoot length.

Table 8.3 Mean square of *in vitro* induced shoot parameters of genoytpes (green- and purple- cocoyam) on MS medium supplemented with different PGRs[a]

Treatment	df	Shoot multiplication parameters/explant		
		Shoot number	Leaf number	Shoot length
Genotypes	1	1.820	0.669	4.630**
PGRs	19	5.650***	4.672***	3.126***
Error	219	0.567	0.513	0.406
Total	239			

[a]PGS-Plant growth regulators: BAP + NAA, BAP + Kn and BAP + NAA + Kn. ** and *** indicate the mean square values of shoot multiplication parameters which were significant at, 0.01 and 0.001 probability level, respectively.

Among the different combinations of BAP with Kn, BAP with NAA and BAP with Kn and NAA, the media containing BAP in combinations with NAA provided relatively higher mean number of shoots per explant than the media containing BAP in combination with Kn and BAP in combination with Kn and NAA (Table 8.4). Maximum mean number of shoot per explant (4.56 $\pm$ 0.35) and (4.83 $\pm$ 0.26) was obtained on MS medium containing 2.5 mg/l BAP in combination with 0.5 mg/l NAA for green- and purple- cocoyam, respectively. The next highest number of shoots per explant (4.39 $\pm$ 0.44) and (4.50 $\pm$ 0.41) was produced on MS medium containing 5.0 mg/l BAP and 1.0 mg/l Kn for green- and purple- cocoyam, respectively.

The media containing BAP in combinations with Kn provided relatively higher mean number of leaves per explant than the media containing BAP + NAA and BAP + NAA + Kn (Table 8.4). Maximum mean number of leaves per explant (4.67 $\pm$ 0.41) and (4.94 $\pm$ 0.50) was obtained on MS medium containing 1.0 mg/l BAP in combination with 0.25 mg/l Kn for green- and purple- cocoyams, respectively. Statistically the same number of leaves per explant was produced on the MS medium containing 2.5 mg/l BAP + 0.5 mg/l NAA, 0.5 mg/l BAP + 0.1 mg/l Kn or 5.0 mg/l BAP + 1.0 mg/l Kn for both cocoyam genotypes.

Among the different combinations of BAP with Kn, BAP with NAA and BAP with Kn and NAA, the media containing BAP + NAA + Kn provided relatively longer shoots for both green- and purple-cocoyams than the media containing BAP + Kn and BAP + NAA (Table 8.4). The longest shoots, 3.92 $\pm$ 0.40 cm and 4.36 $\pm$ 0.46 cm, were obtained on MS medium containing 5.0 mg/l BAP + 0.5 mg/l NAA + 1.0 mg/l Kn for green- and purple- cocoyam, respectively. Statistically, similar result could be achieved on MS media supplemented with 5.0 mg/l BAP + 1.0 mg/l, 2.5 mg/l BAP + 0.5 mg/l NAA, 5.0 mg/l BAP + 1.0 mg/l Kn + 0.25 mg/l NAA and or 5.0 mg/l BAP 1.0 mg/l Kn + 0.5 mg/l NAA for both green- and purple cocoyams. In general, shoots obtained in the medium containing 2.5 mg/l BAP + 0.5 mg/l NAA, looks more comparable with the medium composition (Fig. 8.3).

Table 8.4 Effects of different concentrations of plant growth regulators on shoot multiplication of green-and purple-cocoyams

Combinations PGRs* (mg/l)			Mean shoot number/explant +SE		Mean leaf number/explant +SE		Mean shoot length (cm) + SE	
BAP	Kn	NAA	Green cocoyam	Purple cocoyam	Green cocoyam	Purple cocoyam	Green cocoyam	Purple cocoyam
0	0	0	2.17 ± 0.33^{c}	2.33 ± 0.00^{bc}	2.33 ± 0.17^{e}	2.72 ± 0.10^{bc}	1.69 ± 0.16^{c}	2.08 ± 0.18^{c}
0.5	0.10	0	2.33 ± 0.21^{c}	2.50 ± 0.42^{bc}	3.67 ± 0.44^{abcd}	4.28 ± 0.41^{ab}	1.75 ± 0.16^{c}	2.22 ± 0.14^{c}
1.0	0.25	0	2.28 ± 0.23^{c}	3.78 ± 0.44^{ab}	4.67 ± 0.41^{a}	4.94 ± 0.50^{a}	1.78 ± 0.24^{c}	2.33 ± 0.10^{c}
1.5	0.50	0	2.44 ± 0.50^{c}	2.39 ± 0.25^{bc}	3.11 ± 0.11^{bcde}	3.06 ± 0.34^{bc}	2.39 ± 0.34^{bc}	2.50 ± 0.11^{bc}
2.5	0.75	0	2.17 ± 0.28^{c}	2.72 ± 0.25^{bc}	3.33 ± 0.19^{bcde}	3.00 ± 0.27^{bc}	2.28 ± 0.44^{bc}	2.67 ± 0.20^{bc}
5.0	1.0	0	4.39 ± 0.44^{ab}	4.50 ± 0.41^{a}	3.83 ± 0.42^{ab}	4.11 ± 0.49^{ab}	2.72 ± 0.21^{abc}	3.06 ± 0.57^{abc}
0.5	0	0.25	2.17 ± 0.31^{c}	2.11 ± 0.31^{c}	2.89 ± 0.19^{bcde}	2.50 ± 0.11^{bc}	2.00 ± 0.16^{bc}	2.33 ± 0.28^{c}
1.0	0	0.25	2.23 ± 0.25^{c}	2.61 ± 0.30^{bc}	2.78 ± 0.16^{bcde}	2.72 ± 0.17^{bc}	2.67 ± 0.05^{bc}	2.58 ± 0.13^{bc}
1.5	0	0.25	2.39 ± 0.23^{c}	2.67 ± 0.27^{bc}	2.72 ± 0.13^{bcde}	3.11 ± 0.18^{bc}	2.44 ± 0.11^{bc}	2.75 ± 0.11^{bc}
2.5	0	0.25	3.17 ± 0.07^{abc}	2.89 ± 0.17^{bc}	2.78 ± 0.17^{bcde}	2.94 ± 0.48^{bc}	2.33 ± 0.09^{bc}	2.22 ± 0.31^{c}
5.0	0	0.25	2.17 ± 0.11^{c}	2.39 ± 0.13^{bc}	2.44 ± 0.43^{de}	2.83 ± 0.14^{bc}	2.03 ± 0.10^{bc}	2.11 ± 0.13^{c}
1.0	0	0.5	3.50 ± 0.27^{abc}	2.28 ± 0.22^{bc}	2.94 ± 0.26^{bcde}	3.22 ± 0.31^{abc}	2.50 ± 0.17^{bc}	2.44 ± 0.11^{bc}
1.5	0	0.5	3.22 ± 0.38^{abc}	3.56 ± 0.41^{abc}	2.72 ± 0.20^{bcde}	3.22 ± 0.11^{abc}	2.89 ± 0.44^{bc}	2.47 ± 0.22^{bc}
2.5	0	0.5	4.56 ± 0.35^{a}	4.83 ± 0.26^{a}	3.72 ± 0.18^{abc}	3.83 ± 0.45^{abc}	2.89 ± 0.13^{abc}	3.78 ± 0.78^{ab}
5.0	0	0.5	2.44 ± 0.27^{c}	3.44 ± 0.20^{abc}	2.94 ± 0.18^{bcde}	2.89 ± 0.68^{bc}	2.39 ± 0.37^{bc}	2.67 ± 0.46^{bc}
0.5	1.0	0.25	2.11 ± 0.21^{c}	2.39 ± 0.14^{bc}	2.44 ± 0.25^{de}	2.11 ± 0.17^{c}	2.17 ± 0.15^{bc}	2.61 ± 0.16^{bc}
2.5	1.0	0.25	2.11 ± 0.25^{c}	2.17 ± 0.21^{bc}	2.28 ± 0.11^{e}	2.22 ± 0.22^{c}	2.72 ± 0.23^{abc}	2.53 ± 0.11^{bc}
5.0	1.0	0.25	2.44 ± 0.35^{c}	2.11 ± 0.22^{c}	2.56 ± 0.16^{cde}	3.22 ± 0.46^{abc}	2.69 ± 0.13^{abc}	3.14 ± 0.39^{abc}
2.5	1.0	0.5	3.00 ± 0.22^{bc}	3.39 ± 0.16^{abc}	3.28 ± 0.07^{bcde}	3.06 ± 0.23^{bc}	3.19 ± 0.25^{ab}	3.44 ± 0.10^{abc}
5.0	1.0	0.5	2.24 ± 0.08^{c}	2.22 ± 0.22^{bc}	3.28 ± 0.29^{bcde}	2.94 ± 0.15^{bc}	3.92 ± 0.40^{a}	4.36 ± 0.46^{a}

*PGRs- Plant growth regulators; BAP: 6-Benzylaminopurine; NAA: α-naphthalene acetic acid; Kn: Kinetin. Data are given as means ± standard error of means (SE); means that do not share a letter in superscript within a column are significantly different at $p < 0.05$ by Tukey pairwise comparison.

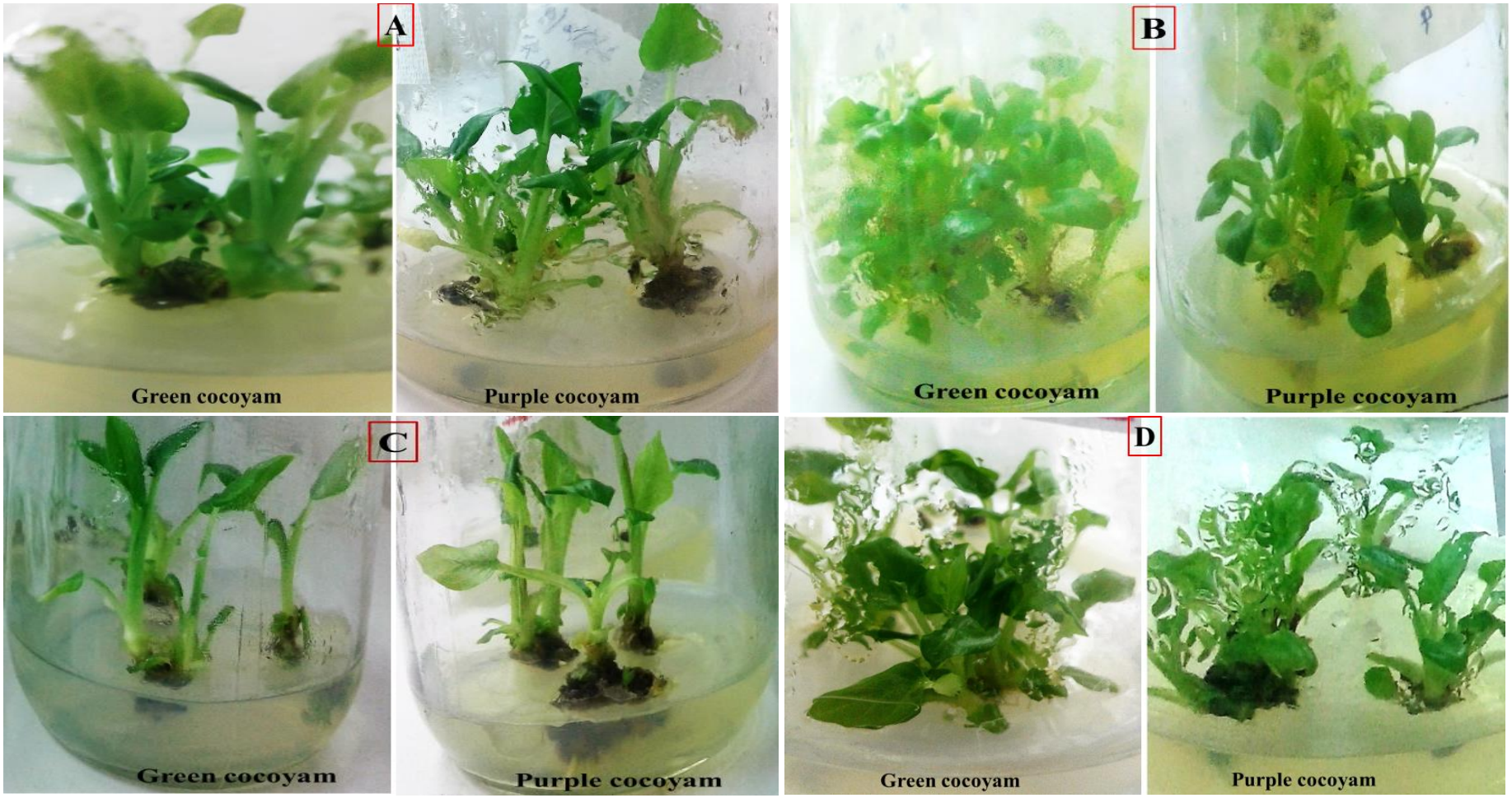

Fig. 8.3 Green- and purple-cocoyams on different multiplication media: (A) 2.5 mg/l BAP + 0.5 NAA; (B)1.0 mg/l BAP + 0.25 mg/l Kn; (C) 5.0 mg/l BAP + 1.0 mg/l Kn + 0.5 mg/l NAA; (D) control (without growth regulator)

8.3.3 Rooting and acclimatization

The influence of genotypes (green- and purple-cocoyams) on the production of roots and root length was not significant. The number of roots per explant and root length were influenced by different concentration of PGRs (IBA or NAA) (Table 8.5).

Table 8.5 Mean square of *in vitro* induced root number and length of cocoyam genotypes (green- and purple-cocoyams) on MS medium supplemented with different concentrations of PGRs (IBA or NAA) [a]

Treatment	df	Root parameters/shoot	
		root number	root length
Genotypes	1	2.45	3.08
PGRs	10	24.61***	25.85***
Error	120	1.658	2.130
Total	131		

[a]PGRs - Plant growth regulators; IBA- Indole-3-butyric acid; NAA- α-naphthalene acetic acid; *** indicates the mean square values of number of roots per explant and root length which were significant at 0.001 probability level

Maximum of 93.3% shoots formed roots on MS medium containing 2.0 mg/l IBA followed by 86.7% on MS medium containing 2.0 mg/l NAA for both green- and purple- cocoyams. Maximum mean number of roots, 6.00 $\pm$ 0.74 and 5.83$\pm$0.49 for green- and purple-cocoyams, respectively, were recorded on MS medium supplemented with 2.0 mg/l IBA. Among the tested concentrations, relatively less number of roots was obtained from IBA and NAA at lower concentration (0.25 mg/l) and NAA at higher concentration (3.0 mg/l). The longest roots (4.06$\pm$0.11 and 6.44$\pm$0.0.99 cm) were produced on medium supplemented with 1.0 mg/l IBA and 2.0 mg/l IBA for green- and purple- cocoyams, respectively (Table 8.6). The roots formed in 2.0 mg/l IBA were shown in Fig. 8.4. Upon acclimatization, 23 (76.7%) of green cocoyam and 25 (83.3%) of purple cocoyam survived. The micropropagated cocoyam after two weeks of acclimatization in greenhouse are shown in Fig. 8.5.

Table 8.6 Effect of IBA and NAA on root induction of green- and purple-cocoyams

IBA	NAA	Root forming shoots (%)		No. of roots/shoot ± SE		Root length (cm) ± SE	
(mg/l)	(mg/l)	Green cocoyam	Purple cocoyam	Green cocoyam	Purple cocoyam	Green cocoyam	Purple cocoyam
0	0	43.3	33.3	0.94±0.34^{b}	0.56±0.07^{c}	0.61±0.22^{c}	0.39±0.10^{b}
0.25	0	66.7	53.3	1.28±0.25^{b}	1.28±0.30^{bc}	2.00±0.48^{bc}	1.67±0.37^{b}
0.50	0	76.8	76.8	2.11±0.85^{b}	2.94±0.67^{b}	2.75±0.26^{b}	2.33±0.29^{b}
1.0	0	83.3	83.3	2.72±0.94^{b}	3.28±0.67^{b}	4.06±0.11^{ab}	3.00±0.45^{b}
2.0	0	93.3	93.3	6.00±0.74^{a}	5.83±0.49^{a}	5.94±0.71^{a}	6.44±0.99^{a}
3.0	0	76.8	76.8	2.39±0.50^{b}	3.28±0.66b	3.94±0.75^{ab}	2.83±0.84^{b}
0	0.25	33.3	23.3	1.00±0.37^{b}	0.50±0.28^{c}	1.61±0.61^{bc}	1.22±0.50^{b}
0	0.50	73.3	60.0	2.06±0.13^{b}	2.00±0.23^{bc}	1.94±0.42^{bc}	1.89±0.21^{b}
0	1.0	76.8	70.8	2.22±0.32^{b}	3.06±0.26^{b}	2.22±0.42^{bc}	2.17±0.45^{b}
0	2.0	86.7	86.7	2.56±0.62^{b}	3.33±0.38^{b}	3.00±0.60^{bc}	2.50±0.58^{b}
0	3.0	70.0	53.3	1.67±0.12^{b}	1.89±0.76^{c}	2.17±0.64^{bc}	2.44±1.11^{b}

Means that do not share a letter in superscript within a column are significantly different at p = 0.05 by Tukey pairwise comparison. Data are given as means ± standard error of means (SE)

Fig. 8.4 *In vitro* rooting of cocoyam shoots on MS medium containing 2.0 mg/l IBA: Green cocoyam (A) and Purple cocoyam (B), the number of roots per plantlet was counted and the root length was measured (cm) (C)

Fig. 8.5 Micropropagated plants after two weeks of acclimatization in the greenhouse

8.4 Discussion

In this study, MS medium containing different concentrations of BAP (1.0, 2.0, 3.0, 4.0 and 5.0 mg/l) resulted in shoot formation in the first culture. The percentage of shoot formation achieved by this study ranged from 83.56% to 100%, which were better achievement than that achieved by Castro (2006) who obtained the highest percentage (83%) initiated shoots of cocoyam from Nicaragua in MS medium containing 0.5 mg/l BAP and 1.0 mg/l IAA. Paul and Bari (2007) achieved the highest percentage of initiated explants from Bangladesh cocoyam (65%) using 0.5 mg/l BAP and 1.0 mg/l IAA. Wokabi (2012) achieved 100% shoot initiation by supplying BAP and IAA, respectively, in concentrations of 2.0 + 0.5, 4.0 + 1.0, 6.0 + 2.0 and 8.0 + 3.0 mg/l. No significant difference in number of shoots per explant and shoot length was observed on MS medium containing different concentrations of BAP. In similar study, Onwubiko *et al*. (2012) reported that five different concentrations (0.25, 0.5 0.75, 1.0 and 1.25 mg/l) BAP did not differentially influence number of shoots per explant and shoot length of two cocoyam varieties from Nigeria. However, Vilchez *et al*. (2009) reported that the use of 2.0, 3.0 and 4.0 mg/l BAP improved the quality of cocoyam shoots in comparison to PGRs free medium. In present study, all explants (100%) induced shoots on MS medium containing 2.0 mg/l BAP, showing that cocoyam shoots can be better initiated by adding BAP alone in MS medium. Analogues to this, Vilchez (2009) reported

the decisive role of incorporation of 2.0 mg/l BAP in culture medium for shoot initiation of cocoyam. Thus, the MS medium containing 2.0 mg/l BAP found to be optimum for *in vitro* shoot initiation as this concentration resulted in 100% initiated quality shoots, which looks more comfortable with the medium composition and turned green within 4 days of culture.

When the initiated shoots were transferred to MS medium containing different concentrations of PGRs, significant difference were observed in terms of number of shoots and leaves per explant and shoot length. Maximum mean number of shoots per explant (4.56 for green cocoyam and 4.83 for purple cocoyam) was obtained on MS medium containing 2.5 mg/l BAP and 0.5 mg/l NAA. Paul and Bari (2007) obtained maximum mean number of cocoyam shoots per explant (1.00) in MS media supplemented with different concentrations of auxins and cytokinins. Sama *et al*. (2012) achieved the average number of cocoyam shoots per explant (9.7) using 4.5 mg/l BAP. Tsehifet Solomon *et al*. (2010) reported 1.5 mg/l BA in combination with 0.5 mg/l IAA was the best concentrations for cocoyam shoot multiplication. The differences in shoot multiplication performance among these studies can be attributed to variations in genotype, type and concentration of culture media compositions and environmental factors such as CO_2 enrichment and light intensity. Among the tested PGRs, the longest shoots were obtained on MS medium supplemented with 5.0 mg/l BAP +1.0 mg/l Kn + 0.5 mg/l NAA. Statistically the same length of shoots but better quality was obtained on MS medium containing 2.5 mg/l BAP and 0.5 mg/l NAA. This indicates the importance of using 2.5 mg/l BAP and 0.5 mg/l NAA on the MS medium for cocoyam shoot multiplication as far as its objective is to obtain highest possible shoot numbers with best length in order to facilitate its management during acclimatization.

The number of roots per shoot and root length were influence by the different concentrations of IBA and NAA. The highest rooting percentage (93.3) was obtained on MS medium

containing 2.0 mg/l IBA. Highest number of roots per shoot and the longest roots were obtained on MS medium supplemented with 2.0 mg/l IBA. Paul and Bari (2007) achieved the highest number of cocoyam roots per shoot (7.9) and root length (6.9 cm) on MS medium supplemented with 0.4 mg/l IAA. Relatively less number of roots was obtained on PGRs free MS medium.

Acclimatization of *in vitro* rooted plantlets was successful, where 76.7% of green cocoyam and 83.3% of purple cocoyam were survived, which were, however, low achievement compared to the reports of Onokpise *et al*. (1992) and Onokpise *et al*. (1999) who obtained 100% survival with acclimatization studies. The relative lower survival percentage observed in this study could be due to the plantlets were kept under a plastic cloche only for three days or due to these cocoyam genotype may be different from the cocoyam genotypes acclimatized by Onokpise *et al*. (1992) and Onokpise *et al*. (1999). Genotype can influence the ability of regenerated plants to withstand the *ex vitro* growing conditions (Hazarika et al., 2006). Regarding the green- and purple- cocoyams, no considerable difference was observed for the shoot multiplication and root induction parameters and acclimatization success. This indicates any perceived difference was due to random error and the genetic variation that existed between the two may be limited to be differentially influenced by the media compositions. In conclusion, the present study results showed that cocoyam (*X. sagittifoulium*) growing in Ethiopia could be propagated *in vitro* by incorporating appropriate concentration of PGR on MS medium for the phases of shoot initiation (2.0 mg/l BAP), shoot multiplication (2.5 mg/l BAP + 0.5 mg/l NAA) and root induction (2.0 mg/l IBA). This protocol can be used to supplement the farmers' conventional methods of propagation of cocoyam in the country. Further work is suggested to evaluate the cormel yields of the multiplied cocoyam plants on field and to investigate growth condition of cocoyam for *ex situ* conservation.

Chapter Nine

Synthesis, Conclusion and Recommendations

9.1 Synthesis

This study employed the techniques of ethnobotanical documentation and genetic characterization using morphological descriptors and molecular markers. The proximate and mineral contents and antinutritional factors were also analyzed along with the development of a micropropagation protocol. Thus, the farmers' knowledge on cocoyam was complemented with the study of genetic diversity using morphological and molecular markers and determination of key nutritional compositions. The problems that farmers face regarding the piecemeal process of cocoyam propagation was taken to the laboratory and a micropropagation technique was developed. The latter could be extended to farmers in due course to promote faster and massive production of cocoyam. Thus, the study on the one hand addressed the knowledge gap that existed in cocoyam, particularly the paucity of scientific data, and on the other hand addressed problems that farmers described in relation to the food quality and those concerned with developing a means for easy propagation of the crop.

The field survey helped to document farmers' knowledge and perceptions of agromorphological traits, uses and management of cocoyam and interpretation in scientific terms. Though cocoyam (*X. sagittifolium*) was considered by farmers as a variety of taro (*C. esculenta*), it is a distinct species in a different genus within the same Araceae family. Different local names (KENI ZHANG, CUBI ZHANG, GOCHELI KIDO, SUDAN KIDO, TEPIYA BOINA, SAMUNA BOINA, GUDETA, AGARFA, DAWURO BOINA, FARANJA BOINA, TONNEKA, BADADIY and FARANJA BOINA) are in use for *X. sagittifolium* in the surveyed areas further affirming its distinctiveness although the term GODERE (Amharic term) has been used for both crops (which is likely more related

to the similarities in growth patterns and mode of utilization). Numerous local names are used for *X. sagittifolium* worldwide (Giacometti and Leon, 1994; Mayo *et al*., 1997; Quero-Garcia *et al*., 2010; Lim, 2015). A multiplicity of names for a given crop usually indicates the usefulness and popularity of the crop among produces and end users. Farmers in the study area also helped with the attempts made in this study towards the reconstruction of the history of the crop in Ethiopia by recalling that cocoyam was introduced into their areas later than taro. They affirmed that it is one of the crops introduced fairly recently into the Ethiopian farming systems. However, the large majority (84%) of farmers, understandably, did not remember the time when cocoyam was introduced into their areas despite the assertion by some farmers from Benchi-Maji and Kefa zones that cocoyam was introduced into their areas in the 1970s. During our survey, we observed that cocoyam was widely distributed in the surveyed areas, growing mainly in the homegarden patches. It is also commonly found in natural ecosystems as escapes from cultivation, on roadsides, in river valleys and as ornamentals mainly in urban centers. This may indicate that cocoyam might have a longer history in Ethiopia.

The knowledge of genetic diversity of plants is a fundamental element to enhance the classification of germplasm, determine conservation plans for existing germplasm and for development and introduction of new varieties. Morphological traits (16 qualitative and 13 quantitative), and SSR and AFLP molecular genetic markers provided insight into a broader context for identifying the genetic diversity and differentiation of cocoyam accessions from Ethiopia. The majority (84.6%) of the quantitative morphological traits showed significant variation among accessions. Both SSR and AFLP molecular markers revealed high genetic diversity across populations, within green morphotype, within purple morphotype as well as when all collections were considered as single population. Solomon Fantaw *et al*. (2014b) characterized 64 cocoyam accessions on the bases of 16 quantitative traits and reported

significant variation among all of the studied quantitative traits. For reason that the exact date of cocoyam introduction into Ethiopia and who introduced it cannot be unequivocally spelt out, the high genetic diversity can fairly indicate that introduction of cocoyam into the country has been made multiple times, through multiple routes and probably by multiple agents. Many introduced plant species have high level of diverse propagules due to multiple introduction events (Rollins *et al*., 2013).

The extent of genetic differentiation and clustering is of great interest in a number of biological fields including evolution, conservation, breeding, and ethnobotany in general including anthropology (Hedrick, 2005). Of 16 qualitative morphological traits, 9 discriminated the cocoyam accessions into two groups. The quantitative morphological traits and SSR markers-based clustering analyses effectively separated the purple cocoyam morphotype from green cocoyam morphotype. The clustering pattern matches with green- and purple-colored cocoyam morphotypes that were observed during field studies. Farmers also identify the two by different local names. In the Qucha Woreda of the Gamo-Gofa Zone, for example, the term TONNEKA is used for purple cocoyam because it has a sour taste whereas the term BADADIYA is used for green cocoyam to indicate its giant size. The color difference could be considered as important for easy identification of cocoyam. Cluster and population structure analyses revealed that the ocoyam accessions that were collected from different zones, districts and kebeles were grouped regardless of the collection sites, indicating that there might have been movement of germplasm between the locations, possibly by farmer-to-farmer planting materials exchange. The fact that as many as 32% of the interviewed farmers did not know how cocoyam was introduced into their garden for the first time also indicates that the crop may be established from cormels and fragments dropped while transporting, irrespective of conscious human planting. A recent article by Carl Zimmer (April 12, 2018) that appeared in the Science column of the New York Times showed that

the sweet potato has travelled a long distance all by itself (www.nytimes.com/2018/04/12/science/sweet-potato-pacific-dna.html). Cocoyam is probably traveling to some distance within Ethiopia without human involvement and its introduction into some gardens could be accidental.

The present survey results showed that both green- and purple-colored cocoyams are mainly used for food and fodder/feed while the purple-colored cocoyam is also used as medicine to treat WULAWUSHIYA (related with hepatitis), BARQA (postpartum depression) and GERGEDA (related with rheumatoid arthritis). However, there is a paucity of data on the nutritional compositions of cocoyam growing in the country. To address this lack of information, proximate and mineral contents and antinutritional factors of green- and purple-colored cocoyams were determined. The analysis showed that both morphotypes of cocoyam can provide nutrient-rich products, with slight differences in the quantities of proximate and minerals contents. However, high antinutritional factors (phytate and tannin) were determined from both morphotypes of cocoyam with significantly higher quantities in purple morphotype, supporting the farmers' response that stated as purple cocoyam has unpleasant smell compared to green cocoyam and that they give the local name SAMUNA BOINA meaning soap taro in *Dawuroto*, *Wolaitato* and *Gofato* languages for purple cocoyam due to its cormel has the smell of soap when cooked. Although cocoyam was appreciated by all respondents for its food values (serving as emergency food), the farmers disliked traits and high antinutritional factors would affect its acceptance because that could impose some adverse effects on its end users. Studies have shown that processing methods such as boiling, fermentation and roasting can significantly reduce contents of antinutritional factors of taro (*C. esculenta*) from Ethiopia (Adane Tilahun *et al*. 2013; Melese Temesgen, 2017; Habtamu Azene and Tesfahun Molla, 2017). Thus, the processing methods should to be tested for the

level of reduction of antinutritional contents of cocoyam in order to obtain healthful and comfortable cocoyam products.

Traditional propagation method of cocoyam was unable to ensure dissemination of its useful aspects, enhance sustainable production and conservation of cocoyam in Ethiopia. The production of a large number of plants of selected germplasm is the prerequisite for establishment of plantations, *ex-situ* conservation and improvement (Siddique and Anis, 2009). A micropropagation protocol was developed for green- and purple- cocoyams growing in the country, by initiating shoot tip explants on MS medium containing 2.0 mg/l BAP, multiplying using MS medium supplemented with 2.5 mg/l BAP and 0.5 mg/l NAA and inducing roots on MS medium containing 2.0 mg/l IBA. This could help to promote faster and massive production of cocoyam to address the problems that farmers face regarding the piecemeal process of cocoyam propagation and for possible wider applications.

9.2 Conclusion

A lot of useful indigenous knowledge on agromorphological traits, uses and management of cocoyam was documented from cocoyam growing areas of Ethiopia. We found that cocoyam is synonymously considered with taro (*C. esculenta*). Two morphotypes of cocoyam were perceived by farmers, which were also distinguished by morphological traits and SSR markers. The study identified qualitative and quantitative traits that will help researchers in recommending traits for morphological identification of cocoyam germplasms. The study revealed high level of genetic diversity, implying genetically diverse cocoyam accessions have been cultivated in the country. This indicates that there might have been multiple introductions of cocoyam into the country although this needs to be investigated. SSR marker-based study revealed that the purple cocoyam was strongly separated from the green cocoyam while the AFLP marker-based study correlated cocoyam accesions neither to geographic locations (populations) nor to morphotypes. This inconsistent result could be attributed to the important features that SSR and AFLP markers differ from each other. The nutritional composition analysis showed that both green- and purple- cocoyams can provide nutrient-rich products although the high antinutritional factors would affect its acceptance. The micropropagation protocol established in this study can be used to supplement the farmers' conventional methods of propagation of cocoyam. The cocoyam growing in Ethiopia can be considered as a crop which can play a significant role in alleviating the household food insecurity and periodic food shortages existing in some families inhabiting cocoyam growing areas.

9.3 Recommendations

Based on the findings of this study, the following recommendations are drawn:

- It has been the farmers who have managed cocoyam to meet their needs. This study is one among the very few pioneer cocoyam researches undertaken in Ethiopia. It did not cover all cocoyam growing areas of the country despite the considerable efforts that have been made to address various aspects. Thus, the future efforts should focus on developing core collections from a wider possible cocoyam growing areas of the country. The cocoyam genotypes should be tested in more than one environment to select elite genotypes for future utilization and conservation.

- The high genetic diversity, detected in this study, should be considered a good opportunity for effective usage and conservation of cocoyam. All applicable conservation strategies should be sought to maintain the existing high genetic diversity of cocoyam accessions in Ethiopia.

- Since some inconsistency has been observed between the results of SSR and AFLP markers regarding clustering of green- and purple-colored cocoyam morphotypes, the results should be interpreted with a degree of caution for a reasonable assessment of true genetic situation of cocoyam in Ethiopia. Full taxonomic study of cocoyam is recommended in order to facilitate investigation of the relationships between its gene pool in Ethiopia and elsewhere in the world including other species of the genus *Xanthosoma*.

- More detailed nutritional analyses including processing and sensory testing are suggested for further study.

- Further work is suggested to evaluate the cormel yields of the tissue culture derived cocoyam plants at a field and to investigate minimum growth condition for *in vitro* storage.

- The existing uses of cocoyam is limited in view of its potential uses. Cocoyam is poorly studied and underutilized crop in spite of its nutritional value and its potential as food crop. Thus, collaborative research intervention involving the development of varieties, making available high quality planting materials for farmers and promoting value chains and market opportunities are valuable for sustainable use of the exisisting diversity and to safeguard the potential end users of cocoyam in the country.

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Apendix 1 Passport data and semi-structured interview guide for cocoyam (*Xanthosoma sagittifolium* (L.) Schott) study in Ethiopia

School of Graduate Studies, Addis Ababa University

Passport data

1. Acquisition date ----------
2. Accession code (include the first letters of Zone and Woreda, year of collection Xs and number**)** (e.g. BS/2014/Xs1 to refer to an accession from Bench-Maji Zone, South-Bench Woreda in 2014 and Xs was given to refer *Xanthosoma sagittifolium* and that was following by number 1 to refer accession number 1----------
3. Scientific name: Genus----------species---------- subspecies or variety---
4. Collector name ----------
5. Country of collection----------
6. Donor name ----------
7. Ethnic group of the individual and/or farmer donating the accession ----------
8. Local name/vernacular name/ of the crop---------- Language: ----
   - Is there any special meaning linked to the name? Yes ----------No------
   - If yes, what is the meaning of the vernacular name? ----------
9. Collection source (home-garden, outfield farm, road side, wild habitat (forest), village market, institution, other) ----------
10. If from farm, status of plantation (backyard garden-------- smallholding (<5 ha) ---- midsize holding (5-10 ha) ----------larger size holding (>10 ha) ---------
11. General distribution of the accessions in the area (rare, limited, widely distributed but scattered small populations, extensive stands of large population) ----------
12. Growing conditions: substrate (wet land (flooded), low land (not flooded), swamp, upland (add approximate slope) ----------
13. Growth conditions: canopy (deep shade, partial sun, full sun) ----------
14. Type of material received (corm, cormel, leaf) ----------
15. Type of maintenance: (vegetative, tissue culture, seeds) ----------
16. Number of plants sampled ----------
17. Location of collection site:
    Kebele---------- District ----------Zone ----------Region ----------
    Altitude ---------- latitude ---------- longitude----------
18. Institution where herbarium specimen will be deposited----------

Semi-structured interview guide

1. Sex and age of each farmer responded to the interview

- Male --------female --------Age--------. For how long had you lived in this area? ---For how long had you grown cocoyam? ----------
2. Description of cocoyam germplasm by farmers

- Do you remember the year when this crop (cocoyam) was introduced into your area? ----------
- Do you remember from where (from whom) you obtained cocoyam for the first time? Yes/No If yes, from where (from whom) ----------
- Are taro and cocoyam the same or different crops? ----------
- How do you differentiate cocoyam from taro? ----------
- How many types of cocoyam do you know/grow? ----------
- If two or more, how do you differentiate them ----------
- Did you observe flower of cocoyam? Yes----------No----------If yes, at which month of cultivation? ----------
  Under what conditions? ----------
3. Present cultivation status: at the time of collection (increasing, decreasing, static, non-cultivated); why ----------
4. Local uses of cocoyam

- For what purposes do you use cocoyam? No use---------- Food---------- Medicinal (witchcraft)---------- Fodder---------- Other (specify) ----------
- If the crop is used for food, Edible: corm---------- cormel---------- petiole------leaves----------other----------What is the mode/method of preparation? ----------
- If cocoyam is used for medicinal purpose, what is the mode of preparation, administration and for the treatment of which disease/s is it used for? ----------
5. Farmers' preferred traits of cocoyam

- Is there a preferred trait of cocoyam? Yes---------- No ----------If yes, would you mention some? ----------
- Is there any aspect of cocoyam that you hate, and that you like to see improved? Yes----- No ------. If yes would you mention some? ----------
6. Farmers' planting material and cropping system

- What part of the crop do you use for planting? Corm -------cormel-------other------which one of these is preferred? ----------why ----------
- How would you cultivate cocoyam? Crop by irrigation ----------By rain-fed-------
- Do you use fertilizer? Yes----------No----------
- How do you crop cocoyam? Mono crop ---------- Cocoyam mixed with other crops----------. If mixed with other crop, with which crop and what is your reason? ----------
- Any antagonism with any other crop? Yes ----------No----------If yes, which crop(s) and how? ----------

7. Time course of land preparation, planting and harvesting of cocoyam

- What is the time course that you prepare land for cocoyam planting? ----------
- What is the time course that you plant cocoyam? ----------
- What is the time course that cocoyam takes to be harvested? ----------

8. Methods that farmers have adopted for cocoyam germplasm conservation
9. Fresh corms buried on farm ----------Fresh cormels buried on farm----------Fresh corms stored at home ---------- Fresh cormels stored at home-------Others (specify) ----------
10. Type of herbarium specimen prepared (leaf, inflorescence, tuber/stem) ----------

Appendix 2 Selected morphological descriptors used to characterize cocoyam (*Xanthosoma sagittifolium*) grown in Ethiopia

S. N	Qualitative character	Character state
1	Plant growth habit	1. Acaulescent 2. Erect above ground stem 3. Reclining aboveground stem
2	Petiole attachment	1. Peltate 2. Subpeltate 3. Non peltate
3	Petiole color (upper 2/3rd)	1. Light green 2. Green 3. Red/Purple 4. Green streaked with red/purple
4	Petiole color (lower 1/3rd)	1. Light green 2 Green 3. Red/Purple 4. Green streaked with red/purple
5	Color of edge of petiole sheath	1. The same as the rest of petiole and sheath 3. Lighter than the rest of petiole and sheath 2. Darker than the rest of petiole and sheath 4. Pink/Red/Purple
6	Lamina orientation	1. One plan - apex up (Erect) 2. One plane - apex down (Droopy) 3. 3-dimentional (cup shaped)
7	Leaf margin color	1. Green to the edge 2. Clear to the edge 3. Purple/Red edge 4. Pale yellow/creamy edge
8	Leaf shape	1. No basal lobes 3. Hastate (basal lobes flared) 4. Sagittate. basal lobes <1/8th the length of leaf 2. Sagittate. basal lobes >1/8th -1/4th the length of leaf 5. Sagittate basal lobes >1/4th the length of leaf
9	Color of upper leaf surface	1. Light green 2. Medium green 3. Dark green 4. Redish/ Purplish green 5. Other
10	Color of lower leaf surface	1. Light green 2. Medium green 3. Dark green 4. Redish/ Purplish green 5. Other
11	Color of veins on upper leaf surface	1. Same as color as lamina 3. Lighter green than lamina 2. Darker green than lamina 4. Red/Purple
12	Color of veins on lower leaf surface	1. Same as color as lamina 2. Darker green than lamina 3. Lighter green than lamina 4. Red/Purple
13	Position of cormel apex	1. Aboveground 2. Underground 3. Both
14	Shape of cormels	1. Globose 2. Ovate 3. Cylindrical 4. Elliptical 5. Mixed (state which of these---)
15	Color of cormel apex	1. White 2. Pink 3. Red
16	Flesh cormel color	1. White 2. Yellow 3. Orange 3. Pink or pale red 4. Purple 5. other
Quantitative trait		**Remak**
1	Over all plant height (cm	Measured from ground level to the top of plant
2	Petiole length (cm)	Length of the longest petiole from the basal zone of the plant to the point of leaf attachment
3	Petiole sheath length (cm	Length of the sheath of longest petiole from the beginning to the end to sheath
4	Leaf length (cm)	The length of the leaf was measured
5	Leaf width (cm)	The width of the leaf was measured
6	Circumference of the above ground stem (cm)	The circumference of pseudostem just above ground measured
7	Number of cormels	The cormels produced by a plant counted
8	Cormel length (cm)	The length of large. medium and small sized cormels measured
9	Cormel diameter (cm	The diameter of large. medium and small sized cormels measured
10	Cormel fresh weight per plant (kg)	The weight of cormels per plant weighed
11	Corm length (cm)	The length of corm measured
12	Corm diameter (cm)	The diameter of corm measured
13	Corm fresh weight (kg)	The fresh weight of corm per plant was weighed

Appendix 3 Mean performances of 13 quantitative traits of 100 cocoyam accessions (65 green- 35 purple morphotypes) from two replications by accession

Accession	Morphotype	PH	PL	PSL	LL	LW	CAGS	NC	CL	CD	CFW	CrL	CrD	CrFw
BS/2014/Xs1	Green	78.25	58.67	31.08	42.78	24.89	32.25	12.83	9.42	3.38	1.90	11.83	6.21	1.25
BS/2014/Xs2	Green	78.08	62.00	33.92	41.56	23.67	31.75	11.83	9.00	3.10	1.40	12.08	5.73	1.20
BS/2014/Xs3	Green	81.33	63.33	33.83	36.42	21.31	29.83	10.25	9.08	2.56	1.25	11.50	5.24	0.93
BS/2014/Xs4	Green	72.42	56.33	29.50	35.06	19.67	25.75	11.83	9.00	2.85	1.33	12.33	5.58	1.25
BS/2014/Xs5	Green	83.58	64.58	39.25	43.17	24.11	32.33	15.33	9.75	2.93	1.88	13.17	6.96	1.23
BS/2014/ Xs5	Green	84.83	67.42	41.42	36.81	21.50	33.00	9.58	11.18	3.10	1.28	13.50	6.31	0.98
BS/2014/Xs7	Green	72.33	54.17	30.42	38.92	23.08	28.08	12.08	10.83	3.04	1.85	11.42	5.75	1.03
BN/2014/ Xs8	Green	80.92	53.83	36.08	40.25	22.89	33.82	13.33	10.58	3.98	2.43	11.33	6.64	1.63
BN /2014/Xs9	Green	74.42	55.50	32.67	35.86	20.53	24.17	10.08	8.92	3.48	1.38	10.33	5.38	0.98
BN/2014/Xs10	Green	75.25	55.83	34.33	36.14	23.22	27.50	8.25	9.08	2.25	0.90	10.50	4.79	2.10
BN/2014/Xs11	Green	79.08	65.58	38.75	39.56	22.56	30.92	11.33	8.92	3.23	1.10	12.92	6.27	1.73
BN/2014/Xs12	Green	67.92	52.25	28.50	35.78	20.89	28.08	7.33	7.08	2.68	1.08	11.33	4.66	0.88
BN/2014/Xs13	Green	66.83	52.58	28.75	32.97	20.31	26.17	10.33	8.33	2.41	1.15	9.33	5.08	0.65
BN/2014/Xs14	Green	73.67	53.42	30.67	33.19	19.94	25.17	9.92	9.83	2.96	1.35	10.25	5.23	0.88
KC/2014/Xs15	Green	68.17	48.92	28.33	31.11	19.14	25.08	9.75	10.33	2.59	1.13	10.83	5.73	0.90
KC/2014/Xs16	Green	66.25	51.50	27.92	35.47	20.19	27.25	12.67	9.50	3.20	1.35	13.08	5.49	0.95
KC/2014/Xs17	Green	75.58	55.25	33.33	38.06	22.75	28.83	11.75	10.67	3.35	1.45	12.50	5.88	1.18
KC/2014/Xs18	Green	72.83	53.17	29.92	33.69	20.81	27.58	11.17	10.33	3.09	1.48	11.83	5.49	1.10
KC/2014/Xs19	Green	74.08	54.33	30.17	38.11	19.92	29.08	12.67	9.50	3.22	1.30	11.71	5.59	0.78
KG/2014/Xs20	Green	68.17	50.17	28.50	29.69	17.28	25.42	5.92	6.58	2.69	0.68	7.42	3.49	0.58
KG/2014/Xs21	Green	66.08	50.00	26.25	36.25	20.47	28.00	12.67	6.75	2.69	0.98	10.83	5.39	1.15
KG/2014/Xs22	Green	70.50	56.92	31.25	36.69	21.03	27.00	11.08	8.83	2.86	1.45	10.50	5.81	0.90
KG/2014/Xs23	Green	75.33	58.58	33.92	33.33	20.00	26.33	9.75	7.42	2.64	1.13	12.58	5.18	0.98
KG/2014/Xs24	Green	65.00	45.92	30.33	30.53	18.42	23.50	8.83	7.17	2.42	0.88	9.92	4.48	1.03
JS/2014/Xs25	Green	74.67	56.33	31.17	35.11	20.86	27.92	9.08	10.25	2.80	1.03	12.00	5.35	0.83
JS/2014/Xs26	Green	71.33	52.58	30.25	39.08	22.97	29.08	13.00	11.83	3.00	1.60	10.00	5.70	1.53
DT/2014/Xs27	Green	76.25	55.67	30.50	38.17	23.36	29.00	9.25	9.67	2.79	1.00	11.92	4.96	0.83
DT/2014/Xs28	Green	69.42	51.75	29.67	34.44	22.39	25.92	11.58	9.75	2.78	1.75	9.75	4.79	1.15
DT/2014/Xs29	Green	77.17	58.58	36.17	35.03	20.44	27.33	13.17	8.58	3.11	1.48	10.50	6.12	1.00
DT/2014/Xs30	Green	74.25	51.92	30.58	37.17	21.58	28.75	6.75	8.42	2.61	0.90	6.08	3.81	0.53
DT/2014/Xs31	Green	75.50	57.83	33.08	36.06	21.22	33.33	10.75	8.42	3.05	0.90	12.00	5.93	1.05
DT/2014/Xs32	Green	77.00	58.50	34.83	37.47	23.06	31.75	11.42	10.00	3.16	1.18	11.92	6.15	1.13
DT/2014/Xs33	Green	67.17	50.25	28.08	35.47	22.14	27.67	8.42	8.33	3.81	1.40	11.50	6.28	0.98
DM/2014/Xs34	Green	68.42	49.33	27.33	36.89	21.89	26.67	8.92	8.08	3.18	0.90	10.92	5.98	1.20
DM/2014/Xs35	Green	67.75	56.50	30.58	35.00	19.97	27.58	10.17	9.42	2.79	0.83	11.58	5.12	0.90
DM/2014/Xs36	Green	71.67	55.08	31.58	39.64	23.06	29.50	10.83	8.83	3.32	1.18	10.92	6.01	0.85
DL/2014/Xs37	Green	74.00	55.33	29.42	40.28	24.50	31.50	11.25	9.50	2.76	1.15	13.17	6.05	0.83
DL/2014/Xs38	Green	70.67	48.33	29.58	34.06	20.33	26.83	10.17	8.58	2.39	1.35	10.67	5.27	1.23
DL/2014/Xs39	Purple	77.42	60.58	32.92	38.61	22.58	29.83	11.33	9.50	3.79	1.38	11.42	5.98	1.63
DL/2014/Xs40	Green	77.83	56.25	33.83	39.25	23.33	31.33	9.50	9.08	3.17	1.30	8.42	5.38	0.90
DL/2014/Xs41	Green	66.08	51.58	27.92	35.06	19.78	25.33	8.83	7.58	2.60	1.13	9.00	4.29	1.00
DL/2014/Xs42	Green	73.00	54.75	31.08	34.33	20.75	27.92	6.92	8.17	3.50	0.95	9.83	5.16	0.90
DL/2014/Xs43	Purple	66.58	54.58	30.75	40.89	22.69	28.25	8.75	9.50	3.46	1.95	13.25	6.46	1.73
DL/2014/Xs44	Green	69.25	52.17	29.83	38.11	22.14	27.67	11.42	8.17	3.16	1.58	10.00	5.78	1.05
DL/2014/Xs45	Purple	69.33	59.33	30.33	43.11	23.11	32.25	7.58	7.42	3.53	1.00	16.58	6.48	2.30
DB/2014/Xs47	Purple	64.75	53.08	29.83	40.28	22.50	34.42	11.08	8.83	3.25	1.25	13.75	5.53	1.68
DB/2014/Xs48	Green	68.50	50.92	28.58	36.08	20.33	28.83	9.42	10.33	2.62	0.75	12.75	5.54	0.90
DB/2014/Xs48	Green	68.33	49.33	27.75	37.17	23.56	27.58	9.67	9.33	3.02	0.80	10.50	5.00	1.18
DB/2014/Xs49	Purple	71.59	53.83	29.75	38.17	23.39	29.08	9.50	9.50	3.68	1.38	12.92	6.37	1.23
DB/2014/Xs50	Green	71.92	50.08	27.92	35.75	21.86	25.83	8.42	9.75	2.90	0.90	8.75	4.41	0.83
DB/2014/Xs51	Purple	67.09	55.50	29.00	38.31	22.83	31.25	8.92	8.42	3.18	1.00	14.08	6.10	1.05
DB/2014/Xs52	Green	73.83	54.42	30.42	35.53	21.03	26.83	10.42	9.08	3.25	1.43	11.33	5.65	1.33
DB/2014/Xs53	Purple	65.75	58.92	38.33	45.25	24.25	30.42	8.67	7.17	3.23	0.95	13.08	5.31	1.50
DB/2014/Xs54	Green	70.25	53.33	25.50	35.47	20.94	27.08	8.17	7.92	2.87	1.10	10.92	5.63	1.08
DB/2014/Xs55	Purple	64.75	52.83	30.67	37.50	22.06	27.75	8.58	8.08	3.68	1.20	15.92	5.70	1.15
WK/2014/Xs56	Purple	71.33	54.33	29.42	38.89	22.33	29.25	9.67	10.25	3.79	1.08	11.17	6.97	1.68
WK/2014/Xs57	Green	75.50	56.33	27.58	38.14	23.08	29.83	9.58	9.42	3.03	1.08	11.33	5.98	0.78
WK/2014/Xs58	Purple	62.59	51.08	29.42	35.67	20.53	27.58	8.92	10.67	3.90	1.25	13.92	6.17	1.48
WK/2014/Xs59	Green	62.33	44.67	26.25	37.92	22.25	28.00	8.67	10.50	2.88	0.93	8.92	5.70	0.88
WK/2014/Xs60	Purple	62.00	49.83	26.17	33.72	19.92	25.00	10.17	8.75	3.48	1.13	11.92	5.33	1.23
WK/2014/Xs61	Green	69.08	48.08	27.92	36.53	22.47	29.00	9.17	9.17	2.94	1.28	9.67	4.90	1.10
WH/2014/Xs62	Purple	74.59	57.92	33.08	40.06	25.47	30.92	11.25	8.25	3.36	1.63	13.83	5.76	1.00
WH/2014/Xs63	Green	75.67	58.67	32.17	35.94	21.42	25.75	9.17	9.00	2.96	1.23	10.33	5.48	1.05

Appendix 3 Continued

WH/2014/Xs64	Purple	70.67	56.58	31.67	40.39	22.50	28.17	8.67	8.33	3.66	1.10	14.50	5.66	1.28
WH/2014/Xs65	Green	63.00	50.08	28.83	32.58	18.11	24.50	9.75	8.08	3.03	1.13	11.25	3.78	0.75
WH/2014/Xs66	Purple	67.08	55.08	33.50	37.42	20.92	30.25	12.58	8.58	4.21	1.55	16.08	6.43	1.35
WH/2014/Xs67	Purple	67.67	54.75	30.75	40.89	22.67	31.08	9.58	8.42	3.54	1.15	14.50	6.95	1.85
WH/2014/Xs68	Purple	57.58	42.75	25.50	38.22	21.83	31.83	11.67	8.75	3.80	1.48	12.42	6.22	1.65
WH/2014/Xs69	Purple	71.42	55.58	31.83	38.83	21.14	28.17	9.00	9.25	4.11	1.20	12.75	6.15	1.65
WS/2014/Xs70	Purple	78.25	63.50	36.33	42.33	24.39	31.33	8.92	9.50	3.93	1.48	12.00	7.28	1.68
WS/2014/Xs71	Purple	65.92	55.58	28.50	37.64	22.44	32.92	11.67	8.58	3.72	1.73	13.92	5.99	1.65
WS/2014/Xs72	Green	71.50	51.58	31.58	40.75	24.19	30.25	13.50	11.25	3.14	1.75	10.08	5.68	0.85
WS/2014/Xs73	Green	75.67	53.50	33.50	36.22	23.50	24.50	12.92	8.42	3.13	1.63	8.92	5.50	1.08
WS/2014/Xs74	Green	75.08	55.17	31.00	38.06	23.33	27.92	10.00	10.00	3.53	1.48	10.67	5.33	1.08
WS/2014/Xs75	Purple	74.75	64.25	36.08	39.28	22.33	30.08	8.67	8.92	3.38	1.15	13.17	5.40	1.05
WS/2014/Xs76	Purple	80.17	66.50	43.92	43.33	23.69	30.08	8.33	9.50	3.95	1.20	14.17	6.51	1.63
WS/2014/Xs77	Green	76.08	53.25	29.83	38.44	22.17	27.75	11.33	8.50	3.26	1.50	11.17	5.63	0.93
WB/2014/Xs78	Purple	72.50	59.75	32.75	40.44	22.67	30.92	8.00	8.92	3.93	1.15	16.33	6.90	1.70
WB/2014/Xs79	Purple	68.58	48.08	28.67	37.25	23.67	28.17	8.25	9.33	4.21	1.35	12.25	6.82	1.45
WB/2014/Xs80	Green	70.92	55.83	27.42	36.86	21.44	24.92	9.25	9.83	3.53	1.05	12.50	6.07	1.08
WB/2014/Xs81	Purple	71.08	54.92	34.08	42.03	23.58	30.75	9.08	7.25	3.48	1.55	16.50	6.93	1.80
WB/2014/Xs82	Purple	76.92	59.42	36.58	39.89	22.08	32.58	8.25	8.17	3.61	0.98	13.17	5.95	1.23
GQ/2014/Xs83	Green	83.42	63.58	35.92	42.42	24.00	30.33	12.92	9.67	3.48	2.00	9.25	4.76	1.05
GQ/2014/Xs84	Purple	69.33	58.83	30.33	40.39	23.42	27.92	10.67	8.08	3.54	1.30	13.50	5.33	1.38
GQ/2014/Xs85	Green	79.67	60.25	32.67	38.50	23.28	28.83	12.58	10.42	3.48	1.50	16.92	5.78	0.88
GQ/2014/Xs86	Purple	68.92	54.92	28.83	40.97	23.75	32.50	9.75	10.17	4.05	1.10	15.25	6.30	1.35
GQ/2014/Xs87	Purple	82.50	67.83	40.50	42.39	23.75	34.00	9.08	8.42	3.94	1.15	13.83	6.74	1.28
GQ/2014/Xs88	Green	79.92	54.33	29.75	36.42	22.67	31.92	9.75	8.25	2.73	1.03	11.08	4.89	0.95
GQ/2014/Xs89	Purple	68.34	58.75	30.33	38.22	21.67	32.08	10.17	8.58	3.73	1.40	13.50	6.67	1.25
GQ/2014/Xs90	Green	57.67	52.17	31.50	36.06	19.19	23.17	9.75	10.75	3.77	1.23	10.83	4.90	0.68
GQ/2014/Xs91	Purple	65.00	55.75	31.67	44.97	25.22	31.67	7.67	7.08	3.55	1.80	16.67	7.73	1.70
GQ/2014/Xs92	Purple	65.00	51.83	30.25	40.31	23.50	32.92	8.58	7.42	3.48	1.35	14.33	5.98	1.40
GQ/2014/Xs93	Purple	68.67	60.08	33.83	38.61	22.86	27.83	9.17	9.08	3.49	1.15	13.83	6.39	1.38
GQ/2014/Xs94	Green	71.92	54.58	29.58	39.42	22.44	27.75	12.67	11.17	3.41	1.73	10.50	5.66	1.23
GD/2014/Xs95	Purple	69.83	57.25	30.58	41.36	23.69	33.08	8.58	8.50	3.67	1.65	12.42	5.64	1.48
GD/2014/Xs96	Purple	67.56	58.75	28.25	35.06	20.08	28.83	9.00	7.67	3.61	1.23	13.33	6.17	1.15
GD/2014/Xs97	Green	75.58	58.25	31.67	43.00	24.83	30.08	7.75	11.08	3.98	1.10	8.75	5.28	0.75
GD/2014/Xs98	Green	61.08	49.25	28.67	32.58	18.67	22.50	6.83	8.50	2.83	1.03	8.33	3.75	0.75
GD/2014/Xs99	Purple	70.92	59.83	34.50	43.83	22.92	33.42	8.00	9.42	3.88	1.13	14.83	7.08	1.85
GD/2014/Xs100	Green	63.50	49.25	25.50	31.22	18.56	21.42	13.92	9.50	3.33	1.38	10.58	5.39	1.05
Grand mean		**71.49**	**55.22**	**31.25**	**37.25**	**22.02**	**28.83**	**10.05**	**9.05**	**3.26**	**1.28**	**11.94**	**5.70**	**1.18**

PH= plant height, PL= petiole length, PSL=petiole sheath length, LL=lamina length, LW=lamina width, CAGS = circumference of above ground pseudo-stem, NC= number of cormels per plant, CL=cormel length, CD=cormel diameter, CFW=cormel fresh weight per plant, CrL= corm length, CrD=corm diameter, CrFW= corm fresh weight per plant.

Appendix 4 Nutrient composition and concentration of MS basal medium

1. Salts				
A. Macro nutrient	mg/l	g/l	**10 x**	**Preparation remark**
1. NH_4NO_3	1650	1.65	16.5 gm	Prepared in 500 ml double distilled water and 50 ml was drawn for 1litre medium (because in 500 ml it was already became 20x), i.e.,1000 ml/20 =50 ml
2. KNO3	1900	1.9	19 gm	
3. Cacl2.2H2O	440	0.44	4.4 gm	Prepare in 250 ml double distilled water and 25ml was drawn for 1-liter medium (because in 250 ml it was already became 40x), i.e., 1000/40 = 25 ml
4. MgSO4.7H2O	370	0.37	3.7 gm	
5. KH2PO4	170	0.17	1.7 gm	
B. Micro nutrient	mg/l	g/l	**1000x**	Prepared in 1 liter double distilled water and 1ml was drawn for 1-liter medium, i.e., 1000/1000 = 1ml
1. H3BO3	6.2	0.006	6.2 gm	
2. MnSO4.4H2O	22.3	0.022	22.3 gm	
3. ZnSO4.7H2O	8.6	0.0086	8.6gm	
4. KI	0.83	0.00083	0.83 gm	
5. $Na_2MoO4.2H2O$*	0.25	0.00025	0.25 gm	
6. CoCl2.6H2O**	0.025	0.000025	0.025 gm	
7. CuSO4.5H2O**	0.025	0.000025	0.25gm	
2. MS Vitamins	mg/l	in 100ml		To prepare 1-liter medium draw
1. Thiamine (HCl)	0.1	100mg	0.1gm	0.1 ml
2. Niacine	0.5	100mg	0.1gm	0.5 ml
3. Pyrodoxine(HCl)	0.5	100mg	0.1gm	0.5 ml
4. Glycine	2.0	100mg	0.1gm	2 ml
5. Myo-inositol	Fresh added	100mg/l	0.1g/l	
3. Growth regulators	??? in required amount			
4. Iron-EDTA Na salt		Fresh added		40mg/l =0.04g/l
5. Sucrose		Fresh added		30 g/l
6. Agar		Fresh added		6 g/l
7. pH		5.8		

Stock solution required for one-liter medium

Stock	ml required for one-liter medium
10 x	100ml/litre
20 x	50 ml/litre
40 x	25 ml/litre
100 x	10ml/litre
200 x	5ml/litre
400 x	2.5 ml/litre
800 x	1.25ml/litre
1000 x	1ml/litre

Appendix 5 Effects of different concentrations of PGRs on shoot multiplication of green- and purple- cocoyams (comparative analysis)

BAP (mg/l)	Kn (mg/l)	NAA (mg/l)	Shoot numbers/explant			Leaf numbers/explant			Shoot length (cm)		
			G	P	p-value	G	P	p-value	G	P	p-value
0	0	0	2.17	2.33	0.636	2.33	2.72	0.088	1.69	2.08	0.233
0.5	0.1	0	2.33	2.50	0.732	3.67	4.28	0.339	1.75	2.22	0.053
1.0	0.25	0	2.28	3.78	0.020	4.67	4.94	0.701	1.78	2.33	0.127
1.5	0.50	0	2.44	2.39	0.924	3.11	3.06	0.884	2.39	2.50	0.766
2.5	0.75	0	2.17	2.72	0.143	3.33	3.00	0.347	2.28	2.67	0.451
5.0	1.0	0	4.39	4.50	0.850	3.83	4.11	0.680	2.72	3.06	0.597
0.5	0	0.25	2.17	2.11	0.892	2.89	2.50	0.112	2.00	2.33	0.327
1.0	0	0.25	2.33	2.61	0.483	2.78	2.72	0.835	2.67	2.58	0.404
1.5	0	0.25	2.39	2.67	0.459	2.72	3.11	0.124	2.44	2.75	0.075
2.5	0	0.25	3.17	2.89	0.363	2.78	2.94	0.751	2.33	2.22	0.743
5.0	0	0.25	2.17	2.39	0.238	2.44	2.83	0.223	2.03	2.11	0.777
1.0	0	0.5	3.50	2.28	0.006	2.94	3.22	0.510	2.50	2.44	0.793
1.5	0	0.5	3.22	3.56	0.556	2.72	3.22	0.065	2.89	2.47	0.155
2.5	0	0.5	4.56	4.83	0.541	3.72	3.83	0.825	2.89	3.78	0.024
5.0	0	0.5	2.44	3.44	0.024	2.94	2.89	0.940	2.39	2.67	0.549
0.5	1.0	0.25	2.11	2.39	0.470	2.44	2.11	0.132	2.17	2.61	0.072
2.5	1.0	0.25	2.11	2.17	0.869	2.28	2.22	0.843	2.72	2.53	0.476
5.0	1.0	0.25	2.43	2.11	0.463	2.56	3.22	0.212	2.69	3.14	0.331
2.5	1.0	0.5	2.00	3.39	0.151	3.28	3.06	0.203	3.19	3.44	0.394
5.0	1.0	0.5	2.24	2.22	0.940	3.28	2.94	0.200	3.92	4.36	0.486

Independent sample t-test. P-values are shown for shoot multiplication parameters of green (G)- and purple (P)- cocoyam. Mean values with $p > 0.05$ are not significantly different their and respective p-values are shown