Department of Crop Sciences
Georg-August University Göttingen

**Muluneh Tamiru Oli**

# Assessing Diversity in Yams (*Dioscorea* spp.) from Ethiopia based on Morphology, AFLP Markers and Tuber Quality, and Farmers' Management of Landraces

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# Assessing Diversity in Yams (*Dioscorea* spp.) from Ethiopia based on Morphology, AFLP Markers and Tuber Quality, and Farmers' Management of Landraces

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Muluneh Tamiru Oli

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"If we knew what it was we were doing, it would not be called research, would it?"

**Albert Einstein**

"I have been impressed with the urgency of doing. Knowing is not enough, we must apply. Being willing is not enough, we must do."

**Leonardo da Vinci**

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## Acronyms and Abbreviations

| | |
|---|---|
| AFLP | Amplified Fragment Length Polymorphism |
| AMOVA | Analysis of Molecular Variance |
| ATP | Adenosine TriPhosphate |
| DM | Dry Matter |
| DNA | DeoxyriboNucleic Acid |
| dNTPs | Deoxynucleotide Triphosphates |
| DTT | Dithiothreitol |
| EDTA | Ethylene Diamine Tetraacetic Acid |
| ESARC | Eastern and Southern African Regional Center (of IITA) |
| FAO | Food and Agriculture Organization |
| GenAlEx | Genetic Analysis in Excel |
| IBCR | Institute of Biodiversity Conservation and Research (currently IBC) |
| ICC | International Association for Cereal Science and Technology |
| IITA | International Institute of Tropical Agriculture |
| IPGRI | International Pant Genetic Resources Institute |
| NTSYSpc | Numerical Taxonomy and Multivariate Analysis System |
| PA | Peasants Association |
| PCA | Principle Components Analysis |
| PCoA | Principal Coordinates Analysis |
| PCR | Polymerase Chain Reaction |
| PGRC/E | Plant Genetic Resource Center of Ethiopia (currently IBC) |
| RAPD | Random Amplified Polymorphic DNA |
| RVA | Rapid Visco Analyzer |
| SNNPRS | Southern Nations, Nationalities and Peoples Regional State |
| TAE Buffer | Tris-Acetate-EDTA |
| TE Buffer | Tris-EDTA |
| UPGMA | Unweighted Pair-Group Method Using Arithmetic Means Algorithm |

# 1. General Introduction

## 1.1. Yam: origin and distribution

Yam belongs to the genus *Dioscorea* in the family Dioscoreaceae. The family is believed to be among the earliest angiosperms and probably originated in Southeast Asia (Coursey 1976). The various *Dioscorea* species apparently followed a divergent evolutionary course in three continents separated by the formation of the Atlantic Ocean and desiccation of the Middle East (Hahn 1995). Accordingly, the major food species originated in three isolated centers: Africa, Southeast Asia and South America (Alexander and Coursey 1969). These centers are also considered areas for independent yam domestication, and represent considerable diversity (Asiedu et al. 1997).

The economically most important yam species include *D. alata*, *D. rotundata* and *D. cayenensis*. *D. alata* originated in Southeast Asia, more specifically in tropical Myanmar and Thailand (Orkwor 1998), and is currently the most diversified and extensively distributed species. The spread of Asiatic yams, mainly that of *D. alata* and *D. esculenta*, took place more than 2000 years ago, reaching Africa around 1000 AD (Coursey 1967). *D. alata* was, then, introduced into tropical America from West Africa around the 16$^{th}$ century by Portuguese and Spanish travelers (Onwueme and Charles 1994).

The species *D. rotundata* and *D. cayenensis* are native to West Africa (Coursey 1976). Of the two, *D. rotundata* is currently the leading species in terms of total area of production worldwide. It is extensively cultivated in West Africa, the West Indies and, to some extent, in East Africa. The introduction of the African species into tropical America is believed to have taken place as early as the 16$^{th}$ century (Coursey 1967). Lamarck gave the first description of *D. cayenensis* in 1792 based on a specimen from French Guiana (and hence the name Cayenne), whereas *D. rotundata* was described in 1813 by Poiret based on a sample from Puerto Rico long before their African origin was established (Hamon et al. 2001). These species, however, had limited eastward movement reaching only as far as East Africa. There is little or no cultivation of the African species in Asia (Onwueme and Charles 1994).

*D. bulbifera*, characterized mainly by the production of bulbils (aerial tubers), is native to both Asia and Africa, where wild forms still exist (Onwueme and Charles 1994). There are, however, appreciable differences between the two continental forms (Alexander and Coursey 1969; Terauchi et al. 1991). The cush-cush yam (*D. trifida*) is the only yam of Tropical American origin to have attained significance as a food crop, but its production is currently restricted to the West Indies (Brücher 1989; Onwueme and Charles 1994). Other yam species of minor economic importance in several tropical regions include *D. dumetorum, D. opposita, D. japonica, D. hispida* and *D. transversa* (Asiedu et al. 1997).

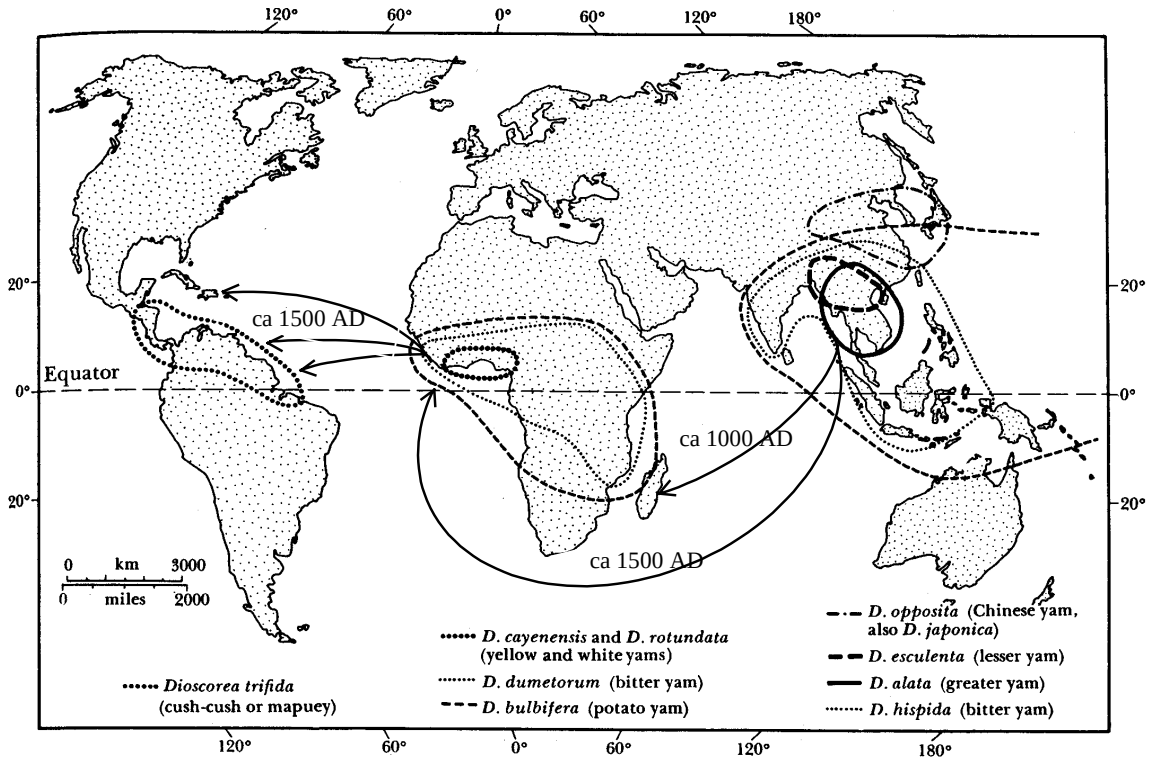



**Figure 1.1.** Approximate areas of origin and times of distribution of the major cultivated yam species (adopted from Coursey 1967; Harris 1972).

## 1.2. Taxonomy and important features of *Dioscorea*

The family Dioscoreaceae is generally classified under the monocotyledons. However, some features in yams such as the presence of a second non-emergent cotyledon and reticulate-veining of the leaves are typical of certain dicotyledonous plants (Purseglove 1972). This has led to the suggestion that the genus *Dioscorea* might have been

derived from plant forms that occurred before the differentiation of monocots and dicots (Degras 1993). Currently, the major *Dioscorea* species are widely distributed in the tropics and sub-tropics although a few species of minor economic importance are found in the warmer regions of the temperate zone (Coursey 1967). About 600 species have been described under the genus *Dioscorea*, making it the largest genus of the family Dioscoreaceae (Alexander and Coursey 1969). However, only few assume importance as crop plants. The genus is subdivided into sections, under which the various species are classified.

The section Enantiophyllum is the largest in terms of number of species, and includes the most important species of *D. alata*, *D. rotundata* and *D cayenensis*. Other members of this section are *D. opposita*, *D. japonica* and *D. transversa* (Asiedu et al. 1997). Vines that twine to the right, i.e. in clockwise direction when viewed from the ground upwards, characterize members of section Enantiophyllum. On the other hand, vines twining to the left distinguish species in sections Lasiophyton (*D. dumetorum* and *D. hispida*), Opsophyton (*D. bulbifera*), Combilium (*D. esculenta*) and Macrogynodium (*D. trifida*) (Onwueme and Charles 1994) (Table 1.1).

Despite the significant progress made over the last couple of decades in understanding the origin, domestication, phylogeny and diversity of the common food yams, their taxonomy remains complicated. For instance, some authors treat the major African species (*D. rotundata* and *D. cayenensis*) as separate (Burkill 1960; Akoroda and Chheda 1983), while others consider them as belonging to the same species (Martin and Rhodes 1978) or a species complex (Ayensu and Coursey 1972). Phylogenic studies based on RFLP[1] markers in chloroplast and nuclear ribosomal DNA[1] indicated common ancestry of the two species, with some evidence suggesting *D. rotundata* as the maternal parent of *D. cayenensis* (Terauchi et al. 1992). More recent findings based on isozyme and molecular markers, however, seem to support the separate identity of the two species (Ramser et al. 1997; Dansi et al. 2000a; Mignouna et al. 2005).

It appears that the process of yam domestication was marked with significant evolutionary changes. Under cultivation, yam is commonly propagated vegetatively by

[1]All abbreviations and acronyms are listed on page -VIII- following list of figures.

the use of either small whole tubers (seed yams) or pieces of large tubers (setts). Sexual reproduction is extremely irregular in cultivated species, the flowering behavior ranging from no flowering to monoecious or dioecious plants, depending on the species and cultivar (Bai and Ekanayake 1998). Even in flowering species or cultivars, seed production is a rare event due to a high degree of flower and ovule abortion (Onwueme 1984).

Yams[2] also exhibit considerable inter and intraspecific variations in ploidy level, which ranges from 2× to 16× based on basic chromosome numbers of either nine or ten (Table 1.1.). For example, three ploidy levels (4×, 6×, 8×) were determined in accessions of *D. rotundata-D. cayenensis* complex from Cameroon (Dansi et al. 2001). Egesi et al. (2002) reported tetraploid and hexaploid plants in accessions of *D. alata* from West Africa. Degras (1993) and Hahn (1995) give detailed review of the chromosomal behavior in yams. In general, intensive vegetative multiplication, reduced fertility and the co-existence of several ploidy levels means that the potential of each clone as well as the relationship between known landraces or cultivars needs to be determined to utilize the available genetic resources in crop improvement programs.

### 1.3. Production status and importance of yams

Yam is a staple food for millions of people in many regions of the tropics including Africa, Asia, the Pacific and Tropical America. It is the fourth most important tuber crop in the world next to potato, cassava and sweet potato (Levand and Shriver 1998, quoted by Mignouna and Dansi 2003). Mean annual production for the period from 1990 to 2005 was estimated at 34 million metric tons, Africa accounting for about 95% of the total output (Table 1.2). Compared to the year 1990, while yield per area nearly remained constant, total production increased by about 88% in 2005. This was mainly brought about by the increase in the total area harvested, which more than doubled over the same period (FAO 2005).

[2]Throughout this thesis, the term ‘yam’ is used to distinguish the crop from the other root and tubers, and ‘yams’ in reference to the various species under genus *Dioscorea*.

**Table 1.1.** The main sections under the genus *Dioscorea* and corresponding cultivated species including their common names, origin and ploidy levels.

| Section | Characteristics | Species | Common Name | Origin | Ploidy |
|---|---|---|---|---|---|
| Enantiophyllum | Vines twining to the right | *D. alata* L. | Water yam; Greater yam; Winged yam | SE Asia | 2n = 20, 30, 40, 50, 60, 70, 80 |
| | | *D. rotundata* Poir. | White Guinea yam; White yam | W Africa | 2n = 40, 80 |
| | | *D. cayenensis* Lam. | Yellow Guinea yam;Yellow yam | W Africa | 2n = 36, 54, 60, 63, 66, 80, 120, 140 |
| | | *D. opposita* Thunb. | Cinnamon yam | China | 2n = 40 |
| | | *D. japonica* Thunb. | Chinese yam | Japan | 2n = 40 |
| | | *D. transversa* R. Br. | | SE Asia | -- |
| Lasiophyton | Vines twining to the left | *D. dumetorum* (Kunth.) Pax | Bitter yam; Trifoliate yam; Cluster yam | Africa | 2n = 36, 40, 45, 54 |
| | | *D. hispida* Dennst. | Asiatic bitter yam | SE Asia India | 2n = 40, 60 |
| Opsophyton | | *D. bulbifera* L. | Aerial yam; potato yam | Africa Tropical-Asia | 2n = 30, 40, 50, 60, 70, 80, 100 |
| Combilium | | *D. esculenta* (Lour.) Burkill | Lesser yam; Asiatic yam | Indochina Oceania | 2n = 30, 40, 60, 90, 100 |
| Macrogynodium | | *D. trifida* L. f. | Cush-cush yam | Tropical-America | 2n = 54, 72, 81 |

**Source**: Coursey 1967; Alexander and Coursey 1969; Purseglove 1972; Rehm and Espig 1991; Degras 1993; Onwueme and Charles 1994; Asiedu et al. 1997.

**Table 1.2.** Mean annual production of yam for the period from 1990 to 2005 (Source: FAO 2005).

| | Area harvested ('000' ha) | Yield (Kg/ha) | Total production ('000' MT) |
|---|---|---|---|
| World | 3,572 | 9,694 | 34,355 |
| Africa | 3,418 | 9,708 | 32,874 |
| Africa (West) | 3,149 | 10,088 | 31,388 |
| Ethiopia[+] | 68 | 4,065 | 277 |

[+]Figures are mean values for the years between 1992 and 2005

The so-called ‘yam belt’ of West Africa, which comprises Cameroon, Nigeria, Benin, Togo, Ghana and Côte d’Ivoire (Hahn 1995), is the principal area of yam production. Within this ’belt’, yam is closely related to socio-cultural life of the inhabitants. For example, for some societies in West Africa, yam is the totem of maleness and also used as a status of wealth based on number, size and diversity of yams offered during feasts, parties and marriage (Hahn et al. 1987). Raynor et al. (1992) described different yam tributes signifying the various events associated with yam harvesting and consumption in Micronesia.

Yam is among the mandated crops of the International Institute of Tropical Agriculture (IITA), which has devoted considerable resources in collecting yam germplasm for purposes of maintenance, crop improvement and distribution on request (Ng 1991). Of about 11,500 accessions of yam collected worldwide, IITA maintains close to 3000 accessions mainly from West Africa (FAO 1996). Yam germplasm from other parts of Africa is hardly represented in the collection. This is the main reason why the status and diversity of yams in other African countries outside the ‘yam belt’ is not known, leading to the perception that yam is only a West African Crop.

### 1.4. Yams in Ethiopia: an overview

Very few reports deal with aspects of yam production and its diversity in Ethiopia. Most of the references on these subjects are often scant and fragmentary. This is the result of research neglect that yam and other traditional crops have been subjected to in the past. Consequently, yams are hardly known to many of the researchers, policy makers and development agents in the country.

Ethiopia is generally considered as ‘an isolated center of yam cultivation’ outside the ‘yam belt’ of West Africa (Norman et al. 1995). Among the first accounts of yams in Ethiopia is the one given by Westphal (1975) who described the various *Dioscorea* species grown in complex farming systems with cereals and other root and tuber crops in Southern, Southwestern and Western parts of the country. Edwards (1991) reported that *Dioscorea* species are widely distributed in Ethiopia, and are one of those crops with wild relatives in the country.

It is widely believed that *D. abyssinica* Hochst. ex Kunth is native to Ethiopia (Coursey 1967; Rehm and Espig 1991), and is currently distributed in the savanna regions of Africa. Nonetheless, little is known about its exact place of origin, production and distribution. In their description of the cultivated and wild yams of Ethiopia, Miége and Demissew (1997) indicated the presence of *D. praehensilis* Benth., which is widely considered as native of West Africa. *D. abyssinica* and *D. praehensilis* are believed to be among the wild species that are ancestors of the cultivated African species (Hahn 1995). These and similar reports contributed a lot in creating awareness and interest about yams in Ethiopia. Nevertheless, the role of yams in the farming systems and local livelihood, their diversity and taxonomic status remain far from clear.

Etissa (1998) reported results from field characterization and evaluation of yam accessions assembled during a collection mission jointly undertaken by Melko Research Center and the then Plant Genetic Resource Center of Ethiopia (PGRC/E) about twenty years before. Although four species could be identified in the collection, several accessions remained unidentified. Gemeda (2000) gave a brief account of the role of yam and other tuber crops in the local livelihood of inhabitants in West Ethiopia. He reported that yam is more productive than the other tuber crops in the area, apparently due to its relative tolerance to drought and termite damage, with an estimated yield of about 20 tons per hectare.

The total annual production of yam in Ethiopia was estimated at about 277,000 metric tons from an area of about 68,000 ha, corresponding to a yield of about 4 tons per hectare (Table 1.2). Although widely referred to, the figures included in the FAO statistics represent a gross underestimation of the production and productivity of yams in Ethiopia compared to those given in some reports (Gemeda 2000). However, this is part of the lack of information on yam, which is also often reflected in national and regional statistics. The recent study by Hildebrand et al. (2002) has been a significant contribution towards exploring the status and potential of yams in Ethiopia. The study describes the traditional knowledge and diversity of yams in Sheko (a remote area in the Southwestern edge of Ethiopia), with emphasis on the role and potentials of the crop in local livelihood and priorities for conservation.

## 1.5. Genetic diversity and its importance

Genetic diversity refers to the amount of genetic variability among individuals of a variety, population or species (Brown 1983), and provides the basis for adaptation to changing environmental conditions and for developing new varieties. This variation can be expressed in differences in morphological characters, physiological properties, biochemical characteristics, or in DNA sequence (Ramanatha and Hodgkin 2002). Consequently, characterization and evaluation of germplasm involves measuring one or a combination of these characters or properties.

Over the last couple of decades, increased studies into aspects of genetic diversity have improved our understanding of the extent and distribution of the diversity present in crops and their wild relatives. In most parts, these studies were triggered by concerns over the loss of valuable genetic resources (Frankel 1974; Harlan 1975) following the introduction of modern crop varieties into centers of crop domestication and diversity (Harlan 1970). This has greatly facilitated the implementation of conservation strategies, both *ex situ* and *in situ,* for the major staple crops (Brush 2000; Scarascia-Mugnozza and Perrino 2002). Nevertheless, many food species can be considered as 'minor', 'underutilized' or 'neglected' (Padulosi et al. 2002) and their status and potential remain mostly unknown (FAO 1996). On the other hand, these crops have national or regional significance as staples, in food supply during certain periods, or for nutritionally balanced diet (Hammer et al. 2001).

It is widely recognized that traditional agro-ecosystems maintain considerable diversity of plants (Bellon and Brush 1994; Brush 2000; Kehlenbeck and Maass 2004) and sustain dynamic evolutionary processes that created this diversity. They also preserve human knowledge that shaped diversity for generations (Bellon 1991). Important elements of crop evolution, thus, are genetic diversity, farmers' knowledge and selection, and exchange of crop varieties (Brush 2000). Individual farmers value diversity in their crops due to heterogeneous environmental and production conditions, risk factors, market demands and requirements as related to the utilization of different products (Bellon 1996). This is often reflected in their decision to grow and maintain diverse crop species and cultivars of the same species. Such human preference and

management have influenced diversity at species and infraspecific levels (Jain 2000) leading to the creation of landraces or traditional varieties.

The concept of a landrace is complex (Zeven 1998). Landraces are often considered as integrated and adapted populations and, more importantly, genetically variable (Harlan 1975). They are also crop populations in balance with their environment, stable over a long period of time and, yet, have a potential for adaptive changes (Frankel and Bennett 1970). Despite the difficulty in defining the term, it is well documented that the diversity present in landraces is very important both in terms of providing the food used by millions throughout the world and as raw materials for breeding modern cultivars (Wood and Lenné 1997).

In general, farmers' decisions and management activities play a central role in determining the availability, composition, distribution and relative abundance of crop species or cultivars in a given agro-ecosystem. This event, referred to as "planned diversity" (Matson et al. 1997), is important both in terms of crop production and in shaping the total biodiversity of an area. It is, therefore, imperative that attempts to study crop diversity in traditional agriculture take into account the role traditional farmers play in creating and managing diversity: an aspect that has been overlooked by many of the endeavors in the field (Thurston et al. 1999; Jain 2000).

### 1.6. Rationale of the study

Although Ethiopia is the center of origin and diversity of a considerable number of crop species (Vavilov and Chester 1951; Harlan 1969; Zohary 1970; Engels et al. 1991), most research works, and subsequent improvement and conservation programs have so far focused mainly on cereals. However, different root and tuber crops, such as enset (*Ensete ventricosum* (Welw.) Cheesman), Oromo potato or *'oromo dinich'* (*Plectranthus edulis* (Vatke.) Agnew), and anchote (*Coccinia abyssinica* (Lam.) Cogn.) were domesticated in Ethiopia. Others such as yam, although believed to have been domesticated elsewhere, developed immense variation in the country (IBCR 2000).

Most of the root and tuber crops did not get a fair share of attention by researchers and policy makers. The curriculum of agricultural colleges and universities also failed to

give appropriate coverage to these crops. This had, and still has, a multiplier effect, as the graduates became researchers, policy makers and extension agents who mainly promote cereals. On the other hand, recent studies on the more traditional crops such as enset have highlighted the extent of existing diversity, significance in farming systems and local livelihood, as well as potentials for improvement (Tsegaye and Struik 2002; Tesfaye and Lüdders 2003).

Little is known about the diversity of yams in Ethiopia and the taxonomic status of most of the species, particularly under cultivation. The scant information available shows that ranges of factors threaten yam production and the available landraces. For example, in Wolayita, one of the major yam-growing areas in Southern Ethiopia, yam production is on the decrease in many localities due to erratic rainfall, shortage of land brought about by increasing population, declining soil fertility, shortage of planting materials and lack of staking materials following shrinking forest areas (Tamiru et al. 2005).

There is a need to broaden the knowledge base of the crop through studies on diversity and use of the available landraces. Detailed analysis of the extent and distribution of the diversity available in yams, and a good understanding of farmers' perceptions and management of local landraces, including criteria for selection and classification, are important in designing conservation and improvement programs.

## 1.7. Objectives of the study

The main objective of the study is to characterize the extent and distribution of yam diversity in the major yam-growing areas of Southern Ethiopia. Its ultimate goal is to provide the basic information needed for future research and development activities. This study may play a significant role in bringing yam to the attention of researchers and policy makers. The research included the following components:

- Assessment of farm-level diversity and distribution of yam landraces, and local management and use of the available diversity;
- Agro-morphological characterization of yam landraces and description of the local classification system;

- Molecular marker-based study of the genetic diversity in yam accessions from Ethiopia, and their relatedness to known genotypes from other African countries with an effort to establish the species identity of local materials; and
- Characterizing the diversity in quality (compositional and functional properties) of yam tubers.

## 1.8. Thesis outline

This introductory chapter will be followed by Chapter 2 describing farm-level diversity of yam landraces in Wolayita and Gamo-Gofa zones, the major yam production areas in Southern Ethiopia. An attempt is made to describe the total number of landraces grown, their abundance and distribution across the districts covered by the study, and assess farmers' management and use of local landraces. Chapter 3 deals with agro-morphological characterization of yam germplasm assembled from different localities in Southern Ethiopia. Results of the morphological characterization are further compared with farmers' classification of yam landraces.

In Chapter 4, Amplified Fragment Length Polymorphic (AFLP) DNA markers are utilized for analysis of genetic diversity in selected yam germplasm from Ethiopia. Furthermore, the diversity in yams from Ethiopia is compared with elite yam genotypes representing the main cultivated *Dioscorea* species obtained from IITA. The diversity in the main tuber constituents and pasting properties is presented in Chapter 5.

In Chapter 6, the main findings of the study are highlighted. Recommendations for future activities, for instance the significance of further investigations into indigenous knowledge of yam in designing improvement and conservation programs, and identifying the species identity of the available landraces through studies on phylogenetic relationships and ploidy levels, are also given.

# 2. Diversity, Distribution and Management of Yam Landraces (*Dioscorea* spp.) in Southern Ethiopia

**Abstract**

*A survey covering 339 farm households and eight districts was conducted in two zones of Southern Ethiopia with the main objective of investigating the diversity and distribution of yam landraces. Methods of data collection included individual interviews using structured and semi-structured questionnaires. A total of 37 named landraces were recorded on-farm. The number of landraces maintained on individual farms ranged from one to six (mean 2.9), and farmers' decision regarding type and number of landraces to plant was influenced by environmental factors, maturity time and market demand. Most of the landraces described had limited distribution and abundance, and only a few dominant landraces were widely grown. There was a considerable variation amongst the districts with respect to diversity, distribution and abundance of the landraces. However, further studies on the local yam classification system, and morphological and molecular marker based analysis of genetic diversity are required to determine the extent and distribution of diversity in these named landraces.*

**Keywords:** *Dioscorea*; Ethiopia; genetic resources; landrace diversity; indigenous knowledge; Yam

## 2.1. Introduction

Yam (*Dioscorea* spp.) is a crop of major economic and cultural importance in sub-Saharan Africa, which accounts for about 95% of the world production (FAO 2005). The so called 'yam zone' or 'yam belt' of West Africa is the principal area of yam production (Coursey 1967; Hahn et al. 1987). Following the establishment of research institutions such as the International Institute of Tropical Agriculture (IITA), yam has attracted much research attention in recent decades. Consequently, progress has been made in understanding the origin, domestication, phylogeny, diversity and production of the major food species. Orkwor et al. (1998) give a review of the recent advances in

yam research. However, the study so far concentrated in the 'yam zone' and, as a result, little is known about the status of yams in the other parts of Africa. This has led to the perception that yam is an important food crop only in parts of West Africa, a view that triggered concerns decades ago but still is largely valid (Ayensu and Coursey 1972; Quin 1998).

Yams in Ethiopia are hardly known to the scientific community, even within the country. The country is only referred to as an isolated center of yam cultivation (Norman et al. 1995), where a number of *Dioscorea* species are grown in complex cropping systems with cereals and other root and tuber crops (Westphal 1975). There has been no systematic study on the diversity, production and use of the crop. Although brief and passing remarks are available in the more general references (Westphal 1975; Engels et al. 1991), most of these materials contain only lists of one or a few of the yam species found in the country.

The recent study by Hildebrand et al. (2002) has been a significant contribution towards exploring the status and potential of yam in Ethiopia. The study reports the local knowledge and diversity status of yam in Sheko (a remote area in the Southwestern edge of Ethiopia), with emphasis on the role and potentials of the crop in local subsistence and priorities for conservation. Miége and Demissew (1997) described eleven *Dioscorea* species, both wild and cultivated, found in the country. These reports indicate that yam is widely distributed in Ethiopia, and is amongst the main root and tuber crops grown by subsistence farmers in the Southern, Southwestern and Western parts of the country. Nevertheless, the extent and distribution of the available inter and intraspecific diversity, particularly under cultivation, is poorly investigated.

In his classification of the major farming systems in Ethiopia, Westphal (1975) described that yam is grown as a co-staple with enset, cereals and other root and tuber crops in Wolayita and the neighboring Gamo-Gofa zone. Subsistent agriculture is the main stay of the local people in these zones, and is constrained by factors such as small landholdings. Yam is one of the traditional crops that have long been cultivated in the area, and is widely adapted to conditions of local agriculture. The extensive production of yam in Wolayita and Gamo-Gofa, its role local subsistence and the existing local knowledge of the crop make the area ideal for diversity studies. It also enables

integration of such studies into the role of indigenous knowledge in the maintenance of local landraces.

In situations where documented data are hardly available, the local farmer is the first source of information to initiate diversity studies. Farmers' perception of local varieties is of utmost attention because it is not only the unit of diversity they recognize but also the unit they actually manage and conserve (Hoogendijk and Williams 2002). The main objective of the present study is, therefore, to investigate farm level diversity and distribution of yam landraces in Wolayita and Gamo-Gofa zones, the major yam production areas in Southern Ethiopia, and to describe how the different landraces are selected, managed and utilized by local farmers.

## 2.2. Materials and methods

### 2.2.1. The study area

The study area is located approximately between latitudes 6°46′ and 7°26′ N, and longitudes 37°01′ and 38°08′ E in the Southern Nations, Nationalities and Peoples' Regional State (SNNPRS) of Ethiopia (Figure 2.1.). Included in the study are Wolayita zone and Kucha district from the neighboring Gamo-Gofa zone (Table 2.1). Wolayita zone is composed of 7 districts and 273 peasant associations (PAs), the lowest administrative unit in Ethiopia. The zone is one of the most densely populated areas in the country with an estimated area of about 4,500 $km^2$ inhabited by around 1.5 million people. This corresponds to an average density of 355 people per $km^2$, which ranges from 141 to 629 people per $km^2$ in Humbo and Damot-Gale districts, respectively (CSA 2000). The district of Kucha, with an estimated area of 1384 $km^2$, was included in the study to consider the distribution of yam landraces beyond Wolayita. Elderly Wolayita farmers credit their neighbors in Gamo-Gofa for introducing yam and its culture into their area.

### 2.2.2. Sampling and data collection

A household-level survey covering the eight districts was conducted from October 2003 to September 2004. A stratified sampling procedure was followed to define the

sampling unit. The area was first stratified in terms of geographic distance and elevation to cover the approximate ecological range of yam so that valid generalizations can be drawn from the findings. Then, 4 to 6 peasant associations (PAs) were selected from each district as the major yam growing areas. Selection of the PAs was made in consultation with district agricultural officers and key informants knowledgeable in the area. Ten households were randomly selected from each PA, bringing the total number of PAs and households covered by the study to 34 and 339, respectively (Table 2.1).

Data were collected through individual interviews with member(s) in each household responsible for management of yam fields, using structured and semi-structured questionnaires. The semi-structured questionnaire was included to enable full consideration of the open-ended questions such as how farmers evaluate and identify the different landraces. Most of the respondents were men even though women farmers were also interviewed in places where they were head of the family or responsible for yam production. Because yam is a crop of much economic and social significance and involves a laborious production system, it is generally considered a man's crop.

Number of landraces per farm was recorded on farm where each farmer was asked to distinguish, name and describe the different landraces he/she was growing. This was conducted during the time of the year when yam plants were still growing in the field to aid identification of the different morphotypes. Data were also recorded on elevation, total farm size, size of land occupied by yams, main problems limiting yam production, uses of the landraces and cultivation practices. Moreover, farmers were asked to verbally report names of landraces they knew and/or heard about besides the ones they were currently growing on their farms.

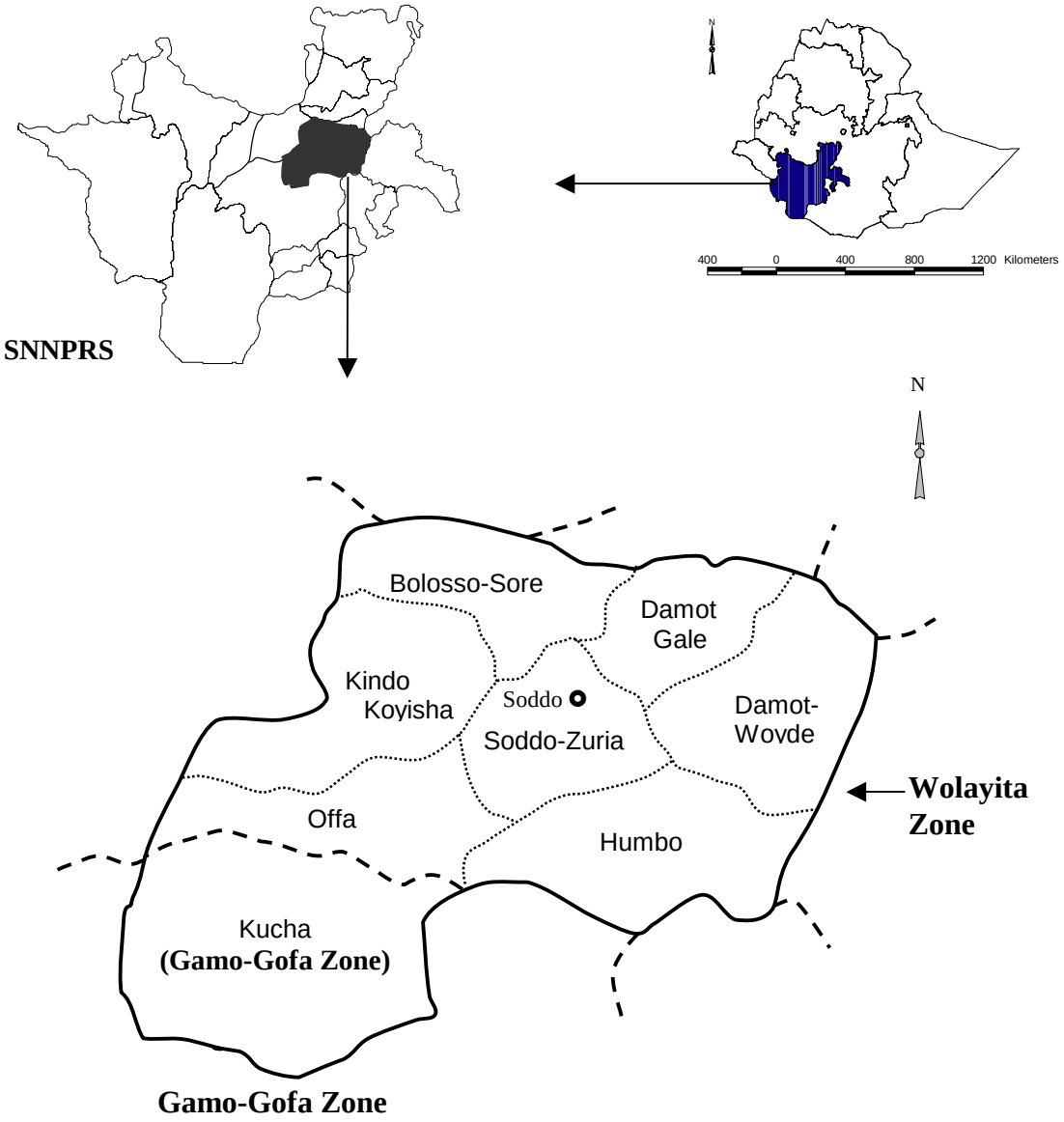



**Figure 2.1.** Location of the study area in Southern Ethiopia, indicating administrative districts and the administrative capital of Wolayita zone, Soddo.

### 2.2.3. Data analysis

For the purpose of this research, a landrace refers to a morphologically distinct population of yam that farmers recognize, name and manage as different. Accordingly, list of all the landraces described throughout the study area was summarized after grouping known synonyms or names that refer to the same landrace in different localities with the help of elderly farmers. Then, the number of different landraces

recorded in each district, without accounting for the number of farms where the landraces were found, was considered as richness of the district.

As measures of diversity that take into account the proportional abundance of landraces (richness and evenness), Simpson and Shannon diversity indices were calculated for all the districts. Simpson's index (D) basically measures the probability that two individuals randomly selected from a sample belong to the same category (Simpson 1949) and, hence, as D increases, diversity decreases. It was, therefore, transformed as 1-D with values ranging from 0 to 1. The index was computed for all the districts using the function:

$$\textit{Simpson's Diversity Index}\ (1-D) = 1 - \sum (n/N)^2$$
$$= \sum \left( \frac{n(n-1)}{N(N-1)} \right)$$

Where, n = the number of farms where a landrace was found, and
N = the total number of farms surveyed in the district.

Shannon diversity index (H´), also referred to as Shannon-Weaver diversity index, takes into account both number and evenness of categories considered, and can be increased either by greater evenness or more unique species or landraces in our case. The index is defined as:

$$\textit{Shanon Diversity Index}\ (H') = -\sum_{i=1}^{S} p_i \ln p_i$$

Where, s = the number of landraces, and
$p$ = proportion of landrace $i$ relative to the total number of landraces (n/N).

Although Shannon's index takes into account evenness of the abundance of landraces, evenness can be calculated separately as a measurer of the observed diversity to the maximum diversity. It is defined by the function E = H´/*ln* S, where H´ is Shannon index and S refers to the number of landraces described in each district. High evenness resulting from all landraces having equal abundance is normally equated with high diversity (Magurran 1988).

Differentiation or beta (β) diversity (Magurran 1988) estimates how different or similar are habitats in terms of diversity of categories under consideration. This can be achieved by employing similarity measures of pair of sites, as is the case with Sørenson's similarity index. In this study, the index was computed based on the presence or absence of landraces (qualitative data) to estimate landrace similarity between pairs of districts as follows:

$$Sørenson's\ Similarity\ Index = \frac{2c}{(a+b)}$$

Where, c = the number of landraces common to both districts,
a = the number of landraces in district A, and
b = the number of landraces in district B.

Frequency distributions, descriptive statistics, correlations and all other relevant data analyses were carried out with the help of the SPSS statistical software (SPSS 12.0.1, SPSS Inc. 2003).

**Table 2.1.** Description of the districts included in the study of Wolayita and Gamo-Gofa zones, Southern Ethiopia.

| District | Zone | Elevation (m asl.) | | Mean population density per km$^{2+}$ | No. of PAs$^{++}$ surveyed | No. of households interviewed |
|---|---|---|---|---|---|---|
| | | Range | Mean | | | |
| Bolosso-Sore | Wolayita | 1830-1980 | 1877 | 491 | 4 | 40 |
| Damot-Gale | Wolayita | 1765-2200 | 1986 | 629 | 4 | 42 |
| Damot-Woyde | Wolayita | 1777-2220 | 1901 | 236 | 6 | 56 |
| Humbo | Wolayita | 1600-1832 | 1774 | 141 | 4 | 42 |
| Kindo-Koyisha | Wolayita | 1660-1730 | 1694 | 224 | 4 | 39 |
| Offa | Wolayita | 1600-1950 | 1685 | 234 | 4 | 40 |
| Soddo-Zuria | Wolayita | 1850-1950 | 1885 | 528 | 4 | 40 |
| Kucha | Gamo-Gofa | 1690-2100 | 1866 | 91 | 4 | 40 |
| **Total** | | | | | 34 | 339 |

$^{+}$ Data source CSA 2000; $^{++}$Peasant Associations

## 2.3. Results

### 2.3.1. Landrace diversity

Overall, local farmers described 37 recognized yam landraces on farm (Table 2.2). Two of these landraces (*bola-boye* and *bunde-buchi*) belong to a well-defined species of aerial yam (*D. bulbifera*), and are apparently identified based on variation in shape and size of bulbils (aerial tubers). However, these characters do not seem to provide a reliable means for identification, as they tend to vary within a landrace or even among bulbils of a single plant. The remaining landraces form a yet unidentified species or group of species. Most of these landraces (70%) are early-maturing types, and are harvested twice (double-harvested). The remaining 30% mature late (9-12 months) and, thus, are harvested only once. Besides, wild yam, widely referred to by the name *sasa*, was encountered in some localities where forest patches still exist.

The number of landraces recorded on individual farms ranged from one to six with a mean and standard deviation of 2.9 and 1.1, respectively. The variation among districts with respect to number of landraces per farm across the farms visited is summarized in Table 2.3. A relatively high number of farms with four or more landraces were found in Kindo-Koyisha, Offa and Kucha districts. As indicated in Table 2.1, most of the farms visited in these districts were located at relatively lower elevations.

The total number of landraces recorded in each district (richness) varied from 8 at Damot-Woyde to 14 at Soddo-Zuria and Damot-Gale districts with a mean and standard deviation of 11.0 and 2.1, respectively (Table 2.4). Both Simpson and Shannon diversity indices revealed that Bolosso-Sore and Damot-Gale were the most diverse districts, while Damot-Woyde was the least diverse. As expected, Shannon diversity index was significantly correlated with landrace number ($r = 0.69$) and number of unique landraces ($r = 0.70$). A similar relationship was observed between Simpson index of diversity and number of total ($r = 0.60$) and unique ($r = 0.62$) landraces.

**Table 2.2.** Yam landraces recorded in the various districts of Wolayita and Gamo-Gofa zones and number of farms where they were encountered.

| | Districts | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Landrace | Bolosso-Sore | Damot-Gale | Damot-Woyde | Kido Humbo | Kido Koyisha | Kido Kucha | Kido Offa | Soddo-Zuria |
| Afra[d] | - | - | - | - | - | - | 2 | 1 |
| Arkiya[d] | - | 7 | - | - | - | - | - | - |
| Ayino or Ayina[s] | 9 | 8 | - | 4 | 1 | 6 | - | 1 |
| Banchuwa[d] | - | - | - | 2 | - | - | - | - |
| Barcha or Barchya[d] | - | - | - | - | 1 | - | 5 | - |
| Barchahuwa[d] | - | - | - | - | - | - | 1 | - |
| Bola-boye[a] | - | - | - | - | - | 1 | - | - |
| Bota-boye[d] | 1 | - | - | - | - | - | - | 3 |
| Buha, Buhe[d] | - | - | - | - | - | 1 | 1 | - |
| Buluwa[d] | - | - | - | - | - | - | - | 1 |
| Buna, Bune, or Buniya[d] | - | - | - | 1 | - | 23 | 8 | - |
| Bunde-buchi[a] | - | - | - | - | - | 2 | - | 1 |
| Chamia[s] | - | 1 | - | - | - | - | - | - |
| Chawula[s] | - | 1 | - | - | - | - | - | - |
| Chichiya[d] | - | 1 | - | - | - | - | - | - |
| Fara, Fura[d] | - | - | - | - | 14 | - | 1 | 4 |
| Gajela[s] | 2 | 20 | - | - | - | - | - | - |
| Gasa[d] | 3 | 1 | 4 | - | 2 | - | - | - |
| Gena[d] | 28 | - | 1 | 2 | 35 | 3 | 12 | 1 |
| Hatiye or Hatiya[d] | 25 | 22 | 53 | 40 | 38 | 40 | 40 | 35 |
| Lohuwa[d] | - | - | - | - | - | - | - | 1 |
| Macha[d] | - | - | - | 1 | - | - | - | - |
| Maleho or Malehuwa[d] | - | - | - | - | - | 3 | 4 | - |
| Martabo[d] | - | 1 | - | - | - | - | - | - |
| Molcha[d] | - | 1 | - | - | - | - | - | - |
| Mortawa or Mortabuwa[s] | 3 | - | - | - | - | - | - | - |
| Natra[d] | - | - | 3 | 1 | - | - | - | 1 |
| Olama or Alama[d] | - | - | - | - | - | - | - | 2 |
| Ochie[d] | - | - | - | - | - | 1 | - | - |
| Oha[d] | 11 | 23 | 56 | 23 | 7 | 15 | - | 26 |
| Sasa[s,w] | - | - | - | - | 2 | - | - | - |
| Suyitiya[d] | - | 10 | - | - | - | - | - | 1 |
| Wadala[s] | 12 | 7 | 31 | 33 | 38 | 40 | 37 | 29 |
| Welluwa[d] | - | - | - | - | - | - | 1 | - |
| Wolabua, Walabo, or Walabuwo[s] | 11 | 5 | 2 | - | - | - | - | - |
| Woyicha[s] | 13 | - | - | - | - | - | - | - |
| Zorewuwa[d] | - | - | 1 | - | 8 | - | - | - |

[d]double harvested; [s]single harvested; [a]aerial yam; [s,w]single harvested and wild

**Table 2.3.** Variation in the number of landraces planted per farm across the districts of Wolayita and in Kucha district of Gamo-Gofa zone.

| No. of landraces per farm | Number of farms | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Bolosso -Sore | Damot Gale | Damot Woyde | Kucha | Humbo | Kindo Koyisha | Offa | Soddo Zuria | Total |
| 1 | 2 | 13 | 0 | 0 | 4 | 0 | 3 | 7 | 29 |
| 2 | 11 | 8 | 11 | 10 | 15 | 2 | 22 | 11 | 100 |
| 3 | 14 | 10 | 32 | 14 | 19 | 20 | 3 | 14 | 126 |
| 4 | 13 | 7 | 2 | 9 | 4 | 4 | 4 | 4 | 47 |
| 5 | 0 | 3 | 1 | 5 | 0 | 12 | 5 | 4 | 33 |
| 6 | 0 | 1 | 0 | 2 | 0 | 1 | 6 | 0 | 4 |
| **Total** | 40 | 42 | 56 | 40 | 42 | 39 | 40 | 40 | 339 |

**Table 2.4.** Yam landrace diversity in the various districts of Woalyita and Gamo-Gofa zones, Southern Ethiopia, expressed as richness, Simpson (1-D) and Shannon (H′) diversity indices, and Evenness.

| District | Richness | % of the total[+] | No. of unique landraces | 1-D | H' | Evenness |
|---|---|---|---|---|---|---|
| Bolosso-Sore | 11 | 29.7 | 2 | 0.85 | 2.08 | 0.87 |
| Damot-Gale | 14 | 37.8 | 6 | 0.85 | 2.14 | 0.81 |
| Damot-Woyde | 8 | 21.6 | 0 | 0.70 | 1.36 | 0.65 |
| Humbo | 9 | 24.3 | 2 | 0.72 | 1.46 | 0.67 |
| Kido-Koyisha | 10 | 27.0 | 1 | 0.79 | 1.76 | 0.76 |
| Offa | 11 | 29.7 | 2 | 0.74 | 1.66 | 0.69 |
| Soddo-Zuria | 14 | 37.8 | 3 | 0.76 | 1.71 | 0.65 |
| Kucha | 11 | 29.7 | 2 | 0.78 | 1.75 | 0.73 |

[+]Calculated on the basis of the 37 landraces described throughout the study area

Although Damot-Gale and Soddo-Zuria were similar in terms of richness, the latter is less diverse partly due to the relatively lower number of unique landraces. The difference between the two districts could also be due to the variation in the abundance of the landraces, which was also reflected in their respective values for evenness. The lowest number of landraces, none of which was unique, represented the least diverse district of Damot-Woyde.

To explore the similarity between districts, Sørenson's similarity index was calculated for all possible pairs of districts, and a similarity matrix was constructed (Table 2.5). Overall, the similarity between two districts varied from 0.16 to 0.67. Damot-Woyde and Kindo-Koyisha were the most similar districts, followed by Damot-Woyde and Bolosso-Sore, and Humbo and Kucha. On the other hand, the most dissimilar districts were Damot-Gale and Offa, Bolosso-Sore and Offa, Damot-Gale and Kucha, and Damot-Woyde and Offa in ascending order of similarity. The result largely reflected the geographic distance between the districts, especially between the dissimilar ones. However, the relationship did not always follow the same general trend, as the most similar districts of Damot-Woyde and Kindo-Koyisha were also among those located farther apart.

### 2.3.2. Distribution and abundance of landraces

There were considerable differences among the landraces with respect to their distribution across the districts covered by this study (Figure 2.2). Eighteen (49%) landraces had a narrow distribution and were specific to a single district. The remaining 21 (51%) were recorded in more than one district. But only two (5%) were ubiquitous, being found in all the districts surveyed. These were the early-maturing *hatiye* (*hatiya*) and the late-maturing *wadala*. The other widespread landraces included *oha, gena, ayino* (*ayina*) and *gasa*.

A similar trend was observed with regard to the abundance (proportion of farms where the landraces were found) of individual landraces. *Hatiye* and *wadala* were the most abundant landraces as they were recorded on 86% and 67% of the farms surveyed, respectively (Figure 2.3). Most of the landraces (70%) were encountered on farms of less than 3% of the farms surveyed. Furthermore, 12 (32%) landraces were recorded on a single farm. As indicated in Table 2.1, landrace abundance also varied across the districts surveyed. Few landraces were well represented in some districts, but virtually missing from the others. For example, *gajela* was encountered on more than 45% of the farms visited in Damot-Gale. Outside this district, it was only found in Bolosso-Sore with a very low abundance. The same was true for *walabua* (*walabo*) and *woyicha* in Bolosso-Sore, *buna* (*bune*) in Kucha and Offa, and *fara* (*fura*) and *zoreuwa*

in Kindo-Koyisha. In general, there was a significant correlation between distribution and abundance of the landraces (r=0.85, $P < 0.01$).

**Table 2.5.** Sørenson similarity estimates of yam diversity between the different districts in Wolayita and Gamo-Gofa zones of Southern Ethiopia on the basis of presence and absence of landraces.

| | Bolosso-Sore | Damot-Gale | Damot-Woyde | Humbo | Kindo-Koyisha | Kucha | Offa | Soddo-Zuria |
|---|---|---|---|---|---|---|---|---|
| Bolosso-Sore | 1.00 | | | | | | | |
| Damot-Gale | 0.56 | 1.00 | | | | | | |
| Damot-Woyde | 0.63 | 0.45 | 1.00 | | | | | |
| Humbo | 0.50 | 0.35 | 0.59 | 1.00 | | | | |
| Kindo-Koyisha | 0.57 | 0.42 | 0.67 | 0.53 | 1.00 | | | |
| Kucha | 0.45 | 0.32 | 0.42 | 0.60 | 0.48 | 1.00 | | |
| Offa | 0.27 | 0.16 | 0.32 | 0.40 | 0.48 | 0.55 | 1.00 | |
| Soddo-Zuria | 0.48 | 0.36 | 0.45 | 0.52 | 0.50 | 0.48 | 0.40 | 1.00 |

The overall distribution of landraces throughout the study area and in two selected districts is summarized in the abundance and frequency matrix given in Figure 2.4. Most of the landraces described in this study were local (found in limited districts) and rare (encountered on a limited number of farms in each district) (Figure 2.4a). The trend in the least diverse district of Damot-Woyde was similar to that for the overall study area (Figure 2.4b). The landraces described in this district were either local and rare (63%) or widespread and common (37%). In the most diverse district of Damot-Gale, the majority of the landraces were fairly distributed with a relatively lower but comparable abundance (Figure 2.4c). This was reflected in the relatively higher evenness of landrace abundance recorded in Damot-Gale (Table 2.2).

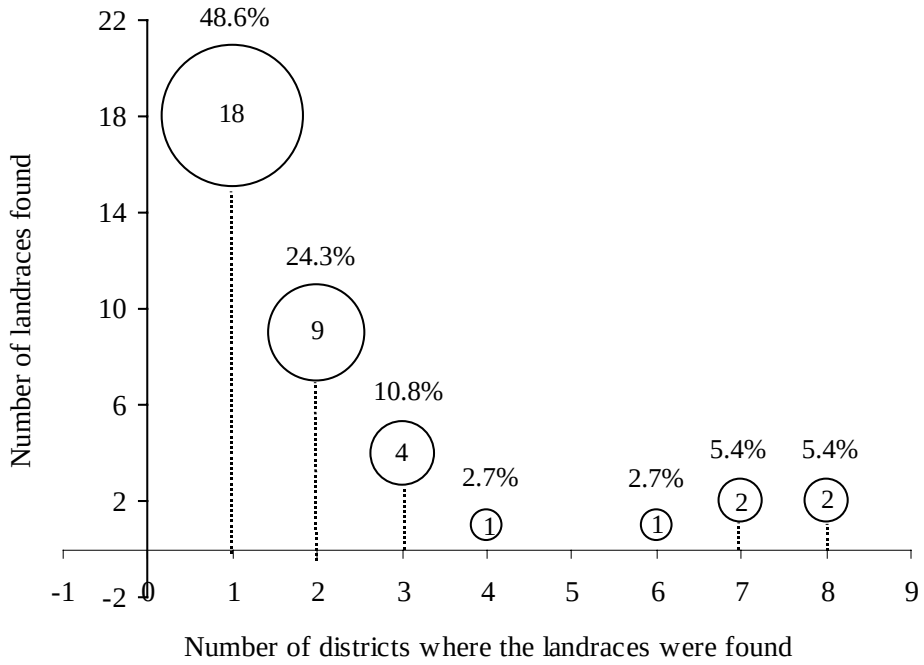



**Figure 2.2**. Distribution range of yam landraces across the districts surveyed in Wolayita and Gamo-Gofa zones of Southern Ethiopia.

The variation among districts with respect to distribution and abundance of landraces was also evident from the number of farms visited and corresponding number of landraces recorded. For example, for the whole study area, it required visits to 112 (33%) farms to capture 51% of the landraces, whereas visits to 278 (82%) farms were enough to record all the landraces (Figure 2.5a). In the least diverse district of Damot-Woyde, 50% of the landraces were already listed after visits to the first 3 (5%) farms, but it took visits to 48 (91%) farms to capture all the landraces described in the district (Figure 2.5b). In Damot-Gale, visits to about 15 (36%) and 37(90%) farms were required to record 50% and 100% of the landraces encountered in the district, respectively (Figure 2.5c).

In addition to those described on their farms, farmers verbally reported some vernacular names of landraces that were no longer found in their community and thought to be lost. Altogether, 46 of such vernacular names were reported throughout the study area. About 59% (25) of these names correspond to those landraces encountered on farms of the other households visited. The widely distributed landraces, such as *hatiye, wadala, oha, gena* and *gassa* were also among those frequently reported verbally. The

remaining 19 vernacular names (41%) were new in the sense that they were never encountered on farmers' fields during the survey. These additional landraces were mostly reported by a single or two and, at most, by six (about 2%) of the households interviewed.

### 2.3.3. Determinants of diversity

The distribution pattern of yam landraces revealed that the type and number of landraces grown by individual farmers were influenced by elevation. The number of landraces grown per farm was negatively correlated ($r = -0.4$; $P < 0.05$) with elevation (Figure 2.6). Although few farms were visited at lower elevations, our observation in most localities was that the number of landraces per farm was generally lower at elevations less than 1700 m asl. Here, yam farms were mostly composed of one or two landraces like *wadala* that are perceived to be tolerant to drought and higher temperatures. Number of landraces per farm was generally higher between 1700 and 1900 m asl. Within this elevation range, farmers have the opportunity to include other landraces that may be less tolerant to drought but permit extended harvesting and generate additional incomes due to their early maturity and high market demand. Number of landraces per farm decreased steadily above 1900 m asl. Although the rainfall amount and distribution at higher elevations is ideal for cultivation of both yam types, farmers mostly prefer the early-maturing landraces, such as *hatiye* and *oha* because of their high market demand.

There was no significant correlation between farm size and number of landraces per farm. Whereas, proportion of the land allocated for yam production was negatively correlated with total farm size (data not shown). This indicates that even those farmers with smaller landholdings allocate a significant share of their land for yam cultivation in order to get a reasonable production and meet family needs.

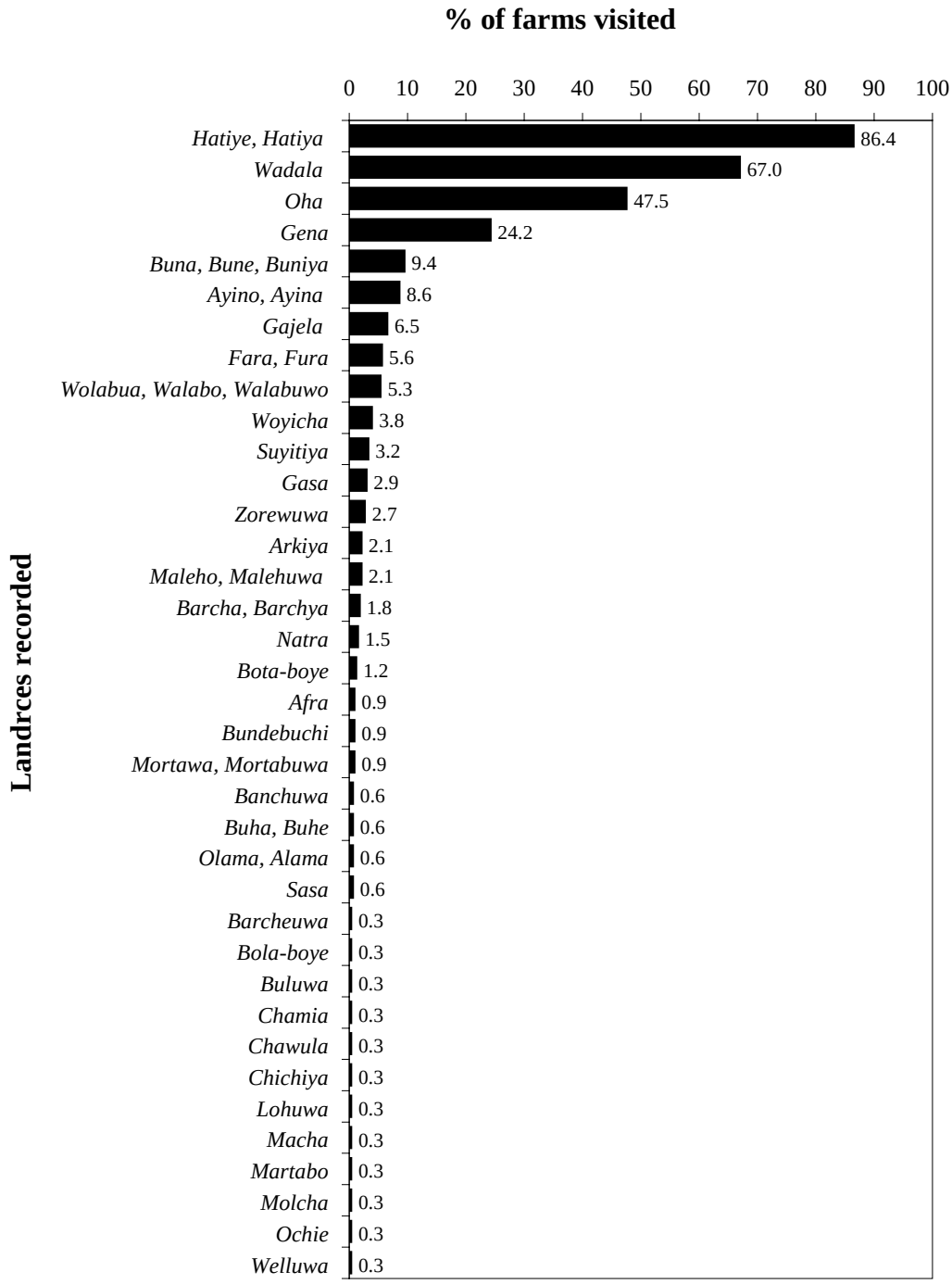



**Figure 2.3.** The relative abundance of yam landraces recorded throughout Wolayita and Gamo-Gofa zones of Southern Ethiopia.

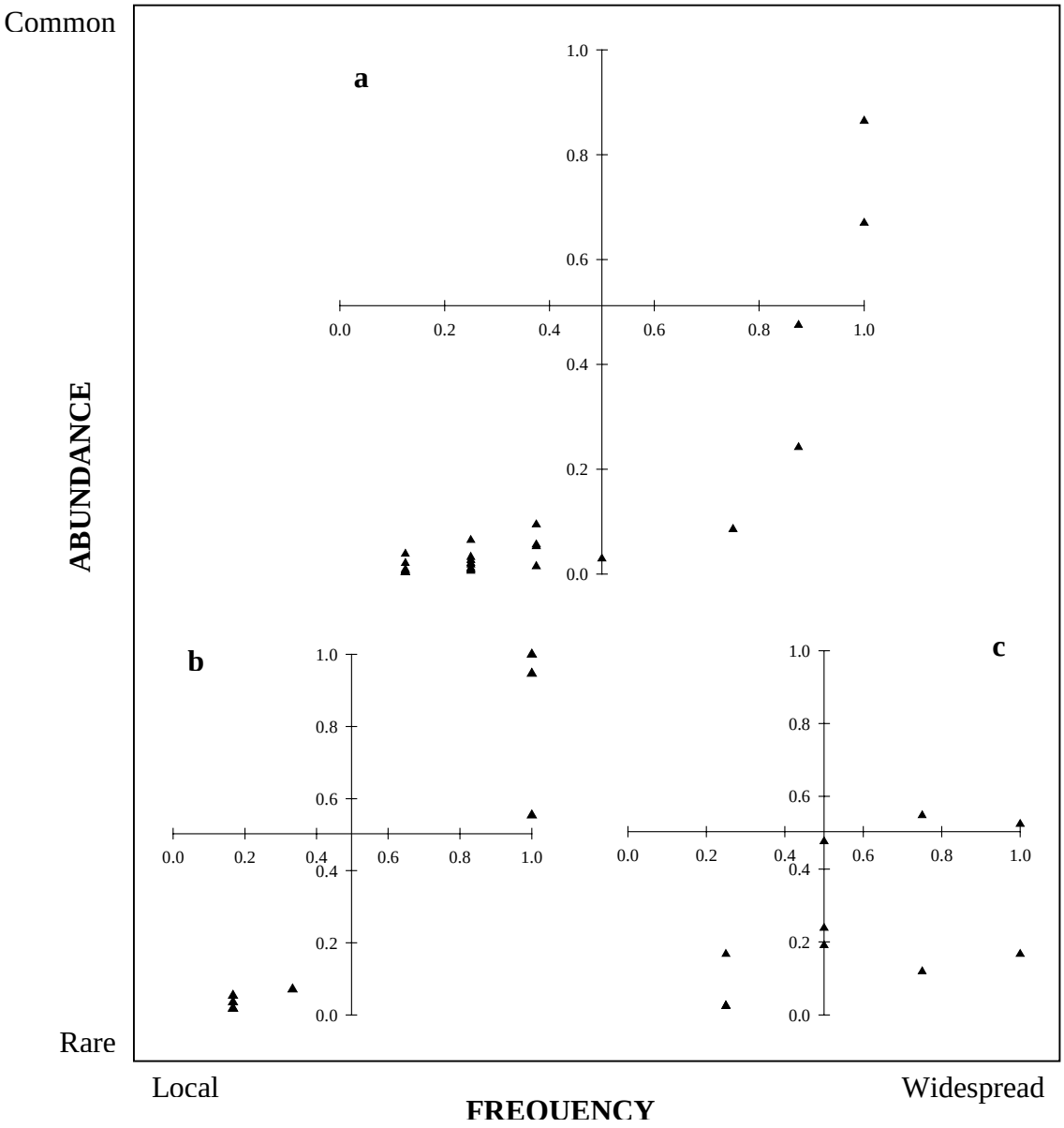



**Figure 2.4.** Frequency and abundance matrix of yam landraces found throughout the study area (a), in the least diverse district of Damot-Woyde (b) and most diverse district Damot-Gale (c).

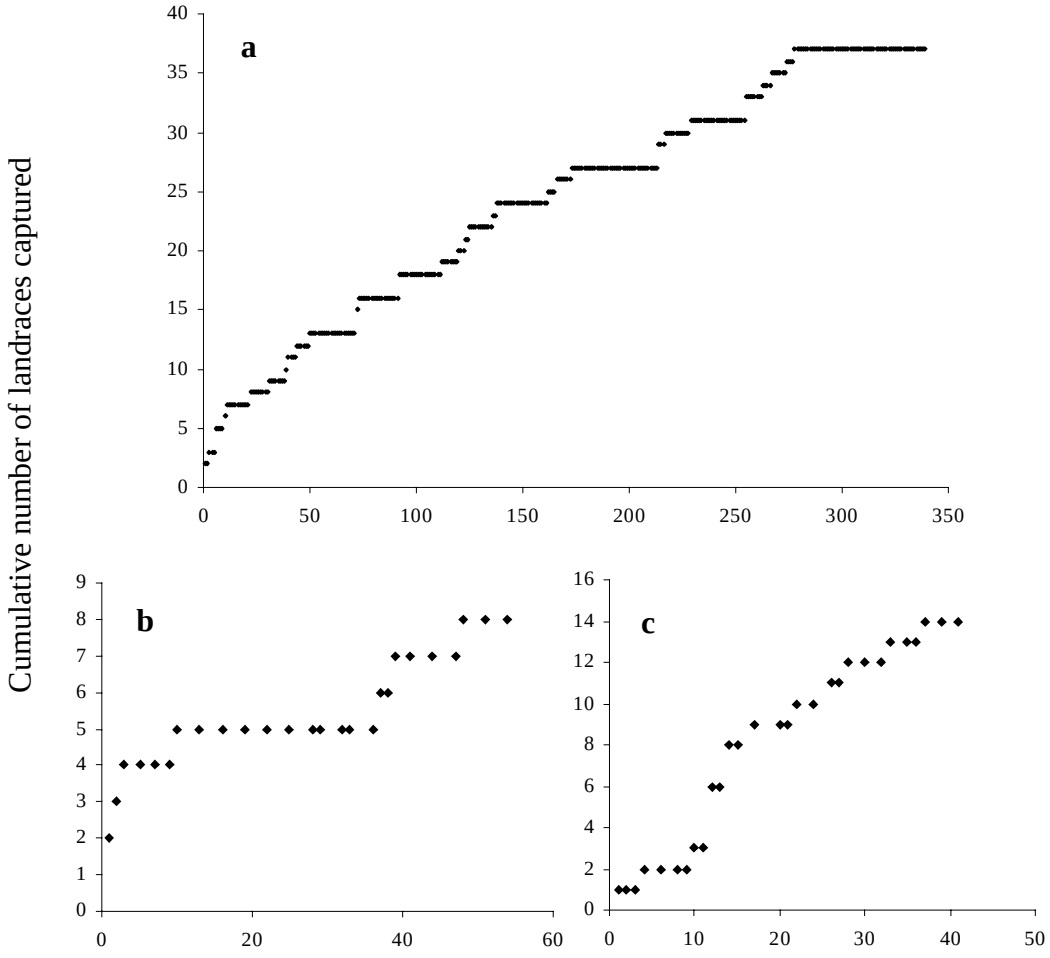



**Figure 2.5.** Number of farms visited and the corresponding number of yam landraces recorded across the study area in Southern Ethiopia (a), in the least diverse district of Damot-Woyde (b) and the most diverse district of Damot-Gale (c).

A significant proportion of farmers in Kindo-Koyisha (87%), Kucha (70%), Offa (63%) and Humbo (33%) districts reported the presence and use of wild yams in their area. The figure was relatively lower in Soddo-Zuria (8%) and Bolosso-Sore (3%), while there was no such report in Damot-Woyde. Wild yam was reported predominantly in localities situated at lower elevations (mainly below 1700 m asl.). These are sparsely populated areas, where patches of forest could still be found. In addition to direct utilization from the natural habitat, wild yam is sometimes manipulated in certain areas. Fore example, in some localities of Kindo-Koyisha, tubers of the wild yam *sasa* (*sesa*) are brought from surrounding forests and planted under big trees on farms, where they

are left to grow for up to three years. The tubers are normally consumed during periods of relative food shortage.

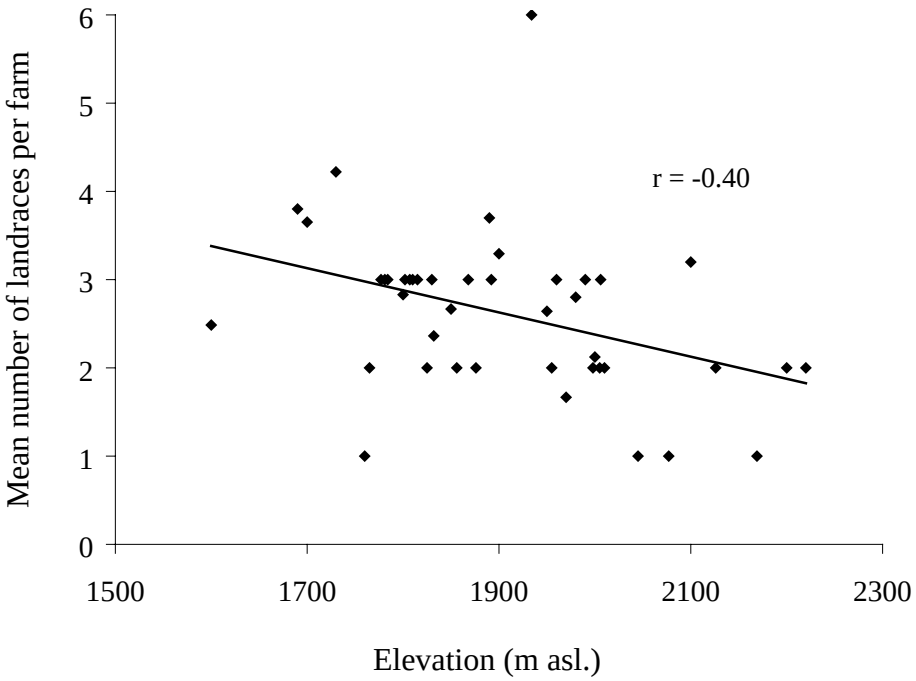



**Figure 2.6.** Mean number of yam landraces per farm related to elevation in Wolayita and Gamo-Gofa zones of Southern Ethiopia.

### 2.3.4. The annual cycle of yam cultivation

In Wolayita and Gamo-Gofa zones, yam is cultivated on an annual cycle of planting in the field starting mainly in October. However, planting is usually delayed till November or December in areas where the dry season is long and severe in order to minimize damage to young plants. Factors such as soil moisture content, intensity of the dry season and anticipated harvesting time are considered in timing field planting. Under normal circumstances, land preparation is carried out when the soil is still moist enough to meet the requirements of yam for loose and deep soils. This also permits planting before the onset of the dry season so that young plants can make use of remaining soil moisture available from the preceding rainy season.

There is no formal seed supply system nor farmers specialize in producing yam planting-materials in the study area. Farmers mostly rely on seed tubers saved from the

preceding cropping season. Some partly meet their demand for seed tubers through purchases from local markets or exchanges with neighbors (Figure 2.7). At the end of each cropping cycle, healthy tubers are selected and stored in shallow pits under shade for one to three months or till required for field planting. For single-harvested landraces that normally produce a single tuber per plant, the head region (proximal end) of each tuber is retained for propagation while the remaining part is consumed. Farmers report that, because of their large open surface wounds, such tuber pieces are susceptible to rotting both under storage and in the field following planting. With double-harvested landraces, a single plant produces multiple tubers following the first harvest. Because of their size, number and presence of root initials on tuber surface, these serve as ideal planting materials.

On about 95% of the farms surveyed, yam was established in monocropping. It is generally perceived that intercropping reduces yield as well as complicates cultural practices. Even those farmers who practiced intercropping shared the same opinion but adopted the system due to scarcity of land. Moreover, there is a common belief among the inhabitants that yam does not appreciate frequent 'visits', as it apparently reduces yield. Frequency of entrance to yam fields is, thus, kept to the minimum, and monocropping appears to be one way to achieve this. Where intercropping was practiced, the crops frequently associated with yam were maize (*Zea mays* L.), sweet potato (*Ipomoea batatas* (L.) Lam.), cabbage (*Brassica* spp.), beans (*Phaseolus* spp.) and, to a lesser extent, coffee (*Coffea arabica* L.)

Yam is chiefly cultivated along rows of stakes, except for wild yam where tubers are brought from surrounding forests and planted near trees for support. Young *Eucalyptus* trees, as well as maize and sorghum (*Sorghum bicolor* (L.) Moench) stalks are among materials widely used for supporting yam plants. Staking commences after tubers have sprouted and produced vines of considerable size. This can be just at planting for those tubers that have already sprouted and had enough time under storage to produce long vines. Each plant is supplied with a vertical stake and trained along it. This system of individual staking was the only type of staking encountered in the study area.

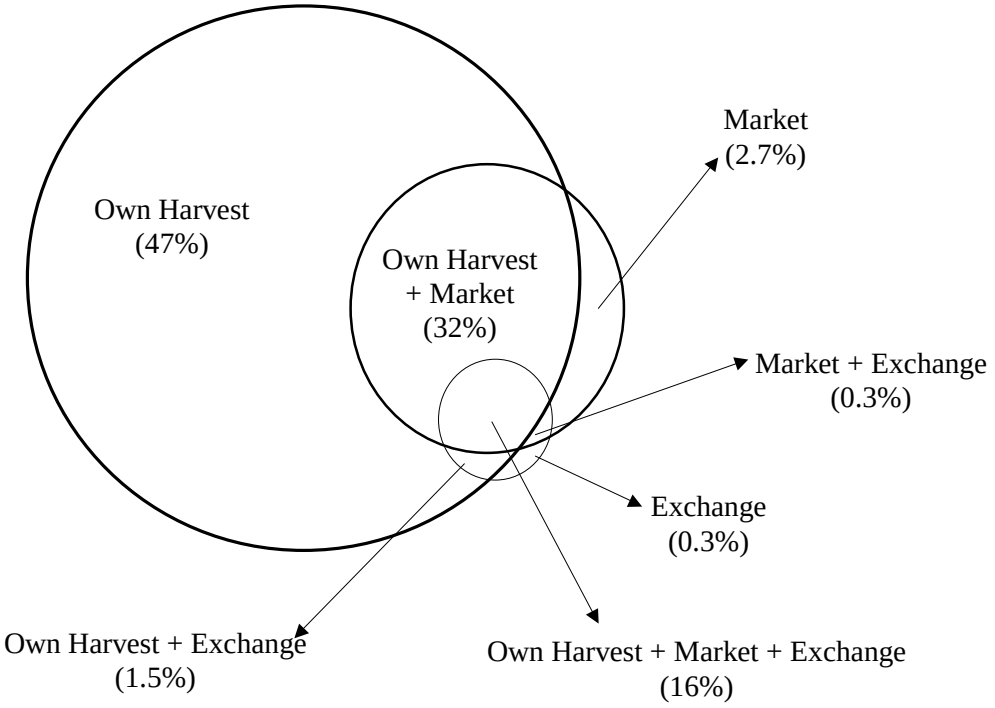



**Figure 2.7.** Major sources of planting-materials (seed tubers) for field planting of yams as reported by farmers in Wolayita and Gamo-Gofa zones of Southern Ethiopia. (Figures in parenthesis are percentage values out of the total 339 farmers interviewed).

Farmers in the study area plant different landraces on their farms. The late and early-maturing landraces usually occupy separate parts of the same plot, while landraces with similar maturity time are planted in mixtures with no regular patterns. Cultivation between rows, to remove weeds and loosen the soil, and training of newly emerging vines are among the most frequent management practices in yam fields. There was no report of the use of commercial fertilizers in yam production. Manure is commonly incorporated into soil during land preparation to maintain soil fertility. Yam is usually planted on relatively fertile plots of land or gets priority when it comes to manure application. Rotation of yam plots on a regular interval of one to fours years, depending on land availability, is also practiced to sustain yields.

Two practices exist with respect to yam harvesting in the study area. The late-maturing landraces are harvested only once (single-harvested) at full senescence, whereas the early-maturing types are harvested twice (double-harvested). For yam planted in October, double harvesting involves a first harvest in May or June. This is achieved by

carefully digging and removing soil to free the tubers, which are then detached at their point of attachment to the corm. Here, the utmost care is taken to avoid damage to the root system. Roots are, then, covered with soil and the plant is left to form more tubers (retuberization). Single harvesting requires less effort as tubers are harvested at the end of the growing season, and no particular care is needed to preserve the root system. Besides, visible onset of senescence is used as a guide for timing harvest. The same applies to the second harvest of double-harvested landraces. However, there is no easy way of determining the optimum time of first harvest. Farmers in Wolayita and Gamo-Gofa are guided by different signals, and one or more of these signals are evaluated to subjectively time first harvesting (Table 2.6). In doing so, the aim is to avoid harvesting too early (lower yield) or too late that compromises the second harvest because plants do not get enough time for re-tuberization.

**Table 2.6.** Criteria employed by farmers in Wolayita and Gamo-Gofa zones of Southern Ethiopia for timing the first harvest of double-harvested yam landraces.

| **Criteria** | Proportion of farmers (%) |
|---|---|
| Senescence of inflorescence | 19.8 |
| Senescence of inflorescence + Flower scent | 13.0 |
| Senescence of inflorescence + Wilting of vine tips | 9.7 |
| Wilting of vine tips | 5.9 |
| Senescence of inflorescence + Digging and checking of tubers | 5.0 |
| Senescence of inflorescence + Flower scent + Wilting of vine tips | 4.7 |
| Time from planting + Wilting of vine tips | 3.8 |
| Senescence of inflorescence + Flower scent + Soil cracking | 3.5 |
| Senescence of inflorescence + Soil cracking + Wilting of vine tips | 3.2 |
| Senescence of inflorescence + Time from planting | 3.2 |
| Time from planting | 2.7 |
| Others[+] | 25.5 |
| Total | 100.0 |

[+]Include the use of the above criteria in various forms of combinations

## 2.4. Discussion

### 2.4.1. Status of yam diversity

Farmers in Wolayita and Gamo-Gofa maintain diverse yam landraces with respect to attributes such as environmental adaptation and length of growing cycle. This finding confirms the salient feature of traditional faming systems in the tropics, where diverse crop species or varieties of the same species are maintained on a single farm (Boster 1983; Clawson 1985; Brush 1995) in response to economic, social, cultural and natural factors (Cox and Wood 1999). Similar observations were made in various traditional faming systems for clonally propagated crops such as enset (Tesfaye and Lüdders 2003; Tsegaye and Struik 2002), banana (*Musa* spp.) (Gold et al. 2002), cassava (*Manihot esculenta* Crantz) (Boster 1985; Salick et al. 1997) and potato (*Solanum tuberosum* L.) (Brush et al.1981). Tsegaye and Struik (2002) recorded a total of 55 named enset landraces in Wolayita, where individual farmers on average maintained eight landraces. They also reported that landrace diversity was affected by factors such as household resources, cultural background, population pressure and agro-ecology.

Two of the landraces described, *bola-boye* and *bunde-buchi*, belong to the species *D. bulbifera*. However, the species identity of the remaining landraces is yet to be established. Preliminary observations based on morphological features seem to indicate that some of the landraces belong to the *D. cayenensis/D. rotundata* species complex as presently understood by most researchers working on yams (e.g. Dansi et al. 1999). Detailed identification works are in progress based on morphological and molecular markers. Most named landraces are morphologically distinct, although this may not be the case for *macha*. On the one hand, the same name is used as a reference to a group of the so-called 'female yams' (*macha boye*), to which all the early-maturing landraces such as *hatiye* and *oha* belong. On the other hand, there are no peculiar characteristics that distinguish the landrace *macha* from the other members of the group *macha*.

The extent of landrace diversity detected in this study is comparable with earlier reports for yams. Hildebrand et al. (2002) described 23 separate indigenous yam types belonging to at least four species of *Dioscorea* in Sheko, Southwest Ethiopia. About

300 different named yam landraces were reported across 10 different ethnic groups throughout Benin (Dansi et al. 1997, quoted by Dansi et al. 1999), while Baco et al. (2004) recorded 88 varieties in the Sienendé district of Benin. Although the number of named landraces recorded in Benin is significantly higher than in our finding, this is not entirely comparable. First, some reports cover an entire region or country, whereas others, including our study, deal with relatively small areas. Furthermore, when conducting such studies across ethnically diverse regions, like in some of the above reports, linguistic polymorphism may lead to overestimation of diversity based on landrace names. The Wolayita language belongs to the Omotic family, and is closely related to *Gamo*, a language of the same family spoken by neighboring farmers in Kucha district. Both languages have a lexical similarity of 79-93% (Girard 2002). This provides a good setting for studying crop diversity in traditional agriculture using named landraces with a minimum influence of language polymorphism.

Yam production in Wolayita and Gamo-Gofa is mainly based on a limited number of widespread landraces such as *hatiye, wadala* and *oha* (Table 2.2 and Figure 2.3). The other landraces described have a rather limited distribution and abundance. This hierarchical nature of spatial distribution, where a limited number of landraces or cultivars are dominant, has been documented for several crop species (Boster 1985; Louette et al. 1997; Tesfaye and Lüdders 2003). This widespread distribution of some landraces also challenges the view that traditional farming systems are isolated and closed, with limited exchange of germplasm. Our finding and those of others mentioned above depict these systems rather as open and dynamic where local networks exist for moving planting materials across wider areas and heterogeneous environments. Yam farmers in the study area acquire part of their planting materials through purchases from local markets or exchanges with neighbors (Figure 2.7). Such networks can cover relatively larger areas, as getting to the next market often involves long distance travels.

It is widely claimed that an advantage of double harvesting is that the first harvesting induces the formation of multiple tubers. Tubers from the second harvest are mostly lignified and fibrous, and posses several visible buds even at harvest (Onwueme and Charles 1994). These are, thus, ideal planting materials. Some farmers prefer to delay or forgo first harvesting, opting for a single harvest of apparently higher yields to

maximize income. These farmers, because they do not have alternative sources of planting materials, purchase seed tubers from local markets for planting the following season. This has created a potential market for seed tubers, where there are now middlemen involved in the business, moving planting materials even over longer distances. This may partly explain the wider distribution of some landraces that are highly preferred by growers and consumers.

This study was an attempt to measure the available yam diversity based on absence and presence of landraces as recognized and named by local farmers. Lack of similar studies in the past makes assessment of changes that might have occurred over time difficult. Farmers recall names of landraces that are either still growing on fields of other farmers, even at distant localities, or do not seem to exist anymore. It might be the case that yam variability has been reduced either in the form of decreased distribution or disappearance of some landraces. The availability of wild yams in areas covered with forest patches indicates possible loss of valuable diversity with shrinking forest areas. Nevertheless, there is no information as to whether there were introductions of new landraces into the area or not. Thus, how much past events influenced the overall yam diversity is far from clear.

About 76 % of the farmers interviewed reported a decreasing trend in yam production due to several environmental and production factors (Tamiru et al. 2005). Yet, production is on the increase in some localities and yam is establishing itself as an important cash crop. Damot-Woyde is one of the districts where yam production is an expanding business (data not shown). It is also the least diverse district in terms of total number of landraces described (Table 2.4). It seems that the increase in production is brought about at the expense of the overall landrace diversity, as farmers are increasingly growing limited number of landraces partly in response to consumer demand. *Hatiye* and *oha* are among the widely cultivated landraces in the district, as they mature early and possess excellent culinary properties. These attributes make them the preferred choice both for farmers and consumers, replacing late-maturing landraces such as *wadala*. As noted by Frankel and Bennett (1970), besides the transition from landraces to advanced cultivars, selection for closely defined objectives can lead to a reduction in genetic variation.

In general, although detailed information is lacking as to the extent of genetic erosion in yams and its implications, genetic vulnerability (Brown 1983) is a legitimate worry in Wolayita and Gamo-Gofa. Farmers are already concerned that yam production is threatened by changing environmental conditions (erratic rains and increasing temperatures). This concern is particularly valid in view of the fact that most of the early-maturing landraces used for increasing production are relatively more prone to drought than the late-maturing ones.

### 2.4.2. Management and use of diversity

Wolayita and Gamo-Gofa farmers are familiar with the diversity available in yams and attributes of each landrace, which are highly valued and utilized accordingly to meet their needs. Unlike other crops, yam is adapted to dry season planting, an attributed widely manipulated by local farmers to ensure household food security. For yams planted in October (beginning of the dry season), the first harvest of early-maturing landraces is expected around May or June. This is a period of relative food shortage in the area, as most of the other crops are still in the field. Thus, yam fills a seasonal gap in food supply. That is why the early-maturing landraces such as *hatiye* and *oha* are widely distributed throughout the study area (Figure 2.3).

Apart from their early maturity, some landraces such as *hatiye* are popular due to their sweet taste and white tuber flesh color, which are preferred for preparation *fichata* (a popular dish made of mashed yam mixed with fermented milk and butter). The white tuber flesh goes well with the milk during mixing. Thus, such landraces are widely distributed across different altitudinal ranges although farmers are aware of the fact that some perform poorly under drier and hotter conditions. *Wadala* is more common at lower elevations, and is highly valued for its sturdy growth, drought tolerance and bigger tubers. It is a late-maturing landrace, and this nature is exploited to extend harvesting into late seasons. Its requirement for more stout staking materials, regular training and, hence, intensive management is usually tolerated because of its acceptable performance under sub-optimal conditions.

There exists a striking similarity between management and use of yams in the study area and other parts of Ethiopia such as Sheko (Hildebrand et al. 2002) and different

African countries (Onwueme 1978; Hahn et al. 1987; Asiedu et al. 1997). This provides an opportunity for sharing experiences mainly with West African countries, where the yam-based agriculture has been supported by research undertakings that have achieved technology delivery and adoption on farms (Quin 1998). On the other hand, Wolayita and Gamo-Gofa farmers employ unique practices with certain degree of sophistication in managing yam. For example, double and single-harvestings are also common features of yam production in other African countries (Onwueme 1978). Among the main problems often mentioned in connection with double harvesting is the lack of a reliable index of maturity to time the first harvesting (Onwueme 1978). Based on experience, farmers in the study area use a range of criteria to subjectively judge time of the first harvesting (Table 2.6). Such practices make the indigenous knowledge of Ethiopian farmers an important aspect of the overall yam diversity.

## 2.5. Conclusions

The high value that Wolayita farmers place on yam is expressed in its continued cultivation despite the lack of any form of support from researchers and policy makers. Yam is still the preferred food compared to the other root and tubers, such as sweet potato, potato and taro, where some improved materials and technological production packages have been provided. Through the use of different landraces of varying attributes, farmers make use of the existing diversity to meet household needs. Given this practical importance of yam in local livelihood, there is an urgent need for research to broaden the knowledge base of the crop.

Analyzing the specific characteristics that local farmers find important in their landraces is vital as it can assist in setting research priorities aimed at conservation and improvement of the crop. These endeavors must also take into consideration the multiple objectives of farmers and the importance of diversity in the physical, economical and cultural context of local agriculture.

In many cultures, different crops are used for rituals or prestige and, hence, provide additional motives for continued maintenance of some crop landraces. Information regarding the role of yam in the social life and believes of the local community can,

thus, give an insight into perceptions and importance of genetic diversity. Accordingly, the indigenous knowledge of yam and its use must be collected, analyzed and properly documented for utilization in research and development undertakings.

Listing names of local landraces is a good start to study crop diversity in traditional farming systems. But detailed analysis of the local classification system, and characterization of the available diversity based on morphological and molecular markers are required to understand the extent of yam diversity in the study area. As parts of an on-going project, these studies are currently under way on yam accessions assembled from the study area, including accessions from more localities in Southern Ethiopia and with reference genotypes from other African countries.

← Staking and training of yam plants in Wondara-Gale (Damot-Gale), Wolayita. (Photo: Muluneh Tamiru)

A well-managed yam field in Girara (Damot-Woyde), Wolayita. (Photo: Muluneh Tamiru) →

A yam plot near Yirgalem (Dale), Sidama. (Photo: Muluneh Tamiru)

A view of yam fields in Girara (Damot-Woyde), Wolayita. Because yam is adapted to dry-season planting, plants have normally attained a considerable size at the start of the rainy season when land is being prepared for planting of the other crops. (Photo: Muluneh Tamiru)

Yam tubers on market at Boditie, (Damot-Gale), Woalyita.
(Photo: Muluneh Tamiru)

# 3. Comparative Analysis of Morphological and Farmers' Cognitive Diversity in Yam Landraces (*Dioscorea* spp.) from Southern Ethiopia.


**Abstract**

*Largely neglected by research and development, much of the knowledge of the genetic diversity in Ethiopian yams is found with the local farmers. Accordingly, the local yam classification system was studied in the major yam growing regions of Southern Ethiopia during the 2003/2004 cropping season through individual and key informant interviews. Data collected included farmers' selection criteria and attributes of each landrace. Besides, 84 accessions were collected and characterized at the experimental station of Awassa College of Agriculture (Ethiopia) based on 32 qualitative morphological traits. Local farmers recognize two major categories of yams: 'hatuma boye' ('male' yam) and 'macha boye' ('female' yam). Female yams mature early, less vigorous in growth, and produce poorly under sup-optimal conditions, while the male ones mature late, vigorous in growth and are drought tolerant. This classification has no reference to the reproductive biology of the plants, although most yam species are dioecious. Individual landraces within each group are identified based on different morphological and growth attributes. Cluster and principal component analyses based on the traits measured gave seven morphological groups. The groups reveled that the overall structure of morphological diversity is largely consistent with farmers' landrace classification, particularly based on maturity time. Nevertheless, there was no morphological difference between some landraces managed as different by farmers. This, together with the fact that the species identity of most of the landraces could not be established using standard morphological descriptors, requires more powerful methods for further characterization of the yam diversity available in Ethiopia.*



**Keywords:** Morphological diversity; local classification; yam; Ethiopia

## 3.1. Introduction

Yams (*Dioscorea* spp.) represent a diverse group of plant species widely grown in the tropics and sub-tropics (Alexander and Coursey 1969). The morphological variability in yam is expressed both in the aerial and underground vegetative apparatus (Asiedu et al. 1997; Hamon et al. 2001). As part of the mandate of the International Institute of Tropical Agriculture (IITA) for research on yams, morphological descriptors have been developed and used for assessing the diversity in the major cultivated species mainly from West Africa (Onyilagha and Lowe 1985; Hamon and Touré 1990b; Dansi et al. 1999; Mignouna et al. 2002a). Accordingly, different species and landraces have been described, their relationships established and identification keys proposed. Progress achieved in this regard has demonstrated the potential of morphological descriptors in the study of yam germplasm.

Recently IITA has extended its program into studying diversity of yam germplasm collected from Eastern Africa, Uganda and Tanzania (IITA 2000). This study in collaboration with ESARC (East and Southern Africa Regional Center) has been launched apparently to investigate if the distinctiveness observed within yam germplasm from Cameroon also extends to East and South Africa. Although Ethiopia is an important center of yam cultivation in the region (Norman et al. 1995), its yam germplasm has not been included in the study.

Yam is exclusively cultivated by subsistence farmers in the densely populated areas of Southern, Southwestern and Western Ethiopia, where it has a considerable importance in local livelihood (Etissa 1996; Gemeda 2000; Chapter 2). It is also found wild in some parts of the country (Etissa 1998) and often collected for food in several localities (Hildebrand et al. 2002; Chapter 2). Ethiopia is the center of origin for yam species such as *D. abyssinica* (Coursey 1967; Zeven and De Wet 1982), which is among the wild species believed to have produced cultivated forms in Africa (Hahn 1995). On this account, Ethiopia may be an important center of yam diversity that can constitute a useful source of materials for genetic improvement of the crop. However, little is known about extent and distribution of the diversity available in the country.

Apart from the knowledge gained through scientific observations and experimentations, knowledge accumulated by local people is fundamental in the study of plant genetic resources. Because traditional societies depend heavily on these resources for survival, they hold vital knowledge (commonly known as traditional or indigenous knowledge) that is crucial in conservation and improvement programs. Nevertheless, researchers often overlook this knowledge and tend to treat plants as unknown genetic packages, particularly during collecting missions (Prain et al. 1995). As Guarino (1995) argues "…collecting landraces while ignoring the dimension of local knowledge cannot but be wasteful at best, hopelessly flawed at worst".

Traditional households' management of diversity includes processes such as selection and local classification systems or folk taxonomy (Hodel et al. 1999). Many researchers have attempted to study local classification systems and relate their findings to the actual genetic diversity available in crop plants. For example, in their investigation of the genetic variability of non-bitter potatoes in the fields of Andean farms, Quiros et al. (1990) found a remarkable degree of correspondence between folk recognition of landraces and results of isozyme analysis. Likewise, farmers in Ethiopia know with considerable accuracy the duration of storability of sorghum landraces and classify them accordingly (Teshome et al. 1999). Good agreement was found between local classification and structure of morphological diversity in cassava (Elias et al. 2001). Sambatti et al. (2001) reported a similar finding in cassava, although the level of variability recognized by farmers appeared to have underestimated the actual genetic diversity.

A recent survey conducted in various localities in Southern Ethiopia highlighted the presence of considerable yam diversity as revealed by the number of named landraces under cultivation (Chapter 2). Although the finding was an important addition to the current knowledge on Ethiopia yams, the question remains as to what level these names represent the actual diversity. Data on how local farmers select and classify their landraces need to be collected, and the available landraces have to be characterized based on the variability in morphological traits and relationships among the various names have to be established.

The main objective of this study was to analyze the extent and structure of morphological diversity among yam accessions collected from the major production areas in Southern Ethiopia. Attempt was also made to relate the result to the level of diversity recognized by local farmers. As yam has long been cultivated in the study area, there might exist a local classification system that is consistent, to some extent, with conventional botanical classification. In this paper, the term 'landrace' refers to clones or populations of yam maintained as distinct by farmers. All yam landraces are, thus, known by their respective vernacular names. 'Accessions' are samples collected during a collecting mission in Ethiopia. A landrace is represented either by a single accession or by two or more accessions sampled from different farms or localities.

## 3.2. Materials and Methods

### 3.2.1. Morphological characterization

#### 3.2.1.1. Planting materials and sampling

A total of 84 yam accessions were considered in this study. Sixty-two accessions were collected from various localities in Gedeo, Sidama, Wolayita and Gamo-Gofa zones of Southern Nations, Nationalities and Peoples' Regional State (SNNPRS) of Ethiopia during the 2003/2004 cropping season (Figure 3.1). The collection covered diverse agro-ecologies within elevation range of 1350 to 2200 m asl., representing one of the major yam production areas in the country. The remaining twenty-two accessions were obtained from Areka Agricultural Research Center, located in Kindo-Koyisha district of Wolayita zone, where a considerable size of yam collection is being maintained. Although the collections at Areka were apparently assembled from Southern and Southwestern Ethiopia, passport data are missing including the exact collection site and time of maturity of each accession. Of all the accessions studied, five belong to a well-defined species of aerial yam (*D. bulbifera*), while the identity of the remaining accessions is yet to be established. Furthermore, most accessions represent different named landraces, while some were collected from various localities under the same vernacular name (Table 3.1).

Key informants drawn from local farmers and district agricultural officers were first consulted to get an overview of yam diversity in the area in terms of named landraces. The collecting was then organized accordingly to cover the most diverse localities. As the tuber is also used for propagation, collecting was undertaken towards harvesting time, but when the aerial vegetative plant parts were still green to aid identification of the different morphotypes. Here, a morphotype represents a phenotypically similar plant group or population in a field of yam composed of mixture of landraces, and considered as a sampling unit in this study. It does not, however, imply any genetic identity.

In Wolayita and Gamo-Gofa zones, the early and late-maturing landraces occupy separate rows on the same plot, whereas landraces in the same maturity group are planted in mixtures with no particular pattern. The latter was ubiquitous in Sidama and Gedeo where only early-maturing landraces are cultivated. Since random sampling in such fields may lead to overestimation of abundant clones at the expense of rare ones (Huaman et al. 1995), the available morphotypes were selectively sampled to capture as much diversity as possible. Accordingly, single plants were sampled from those early-maturing landraces that normally produce multiple tubers following the first harvest. For late-maturing landraces, where a single tuber per plant is expected at the end of the season, tubers were bulked from 2-4 plants representing a distinct morphotype. However, during morphological characterization, any plant with distinct morphological features from the rest in the sample was re-entered as a new accession.

Lack of easily recognizable aerial morphological features to distinguish some early-maturing landraces was among the problems encountered during sampling in fields where such landraces were established in mixtures. Farmers could only distinguish these landraces after digging out the tubers and inspecting tuber flesh color. Thus, sampling on local markets proved a more effective way for collecting the different morphotypes.

#### 3.2.1.2. Field planting

The accessions were characterized under field conditions at the experimental fields of Awassa College of Agriculture (Debub University) in Southern Ethiopia. The site is

located at latitude 7º03 N, longitude 38º28 E, and an elevation of about 1670 m asl. The experimental plot was plowed three weeks in advance of the actual planting time during which well-decomposed manure was applied. The experiment was laid out in a randomized block design with two replications, and planting was carried out on 15 November 2003. Depending on availability of seed tubers, 1-3 tubers were planted in single row plots, making the overall number of tubers per accession in both replications 2 to 6. The tubers were planted at a spacing of 1 m between them in each row, while the rows were spaced 1.5 m apart to avoid intermingling of the trailing yam vines. Plants were supported by individual stakes of eucalyptus about 2.5 m aboveground to induce good canopy development. The plots were irrigated as deemed necessary throughout the dry season; however, no irrigation was required after onset of the rainy season.

#### 3.2.1.3. Morphological descriptors

The morphological descriptors used in this study were modified from those previously employed for characterizing cultivated yams in West Africa (Hamon and Toré 1990b; Lebot et al. 1998; Dansi et al. 1999; Mignouna et al. 2002a) and recommended for describing *Dioscorea* species (IPGRI/IITA 1997). Characterization of the aerial vegetative parts started shortly after planting, depending on the rate of sprouting and early growth of individual accessions, and was regularly monitored throughout the growing period. For both the early (double-harvested) and late (single-harvested) maturing landraces, tubers were harvested only once at full senescence and subsequently described.

#### 3.2.1.4. Data collection, treatment and multivariate analysis

Data was recorded on different types of qualitative morphological traits such as binomial, ordinal and multi-state (non-ordinal) with varying scales of measurement (Table 3.2). For measurements involving leaves, at least ten leaves per plant and two plants in each replication were assessed, and mean values for the two replications were considered. Young stems and leaves were assessed after about 20-30 days from emergence, while mature stems were described at full maturity but before senescence (IPGRI/IITA 1997). Traits related to color were determined with the help of Munsell

color chart for plant tissues. Overall, 42 different morphological characters (12 of stem, 23 of leaf, 4 of tuber, and 3 of inflorescence) expressed in 143 character states were used in characterizing the accessions (Table 3.2).

In cluster analysis, the presence of different types of variables and dealing with situations where a few qualitative characters have the highest discriminatory effects pose a major problem (Franco et al. 1997). As the available distance measures mostly depend on type of variable and scale of measurement (Mohammadi and Prasanna 2003), it is essential that the data set is standardized to eliminate scale differences and give equal weight to the contribution of all characters in the final output (Franco et al. 1997). In this study, dividing each observed value with its respective range standardized the data set. Then, a matrix of similarity was generated based on Euclidean distance. The matrix was subjected to cluster analysis using the UPGMA (Unweighted Paired Group Method using Arithmetic Averages) hierarchical clustering algorithm to generate a dendrogram of relatedness. To measure the goodness of fit of the cluster analysis, the cophenetic correlation coefficient between the original data, based on which the clustering was made, and the cophenetic values was estimated.

Principal component analysis (PCA) was performed to further reveal patterns within the data matrix. Both cluster and principal component analyses were based on those morphological traits that showed variations between the accessions studied. The analyses were made using the computer program NTSYpc, version 2.1 (Rohlf, 2000) and the data reduction function of SPSS for Windows (version 12.0, 2003).

### 3.2.2. Local classification system

The local classification system of yam was assessed during the collecting mission by asking farmers to describe the distinguishing features, selection criteria and attributes of each landrace that are important in their decision to maintain the landrace. Data collection methods included listing of local names and description of each landrace through individual and key informant interviews. Accordingly, information was compiled regarding the various landraces and their attributes that are highly valued by local farmers. Data collected during the collecting mission was also complemented with additional data obtained during the farm-level survey conducted from October

2003 to September 2004 to investigate landrace diversity. This permitted description of the main features of local classification system, which was also compared with results of the morphological characterization work.

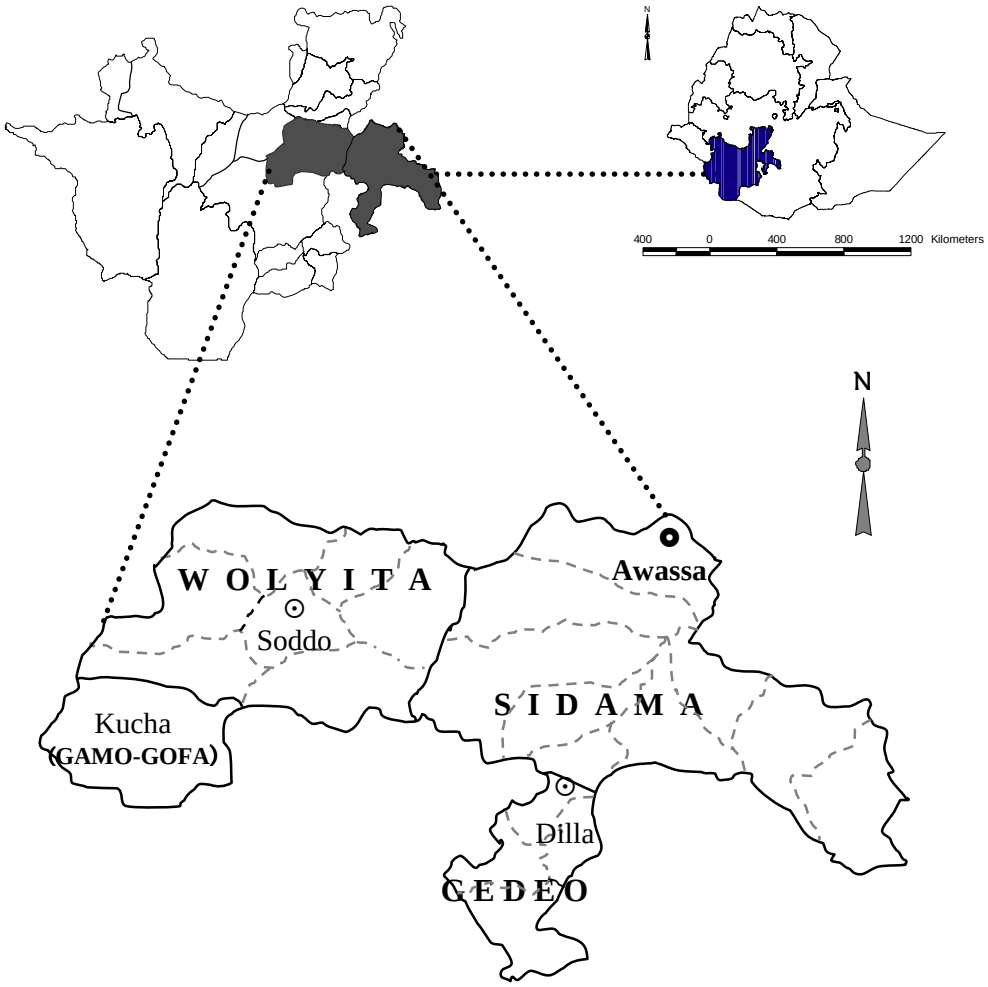



**Figure 3.1.** Map of collecting areas in Southern Ethiopia.

**Table 3.1.** Origin and local names of the 84 yam accessions (*Dioscorea* spp.) considered in the study.

| No. | Accession Code | Local name | Area of collection | Altitude (m asl.) | Lat. (N) | Long. (E) | No. | Accession Code | Local name | Area of collection | Altitude. (m asl.) | Lat. (N) | Long. (E) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | GGF 001 | *Wadala* | Gamo-Gofa | 1375 | 6°28´ | 37°27´ | 34 | WOL 026 | *Boye* | Wolayita | 2045 | 7°02´ | 37°55´ |
| 2 | GGF 002 | *Wadala* | Gamo-Gofa | 1714 | 6°25´ | 37°28´ | 35 | WOL 027 | *Hatiye* | Wolayita | 2077 | 6°51´ | 37°45´ |
| 3 | GGF 003 | *Hatiye* | Gamo-Gofa | 1715 | 6°25´ | 37°28´ | 36 | WOL 028 | *Oha* | Wolayita | 1967 | 7°03´ | 37°55´ |
| 4 | GGF 004 | *Buna (Bune)* | Gamo-Gofa | 1715 | 6°25´ | 37°28´ | 37 | SID 001 | *Ado* | Sidama | 1782 | 6°46´ | 38°24´ |
| 5 | WOL 001 | *Wadala* | Wolayita | 1681 | 6°44´ | 37°36´ | 38 | SID 002 | *Goloma* | Sidama | 1783 | 6°46´ | 38°24´ |
| 6 | WOL 002 | *Hatiye* | Wolayita | 1682 | 6°44´ | 37°36´ | 39 | SID 003 | *Ganticho* | Sidama | 1742 | 6°46´ | 38°25´ |
| 7 | WOL 003 | *Oha* | Wolayita | 1865 | 6°51´ | 37°52´ | 40 | SID 004 | *Ado* | Sidama | 1743 | 6°46´ | 38°25´ |
| 8 | WOL 004a | *Wadala* | Wolayita | 1866 | 6°51´ | 37°52´ | 41 | SID 005 | *Ado* | Sidama | 1813 | 6°45´ | 38°27´ |
| 9 | WOL 004b | *Wadala* | Wolayita | 1866 | 6°51´ | 37°52´ | 42 | SID 006 | *Ado* | Sidama | 1870 | 6°37´ | 38°25´ |
| 10 | WOL 005 | *Oha* | Wolayita | 1802 | 6°50´ | 37°52´ | 43 | SID 007 | *Ado* | Sidama | 1823 | 6°30´ | 38°23´ |
| 11 | WOL 006 | *Zoreuwa* | Wolayita | 1803 | 6°50´ | 37°52´ | 44 | SID 008 | *Ganticho* | Sidama | 1823 | 6°30´ | 38°23´ |
| 12 | WOL 007 | *Hatiye* | Wolayita | 1777 | 6°50´ | 37°52´ | 45 | SID 009 | *Ado* | Sidama | 1823 | 6°30´ | 38°23´ |
| 13 | WOL 008 | *Oha* | Wolayita | 1778 | 6°50´ | 37°52´ | 46 | SID 010 | *Ado* | Sidama | 1765 | 6°28´ | 38°20´ |
| 14 | WOL 009 | *Hatiye* | Wolayita | 1767 | 7°04´ | 37°42´ | 47 | SID 011 | *Ado* | Sidama | 1714 | 6°28´ | 38°19´ |
| 15 | WOL 010 | *Hatiye* | Wolayita | 1768 | 7°04´ | 37°42´ | 48 | SID 012 | *Ganticho* | Sidama | 1715 | 6°28´ | 38°19´ |
| 16 | WOL 011 | *Mortawa* | Wolayita | 1769 | 7°04´ | 37°42´ | 49 | SID 013 | *Ganticho* | Sidama | 1642 | 6°27´ | 38°18´ |
| 17 | WOL 012 | *Ayina* | Wolayita | 1770 | 7°04´ | 37°42´ | 50 | SID 014 | *Ado* | Sidama | 1643 | 6°27´ | 38°18´ |
| 18 | WOL 013a | *Gajela* | Wolayita | 1935 | 6°58´ | 37°44´ | 51 | SID 015 | *Ganticho* | Sidama | 1865 | 6°35´ | 38°20´ |
| 19 | WOL 013b | *Gajela* | Wolayita | 1935 | 6°58´ | 37°44´ | 52 | SID 016 | *Ado* | Sidama | 1866 | 6°35´ | 38°20´ |
| 20 | WOL 014 | *Macha* | Wolayita | 1936 | 6°58´ | 37°44´ | 53 | SID 017 | *Ganticho* | Sidama | 1867 | 6°35´ | 38°20´ |
| 21 | WOL 015 | *Wadala* | Wolayita | 1930 | 6°54´ | 37°43´ | 54 | SID 018 | *Ganticho* | Sidama | 1868 | 6°35´ | 38°20´ |
| 22 | WOL 016 | *Macha* | Wolayita | 1931 | 6°54´ | 37°43´ | 55 | GED 001 | *Ganticho* | Gedeo | 1810 | 6°09´ | 38°11´ |
| 23 | WOL 017* | *Bakiche* | Wolayita | 1561 | 6°52´ | 37°32´ | 56 | GED 002 | *Ganticho* | Gedeo | 1840 | 6°12´ | 38°12´ |
| 24 | WOL 018a | *Wayia* | Wolayita | 1562 | 6°52´ | 37°32´ | 57 | GED 003 | *Toracho* | Gedeo | 1687 | 6°00´ | 38°09´ |
| 23 | WOL 018b | *Wayia* | Wolayita | 1562 | 6°52´ | 37°32´ | 58 | GED 004 | *Ganticho* | Gedeo | 1688 | 6°00´ | 38°09´ |
| 26 | WOL 019 | *Gena* | Wolayita | 1563 | 6°52´ | 37°32´ | 59 | GED 005 | *Toracho* | Gedeo | 1689 | 6°00´ | 38°09´ |
| 27 | WOL 020 | *Hatiye* | Wolayita | 1564 | 6°52´ | 37°32´ | 60 | GED 006 | *Ganticho* | Gedeo | 1690 | 6°00´ | 38°09´ |
| 28 | WOL 021* | *Bundi- Buchi* | Wolayita | 1594 | 6°42´ | 37°46´ | 61 | GED 007 | *Toracho* | Gedeo | 1800 | 6°11´ | 38°03´ |
| 29 | WOL 022 | *Hatiye* | Wolayita | 2126 | 6°56´ | 37°49´ | 62 | GED 008 | *Toracho* | Gedeo | 1740 | 6°19´ | 38°15´ |
| 30 | WOL 023a | *Walabo* | Wolayita | 2126 | 6°56´ | 37°49´ | 63 | AKA 000* | *Unknown* | AARC | | | |
| 31 | WOL 023b | *Walabo* | Wolayita | 2126 | 6°56´ | 37°49´ | 64 | AKA 002* | *Unknown* | AARC | | | |
| 32 | WOL 024 | *Hatiye* | Wolayita | 2169 | 6°56´ | 37°48´ | 65 | AKA 003* | *Unknown* | AARC | | | |
| 33 | WOL 025 | *Macha* | Wolayita | 1910 | 7°02´ | 37°55´ | 66-84 | AKA005-AKA022 | *Unknown* | AARC | | | |

GGF = Gamo-Gofa; WOL = Wolayita; SID = Sidama; GED = Gedeo zone; AARC= Areka Agricultural Research Center; AKA = Areka; and *aerial yams (*Dioscorea bulbifera*)

**Table 3.2.** Morphological descriptors used for characterization of yam accessions (*Dioscorea* spp.) collected from Southern Ethiopia.

| Descriptor | Descriptor state |
|---|---|
| *Young stem* | |
| Spines | 0=none, 1=few, 2=many |
| Wings | 0=absent, 1=present |
| Hairs | 0=absent, 1=present |
| Barky patches | 0=absent, 1=present |
| Stem color* | 1=green, 2=brownish green, 3=purplish green, 4=purple |
| Color at spine base* | 0=absent, 1=present |
| *Mature stem* | |
| Twining direction* | 1=clockwise, 2=anticlockwise |
| Stem color* | 1=green, 2=brownish green, 3=purplish green, 4=purple |
| Spines at stem base* | 0=none, 1=few, 2=many |
| Spines at stem above base* | 0=none, 1=few, 2=many |
| Spine shape* | 0=none, 1=straight, 2=curved |
| Waxinness | 0=absent, 1=present |
| Wings | 0=absent, 1=present |
| *Young leaf* | |
| Leaf color* | 1=light green, 2=green, 3=pale green, 4=brownish green, 5=purplish green, 6=purplish brown, 7=purple |
| Leaf margin color* | 1=pale green, 2=green, 3=green with brown tip, 4=green with purple tip, 5=brownish green, 6=purplish green, 7=purplish brown, 8=purple |
| Vein color* | 1=yellowish green, 2=pale green, 3=green, 4=brownish green, 5=purplish green, 6=purplish brown, 7=pale purple, 8=purple |
| Petiole wing* | 0=absent, 1=present |
| Hairiness of leaf surface | 0=absent, 1=present |
| Petiole color* | 1=light green, 2=pale green, 3=green, 4=green with brown base, 5=green with purple base, 6=green with purple leaf junction, 7=brownish green with brown base, 8=brownish green with purple base, 9=purplish green, 10=purplish green with purple base, 11=purplish green with purple leaf junction, 12=purplish brown, 13=pale purple with purple base, 14=purple |
| Petiole wing color* | 0=not applicable, 1=light green, 2=pale green, 3=green, 4=green with purple edge, 5=brownish green, 6=purplish green, 7=purplish brown, 8=purple |
| *Mature leaf* | |
| Leaf position | 1=alternate, 2=opposite |
| Leaf type | 1=simple, 2=compound |
| Leaf margin | 1=entire, 2=serrate |
| Leaf margin color* | 1=light green, 2=green |
| Leaf lobation* | 1=shallow, 2=deep |
| Leaf color* | 1=light green, 2=pale green, 3=green, 4=dark green |
| Vein color, upper surface* | 1=yellowish, 2=light green, 3=pale green, 4=green |
| Vein color, lower surface* | 1=light green, 2=green |
| Leaf shape* | 1=cordate, 2=cordate long, 3=cordate broad |

**Table 3.2.** Continued.

| Descriptor | Descriptor state |
|---|---|
| *Mature leaf (con.)* | |
| Leaf apex shape | 1=obtuse, 2=acute |
| Distance between lobes | 1=intermediate, 2=distant |
| Downward arching along main vein* | 0=absent, 1=present |
| Upward folding of leaves | 0=none, 1=weak, 2=strong |
| Leaf tip color* | 1=light green, 2=green |
| Petiole color* | 1=green, 2=green with brown base, 3=green with brown at both ends, 4=green with purple base, 5=green with purple leaf junction, 6=green with purple at both ends, 7=purple green with purple at both ends |
| Petiole wing color* | 0=not applicable, 1=green |
| *Tuber* | |
| Type of tuber* | 1=underground, 2=aerial |
| Tuber shape* | 1=cylindrical, 2=irregular |
| Tendency to branch* | 0=none, 1=slightly branched, 2=branched, 3=highly branched |
| Tuber flesh color, proximal end* | 1=white, 2=white with purple, 3=purple with white |
| Tuber flesh color, middle section* | 1=white, 2=white with purple, 3=purple with white |
| Tuber flesh color, distal end* | 1=white, 2=white with purple, 3=purple with white |
| *Inflorescence* | |
| Flowering* | 0=no, 1=yes |
| Sex* | 1=female, 2=male |
| Type of inflorescence* | 1=spike, 2=raceme, 3=panicle |

*Traits used for clustering and principal component analysis

## 3.3. Results

### 3.3.1. Farmers' classification of yam landraces

Farmers in the study area use a combination of different criteria to classify yam landraces. While *boye* is the local name for yams in Wolayita and Gamo-Gofa zones, those that produce aerial tubers or bulbils (*D. bulbifera*) are referred to by the name *bola-boye* (which literally means 'aboveground yam') in order to distinguish them from species producing underground tubers. Farmers use a common set of criteria for further classification of their landraces into different groups (Table 3.3). Nevertheless, the number of criteria used and the relative importance of each criterion appeared to vary from place to place.

Wolayita and Gamo-Gofa farmers recognize two major categories of yam landraces with underground tubers: *hatuma boye* (‘male’ yam) and *macha boye* (‘female’ yam). Designation of the two as ‘female’ and ‘male’ has no reference to the reproductive biology of the landraces, notwithstanding the fact that most yam species are dioecious. It also became apparent during the survey that local farmers are not aware of the existence of such a phenomenon in yam. The categorization is rather based on maturity time, morphological traits, growth and other related attributes (Table 3.4). Besides, this classification takes into account a certain degree of ecological adaptation. While the *macha* group requires optimum environmental conditions for growth and reasonable yields, the *hatuma* are generally tolerant to drought and higher temperatures.

Within each group, morphological attributes such as stem color, presence or absence of spines, leaf color and shape, and tuber flesh color are principal criteria for identifying individual landraces. For example, the main varietal classes within the *macha* group are the ‘white’ and ‘variegated’ (white and purple with varying intensity of the purple coloration) flesh-colored types. *Hatiye* has a uniform white tuber flesh, while *oha* exhibits a predominantly white tuber flesh color mixed with purple. However, the identification of individual landraces within the *hatuma* group combines various attributes more than just morphological characteristics (Table 3.5).

Yam is referred to as *bohe* or *boyina* in Sidama and Gedeo zones, where production is based exclusively on early-maturing landraces. Here, tuber flesh color is the principal criterion for classifying available landraces. Based on farmers’ account of their main distinguishing features, landraces found in Sidama and Gedeo roughly correspond to the *macha* group in Wolayita and Gamo-Gofa zones. Although the landraces are named differently in the local languages, linguistic reflection of key morphological features is similar.

**Table 3.3.** Farmers' criteria used for local classification of yam landraces in Southern Ethiopia.

| Descriptor | |
|---|---|
| Morphological characters | – Stem color<br>– Presence or absence of spines on stems and tuber surfaces<br>– Leaf size, color, and shape |
| Growth attributes | – Vigor<br>– Maturity time (duration) |
| Organoleptic or utilization qualities | – Taste<br>– Firmness of tuber<br>– Tuber flesh color |
| Ecological adaptation | – Tolerance to drought and high temperature conditions |

**Table 3.4.** Characteristics of *hatuma* ('male') and *macha* ('female') *boye* (yam) in Wolayita and Gamo-Gofa zones of Southern Ethiopia.

| Characteristics | Category | |
|---|---|---|
| | *Macha boye* | *Hatuma boye* |
| Maturity time | Early | Late |
| Harvesting | Twice | Once |
| Plant vigor | Less vigorous | Vigorous |
| Number of tubers per plant | Multiple following the first harvest | Mostly one or two |
| Yield per plant | Relatively low | High |
| Tolerance to drought and unfavorable growth conditions | Susceptible | Tolerant |
| Tuber quality | Mostly sweet | Bitter at early stage of maturity and only consumed at full maturity |

**Table 3.5.** Attributes of yam landraces as described by farmers in Southern Ethiopia.

| ***Hatuma boye* ('male' yam)** | |
|---|---|
| Landrace | Attributes |
| *Ayina (Ayino)* | – Brownish and thorny vines<br>– Dark brownish leaves<br>– Large tubers with purplish white flesh color<br>– High-yielding<br>– Soft tuber flesh that cooks easily<br>– Late-maturing but earlier than most of the other landraces in this group<br>– Harvested twice in some localities of Damot-Woyde |
| *Bune* | – Thorny vines even at early stage of growth<br>– Pale green and soft leaves<br>– Highly branched tubers (like 'dogs feet') with horizontal growth habit<br>– White tuber flesh color<br>– Flower-like structure with three angles<br>– Matures very late in the dry season (November-December)<br>– Tubers can be left in the soil for a couple of months<br>– Highly drought-tolerant |
| *Gajela* | – Brownish green leaves<br>– Relatively big tubers<br>– Deep purplish tuber flesh color |
| *Gena* | – Purplish leaves and vines<br>– Variegated tuber flesh color (purple and white)<br>– Large tubers<br>– Follows the early landraces in maturity |
| *Moratawa* | – Broad leaves with pale green color<br>– Thick vines<br>– White tuber flesh color |
| *Wadala* | – Thick vines and vigorous growth<br>– Large dark green leaves<br>– Large tubers<br>– Thorns on tuber surface<br>– Late-maturing<br>– Drought tolerant |
| *Waiya* | – Relatively small pale green leaves<br>– Vigorous growth and spiny vines<br>– White tuber flesh color<br>– |
| *Walabo* | – Very large tubers<br>– Slow to form tubers<br>– Slow-cooking type |
| *Zoreuwa* | – Dark green leaves<br>– Vines with many thorns<br>– Mostly white but slightly variegated tuber color |

**Table 3.5.** Continued.

| ***Macha boye* ('female' yam)** | |
|---|---|
| Landrace | Attributes |
| *Ado* | – Purplish vine color<br>– White tuber flesh color |
| *Ganticha* | – Vigorous growth and large tubers<br>– Thorns on vines<br>– Variegated tuber color<br>– Tuber with slightly bitter taste |
| *Hatiye* | – Small shiny leaves<br>– White tuber flesh color<br>– Sweet taste<br>– Early maturity |
| *Oha* | – Thorns on vines and, sometimes, tuber surface<br>– Purplish tuber flesh color |
| *Toracho* | – Sweet tuber taste<br>– White tuber flesh color |

### 3.3.2. Morphological diversity assessed by cluster analysis

The accessions studied showed considerable variation both in the underground and aerial morphological parts. Examples of variation in tuber and inflorescence morphology are given in Figure 3.2. Analysis of morphological variability based on Euclidean distance and UPGMA clustering gave seven major groups (Figure 3.3). The cophenetic correlation value of r = 0.84 also indicated a good fit for the cluster analysis (Rolf 2000). Cluster 1 included the accessions GGF 001 and GGF 002 that were collected under the same vernacular name (*wadala*) from different localities in Kucha district of Gamo-Gofa zone. Twelve of the fourteen accessions representing the late-maturing landraces from Wolayita were grouped together in cluster 2. Within this cluster, the accessions WOL 011 and WOL 012, representing the landraces *moratawa* and *ayina* (*ayino*), respectively formed a sub-group. Cluster 3 contained AKA 014, the only non-flowering accession in the collection.

Cluster 4 was the largest and constituted by 61 accessions. It included all the accessions from Gedeo (GED) and Sidama (SID), and some accessions from Woalyita

(WOL) and Gamo-Gofa (GGF) zones. Almost all accessions obtained from Areka Agricultural Research Center (AKA), except those of aerial yam and the non-flowering AKA 014, were grouped here. This cluster represented all the early-maturing landraces, except the two late-maturing landraces from Wolayita (WOL 001 and WOL 006), although data was not available on maturity time of the accessions from Areka (AKA). This cluster was further subdivided into two. Nevertheless, the morphological descriptors responsible for the sub-grouping could not be identified from the clustering. It was, however, clear that tuber flesh color did not show association with this sub-grouping as landraces with differing tuber colors were found in both sub-groups.

Cluster 5 represented GGF 004 (*bune*), one of the very few accessions with female inflorescence. This landrace is highly valued by farmers in some localities of Wolayita and Gamo-Gofa for its maturity late in the season (November and December), when the harvesting season is already over even for late-maturing landraces. Cluster 6 included WOL 022 and WOL 025. WOL 022 (*hatiye*) clustered separately from the other accessions known by the same vernacular name in cluster 4. WOL 025 was collected under the name *macha*, which is also a designation for the so-called 'female' yams (*macha boye*) in the study area. Cluster 7 consisted the accessions WOL 017, WOL 021, AKA 001, AKA 002 and AKA 003 that belong to a species of aerial yam.

### 3.3.3. Morphological diversity assessed by principal components analysis

Patterns of variation and the relative importance of each descriptor in explaining the observed variability were assessed through principal component analysis (PCA). The first nine principal components explained 77% of the variation, while the first principal component (PC 1) alone accounted for 23% of the total variability (Table 3.6). Descriptors such as leaf shape, direction of twining, leaf lobation, leaf folding, petiole wing color, stem color (both young and mature) and color of the young leaf had the highest loadings on PC 1. The second principal component (PC 2), explaining 18% of the total variation, was highly correlated with presence or absence of spines on stems, spine shape and tuber flesh color.


WOL-015 (*Wadala*) GGF-004 (*Bune*) AKA-017 WOL-013b (*Gajela*)


WOL-013b WOL-O14 GED-006 WOL-018b WOL-001 WOL-011 GGF-002 AKA-017 SID-001

**Figure 3.2.** Variation in tuber morphology (a) and inflorescence type (b) for selected yam accessions collected from Southern Ethiopia. [Staminate inflorescence with increasing number of spikes (SID-001, WOL-001, WOL-011, GGF-002, WOL-014 and GED-006), panicle type staminate inflorescence (WOL-013b, WOL-018b), and pistilate inflorescence showing fruit capsule (AKA-017)].

To assess the score of individual accessions, PC 1 and PC 2 were plotted (Figure 3.4). The aerial yam accessions occupied the top left corner of the plot with the lowest scores for PC1 and the highest positive values for PC2. All the late-maturing landraces, except WOL 001 and WOL 006, had the highest positive scores for both components and grouped to the right top corner of the plot. The third biggest set, representing the early-maturing landraces and those from Areka, was grouped in the middle. This finding is consistent with the separation of accessions into three main groups (aerial, early-maturing, and late-maturing landraces) by UPGMA clustering (Figure 3.2). It also roughly corresponded with the two major categories recognized by local farmers.

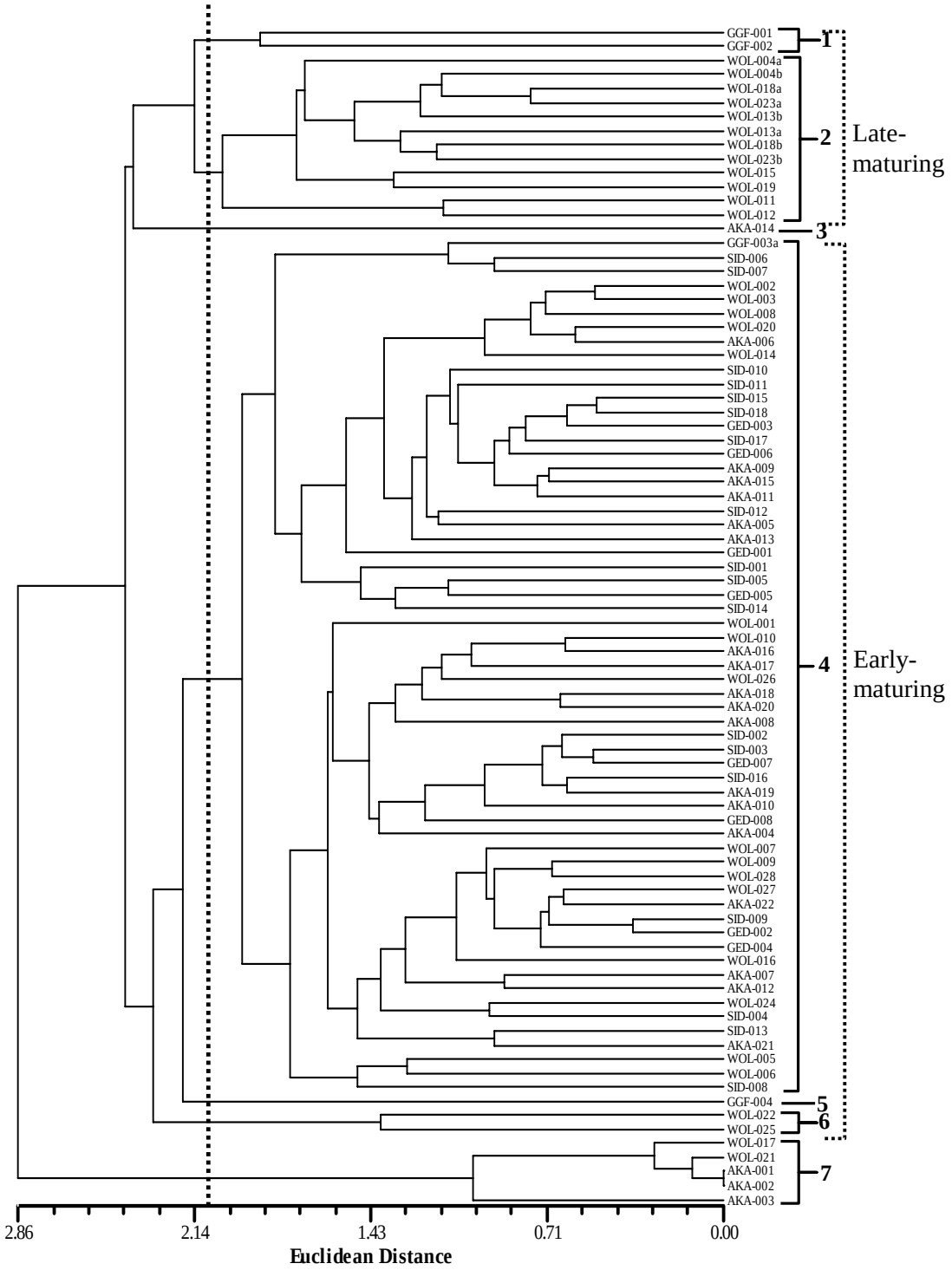



**Figure 3.3.** Relationship of 84 yam accessions (*Dioscorea* spp.) from Southern Ethiopia based on Euclidean distance and UPGMA clustering using 29 qualitative morphological characters.

The third principal component (PC 3) was highly correlated with presence or absence of petiole wings, petiole wing color, leaf margin color and tuber shape (Table 3.6). The

plot of PC2 and PC3 detected two sub-groups within the accessions representing the early-maturing landraces (Figure 3.5). Accordingly, it revealed the main morphological traits responsible for the sub-groups detected within cluster 4 by UPGMA clustering (Figure 3.2).

**Table 3.6.** Eigenvalues, variance, cumulative variance and component scores (eigenvectors) of the first 9 principal components (PC) for morphological divergence in 84 accessions of *Dioscorea* spp. from Ethiopia.

| | Component scores | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | PC 1 | PC 2 | PC 3 | PC 4 | PC 5 | PC 6 | PC 7 | PC 8 | PC 9 |
| Eigenvalues | 7.25 | 5.89 | 2.43 | 2.12 | 1.92 | 1.56 | 1.18 | 1.13 | 1.04 |
| Variance (%) | 22.67 | 18.40 | 7.59 | 6.64 | 5.99 | 4.88 | 3.70 | 3.53 | 3.27 |
| Cumulative (%) | 22.67 | 41.07 | 48.66 | 55.30 | 61.28 | 66.16 | 69.86 | 73.40 | 76.66 |
| Stem color, young | **0.63** | 0.27 | 0.01 | -0.05 | 0.25 | 0.15 | -0.27 | 0.05 | 0.03 |
| Spines on young stems | 0.13 | **-0.61** | 0.13 | 0.10 | -0.13 | 0.02 | -0.05 | -0.01 | 0.28 |
| Direction of twining | **-0.87** | 0.40 | 0.15 | 0.10 | -0.04 | 0.14 | 0.03 | 0.03 | 0.03 |
| Stem color, mature stem | 0.60 | -0.05 | 0.07 | 0.01 | -0.11 | 0.31 | -0.45 | -0.13 | 0.29 |
| Spines at stem base | 0.22 | **-0.73** | 0.16 | 0.26 | -0.17 | 0.11 | 0.32 | -0.14 | 0.00 |
| Spines at stem above base | 0.11 | **-0.69** | 0.12 | 0.18 | -0.15 | 0.15 | 0.40 | -0.07 | -0.08 |
| Spine shape | 0.32 | **-0.67** | 0.13 | 0.30 | -0.23 | 0.21 | 0.18 | -0.02 | -0.07 |
| Color of young leaves | 0.61 | 0.57 | 0.16 | 0.06 | 0.04 | -0.05 | 0.17 | 0.02 | 0.10 |
| Leaf margin color, young leaves | 0.60 | 0.47 | 0.31 | 0.08 | 0.00 | -0.05 | 0.06 | 0.16 | 0.08 |
| Vein color, young leaves | 0.57 | 0.49 | 0.29 | 0.06 | 0.01 | 0.14 | 0.14 | 0.04 | 0.09 |
| Petiole color, young leaves | 0.59 | 0.52 | 0.29 | 0.07 | 0.03 | -0.04 | -0.05 | 0.00 | 0.11 |
| Petiole wing, young leaves | -0.21 | 0.09 | **0.76** | 0.20 | 0.18 | -0.37 | -0.11 | -0.08 | -0.14 |
| Petiole wing color, young leaves | 0.10 | -0.11 | **0.80** | 0.15 | 0.16 | **-0.41** | -0.10 | -0.03 | -0.11 |
| Leaf margin color, mature leaves | 0.08 | -0.06 | 0.46 | 0.19 | -0.21 | **0.51** | 0.04 | -0.20 | 0.36 |
| Leaf lobation | **-0.87** | 0.40 | 0.15 | 0.10 | -0.04 | 0.14 | 0.03 | 0.03 | 0.03 |
| Leaf color, mature leaves | -0.11 | -0.22 | 0.13 | 0.14 | **-0.57** | 0.08 | **-0.49** | 0.10 | -0.07 |
| Leaf vein color, upper surface | 0.08 | -0.26 | 0.33 | -0.10 | -0.26 | -0.13 | 0.26 | **0.58** | -0.04 |
| Leaf vein color, lower surface | 0.00 | 0.15 | 0.19 | **-0.51** | 0.13 | -0.01 | 0.17 | 0.25 | **0.46** |
| Leaf shape | **-0.88** | 0.27 | 0.06 | 0.03 | -0.02 | 0.15 | 0.02 | 0.06 | 0.09 |
| Upward folding of leaves | **0.86** | -0.38 | -0.14 | -0.13 | 0.11 | -0.04 | -0.07 | 0.03 | 0.01 |
| Leaf tip color | -0.03 | 0.13 | 0.04 | -0.17 | 0.49 | -0.04 | 0.29 | -0.55 | 0.13 |
| Petiole color | 0.36 | 0.43 | 0.12 | -0.27 | 0.11 | 0.36 | 0.11 | 0.24 | -0.10 |
| Petiole wing color | **-0.72** | 0.22 | 0.09 | 0.11 | -0.06 | 0.14 | 0.01 | -0.03 | 0.16 |
| Type of tuber | **-0.87** | 0.40 | 0.15 | 0.10 | -0.04 | 0.14 | 0.03 | 0.03 | 0.03 |
| Shape of tuber | -0.16 | -0.44 | 0.44 | -0.33 | 0.25 | 0.28 | -0.12 | -0.10 | -0.33 |
| Tendency of tubers to branch | -0.17 | -0.47 | 0.28 | -0.34 | 0.34 | 0.34 | -0.20 | -0.06 | -0.16 |
| Tuber flesh color, upper section | 0.28 | **0.66** | -0.11 | 0.14 | -0.25 | 0.09 | 0.13 | -0.22 | -0.12 |
| Tuber flesh color, middle section | 0.33 | **0.68** | -0.04 | 0.10 | -0.28 | 0.11 | 0.06 | -0.11 | -0.31 |
| Tuber flesh color, lower section | 0.36 | **0.64** | 0.12 | 0.10 | -0.10 | 0.26 | 0.04 | -0.04 | -0.33 |
| Flowering capacity | -0.06 | -0.18 | -0.10 | **0.73** | 0.45 | 0.05 | -0.09 | 0.18 | 0.09 |
| Sex of plants | 0.03 | -0.13 | -0.16 | 0.31 | **0.59** | 0.45 | 0.07 | 0.34 | -0.15 |
| Type of inflorescence | 0.13 | 0.21 | -0.16 | **0.68** | 0.25 | -0.14 | -0.09 | -0.01 | 0.09 |

Coefficients in bold indicate descriptors that are highly correlated with the corresponding principal component.

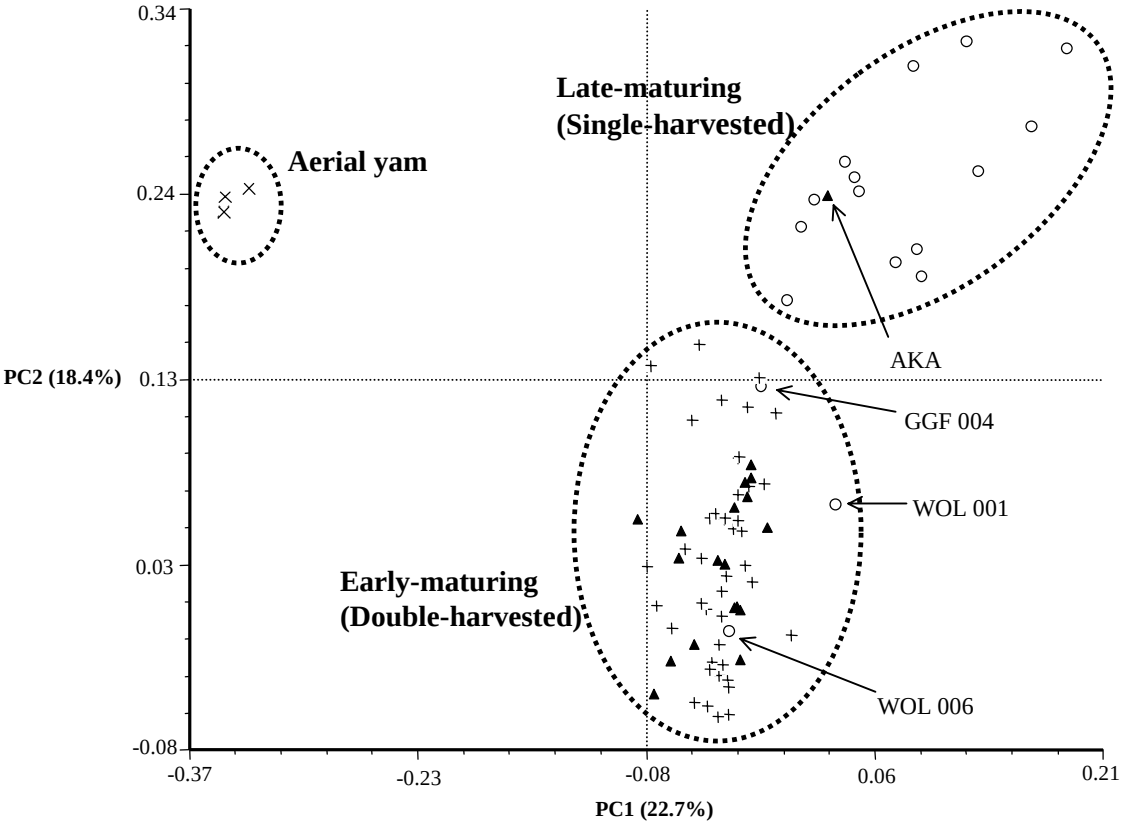



**Figure 3.4.** Plot of the first (PC1) and second (PC2) principal components for 84 yam accessions collected from Southern Ethiopia based on 29 qualitative morphological traits (o = *hatuma boye*, + = *macha boye*, × = *bola boye*, and ▲ = accessions obtained from Areka Agricultural Research Center).

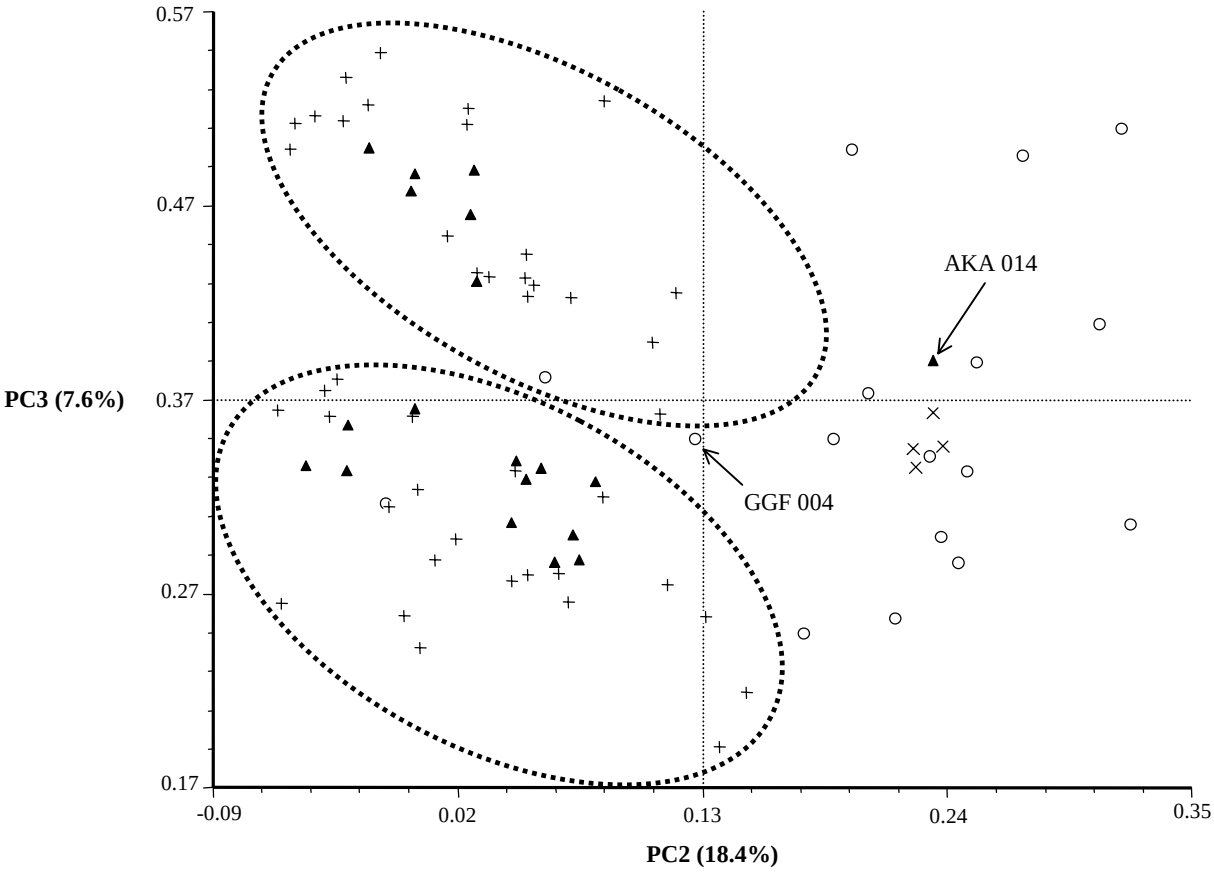



**Figure 3.5.** Plot of the second (PC 2) and third (PC 3) principal components indicating the sub-grouping within early-maturing yam landraces (○ = *hatuma boye*, + = *macha boye*, × = *bola boye*, and ▲ = accessions obtained from Areka Agricultural Research Center)

## 3.4. Discussion

### 3.4.1. Yam diversity recognized by local farmers

Farmers' selection and management of crop diversity is facilitated through a complex local classification system (Brush et al. 1981), which is developed as it suits their needs without reference to any code of nomenclature. As selections are often made in terms of known categories, farmers manage not only crop plants but also their classification scheme (Boster 1985). The local classification system reported here shows a pattern of hierarchy in that it starts with a more general grouping of the available landraces into those that produce either aerial or underground tubers. At this level, it is akin to the

conventional botanical taxonomy, where the major cultivated species are classified into sections within the genus *Dioscorea* mainly based on direction of vine twining.

Landraces with underground tubers are further separated into two groups as 'male' (*hatuma*) and 'female' (*macha*). This is followed by a further distinction whereby individual landraces are identified on account of various morphological traits as well as other plant attributes. While groupings at the higher categories recognized by the farmer was relatively easier, identification of individual landraces often required a good understanding of the various closely related plant attributes. These features, rather typical for folk taxonomy, were reported for potato in the Andes (Brush et al. 1981; Quiros et al. 1990), cassava in Peru (Boster 1985), and maize in Mexico (Hernández-Xolocotzi 1985), where farmers manage a considerable diversity of local varieties.

The categorization of yam landraces as 'female' and 'male' is an interesting aspect of the local classification system. Careful scrutiny of farmers' account of the two categories indicates that this grouping reflects more than mere differences in agro-morphological traits and ecological adaptation. It appears that the system has also a bearing on the society's perception of gender and its role. The *hatuma* group is vigorous in growth, tolerant to sub-optimal conditions and produces bigger tubers. These attributes in a way depict what is expected of a man in the society: to be strong and endure hardship. Conversely, the *macha* yams are the first to be harvested and, thus, fill a seasonal gap in food supply. They also require care and optimum growth conditions, and produce tubers with excellent eating quality. As farmers often put it "*macha boye* sustain life of the family during critical period of the year, give multiple tubers at the second harvest that makes propagation easier, and produce sweet tubers. Besides, they require good care like our ladies".

This gender-related categorization of crop landraces in the study area is not peculiar to yam. A similar system also exists for enset in Sidama and Wolayita (personal observation), where local landraces are separated as 'male' and 'female' partly based on the shape of the pseudostem. Negash and Nieof (2004) recently reported a gender-based classification of enset landraces in Keffa-Sheka zone, Southern Ethiopia. Following a detailed description of the major attributes of the two groups (female vs. male), they pointed out that such categories reflect the fact that men and women prefer

different qualities in their landraces. Moreover, the 'female' characters are related to consumption qualities, highlighting the role of women in household food security. These findings further reveal the complex nature of folk taxonomy and support the view that crops are biological as well as cultural entities, which makes the study of both evolution and society possible through diversity (Brush 2004).

Many non-adaptive plant characters, because they are perceptually salient, are widely used in local classification systems (Boster 1985). Pigmentations associated with some morphological traits are among such characters commonly utilized in identifying local varieties in many crops (Brush et al. 1981; Boster 1985; Teshome et al. 1999) including yams (Onyilagha and Lowe 1985; Hamon and Touré 1990b; Mignouna et al. 2002a). The role of tuber flesh color for identification of yam landraces in the study area is also apparent in many names. Examples include *ado* (white or milk) and *ganticho* (variegated) in Sidama, and *toracho* (white) in Gedeo. Sidama and Gedeo languages belong to the Cushitic language family and share lexical similarity of about 60% (www.ethnologue.com). Even when not directly reflected in the names, tuber flesh color is a key variable in distinguishing many landraces. Nevertheless, how much of this color variation in yam is represented by differences at DNA level remains to be investigated.

Although traditional farmers in the tropics often have access to a range of plant species, a system of local classification is known for a limited number of crops that are long associated with and have a significant role in the livelihood of a given society (Berlin 1992). Our observation is in agreement with this general fact. For example, although sweet potato is currently one of the major crops in Wolayita both in terms of area of production and in maintaining household food security, it has a relatively recent production history. Thus, cultivars under production are mainly known by names given by researchers (personal observation). On the other hand, there exists a well-defined local classification system for enset, one of the most important indigenous root crops in Sidama and Wolayita (Tsegaye 2002; Tesfaye 2003; Negash and Nieof 2004).

### 3.4.2. Patterns of morphological diversity and their correspondence with folk taxonomy

The analysis of morphological variation through cluster and principal component analyses gave well-defined groups. The PCA confirmed that characters such as direction of twining, type of tubers and leaf characteristics (shape, lobation, petiole wing color and shape) are important to distinguish accessions of aerial yam from those that produce underground tubers. Direction of twining is considered a key morphological feature to classify yam species into various sections (Onwueme and Charles 1994). Major groups constituted by cluster analysis were also detected in the PCA, the most important morphological traits being those with high loadings on the first and second principal components (Table 3.6).

Accessions with underground tubers were basically clustered into two groups on the basis of differences in presence/absence of spines, leaf shape and pigments associated with various plant parts. These clusters also mainly reflected the two maturity groups recognized by local farmers, a result congruent with earlier findings for West African yams. In their classification of 393 accessions of the *D. cayenensis-rotundata* complex, Hamon and Touré (1990b) found two major morphological groups constituted partly based on variation in length of the vegetative cycle. Dansi et al. (1999) also reported two main classes in 560 accessions of yam collected from Benin Republic on the basis of maturity time, while further sub-groupings took into account the variability expressed in different morphological traits.

The two accessions from Gamo-Gofa (GGF 001 and GGF 002) were clustered separately from other accessions of similar maturity group from Wolayita, some of which are also known by the same vernacular name. This represents a case where different materials are named similarly and, hence, farmers' classification underestimates the actual diversity. If the claim that yam was originally introduced from Gamo-Gofa into Wolayita long time ago is valid, this variation could be a result of mutation accumulated under cultivation since the introduction. Various domesticates become partitioned for several reasons, such as difference in use by man and adaptation to different environments. These sub-groups often have predictive values associated with good natural groups (Pickersgill 1986). However, caution is

needed in interpreting the result of this study as the number of landraces or accessions of each landrace that represented the two zones significantly differ. Only two accessions of *wadala* were included from Gamo-Gofa.

While there was some evidence of correlation between clustering of the late-maturing accessions and geographic area, there was no geographic differentiation within the early-maturing accessions. The latter types are highly valued for their early maturity, relatively higher multiplication ratio and excellent organoleptic qualities. These attributes, which made them popular among the different ethnic groups, must have also facilitated their spread across wider geographic areas. In all the localities covered in this study, identification and farmers' evaluation of these landraces is made in the same way based on similar key traits. These landraces are the most preferred types in the study area and are being utilized for expanding production in most localities. A similar case, where landraces with more desirable attributes are distributed across a larger area, has been reported for yam in the Republic of Benin (Dansi et al. 1999).

The lack of geographic pattern of variation within the early-maturing landraces is probably due to the fact that similar selection forces, based on farmers' criteria, are operating under cultivation in the different areas. For example, landraces with white tuber flesh color are the popular choice across the study area and particularly preferred in Wolayita for preparation of *fichata*. This human selection against pigmented tubers appears to be responsible for the high frequency of early-maturing landraces with white tubers such as in *hatiye*, *ado*, and *toracho*. This supports the claim by some elderly Sidama farmers that settlers from Wolayita ethnic group have introduced yam and its culture into their area. Moreover, the level of diversity present, depth in the local classification system, and the role of yam in the socio-cultural life of the inhabitants in Wolayita and Gamo-Gofa indicate that yam cultivation in these areas has longer history than in Gedeo and Sidama.

Another finding of this study relates to the extent to which farmers consider reproductive morphological traits for identification of yam landraces. Almost all accessions studied have a spike type of staminate inflorescence except GGF 004, AKA 004, AKA 013 and AKA 017, which have pistilate inflorescences. However, WOL 004b, WOL 013b, WOL 018b and WOL 023b that were originally registered with the

code WOL 004, WOL 013, WOL 018 and WOL 023, respectively, were later treated as separate accessions owing to their peculiar (panicle type) staminate inflorescence compared to the other plants in the samples (Figure 3.2b). Farmers do not perceive these as different. It is, thus, common to see individual plants with both types of inflorescence within populations managed as similar. The farmers recognize the pistilate inflorescence of GGF 004 (*bune*), which they described as "flower-like structure with three wings" in reference to shape of the fruit capsule. This appears to be related to the popularity of the landrace for its maturity late in the season.

Classification of local landraces according to inflorescence morphology is known in vegetatively propagated crops like banana (Ortiz et al. 1998). Similarly, Jianchu et al. (2001) reported a case in China where inflorescence morphology is becoming a key criterion in local classification of taro landraces due to its increasing use as a vegetable. Such reports seem to suggest that traditional farmers consider variation in inflorescence morphology only when it is related to parts used for consumption or multiplication. However, such a generalization would be a gross oversimplification of the complex nature of local classification and management of diversity.

In general, our findings demonstrated that morphological groups obtained by multivariate statistics are consistent to a considerable extent with yam categories recognized by local farmers. Although maturity time was not among the criteria considered in the multivariate analysis, the finding roughly corresponds with farmers' classification of their landraces as *hatuma* and *macha boye* (Figure 3.4). This shows that agronomic traits are reflected in morphological characters. Thus, the relationship between local classification system and morphological diversity is relevant at least for two reasons. First, if there exists any relationship between maturity time and genetic identity, as earlier reports indicate for yam (Hamon et al. 2001), such a classification has clear biological implications. Second, as farmers' exchanges of germplasm are often made according to locally known names, the existing classification system has a bearing on the distribution of the various landraces.

## 3.5. Conclusions and future prospects

Findings of this study, together with our earlier report on farm level landrace diversity, management and use of yam in the present study area, gives a good overview of the structure and distribution of diversity in yams. Main conclusions and issues that warrant further attention include:

- Gamo-Gofa and Wolayita zones represent important areas of yam diversity as indicated not only in terms of number of landraces grown and structure of morphological diversity but also in the depth of farmers' knowledge of their landraces. The lack of a geographical pattern of variation within the early-maturing landraces that are also well distributed in Sidama and Gedeo zones, as well as the oral history on distribution of yams in the study area, confirm the importance of yam diversity in Wolayita and Gamo-Gofa zones. This finding is important in planning conservation and development programs.

- Selection for desirable agro-morphological traits, as well as socio-cultural factors appear to be the major forces behind the dynamics of yam diversity in the study area. Such an observation is central in designing an appropriate conservation strategy. Apart from the fact that long-term *ex situ* conservation of yam tubers, like in many vegetative species and those with non-orthodox seeds, is challenging, *in situ* conservation is appropriate to preserve the dynamic evolutionary processes in the field. This approach can draw on and also ensure conservation of the local indigenous knowledge about yams.

- Early-maturing landraces are the most popular as they fit well into the local subsistence agriculture and, thus, currently are the preferred choice for expanding yam production. Thus, urgent attention is required to conserve the late-maturing landraces, whose production is being threatened by the expansion of the early-maturing ones.

- Despite the potential of morphological characterization in diversity studies, the expression of morphological characters is partly subjected to environmental variation and, thus, provides limited genetic information. This was apparent from

the lack of association between the morphological groups detected and some landrace names particularly within late-maturing types. Thus, a detailed characterization entails DNA-based techniques to reveal the extent of the existing diversity. The presence of some accessions with peculiar type of inflorescence in the collection and the fact that the accessions could not be unequivocally identified with known species make taxonomic and phylogenetic studies necessary. In light of the present confusion regarding the taxonomic status of the African species, taxonomic and phylogenic studies are also invaluable additions to the current knowledge of yams.

Views from the experimental plot established for morphological characterization of yam accessions.
(Photo: Muluneh Tamiru)

↑ Yam morphological diversity in both the aerial and underground vegetative parts (Photo: Muluneh Tamiru)

*D. bulbifera* (aerial yam) plant with aerial tubers (bulbils). (Photo: Muluneh Tamiru)

# 4. Genetic Diversity in Yam Germplasm (*Dioscorea* sp.) from Ethiopia and their Relatedness to the main Cultivated *Dioscorea* Species Assessed by AFLP Markers


**Abstract**

*Farmers in Southern Ethiopia maintain considerable number of named yam landraces, most of which are morphologically distinct. Nonetheless, the diversity in some morphological groups and the species identity of most of the landraces are yet to be established. In the present study, AFLP markers were used to investigate the extent of genetic diversity in selected Ethiopian yam germplasm accessions, and compare their relationships with the commonly cultivated yam species such as D. alata, D. bulbifera, D. cayenensis and D. rotundata. Inter and intraspecific genetic similarities were estimated using Jaccard Coefficient based on ten AFLP primer combinations that generated 900 fragments, of which 97% were polymorphic. Cluster and principal coordinate analyses, separating the accessions into their respective taxa, revealed the distinctiveness of the germplasm from Ethiopia. The Ethiopian materials were genetically closer to D. cayenensis and D. rotundata than to the other species. A separate analysis of the Ethiopian materials gave six clusters representing mostly the various maturity groups, and the only non-flowering accession in the collection. It also revealed that some accessions collected under the same local name are genetically distant. Analysis of molecular variance (AMOVA) showed that 81% of the variation detected was found within collecting areas, while the variation among collecting areas contributed only 19%. The groups detected by AFLP markers were highly consistent with farmers' landrace classification based on time of maturity. To a large extent, they also reflected the structure of morphological diversity. Further studies are required to establish the species identity of Ethiopian yams. This will significantly contribute to our understanding of the current puzzle surrounding taxonomy of the major African species.*



**Keywords**: AFLP analysis; *Dioscorea*; Ethiopia; genetic diversity

## 4.1. Introduction

Yam belongs to the genus *Dioscorea* in the family Dioscoreaceae, and represents a multi-species tuber crop widely distributed throughout the humid and sub-humid tropics (Coursey 1967). It is believed that the major food species originated in three isolated regions: Southeast Asia, West Africa and Tropical America, which are also considered as centers of yam domestication and diversity (Asiedu et al. 1997). Yam has a significant economic and social importance for millions of people in sub-Saharan Africa, where more that 95% of the world yam is produced (Degras 1993). Ethiopia is an important center of yam cultivation in East Africa, and the crop plays a significant role in local livelihood particularly in the densely populated areas of Southern, Southwestern and Western parts of the country.

Yam shows considerable diversity both at inter and intraspecific levels (Okoli 1991). The diversity under cultivation is further enhanced by the on-going domestications of wild yams in many countries (Mignouna and Dansi 2003; Scarcelli et al. 2006). Similar trends exist in some localities in Ethiopia, where transplanting of wild yam tubers to farmers' fields forms part of the yam cultivation practice (Hildebrand 2003; Chapter 2). Nevertheless, the extent of genetic diversity in many *Dioscorea* species and their relationships is yet to be investigated in detail. Attempts to classify yam using morphological (Hamon and Touré 1990b; Dansi et al. 1999) and isozyme (Hamon and Touré 1990a; Dansi et al. 2000a) markers did not give conclusive results due to their high degree of variability. Chromosome counts are also variable in yams, ranging from 2n = 20 to 2n = 140 in the common food species (Hahn 1995).

Different molecular markers are available for measuring diversity directly at DNA level and, hence, avoid the inherent limitations associated with morphological and biochemical markers. Recently, these markers have been used in many food species including clonally propagated crops such as cassava (Chavrriaga-Aguirre et al. 1999), enset (Negash et al. 2002), sweet potato (Zhang et al. 2000), and *Musa* spp. (Ude et al. 2003) for purposes of germplasm acquisition, maintenance and improvement. Techniques such as RFLP (Restriction Fragment Length Polymorphism), RAPD (Random Amplified Polymorphic DNA) and AFLP (Amplified Fragment Length Polymorphism) have also been applied in yams for taxonomic, phylogenetic and

diversity studies (Terauchi et al. 1992; Asemota et al. 1996; Ramser et al. 1996, 1997; Mignouna et al. 1998; Dansi et al. 2000b). Most of the markers assayed were able to detect differences among cultivars that were considered similar based on morphological and isozyme markers, demonstrating their usefulness as discriminative tools in yam. Efforts are now under way for the broader application of molecular markers for genetic improvement of the crop (Mignouna et al. 2003a).

The AFLP technique, developed by Vos et al. (1995), belongs to the category of selective restriction fragment amplification technique that combines the use of restriction enzymes and polymerase chain reaction (PCR). Since its development, AFLP has established itself as a popular DNA marker with a high degree of reproducibility and discriminative power (Savelkoul et al. 1999). As it requires no previous sequence information, it is applicable to a broad range of organisms (Weising et al. 2005).

Estimates of genetic relationships based on AFLP patterns provide useful information about genetic diversity (Negash et al. 2002), phylogeny (Sharma et al. 1996) and the geographic origin of genotypes and gene pools of plants (Paul et al. 1997; Anthony et al. 2002). This method has been successfully used in yams for diversity studies (Mignouna et al. 1998; Malapa et al. 2005) and construction of genetic linkage maps (Mignouna et al. 2002b). Compared to other markers such as RAPD, the AFLP technique generates more polymorphic markers and, thus, proved to be a sensitive and robust DNA fingerprinting technique for genomic analysis in yams, including the detection of duplicates in germplasm collections (Mignouna et al. 2003b).

The diversity in Ethiopian yams, particularly under cultivation, is poorly understood. A previous study revealed the presence of a substantial number of landraces with varying degree of abundance and distribution in Wolayita and Gamo-Gofa zones of Southern Ethiopia (Chapter 2). Accessions collected from the same zones and additional localities in Southern Ethiopia were also characterized based on key morphological traits (Chapter 3). Our finding was that named landraces often represent phenotypically distinct materials, and the overall structure of morphological diversity is largely consistent with farmers' classification of their landraces. The actual diversity at DNA level is, however, yet to be investigated. Moreover, the use of standard descriptors did

not allow accurate classification of the accessions into any of the known cultivated *Dioscorea* species.

The main objective of this study was to use AFLP markers for a detailed analysis of genetic diversity among yam accessions collected from Southern Ethiopia. It also aims at ascertaining species identity of the accessions by including elite yam genotypes from the main cultivated *Dioscorea* species as reference materials. Most previous works involving the use of molecular markers in diversity studies were on yams from West Africa. Therefore, as the first of its kind on Ethiopian yams, the study strives to generate information that is crucial in guiding improvement and conservation programs in the country.

## 4.2. Materials and methods

### 4.2.1. Plant material

Sixty-two yam accessions were considered in this study (Table 4.1), and 53 of them were selected among the collection assembled from the major yam growing areas in Southern Ethiopia and previously used for morphological characterization (Chapter 3). They represent the variability in morphological traits, maturity time, and landrace names. Of these, five accessions belong to the species *D. bulbifera* (aerial yam), while the remaining 48 accessions could not be clearly identified based on conventional taxonomic procedures for *Dioscorea* species. Additional nine elite genotypes, representing three major cultivated yam species, were obtained from the International Institute of Tropical Agriculture (IITA) in Nigeria and included as reference materials.

The Ethiopian accessions were transported to Germany as seed tubers, and were established in the greenhouse of the Department of Crop Sciences at Georg-August-University Göttingen. The genotypes from IITA were received as tissue culture plantlets, and were transferred to fresh medium for furnishing young leaves for DNA extraction.

### 4.2.2. DNA isolation

Nucleon PhytoPure plant and fungal DNA extraction kit from Amersham (Amersham™ Biosciences, Freiburg, Germany), was used for extraction of total genomic DNA. Approximately 0.1 g of fresh young leaves were harvested from single plants into a 1.5 ml eppendorf tube, immediately frozen with liquid nitrogen and stored at -20ºC. DNA was extracted following the extraction and purification protocols of the manufacturer with the following minor modifications. Mercaptoethanol and RNase were added to Reagent 1 at concentrations of 10 mM and 20 µg/ml, respectively. Following precipitation of the DNA with cold isopropanol, samples were stored over night at 4ºC. After DNA was re-suspended in TE buffer (10 mM trisHCl pH 8.0, 1mM EDTA) samples were incubated at 65ºC in a shaking water bath for 1 hr to ensure a good re-suspension.

DNA concentration was measured by a fluorescent DNA quantification method using a Versa Fluro™ flurometer (Bio-Rad laboratories, Hercules, USA) with the flurochrome dye Hoechst 3325. DNA quality was checked on 1% agarose gels prepared with TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0). The gels were run for 2 hrs at 100 V. Samples were finally diluted to a standard concentration of 50 ng/µl with TE buffer.

### 4.2.3. AFLP analysis

#### 4.2.3.1. Restriction-Ligation

AFLP analysis was performed following the procedures described by Vos et al. (1995). Approximately 250 ng DNA samples were digested with 4 units of both the restriction endonucleases *Eco*RI and *Mse*I for 1:30 hr at 37ºC in a final reaction volume of 30 µl containing 1× restriction-ligation (RL) buffer (10 mM Tris-acetate acid, 10 mM Magnesium Acetate, 50 mM Potassium Acetate, 5 mM DTT, pH 7.5). Ligation of adapters followed immediately using 5 pmoles *Eco*RI and 50 pmoles *Mse*I adapters. The adapter ligation mixture contained 30 µl of the restriction digestion aliquot to which 1 unit of $T_4$ DNA Ligase (Promega GmbH, Germany), 1× RL buffer, PCR grade water, and 0.25 mM ATP were added giving a final reaction volume of 40 µl.

The ligation of adapters was carried out in a Biometra T-Gradient thermo cycler (Biometra, Göttingen, Germany) with a program of 37ºC/3 hr and 10 min.; 33.5ºC/3 min.; 30ºC/3 min.; 26ºC/4 min.; and 22ºC/15 min. The program was designed to maintain the optimum activity of *Eco*RI and *Mse*I enzymes for the first 3 hrs and 10 sec. in order to restrict fragment-to-fragment ligation. The activity of $T_4$ DNA Ligase was kept optimal by maintaining the temperature at about 22ºC over the final 15 min. of the reaction. An aliquot of the digested-ligated template DNA was diluted 1:5 with TE buffer, and 8 µl of the dilution was used as a template for the preamplification reaction.

#### 4.2.3.2. Preamplification

To increases the amount of template available for fingerprinting and ensure complete selectivity of the final amplification, preamplification reactions were carried out with primers having single selective nucleotides at their 3´ end (E01 and M02) (Table 4.2). The 20 µl reaction mix was made of 8 µl of the digested and ligated template DNA and 12 µl preamplification mix containing 0.3 mM dNTPs, 1.5 U *Taq*-DNA-Polymerase (Solis BioDyne, Estonia), 1× PCR buffer (10 mM Tris-HCl, 1.5 mM $MgCl_2$, 50 mM KCl, pH 8.3), 10 pmoles *Eco*RI (A-3), and 8.7 pmoles *Mse*I (C-3). 2.5 mM $MgCl_2$ was added from 25 mM stock solution bringing the final concentration of $MgCl_2$ to 4 mM. The temperature/time profile of the cycles was an initial 94ºC/30 sec. for denaturing DNA, and then 20 cycles of 94ºC/30 sec. denaturing, 56ºC/30 sec. annealing, and 72ºC/60 sec. extension. A final step of 5 min. extension and incubation was carried out at 72ºC. The preamplification product was finally diluted 1:10 with TE buffer and used in the amplification reaction.

#### 4.2.3.3. Amplification

Ten primer combinations, including those previously used in yam genome analysis (Mignouna et al. 1998; 2002b; 2003b), were employed in this study. Amplification reactions were carried out using 2 pmoles and 7 pmoles *Eco*RI and *Mse*I primers, respectively, having three selective nucleotides at their 3´ ends (Table 4.2). The 20 µl reaction mixture contained 14 µl of amplification mix (0.24 mM dNTPs, 0.6 U *Taq*-DNA-polymerase, 1× PCR-buffer, and 4 mM $MgCl_2$) and 6 µl of the diluted

preamplification product as a template. The PCR was performed with initial denaturation at 94ºC for 30 sec., one cycle with a temperature/time profile of 94ºC/30 sec. (denaturing), 65ºC/30 sec. (annealing) and 72ºC/60 sec. (extension) followed by 11 cycles of touch down protocol with a similar temperature/time profile as in the previous cycle but with the annealing temperature being lowered by about 0.7ºC in each cycle. This was followed by a further 24 cycles with a temperature/time profile of 94ºC for 30 sec., 56ºC for 30 sec. and 72ºC for 60 sec. (extended by 1 second per cycle).

#### 4.2.3.4. Gel electrophoresis

Gel electrophoresis and detection of the AFLP amplification products were carried out on an automated DNA sequencer (Li-Cor 4200 *IR*$^2$, Li-Cor Inc, Nebraska, USA). The AFLP fragments were mixed with a loading dye [98% (v/v) formamide, 10 mM EDTA, 0.025% bromophenol blue, and 0.025% xylene cyanol] at a 2:1 ratio. The mixtures were denatured for 4 min. at 95ºC and then quickly cooled on ice before loading. The fragments were resolved on 6% denaturing polyacrylamide gels (25 cm x 0.2 mm) containing polyacrylamide (acrylamide/bisacrylamide), 1.386 M Urea (NF-urea Rotiphore®), 10× TBE buffer (1.34 mM Tris-HCl, 450 mM Borci Acid, 25 mM EDTA, pH = 9.2), 12% Long Ranger™ (50% gel solution). Polymerization was started by the addition of 0.01% TMED. The gels were pre-run ($T^o$ = 45°C, Voltage = 1000 V, and Current = 37 mA) for 15 min. before loading the samples. About 1.4 µl of each sample was loaded and fragment mobility measured by a real-time laser for 6:30 hr using the same temperature, current and voltage profiles as in the pre-run. All 62 samples were run on the same gel, thus, one gel was used per primer combination. To facilitate data scoring, a 50-700 bp DNA sizing standard (LI-COR® Biotechnology, USA) was used. Gel images were stored electronically for further analysis.

### 4.2.4. Data scoring and analysis

Polymorphic bands were scored as 1 (present) and 0 (absent). A band was considered polymorphic if it was present in at least one accession and absent in others. Mostly, clearly scorable bands were considered. But in very few cases where it was not possible to clearly decide whether a band was present or absent mainly due to a low intensity, it was scored as 9 and later considered as missing data point in the analysis.

Fragment scoring was performed manually with the help of the Adobe Photoshop software (Adobe®photoshop®7.0).

The data matrix for all the 62 accessions and the 10 primer combinations used, excluding monomorphic bands, was used to calculate pair-wise genetic similarity based on Jaccards Coefficient ($GS_J$). The resulting similarity matrix was subjected to clustering using the unweighted pair group method using arithmetic means (UPGMA) algorithm, and principal coordinate analysis with the help of the computer program NTSYSpc Version 2.1 (Rohlf 2000). The matrix was also subjected to bootstrapping using the software WinBoot (Yap and Nelson 1996), whereby 1000 randomly drawn samples were used to assess the solidity of the genetic relationships among the groups constituted by cluster analysis. To test the goodness of fit between the UPGMA dendrogram (cophenetic matrix) and the original similarity matrix, the cophenetic correlation coefficient was calculated by means of the MXCOMP function of the NTSYSpc. The unidentified accessions from Ethiopia were further subjected to similar analyses, separately. For these accessions, analysis of molecular variance (AMOVA) was also performed with the help of the software GenAlEx6 (Peakall and Smouse 2006) using collecting area (i.e., zones) as a grouping criterion.

## 4.3. Results

### 4.3.1. AFLP polymorphism

AFLP fingerprinting of the 62 accessions/genotypes with ten *Eco*RI/*Mse*I primer combinations resulted in the amplification of a total of 900 scorable fragments. Of these, 877 (97.4%) were polymorphic. The number of total fragments scored per primer combination varied from 73 to 119 with a mean of 90 fragments (Table 4.3). All the primer combinations used detected considerable diversity, the proportion of polymorphic fragments per primer pair varying from 94.5 to 100%. Overall, the size of fragments scored ranged from about 50 to 600 nucleotides.

The polymorphism detected within the different *Dioscorea* groups studied is summarized in Table 4.4. On average, the primer pairs used revealed a relatively

higher proportion of polymorphic bands within the accessions from Ethiopia (43.8%), ranging from 34.2% to 61.8%. This was followed by *D. rotundata* (39.7%). Very low polymorphism was detected within the accessions of *D. bulbifera* (6.1%).

### 4.3.2. Genetic diversity within and between groups

The comparison of pair-wise genetic similarity values revealed broad genetic diversity among the 62 accessions/genotypes studied (Figure 4.1). About 52% of the pair-wise comparisons represented genetic similarity greater than 0.8, 26% showed similarities less than 0.4, while the remaining 22% had genetic similarities values between 0.4 and 0.8. Overall, pair-wise genetic similarity values ranged from 0.088 to 1.000 in all the accessions considered, with a mean value of 0.645. The accessions AKA 001 (*D. bulbifera*) and TDr-932 (*D. rotundata*) were the most dissimilar, whereas WOL 014 and WOL 016, and SID 011, SID 017 and AKA 004 were genetically very close.

The mean genetic similarities within and among the various *Dioscorea* species studied are presented in Table 4.5. The most similar species were *D. rotundata* and *D. cayenensis* with mean similarity coefficient of 0.552. The species *D. bulbifera* was genetically the most distant from all the other species and the accessions from Ethiopia. The Ethiopian accessions were genetically closer to *D. rotundata* (0.419) and *D. cayenensis* (0.362), while *D. bulbifera* was relatively closer to *D. alata* (0.275) than to any of the other species studied. The mean intraspecific genetic similarity varied between 0.734 and 0.973 for *D. cayenensis* and *D. bulbifera* accessions, respectively. The accessions from Ethiopia exhibited a relatively higher within group genetic variability.

**Table 4.1.** Yam accessions studied.

| Accession | Country of origin | Collecting zones | Source | Type of material | Species | Accession | Country of origin | Collecting zones | Source | Type of material | Species |
|---|---|---|---|---|---|---|---|---|---|---|---|
| GGF 001 | Ethiopia | Gamo-Gofa | Farmers' field | Landrace | Unknown | SID 008 | Ethiopia | Sidama | Farmers' field | Landrace | Unknown |
| GGF 002 | Ethiopia | Gamo-Gofa | Farmers' field | Landrace | Unknown | SID 009 | Ethiopia | Sidama | Farmers' field | Landrace | Unknown |
| GGF 003 | Ethiopia | Gamo-Gofa | Farmers' field | Landrace | Unknown | SID 011 | Ethiopia | Sidama | Farmers' field | Landrace | Unknown |
| GGF 004 | Ethiopia | Gamo-Gofa | Farmers' field | Landrace | Unknown | SID 012 | Ethiopia | Sidama | Farmers' field | Landrace | Unknown |
| WOL 001 | Ethiopia | Wolayita | Farmers' field | Landrace | Unknown | SID 016 | Ethiopia | Sidama | Farmers' field | Landrace | Unknown |
| WOL 002 | Ethiopia | Wolayita | Farmers' field | Landrace | Unknown | SID 017 | Ethiopia | Sidama | Farmers' field | Landrace | Unknown |
| WOL 004a | Ethiopia | Wolayita | Farmers' field | Landrace | Unknown | GED 001 | Ethiopia | Gedeo | Farmers' field | Landrace | Unknown |
| WOL 004b | Ethiopia | Wolayita | Farmers' field | Landrace | Unknown | GED 002 | Ethiopia | Gedeo | Farmers' field | Landrace | Unknown |
| WOL 006 | Ethiopia | Wolayita | Farmers' field | Landrace | Unknown | GED 005 | Ethiopia | Gedeo | Farmers' field | Landrace | Unknown |
| WOL 007 | Ethiopia | Wolayita | Farmers' field | Landrace | Unknown | GED 008 | Ethiopia | Gedeo | Farmers' field | Landrace | Unknown |
| WOL 008 | Ethiopia | Wolayita | Farmers' field | Landrace | Unknown | AKA 004 | Ethiopia | Areka | Research station | Landrace | Unknown |
| WOL 009 | Ethiopia | Wolayita | Farmers' field | Landrace | Unknown | AKA 005 | Ethiopia | Areka | Research station | Landrace | Unknown |
| WOL 010 | Ethiopia | Wolayita | Farmers' field | Landrace | Unknown | AKA 006 | Ethiopia | Areka | Research station | Landrace | Unknown |
| WOL 011 | Ethiopia | Wolayita | Farmers' field | Landrace | Unknown | AKA 013 | Ethiopia | Areka | Research station | Landrace | Unknown |
| WOL 012 | Ethiopia | Wolayita | Farmers' field | Landrace | Unknown | AKA 014 | Ethiopia | Areka | Research station | Landrace | Unknown |
| WOL 013a | Ethiopia | Wolayita | Farmers' field | Landrace | Unknown | AKA 016 | Ethiopia | Areka | Research station | Landrace | Unknown |
| WOL 013b | Ethiopia | Wolayita | Farmers' field | Landrace | Unknown | AKA 019 | Ethiopia | Areka | Research station | Landrace | Unknown |
| WOL 014 | Ethiopia | Wolayita | Farmers' field | Landrace | Unknown | TDr 95/18531 | Nigeria | | IITA | Advanced clone | *D. rotundata* |
| WOL 015 | Ethiopia | Wolayita | Farmers' field | Landrace | Unknown | TDr 93-2 | Nigeria | | IITA | Landrace | *D. rotundata* |
| WOL 016 | Ethiopia | Wolayita | Farmers' field | Landrace | Unknown | TDr 93-50 | Nigeria | | IITA | Landrace | *D. rotundata* |
| WOL 018 | Ethiopia | Wolayita | Farmers' field | Landrace | Unknown | TDr 95/19156 | Nigeria | | IITA | Advanced clone | *D. rotundata* |
| WOL 020 | Ethiopia | Wolayita | Farmers' field | Landrace | Unknown | TDr 98/00328 | Nigeria | | IITA | Advanced clone | *D. rotundata* |
| WOL 023a | Ethiopia | Wolayita | Farmers' field | Landrace | Unknown | TDc 95-293 | Ghana | | IITA | Landrace | *D. cayenensis* |
| WOL 023b | Ethiopia | Wolayita | Farmers' field | Landrace | Unknown | TDc 95-294 | Uganda | | IITA | Landrace | *D. cayenensis* |
| WOL 025 | Ethiopia | Wolayita | Farmers' field | Landrace | Unknown | TDc 98-136 | Ghana | | IITA | Landrace | *D. cayenensis* |
| WOL 026 | Ethiopia | Wolayita | Farmers' field | Landrace | Unknown | TDa 98/01174 | Nigeria | | IITA | Advanced clone | *D. alata* |
| WOL 028 | Ethiopia | Wolayita | Farmers' field | Landrace | Unknown | WOL 017 | Ethiopia | Wolayita | Farmers' field | Landrace | *D. bulbifera* |
| SID 001 | Ethiopia | Sidama | Farmers' field | Landrace | Unknown | WOL 021 | Ethiopia | Wolayita | Farmers' field | Landrace | *D. bulbifera* |
| SID 002 | Ethiopia | Sidama | Farmers' field | Landrace | Unknown | AKA 001 | Ethiopia | Areka | Research station | Landrace | *D. bulbifera* |
| SID 005 | Ethiopia | Sidama | Farmers' field | Landrace | Unknown | AKA 002 | Ethiopia | Areka | Research station | Landrace | *D. bulbifera* |
| SID 007 | Ethiopia | Sidama | Farmers' field | Landrace | Unknown | AKA 003 | Ethiopia | Areka | Research station | Landrace | *D. bulbifera* |

Analysis of molecular variance (AMOVA) for the unidentified Ethiopian accessions, using collecting area as a grouping criterion, revealed that within group variance accounted for 81% of the total variance, while the variation among collecting areas contributed only 19% (Table 4.6). The genetic similarity within and among collecting areas was analyzed using Jaccard similarity coefficient to further reveal the pattern of diversity in the various areas (Table 4.7). The accessions from Gedeo and Sidama exhibited the highest within and between-group similarities, while those from Gamo-Gofa, Wolayita, and Areka were highly diverse.

**Table 4.2.** Adapters and primers used in the study of *Dioscorea* spp. and their sequences.

| Adapters/primers | Sequence |
|---|---|
| *Eco*RI Adapter | 5′ CTC GTA GAC TGC GTA CC 3′<br>3′ CTG ACG CAT GGT TAA 5′ |
| *Mse*I Adapter | 5′ GAC GAT GAG TCC TGA G 3′<br>3′ TA CTC AGG ACT CAT 5′ |
| *Eco*RI Primer E01 | 5′ CTG CGT ACC AAT TCA 3′ |
| *Mse*I Primer M02 | 5′ GAT GAG TCC TGA GTA AC 3′ |
| *Eco*RI + 3 | |
| E32 | 5′ CTG CGT ACC AAT TCA AC 3′ |
| E33 | 5′ CTG CGT ACC AAT TCA AG 3′ |
| E35 | 5′ CTG CGT ACC AAT TCA CA 3′ |
| E38 | 5′ CTG CGT ACC AAT TCA CT 3′ |
| *Mse*I + 3 | |
| M48 | 5′ GAT GAG TCC TGA GTA ACA C 3′ |
| M49 | 5′ GAT GAG TCC TGA GTA ACA G 3′ |
| M50 | 5′ GAT GAG TCC TGA GTA ACA T 3′ |
| M51 | 5′ GAT GAG TCC TGA GTA ACC A 3′ |
| M59 | 5′ GAT GAG TCC TGA GTA ACT A 3′ |
| M60 | 5′ GAT GAG TCC TGA GTA ACT C 3′ |
| M62 | 5′ GAT GAG TCC TGA GTA ACT T 3′ |

**Table 4.3.** Total number of fragments scored and level of polymorphic bands detected in *Dioscorea* spp. according to the primer combinations used.

| Primer combinations | Total number of fragments scored | Number of monomorphic fragments | Polymorphic Fragments(%) |
|---|---|---|---|
| E-AAC/M-CAC | 78 | 1 | 98.7 |
| E-AAC/M-CAG | 88 | 0 | 100.0 |
| E-AAC/M-CAT | 74 | 4 | 94.5 |
| E-AAC/M-CTC | 73 | 2 | 97.2 |
| E-AAG/M-CCA | 119 | 0 | 100.0 |
| E-AAG/M-CTC | 75 | 4 | 94.6 |
| E-ACA/M-CAT | 115 | 0 | 100.0 |
| E-ACA/M-CTC | 90 | 4 | 95.5 |
| E-ACT/M-CTA | 114 | 4 | 96.4 |
| E-ACT/M-CTT | 74 | 4 | 94.6 |
| Total | 900 | | |
| Mean | | | 97.4 |

### 4.3.3. Phenetic analysis

Cluster analysis based on Jaccard similarity coefficient and UPGMA separated the 62 accessions into five distinct clusters at about 60% of genetic similarity (Figure 4.2). Each cluster was supported by a bootstrap value greater than 99% based on 1000 permutations. The existence of very distinct clusters was also reflected in the high cophenetic correlation coefficient (r = 0.99). The unidentified accessions from Ethiopia formed a distinct group, first cluster. The second cluster represented all the genotypes of *D. rotundata*, whereas the *D. cayenensis* genotypes were clearly separated into the third cluster. The single accession of *D. alata* was distinct from the others, and was represented by the fourth cluster. Accessions of aerial yam (*D. bulbifera*) were grouped separately into the fifth cluster.

A separate clustering of the 48 unidentified accessions from Ethiopia gave six distinct clusters at about 70% genetic similarity levels, and three of these clusters represented individual accessions (Figure 4.3). All the clusters where supported by high bootstrap values (>99%). Interestingly, cluster 1 included 12 of the 14 accessions representing the late-maturing (single-harvested) landraces. Accession WOL 012, known by the

local name *ayina*, was clearly distinct from the other late-maturing accessions and formed cluster 2. The same was true for accession GGF 004, representing cluster 3. This cluster was closely related to cluster 4 that comprised all accessions of the early-maturing landraces. Cluster 4 also included five of the accession from Areka Agricultural Research Center (AKA), for which data were not available on maturity time. Cluster 5 represented WOL 004b, one of the accessions with distinct panicle-type inflorescence, and AKA 013, one of the three accessions with female inflorescence. The other two accessions with female inflorescence are GGF 004 (cluster 3) and AKA 004 that was groped in cluster 4. The only non-flowering accession in the collection, AKA 014, was clearly separated from the rest and represented by cluster 6. Of the 165 polymorphic AFLP bands used for this clustering, 25 were specific to AKA 014.

**Table 4.4.** The level of polymorphism revealed with 10 AFLP primer combinations within the unidentified yam accessions from Ethiopia and three known *Dioscorea* species.

| Primer combinations | Total number of bands scored per primer combination, and number and proportion of polymeric bands | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Ethiopian accessions | | *D. rotundata* | | *D. cayenensis* | | *D. bulbifera* | |
| | Total | Polym. (%) | Total | Polym. (%) | Total | Polym. (%) | Total | Polym. (%) |
| E-AAC/M-CAC | 36 | 20 (55.6) | 37 | 17 (45.9) | 35 | 9 (25.7) | 27 | 3 (11.1) |
| E-AAC/M-CAG | 34 | 21 (61.8) | 34 | 15 (44.1) | 45 | 19 (42.2) | 26 | 3 (11.5) |
| E-AAC/M-CAT | 29 | 13 (44.8) | 32 | 12 (37.5) | 40 | 15 (37.5) | 28 | 1 (3.6) |
| E-AAC/M-CTC | 34 | 14 (41.2) | 30 | 10 (33.3) | 42 | 13 (31.0) | 24 | 0 (0.0) |
| E-AAG/M-CCA | 44 | 21 (47.7) | 50 | 22 (44.0) | 63 | 23 (36.5) | 34 | 3 (0.8) |
| E-AAG/M-CTC | 35 | 15 (42.9) | 31 | 11 (35.5) | 37 | 12 (32.4) | 24 | 2 (8.3) |
| E-ACA/M-CAT | 46 | 17 (37.0) | 35 | 14 (40.0) | 63 | 23 (36.5) | 36 | 3 (8.3) |
| E-ACA/M-CTC | 37 | 13 (35.1) | 51 | 19 (37.3) | 53 | 10 (32.4) | 32 | 2 (6.3) |
| E-ACT/M-CTA | 43 | 18 (41.9) | 51 | 18 (35.5) | 56 | 37 (66.1) | 39 | 1 (2.6) |
| E-ACT/M-CTT | 38 | 13 (34.2) | 39 | 17 (40.0) | 37 | 16 (43.2) | 29 | 0 (0.0) |
| Total | 376 | 165 (43.8) | 390 | 155 (39.7) | 471 | 177 (37.6) | 299 | 18 (6.0) |

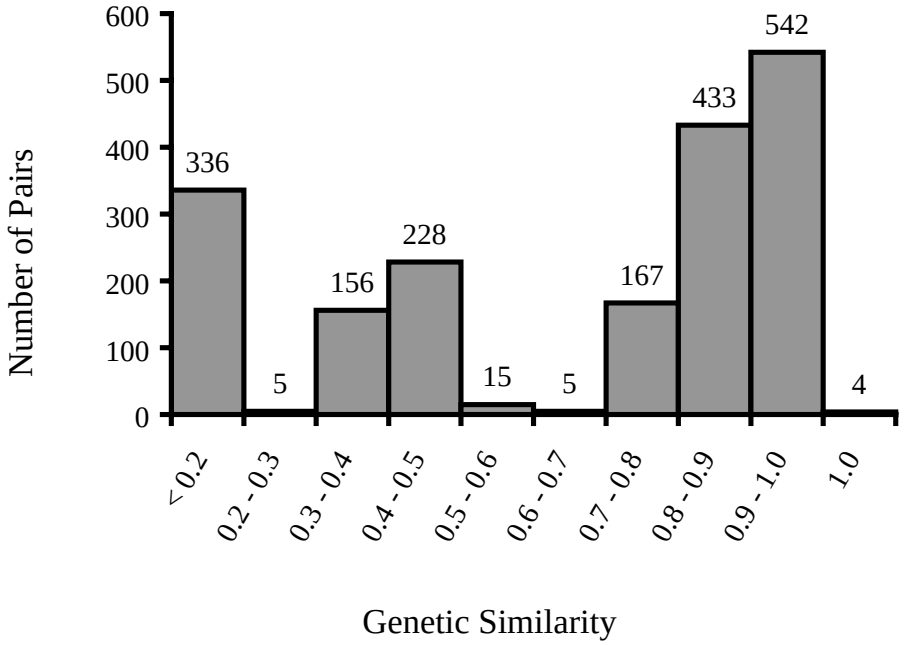



**Figure 4.1.** Distribution of pair-wise genetic similarities (Jaccard coefficient) among the 62 *Dioscorea* accessions studied.

**Table 4.5** Matrix of mean genetic similarity estimates (Jaccard coefficient) among and within the unidentified accessions from Ethiopia and the other *Dioscorea* species studied (figures in parenthesis refer to within group genetic similarity ranges).

| | Ethiopian accessions | *D. alata* | *D. bulbifera* | *D. cayenensis* | *D. rotundata* |
|---|---|---|---|---|---|
| Ethiopian accessions | 0.884 (0.696-1.000) | | | | |
| *D. alata* | 0.149 | ---- | | | |
| *D. bulbifera* | 0.116 | 0.275 | 0.973 (0.949-0.987) | | |
| *D. cayenensis* | 0.362 | 0.166 | 0.109 | 0.734 (0.626-0.934) | |
| *D. rotundata* | 0.419 | 0.157 | 0.100 | 0.552 | 0.775 (0.722-0.801) |

Number of accession studied: Ethiopian accessions = 48, *alata* = 1, *bulbifera* = 5, *cayenensis* = 3 and *rotundata* = 5

**Table 4.6.** Analysis of molecular variance (AMOVA) of 165 polymorphic AFLP markers from the 48 unidentified yam accessions from Ethiopia.

| Source of variance | df | SS | CV | % Total | P-value | P |
|---|---|---|---|---|---|---|
| Among collecting areas | 4 | 165.66 | 3.29 | 19 | | |
| Within collecting areas | 43 | 605.92 | 14.09 | 81 | 0.189 | 0.016 |

**Table 4.7.** Genetic similarity estimates (Jaccard coefficient) within and among yam accessions from different collecting areas in Southern Ethiopia. (Values in parenthesis refer to range of genetic similarity).

| Zones | Areka | Gamo-Gofa | Gedeo | Sidama | Wolayita |
|---|---|---|---|---|---|
| Areka | 0.536 (0.173-0.923) | | | | |
| Gamo-Gofa | 0.467 (0.175-0.942) | 0.552 (0.359-0.983) | | | |
| Gedeo | 0.684 (0.165-0.954) | 0.541 (0.346-0.891) | 0.890 (0.803-0.955) | | |
| Sidama | 0.683 (0.116-1.000) | 0.554 (0.320-0.981) | 0.906 (0.796-1.000) | 0.906 (0.796-1.000) | |
| Wolayita | 0.502 (0.170-0.944) | 0.591 (0.330-0.983) | 0.609 (0.322-0.977) | 0.611 (0.293-0.982) | 0.591 (0.330-0.983) |

Number of accessions studied: Areka = 7, Gamo-Gofa = 4, Gedeo = 4, Sidama = 10 and Wolayita = 23.

The relationship among the 62 accessions studied was further illustrated by results of the principal coordinate analysis. The first three principal coordinates accounted for 40.8%, 18.9% and 12.6% of the total variance. Plotting of the first and second coordinates clearly separated accessions of *D. alata*, *D. bulbifera* and those from Ethiopia (Figure 4.4) and, thus, was highly consistent with results of the cluster analysis (Figure 4.2). Although the species *D. rotundata* and *D. cayenensis* were distinct from the other groups, the separation between the two was relatively weak.

Following principal coordinate analysis of the 48 Ethiopian accessions, a plot of the first and second principal coordinates (Figure 4.5) detected the same groups revealed by cluster analysis (Figure 4.3) although clusters 5 and 6 were not clearly separated.

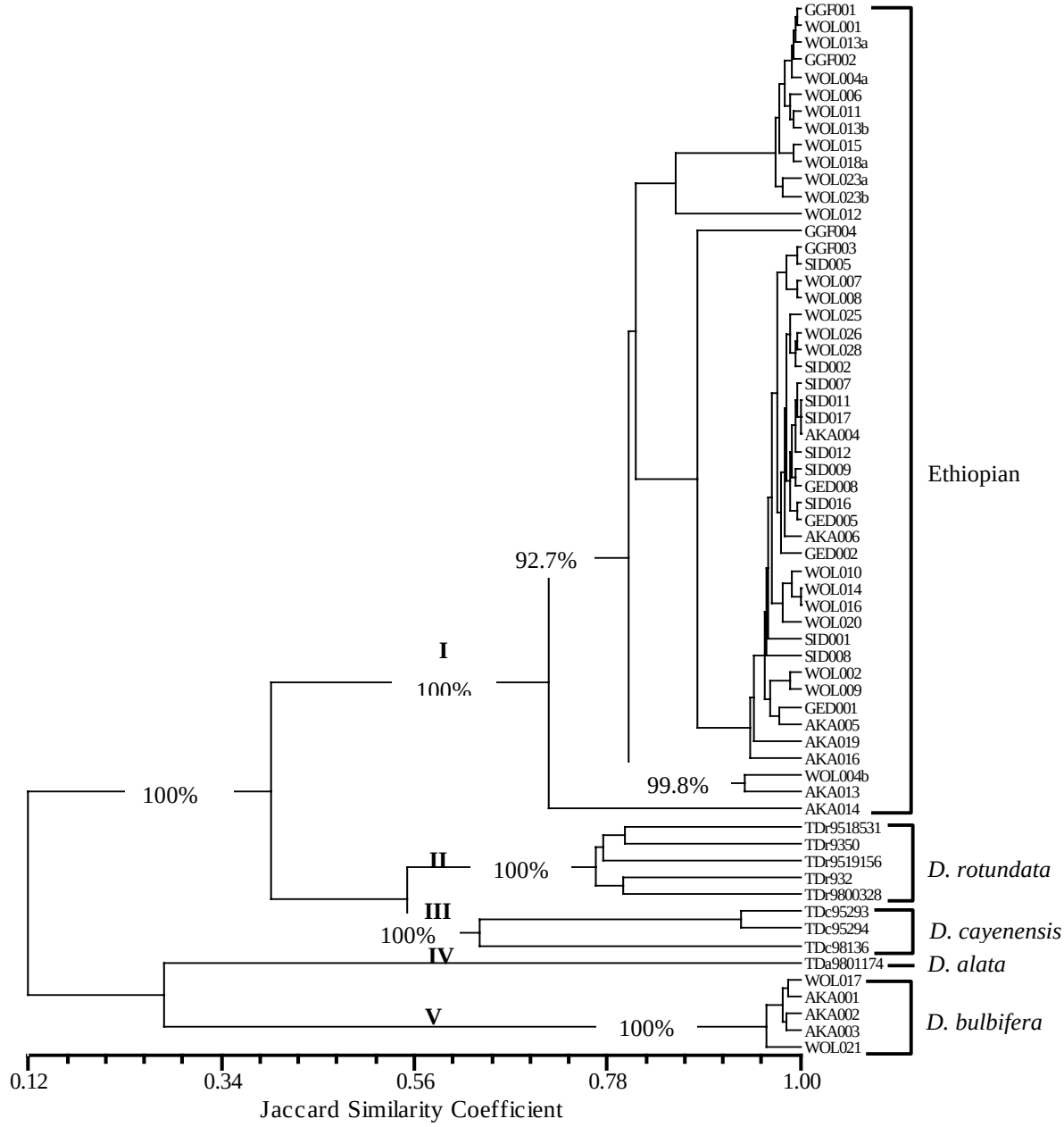



**Figure 4.2.** Dendrogram of 62 *Dioscorea* accessions evaluated based on Jaccard similarity coefficient and UPGMA clustering (Percentage figures indicate bootstrap values based on 1000 replicate analyses; cophenetic correlation coefficient (r) = 0.99).

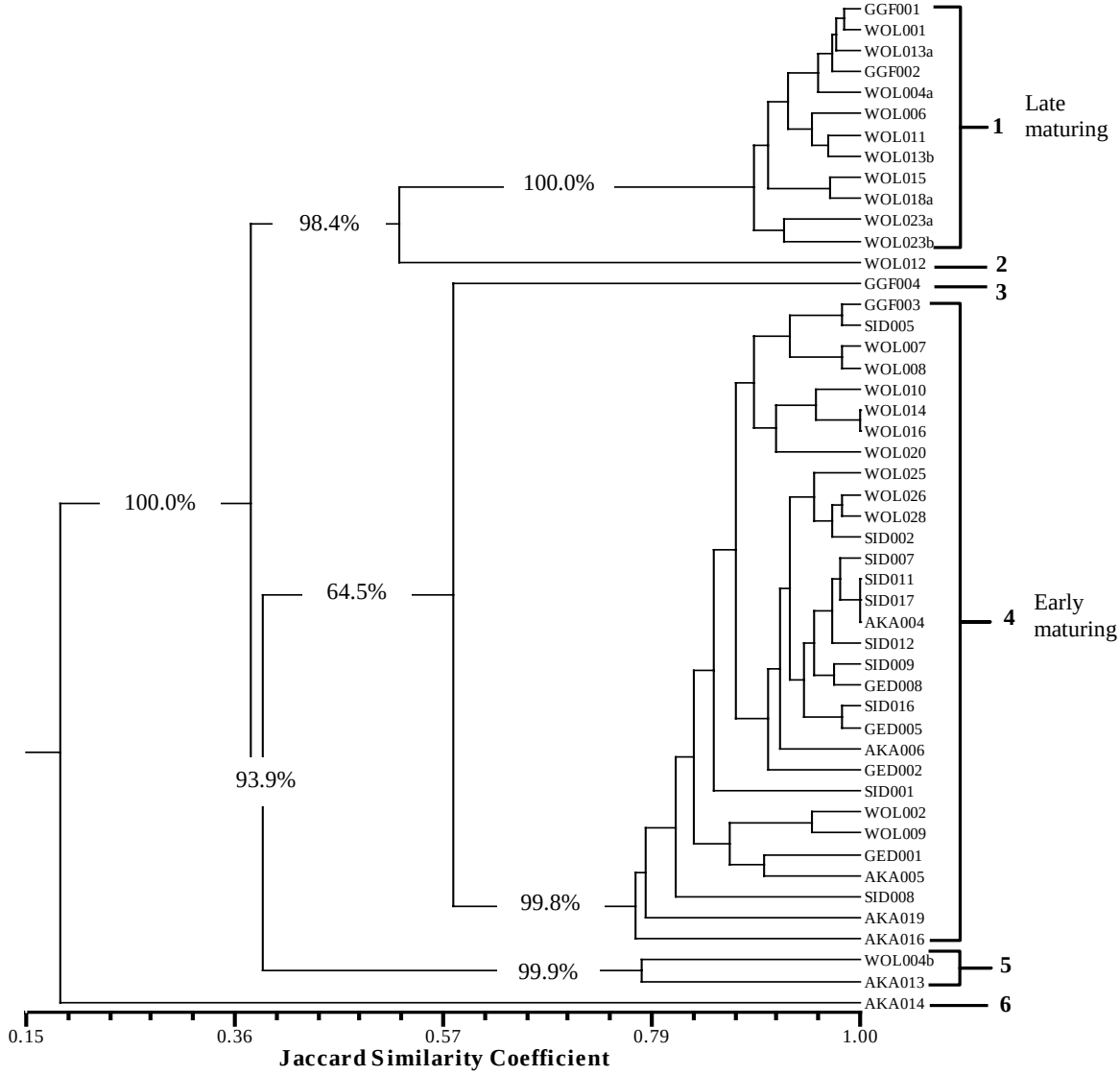



**Figure 4.3.** Dendrogram of 48 unidentified *Dioscorea* accessions from Ethiopia based on Jaccard similarity coefficient and UPGMA clustering (Percentage figures indicate bootstrap values based on 1000 replicate analyses; cophenetic correlation coefficient (r) = 0.99).

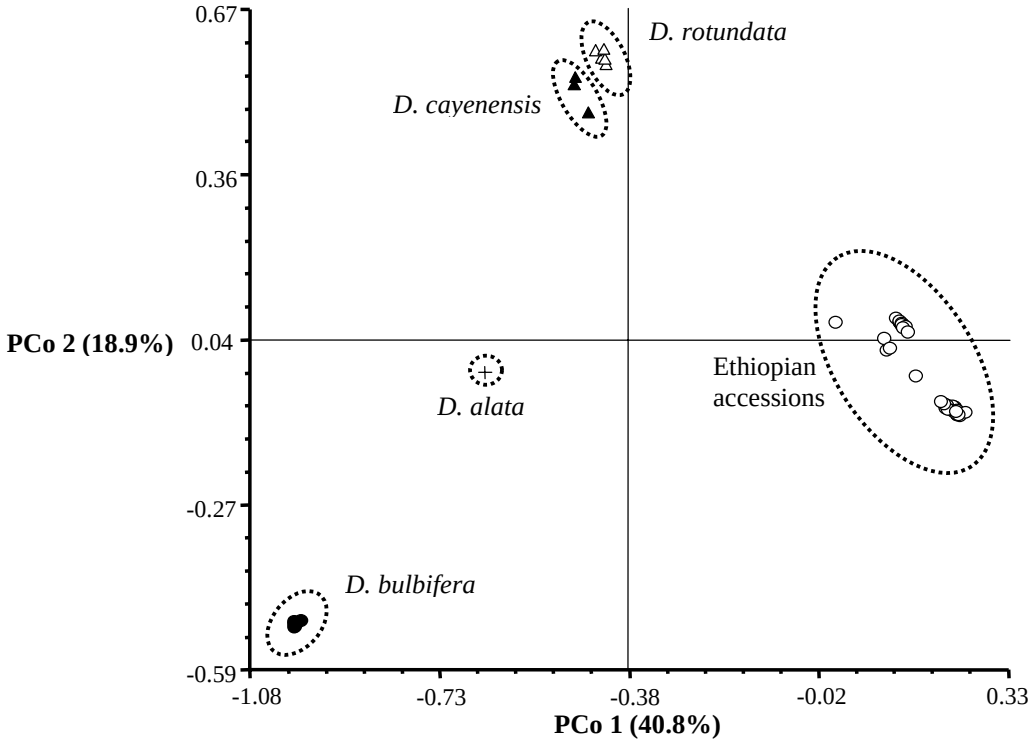



**Figure 4.4.** Plot of the first and second principal coordinates for 62 *Dioscorea* accessions based on 877 polymorphic bands derived from 10 AFLP primer pairs.

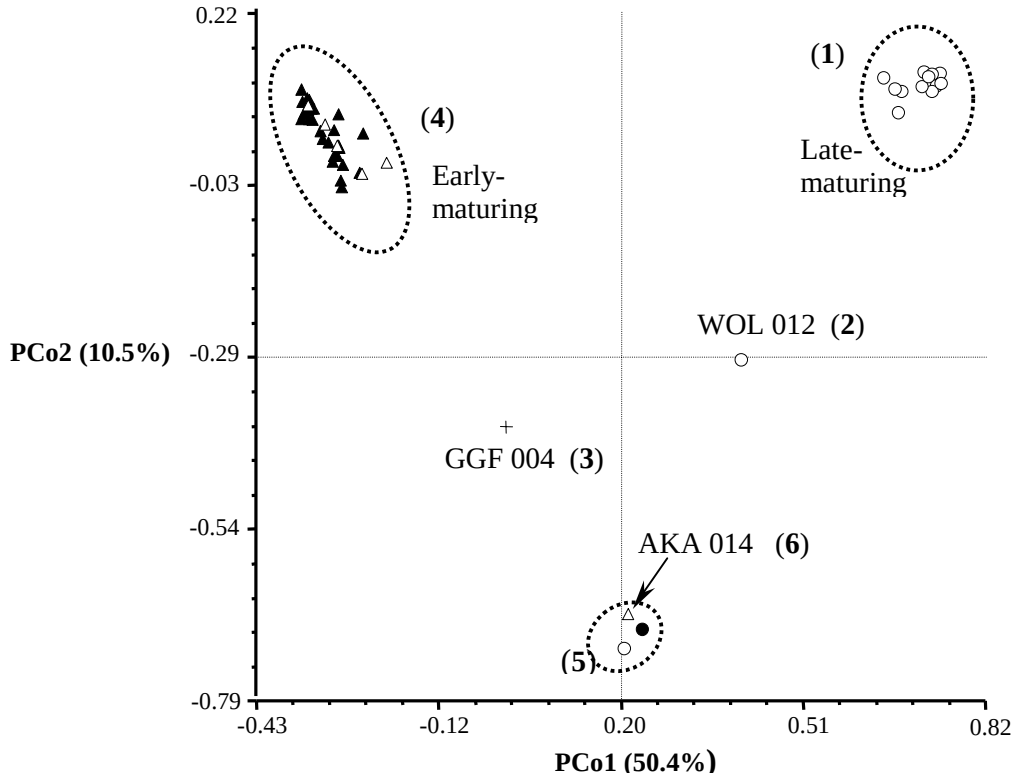



**Figure 4.5.** Plot of the first and second principal coordinates for 48 unidentified Dioscorea accessions from Ethiopia based on 165 polymorphic bands derived from 10 AFLP primer pairs. (Numbers in parenthesis refer to the clusters from UPGMA analysis; Δ refer to accessions obtained from Areka Agricultural Research Center for which data on maturity time is not available).

## 4.4. Discussion

### 4.4.1. Interspecific genetic variability

The genetic relationships revealed by AFLP analysis among the different yam species are generally consistent with the established taxonomy of common *Dioscorea* species. The species *D. rotundata*, *D. cayenensis*, and *D. alata* belong to section Enantiophylum, whereas *D. bulbifera* belongs to section Opsophyton under the genus *Dioscorea* (Onwueme and Charles 1994). *D. bulbifera* is also a distinct member of the genus in that it produces edible bulbils (aerial tubers), and is the only species that has

wild forms both in Asia and Africa (Ramser et al. 1996). Thus, the lower genetic similarity detected between *D. bulbifera* and the other species was not unexpected.

This finding shows that *D. alata* is genetically closer to *D. bulbifera* than to the other species in the same section. This contradicts earlier reports involving both species (Malapa et al. 2005). Although our finding needs to be further confirmed by including more accessions from different geographic origins, the fact that some cultivars of *D. alata* produce aerial tubers lends support to its apparent closeness to *D. bulbifera*. In situations where genetic exchange is severely limited, observed genetic similarities reflect more common ancestry than on-going genetic exchange (Schaal et al. 1998).

The relationship between *D. rotundata* and *D. cayenensis* has been a subject of much debate for close to half a century. Although the two species have been considered as separate taxa based on morphological traits (Burkill 1960; Akoroda and Chheda 1983; Onyilagha and Lowe 1985), the presence of intermediate forms has led to their classification as varieties within the same species (Martin and Rhodes 1978) or as a species complex (Hamon and Touré 1990b). On the basis of phylogenic studies using RFLP analysis in chloroplast and nuclear ribosomal DNA, it was suggested that *D. cayenensis* is a variety of *D. rotundata* (Terauchi et al. 1992). Nevertheless, the more recent studies based on isozyme (Dansi et al. 200a) and molecular markers (Ramser et al. 1997; Mignouna et al. 1998; Mignouna et al. 2005) support the separate identity of the two species. Our finding also seems to confirm that the two are distinct species, although this was not clear from result of the principal coordinate analysis.

Both cluster and principal coordinate analyses confirmed the distinctiveness of the Ethiopian accessions from the other species. Miége and Demissew (1997) described eleven *Dioscorea* species found in Ethiopia including *D. abyssinica* and *D. praehensilis*, the wild species believed to be among those that gave rise to cultivated forms in West Africa (Terauchi 1992). In their description of Sheko (Southwest Ethiopia) yam landraces, Hildebrand et al. (2002) used the designation '*D. cayenensis* complex' for all 'the poorly understood set of species: *D. cayenensis, D. rotundata, D. abyssinica, D. praehensilis* and *D. sagittifolia*. Members of this 'complex' are subjected to 'adoptive transplantation' (Hildebrand 2003), a process of yam domestication whereby wild yam tubers are transplanted to farmers' fields. These,

together with our finding that the Ethiopian accessions are genetically closer to *D. rotundata* and *D. cayenensis* than the other species studied, at least based on pair-wise genetic similarity values between groups, suggest the possible involvement of similar wild species in the process of yam domestication both in Ethiopia and West Africa. The distinctiveness of yams from Ethiopia may represent a divergent evolutionary pathway isolated from the widely known center of diversity in West Africa.

### 4.4.2. Diversity within the accessions from Ethiopia

The present study revealed considerable variation within the unidentified accessions from Ethiopia. Both cluster and principal coordinate analyses were consistent in their grouping of the accessions into the various clusters although clusters 5 and 6 were not clearly separated in the latter. The two major groups, cluster 1 and 4 (Figure 4.3 and 4.5), roughly correspond to the main yam categories recognized by local farmers: *hatuma boye* ('male' yam) and *macha boye* ('female' yam). The 'female' yams include all the early-maturing (7-8 months) landraces that are harvested twice (double-harvested), while the so-called 'male' yams mature late (9-11 months) and are harvested only once. This observation is partly in agreement with earlier findings based on morphological characterization of the same accessions (Chapter 2), and suggests that the categories represent genetically distinct groups of landraces. Although these clearly distinct yam types are well known in African yam species (Onwueme and Charles 1994), their variation has not been well investigated at DNA level. Dansi et al. (2000b) found that grouping of Guinea yam cultivars based on RAPD patterns reflected more relatedness with respect to agronomic and organoleptic characteristics than with morphological traits.

The separate clustering of WOL 012, representing the landrace *ayina* or *ayino,* further substantiates the relationship between the variation in maturity time and the actual diversity at DNA level. *Ayina* is harvested only once in most localities across the study area. It is also morphologically very close to the late-maturing landraces (Chapter 2). Nevertheless, it matures earlier than all the landraces in this maturity group. That is why some farmers in Damot Woyde district of Wolayita zone manage *ayina* as an early-maturing landrace with two harvests per cropping season. In effect, this landrace

is an intermediate type with respect to maturity time, which corresponds to its value for the first principal coordinate for molecular difference (Figure 4.6).

The differentiation of GGF 004, WOL 004b and AKA 013 merits further discussion. GGF 004, known by the local name *bune* or *buna*, is among the landraces with very limited distribution, but widely grown in Kucha district of Gamo-Gofa zone (Chapter 1). It is perceived to be highly drought-tolerant, and the tubers store well in the soil for a long time. Accordingly, it is usually harvested late in the season (November to December) after the harvesting season is over even for the late-maturing landraces. Farmers also distinguish *bune* by its highly branched tubers, having a shape of 'dog's feet', and the distinct female inflorescence (Chapter 2).

Accessions WOL 004b and AKA 013 are distinct with respect to inflorescence morphology. WOL 004b has a panicle type of inflorescence, contrary to the common raceme type, while AKA 013 possesses female inflorescence. Nonetheless, this cannot fully explain their separate grouping from other accessions with similar inflorescence types. On the one hand, WOL 013b and WOL 023b, despite having similar inflorescence as WOL 004b, were grouped in cluster 1. Likewise, AKA 004 and GGF 004, the other accessions with female inflorescence, were separated from AKA 013. Similar reports are available in clonally propagated crops such as plantain (Ude et al. 2003), where inflorescence morphology, although a key trait in conventional taxonomy, did not show association with observed groupings of genotypes based on molecular markers.

Previously, we reported that most named landraces are morphologically distinct (Chapter 3). But, there were also cases where no obvious morphological differentiations were observed between landraces known by different local names. This is more common among landraces within the early-maturing group. The structure of genetic diversity revealed in the present study also confirms this general fact. Landraces within the late maturing group are distinguished on the basis of various morphological and growth attributes. For example, GGF 001, GGF 002, WOL 001, WOL 004a and WOL 004b, representing the landrace *wadala*, were collected from different localities. Their groping within cluster 1, except WOL 004b, confirms their distinct identity. WOL 004b, which was sampled from the same field with WOL 004a,

is well separated from this group, supporting the fact that landraces are often genetically heterogeneous (Harlan 1975). This also represents a case where farmers' classification underestimates the actual genetic diversity.

Tuber flesh color is among the traits widely used to distinguish individual landraces particularly within the early-maturing group. Such traits are salient to the local farmers and frequently used in categorization of crop landraces. Nevertheless, the result shows no distinct grouping of these landraces within cluster 4. For example, GGF 003, WOL 002, WOL 007 and WOL 005 are all known by the same vernacular name *hatiye* and distinguished by white tuber flesh color. The same is true for SID 001 and SID 005 that are referred to by the local name *ado* in the Sidama language due to the white tuber color. It is known that significant morphological variations may be the result of differences only in few genes (Bradley et al. 1997).

There appears to be no geographic pattern of diversity within the accessions from Ethiopia. The early-maturing landraces are distributed throughout the collecting area. However, their grouping within cluster 4 does not relate to their area of origin. This observation is supported by the partitioning of genetic variation by AMOVA (Table 4.6), which showed very low variability among the collecting areas. This is also in line with the oral history that yam and its culture was originally introduced from Gamo-Gofa to Wolayita, and then to Sidama and Gedeo zones (Chapter 1). This might have followed extensive exchange of germplasm among the various areas. The lower genetic similarity observed between some areas, such as Wolayita and Gedeo, and Gamo-Gofa and Gedeo is probably due to the absence of late-maturing landraces in Sidama and Gedeo.

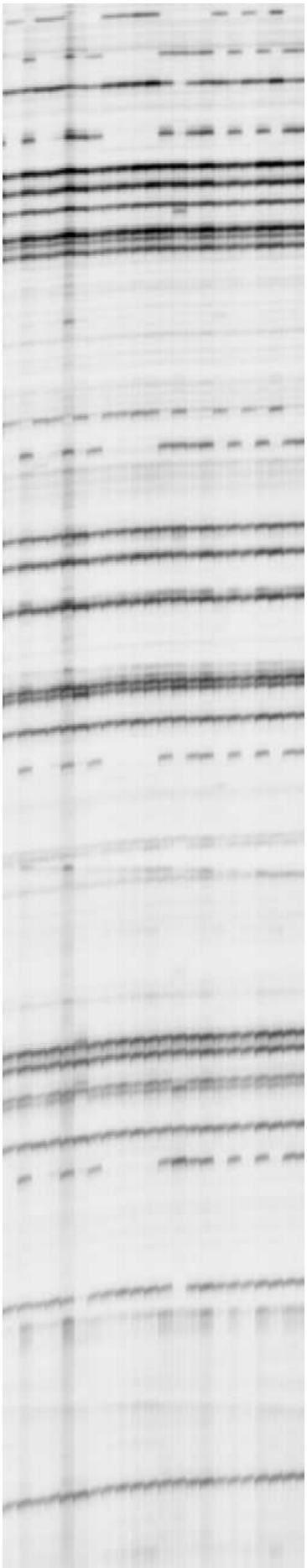
### 4.4.3. Implications for conservation and improvement

A clear understanding of the identity and genetic diversity of accessions is a prerequisite for efficient management as well as effective utilization of crop germplasm. The pattern of genetic diversity revealed in this study is generally consistent with farmers' classification of local landraces and structure of morphological diversity, demonstrating the vital role traditional knowledge can play in future management and improvement of yam landraces. The distinct clustering of some

landraces provides an opportunity for their use in further studies striving to improve the crop.

The distinctiveness of Ethiopian yams from commonly cultivated yam species is an important finding of this study, indicating the need to further resolve their species identity. Future studies have to consider the use of co-dominant molecular markers, such as RFLP and microsatellites that allow the detection of phylogenic relationships, and also include cultivated and wild species both from Ethiopia as well as other African countries. Determination of ploidy level may provide crucial information regarding their genome organization. If the distinctiveness of these materials is further confirmed by such studies, given the fact that different wild types exist in the country, it may lead to the conclusion that Ethiopia represents a distinct center of yam diversity.

The pattern of diversity across the study area revealed that Gamo-Gofa and Woalyita zones host considerable levels of yam diversity. Accordingly, future collections activities must consider more sampling in these areas and also extend to other localities in the Southern and Southwestern parts of the country that are not covered by this study, in order to capture as much diversity as possible.



Ariel yam (*D. bulbifera)* inflorescence →

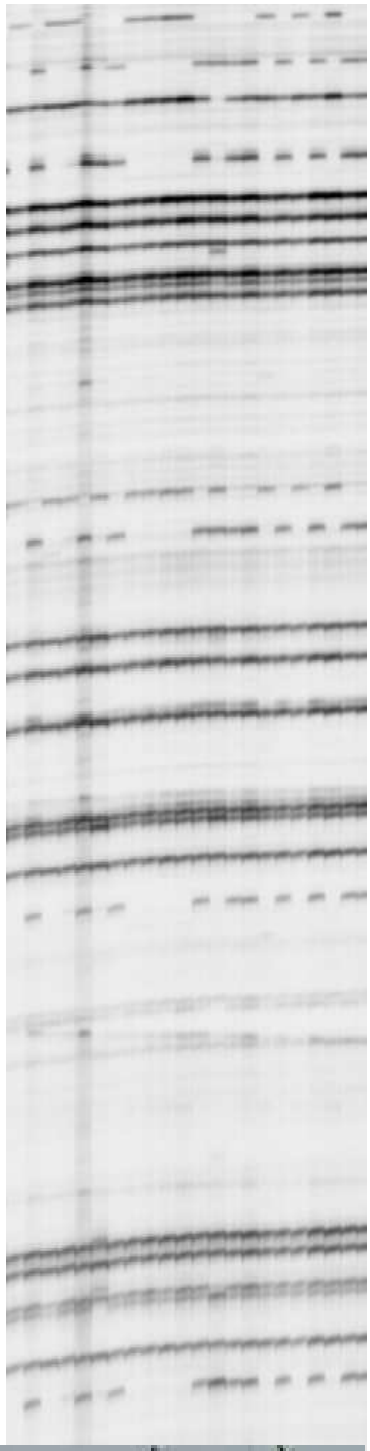
Direction of twining

← Anti-Clockwise

↓ Clockwise

Yam fruit capsule after full senesce of the plant. (Photo:Muluneh Tamiru)

↑ Yam in full bloom. (Photo: Muluneh Tamiru)



Diversity in yam inflorescence morphology. ↓
(Photo: Muluneh Tamiru)

# 5. Characterizing Diversity in Composition and Pasting Properties of Tuber Flour in Yam Germplasm (*Dioscorea* spp.) from Southern Ethiopia

**Abstract**

*The quality and suitability of starch or starch-containing materials for food and non-food applications depend mainly on their composition and functional properties. Studies on these aspects are important to detect differences among genotypes, and facilitate utilization, improvement and conservation of crop genetic resources. The diversity in composition and pasting properties of 65 yam accessions collected from Southern Ethiopia were investigated. Parameters measured included contents of protein and starch, amylose fraction, and pasting properties of freeze-dried tuber flour material. Type of tuber (aerial vs. underground), maturity time (early vs. late), and sex of the plant mostly showed significant influences. Starch content varied from 65.2 to 76.6% DM (mean 70.9%), while protein content ranged between 6.4% and 13.9% (mean 9.7%). Amylose represented between 7.1% and 30.6% of the starch fraction. It also showed the highest variability compared to protein and starch contents, and was the only parameter significantly influenced by the geographic origin of accessions. Furthermore, a significant negative correlation ($P < 0.01$) was observed between starch content and its amylose fraction. Clustering based on Euclidean distance and principal component analysis clearly distinguished the aerial yam accessions from those producing underground tubers. Further studies on similar parameters under different environmental conditions and on physico-chemical properties of isolated yam starch will be useful to reveal the potential of yam both for food and non-food applications.*

**Keywords:** *Dioscorea*; Ethiopia; tuber; starch; amylose; pasting property

## 5.1. Introduction

Root and tuber crops such as sweet potato (*Ipomoea batatas* (L.) Lam.), yams (*Dioscorea* spp.), potato (*Solanum tuberosum* L.), cocoyams (*Colocasia* spp. and *Xanthosomas* spp.) and cassava (*Manihot esculenta* Crantz) are important sources of carbohydrates in the world's food next to cereals. They are staple foods for over one billion of the world population (Millstone and Lang 2003) mainly comprising the lower socio-economic groups (Chandra 1994). Africa heavily depends on these crops, which play vital roles in food security both at household and national levels and, hence, often referred to as 'insurance crops' (Onwueme and Charles 1994). Consequently, improved production of root and tubers is considered one of the options for alleviating the current food crisis facing the growing African population.

Yam constitutes a major starchy food source in a number of regions across the humid and sub-humid tropics. The plant has a tremendous sink capacity to store food reserves, with individual tubers weighing up to 20-30 kg (Asiedu et al. 1997). Dormancy in both aerial and underground tubers maintains organoleptic quality and ensures extended supply of tubers on markets (Craufurd et al. 2001). In contrast to the low contents in most root and tuber crops, some species of yam are good sources of protein (Hahn et al. 1987), while the alkaloids (sapogenins) present in tubers of some species are utilized in many pharmaceutical preparations (Degras 1993). Besides, yam has both cultural and social significance in many societies (Hahn et al. 1987). These are among the attributes that make yam a crop of immense potential.

A recent study conducted in the major yam growing areas of Southern Ethiopia show that farmers grow diverse yam landraces, which play important roles in local livelihood (Chapter 2, 3 and 4). Yam is planted at the onset of the dry season, mainly in October, and early harvests fill a seasonal gap in food supply during the months of May and June. It is also the preferred food for honored guests, and traditional meals made of yam are served during the main traditional and religious festivals. Accordingly, yam tubers fetch relatively higher prices compared to the other root and tuber crops. Hence, yam is important not only for household food security but also as a source of cash income.

Yam requires 7 to 11 months from planting to harvesting. The supply of fresh tubers to local markets in Ethiopia is limited to the period from May to September. Moreover, yam is not processed in any form in the country. Tubers are mostly consumed boiled. In Wolayita, boiled yam is mashed and mixed with butter and fermented milk to prepare the popular dish known as *fichata*. Roasting of tubers is also known in some localities although practiced rarely. In contrast, yam is utilized in different ways in West Africa, a known center of yam diversity and production. In addition to boiled, mashed, fried, roasted and baked yam, pounded yam or 'fufu' is a very popular form of yam consumption. Tubers are also processed into flour, flakes, chips and dry roasted slices (Onwueme and Charles 1994). Other uses include preparation of local beer from detoxified poisonous varieties (Kay 1973).

Yam shows considerable intra and interspecific variations with regard to compositional and other physico-chemical properties of the tuber (Egbe and Treche 1983; Muzac-Turker et al. 1993; Farhat et al. 1999; Rolland-Sabaté et al. 2003; Amani et al. 2004). Egbe and Treche (1983) studied 98 cultivars representing eight *Dioscorea* species, and found high variability for mineral, protein and lipid contents, as well as cell wall constituents. Rolland-Sabaté et al. (2003) reported the diversity in macromolecular characteristics of starch obtained from four *Dioscorea* species. Gebre-Mariam and Schmidt (1998) similarly attempted to investigate the compositional and physico-chemical properties of isolated starch in Ethiopian yam. As they only studied tubers from a single location in Southern Ethiopia, the existing diversity of these tuber parameters throughout the country and in different landraces remains unknown.

The eating quality and suitability of yam tuber for various preparations is affected by its composition and functional properties (Lebot et al. 2005). Functional properties such as pasting are, in turn, influenced by composition and structure of the genotype under investigation. The organization between the different starch granules, the botanic origin of the starch, and its concentration also significantly influence its properties (Amani et al. 2004). Thus, studies on the composition and functional properties of starch and starch-containing materials such as flour are useful for detecting differences among genotypes. This approach has been used to screen materials for different food applications (Panozzo and McCormick 1993). A good understanding of these properties also increase utilization of the materials both for food and non-food

applications, such as in adhesives, cosmetics, plastics and textiles, facilitating conservation and improvement of the available germplasm.

The main objective of this study is to investigate the variation in composition and pasting properties of tuber flour within yam germplasm collections from Southern Ethiopia. It aims at assessing potentials of the accessions for increased supply of the main tuber constituents through selection or improved breeding techniques. Furthermore, it attempts to generate information on suitability of yam tuber for processing into different food products as well as for use in non-food applications. This can partly address the current problem associated with seasonal supply of fresh yam tubers.

## 5.2. Materials and methods

### 5.2.1. Plant materials and sample preparation

Sixty-five yam accessions collected from various localities in Southern Ethiopia were considered in this study. These were selected among those previously used for morphological characterization, and represent either morphologically distinct landraces or landraces collected under the same vernacular name from different localities (Table 5.1). Further details on experimental conditions during morphological characterization are provided in chapter 3. For both the early and late-maturing landraces, one tuber was harvested from single plants at full senescence. Following washing and peeling, slices were taken from the distal, middle and proximal sections of the tuber to avoid longitudinal and radial gradients. Samples were stored overnight in a refrigerator and then transported in icebox to the International Livestock Research Institute (ILRI) in Addis Ababa (Ethiopia) for freeze-drying. Samples were lyophilized to minimum moisture content using LABCONCO freeze-drying machine (LABCONCO Corporation, Missouri, USA). Lyophilized chips were transported in airtight plastic bags to Germany for use in the various analyses.

### 5.2.2. Determination of dry matter content

The samples were ground into powder using a grinder with screen aperture size of 1 mm diameter. Before the analyses commenced, sample dry matter content was determined according to ICC standard No. 110/1 (ICC 1976). 2.5 g flour samples were weighed into a crucible and oven-dried at about 110ºC for up to 4 hours, and this was repeated until a constant weight was obtained. Duplicate measurements were made simultaneously and mean values were used for determination of sample dry matter.

### 5.2.3. Determination of protein content

Protein content was determined using LECO®CNS-2000 Carbon, Nitrogen and Sulfur analyzer (LECO Instruments GmBH, München, Germany). About 600-700 mg flour samples and 200 mg of the control (EDTA, 9.75% N) were weighed into ceramic sample boats, which were inserted into the combustion chamber in the analyzer. The nitrogen content of the samples was determined following a combustion process (at about 900ºC in the presence of $O_2$) that converts elemental Nitrogen to $N_2$, $NO_x$ gases (that are further reduced to $N_2$ in the catalyst heater), and $CO_2$. The total nitrogen (N%) content was, then, multiplied by a protein coefficient 6.25 to estimate protein content of the samples.

### 5.2.4. Determination of starch content

Starch content was determined polarimetically by hydrochloric acid dissociation (ICC 1994) as follows: 2.5 g flour sample was weighed into 100 ml volumetric flasks with 25 ml of 1.124% HCl. After continuous hand shaking for 2 min., a further 25 ml of HCl was added and samples were hydrolyzed on a shaking water bath at 100ºC for 15 min. 40 ml of distilled water was added into each sample, which was then cooled under running water. Following addition of 5 ml of phosphoric acid, samples were homogenized by shaking, and distilled water was added to the 100 ml mark. Then, they were filtered through a dry filter paper ($593^{1/2}$ Schleicher and Schüll, Germany) into a 100 ml conical flask. The optical rotation of the filtrate was measured in a polarimeter (Carl Zeiss, Germany). Three readings were taken for each measurement (duplicate

measurements were made for each sample) and the starch content was determined according to the following function:

$$\textit{Starch content}\ (\%) = \frac{\alpha \times 10^{6}}{[\alpha]D^{20} \times E \times DM \times l}$$

Where, α = measured angle in degrees
$[\alpha]_D^{20}$ = specific value of rotation of the starch (degree ml/g dm)
E = weight of sample in g
DM = sample dry matter content
*l* = length of polarimeter tube in dm (1.901 dm)

As the specific value of rotation for yam starch ($[\alpha]_D^{20}$) was not known, the value for cassava starch, 184.6 (ICC 1994), was used in the above computation.

### 5.2.5. Determination of amylose content

Amylose was estimated according to the two-wavelength spectrophotometeric method of Hovenkamp-Hermelink et al. (1988) that involves starch staining with $I_2$-KI solutions and measuring absorption at 550 nm and 618 nm. First, stock solutions of amylose and/or amylopectin were prepared by dissolving 25 mg of both pure amylose and amylopectin extracted from potato starch (Sigma, Germany) in 10 ml of 45% $HClO_4$ (perchloric acid). After about 4 min. of reaction, water was added to a final volume of 100 ml and the samples were diluted to obtain starch solutions with 10, 20 and 60% amylose. These were further diluted to final starch concentrations of 0.2, 0.4, 0.6 and 0.8 mg/100 ml. Iodine and KI staining was performed by mixing 4 ml of the standard solutions and 5 ml diluted Lugol's solution (2 g KI and 1 g Iodine in 300 ml water, then diluted to a concentration of 1:2 with distilled water) in a cuvette, and absorbance was immediately measured in a spectrophotometer (Hewlett Packard 8453, Germany). The absorption spectra were determined against a blank sample containing 4 ml $HClO_4$ and 5 ml diluted Lugol's solution. The result was used to generate a calibration curve (Figure 5.1) based on which the amylose in yam flour was estimated as per the following procedure.

20 mg flour samples were mixed with 0.5 ml of 45% $HClO_4$. Following a 4 min. reaction, 8 ml of distilled water was added to each sample, which was mixed and

filtered. The filtrate was further diluted with water at a concentration of 0.5 ml filtrate and 9.5 ml distilled water. 4 ml of the diluted sample was mixed with 5 ml of diluted Lugol's solution and the absorbance was immediately measured in the spectrophotometer at 550 nm and 618 nm.

The ratio (*R*) of the absorbencies at 618 and 550 nm was calculated after Hovenkamp-Hermelink et al. (1988) as:

$$R = \frac{P \times G \times a(am\,618) + (1-P) \times G \times a(ap\,618)}{P \times G \times a(am\,550) + (1-P) \times G \times a(ap\,550)} = \frac{P \times 7.42 + (1-P) \times 2.04}{P \times 5.17 + (1-P) \times 3.11}$$

Where, P = fraction of amylose
G = starch concentration
*a* = absorption coefficient (calculated from the calibration curve)
*am* = amylose
*ap* = amylopectin

Thus, the fraction of amylose (P) was calculated from the above equation following substitution of the *R-values* from the calibration curve as:

$$P = \frac{3.23 - 4.52 \times R}{1.37 \times R - 5.56}$$

5.2.6. Measurement of viscosity

Pasting properties of the flour were determined in a Rapid Visco Analyzer (RVA) (model RVA-3 Newport Scientific Pty. Ltd., Sydney, Australia) according to the instruction manual of the supplier. Each sample (flour weight corrected for moisture content using 3.5 g at 14% moisture basis) was mixed with de-ionized water to get a final net weight (flour + water) of about 28 g in RVA sample canisters. The test was performed according to the following temperature profile: Initial holding at 50ºC for 1 min., heating to 95ºC over the next 3.4 min., holding at 95ºC for a further 2.7 min., then a constant ramping down to 50ºC over the next 3.8 min., after which the temperature was held constant until the12.5$^{th}$ min. The rate of heating and cooling was 12ºC per

min. The speed of the puddle was constant at 160 rpm throughout the test, following an initial speed of 960 rpm for 10 sec. to mix the samples.

### 5.2.7. Statistical analysis

The data set, containing duplicate measurements of each sample both for the compositional and pasting property parameters, was subjected to descriptive statistics. Pearson correlation coefficient (r) was calculated to determine relationships among the various parameters. Using type of tuber (aerial vs. underground), sex of the plant (male vs. female) and maturity time (early vs. late) as grouping criteria, equality of mean values was tasted using *t-test* for independent samples. The mean values for the groups formed using collecting area were compared using the Duncan test provided in the one-way analysis of variance function. Groups formed based on collecting areas and sex contained all landraces with underground tubers, while those formed based on maturity time excluded the landraces from Areka as information was lucking on this aspect. All computations were carried out using the SPSS software version 12.0.1 (SPSS Inc. 2003, Chicago, USA). The entire data set, following standardization by diving each variable with its respective range, was also subjected to clustering (based on unweighted pair group method using arithmetic means (UPGMA) and Euclidean distance) and principal component analyses (PCA) using the computer program NTSYpc, version 2.1 (Rholf 2000). Factor analysis was carried out with the help of the data reduction function in SPSS to assess the correlation between the principal components and the parameters investigated.

## 5.3. Result

### 5.3.1. Flour composition

A considerable variability was found among accessions with respect to the major tuber constituents (Table 5.2). Starch constituted between 65.2% and 76.6% DM with a mean and standard deviation of 70.9 and 2.9, respectively. Comparison of mean values revealed that aerial yam accessions had significantly lower starch contents than those with underground tubers. There were also significant differences between late and early maturing landraces with underground tubers, and between accessions from

different geographic origins (Table 5.3). The mean starch content of tubers from male plants was not significantly different from those from female plants.

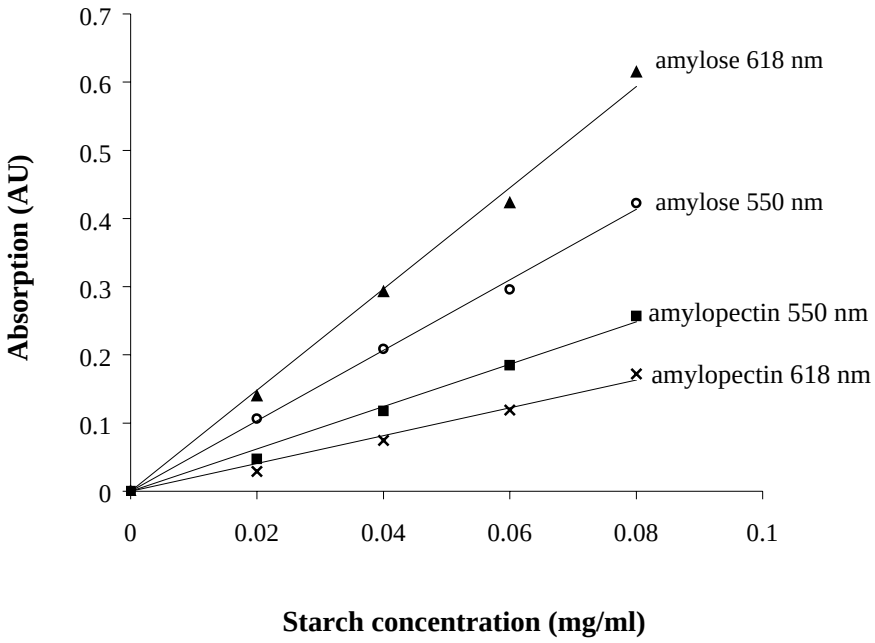



**Figure 5.1.** Absorption values of amylose and amylopectin solutions of various concentrations [the absorption coefficient (a) for amylose (618 nm) = 7.42, amylose (550 nm) = 5.17, amylopectin (618 nm) = 2.04, and amylopectin (550 nm) = 3.11].

In terms of observed range (7.1-30.6%) and coefficient of variation (16.8%), amylose exhibited the highest variability compared to starch and protein contents (Table 5.2). Overall, the aerial yam accessions had significantly higher amylose content (27.0%, $P < 0.05$) than those with underground tubers (Table 5.3). Interestingly, amylose content significantly varied among groups collected from different zones. The accessions from Areka had the highest amylose content (20.5%) followed by those from Gedeo (18.2%), Gamo-Gofa (18.0%) and Sidama (16.3%) zones. On average, accession from Woalyita had the lowest amylose (14.2%).

Protein content varied between 6.4% and 13.9% DM with a mean and standard deviation of 9.7 and 1.8, respectively. Time of maturity and type of tuber (aerial vs. underground) did not impart significant influences on protein content, although aerial

yam accessions had a slightly higher mean value for protein (Table 5.3). There was no significant geographic pattern of variation in protein content. However, male plants had significantly higher protein content than the female ones. A linear correlation test was made to investigate the association among the various variables measured (Table 5.4). Pearson's correlation coefficient of -0.51 indicated a significantly negative ($P < 0.01$) correlation between starch and protein contents. Although not equally strong, a similar correlation was detected between starch and amylose contents. Accessions with higher starch values generally had lower amylose contents (Figure 5.2).

### 5.3.2. Pasting properties of yam flour

The RVA results showed considerable variation in pasting properties among the accessions studied (Table 5.2). Maturity time and type of tuber significantly influenced some of the variables measured (Table 5.3). For example, the early-maturing landraces attained significantly higher peak and trough viscosities, and took longer time to reach peak viscosity than the late-maturing ones. Accessions of aerial yam exhibited significantly lower peak, breakdown and setback viscosities and pasting temperature but attained the highest trough viscosity compared to those with underground tubers.

There were no significant variations between accessions obtained from different geographic origin with respect to pasting properties of flour based on group mean value, except that the collections from Gedeo attained a significantly higher setback viscosity than those from Gamo-Gofa. Peak viscosity attained by male plants and the time taken to reach it were significantly higher ($P < 0.01$) than for female plants (Table 5.3). Yet, there were no significant differences between male and female plants with respect to the remaining pasting properties.

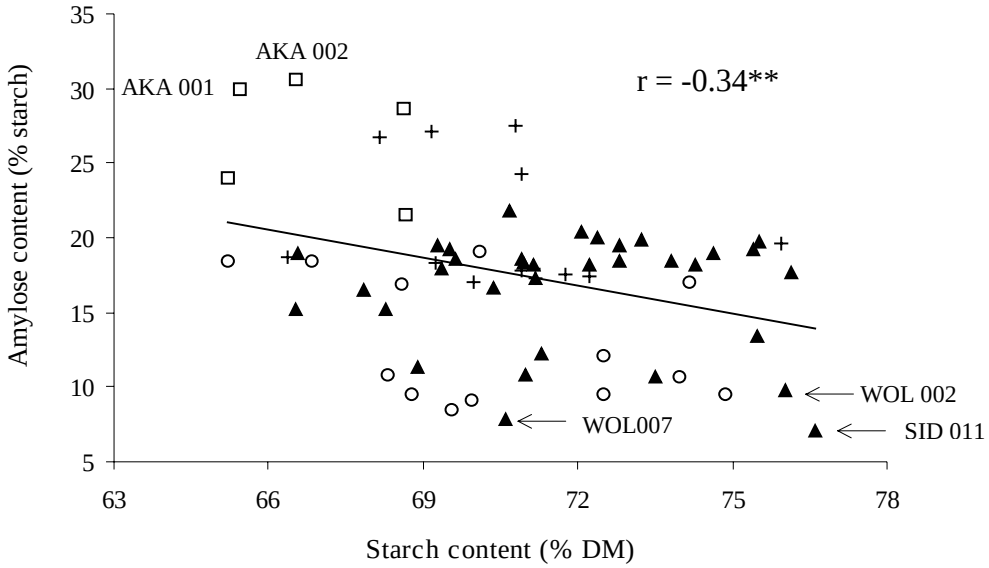



**Figure 5.2.** The correlation between starch and amylose contents in 65 yam accessions (*Dioscorea* spp.) collected from Southern Ethiopia (□ = aerial yam; ▲ = early-maturing; ○ = late-maturing; and + = accession obtained from Areka Agricultural Research Center).

**Table 5.1.** Description of the 65 Ethiopian yam (*Dioscorea* spp.) accessions studied.

| Acc No. | Accession Code | Local name | Type of Tuber | Sex | Time of Maturity | Acc No. | Accession Code | Local name | Type of Tuber | Sex | Time of Maturity |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | AKA 001 | Unknown | Aerial | Hermaphro. | -- | 34 | SID 012 | *Ganticho* | Underground | Male | Early |
| 2 | AKA 002 | Unknown | Aerial | Hermaphro. | -- | 35 | SID 013 | *Ganticho* | Underground | Male | Early |
| 3 | AKA 003 | Unknown | Aerial | Hermaphro. | -- | 36 | SID 015 | *Ganticho* | Underground | Male | Early |
| 4 | AKA 004 | Unknown | Underground | Female | -- | 37 | SID 018 | *Ganticho* | Underground | Male | Early |
| 5 | AKA 005 | Unknown | Underground | Male | -- | 38 | WOL 001 | *Wadala* | Underground | Male | Late |
| 6 | AKA 008 | Unknown | Underground | Male | -- | 39 | WOL 002 | *Hatiye* | Underground | Male | Early |
| 7 | AKA 009 | Unknown | Underground | Male | -- | 40 | WOL 003 | *Oha* | Underground | Male | Early |
| 8 | AKA 011 | Unknown | Underground | Male | -- | 41 | WOL 004a | *Wadala* | Underground | Male | Late |
| 9 | AKA 013 | Unknown | Underground | Female | -- | 42 | WOL 004b | *Wadala* | Underground | Male | Late |
| 10 | AKA 014 | Unknown | Underground | Male | -- | 43 | WOL 005 | *Oha* | Underground | Male | Early |
| 11 | AKA 016 | Unknown | Underground | Male | -- | 44 | WOL 006 | *Zoreuwa* | Underground | Male | Late |
| 12 | AKA 017 | Unknown | Underground | Female | -- | 45 | WOL 007 | *Hatiye* | Underground | Male | Early |
| 13 | AKA 018 | Unknown | Underground | Male | -- | 46 | WOL 008 | *Oha* | Underground | Male | Early |
| 14 | AKA 019a | Unknown | Underground | Male | -- | 47 | WOL 009 | *Hatiye* | Underground | Male | Early |
| 15 | AKA 019b | Unknown | Underground | Male | -- | 48 | WOL 011 | *Mortawa* | Underground | Male | Late |
| 16 | AKA 022 | Unknown | Underground | Male | -- | 49 | WOL 012 | *Ayina* | Underground | Male | Late |
| 17 | GED 001 | Ganticho | Underground | Male | Early | 50 | WOL 013a | *Gajela* | Underground | Male | Late |
| 18 | GED 003 | *Toracho* | Underground | Male | Early | 51 | WOL 013b | *Gajela* | Underground | Male | Late |
| 19 | GED 004 | *Ganticho* | Underground | Male | Early | 52 | WOL 015 | *Wadala* | Underground | Male | Late |
| 20 | GED 005 | *Toracho* | Underground | Male | Early | 53 | WOL 016 | *Macha* | Underground | Male | Early |
| 21 | GED 006 | *Ganticho* | Underground | Male | Early | 54 | WOL 017 | *Bakiche* | Aerial | Hermaphro. | -- |
| 22 | GED 007 | *Toracho* | Underground | Male | Early | 55 | WOL 018a | *Wayia* | Underground | Male | Late |
| 23 | GED 008 | *Toracho* | Underground | Male | Early | 56 | WOL 018b | *Wayia* | Underground | Male | Late |
| 24 | GGF 001 | *Wadala* | Underground | Male | Early | 57 | WOL 019 | *Gena* | Underground | Male | Late |
| 25 | GGF 002 | *Wadala* | Underground | Male | Early | 58 | WOL 020 | *Hatiye* | Underground | Male | Early |
| 26 | GGF 003 | *Hatiye* | Underground | Male | Early | 59 | WOL 021 | *Bundi-buchi* | Aerial | Hermaphro. | -- |
| 27 | GGF 004 | *Buna* | Underground | Female | Early | 60 | WOL 023b | *Walabo* | Underground | Male | Late |
| 28 | SID 001 | *Addo* | Underground | Male | Early | 61 | WOL 024 | *Hatiye* | Underground | Male | Early |
| 29 | SID 002 | *Goloma* | Underground | Male | Early | 62 | WOL 025 | *Macha* | Underground | Male | Early |
| 30 | SID 005 | *Addo* | Underground | Male | Early | 63 | WOL 026 | *Boye* | Underground | Male | Early |
| 31 | SID 007 | *Addo* | Underground | Male | Early | 64 | WOL 027 | *Hatiye* | Underground | Male | Early |
| 32 | SID 010 | *Addo* | Underground | Male | Early | 65 | WOL 028 | *Oha* | Underground | Male | Early |
| 33 | SID 011 | *Addo* | Underground | Male | Early | | | | | | |

GGF = Gamo-Gofa; WOL = Wolayita; SID = Sidama; and GED = Gedeo zones. AKA= Areka Agricultural Research Center (data is missing on maturity time of the accessions obtained from Areka).

**Table 5.2.** Composition and pasting properties of yam (*Dioscorea* spp.) flour in 65 accessions collected in Southern Ethiopia.

| | Composition | | | Pasting properties | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Accession | Starch content (%DM) | Amylose content (%) | Protein Content (%DM) | Peak viscosity (cP) | Trough Viscosity (cP) | Breakdown[1] Viscosity (cP) | Final Viscosity (cP) | Setback[2] (cP) | Peak[3] Time (min.) | Pasting Temperature (ºC) |
| AKA 001 | 66.5 | 30.6 | 10.4 | 3725.5 | 3639.0 | 86.5 | 5278.0 | 1639.0 | 6.7 | 71.1 |
| AKA 002 | 65.5 | 30.0 | 12.0 | 4786.0 | 3261.0 | 1525.0 | 4296.5 | 1035.5 | 4.7 | 67.7 |
| AKA 003 | 68.6 | 28.6 | 9.8 | 5009.0 | 2489.5 | 2519.5 | 3463.5 | 974.0 | 4.2 | 67.0 |
| AKA 004 | 70.9 | 24.3 | 8.0 | 4522.5 | 1890.5 | 2632.0 | 5229.5 | 3339.0 | 4.6 | 73.4 |
| AKA 005 | 69.2 | 27.2 | 11.0 | 5270.3 | 1926.3 | 3344.0 | 4795.8 | 2869.5 | 4.9 | 74.8 |
| AKA 008 | 68.2 | 26.7 | 11.7 | 6649.0 | 3177.5 | 3471.5 | 5538.5 | 2361.0 | 4.9 | 73.9 |
| AKA 009 | 70.8 | 27.4 | 10.0 | 6236.5 | 2349.0 | 3887.5 | 5602.0 | 3253.0 | 5.1 | 75.9 |
| AKA 011 | 70.9 | 17.8 | 10.4 | 5905.0 | 2211.0 | 3694.0 | 4598.0 | 2387.0 | 5.1 | 74.8 |
| AKA 013 | 71.3 | 17.6 | 7.8 | 4682.7 | 2005.0 | 2677.7 | 5213.7 | 3208.7 | 4.7 | 73.7 |
| AKA 014 | 66.4 | 18.7 | 8.1 | 6091.0 | 3218.0 | 2873.0 | 4150.5 | 932.5 | 5.0 | 75.5 |
| AKA 016 | 72.2 | 17.4 | 8.8 | 5813.0 | 2251.3 | 3561.7 | 5637.7 | 3386.3 | 5.0 | 74.6 |
| AKA 017 | 75.9 | 19.6 | 8.6 | 3844.5 | 1668.0 | 2176.5 | 4677.0 | 3009.0 | 4.6 | 73.5 |
| AKA 018 | 70.0 | 17.0 | 9.5 | 5911.0 | 2674.3 | 3236.8 | 5412.0 | 2737.8 | 5.1 | 74.1 |
| AKA 019a | 71.8 | 17.5 | 8.4 | 4766.0 | 2144.0 | 2622.0 | 5894.0 | 3750.0 | 4.9 | 75.6 |
| AKA 019b | 71.2 | 17.3 | 9.9 | 6172.5 | 2996.5 | 3176.0 | 4990.0 | 1993.5 | 5.2 | 77.1 |
| AKA 022 | 69.2 | 18.4 | 9.7 | 7336.0 | 3661.0 | 3675.0 | 5239.5 | 1578.5 | 5.2 | 75.2 |
| GED 001 | 66.6 | 19.0 | 13.9 | 4215.3 | 1799.7 | 2415.7 | 5166.3 | 3366.7 | 4.7 | 76.4 |
| GED 003 | 73.2 | 19.9 | 8.4 | 5586.3 | 1849.0 | 3737.3 | 5268.0 | 3419.0 | 4.9 | 74.4 |
| GED 004 | 70.9 | 18.3 | 8.8 | 6025.0 | 2652.0 | 3373.0 | 5676.0 | 3024.0 | 5.0 | 77.9 |
| GED 005 | 71.1 | 18.2 | 13.9 | 5854.5 | 2118.5 | 3736.0 | 4750.0 | 2631.5 | 4.8 | 75.5 |
| GED 006 | 68.3 | 15.2 | 9.4 | 5930.0 | 2012.7 | 3917.3 | 5792.3 | 3779.7 | 4.9 | 74.0 |
| GED 007 | 72.1 | 20.4 | 7.5 | 4477.0 | 1492.0 | 2985.0 | 4950.0 | 3458.0 | 4.7 | 72.6 |
| GED 008 | 70.4 | 16.6 | 12.0 | 5713.0 | 2154.5 | 3558.5 | 5234.0 | 3079.5 | 5.0 | 74.5 |
| GGF 001 | 66.5 | 15.2 | 13.8 | 5653.3 | 1733.3 | 3920.0 | 5324.0 | 3590.7 | 4.6 | 73.6 |
| GGF 002 | 70.9 | 18.5 | 11.0 | 5994.7 | 2927.3 | 3067.3 | 4857.7 | 1930.3 | 4.5 | 72.9 |
| GGF 003a | 74.6 | 18.9 | 8.8 | 5978.0 | 3552.3 | 2425.7 | 4423.7 | 871.3 | 5.1 | 74.9 |
| GGF 003b | 72.8 | 19.5 | 8.2 | 5055.0 | 1883.0 | 3172.0 | 5158.5 | 3275.5 | 4.7 | 74.4 |
| SID 001 | 69.5 | 19.2 | 10.2 | 6808.8 | 2994.5 | 3814.3 | 5272.3 | 2277.8 | 5.0 | 73.4 |

**Table 5.2.** Continued

| | Composition | | | Pasting properties | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Accession | Starch content (%DM) | Amylose content (%) | Protein Content (%DM) | Peak viscosity (cP) | Trough Viscosity (cP) | Breakdown[1] Viscosity (cP) | Final Viscosity (cP) | Setback[2] (cP) | Peak[3] Time (min.) | Pasting Temperature (ºC) |
| SID 002 | 67.8 | 16.5 | 10.9 | 5516.0 | 2162.5 | 3353.5 | 5030.5 | 2868.0 | 5.0 | 77.5 |
| SID 003 | 76.1 | 17.6 | 6.8 | 5251.0 | 2724.7 | 2526.3 | 5620.0 | 2895.3 | 5.0 | 77.0 |
| SID 005 | 71.2 | 17.3 | 11.9 | 6278.5 | 2622.3 | 3656.3 | 4950.8 | 2328.5 | 5.0 | 75.3 |
| SID 007 | 72.2 | 18.2 | 7.4 | 5269.0 | 2007.0 | 3262.0 | 6092.5 | 4085.5 | 5.2 | 74.4 |
| SID 010 | 71.3 | 12.2 | 13.5 | 5347.0 | 2240.0 | 3107.0 | 5092.3 | 2852.3 | 5.0 | 72.9 |
| SID 011 | 76.6 | 7.1 | 8.0 | 5382.5 | 3876.5 | 1506.0 | 6028.5 | 2152.0 | 4.9 | 73.9 |
| SID 012 | 73.5 | 10.7 | 8.1 | 4749.3 | 2268.8 | 2480.5 | 4662.0 | 2393.3 | 4.7 | 73.3 |
| SID 013 | 75.4 | 19.3 | 8.6 | 5908.0 | 2231.3 | 3676.7 | 5362.3 | 3131.0 | 5.0 | 75.6 |
| SID 015 | 69.3 | 19.5 | 9.3 | 4916.0 | 1778.5 | 3137.5 | 4394.5 | 2616.0 | 5.1 | 73.4 |
| SID 018 | 70.7 | 21.9 | 11.2 | 6300.3 | 2254.8 | 4045.5 | 5864.8 | 3610.0 | 4.8 | 72.8 |
| WOL 001 | 72.5 | 12.0 | 9.4 | 4535.5 | 1835.5 | 2700.0 | 4500.5 | 2665.0 | 4.5 | 74.7 |
| WOL 002 | 76.0 | 9.8 | 8.6 | 6025.5 | 2186.5 | 3839.0 | 5764.0 | 3577.5 | 5.1 | 74.3 |
| WOL 003 | 73.8 | 18.4 | 9.9 | 6220.5 | 2475.5 | 3745.0 | 4431.0 | 1955.5 | 5.0 | 74.3 |
| WOL 004a | 68.3 | 10.9 | 8.3 | 4260.5 | 1755.5 | 2505.0 | 4923.0 | 3167.5 | 4.5 | 72.6 |
| WOL 004b | 68.8 | 9.5 | 7.8 | 4268.0 | 1554.3 | 2713.7 | 4908.3 | 3354.0 | 4.3 | 74.4 |
| WOL 005 | 68.9 | 11.4 | 11.9 | 4759.3 | 1888.0 | 2871.3 | 4770.3 | 2882.3 | 4.8 | 72.7 |
| WOL 006 | 69.6 | 8.5 | 9.3 | 4525.5 | 1803.5 | 2722.0 | 4983.5 | 3180.0 | 4.6 | 74.4 |
| WOL 007 | 70.6 | 7.8 | 12.8 | 5940.3 | 2877.0 | 3063.3 | 4875.7 | 1998.7 | 5.1 | 76.3 |
| WOL 008 | 75.5 | 13.5 | 10.6 | 4603.5 | 2049.5 | 2554.0 | 4890.5 | 2841.0 | 4.9 | 78.7 |
| WOL 009 | 71.0 | 10.8 | 9.7 | 6219.5 | 2013.5 | 4206.0 | 4491.0 | 2477.5 | 4.9 | 74.3 |
| WOL 011 | 69.9 | 9.2 | 9.6 | 5285.5 | 1953.0 | 3332.5 | 5442.0 | 3489.0 | 4.8 | 75.1 |
| WOL 012 | 74.0 | 10.7 | 9.1 | 3940.0 | 1961.0 | 1979.0 | 5718.5 | 3757.5 | 4.8 | 73.5 |
| WOL 013a | 74.9 | 9.6 | 7.0 | 5207.0 | 1763.0 | 3444.0 | 4888.0 | 3125.0 | 4.4 | 72.7 |
| WOL 013b | 72.5 | 9.5 | 7.9 | 5334.3 | 2358.3 | 2976.0 | 4751.7 | 2393.3 | 4.6 | 72.7 |
| WOL 015 | 65.2 | 18.4 | 10.2 | 5418.0 | 1684.0 | 3734.0 | 4718.0 | 3034.0 | 4.4 | 73.4 |
| WOL 016 | 75.5 | 19.8 | 8.3 | 5736.3 | 2516.0 | 3220.3 | 4429.0 | 1913.0 | 5.2 | 77.3 |
| WOL 017 | 65.2 | 24.0 | 11.8 | 4544.0 | 3004.5 | 1539.5 | 3942.5 | 938.0 | 4.8 | 66.7 |
| WOL 018a | 70.1 | 19.1 | 9.5 | 4641.7 | 1616.0 | 3025.7 | 4251.0 | 2635.0 | 4.4 | 73.2 |

**Table 5.2.** Continued

| | Composition | | | Pasting properties | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Accession | Starch content (%DM) | Amylose content (%) | Protein Content (%DM) | Peak viscosity (cP) | Trough Viscosity (cP) | Breakdown[1] Viscosity (cP) | Final Viscosity (cP) | Setback[2] (cP) | Peak[3] Time (min.) | Pasting Temperature (ºC) |
| WOL 018b | 68.6 | 16.8 | 7.8 | 5468.3 | 2505.3 | 2963.0 | 4858.0 | 2352.7 | 4.5 | 73.4 |
| WOL 019 | 66.8 | 18.4 | 11.9 | 4018.5 | 1288.0 | 2730.5 | 3424.5 | 2136.5 | 4.0 | 74.4 |
| WOL 020 | 69.6 | 18.6 | 9.3 | 5613.7 | 2012.7 | 3601.0 | 4675.7 | 2663.0 | 5.0 | 74.3 |
| WOL 021 | 68.6 | 21.6 | 8.4 | 4156.0 | 3968.0 | 188.0 | 5703.0 | 1735.0 | 4.7 | 67.1 |
| WOL 023b | 74.2 | 17.1 | 6.4 | 4713.5 | 1788.5 | 2925.0 | 5249.0 | 3460.5 | 4.5 | 72.6 |
| WOL 024 | 72.8 | 18.5 | 9.5 | 5712.3 | 2258.0 | 3454.3 | 4659.0 | 2401.0 | 4.9 | 72.8 |
| WOL 025 | 74.3 | 18.2 | 7.6 | 5463.5 | 2151.0 | 3312.5 | 4922.5 | 2771.5 | 5.0 | 71.8 |
| WOL 026 | 72.4 | 20.0 | 8.8 | 4778.0 | 1940.0 | 2838.0 | 3930.0 | 1990.0 | 5.0 | 73.9 |
| WOL 028 | 69.4 | 17.9 | 11.4 | 6128.5 | 2802.0 | 3326.5 | 5611.5 | 2809.5 | 5.2 | 75.9 |
| Mean | 70.9 | 17.5 | 9.7 | 5329.9 | 2321.6 | 3008.3 | 4995.4 | 2678.8 | 4.9 | 73.9 |
| Std. Dev. | 2.9 | 5.3 | 1.8 | 784.1 | 607.6 | 793.0 | 575.5 | 766.1 | .35 | 2.3 |
| CV (%) | 4.1 | 16.8 | 7.8 | 14.7 | 26.2 | 26.4 | 11.5 | 28.6 | 7.2 | 3.1 |
| Range | 65.2-76.6 | 7.1-30.6 | 6.4-13.4 | 3725.5-7336.0 | 1288.0-3968.0 | 86.5-4206.0 | 3424.5-6092.5 | 871.3-4085.0 | 4.0-6.7 | 66.7-78.7 |

[1]Peak viscosity – trough viscosity
[2]Fnal viscosity – trough viscosity
[3]Time at which peak viscosity is reached

**Table 5.3.** Mean comparison of composition and pasting properties of yam (*Dioscorea* spp.) tuber flour among 65 Ethiopian accessions differing with respect to type of tuber, sex, maturity time and geographic origin.

| | | Composition | | | Pasting Properties | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Type of tuber** | N | Starch content (%DM) | Amylose content (%DM) | Protein content (%DM) | Peak Viscosity (cP) | Trough Viscosity (cP) | Breakdown Viscosity (cP) | Final Viscosity (cP) | Set-back Viscosity (cP) | Peak time (min.) | Pasting T$^{o}$ ($^{o}$C) |
| Aerial yam | 5 | 66.9$^{a}$ | 27.0$^{b}$ | 10.5$^{a}$ | 4444.1$^{a}$ | 3272.4$^{b}$ | 1771.7$^{a}$ | 4536.7$^{a}$ | 1264.3$^{a}$ | 5.0$^{a}$ | 67.9$^{a}$ |
| Underground | 60 | 71.2$^{b}$ | 16.7$^{a}$ | 9.6$^{a}$ | 5403.8$^{b}$ | 2242.4$^{a}$ | 3161.8$^{b}$ | 5033.6$^{a}$ | 2791.2$^{b}$ | 4.8$^{a}$ | 74.4$^{a}$ |
| **Sex** | | | | | | | | | | | |
| Male | 56 | 71.1$^{b}$ | 16.4$^{a}$ | 9.7$^{b}$ | 5466.4$^{b}$ | 2269.6$^{a}$ | 3196.9$^{a}$ | 5031.0$^{a}$ | 2761.4$^{a}$ | 4.8$^{b}$ | 74.5$^{a}$ |
| Female | 4 | 72.2$^{b}$ | 20.3$^{a}$ | 8.2$^{a}$ | 4526.2$^{a}$ | 1861.6$^{a}$ | 2664.5$^{a}$ | 5069.7$^{a}$ | 3208.0$^{a}$ | 4.6$^{a}$ | 73.8$^{a}$ |
| **Maturity time** | | | | | | | | | | | |
| Early | 32 | 72.0$^{b}$ | 16.6$^{b}$ | 9.8$^{a}$ | 5555.0$^{b}$ | 2307.6$^{b}$ | 3247.4$^{a}$ | 5070.0$^{a}$ | 2762.4$^{a}$ | 4.9$^{b}$ | 74.3$^{b}$ |
| Late | 15 | 70.2$^{a}$ | 13.6$^{a}$ | 9.3$^{a}$ | 4884.3$^{a}$ | 1901.8$^{a}$ | 2982.5$^{a}$ | 4853.2$^{a}$ | 2951.4$^{a}$ | 4.5$^{a}$ | 73.6$^{b}$ |
| **Collection area** | | | | | | | | | | | |
| Areka | 13 | 70.0$^{a}$ | 20.5$^{b}$ | 9.4$^{a}$ | 5630.8$^{a}$ | 2474.8$^{a}$ | 3156.0$^{a}$ | 5152.2$^{a}$ | 2677.4$^{ab}$ | 4.9$^{a}$ | 74.8$^{a}$ |
| Gamo-Gofa | 4 | 71.2$^{a}$ | 18.0$^{ab}$ | 10.5$^{a}$ | 5670.3$^{a}$ | 2524.0$^{a}$ | 3146.3$^{a}$ | 4941.0$^{a}$ | 2417.0$^{a}$ | 4.7$^{a}$ | 73.9$^{a}$ |
| Gedeo | 7 | 70.4$^{a}$ | 18.2$^{ab}$ | 10.6$^{a}$ | 5400.2$^{a}$ | 2011.2$^{a}$ | 3389.3$^{a}$ | 5262.4$^{a}$ | 3251.2$^{b}$ | 4.8$^{a}$ | 75.0$^{a}$ |
| Sidama | 11 | 72.1$^{a}$ | 16.3$^{ab}$ | 9.6$^{a}$ | 5611.5$^{a}$ | 2469.2$^{a}$ | 3142.3$^{a}$ | 5306.4$^{a}$ | 2837.2$^{ab}$ | 4.9$^{a}$ | 74.5$^{a}$ |
| Wolayita | 25 | 71.1$^{a}$ | 14.2$^{a}$ | 9.3$^{a}$ | 5152.7$^{a}$ | 2041.4$^{a}$ | 3111.3$^{a}$ | 4802.7$^{a}$ | 2761.2$^{ab}$ | 4.7$^{a}$ | 74.1$^{a}$ |

Values in the same column with different letter superscripts are significantly different.

**Table 5.4.** Pearson's linear correlation coefficient for the various chemical and pasting properties of yam (*Dioscorea* spp.) flour for accessions collected in Southern Ethiopia.

| | Starch Content | Protein Content | Amylose Content | Peak Viscosity | Trough Viscosity | Breakdown Viscosity | Final Viscosity | Setback Viscosity | Peak Time |
|---|---|---|---|---|---|---|---|---|---|
| Protein content | -0.51** | | | | | | | | |
| Amylose content | -0.34** | 0.15 | | | | | | | |
| Peak viscosity | 0.07 | 0.17 | 0.02 | | | | | | |
| Tough viscosity | -0.07 | 0.06 | 0.25* | 0.37** | | | | | |
| Breakdown viscosity | 0.12 | 0.12 | -0.18 | 0.70** | -0.40** | | | | |
| Final viscosity | 0.24 | -0.16 | -0.17 | 0.21 | 0.16 | 0.08 | | | |
| Setback viscosity | 0.24 | -0.17 | -0.33** | -0.14 | -0.67** | 0.38** | 0.62** | | |
| Peak time | 0.07 | 0.08 | 0.24 | 0.27* | 0.49** | -0.11 | 0.32** | -0.14 | |
| Pasting temperature | 0.32** | 0.02 | -0.33** | 0.34** | -0.18 | 0.48** | 0.27* | 0.35** | 0.22 |

** Correlation is significant at $P < 0.01$

* Correlation is significant at $P < 0.05$

RVA pasting curves were also considered to further elucidate the variability in pasting properties. For example, the pasting profile of aerial yam accessions revealed the intraspecific variability for peak, trough and final viscosities (Figure 5.3). AKA 001 and WOL 021 attained higher trough and final viscosities than AKA 003, AKA 002 and WOL 017. They also attained final viscosities higher than their respective peak viscosities. This was partly reflected in their very low breakdown viscosity values (Table 5.2). The variability among selected landraces, including aerial yam, was also visualized for their RVA pasting profile (Figure 5.4). The aerial yam accession showed lower peak and final viscosities compared to the other landraces with underground tubers.

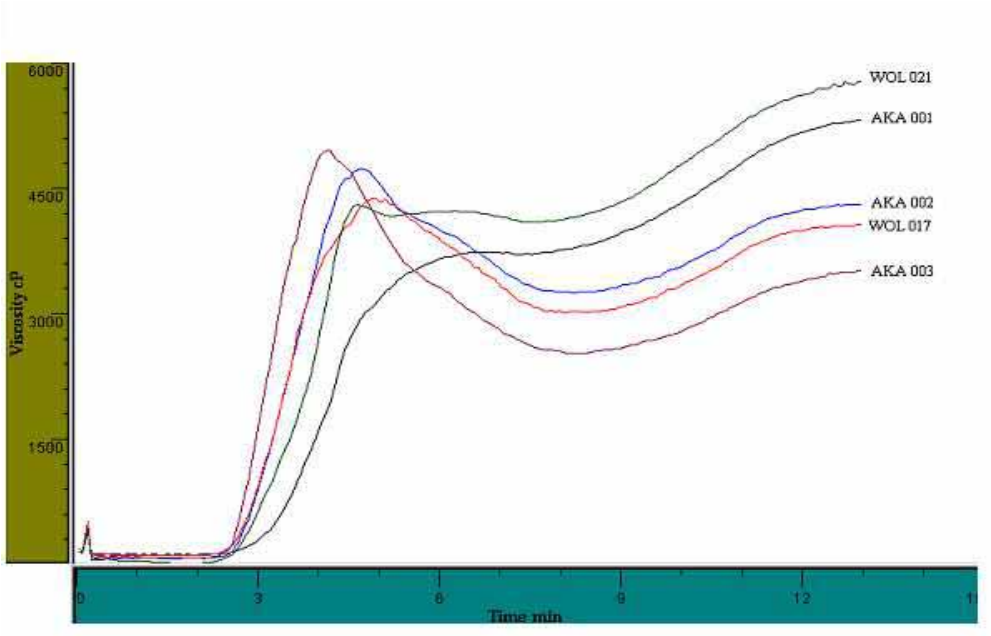



**Figure 5.3.** RVA pasting profiles of yam flour for five accessions of aerial yam (D. bulbifera) collected from Southern Ethiopia.

Pearson correlation analysis was used to investigate the effect of the main flour constituents on its pasting properties, and to explore interrelationships amongst the various RVA pasting attributes (Table 5.4). In general, starch content was not significantly correlated with flour pasting properties except for its positive correlation with pasting temperature. Amylose content was positively correlated with trough viscosity ($P < 0.05$), while it was inversely correlated with setback viscosity ($P < 0.01$) and pasting temperature ($P < 001$). Various pasting properties were also significantly

correlated among each other (Table 5.4). For example, peak viscosity was positively correlated with trough viscosity ($P < 0.01$), breakdown viscosity ($P < 0.01$), peak time ($P < 0.05$) and pasting temperature ($P < 0.01$).

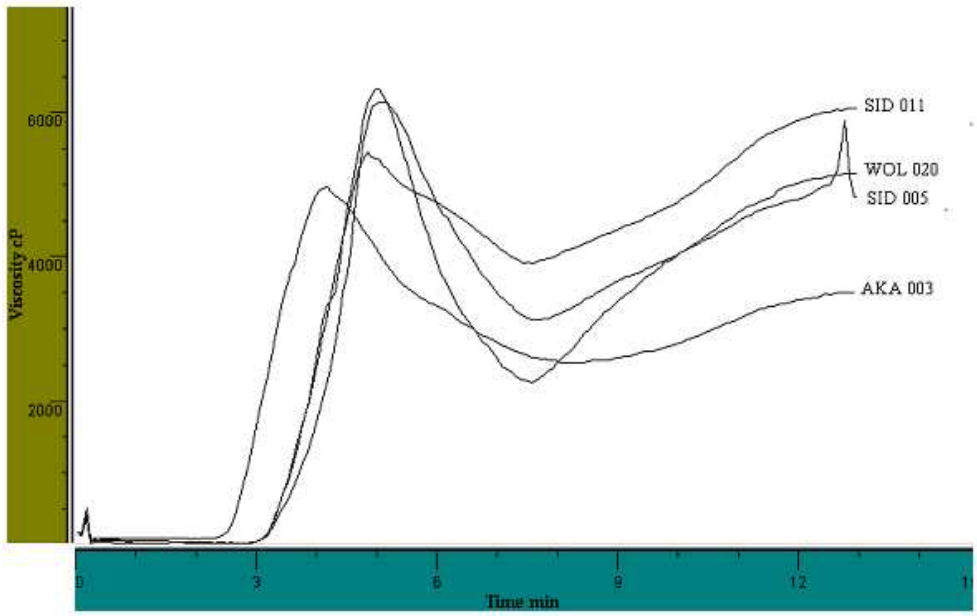



**Figure 5.4.** RVA viscograms of yam flour of four selected yam landraces collected from Southern Ethiopia (AKA 003 = aerial yam, SID 005 and SID 011 = ado, and WOL 020 = hatiye).

### 5.1.1. Phenetic analysis

To visualize the association among the accessions studied with respect to all the 10 parameters measured, the data was subjected to both cluster and principal component (PCA) analyses. UPGMA clustering, while clearly separating the accessions into those producing aerial and underground tubers, gave four distinct clusters (Figure 5.5). The first two clusters represented aerial yam accessions. Cluster 1 contained AKA 001 and WOL 021, while the three remaining aerial yam accessions (AKA 002, AKA 003 and WOL 017) were grouped together in the second cluster. Cluster 3 was constituted by 59 accessions, including both early and late-maturing landraces. This cluster was further divided into four sub-groups: one major sub-group with 52 accessions, and three other minor sub-groups with 3 (GED 001, GGF 001 and WOL 015), 1 (WOL 019) and 3 accessions (AKA 014, GGF 003 and WOL 016). Cluster 4 represented SID 011, the

accession with the lowest amylose content and one of the lowest values for breakdown viscosity.

The PCA provided ten principal components, eight of which accounted for the total variability observed among the accessions studied (Table 5.5). The first four principal components, accounting for about 78% of the total variability, were highly correlated with pasting properties of the flour. The fifth, sixth and seventh components accounted for 8, 7 and 4% of the total variability, and had the highest loadings on amylose, protein and starch contents, respectively. The pattern of variation explained by the first two principal components is given in Figure 5.6. As expected, aerial yam accessions were clearly separated from those with underground tubers. Plot of the first and third principal components further revealed the two clusters (cluster 1 and 2) within the aerial yam accessions, and the fourth cluster representing SID 011 (Figure 5.7). GGF 003, AKA 014, WOL 019 and GGF 001, the accessions that could not be unequivocally grouped with others, were among those represented by the three minor sub-groups detected within cluster 3 by UPGM clustering (Figure 5.5).

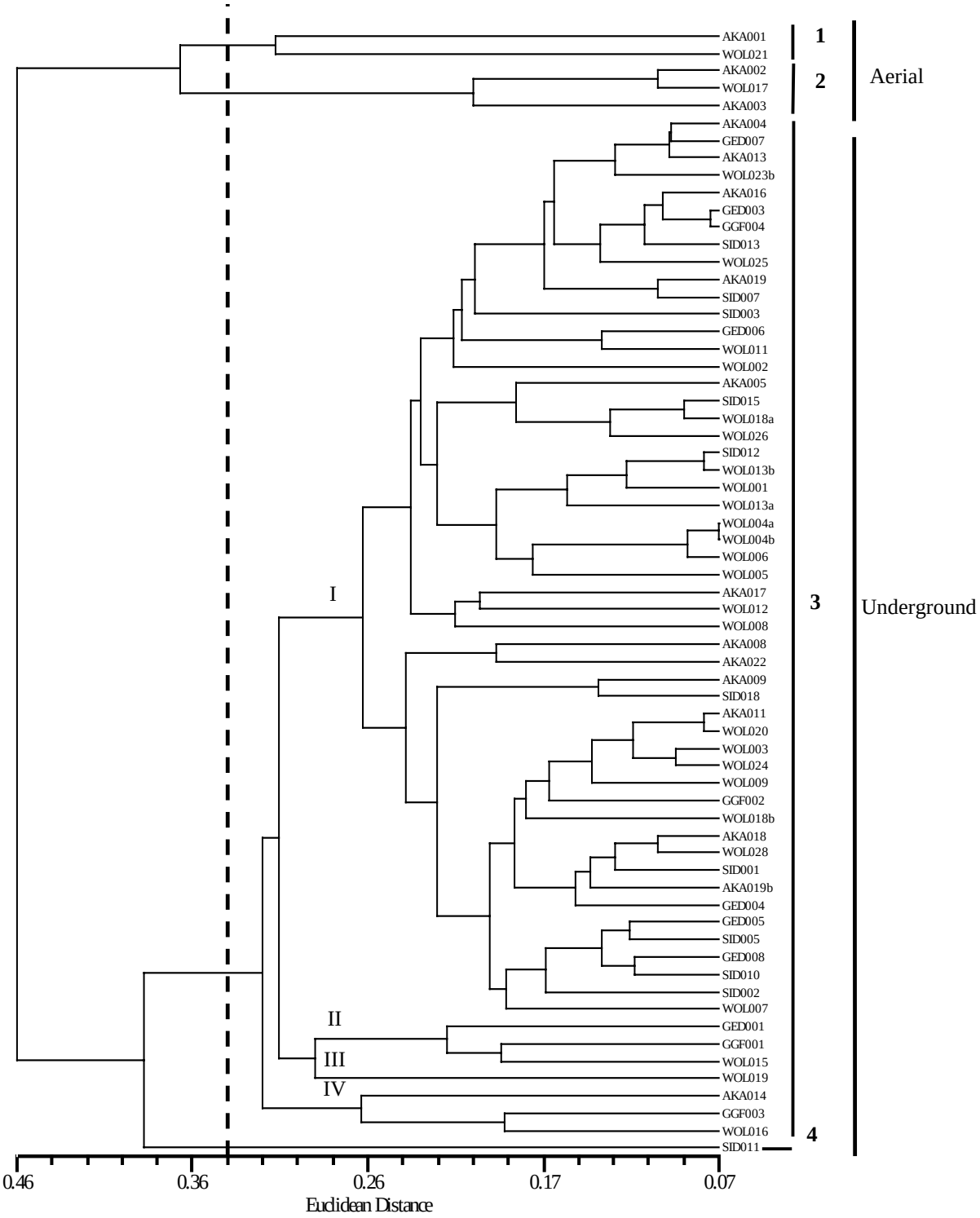



**Figure 5.5.** UPGMA dendrogram (Euclidean distance) for 65 yam accessions (*Dioscorea* spp.) collected in Southern Ethiopia on the basis of 10 compositional and pasting property parameters of the flour.

**Table 5.5.** Eigenvalues, variance, cumulative variance and factor loadings for the first eight principal components (PC) for the variability in 65 accessions of *Dioscorea* spp. based on composition and pasting properties of tuber flour.

| | Factor loadings | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | PC 1 | PC 2 | PC 3 | PC 4 | PC 5 | PC 6 | PC 7 | PC 8 |
| Eigenvalues | 2.85 | 2.14 | 1.67 | 1.15 | 0.80 | 0.71 | 0.38 | 0.31 |
| Variance (%) | 28.45 | 21.43 | 16.66 | 11.55 | 7.97 | 7.06 | 3.78 | 3.10 |
| Cumulative (%) | 28.45 | 49.88 | 66.54 | 78.09 | 86.06 | 93.12 | 96.90 | 100.00 |
| Starch Content | -0.04 | 0.05 | 0.11 | 0.04 | -0.16 | -0.28 | **0.93** | 0.14 |
| Protein Content | 0.03 | 0.10 | -0.08 | 0.05 | 0.05 | **0.96** | -0.25 | 0.03 |
| Amylose Content | 0.12 | -0.03 | -0.10 | 0.13 | **0.95** | 0.05 | -0.15 | -0.14 |
| Peak Viscosity | 0.34 | **0.91** | 0.09 | 0.12 | 0.02 | 0.07 | 0.03 | 0.13 |
| Tough Viscosity | **0.96** | 0.02 | 0.08 | 0.25 | 0.10 | 0.02 | -0.02 | -0.09 |
| Breakdown Viscosity | -0.39 | **0.89** | 0.03 | -0.07 | -0.06 | 0.06 | 0.04 | 0.20 |
| Final Viscosity | 0.06 | 0.08 | **0.97** | 0.18 | -0.07 | -0.07 | 0.09 | 0.09 |
| Setback Viscosity | **-0.72** | 0.04 | **0.66** | -0.06 | -0.13 | -0.07 | 0.08 | 0.14 |
| Peak Time | 0.25 | 0.04 | 0.15 | **0.94** | 0.14 | 0.05 | 0.03 | 0.13 |
| Pasting Temperature | -0.14 | 0.27 | 0.13 | 0.14 | -0.16 | 0.03 | 0.15 | **0.91** |

Coefficients in bold indicate the variables with the highest loadings on the corresponding component.

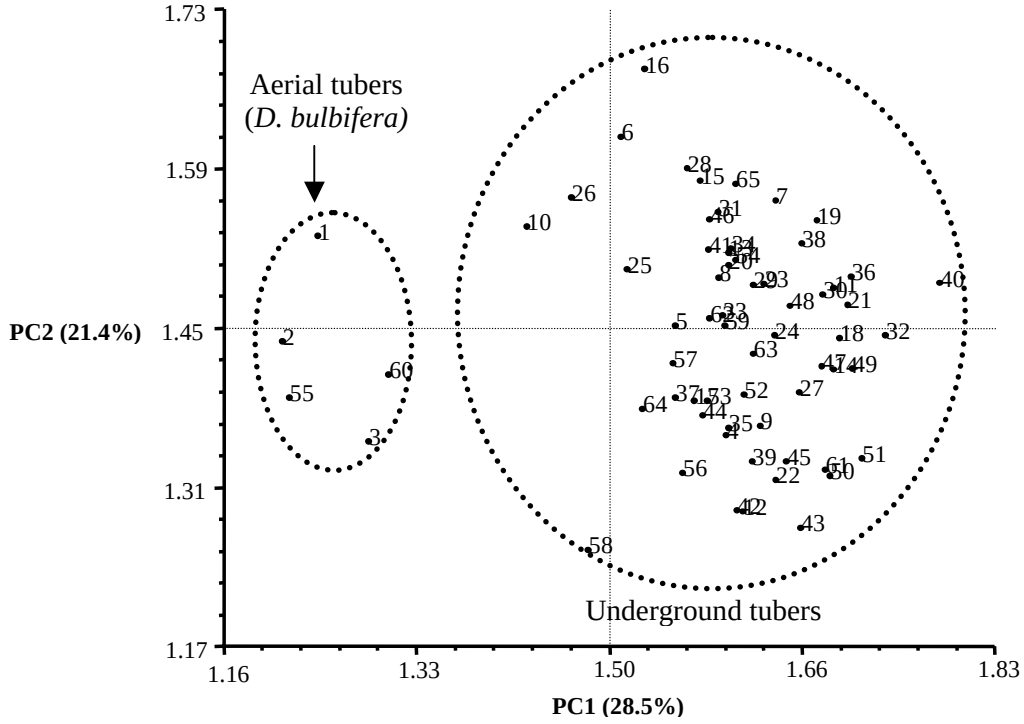



**Figure 5.6.** Plot of the first and second components scores for 65 accessions of *Dioscorea* spp. based on 10 compositional and pasting properties of the flour (the numbers refer to list of accessions in Table 5.1).

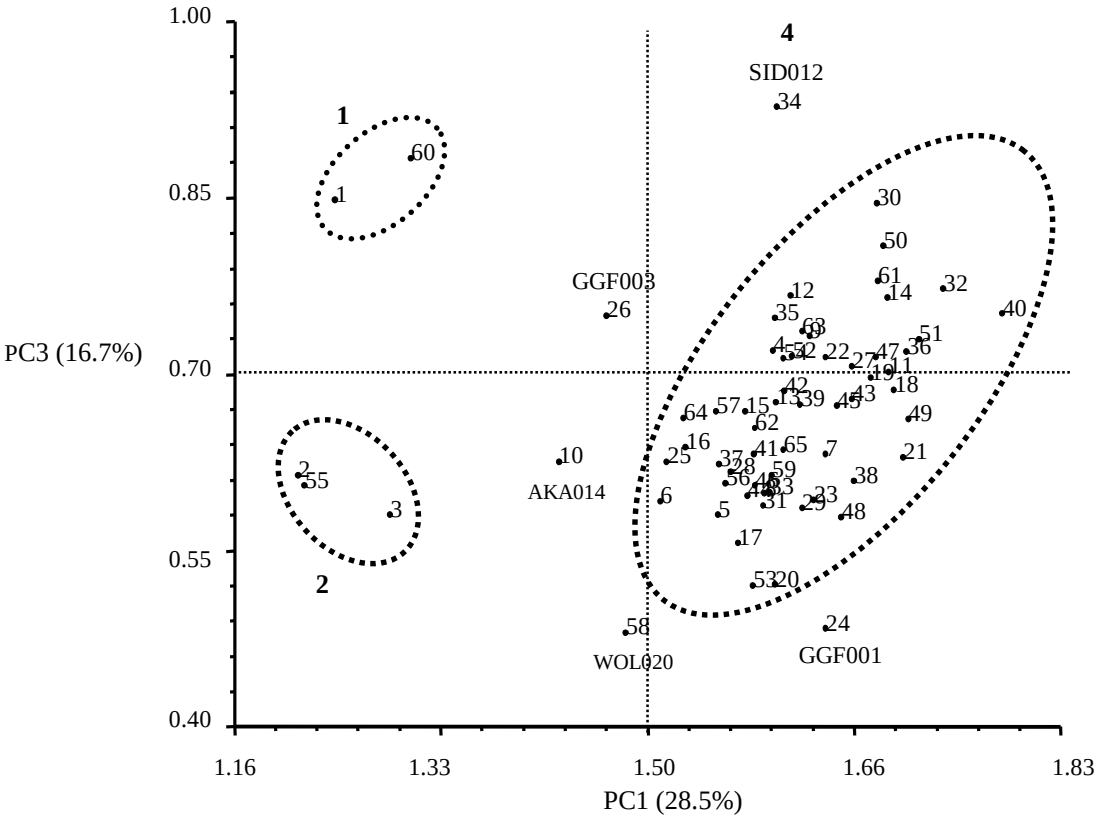



**Figure 5.7.** Plot of the second and third component scores for 65 accessions of Dioscorea spp. based on 10 compositional and pasting properties of the flour (Numbers 1, 2 3, and 4 refer to the clusters in Figure 5.5).

## 5.2. Discussion

### 5.2.1. Variability in chemical composition

Considerable diversity existed among the accessions studied with respect to the major tuber constituents and pasting properties of the flour. The protein content result confirmed previous findings involving yams from different botanical sources (Martin 1979; Egbe and Treche 1983; Lebot et al. 2005), but was higher than reported values for various yam species from Jamaica (Muzac-Tucker et al. 1993). Martin (1979) recorded protein contents ranging from 6.3% to 15.5% (mean = 9%) in 47 varieties belonging to five *Dioscorea* species. He further noted that higher protein contents are characteristics of vigorous cultivars with larger tubers. But, our finding does not support this observation, as protein content of the late-maturing landraces, which are

vigorous and often produce larger tubers, was not significantly different from that of early-maturing ones (Table 5.2).

Although aerial yam accessions on average had relatively higher protein than the other accessions, the difference was not statistically significant. It appears that, on the basis of individual accessions, the highest protein contents were recorded in those accessions with underground tubers (Table 5.1). However, this can be due to the fact that more accessions were considered from the later group. Bhandari et al. (2003) found higher protein contents in aerial yam compared to other wild yam species found in Nepal. Still their finding contradicts that of Egbe and Treche (1983) who recorded a lower protein content in aerial yam accessions collected from Cameroon. Aerial yam is the only species native to both Asia and Africa, widely distributed in different regions, and shows considerable diversity (Degras 1993). It is grown either for the bulbils (aerial tubers), underground tubers, or both (Onwueme and Charles 1994). It is, therefore, important that the diversity in compositional and functional properties is assessed in the part(s) utilized for human consumption.

The mean protein content of the accessions investigated in this study, 9.7%, is higher than reported values for sweet potato, potato, cassava, taro and plantain (Woolfe 1992; Rehm and Espig 1991). This confirms earlier reports that yam contains more protein than the other root and tuber crops (Hahn et al.1987). Taking into account the protein-poor diets of most inhabitants of the tropical region and the higher yam consumption rates particularly during peak harvesting time, this finding shows the potential of yam as important protein source. The difference (more than double) between the accessions with the lowest and highest protein contents may also provide scope for improving the protein supply by yam through selection of landraces or clones with high protein contents.

Starch is the major storage carbohydrate in most plants and is primarily found in seeds and underground storage organs such as in roots and tubers. Yam tubers contain about 50-80% starch on dry matter basis, which is fairly distributed throughout the tuber (Degras 1993). Our finding (65.2% to 76.6%) also falls within the frequently reported values for the major cultivated yam species. Although no significant geographic pattern of variation was observed with regard to starch content, the aerial yam

accessions had significantly lower starch contents than the rest, supporting Martin's (1979) finding. Overall, the variation in starch content is less compared to that of the other parameters analyzed.

The relative proportion and characteristics of the two principal components of starch, amylose and amylopectin, greatly affect its properties (Ellis et al. 1998). This, in turn, has a marked influence on food quality. The variation in amylose content (7.1%-30.6%) and the significantly high values for aerial yam accessions reported here is in agreement with the available literature for yam starch from different botanical sources (Martin 1979; Farhat et al. 1999; Amani et al. 2004; Brunnschweiler et al. 2005). It is also comparable with that of potato and cassava starch (Moorthy 200), the two widely utilized tuber starches for food and non-food applications (Ellis et al. 1998).

It appears that reports on factors that affect the variation in amylose content are very limited, and this topic has not been properly investigated in yams. The negative correlation observed between amylose and starch contents in this study contradicts the report by Lebot et al. (2005), who found a positive correlation between the two parameters in *D. alata* tubers. Brunnschweiler et al. (2005) found no significant variation in amylose content among the various cultivars considered in their study, despite the amylose-content gradient across different sections of individual tubers. Variation in amylose content may be related to factors affecting the activity of GBSS (granule-bound starch synthase), the key enzyme involved in amylose synthesis, which can be altered without significant effects on the total amount of starch produced (Visser and Jacobsen 1993).

### 5.2.2. Diversity in pasting properties of yam flour

The accessions studied displayed a wide range of pasting properties, which also explained much of the observed variability (Table 5.5). The lower values in aerial yam accessions for most of the parameters measured (peak viscosity, breakdown, setback, final viscosity and pasting temperature) are most likely due to their significantly lower starch content compared to those with underground tubers (Figure 5.2). Their relatively higher amylose and protein contents may also offer some explanation, as these constituents are known to affect pasting properties even in isolated starch. For

example, increased protein contents in chickpea were associated with reductions in peak, breakdown and final viscosities (Sayar et al. 2005).

Alves et al. (2002) conducted a comparative analysis of yam flour and isolated starch with respect to various functional properties, and found a lower viscosity in flour samples. In addition to the relatively lower starch content of the flour, the difference was attributed to the presence of other components such as fiber, lipids, proteins and minerals that influence pasting. Even in isolated starch, the non-starch components of starch granules such as ash, cellulose, proteins and lipids affect its functional properties (Moorthy 2002). Although these components are generally low in yam (Gebre-Mariam and Schmidt 1998; Amani et al. 2004) and, thus, impart minor influences, the interspecific variation in starch granules size and shape significantly affect pasting properties (Răsper 1971; Farhat et al. 1999; Amani et al. 2004)

As was evident from the relationships among the attributes after peak viscosity (Table 5.4), breakdown viscosity was negatively correlated with trough viscosity, while showing positive correlation with setback viscosity. High breakdown viscosity is the result of a high degree of collapse of swollen starch granules, leading to lower trough viscosity. The collapse in starch granules is also accompanied by a significant release of the more soluble amylose into the solution. Upon cooling, re-association among starch molecules (retrogradation), particularly amylose, brings about a rise in viscosity to a final level. The higher final viscosities attained by AKA 001 and WOL 021 relative to their respective peak viscosities show the high tendency of the material to retrograde (Figure 5.3). This was also apparent from the lack of noticeable breakdown in viscosity on heating and steering. Furthermore, the lack of a clear peak or rise in viscosity even beyond 95ºC in AKA 001 means that not all granules gelatinized at 95ºC and some continued to gelatinize during the holding period (Moorthy 2002).

Pasting profiles have important bearings on suitability of a material for different applications. For example, pasting temperature, i.e., the temperature at the onset of rise in viscosity, is related to resistance of a material to swelling. It indicates the maximum temperature required for cooking and the associated energy cost (Singh et al. 2004). Breakdown viscosity, a measure of the susceptibility of cooked starch to disintegration, is also an important factor for many processes. Starch with a high setback viscosity is

suitable for use in heat-processed food, however, has restricted applications where low syneresis or weeping is required such as in frozen or refrigerated food (Singh et al. 2004).

Previous studies on pasting properties of starchy foods mostly involved isolated starch, and few data are available on properties of flour materials. This makes comparison of our finding with that of the other root and tuber crops is hardly possible. Nevertheless, the overall viscosity profile of the aerial yam accession is similar to those reported for other *Dioscorea* species, while that of the accessions with underground tubers confirms to the profile for potato starch (Farhat et al. 1999). It has been shown that yam starch generally attains a lower viscosity compared to potato (Farhat et al. 1999) but exhibit a similar pasting profile to that of cassava starch (Moorthy 2002).

There was no significant effect of the geographic origin of accessions on most the variables measured. This was evident from both the cluster analysis (Figure 5.5) and PCA (Figure 5.6), where groupings mainly reflected differences in type of tuber and maturity time. This is congruent with the findings of Lebot et al. (2005). However, reports on the effect of geographic origin are often inconclusive. In the review by Shannon and Garwood (1984), the effects of growing conditions on starch composition are less compared to those associated with genotype and organ maturity. On the other hand, Ellis et al. (1998) refer to several findings in which growing condition significantly influenced starch composition and properties. For example, growing condition significantly influenced amylose content and gelatinization temperature in potato (Cottrell et al. 1995). Thus, the properties of each known genotype should be investigated under various conditions if the effects of genotype-by-environment interactions are to be fully understood.

In general, the finding shows the presence of a considerable variability among accessions with respect to the parameters considered. Results of the cluster and principal component analyses were also consistent in revealing the main pattern of variation among the accessions. Any progress towards the potential use of yam flour or starch in both food and non-food applications can add value to this traditional crop thereby increasing its improvement and utilization. The recent reports on a sweet

potato variety with low gelatinization property (Katayama et al. 2002; 2004) and a new starch type from *Dioscorea opposita* (Shujun 2006) are examples of such a progress.

## 5.3. Conclusion

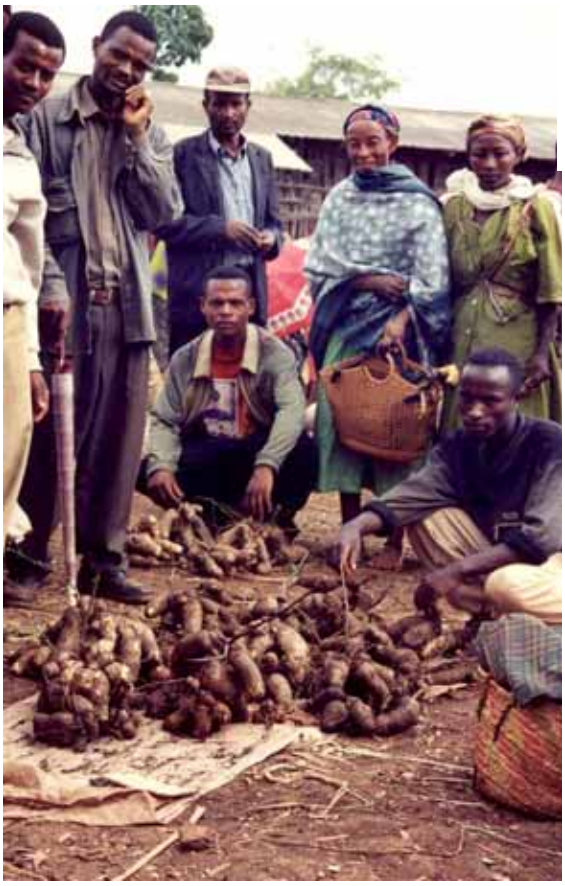
The broad ranges of properties observed indicate the available potential in selecting genotypes superior for desirable traits. This can be one way of improving yam in the short term. The selected materials may also form the basis for future improvement of the crop.

In this study, attempt was made to compare the two maturity groups (early vs. late) recognized by local farmers on the basis of the parameters measured. The early-maturing landraces are first harvested in May or June and, then, the second harvesting is carried out from August to September. In this study, however, tubers were sampled only once for all accessions at the end of the growing season. For detailed assessment of the effect of such practice on the chemical and functional properties of the flour, it is imperative that samples are colleted twice for early-maturing landraces as practiced by local farmers. Any effect of sex of the plant on its product properties also needs to be clearly established by including more female plants in future studies.

The aerial yam accessions have shown distinct properties compared to those with underground tubers, indicating the need for further studs o this species. This species is only cultivated to a lesser extent in the study area, although widely known in other parts of the country. Considering its high multiplication ratio, there is a potential for its expansion in the study area and introduction into other regions if the need arises. However, its acceptance by local farmers and consumers is an issue that needs the attention of researchers in relevant fields of study.

Recommendations regarding the potential use of yam from Ethiopia in both food and non-food applications significantly benefit from additional knowledge on the morphological, functional and physico-chemical properties of isolated starch compared to flour material. Information on starch granule size and shape, content of minor constituents such as lipids, proteins and non-starchy carbohydrates, and their effect on

physical and pasting properties of starch will shade more light on the potential applications of yam starch and the modifications needed for various purposes.



← Yam seed tubers on market at Chelelektu (Kochore), Gedeo. (Photo: Muluneh Tamiru)

Yam plant trained along a tree on a farm near Kebado (Dara), Sidama. → (Photo: Muluneh Tamiru)

A fenced yam plot near Humbo, Wolayita. (Photo: Muluneh Tamiru)

↑

Yam planted alongside sweet potato plot in Wolayita. (Photo: Muluneh Tamiru)

Yam planted in old coffee plantation for support in Dara, Sidama.

↓

Sorghum stalk used for yam staking in Wolayita.

↓

# 6. Outlook and Conclusions

In the preceding chapters, findings of the various investigations conducted on topics related to yam diversity in the major yam-growing areas of Southern Ethiopia have been presented and discussed. Main conclusions of the study and key issues for future considerations are given below:

- The number of landraces described in the study area, their distribution and abundance, role as a source of food and cash income, as well as the existing local classification system all reflect the significance of yam in local subsistence. Its continued production despite the lack of any support from researchers and policy makers suggests that yam is adapted to local agriculture, where different crop species or cultivars and cultivation practices are carefully selected to fit the prevailing environmental, economical and socio-cultural conditions. This reality calls for a coordinated research program to uncover the full diversity and potential of existing landraces in order to promote their conservation, so that they can be harnessed for crop improvement.

- Farmers' decision-making processes in response to environmental, economical and socio-cultural conditions to meet household demands are the main factor behind the dynamics of yam diversity. Ecological adaptation, maturity time, organoleptic properties and market demand are among factors that affect the number and composition of landraces maintained by individual households. The current preferences towards double-harvested landraces because of early harvest and high market demand have implications in setting priorities for future activities. For instance, conservation programs can take on the task of safeguarding the single-harvested landraces that are presently being replaced by the expanding production of early-maturing ones, while tackling the major constraints facing the production of the latter group ought to be a priority in a crop improvement program.

- Farmers in the study area possess considerable knowledge about the diversity present in yams and the attributes of each landrace. Owing to past research neglect, farmers are often the only sources of information concerning yams in

Ethiopia. The local yam classification system, representing an important aspect of farmers' management of existing diversity, is typical of crop species that have significant importance in faming systems and are closely associated with socio-cultural life of traditional societies. Some of the criteria that are commonly considered for categorizing local yam varieties (such as tolerance to drought, maturity time and tuber quality) represent those traits that farmers value the most in their landraces. Consequently, a thorough analysis of the indigenous knowledge system, farmers' participation in designing and implementing conservation as well as improvement programs is critical to bring practical solutions to problems of immediate concern to them.

° Most named landraces are morphologically distinct. Nevertheless, the structure of morphological diversity is not always consistent with farmers' landrace classification. This is partly due to the fact that variation in morphological traits is only one of the many attributes considered in local classification of yam landraces. Local classification systems are holistic in approach and often reflect the multiple objectives of traditional households. It is, therefore, imperative that any assessment of diversity in local varieties and interpretation of results take full note of how diversity is perceived and managed, and its importance in local agriculture.

° As revealed by AFLP analysis of genetic diversity, the Ethiopian yam germplasm is genetically distant from the commonly cultivated *Dioscorea* species. The distinct identity of the Ethiopian materials presents both challenges and opportunities for future works. Of immediate interest is the need to establish the species identity of these materials by including more reference materials. The occurrence of wild species in Ethiopia that are believed to be among those that produced cultivated forms in West Africa requires the inclusion of more genotypes (both cultivated and wild) from other regions of Ethiopia and known African centers of yam diversity in such studies. This is important to further elucidate the evolutionary dynamics and domestication of yams in the country in particular, and to address the taxonomic puzzle surrounding African yam species in general.

- Time of maturity is a principal criterion employed in local classification of yam landraces. The fact that the major groups constituted based on morphological traits largely reflected differences in maturity time suggests that agronomic traits are well represented in the variations of morphological characters. Interestingly, the clusters formed using genetic similarities based on AFLP markers further show that variations in maturity time reflect significant differences at DNA level. Thus, maturity time could offer a useful guide in sampling genotypes on farmers' fields and in subsequent management of accessions.

- The investigation on composition and pasting properties of yam flour clearly indicated the presence of considerable diversity among the accessions studied. For instance, the range of values recorded for protein provides an opportunity for selection of superior genotypes to improve protein supply by yam. However, the general lack of clear groups except that of accessions with aerial vs. underground tubers suggests that the attributes and potential of each landrace should be assessed under different environmental conditions to come up with conclusive remarks. Moreover, studies on composition, texture and functional properties of isolated yam starch are important to establish its potential use for processing yam into different forms, or for use in non-food applications such as textiles, cosmetics or agro-chemicals.

- The level of diversity detected and depth of local knowledge show that Woalyita and Gamo-Gofa zones represent important areas of yam diversity. Thus, they must be given due emphasis when further collections and *in situ* conservation programs are stipulated. Germplasm collection and similar research in areas not covered by this study are also important to reveal the extent of yam diversity in the country.

Ethiopia is endowed with a great diversity of plants. But their contribution to local diet and the country's economy is mostly minimum. Apart from the conservative food habit of the society at large, lack of understanding about the potentials of these crops and their importance in human diet are among the factors that contributed to the current state of affairs. On this account, this study has made significant contributions in our

understanding of the diversity available in one of the traditional food crops of Ethiopia. The presence of different landraces, which are often composed of genetically variable crop populations, in traditional farming systems in the tropics are often attributed to adaptations to heterogeneous environmental conditions. On the other hand, factors such as market demand, customs and traditions, curiosity and aesthetic value can affect crop diversity in many regions. Accordingly, identifying traits that farmers find important in their landraces and assessing needs for crop improvement and conservation should be among the objectives for studying crop genetic resources in traditional farming systems.

## Summary

Ethiopia is a center of origin and domestication for a range of crop species. This geographic importance of the country has, in the recent past, attracted several studies on topics related to crop genetic resources. Nevertheless, the diversity present in some of the country's widely cultivated food species is still poorly understood. Yam (*Dioscorea* spp.) is one of the major root and tuber crops grown in the densely populated areas of Southern, Southwestern and Western Ethiopia, and has a considerable importance in local livelihood. Conversely, little is known about yam diversity and production status in the country due to past research neglect. Hence, issues pertinent to improved utilization and conservation of the available genetic resources are yet to be addressed.

This study was initiated with the main objective of characterizing the extent and distribution of yam diversity in the major yam-growing areas of Southern Ethiopia. It also aimed at identifying the role of yam in local subsistence as well as factors that are important in farmers' maintenance of yam diversity, assessing implications for future research, conservation and development programs. Its ultimate goal is to bring yam to the attention of researchers and policy makers through broadening the knowledge base of the crop. To this end, four different research topics were considered and their respective results have been presented in four articles (chapter 2 to chapter 5). The major findings of the study were highlighted in chapter 6.

The diversity, farmers' knowledge, management and use of yam landraces were investigated through a farm-level survey that covered 339 households in eight districts of Wolayita zone and in Kucha district of the neighboring Gamo-Gofa zone during the 2003/2004 cropping season. The study area was stratified according to geographic distance and elevation to cover the ecological range of yam. Overall, 37 named landraces were described on farm, revealing considerable diversity in the region. Most of the landraces described had limited abundance and distribution, and up to 32% of the landraces were recorded on single farms. Production is, thus, based mainly on a few widely distributed dominant landraces that are perceived to be superior with respect to various attributes. The number of landraces maintained on individual farms ranged

from one to six, with a mean of 2.9. Farmers' decisions regarding what to plant are influenced by environmental factors (for example, drought tolerance), maturity time, tuber quality and existing market demand.

There was considerable variation amongst the districts surveyed with respect to number, distribution and abundance of landraces. Damot-Gale of Wolayita zone was the most diverse district both in terms of total and unique number of landraces recorded. The level of similarity/differences between districts with regard to named landraces mainly reflected their geographic distance. This can partly be explained by the existing local seed supply system. Farmers rely on seed tubers saved from the preceding harvest, while local markets and exchanges with neighbors represent secondary sources that partly cover the need for planting materials. Still, these seed exchange systems can cover considerable distances. The importance of yam in the study area is twofold. First, as it is adapted to dry-season planting, early harvests fill a seasonal gap in food supply. This is crucial for household food security. Second, yam is traditionally preferred over the other root and tuber crops such as sweet potato, potato, cassava and taro, and thus fetches higher prices on local markets. As a result, yam is establishing itself as an important cash crop in many localities. It is worth noting that the areas covered by this study are not known for other cash crops, such as coffee.

Overall, yam production is on the decline in most areas due to a range of factors. On the other hand, production is on the increase in some localities based on a few selected landraces. It appears that early maturity, combined with excellent organoleptic quality and market demand, has made the double-harvested (early-maturing) landraces the preferred choice in areas where production is on the increase. This trend has important implications that need to be taken into consideration in any effort to conserve or improve existing landraces. On the one hand, the double-harvested landraces are prone to drought, the most important limiting factor to crop production in the area. On the other, there is a threat to the late-maturing landraces that are being replaced by the early-maturing ones in response to the current demand.

The morphological variation in 84 accessions collected from Gamo-Gofa, Gedeo, Sidama and Wolayita zones was assessed based on 32 quantitative morphological traits.

The accessions were characterized on experimental plots established at Awassa College of Agriculture, Ethiopia. Despite the considerable variation observed among individual accessions, the major morphological groups represented the two main yam types (aerial vs. underground) and maturity groups (early vs. late) to which the accessions belong. Most named landraces were morphologically distinct. However, no significant morphological variations were detected among some accessions collected under different vernacular names. The structure of morphological diversity was further compared to the local classification system, which was studied in the same areas through individual and key informant interviews. Data collected include farmers' selection criteria and attributes of each landrace.

There is a well-developed local yam classification system that widely reflects variations in morphological and agronomic traits, ecological adaptation as well as existing social values. Following grouping available landraces into those that produce aerial (*bola boye*) and underground tubers, local farmers recognize two major categories within the latter group: *hatuma boye* ('male' yam) and *macha boye* ('female' yam). Although yam is mostly dioecious, this classification has no reference to the reproductive biology of the plants. It is rather based on maturity time, vigor and ecological adaptation, as well as reflects the society's perception of gender and its role. Individual landraces within both groups are further identified based on differences in morphological and growth attributes. Although maturity time was not included in the characterization work, hierarchical clustering and principal component analysis gave morphological groups that are largely consistent with farmers' landrace classification according to maturity time.

Lack of significant morphological variation among some landraces managed as different and the inability of standard morphological descriptors in revealing the species identity of landraces with underground tubers justified the use of molecular markers for further analysis of the available diversity. Consequently, 48 selected Ethiopian yam germplasm collections representing the variability in landrace names, geographic origin and morphological traits were subjected to genetic diversity analysis based on AFLP (Amplified Fragment Length Polymorphism) markers. Relationships of these materials with the commonly cultivated species *D. alata*, *D. bulbifera*, *D. cayenensis* and *D. rotundata* were investigated by including in the study elite genotypes

from West Africa. Ten AFLP primer combinations generated 900 fragments, of which 97% were polymorphic. Both cluster and principal coordinate analyses separated the accessions into their respective taxa, whereas the Ethiopian materials constituted a cluster distinct from any of the investigated species. Interspecific genetic similarity values based on Jaccard Coefficient have shown that the Ethiopian accessions are genetically closer to *D. cayenensis* and *D. rotundata* than to the other species studied. Further analysis of diversity within the Ethiopian materials using cluster and principal coordinate analyses gave six distinct clusters, revealing the variation between accessions mainly with regard to maturity time. The only non-flowering accession in the collection was also detected, while some accessions collected under similar vernacular names were found to be genetically different. The overall findings are consistent with farmers' landrace classification based on time of maturity and structure of morphological diversity.

Assessing the quality and suitability of starch and starch-containing materials for food and non-food applications is important to detect the potential of genotypes. This can lead to increased utilization, thereby facilitating improvement and conservation of crop genetic resources. 65 yam accessions representing distinct landraces or landraces collected from different localities under the same name were characterized for diversity in composition and pasting properties of tuber flour. Comparison of mean values indicated that accessions of aerial yam and those with underground tubers significantly differed with respect to most of the parameters measured. On the other hand, groups formed based on type of tubers and maturity time showed significant differences only for some of the parameters. Starch content varied from 65.2% to 76.6% of DM (mean 70.9%), while protein content ranged between 6.4% and 13.4%. Amylose, accounting for 7.1% to 30.6% of starch, showed the highest variability compared to protein and starch contents, and was the only parameter significantly influenced by geographic origin of the accessions. Furthermore, a significant negative correlation ($P < 0.01$) was observed between starch content and its amylose fraction. However, the biological or environmental basis of this relationship is far from clear. Cluster and principal component analyses (PCA) clearly distinguished the aerial yam accessions from those producing underground tubers. The first four principal components generated by PCA, explaining about 78% of the total variability, were highly correlated with pasting property parameters. It generally appears that, the clusters constituted based on

compositional and pasting property parameters for the accessions producing underground tubers did not confirm to the clusters constituted based on both morphological and AFLP markers in the previous studies.

In general, this study has contributed significantly to our understanding of the diversity and importance of yams in the parts of Ethiopia covered. Its findings lay an important foundation for further studies. They also provide the basis for improved utilization and conservation of yam germplasm. Similar investigations in areas not covered here would provide additional information on the extent of yam diversity in Ethiopia. In future studies, attention should be given to wild *Dioscorea* genetic resources to further elucidate the evolution, domestication and species identity of Ethiopian yams. So far, yam has survived the neglect by researchers and policy makers because of its crucial role in local subsistence. However, in the face of changing environmental, social, economical, and policy factors, it is difficult to ascertain the continued maintenance of yam landraces by local farmers. This requires detailed studies on the underlying factors and possible implications. Collection, analysis and documentation of the local knowledge of yam are as crucial as conservation of the germplasm. Studies on composition and properties of isolated starch would compliment our findings and further reveal the potential of yam in food and non-food applications. Equally important is the need for research in applied agronomy to deal with the main constraints in yam production, such as low multiplication ratio, scarcity of staking materials, declining soil fertility, among many others. These can certainly improve the role of yam in addressing food security both at households and national levels.

## Zusammenfassung

Äthiopien ist ein Genzentrum für eine Reihe von Kulturpflanzen. Daher wurden in diesem Land in der Vergangenheit zahlreiche Untersuchungen zur Bedeutung genetischer Ressourcen durchgeführt. Bei einigen der Hauptnahrungspflanzen in Äthiopien ist aber das Ausmaß der vorhandenen genetischen Diversität noch weitgehend unbekannt. Obwohl Yam (*Dioscorea* spp.) zu den wichtigsten Knollenpflanzen in den dicht bevölkerten Regionen im Süden, Südwesten und Westen Äthiopiens gehört und eine große Rolle in der lokalen Ernährung spielt, wurde diese Fruchtart in der Forschung bisher vernachlässigt und wenig ist über ihre Diversität und Anbauweise bekannt. Daher sind viele Fragen zur besseren Nutzung und Erhaltung der genetischen Ressourcen bisher nicht untersucht.

Ziel der Arbeit ist es, die Diversität von Yam in seinem Hauptanbaugebiet im Süden Äthiopiens zu charakterisieren. Von besonderer Bedeutung ist dabei die Frage, wie die Landwirte in der Subsistenzwirtschaft die Diversität erhalten. Durch die Arbeit soll ein stärkeres Interesse für Yam in der Forschung geweckt werden.

Das Wissen der einheimischen Bevölkerung über Diversität und Anbau von Yam wurde durch eine Befragung von 339 Haushalten in acht Bezirken in Wolayita und dem Bezirk Kucha in Gamo-Gofa während der Anbausaison 2003/2004 untersucht. Das Untersuchungsgebiet wurde so unterteilt, dass die geographische Verteilung und die unterschiedlichen Höhenlagen gut repräsentiert waren. Ingesamt wurden 37 unterschiedlich benannte Landsorten erfasst. Die meisten der Landsorten hatten nur eine eingeschränkte Verbreitung, 32 % aller Landsorten wurden sogar nur auf einer Farm angetroffen. Die Yam-Produktion beruht daher vor allem auf wenigen, sehr weit verbreiteten Landsorten mit besonderer Anbaueignung. Die Anzahl der je Farm angebauten Landsorten lag zwischen einer und sechs, mit einem Mittelwert von 2,9. Als Gründe für den Anbau bestimmter Landsorten gaben die Landwirte die Anpassung an Umweltfaktoren (z.B. Trockenheit), die Reifezeit, Qualitätseigenschaften sowie Vermarktungsmöglichkeiten an.

Die Landwirte verwenden überwiegend eigenes Pflanzgut, während lokale Märkte und der Austausch mit Nachbarn eine geringere Rolle spielen. Yam hat im Untersuchungsgebiet vor allem aus zwei Gründen eine große Bedeutung. Zum einen ist diese Fruchtart an den Anbau in der Trockenzeit angepasst und kann daher bereits früh in der kritischen Jahreszeit einen Ertrag liefern, um die Versorgung der Haushalte mit Nahrungsmitteln zu sichern. Zum anderen wird Yam von den Verbrauchern traditionell gegenüber anderen Knollenfrüchten wie Süßkartoffeln, Kartoffel, Cassava und Taro bevorzugt und ist daher eine wichtige Marktfrucht. Dies ist von besonderer Bedeutung, da in dem untersuchten Gebiet keine anderen Marktfrüchte, wie z.B. Kaffee, angebaut werden.

Es wurde sowohl ein Rückgang des Yamanbaus als auch der Trend zum Anbau einer geringeren Anzahl von Landsorten beobachtet. Besonders die frühreifen Landsorten, die zweimal beerntet werden, nehmen an Bedeutung zu. Allerdings sind diese relativ anfällig gegen Trockenstress, und daher ist es wichtig, auch die spätreifen Landsorten zu erhalten.

Die morphologische Variation in 32 quantitativen Merkmalen wurde an 84 Herkünften aus Gamo-Gofa, Gedeo, Sidama und Wolayita untersucht. Die Untersuchung wurde auf dem Versuchsfeld des Awassa College of Agriculture durchgeführt. Trotz einer großen Variation in allen Merkmalen konnten mit Hilfe von multivariaten statistischen Methoden als wesentliche Gruppen die beiden Typen mit ober- bzw. unterirdischen Knollen sowie die beiden Reifegruppen früh und spät klar unterschieden werden, und dieses obwohl die Reifezeit nicht in die statistische Berechnung mit einbezogen worden war. Landsorten mit unterschiedlichen Namen ließen sich meist auch morphologisch unterscheiden, wobei jedoch einige Ausnahmen auftraten; z.B. wurden verschiedentlich morphologisch unterschiedliche Landsorten unter demselben Namen gesammelt.

Die Einteilung verschiedener Typen durch die Farmer aufgrund der Reifezeit stimmte weitgehend überein mit den während der Charakterisierung im Feldanbau beobachteten Unterschieden in morphologischen und agronomischen Eigenschaften. Es wird zunächst der Typ mit oberirdischen Knollen (*bola boye*) unterschieden, und die Formen mit unterirdischen Knollen werden in „männliche“, spätreife (*hatuma boye*) und „weibliche“, frühreife (*macha boye*) Sorten unterteilt. Obwohl Yam meist diözisch ist,

bezieht sich diese Einteilung nicht auf das Geschlecht der Blüten, sondern auf Reifezeit und Wuchstyp.

Da jedoch in einigen Fällen zwischen verschieden bezeichneten Landsorten keine eindeutigen morphologischen Unterschiede auftraten, und da zusätzlich die Herkünfte mit unterirdischen Knollen nicht einer definierten botanischen Art zugeordnet werden konnten, wurden zur Klassifizierung auch molekulare Marker verwendet. Dazu wurden 48 Herkünfte, die für das Untersuchungsmaterial repräsentativ waren, mit AFLP (Amplified Fragment Length Polymorphism) Markern charakterisiert. Zum Vergleich wurden bekannte Herkünfte der üblicherweise angebauten Arten *D. alata, D. bulbifera, D. cayenensis* und *D. rotundata* mit einbezogen. Die AFLP Analyse zeigte etwa 900 Marker, von denen 97 % polymorph waren. Sowohl mit der Cluster- als auch mit der Hauptkomponenten-Analyse konnten die zum Vergleich verwendeten botanischen Arten klar abgegrenzt werden, während die äthiopischen Herkünfte keiner der untersuchten gängigen Arten eindeutig zugeordnet werden konnten. Mit den angewandten statistischen Methoden fielen die äthiopischen Herkünfte in sechs Gruppen, die sich u.a. deutlich in der Reifezeit unterscheiden.

Für die Unterscheidung und Beurteilung der verschiedenen Herkünfte sind auch Qualitätsmerkmale von großer Bedeutung. Im Vordergrund steht dabei die Eignung der Stärke und von Stärkeprodukten für Anwendungen im Food- und Non-Food Bereich. Bei der Untersuchung von 65 Herkünften wurde eine große Variation in der Zusammensetzung der Knollen und in ihrer Verarbeitungseignung beobachtet. Besonders deutlich war der Unterschied zwischen Herkünften mit ober- bzw. unterirdischen Knollen. Der Stärkegehalt der untersuchten Herkünfte variierte zwischen 65 und 77 %, und der Proteingehalt zwischen 6,4 und 13,4 %. Eine besonders hohe Variabilität wurde für den Amyloseanteil an der Stärke beobachtet, der zwischen 7,1 und 30,6 % lag. Sowohl Cluster- als auch Hauptkomponentenanalyse der Qualitätsdaten führte zu einer klaren Differenzierung zwischen Formen mit ober- bzw. unterirdischen Knollen.

Bei zukünftigen Untersuchungen sollten verstärkt Wildarten einbezogen werden. Von großer Bedeutung ist auch die Erhaltung, Sammlung, und Evaluierung lokaler Landsorten. Diese Untersuchungen sollten von Studien zur Verbesserung der Qualität

und zu verbesserten Anbautechniken begleitet werden. Dadurch würde Yam einen noch größeren Stellenwert für die Sicherung der Ernährung Äthiopiens erlangen.

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# Curriculum Vitae

## Personal


| | |
|---|---|
| Name: | Muluneh Tamiru Oli |
| Sex: | Male |
| Date: | 15.02.1970 |
| Place of Birth: | Nekemte (Wollega), Ethiopia |
| Nationality: | Ethiopian |

Current Address:
Theodor-Heuss-St. 11
Zimmer 411
37075 Göttingen
**Germany**
**Email:** mtamiru1@yahoo.com

Permanent Address:
Debub University
P. O. Box – 5
Awassa
**Ethiopia**


## Education and Training

1976-1983: Elementary school, Nekemte (Wollega), **Ethiopia**.

1983-1987: Secondary school, Arjo (Wollega), **Ethiopia**.

1987-1989: Awassa College of Agriculture, **Ethiopia**, Diploma in Plant Sciences and Technology.

1993-1996: The University of Reading, **UK**, M.Sc. in Horticulture

August-November 2000: International Course on Vegetable Production, International Agricultural Center, Wageningen, **The Netherlands**.

February-March 2002: International Course on Research and Development in Postharvest Biology and Technology. Institute of Technology and Storage of Agricultural Products, Department of Postharvest Science of Fresh Produce, The Volcanic Center, **Israel**.

April 2003–present: PhD student, Georg-August University Göttingen, Göttingen, **Germany**.

## Professional career

1989-1993: Technical Assistant, Section of Horticulture, Awassa College of Agriculture, Debub University, Ethiopia.

1996 - 2002: Lecturer, Department of Plant Production and Dryland Farming, Awassa College of Agriculture, Debub University, Ethiopia.

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