STUDIES ON MOLECULAR GENETIC DIVERSITY and USEFUL GENOMIC TRAITS of YAM (*DIOSCOREA* SPP) GERMPLASM COLLECTIONS FROM ETHIOPIA

A THESIS SUBMITTED TO

THE SCHOOL OF GRADUATE STUDIES FACULTY OF LIFE SCIENCES

ADDIS ABABA UNIVERSITY

BY

ATNAFUA BEKELE

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE

DOCTOR OF PHILOSOPHY (PhD) IN BIOLOGY

(APPLIED GENETICS)

ADDIS ABABA JUNE 2014

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SUPERVISOR: - PROF. ENDASHAW BEKELE

ADDIS ABABA **JUNE 2014**

DECLARATION

I declare that the thesis hereby submitted by me for the Degree Doctor of Philosophy (PhD) in Biology (Applied Genetics) to the School of Graduate Studies of Addis Ababa University is my own independent work and has not previously been submitted by me or anybody else at another university. The materials obtained from other sources have been duly acknowledged in the thesis.

Signed on June 2014, The School of Graduate Studies, Faculty of Life Sciences, Addis Ababa University, Arat Kilo.

PhD Candidate ______________________________
Atnafua Bekele

Supervisor ______________________________
Prof. Endashaw Bekele

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Dedication

To my late father Ato Bekele Damtew and my Daughter Ruth

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Acronyms and Abbreviations

AAU =Addis Ababa University

Ae =Effective number of alleles

AFLP =Amplified Fragment Length Polymorphism

AMOVA =Analysis of Molecular Variance

APS =Amonium Peri Sulfate

ARC =Areka Agricultural Research Center

CTAB =Cetyltriethyl Ammonium Bromide

DNA =DeoxyriboNucleic Acid

dNTPS =Deoxy nucleotide Triphosphates

EST =Expressed Sequence Tag

EHNRI =Ethiopian Health and Nutrition Research Insititute

FAAS =FLAME ATOMIC ABSORPTION SPECTROMETRY

FAO =FOOD AND AGRICULTURAL ORGANIZATION

H =Nei's Genetic Diversity

He =Expected Heterozygosity

UHe =Unbiased Expected Heterozygosity

GENALEX =Genetic Analysis in Excel

IITA =International Institute of Tropical Agriculture

IBC =Institute of Biodiversity Conservation (Currently IBCR)

IPGRI =International Plant Genetic Resources Institute

MG/100 G =milligram per hundred gram

MT =METRIC TON

NTSYpc =Numerical Taxonomy and Multivariate Analysis System

NA =Number of Alleles

NE =Number of Effective Alleles

% P =Percent Polymorphism

PAGE =Polyacrylamide Gel Electrophoresis

PCA =Principal Component Analysis

PCs =Principal Components

PCR =Polymorphic Chain Reaction

PCO =Principal Coordinate

PIC =Polymorphic Information Content

r =Correlation Coefficients

RFLP = Restriction Fragment Length polymorphism

RNA =Ribose Nucleic Acid

I =Shannon's Information Index

SSRs =Simple Sequence Repeats

SNP =Single Nucleotide Polymorphism

SNNPRS =Southern Nation Nationalities People Regional State

Ta =Annealing Temperature

TE =TRIS-EDTA

TEMED = Tetra-Methyl Ethylene Di-Amine

TBE =Tris Borate EDTA
UPGMA =Un-Weighted Pair-Group Method Average

Abstract

Yam (*Dioscorea* spp L.) is one of the underutilized tuber crops in Ethiopia. It is cultivated mainly in South and Southwestern part of the country. Sixty yam accessions (Based on availability 6-15 sprouted tubers of each sample) from ten different geographic origins of Southern Nations Nationalities & People's Regional State and Oromiya region of the country were used in this study. The sprouted tubers of some accessions were received from Research Centers where others were directly collected from farmers' fields during early March, 2010. These collected yam genotypes were planted in two different experimental sites at Hawasa and Wonago.

Under morphological variability study the collected yam (*Dioscorea* spp) were identified in to six different *Dioscorea* species. These were *D. bulbifera* L., *D. abyssinica* Hochst., *D. cayenensis* Lam*.*, *D. rotundata* Poir *, D. praehensilis* Benth*. and D. alata* L. These identified *Dioscorea* species were subjected to morphological characterization study to observe their diversity relatedness among and within the genotypes by observing only one type of species from a plot. Based on Shan UPGMA cluster analysis, all sixty yam genotypes included in the study were grouped in to five clusters. In this analysis the cophenetic correlation coefficient r=80 revealed the efficiency of the dendrogram.

Forty two different acessions of six different species of yam tubers *D. bulbifera, D. abyssinica, D.cayenensis, D.rotundata D. praehensilis, and D. alata* from six different major growing regions of the country Gedio, Gamogofa, Wolita, Dauro, Wolega and Jima were selected randomely for nutritional analysis from the expermental field where yam morphological diversity was studied. The result from this study showed that yam is the potential tuber crop with respect to both proximate composition and mineral contents.

In the present study, different *Dioscorea* species were observed within most yam accession found in same vernacular name. Hence, a total of two hundred and fifty eight yam leaf samples from six different *Dioscorea* species (one to five yam plants from experiemental plot) were observed separetly using twenty SSR loci. Among these, two hundred and ten yam landraces were taken independently to each species and region based statistical analysis from twelve SSR loci which showed clear electrophoretic bands. Distance matrix derived un-rooted Phylogram of 210 accessions based on six *Dioscorea* species indicated the separation of *D. cayenensis, D. abyssinica* and *D. bulbifera* by grouping them in to four different clusters. One cluster group separated the *Dioscorea* species (*D. praehensilis* and *D. abyssinica*) from those of cultivated species. Similarly two clusters grouped *D. praehensilis* and *D.rotundata* together whereas two sub cluster groups were composed of sixty four yam acessions overlapped five species together by excluding *D.cayenensis.* A similarity matrix derived UPGMA cluster analysis based on geographical sites showed nine distinct clustering groups which indicated presence of relationship between genetic distances to that of geographical distance in most of clustering groups. These cluster grouping is supported by Analysis of Molecular Variance (AMOVA) which showed the significance genetic variation of 210 yam accessions within and among ten collection sites .

Combined data analysis was also made for 42 yam accessions which have common nutritional, morphological and microsatellites data set. The dendrogram obtained based on combined analysis grouped four different yam (*Dioscorea* spp) from different geographic origins separately within five clusters. Generally SSR markers were powerful to demonstrate diversity of Ethiopian yam (*Dioscorea* spp) with respect to their species groups and geographic location. The Result of morphological, nutritional composition and combination of all data set analysis gave some results in agreement with result of SSR marker. Therefore, such type of study is vital to drag out different type of information and helps to enhance productivity of the crop in different aspects.

Key Words: Ethiopia, *Dioscorea* species, Morphology, Proximate Composition, Mineral Contents, Simple Sequence Repeats (SSR)

1. General Introduction

Roots and tubers refer to any growing plant that stores edible material in subterranean root, corm and tuber. They are the most important food crops after cereals and are of very ancient origin in the tropics and subtropics (Asha and Nair, 2002). These crops have contributed to the human existence, survival, and socio economic history. North Eastern Himalayas and Western Ghats are hot spots of global diversity and are rich in wild relatives of tropical root and tuber crops (Burkill, 1960). The Indo Burma region is the centre of origin of taro and Asiatic edible yams (Leon, 1976; Kambaska, 2009).

Tuber crops have the highest rate of dry matter production per day and are major calorie contributors. They have an important place in the dietary habits of small and marginal farmers especially in the food security of indigenous populations. Tuber crops not only enrich the diet of the people but also possess medicinal properties to cure many ailments or check their incidence. Many tropical tuber crops are used in the preparation of stimulants, tonics, carminatives and expectorants. Tuber crops are rich in dietary fibre and carotenoids (Edlson *et al.,* 2006)

Yam belongs to the oldest monocotyledonous family *Dioscoreaceae* (Coursey, 1967). The family *Dioscoreaceae* is a natural group of tuber forming tropical vines and is usually grouped with Liliales (Burkill, 1960). The areal storage organ of *Dioscoreaceae* is the bulbil. They are perennial plants with a strongly marked annual cycle of growth (Coursey, 1983). Yams are dioecious, with male and female flowers borne on separate plants. Cultivars may be male, female or sterile.

The term ‘yam’ refers to all members of the genus *Dioscorea*, which contains over 600 species (Hodeba *et al.*, 2008). Many wild yam species contain toxic and/or other bioactive chemicals and some of these are cultivated for pharmaceutical products (Coursey, 1967).

About 10 species of yam are commonly cultivated for food, while a number of others are harvested from the wild in times of food scarcity (Bhandari *et al*., 2003). Cultivated species include *D. alata* L*.,* *D. cayenensis* Lam., *D. rotundata* Poir. *D. esculenta* (Lour) Burk, *D. bulbifera* L., *D. nummularia* Lam*., D. pentaphylla* L*., D. hispida* Dennst*, D. trifida* L.F and *D. dumetorum* (Kunth) Pax. They are annual or perennial herbaceous vines with edible underground tubers and are the world’s second most important tuber crop (Nweke *et al*., 1991). It is indicated that approximately 200 species of yam are distributed throughout the tropics and subtropics (Ayensu, 1972).

Although highly variable in appearance both between and within species, all yams share a common growth habit of thin, twining vines and a shallow, widely radiating root system, both of which die and are renewed each year. All economically important species are tuberous, producing one or more underground tubers.

The nutritional value of roots and tubers lies in their potential ability to provide one of the cheapest sources of dietary energy in the form of carbohydrates in developing countries (Ugwu, 2009). Yam is cultivated mainly for its tuber. The tubers have a dual agricultural function: first, as source of food, it is a major source of nourishment to millions of people and secondly as a planting material (Hahn, 1995; Craufurd *et al*., 2006). It is a major source of carbohydrates and nutrient energy for many people in tropical countries including east and west Africa, the Carribean, South Africa, India and South East Asia (Yang *et al*., 2009; FAO, 1991) whereas 95% of the world production occurs in West Africa (FAO, 1991). The most common use of yam is as a boiled vegetable with some kind of sauce, but the skin is not eaten. It may be removed before or after boiling. In West Africa, yam is often pounded into a thick paste after boiling and is eaten with soup (Orkwor *et al*., 1998). Yam is also processed into flour. It may also be baked, fried, roasted or mashed to suit regional tastes and customs. However, other specific ways of preparing yam can be found in other regions (Okaka *et al*., 1991).

Yam contains approximately four times as much protein as cassava, and is the only major tuber crop that exceeds rice in protein content in proportion to digestible energy (Bradbury and Holloway, 1988; Jane, 2010). The dry matter is composed mainly of carbohydrate, vitamins as well as protein and minerals. Nutrient content varies with species and cooking procedure (Osagie, 1992).

Yams are grown on about5 million hectares of land in about 47 countries of tropical and subtropical regions of the world with Nigeria as the leading world producer (FAO, 2005). In West and Central Africa farmers had selected yam genotypes that best suited their needs. This has produced a large number of traditional cultivars so its' production has increased steadily (FAO, 2006). This increase has been achieved mainly through the planting of traditional landraces with rapid increase in acreage of yam fields in to marginal lands and in to non-traditional yam growing areas (Hodeba, 2008). Nigeria's yam production was 35 million metric tonnes in 2008 (FAO, 2012). The world annual production is approximately 50 million metric tonnes of fresh tubers. More than 96% of this is being cultivated in Africa, where four countries (Nigeria, Ivory Coast, Ghana and Benin) account for 90% of production thus contributing morethan 45 million t/year. *Dioscorea rotundata* Poir., *D .alata* and *D. cayenesis* are by far the major cultivated species, while *D. dumetorum* (Kunth) , *D. bulbifera* L. , *D. trifida* L., *D. esculenta* (Lour.), *D. opposite* Kunth or *D.Japonica* L. (cinnamon yam) are referred to as the minor yams (Lebot, 2009).

Ethiopia is the seventh largest yam producing country in Africa with about 62,000 ha of land. The crop plays a vital role in local livelihood particularly in the densely populated areas of southern, southwestern, and western parts of the country (Hildebrand, *et al*., 2003; Muluneh Tamru, 2006).

The country is considered to be the center of origin for *Dioscorea abyssinica* Hochst*,* which is widely distributed in the savanna region of West Africa (Coursey, 1967) and one of the yam species *D. gilletti.,* is a near endemic occurring in Southeast Ethiopia and Northern Kenya bordering Ethiopia (Sebsebe Demissew *et al*., 2003; Sebsebe and Inger, 2010). All the cultivars of *D. cayenensis- D. rotundata* complex are domesticated from the related wild species *Dioscorea abyssinica* , *Dioscorea praehensilis* Benth and *Dioscorea burkilliana* J. Miège (Mignouna and Dansi, 2003; Dumont, *et al., 2*006) which are collected in the bushes (Forests, savannah and gallery forests) or in the ancient fallows (Dansi *et al.*, 2013) . Hence, In West Africa, guinea yam (*D. cayenensis and D. rotundata* complex) had considerable varietal and genetic diversity due to the continuous process of domestication (Mignouna and Dansi, 2003; Dansi *et al.*, 2013).

Ethiopian farmers particularly in many areas of south, southwest, and western regions cultivate yams (Edossa Etisa, 1998). About nine types of yam have been reported to grow in Ethiopia indicating the diversity of the species in the country (Westpal, 1975).

Yam is a crop that has attracted little attention by agricultural researchers. Yam producing countries of the world are in the tropics and have poor economies that support little or inadequate agricultural research (Chukwu and lkewelle, 2000). Yams thus belong to a group of crops labeled “orphaned crops”, which have not received research attention for a long period of time and very little improvement, has been made to the crop (Otoo, 2009).

The present observed research problems with regard to Ethiopian yam (*Dioscorea* spp) improvement activities are mainly due to gaps in information on morphological variability among species, nutritional value, degree of genetic relatedness and divergence among and within population and species.

Characterization and evaluation study using Amplified Fragment Length Polymorphism (AFLP) on forty three yams (*Dioscorea* spp) of Ethiopia did not reveal clear species boundaries (Wendawok Abebe *et al.*, 2012) and a recommendation that the taxonomy of

Dioscorea species needs to be studied using other markers was suggested. Additionally, Wendawok Abebe *et al.*, 2012 suggested that future studies should be done at the population scale and in a broad range of geographical regions taking the diversity within each member of the Guinea yams.

Similarly based on sixty two yam (*Dioscorea* spp) accessions diversity study using AFLP markers, (Muluneh Tamru, 2006) indicated Ethiopian yams are distinct from West African yams. He also suggested further studies on cultivated and wild yam species using co-dominant molecular markers by including more samples from Wolayita, Gamogofa and also extending to other localities in the southern and south western parts of the country to cover areas that were not covered by his study.

The present study considers the recommendations of earlier studies and existing research gaps of this crop. Morphological and Simple Sequence Repeats (SSR) markers were used to study the diversity of yam (*Dioscorea* spp) by adding additional locations that were not covered in earlier obeservations. Nutritional composition of Ethiopian yam (*Dioscorea* spp) is also considered since information on this aspect is quite limited except to Muluneh Tamru's, (2006) report on contents of protein and starch, amaylose fraction and pasting properties of this crop. Thus, the present study includes observation on nutritional content and variability among Ethiopian yam (*Dioscore*a spp) particularly with respect to their proximate composition and minerals content. The outcome of such study enables to better use the existing variability through selection and breeding in order to ensure food security particularly for yam producing farmers of the country.

2. Literature Review

Dioscoreaceae are herbaceous plants with ten genera. *Dioscorea* is the largest and most important genus (Muzac *et al*., 1993). They possess rhizomes and tubers which are edible. Yam is a monocotyledonous angiosperm, which belongs to the order *Liliflorae*, family *Dioscoreaceae,* and genus *Dioscorea.* The name yam is derived from the African tribe ‘niam’ or ‘enyame’. Yam is originated in the Far East, the genus had spread worldwide by the end of the Cretaceous period around seventy five million years ago (Alexander and Coursey, 1969). The genus was dispersed worldwide at the end of the Cretaceous period, evolved in different directions throughout the new and old world, and resulted in distinct species (Lebot, 2009).

Table 1: Yam producing countries in the world with their ranked production data (FAOSTAT, 2013)

Country	Total production (x1000 MT)	Area Harvested (Hectares)
Nigeria	37,115.5	2,889,050
Ghana	6,295.4	403,798
Côte d'Ivoire	5, 539.8	834,369
Benin	2,366	182,203
Togo	727.7	71,226
Cameroon	510	46,000
Central African Republic	452.4	57,288
Chad	400	42,000
Papua new Guinea	395	19,245
Ethiopia	339.7	42,271
Gabon	204	32,735
Sudan	156	62,485
Congo	93	23,530
Mali	92	4,000
Cuba	349.7	50.475
Colombia	346	33,426
Brazil	244	25,035
Jamaica	134	8,323
Venezuela	102	9,662
Japan	177.9	7,486
Philippines	17.8	2,974

Source:-2010-2013 Factfish

2.1 Taxonomy and geographical distribution of yam (*Dioscorea* spp)

Agriculture originated in at least six different areas of the world namely Mesoamerica, the Andes of South America, Southwest Asia, Africa, Southern China, and Southeast Asia (Smartt and Simmonds, 1995 quoted in Paul, 2004). They are located in tropical or subtropical regions generally between 35° N. and 35° S. Lat. One can guess that this type of environment at the time of domestication would have harbored a wider range of resources than areas that are located at higher or lower altitudes. In turn, this abundance of resources would have allowed early farmers to continue procuring food through the old methods of hunting and gathering. It would also have allowed them to more easily identify plants or animals that were inclined to domestication (Paul, 2004).

Yam was believed to be indigenous to West Africa (Coursey, 1967 quoted in Muluneh Tamru, 2006), but its origins are variable due to a large species diversity of the genus *Dioscorea* (Malaurie *et al*., 1998).

Generally major cultivated *Dioscorea* species appear to be originated from tropical areas in three different continents Africa *(D. caynenensis* complex), South America (*D. trifida* L.F.), South East Asia and south pacific (*D. alata* and *D. esculanta* (Lour.) Burkill) (Coursey, 1967, Alexandre and Coursey, 1969; Orkwor *et al*., 1998 and Edison *et al.,* 2006). *D. rotundata* , *D. cayenensis* and *D. dumetrum* were domesticated in eastern Nigeria and from land tracts adjoining the Niger and Benue Rivers in West Africa (Coursey, 1967) while the water or greater yam (*D. alata*) probably originated from the southeast Asian Oceanian region in tropical Myanmar and Thailand (Malapa *et al*., 2005).

The minor species *D. trifida* is of South American origin (Brucher, 1989). There was not any migration of African species to Asia (Coursey, 1976) until very recent times when *D. rotundata* was introduced from the International Institute of Agriculture (IITA) to India (Sen and Das, 1991; Craufurad *et al.,* 2000).

Indo-Malayan centre of origin for *D. alata was* hypothesized based on the widespread varieties found there. That great numbers of varieties are known from India to the islands of the South Pacific, many of which are quite local in distribution, suggests that the species was domesticated and widely distributed very early (De Candolle, 1886; Franklin, 1976).

The occurrence of distinct but closely related varieties on somewhat isolated islands suggests that it has continued to evolve in areas where it has been introduced (De Candolle, 1886; Franklin, 1976).

It is supposed that *D. trífida* has been domesticated in South America. It presently ranges from eastern Peru through tropical Brazil, to the Guyanas, and beyond to the islands of Trinidad and Tobago, and all of the West Indies (Brucher, 1968). A limited number of named varieties are recognized in the western most parts of the range (eastern Peru). For example, nine named clones are to be found in the gardens of the Aguaruna Jivaros (a tribe of Peru). In the Guyanas, a wide range of cultivated as well as wild forms exists, and this area may be thought of as the probable centre of origin (Franklin and Lucien, 1978).

Many of the *Dioscorea* species serve as a ‘life saving’ plant group for the marginal farming and forest dwelling communities, during periods of food scarcity (Arora and Anjula, 1996). The genus is considered to be among the most primitive of the angiosperms and contains over 600 species, of which only about ten are considered as edible (Govaerts and Eilkin, 2007) while many of the wild yams are also important plants in times of food scarcity.

The most cultivated species in Africa are *D. alata L.*, *D. bulbifera L.*, *D. cayenesis Lam. D. esculenta (Lour.) Burk*, *D. rotundata Poir.* and *D. trifida L. (*Craufurd *et al., 2006).* Among these species *D. rotundata* and *D. alata* are the most important and they cover about 90% of world production of food yams (Alexander and Coursey, 1969).

2.2 Some yam (*Dioscorea* species) and their origin

The cultivated Guinea yams *Dioscorea rotundata* and *D. cayenensis* are the most popular and economically important yams in West and Central Africa where they are indigenous, while *D. alata* is the most widely distributed species globally (Mignouna and Dansi., 2003).

***D. abyssinica:* -** The species has long been regarded as same species with *D. togoensis* (Mie’ge, 1952). However, based on morphological characters Mie’ge (1982) indicated that *D.abyssinica*, *D. lecardi a*nd *D. sagitifolia* are morphologically similar and that have no justification for treat each of them as a separate species. In west Africa *D. Abyssinica’s* is distributed throughout the area where *D. rotundata* yams are domesticated

(Miege, 1968). In the case of Ethiopia, *D. abyssinica* is widely distributed in southern, western and northern parts of the country between 1000m and 1800 m above sea level (Mie'ge and Sebsebe Demsew, 1997) and the country is considered to be the centre of origin for the species (Franklin and Lucien, 1978) (Figure 6a).

***D. preahensilis:* -** This species has a wide geographical range in Africa and occurs throughout the Western, Central and Eastern parts of the continent (Dumont *et al*., 2006). Mie'ge, (1952) indicated that *D. abyssinica*, *D. sagitifolia*, *D. preahensilis*, *D. liebrechstiana*, *D. mangenotiana* and *D. lecardi* are possible ancestors of Guinea yams (*D. rotundata* and *D. Cayenensis*). Later Miege, (1982) proposed that *D. abyssinica*, *D. lecardi, and D. sajitifola* are morphologically similar as it is not possible to separate them as different species. In addition *D. liebrechstiana* is morphologically very close to *D. preahensilis* (Dumont *et al*., 2006). Coursey (1976) showed that *D. praehensillis* is the possible ancestor for the cultivated Guinea yams (*D. cayenensis* Lam. and *D. rotundata* Poir.). Similarly according to Dumont *et al*., (2006) *D. cayenensis* and *D. rotundata* are yams domesticated from wild *Dioscoreaceae* of the Enantiophyllum Uline section that have speciated in Africa. The wild species of this section, such as *D. abyssinica* and *D. praehensillis*, are considered to be the major source of variability (Figure 6e).

***D. rotundata*:** - The species is also referred to as White Guinea yam/White yam (*D. rotunda*) (Figure 6b). It is considered to be one of the four most important food yam species or complexes of species namely (*D. cayenensis*/*D. rotundata* complex, *D. alata* and *D. bulbifera*). *Dioscorea rotunda* is also known by a host of local names, and is quite similar to water yam but more solid. Other yam species of minor economic importance in several tropical regions include *D. dumetorum*, *D. opposite*, *D. japonica*, *D. hispadda* and *D. transversa* (Asiedu *et al*., 1997).

***D. cayenensis* :-** Also known as yellow Guinea yam and Yellow yam is its other names and it is indigenous to the forest zone of West Africa. It derives its common name from its yellow flesh and is very similar to white yam in appearance. Its yellow flesh (Figure 6d) is caused by the presence of carotenoids. It is not as popular as the white yam nor does it store well. It requires longer period of vegetation and a shorter dormancy than the white yam (Mignouna and Dansi, 2003).

***Dioscorea alata L.*:-** Also called water yam is an important food in Africa and the Caribbean (Lebot *et al.*, 2005).It is believed to have originated and first cultivated in Southeast Asia, but is now widely grown throughout the humid tropics. It is second only to white yam in popularity. According to Lebot *et al.,* 2005, it is the most widely distributed species in the humid and semi-humid tropics. It is a vigorously growing, twining, herbaceous vine reaching 10-20 m in length (Brunnschweiler *et al*., 2004). The fingered yam is a cultivar which has finger like bulge, dark brown skin, white flesh and watery flesh that is sometimes tinged with pink (Figure 6f).

D. bulbifera: - It is also referred to as potato yam, aerial yam or air potato. It is the only yam species that has independent domestication, Africa and Asia including Ethiopia (Miege and Sebsbe Demessew 1997). It is the oddest yam as it has poor underground tubers (Figure 6c and 7c-8) but bears small tubers above ground on its long, climbing vines. There are a few other species that also have aerial tubers. The aerial tubers usually weigh between one and four pounds and may contain a toxic substance. The underground varieties are hard and bitter. The species found in Africa and Asia have slight morphological differences in (bulbils shape, size, bulbils colour, and weight (Jayasurya, 1984; Wilkin, 1998).

The Asiatic tubers are less toxic than the African varieties. Significant quantities of *D. bulbifera* are also grown in the Caribbean, and virtually all humid or sub-humid parts of Asia with some morphological differences in bulbils, tubers and leaves (Burkill, 1960; Miege, 1982).

Dioscorea dumetorum: African bitter yam is its alternative name. It contains a bitter toxic alkaloid called dihydrodioscorine, which is removed by soaking and boiling in changes of water. Some cultivated forms had most of this dangerous substance bred out of them. It is also called trifoliate yam because of its leaves. The species originates in Africa where wild cultivars also exist. One marked characteristic of the bitter yam is the bitter flavour of its tubers. Another undesired characteristic is that the flesh hardens if not cooked soon after harvest. Some wild cultivars are highly poisonous (Mignouna *et al.*, 2004).

D. trifida: Also called Cush-cush yam is the only yam indigenous to Guyana region of South America but nowadays its production is restricted to West Indies (Onwueme, I.C.

and Charles, W.B. 1994). It is one of the four most important food yams of the world. This type bears clusters of up to a dozen tubers of good quality. There are also dark fleshed types. When boiled or steamed, they emit an unexpected odour of bacon and eggs. The starchy texture is much softer and lighter than other yams. The flavour suggests a slightly sweet, smoky baking potato, but a firmer and drier texture. Because of their relative ease of cultivation and their good flavour they are considered to have a great potential for increased production (Kay, 1987).

D. opposite* or *D. batatas: - It is also known as Chinese yam, Cinnamon yam, lesser yam and also called by numerous other local names. It is considered as one of the most neglected species of *Dioscorea* because of the presence of poisonous alkaloids known as dioscorine (Kambaska, 2008). It originated in Indochina and is fairly uncommon in the West. It was successfully grown in Europe, for a time, as a potato substitute after the potato famine in the 1840s. It is a good all purpose yam that blends in well with almost anything, much like the potato. It is cultivated in China, Japan, Korea, Taiwan and India. It is a cultivated form of another species which grows wild in China and Japan, and is more resistant to cold than other yams. The tubers can be over five feet long, and is the variety most often eaten in Southeast Asia. It is difficult to harvest, however, as the tubers descend vertically. It has been used in traditional Chinese medicine for many years, to strengthen stomach function, alleviate anorexia, and cure diarrhea, and used as a delicious food in Chinese diets. It contains many chemical components such as mannan, allantoin, dopamine, batatasine, phytic acid, abscisin II, amino acids, glucoprotein, choline, ergosterol, campesterol, saponins, starch, non-starch polysaccharides and minerals (K, S, Ca, Mg, Fe, Zn, Cu, Mn) and so on Wang *et al*., (2008).

D. japonica: - It is also known as Japanese yam. The Japanese yam, AKA mountain yam, is a sweet yam variety. It is native to regions of China and Japan. It can sometimes reach lengths of six feet. The skin is brown and the flesh is white. Because it is slim and brittle, digging it up requires a high degree of expertise and when accomplished, it is transported with great care coddled in straw. It has always been considered a particularly nourishing food whose energy giving qualities important. It does contain a large amount of the enzyme diatase, which helps the digestion. It is almost always eaten raw.

***D. esculenta*: -** It is also known as lesser yam. It is native to south East Asia and is the third most commonly cultivated species in this region. All yams, except this species, contain the toxin, dioscorine, which is destroyed when cooked. It is especially small and aromatic and cooked like potatoes. There are single and bunched varieties. The tubers are eaten baked, boiled, or fried much like potatoes. Because of the small size of the tubers mechanical cultivation is possible. Its easy preparation and good flavour, could help the lesser yam to become more popular in the future (Kay, 1987)

2.3 Cultivation & socio economic importance of yam *(Dioscorea spp)*

Yams (*Dioscorea* spp.) are primary agricultural commodities and major staple crops in Africa, where yam cultivation began 11,000 years ago (IITA, 2010). They are tuberous plants. The tuber, an underground stem structure accumulates starch and nutrients during the rainy season when a yam plant grows. During the dry season the foliage of the yam plant dies back and the tuber remains dormant until the growth of the new stems.

Because of this storage system, yams have constituted an important source of food to humans during the late rainy season and early to mid dry season in tropical & sub tropical areas (Coursey, 1967). Food yams are grown principally for the carbohydrate they provide. The tubers have a tremendous capacity to store food reserves. They broaden the food base and bring food security to 300 million people in the low income, food deficient Countries of the tropics, providing them with about 200 kilocalories daily (Hahn *et al* ., 1987; FAO, 1999).

West Africa accounts for about 95 % of world production and 96 % of the area. Yam production globally reached 39.85 million Mt. harvested from 4.44 million hectare in 2005 (FAO, 2006). The largest producer was Nigeria with 26.59 million Mt. followed by Ghana (3.89), Coted'Ivoire (3.00), and Benin (2.56) (Nweke *et al.,* 1991).

The domestic market is developed in the yam producing areas making the crop the main source of cash for a large majority of small scale farmers (Asiedu and Sarite, 2010). In West Africa they are major sources of income and have high cultural value.

They are used in marriage ceremonies, and a festival is held annually to celebrate its harvest. Consumer demand for yam is generally very high in this sub-region and yam cultivation is very profitable despite high production costs (IITA, 2010).

2.4 Yam species in Ethiopia

Ethiopia is located in the tropics in the horn of Africa between 3º and 15º N, 33º and 48º E, bordering Somalia, Sudan, Djibouti, Kenya and Eritrea. It has diverse physiogeographic features with high and rugged mountains, flat topped plateaux, deep gorges, incised river valleys and rolling plains. The altitudinal variation ranges from 116m below sea level in the Dalol depression in the Afar region to 4,620 masl at Ras Dashen. The Great Rift Valley runs from northeast to southwest of the country and separates the western and south eastern highlands. The highlands on each side of the rift valley gives way to extensive semi-arid lowlands to the east, south and west of the country (IBC, 2008).

The existence of diverse farming systems, socio-economics, cultures and agro-ecologies have endowed Ethiopia with a diverse biological wealth of plants, animals, and microbial species, especially crop diversity. Crop plants such as coffee (*Coffea arabica),* safflower (*Carthamus tinctorius*)*,* tef (*Eragrostis tef*)*,* noug (*Guizotia abyssinica),* anchote (*Coccinia abyssinica)* and enset *(Ensete ventricosum)* originated in Ethiopia (Vavilov, 1951 quoted in Muluneh Tamru, 2006).

High genetic diversity is found in major food crops (wheat, barley, sorghum and peas); industrial crops (linseed, castor and cotton); cash crop (coffee); food crops of regional and local importance (tef, noug, Ethiopian mustard, enset, finger millet, cowpea, lentil) and in a number of forage species of world importance (clovers, medics, oats). There are several indigenous cultivated or semi-cultivated root and tuber crops in Ethiopia. These crops have an important place in the diet of the population (IBC, 2008)

It is reported that Ethiopia is the seventh largest yam producing country in Africa with about 62,000 ha of land under yam cultivation (Edossa Etissa, 1998). Also total annual production of yam in 2011 was estimated at about 339,740 metric tonnes from an area of about 1,104,300 Sq.Km (FAOSTAT, 2013 quoted in Factfish, 2010-2013).

Yams (*Dioscorea* spp.) are among the most important tuber crops in the country, especially in humid areas where there is heavy year round precipitation (Kay, 1987). It is known by different vernacular names in different locations of the country such as Boye, by Dauro people; Kocho or Wocheno, Oromo; Bohe, Wolayita; Kuso, Yem; and Boina, Sidama (Edosa Etisa, 1996). There are various ways of yam preparation for food in the different growing regions of the country. For example, in the Sheko districts of south west of Ethiopia cooking methods relate to the gender of the cooker. Men prepare a yam, either in the field or at home. First he cleans the tuber and roasts up the wild yam that he found in the field as a full meal if he is single, or prepare it as a late afternoon snack for his wife and children. In contrast, women prepare yams by steaming. To get a family meal, a woman will harvest one or two large stake yams and peel them (Elisabeth, 2003).

Several species of *Dioscorea* grow in different parts of Ethiopia; not all are edible. Eleven described *Dioscorea* species, (both wild and cultivated), are found in the country (Mie'ge and Sebsebe Demissew, 1997) indicating the wide diversity of the species in the country.The species *D. alata* L is not indigenous to Ethiopia but originated in Southeast Asia. In Ethiopia, it is sterile and grows only in cultivation.

The *D. cayenensis* complex is a provisional name for a set of sub Saharan yam species. The set of species is composed of *D. Cayenesis, D. rotundata, D. abyssinica, D. praehensilis and D. sagitifolia* (Wilkin *et al.,* 2001). These species are indigenous to Ethiopia and occur all over sub Saharan Africa from 500m to 1800m altitude, especially in seasonally dry areas (Miège and sebsebe Demisew 1997).

2.5 Previous researches on Ethiopian yam (*Dioscorea* spp) diversity

Root and tuber crops are widely cultivated in southern Ethiopia, and these crops support a considerable portion of the country's population as source of food. Prominent among these are: potato (*Solanum tuberosum* L.), sweet potato (*Ipomea batatas* L.), yams (*Dioscorea* spp.), Oromo dinch (*Coleus parviflorus* Benth), Koteharrie *(Diaspora bulbiferous*) and anchote (*Coccinia abyssinica* (Lam.) Cogn.) (Melaku *et al*, 2000).

Farmers, particularly in south, south west and western regions of the country cultivate yams (Edossa Etissa, 1998). In these regions yam has considerable importance in the local livelihood. It is highly valued by the farmers and managed accordingly to meet their needs.

Yams have become an important cash crop in most localities and are of preferred food for respected guests. Yams are also served during the traditional celebration which coincides with the peak harvesting time and thus allowing farmers to earn profit from the market. Hence, yam is important not only for household food security, but also as a source of cash income (Muluneh Tamru 2006).

Yams grow in the altitude range of 1140 to 2200 and in a wide range of soils mainly in clay, clay loam, sandy and sandy loam types. Yam is planted in October (in most parts of Southern Ethiopia), November & December (in South Western and Western part of the country) (Hildebrand, 2003) at the onset of the dry season making use of soil moisture reserves from the preceding rains. Early maturing cultivars of yam are ready for harvest starting from May, which is the main food deficient period. The fact that it is harvested in the season where cereal crops are not enables yams to fill the gap in food shortage and hence play a vital role in food security. Therefore yams development of early maturing varieties in these crops has significantly contributed to increased production, because early varieties can fit well in the multiple cropping system leading to an increase in the over all production (Muluneh Tamiru 2006).

According to Muluneh Tamru (2006), the morphological variation of eighty four yam (*Dioscorea* spp) accessions collected from Gamo Gofa, Gedio, Sidama and Wolayita zones were assessed based on thirty two qualitative morphological traits.

The author reported that morphological groups revealed through cluster and principal component analysis were largely consistent with farmers' landrace classification, mainly to maturity time. However, there was no morphological difference between some landraces managed as different by farmers.

In the same study, Muluneh used AFLP (Amplified Fragment Length Polymorphism) markers for genetic diversity analysis on forty eight selected unidentified Ethiopian yam *Dioscorea* accessions representing the variability in land race names, geographic origin and morphological traits. Relationships of these materials with the commonly cultivated species *D.alata*, *D. bulbifera, D. cayenesis* and *D. rotundata* were investigated by including in the study elite genotypes from West Africa. Both cluster and principal coordinate analysis separated the accessions into their respective taxa, whereas the Ethiopian materials constituted a cluster distinct from any of the investigated species. He suggested that the distinctiveness of the Ethiopian Guinea yams may represent a divergent evolutionary pathway isolated from the widely known centre of diversity in West Africa. Interspecific genetic similarity values using AFLP on Jaccared coefficient have shown that the Ethiopian accessions are genetically closer to *D. cayenensis* and *D. rotundata* than to the other species studied.

The overall findings were consistent with farmers' landrace classification based on time of maturity and structure of morphological diversity. Based on these results, Muluneh suggested that further studies are required on the yam classification system, morphological and molecular marker based analysis of genetic diversity including covering areas which were not included in his studies. This will help to determine the extent and distribution of diversity in these named land races of Ethiopian yam. In addition, he recommended giving attention to wild *Dioscorea* genetic resources to further elucidate the evolution, domestication and species identity of Ethiopian yams.

Wendawek Abebe (2008) conducted research on the genetic diversity of Yam (*Dioscorea* spp) using morphometric analysis, molecular markers namely AFLP & microsatellites. The plant materials used for morphometry were 40 dried herbarium specimens of *D. abyssinica, D. praehensilis, D. bulbifera* and *D. cayenensis* whereas for AFLP studies he used yam (*Dioscorea* spp*)* consisting of 46 accessions which were collected from Sheko region and its environs of southwest Ethiopia. In these studies *D. abyssinica, D. praehensilis, D. cayenensis* were considered. In addition two accessions of *D. schimperiana* and one accession of *D. bulbifera* were included for comparative studies.

Similarly Microsatellite analysis was conducted using species *D.abyssinica, D.praehensilis D. rotundatam and D.cayenensis* (Collection area in sidama, kefa and wolega). Based on this research, he indicated that phonogram PCO and PCA scatter plot based on morphological, AFLP and microsatellite markers failed to produce a clear partitioning of the individuals into discrete taxa that could be interpreted according to the existing classification system. However, a large geographically structured clustering pattern was observed in microsatellite markers genetic diversity analysis.

2.6 Statement of the problem and objective of the present study

The observed research problems towards Ethiopian yam (*Dioscorea* spp) improvement activities is mainly due to absence of adequate information on its' morphological variability, nutritional value and extent of genetic diversity among it's' genotypes.

The extent of research that has been conducted on Ethiopian yam is minimal compared to the research that has been conducted on other crops grown in the country as well as that indicated on the West African yam. Earlier studies indicated the need for further research to better understand the extent of existing diversity of yam genotypes in Ethiopia.

Hence, the present study takes into account recommendation of previous research and the existing research problems. The results obtained from this study are expected to play a vital role in increasing production, productivity and nutritive value of the crop through selection, breeding and effective conservation activities for future use.

2.7 Objectives

2.7.1 General objective of the study are

- To conduct morphological variability studies among collected Ethiopian yam accessions
- To interprate statistical analysis results drawn from raw datas of morphological characterization, molecular characterization, proximate compositions and mineral contents of Ethiopian yam (*Dioscorea* spp)
- To demonstrate extent of Ethiopian yam (*Dioscorea* spp) genetic diversity and nutrient contents variability for its future conservation and improvement activities

2.7.2 Specific objectives

- To conduct morphological variability studies on the landraces of Ethiopian yam (*Dioscorea* Spp) landraces collected from ten major producing areas of the country
- To investigate molecular characterization of Ethiopian yam (*Dioscorea* spp) germplasms using Simple Sequence Repeats (SSR) markers
- To conduct nutritional evaluation of Ethiopian yam (*Dioscorea* spp) species growing in southern, south western and south eastern parts of the country
- To compare the correlation of morphological variation with that obtained study and results from molecular variation studies

3. Materials and methods

3.1. Morphological characterization and yam landraces

Sixty yam accessions (Based on availability 6-15 sprouted tubers of each sample) from ten different geographic origins of Southern Nations Nationalities & People's Regional State and Oromiya region of the country were used for this study. The sprouted tubers of some accessions were obtained from Research Centers whereas others were directly collected from farmers' fields during early March, 2010. The collection sites were Gamogofa, Sidama, Dauro, Gedio, East Wolega, Jima, Kafa, Areka Agricultural Research Center (ARC), Wolayita and Kembata (Table 2 and Fig. 1). However, yam accessions obtained from Areka Agricultural Research Center had no locality data hence it was difficult to identify their area of collection of whether they were collected from the same origin or not. Therefore in the present study, these accessions were considered as ARC collections.

Table 2: Ethiopian yam germplasms with their orign site, species group and respective altitudes used in the study

SN	Accessio -ns name	Place of collection			Altitude
		Zone	Woreda (Local	Species	
1	Jima	Jima	-	Cay., al., prh., rot.	1780
2	Ged 04	Wonago	Dila	Rot., aby	1940
3	Wol 11	Wolayita	-	Rot., Aby.	1940
4	GG01	Gamo Gofa	Bonke (Kemba)	aby., prh.	1540
5	BD/065	Kefa	-	Prh., cay. aby.	1600
6	BD055	Kefa	-	Aby., prh.	1600
7	GG02	Gamo Gofa	Arbaminch (Hatiya-	Rot., pr.	1140
8	GG03	Gamo Gofa	Breda (Bunne-2)	Prh.	1655
9	Aw 1	Areka	-	Rot., cay, .prh.	1780
10	Aw 5	Areka	-	Prh.,Rot., cay	1780
11	Sida 08	Sidama	-	Rot., cay.	
12	Wol13	North omo	Kedida Gamela	Aby., Rot., cay.,bu	1970
13	Ged 05	Gedio	Wonago	Aby., Rot., cay.	1770
14	Wol 12	North omo	Alabana tembar	Aby., Rot., cay.,al.,bu.	1630
15	Ged 06	Gedio	Wonago	Aby., Rot., cay.	1610
16	Wol 14	North omo	Wancharo	cay.	
17	GG04	Gamogofa	Gofa (Tolla)	Prh.,	1340
18	GG05	Gamo Gofa	Arba minch (Bunne-	Prh.,cay., rot.	2070
19	GG06	Gamo Gofa	Kucha (Bunne-3)	prh.., rot., cay.	1500
20	Sid 10	Sidama	-	Rot., cay.	
21	Sida01	Sidama	Dalle (Gellawcho)	Rot., pr.	1940
22	No 6	Aw 6	Areka Research	Al.	1780
23	No 2	Aw 2	Areka Research	Aby., Rot., cay.	1780
24	No 61	Aw 61	Areka Research	Prh., rot., cay.	1780
25	No 62	AW 62-	Areka Research	Rot., cay.,	1780
26	GG07	Gamo Ggofa	Bonke (Arfa-01)	cay.	2070
27	Aw/27	Areka	Areka Research	Bul.	1780
28	Sida 02	Sidama	Dale (Genticha)	Rot., cay.	1940

29	Sida 03	Sidama	Dale (Midasho)	Rot., cay.	1940
30	Sida 04	Sidama	Dale (Ouwisho)	Bu.	1940
31	Sida 05	Sidama	Dale (Adameado)	Rot.,	1940
32	46/87	Jim 04	Jima (JRC)	Rot., al.	1780
33	Sida 07	Sidama	Dale (Wnedu)	Bul., prh.	1940
34	Wol 01	Wolayita	Dwoyde (Oha)	Bul	1780
35	Wol 02	Wolayita	Dgalle (Arkiya)	Prh.,	2200
36	Wol 03	Wolayita	Dgalle (Gassa)	Rot.,	1950
37	Dau 01	Dauro	Mareka (Dorsita)	Bul.,	1580
38	Dau 02	Dauro	Konta (Gebiche)	Aby.,	1900
39	Aw/010	Wolayita	Areka (Gaffela)	Prh.	1870
40	Kem01	Kembata	Hadero (Makawa)	Prh.,	1140
41	Ged 07	Gedio	-	Aby.,pr., Rot., cay.	1590
42	GG 08	Gamogofa	Bonke (Afra-2)	prh.,aby	1900
43	Wol 05	Wolayita	Dwoyde (Wiyacha)	Aby.,	1780
44	Wolg 01	East Wolega	Sasiga (Gudina)	Aby., Rot.	1750
45	Wolg02	East Wolega	Diga (Msreta)	cay.	1650
46	Wolg 03	East Wolega	Sasiga (Haro)	cay.	1750
47	Wolg04	East Wolega	Diga (Lalo)	Rot.,	1650
48	Jim 02	Jima	--	Al. , aby., prh.	1780
49	Jim 03	Jima	-	Bu.	1780
50	Wolg 03	East Wolega	Diga (Dhoknuma)	Bu.	1650
51	Wolg 04	East Wolega	Diga (Roba)	Bu.	1650
52	Jim 04	Jima	--	Al.	1780
53	Wol 06	North Omo	Kokate (Cheyae,	Bu.,	
54	Wol 07	North Omo	Sodo zuria (Oha 2)	Rot., cay.	1900
55	Wol 08	North Omo	Sodo zuria (Wdela	Bu., rt.	1850
56	Wol 09	North Omo	Sodo zuria (Oha 1)	Rot., bul.	1860
57	Wol 10	North Omo	Sodo zuria	Rot., cay.	1850
58	No 63	-	Areka Research	Rot., cay.	1780
59	Ged 02	Gedio	Wonago (Ganticho)	Bu., aby.	1770
60	Ged o3	Gedio	Wonago (Nifo)	Bu.	1770

Note: - Aby. Rot., cay., Bu., Prh. and al. are *Dioscorea* species *D. abyssinica, D. rotundata, D. cayenensis, D. bulbifera, D. praehensilis and D. alata* respectively -----:- *Dioscorea* species of each plot considered during morphological characterization

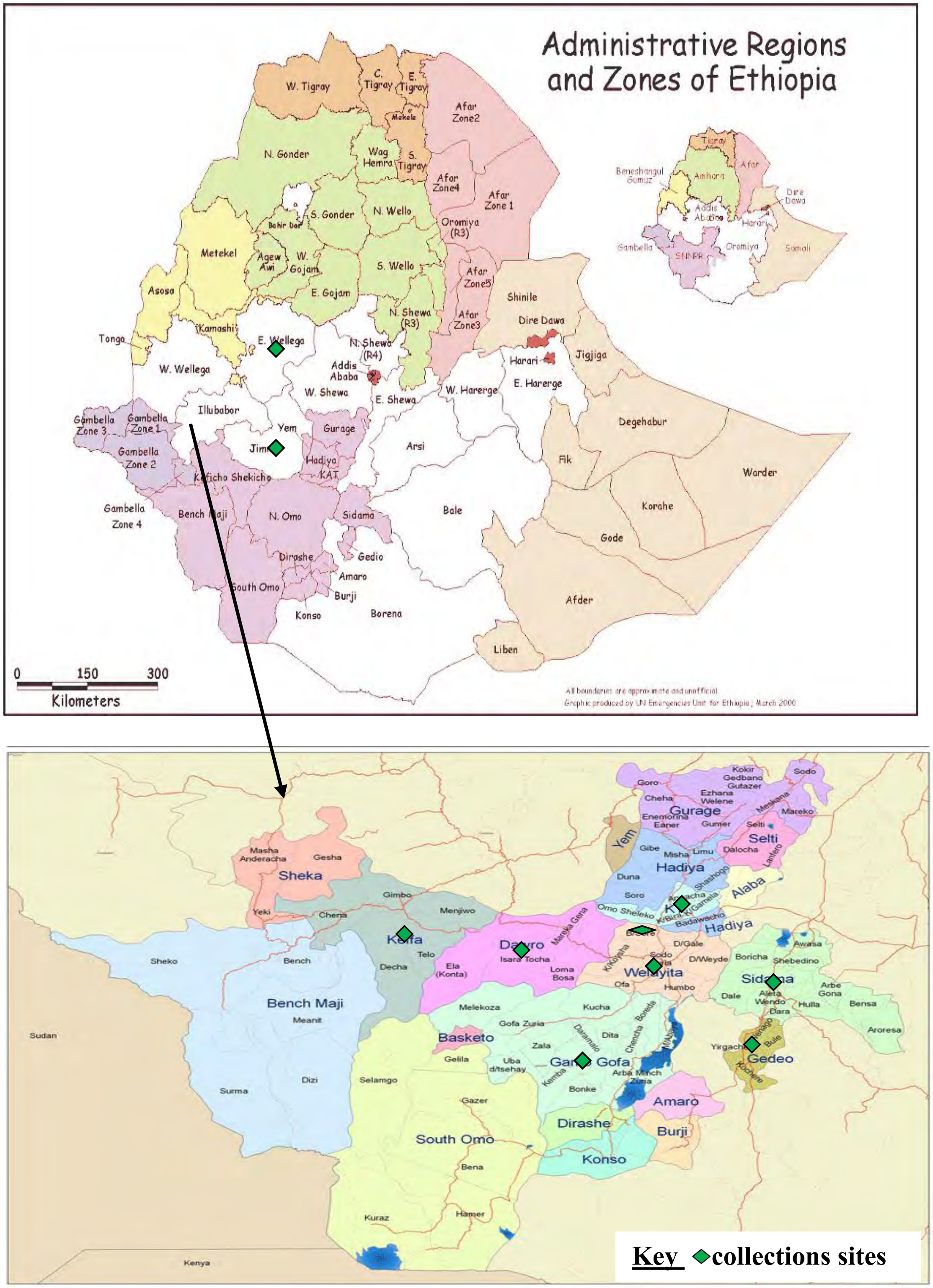


Figure 1: Locations of the study areas in Oromiya and Southern (SNNPRS) regions of Ethiopia (Source: Map of the world)

The samples collected were planted in complete randomized block design at two research sites (namely at Hawasa and Wonago) which fall under Southern Agricultural Research Institute at the end of April 2010 (Figure 2). All important cultural practices such as staking, weeding and irrigation were done starting from planting till harvesting.

Among 45 morphological characters (Table, 3) observed in the study presence and absence of spines on stems & roots , number of male and female inflorescence, stem length, twining direction, and flesh color were the major traits considered for species identification. The species identified were confirmed using Keys developed by Miege and Sebsebe (1997). Herbarium samples were collected from the experiemental field at the time of flowering for taxonomic identification and reference use with respect to further studies.

These identified *Dioscorea* species were subjected to qualitative morphological characterization by observing an accession of one species group from a plot. The qualitative morphological characterizations were carried out to determine the level of variability and the relationship among accessions using IPGRI/IITA (1997) descriptors. Characters were observed on at least five different healthy plants per accession. The color aspect of the plant was recorded using Munsell color chart for plant tissues. The farmers' perceptions were taken at the time of collection and sensory evaluation at the time of harvesting for both boiled and un-boiled yam tubers.

In total forty five morphological characters (including eight characters taken based on farmers perception to boiled yam tubers) were recorded at the time of young, flowering, maturity stage of the plant and at the time of harvesting using IPGR 1997 descriptor list (Table 3 and 4). The collected raw data were subjected to statistical analysis using computer software packages for the diversity and relatedness study among yams (*Dioscorea* spp).

Figure 2: General appearance of the field experiment of yam (*Dioscorea spp*) at Hawasa Agricultural Research Center

Table 3: The qualitative morphological characters and their score code of young, mature leaf and stem and tuber of yam *(Dioscorea* spp) germplasms collected during field experiment

Young mature leaf and stem and tuber	Characters	Score code/ Descriptor state
young leaf	Leaf Colour	1 Yellowish, 2 Pale green, 3 Dark green, 3 purplish green, 4 purple, 99 other
	Leaf Margin Colour	1 Green, 2 Purple, 3 Other
	Vein Colour	1 yellowish, 2 Green, 3 Pale purple, 4
	petiole colour	1 Green, 2 Green with purple edges, 3 Purple, 99 Others (specify in descriptor 7.7)
	petiole wing colour	1 Green, 2 Green with purple edges, 3 Purple, 99 Others (specify in descriptor 7.7)
	leaf hairiness upper/lower part	1 Green, 2 Green with purple edges, 3 Purple,
Mature leaf	position of widest part of leaf	1 Third upper, 2 middle, 3 third lower
	absence and presence of waxiness	0 Absent, 1 Present
	hairiness of petiole	3 sparse, 5dense
	petiole colour	1 All green with purple base, 2 All green with purple leaf junction, 3 All green with purple at both ends, 4 All purplish green with purple base, 5 All purplish green with purple leaf junction, 6 All purplish green with purple at both ends, 7 Green, 8 Purple 9 Brownish green, 10 Brown, 11 Dark brown 99 Other (specify in descriptor 7.7 Notes
	petiole wing colour	Green, Green with purple edge, Purple, other
	Young stem colour after emergence	1 Green, 2 purple green, 3 Brownish green, 4 Dark Brown, 5 Purple, 99 other
	absence or presence barky patches	0 Absent, 1Present
	Absence or presence of spines	0 Absent, 1Present
Mature	leaf density	3 Low, 5 intermediate, 7 High

	leaf type	1 Simple, 2 compound
	Leatherness	0 no, 1 yes
	waxiness of upper/lower surface	1 Waxy upper surface, 2 waxy lower surface, 3 both
	leaf apex shape	1 Obtuse 2 Acute 3 Emarginate 99 Other (specify in descriptor 7.7 Notes
	position of widest part of leaf	1 Third upper, 2 middle, third lower
	Distance b/n lobs	1 No measurable distance, 5 intermediate, 9 very distant
	up ward folding of leaf along main vein	3 Weak, 7 Strong
	Dawn ward folding of leaf along main vein	3 Few, 7 many
Mature stem	Plant type	1 Dwarf, 2 shrub like, 3 climbing
	Twining habit	0 no, 1 yes
	Twining Direction	1 Clockwise (climbing to the left), 2 .Antilock Wise (climbing to the right)
	Mature Stem Colour	1 Green, 2 Purplish green, 3 Brownish green, 4 dark brown, 5 purple, 99 other
Under ground Tubers	Corm Size	3 Small, 5 intermediate, 7 large
	Absence or presence of under ground tuber	0 no, 1 yes
	corm type	1 Regular, 2 Transversally elongated, 3 branched
	Tendency of tuber to branch	3 Slightly branched, 5 Branched, 7 Highly branched
	Ab/pr of cracks on tuber surface	3 Sparse, 7 dense
	Spines of roots	3 Few, 7 many
	Root on the tuber surface	0 No, 3 Few, 7 Many
	Place of root on the tuber	3 Few, 7 Many
	Uniformity of flesh colour	0 no, 1 yes

	maturity tuber after emergency	1. Up to 6 months, 2. 7-8 months, 3. 9-10 months

3.2. Characters evaluated by farmers

Fifteen farmers were selected from Wonago district near the research site. Following their consent an introduction was given to them by the researcher on the sensory parameters of interest. Yam tubers from each accession were taken washed and boiled by farmers as they would traditionally prepare them for consumption. Sensory parameters that farmers had difficulty in evaluating correctly were reviewed. The same accessions were evaluated again and again by farmers and discussed until they agreed with the sensory values. The sensory values decided by farmers were recorded by the researcher as indicated in Table 4 using IPGRI descriptor list.

Table 4: Characters assessed by farmers

	Character	**Score code/ Descriptor state**
Cooked tuber	Stickiness of cooked tuber	Not sticky 2. Sticky, 2 Very sticky
	Flavor of cooked tuber	0 Not acceptable, 1 Acceptable, 2 Very acceptable
	Absence/presence of moisture on cooked tuber	0 Absent , 1 Present
	Bitterness of cooked tuber	0 Not bitter, 1 Bitter, 2 Very bitter
	Sweetness of cooked tuber	0 Not sweet, 1 Sweet, 2 Very sweet
	Appearance of tuber after cooking	2 Poor, 5 Fair , 7Good
	Color of tuber after cooking	1 White not colored, 9 highly colored
	Overall assessment of cooked tuber	3 Low , 5 Intermediate, 7 High

3.3. Statistical analysis for morphological characterization

Statistical analysis was done using Numerical Taxonomy and Multivariate Analysis System Version 2.02 NTSYpc software program (Rolf,1998) and the data reduction function of SPSS for windows (version 12.0, 2003). The standardized data for qualitative characters were subjected to multivariate analysis and principal component analysis to identify the most discriminating morphological characters. The matrix of similarity was generated based on distance coeficents. The distance matrix was subjected to hierarchical cluster analysis using Shan through an un-weighted pair-group method average (UPGMA) as defined by Sneath and Sokal (1973). Cophenetic value (ultrametric) matrix was used to compute the cophenetic correlation as a measure of goodness of fit.

3.4. Samples preparation for proximate and minerals content analysis

Six different species of yam tubers *D. bulbifera, D. abyssinica, D. cayenensis, D. rotundata D. praehensilis, and D. alata* from six different major growing regions of the country (Gedeo, Gamogofa, Wolayita, Dauro, Wolega and Jima) were selected randomly for nutritional analysis from the expermental field where yam morphological diversity was studied (Table 2). The selected yam samples with their respective collection area, vernacular name and species are indicated in Table 5.

Table 5: Yam (*Dioscorea* spp) accessions considered for nutitional composition study

SN	Accession Code	Vernacular Name	Species	Origns
1	Ged 01	-	*D. abyssinica*	Gedio
2	Wol 10	Boyebene	*D. abyssinica*	Wolayita
3	GG01	Tola-1	*D. abyssinica*	Gamogofa
4	GG02	Tola-2	*D. abyssinica*	Gamogofa
5	GG03	Bune-1	*D. abyssinica*	Gamogofa
6	GG04	Bune-2	*D. abyssinica*	Gamogofa
7	GG05	Bune-3	*D. abyssinica*	Gamogofa
8	GG06	Bune-4	*D. abyssinica*	Gamogofa
9	GG07	Bune-5	*D. cayenensis*	Gamogofa
10	GG08	Bune-6	*D. cayenensis*	Gamogofa
11	Wol 01	Oha-1	*D. rotundata*	Wolayita
12	Wol 02	Oha-2	*D. rotundata*	Wolayita
13	Wol 03	Arkiya-1	*D. Praeihensilis*	Wolayita
14	Wol 04	Arkiya-2	*D. Praeihensilis*	Wolayita
15	Wol 05	Gassa	*D. rotundata*	Wolayita
16	Dau 01	Dorsita-1	*D. bulbifera*	Dauro
17	Dau 02	Dorsita-2	*D. bulbifera*	Dauro
18	Dau 03	Gebiche-1	*D. abyssinica*	Dauro
19	Dau 04	Gebiche-2	*D. abyssinica*	Dauro
20	Wol 06	Gafela-1	*D. praeihensilis*	Wolayita
21	Wol 07	Gafela-2	*D. praeihensilis*	Wolayita
422	GG 09	Bonke	*D. praeihensilis*	
23	Wol 08	Wiyacha-1	*D. abyssinica*	Wolayita
24	Wol 09	Wiyacha-2	*D. abyssinica*	Wolayita

25	Wolg 01	Gudina-1	*D. abyssinica*	Wolayita
26	Wolg 02	Gudina-2	*D. rotundata*	Wolayita
27	Jim 01		*D. alata*	Jima
28	Jim 02		*D. alata*	Jima
29	Wolg 03	Lalo-1	*D. rotundata*	Wolega
30	Wolg 04	Lalo-2	*D. rotundata*	Wolega
31	Jim 03		*D .bulbifera*	Jima
32	Jim 04		*D .bulbifera*	Jima
33	Wolg 05	Dhoknuma-1	*D .bulbifera*	Wolega
34	Wolg 06	Dhoknuma-2	*D .bulbifera*	Wolega
35	Jim 05		*D. alata*	Jima
36	Jim 06		*D. alata*	Jima
37	Ged 02	Ganticho-1	*D.bulbifera*	Gedio
38	Ged 03	Ganticho-2	*D.bulbifera*	Gedio
39	Ged 04	Nifo-1	*D.bulbifera*	Gedio
40	Ged 05	Nifo-2	*D.bulbifera*	Gedio
41	Wolg 07	Roba-1	*D.bulbifera*	Wolega
42	Wolg 08	Roba-2	*D.bulbifera*	Wolega

Note:- Wol=wolayita, GG=GAmogofa, Sida= Sidama, Ged=Gedio,Wolg=Wolega, Dau=Dauro,Jim= Jima

Tubers weighing 100 g were peeled, cut into small cubes. These samples were dried in an air convection oven at 60° C for 72 hours (Lape and Treche, 1994) and kept at -20° C refrigerator. After drying, the samples were ground to powder and stored in air tight bottles at room temprature before analysis. Proximate composition (ash, crude fat, crude protein, and crude fibber) and minerals content (calcium, phosphorous, zink and iron) were determined at the Ethiopian Health and Nutrition Research Insititute (EHNRI).

3.4.1. Moisture determination

Before drying 5-10 g. of each fresh sample was weighed in a previously dried glass box. The samples were dried in a thermostatically controlled oven at 105 ^{o}C for 24 hours. The dried samples in a glass box were placed in a desiccator to cool and their weight was recorded. The moisture content was calculated and expressed as a percentage of the initial weight of samples as indicated below.

$$\%\ \text{moisture} = \frac{(W1-W2)}{SW} \times 100$$

Where, W1: weight of glass box and fresh sample, W2: weight of dry sample and glass box, Sw: where sample weight consists of fresh sample weight plus glass weight

3.4.2. Protein determination

In the present study, nitrogen content was estimated by Kjeldhal analysisis and Crude protein content was calculated by multiplying the nitrogen content by a factor of 6.25.

On the basis of early determinations, the average nitrogen (N) content of proteins was found to be about 16 percent, which led to use of the expression N x 6.25 (1/0.16 = 6.25) to convert nitrogen content into protein content. This approach was based on two assumptions namely that dietary carbohydrates and fats do not contain nitrogen and secondly that nearly all of the nitrogen in the diet is present as amino acids in proteins (FAO, 2003).

This use of a single factor of '6.25' is however confounded by two considerations. First, not all nitrogen in foods is found in proteins. Nitrogen is also contained in variable quantities of other compounds not only in proteins. Second, the nitrogen content of specific amino acids varies according to the molecular weight of the amino acid and the number of nitrogen atoms it contains. Because of this, the nitrogen content of proteins actually varies from about 13 to 19 percent. This would equate to nitrogen conversion factors ranging from 5.26 (1/0.19) to 7.69 (1/0.13). In response to these considerations, It is suggested that N x 6.25 be abandoned and replaced by N x a factor specific for the food in question (Jones, 1941 quoted in FAO, 2003) . This specific factor is referred to as the "Jones factor". This factor for animal proteins and for the vegetable proteins is generally in the range of between 6.25 and 6.38 and 5.7 to 6.25 respectively (FAO, 2003).

3.4.3. Fiber determination

Crude fiber content was determined by Weende scheme according to the method described by Rosalinda and Vinginia, 1996. The scheme named after Weede experimental station in Germany which estabilished the analytic method in 1865 . It is a method of analyzing food and other biological materials according to their molecular components (Rosalinda and Viginia, 1996).

The dried sample was boiled for 30 minutes in dilute sulphuric acid and filtered. This residue was again boiled in sodium hydroxide.The insoluble residue consisted of crude fiber and ash. This resdue was burned and the weight difference was taken as crude fiber.

3.4.4. Fat and minerals determinations

Fat content in food sample was determined by solxet (Diethyl ether) method. The dried sample was extracted with ether. This ether extract gave crude fat. The amount of trace elements such as calcium, phosphorous, iron and zinc in yam samples was determined by flame atomic absorption spectrometry (FAAS) (Walchs and coworkers, 1957). In this method, the organic material was removed by dry ashing. The residue was dissolved in dilute acid. The solution was sprayed in to the FAAS and the absorbtion of the metal to be analyzed was measured at a specific wavelength.

Atomic Absorption Spectrometry (AAS) is a technique for measuring quantities of chemical elements by measuring the absorbed radiation through chemical element of interest. This is done by reading the spectra produced when the sample is excited by radiation. Atomic absorption methods measure the amount of energy in the form of photons of light that are absorbed by the sample. A detector measures the wavelengths of light transmitted by samples and compares to the wavelengths which originally passed through them. The energy required for an electron to leave an atom is known as ionization energy and is specific to each chemical element. When an electron moves from one energy level to another within the atom, a photon is emitted with energy. Atoms of an element emit a characteristic spectral line. Every atom has its own distinct pattern of wavelengths at which it will absorb energy due to the unique configuration of electrons in its outer shell. This enables the qualitative analysis of a sample. Absorbance is directly proportional to the concentration of the analyte absorbed for the existing set of conditions (García and Báez, 2012).

3.5. Statistical analysis for proximate compositions and mineral contents

The Pearson correlation coefficient was used to estimate the relationships among average stem height, average number of leaves at 30 days of emergence, average tuber yield, proximate compositions and mineral contents of the 42 yam germplasms using the computer program Gen Stat (discovery edition 3) and graphical representation was made using excell program. The distance matrix was generated based on euclidian distance coeficients using Multivariate Analysis System Version 2.02 NTSYpc software program (Rolf, 1998). The distance matrix was subjected to hierarchical cluster analysis using Shan through an un-weighted pair-group method average (UPGMA) as defined by Sneath and Sokal (1973) and Latent vectors was analyzed using Gen Stat (discovery edition 3).

Table 6: Nutritional parameters considered for cluster analysis

Nutritional parameters		
1	Crude Fat	Percent of total
3	Crude Protein	Percent of total
4	Crude Fiber	Percent of total
5	Moisture	Percent of total
6	Ash	Percent of total
7	Iron	Percent of total
8	Zink	Percent of total
9	Calcium	Percent of total

10	Phosphorous	Percent of total

3.6. Microsatellites (SSR)

3.6.1. DNA extraction

In the present study, different *Dioscorea* species were observed within most yam accession found in the same vernacular name. Hence, a total of two hundred fifty eight yam leaf samples with six different *Dioscorea* species (one to five yam plants from experiemental plot) were observed separetly using twenty SSR markers. Young and clean leaves 0.2g -0.5g were collected per plant and dried in silica gel. From these dried leaves 0.2g was taken from each sample and ground with sterile pestle and mortar with addition of liquid nitrogen. Genomic DNA was isolated from about 0.2 g of pulverized leaf sample using modified triple Cetyl Trimethyl Ammonium Bromide (CTAB) extraction technique as describe by Borsch *et al.,* (2003). The yield of DNA was assessed by running 3 μl of freshly extracted genomic DNA samples on 1% agarose gel stained with 3 μl of ethidium bromide and visualized under an ultraviolet transiluminator at the Genetics Research laboratory of Addis Ababa University, Addis Ababa, Ethiopia (Figure 3). The quality and concentration of all DNA samples were determined using NanoDrop spectrophotometer (NanoDrop Technologies, Wilming ton, DE) at the International Institute of Tropical Agriculture (IITA), Ibadan Nigeria.

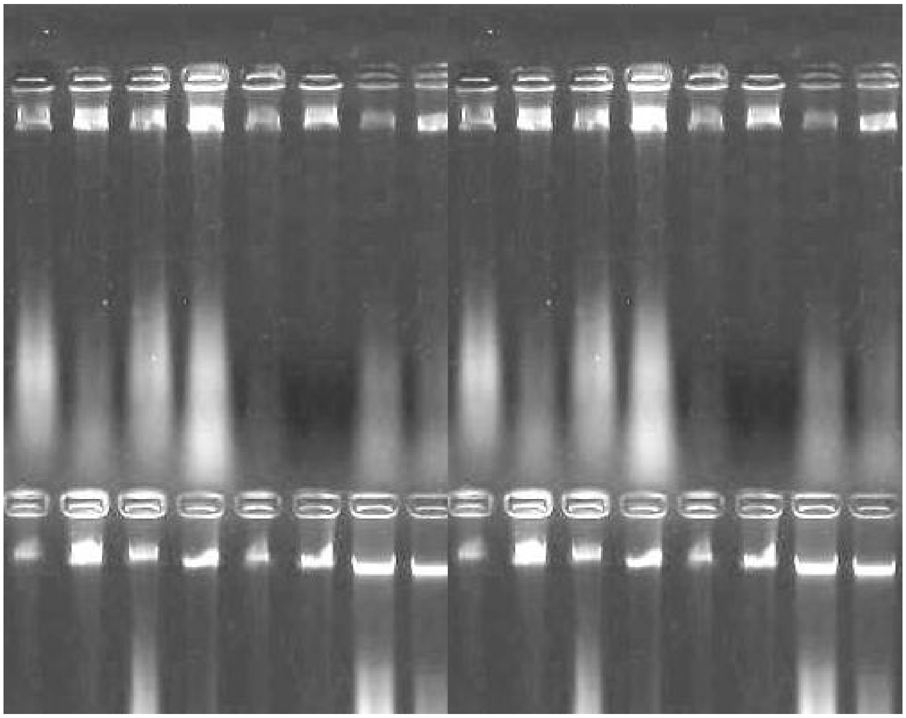


Figure 3. Test gel of some diluted DNA samples extracted from Ethiopian yam (*Dioscorea* spp) collections

3.6.2. PCR amplification

All the DNA samples were diluted to a standard concentration of 25 ng/µl with ultra pure water for PCR amplification. Polymerase chain reactions (PCRs) were carried out in a total volume of 10 µl, containing 2µl of 25 ng of genomic DNA, 1µl of 5mM forward primer, 1µl of 5mM of reverse primer, 1µl 10X NH4 reaction buffer, 0.4 µl of 50 mM $Mgcl_2$, 0.4 µl of 5 mM $dNTP_S$, and 0.06µl (5u/µl, Bioline) Biotaq ™ DNA polymerase. Touchdown PCR was performed using Applied Biosystems verti 96 well thermal cycler.

In this program the anealing temprature was gradually decreased by 1^0 C during the cycling program in order to favour only specific product amplification. The PCR cycle consisted of initial denaturation at 95^0c for 2 minutes followed by 35 cycles for 65^0 C anealing for 20 seconds then with 1^0c reduction in temprature per cycle for 10 cycles and the last extension step was 72 ^{0}c for 5 minute. The PCR products were stored at 4^0C until used. Touchdown PCR is a modification of PCR in which the intial annealing temprature is higher than the optimal and is gradually reduced over subsequent cycles until the Tm temprature or "touchdown temprature" is reached. The annealing temperature is decreased by 1^0 C every other cycle to approximately 10^0 C below the calculated Tm to permit exponential amplification (Rous, 1995).

3.6.3. Microsatellite markers and detection of PCR products

In this procedure, two cleaned balanced plates were taken and at the edges of the plates, polyacrylamide gel was poured immediately . After the gel became polymerized, the plates were fixed to the tanker and 1x TBE was added at its upper side. The tank was connected to a circute and left until it became warm. The denatured PCR products using formamide dye were loded in to gel with a approximately 2.5 ul inside each well. This was allowed to run at 80 Watt for one and a half hours to two and a half hours according to the molecular weight of the primers. Thereafter, the gel was subjected to fixing solution for 20-30 minutes, silver staining solution for 30 minutes, distilled water for about 5 seconds , developing solution and it was shake by hand until the bands were visible. At the end the bands were fixed using fixing solution in 200ml of glacical acetic acid and 1800 ml of ultra pure water.

3.6.4. Details of germplasm studied and Micro Satellite Primers

A total of two hundred and fifty eight yam accessions with six different *Dioscorea* species (one to five yam plants from each experiemental plot) was observed separately using twenty SSR loci. A total of 20 (thirteen DNA microsatellite markers and seven EST derived) markers were used in this study, of which 14 (eleven DNA microsatellite markers and three EST derived) microsatellite markers produced electrophoretic bands (Table 7). However, among these twelve (ten DNA microsatellite markers and two EST derived) microsatellite markers produced clear electrophoretic bands for all studied species and these SSR markers were considered for diversity analysis (Table 8).

Loading of the DNA samples on polyacrylamide gel were done in two different phases. First 96 denatured DNA samples were loaded on plate 1 and depending on molecular weight of the primer with 20-30 minutes gap the second denatured DNA samples were loaded on plate 2. The rest 66 samples were loaded on another third plate as shown in Figures 4 and 5.

Table 7: The list of Microsatellite primers used in this study

SN	SSR Primers	Electrophoretic bands	SN	SSR Primers	Electrophoretic bands
1	YM 13	*	11	D9	*
2	Da1 C12	*	12	Dpr3F12	X
3	Da1 A01	*	13	Dpr3B12	*
4	Dpr3 D06	*	14	D95	X
5	Da1F08	*	15	D58	X
6	DA1D08	*	16	D 25	X
7	Dab2 C12	*	17	YM 26	XX
8	Ym30	*	18	DPR3F04	*
9	D83	*	19	D91	X
10	D55	*	20	Dab2C05	*

Note: -SN=serial number; *= SSR primers showed electrophoretic bands to all studied genotypes;x = SSR primers did not show electrophoretic bands to most studied genotypes; XX= = SSR primers did not show electrophoretic bands to all studied genotypes

Table 8: Primer sequences (forward and reserve) used in the SSR analyses and their annealing temperature (Ta)

Locus	Primer sequences (5′−3′)	Annealing temprature (ºC)
Dalc12	F: GCCTTTGTGCGTATCT R: AATCGGCTACACTCATCT	53
Ym30	F:GGTCCTCTTCTATCCCAACAA R:CACGTATTAACTCCATCATCC	52
Da1Do8	F:GATGCTATGAACACAACTAA R: TTTGACAGTGAGAATGGA	51
Dpr3fo4	F: AGACTCTTGCTCATGT R: GCCTTGTTACTTTATTC	51
D55	F:TGGACTAACGTGGTGTAGG R:CTAACAACACACACACGGG	54
D83	F:AGCTGAGATGGGAGGATCAA R:AGGAGGAGGTGGAGGACTTC	55
Da1ao1	F: TATAATCGGCCAGAGG R: TGTTGGAAGCATAGAGAA	51
dab2c12	F: GCCTTTGTGCGTATCT R: AATCGGCTACACTCATCT	47
Da1fo8	F: AATGCTTCGTAATCCAAC R: CTATAAGGAATTGGTGCC	51
YM13	F:TTCCCTAATTGTTCCTCTTGTT R:GTCCTCGTTTTCCCTCTGTGT	58
Dpr3D06	F: ATAGGAAGGCAATCAGG R: ACCCATCGTCTTACCC	51
Dpr3b12	F: CATCAATCTTTCTCTGCTT R: CCATCACACAATCCATC	51

Note:- F=forward primer sequence R= reverse primer sequence

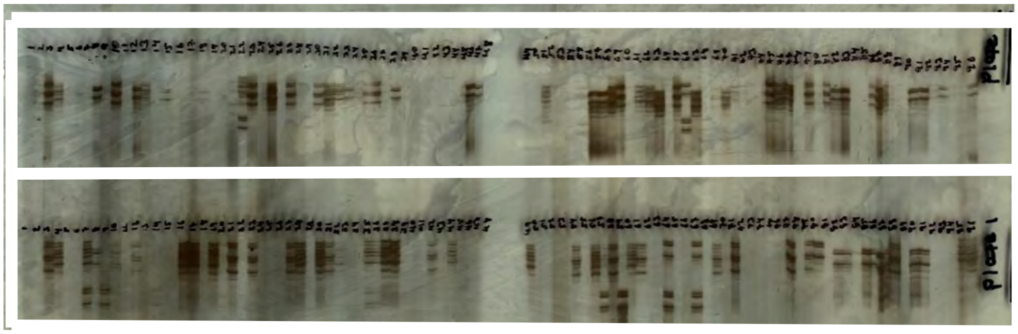


Figure 4. Polyacrylamide gel containing ethidium bromide stained SSR primer DPr3B12 fragments obtained from 192 accessions of six Ethiopian yam (*Dioscorea*) species

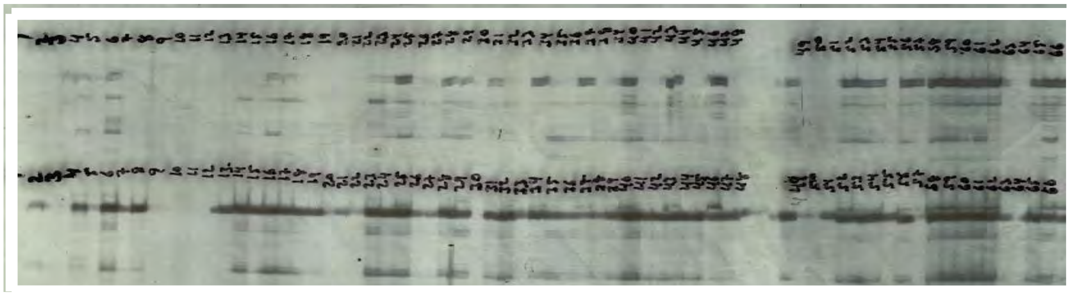


Figure 5. Polyacrylamide gel containing ethidium bromide stained EST drived SSR primers D83 and D55 fragments obtained from 66 accessions of six Ethiopian yam (*Dioscorea*) species

3.6.5 Data scoring and statistical data analysis

Among 258 yam accessions, two hundred and ten acessions were observed independently to each species and region based statistical analysis from twelve SSR loci which gave clear electrophoretic bands (Table, 9). Data were recorded as 1 for the presence and 0 for the absence of each amplified fragment. A 50-basepair ladder was used to estimate the size of the fragments.

Analysis of Molecular Variance was computed following detection of PCR products and data scoring using the computer program GenAlex (Peakall and Smouse, 2006). Free Tree 0.9.1.50 (Pavlicek *et al.,* 1999) software's were used to determine the genetic parameters Jaccard's coefficient of similarity as indicated bellow.

$$S_{ij} = \frac{a}{a+b+c}$$, where S_{ij} is Jaccard's similarity coefficient

Where, a= the total number of bands shared between individuals i and j

B= the total number of bands present in individual i but not in individual j

C= the total number of bands present in individual j but not in individual

The POPGENE 1.32 program (Yeh & Boyle, 1997) was used to calculate the genetic diversity parameters: allele frequencies at each locus the number of observed alleles, the number of effective alleles, polymorphism percentage and gene diversity (*H*) according to Levene (1949).

Effective number of alleles (Ae) is the number of alleles that can be present in a population. It is calculated as $Ae = 1/ (1 – h) = 1/\Sigma pi^2$ where, pi = frequency of the ith allele in a locus.

One of the most frequently used measures of genetic variation is Nei's gene diversity. The gene diversity 'H' is calculated per locus as $H=1-\sum pi^2$. Where gene diversity is summed from i = 1 to n and pi is the frequency of the ith allele and n is the number of alleles.

The distance and DICE coefficient of similarity matrices with Unweighted Pair Group Method with Arithmetic mean (UPGMA) (Sneath and Sokal, 1973) were generated using Free Tree 0.9.1.50 Software (Pavlicek *et al.,* 1999) and trees were then viewed using

TREEVIEW 0.9.1.50 Software (Pavlicek *et al.,* 1999) of Tree un rooted and slanted methods for species based and site based populations respectively.To further examine the patterns of variation among Yam population on 2D, a principal coordinated analysis (PCO) was performed using NTSYS-pc version 2.2 (Rohlf, 2006) based on Nei's unbiased genetic distance (Nei 's, 1978).

3.7. Combined data analysis

3.7.1 Combined data sets and statistical data analysis

It is not only reasonable to analyze data sets separately on the basis of different modes of inheritance but also, it is essential to analyze raw data in different ways to possibly draw sensible conclusions (Pedersen *et al*, 1996; Mohammadi and Prasanna, 2003).

Multivariate methods such as cluster analysis can be carried out using morphological (qualitative), biochemical, and molecular marker data or combinations of such data. In the present study, binary (scored as 1 or 0) data were used for molecular markers data. Some characters of morphological (qualitative) data were in binary mode and some other traits were in categorical trend. The categorical data types were converted to binary form by assuming each category as a separate locus. Nutritional contents were recorded in percentage value hence similar to binary data percentage values have also non normal distribution.

In the present study, combined analysis was made for 42 plants which have all nutritional, morphological and microsatellites data set (Table, 9). Thirty qualitative morphological characters, data derived from twelve SSR markers, proximate composition (moisture, ash, crude fat, crude protein and crude fiber) and minerals content (calcium, phosphorous, zink and iron) were considered in combined data set analysis as indicated in Table 9

The statistical analysis were computed using two computer statistical software packages, Free Tree program (Pavlicek *et al.*, 1999) and SAS 1999 respectively through an approach which allows data to be combined for statistical analysis (Rodgers *et al.,* 1997). After generating the combined dissimilarity matrix, yam genotypes cluster groups were studied with the technical details of the pseudo-t2 statistic (SAS, 1999).

Analysis of Molecular Variance (AMOVA) (Tables 31, 32) was computed from microsatellite (SSR) data (yam accessions considered in combined analysis) using the computer program GenAlex 6.2 based on 999 permutations (Peakall and Smouse, 2006). AMOVA allows for a partitioning of molecular variance within and among populations and tests the significance of partitioned variance components using permutational testing procedures (Excoffier *et als.*, 1992). ΦPT values, similar to Fst when data are haploid or binary, are calculated in an AMOVA and represent the proportion of the total variance that is partitioned between populations (Peakall and Smouse, 2006).

Table 9: Forty two Ethiopian yam genotypes with their respective origin evaluated in combined analysis of nutritional, morphological and molecular markers

SN	AccessionCode	Vernacular Name	Species	Origns
1	Ged 05	-	*D. abyssinica*	Gedio
2	Wol 10	Boyebene	*D. abyssinica*	Wolayita

3	GG02	Tola-1	*D. abyssinica*	Gamogofa
4	GG03	Tola-2	*D. abyssinica*	Gamogofa
5	GG04	Bune-1	*D. abyssinica*	Gamogofa
6	GG05	Bune-2	*D. abyssinica*	Gamogofa
7	GG06	Bune-3	*D. abyssinica*	Gamogofa
8	GG07	Bune-4	*D. abyssinica*	Gamogofa
9	GG08	Bune-5	*D. cayenensis*	Gamogofa
10	GG09	Bune-6	*D. cayenensis*	Gamogofa
11	Wol 01	Oha-1	*D. rotundata*	Wolayita
12	Wol 02	Oha-2	*D. rotundata*	Wolayita
13	Wol 03	Arkiya-1	*D. Praeihensilis*	Wolayita
14	Wol 04	Arkiya-2	*D. Praeihensilis*	Wolayita
15	Wol 05	Gassa	*D. rotundata*	Wolayita
16	Dau 01	Dorsita-1	*D. bulbifera*	Dauro
17	Dau 02	Dorsita-2	*D. bulbifera*	Dauro
18	Dau 03	Gebiche-1	*D. abyssinica*	Dauro
19	Dau 04	Gebiche-2	*D. abyssinica*	Dauro
20	Wol 06	Gafela-1	*D. praeihensilis*	Wolayita
21	Wol 07	Gafela-2	*D. praeihensilis*	Wolayita
22	GG 01	Bonke	*D. praeihensilis*	Gamogofa
23	Wol 08	Wiyacha-1	*D. abyssinica*	Wolayita
24	Wol 09	Wiyacha-2	*D. abyssinica*	Wolayita
25	Wolg 01	Gudina-1	*D. abyssinica*	Wolayita
26	Wolg 02	Gudina-2	*D. rotundata*	Wolayita
27	Jim 02	-	*D. alata*	Jima
28	Jim 03	-	*D. alata*	Jima
29	Wolg 03	Lalo-1	*D. rotundata*	Wolega
30	Wolg 04	Lalo-2	*D. rotundata*	Wolega
31	Jim 04	-	*D .bulbifera*	Jima
32	Jim 05	-	*D .bulbifera*	Jima
33	Wolg 05	Dhoknuma-1	*D .bulbifera*	Wolega
34	Wolg 06	Dhoknuma-2	*D .bulbifera*	Wolega
35	Jim 06	-	*D. alata*	Jima
36	Jim 07	-	*D. alata*	Jima
37	Ged 02	Ganticho-1	*D.bulbifera*	Gedio
38	Ged 03	Ganticho-2	*D.bulbifera*	Gedio
39	Ged 04	Nifo-1	*D.bulbifera*	Gedio
40	Ged 05	Nifo-2	*D.bulbifera*	Gedio

41	Wolg 07	Roba-1	*D.bulbifera*	Wolega
42	Wolg 08	Roba-2	*D.bulbifera*	Wolega

Table 10: Qualitative traits and nutritional parameters measured from yam (*Dioscorea* spp) germplasms

SN	Qualitative Morphological Characters	Score code/ Descriptor state
1	Leaf Color	1 Yellowish, 2 Pale green, 3 Dark green, 3 purplish green, 4 purple, 99 other
2	Leaf Margin Color	1 Green, 2 Purple, 3 Other
3	Vein Color	1 yellowish, 2 Green, 3 Pale purple, 4 Purple, 99 other
4	Young petiole color	1 Green, 2 Green with purple edges, 3 Purple, 99 Others (specify in descriptor 7.7)
5	Young petiole wing color	1 Green, 2 Green with purple edges, 3 Purple, 99 Others (specify in descriptor 7.7)
6	stem color after emergence	1 Green, 2 purple green, 3 Brownish green, 4 Dark Brown, 5 Purple, 99 other
7	Mature petiole color	1 All green with purple base, 2 All green with purple leaf junction, 3 All green with purple at both ends, 4 All purplish green with purple base, 5 All purplish green with purple leaf junction, 6 All purplish green with purple at both ends, 7 Green, 8 Purple 9 Brownish green, 10 Brown, 11 Dark brown 99 Other (specify in descriptor 7.7 Notes
8	leaf density	3 Low, 5 intermediate, 7 High
9	Leatherness	0 no, 1 yes
10	waxiness of upper/lower surface	1 Waxy upper surface, 2 waxy lower surface, 3 both
11	Distance b/n lobs	1 No measurable distance, 5 intermediate, 9 very distant
12	Up-ward folding of leaf along main vein	3 Weak, 7 Strong
13	Twining Direction	1 Clockwise (climbing to the left), 2 Anticlock Wise (climbing to the right)
14	Stem Color	1 Green, 2 Purplish green, 3 Brownish green, 4 dark brown, 5 purple, 99 other
15	Corm Size	3 Small, 5 intermediate, 7 large
16	Spines of roots	3 Sparse, 7 dense
17	Tuber width at middle part	1 Regular, 2 Transversally elongated, 3 branched
18	Absence or presence cracks on tuber surface	0 No, 1 Yes

19	Root tuber surface	0 No, 3 Few, 7 Many
20	place of root on the tuber	3 Few, 7 Many
21	Uniformity of flesh color	0 no, 1 yes
22	maturity tuber after emergency	1. Up to 6 months, 2. 7-8 months, 3. 9-10 months
23	flesh color lower part tuber	
24	**Absence or presence of spines**	**0 Absent, 1Present**
25	**Dawn ward folding of leaf along main vein**	**3 Few, 7 many**
	Traits evaluated by Farmers	
26	Absence or presence of moisture in Cooked	0 Absent , 1 Present
27	Bitterness of cooked tuber	0 Not bitter, 1 Bitter, 2 Very bitter
28	Flavor of cooked tuber	0 Not acceptable, 1 Acceptable, 2 Very acceptable
29	After cooking appearance	2 Poor, 5 Fair , 7Good
30	color of tuber after cooking	1 White not colored, 9 highly colored
	Nutritional parameters	
31	Crude Fat	Percent of total
32	Crude Protein	Percent of total
32	Crude Fiber	Percent of total
33	Moisture	Percent of total
34	Ash	Percent of total
35	Iron	Percent of total
36	Zink	Percent of total
37	Calcium	Percent of total
38	Phosphorous	Percent of total

Note: SN=Serial number

4. Results

4.1 Morphological variability based species identification

Based on presence and absence of spines on stems & roots , number of male and female inflorescence, stem length, twining direction, and flesh color 60 yam germplams from ten major growing areas of the country were grouped into six species namely *D. alata*, *D.bulbifera*, *D. abyssinica*, *D. praehensilis*, *D. rotundata* and *D. cayenensis*.

The two species *D. rotundata* and *D. cayenensis* were observed to have 1-3 male inflorescences per spike and after cutting revealed white and yelowish flesh color. During this study the yellowish colour was grouped to *D. cayenensis* whereas the white flesh colour was grouped to *D. rotundata* as indicated in Figures 6d and 6b respectively.

The species *D. alata* was differentiated from the other groups based on its four angled stem. The average stem length of this species under study were 10m long, it had different tuber shape apperance (Figure 6f) whereas *D. abyssinica* had an average climber stem of 2-5m and the number of male infloresences observed was 3-6.

Under this study most of the *D. abyssinica* species had male infloresecence. Spines were observed on stem of this species (Figure 6a) . Whereas, the average stem length recorded from *D. praehensilis* was 10 m and spines were observed both on roots and stems of this species. The number of male and female inflorescences observed in *D. praehensilis* were 3-5 and 1-2 respectively (Figure 6e). Generaly the flesh color of both *D. praehensilis* and *D. abyssinica* revealed three types viz: purplish colour, a central white colour surounded by purplish colour and the mixure of white and purplish colours (Figures 6a and 6e).

The species whose stems were climbing to the left or in a clock wise direction were grouped in to *D.bulbifera* as it is indicated in Figure 6c. All the other species under study displayed twining direction an anti clock wise. The average stem length was 3-10 m with 3-5 male inflorescences. The tuber flesh color was mostly white and rarely a mixture of white & purplish color was observed.

Figure 6. Morphological variations (Male and female inflorescences, fruits, twining habit and tuber flesh color) among six yam (*Dioscorea* spps) germplasm observed in this study

6a. Collection area: - Jima (South west of Addis Ababa)
Altitude: - 1753 m.a.s.l Species: - *D. abyssinica*

6b. Collection: - Wonago Altitude: - 1940 Species: - *D. rotundata* (White yam)

6 c. Collection area: - Wolega Vernacular name:- Dhokunma
Altitude: - Species: - *D.bulbifera*

6d. Collection: - Kefa Species: - *D.cayenensis*

6e. Collection area: - Gamogofa **Altitude:-1140**
Vernacular name: - Hatiya-2 **Species: - *D. Praehensilis***

6f. Collection area: - Jima **Species: - *D.alata***

Figure 7. Tuber shape similarities and differences among six different species of yam (*Dioscorea* spp) germplasm with their respective collection area (local name and altitude)

7a-1 7a- 2 7a- 3 7a- 4 7a- 5

7a-1.Wolayita (Chocha , 1850masl), 7a-2. Gedio (Local Gedio), 7a-3. Sidama (Gellawcho, 1940 masl), 7a-4. Sidama (Adame Ado) and 7a -5. Jima
Species: - *D.rotunda*

7b- 1 7b- 2

7b-1:-Jima (1753 masl), Kembata 7b- 2:- (1630masl)
Species: - *D. abyssinica*

7c- 1 7c- 2

7c- 1: - Gedio (Ganticho), and 7c- 2 Wolayita (Oha, 1780) Species:-*D.bulbifera*

7d-1 7d-2

7d-1 Wolayita and 7d-2 Jima , Species: *D.alata*

. 7e-1 7e-2 7e-3

7e-1- Gamogofa (Bunne 2, 1655), 7e-2 Awasa (Bunne 1) ,7e-3 Gamogofa (Bunne 1) Species: - *D. perhensilies*

4.2 Morphological diversity data of yam determined based on cluster analysis

Among 45 qualitative morphological characters recorded on six *Dioscorea* species namely *D. abyssinica*, *D. praeihensilis*, *D. cayenensis*, *D. rotundata*, *D. alata* and *D.bulbifera* 12 traits did not reveal phenotypic variation among the 60 yam germplasm studied. These traits were not included for clustering and principal component analysis. Based on the relative magnitude of distance similarity matrix and dendrogram using Shan UPGMA cluster analysis, all sixty yam genotypes included in the study were grouped into five clusters (Fig. 8).

At the similarity distance 1.81 the dendrogram identified three clusters 1 and 2 which contain eight to eighteen accessions per cluster (Table 11 and Table 12). Cluster 1 is the second largest group and contains 18 yam accessions with green mature petiole colour. Cluster 2 contained accessions with pale purple young petiole colour, green mature petiole colour, high leaf density, presence of cracks on tuber surface, intermediate corm size, highly coloured tuber after cooking and cooked tuber sweetness

The dendrogram at the similarity distance 1.6 identified clusters 3. This cluster group is characterized by dark brown stem color after emergency, green mature petiole color, intermediate corm size and presence of cracks on the cooked tuber.

The dendrogram with similarity distance 1.71 identifies one cluster group 4. This cluster is the largest and is composed of 19 accessions.This group is characterized by yellow mature petiole color, green stem color and absence of cracks on the tuber. Cluster 5 contained two cluster groups cluster 5a and cluster 5b. Cluster 5a is a group of *D. bulbifera* species with yellow maturity petiole color, green stem color, twining direction to left, intermediate corm size and small tuber width at middle part. Cluster 5b is a group of *D. rotundata* composed from three yam accessions. This group is characterized by absence of barky patches on the stem, green stem color, and yellow mature petiole color, small tuber width at middle part, absence of tuber cracks, late maturity group and white tuber color after cooking. In this analysis the cophenetic correlation coefficient result was r=80 which revealed the efficiency of the dendrogram.

Table 11: Clustering groups of six different Ethiopian *Dioscorea* species derived from distance similarity coefficient based on their morphological diversity data

Cluster name	Sub clusters	Number of accessions	Species name and their frequencies		Comments
C1		18	*D. abyssinica*	6	green mature petiole colour
			D.preahensilis	5	
			D. rotundata	3	
			D. cayenensis	6	
C2		8	*D. alata*	3	pale purple young petiole colour, green mature petiole colour, high leaf density, presence of cracks on tuber surface, intermediate corm size and cooked tuber sweetness and
			D. rotundata	1	
			D. bulbifera	2	
			D. abyssinica	2	
C3		7	*D. preahensilis*	2	dark brown stem color after emergency, green mature petiole color, intermediate corm size and presence of cracks on cooked tuber
			D. abyssinica	1	
			D. cayenensis	1	
			D. rotundata	1	
			D. bulbifera	2	
C4		19	*D. abyssinica*	2	yellow mature petiole color, green stem color and absence of cracks on the tuber
			D. preahensilis	4	
			D. cayenensis	4	
			D. bulbifera	1	
			D. rotundata	5	

C5	C5a	5	*D. bulbifera*	5	yellow maturity petiole color, green stem color, twining direction to left, intermediate corm size and small tuber width at middle part
	C5b	3	*D. rotundata*	3	absence of barky patches on the stem, green stem color, yellow mature petiole color, small tuber width at middle part, absence of tuber cracks, late maturity group , white tuber color after cooking and twining direction to right

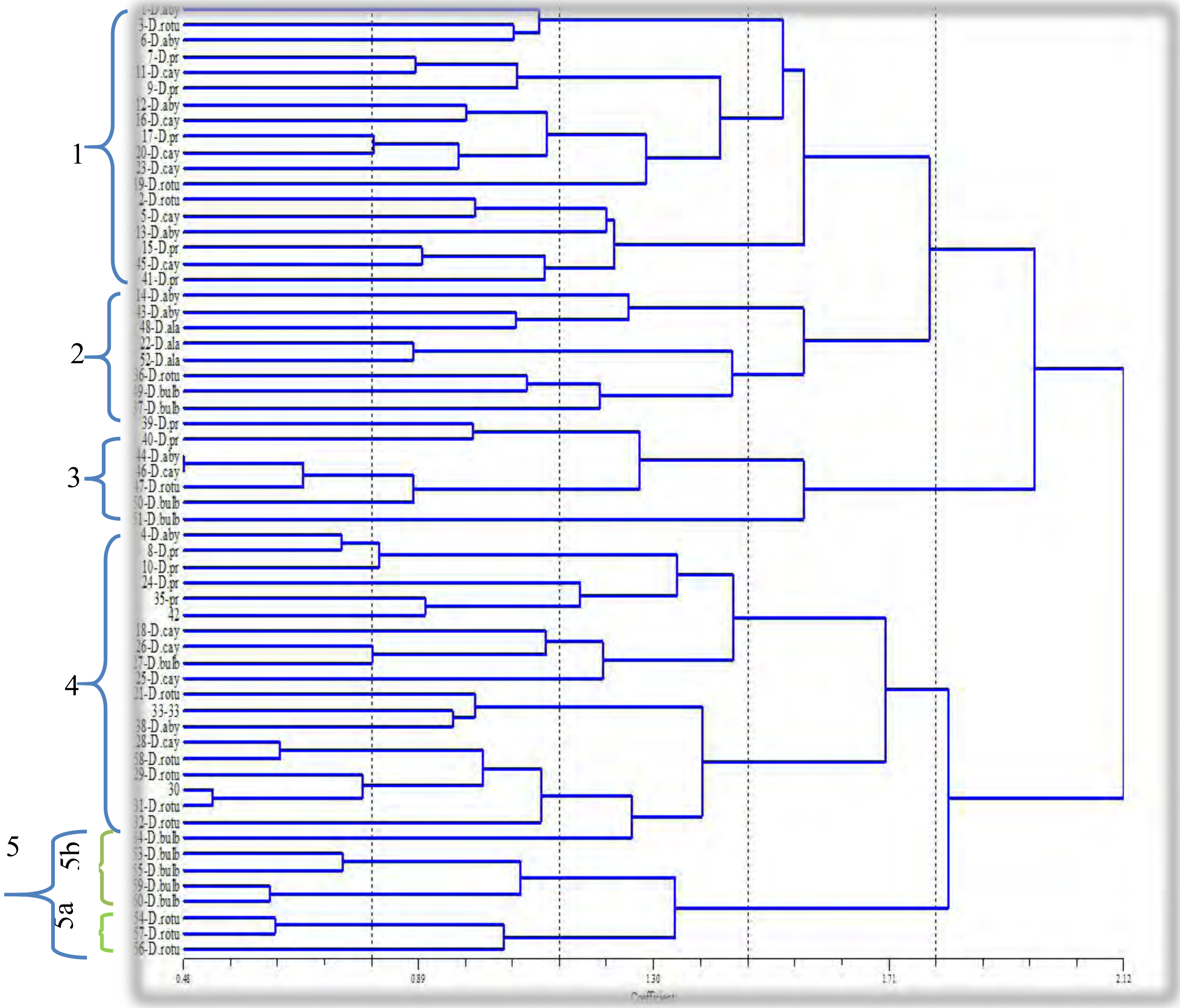


Figure 8. Relation ship of six yams (*Dioscorea* species) species collected from 10 major growing areas of Ethiopia based on distance UPGMA hierarchical cluster analysis

Table 12: Clustering pattern of yam (*Dioscorea spp*) accessions derived from the similarity matrix of 60 accessions based on their morphological traits

Cluster name	Sub clusters	Number of accessions	Accessions included (SN)	Species name	Origin
C1		18	(1,3,6,7,11,9,12,16,17,20,23 19,2,5,3,15,45 41)	*D.abyssinica* *D.preahensilis* *D. rotundata* *D. cayenensis*	Ji,Wo,Kf,Ga,Ga,Ar,Wo,Wo,Ga,Si,Ar,Ga,Ge,Kf,Ge,Ge,Wo,Ge,Wo,Wo)
C2		8	(14,43,48,22,52,36,49,37)	*D. alata* *D. rotundata* *D. bulbifera* *D.abyssinica*	(Ji, Ar, Ji,Wo,Ji, Da)
C3		7	(39,40,44,46,47,50,51,	*D.abyssinica* *D.preahensilis* *D. rotundata* *D. cayenensis* *D. bulbifera*	(Wo,Km,Wg,Wg,Wg,Wg,Wg,
C4		19	(4,8,10,24,35,42,18,26,27,25,21,33,38,28,58,29,30,31,32)	*D. cayenensis* *D. rotundata* *D. bulbifera* *D. preahensilis* *D. abyssinica*	Ga,Ga,Ar,Ar,Si,GaGa,Ga,Ar, Ar,Si, Da,Si, Un,Si,Si,Si, Ji)
C5	C5a	5	(34,53,55,59 , 60)	*D. bulbifera*	(Wo,Wo,Wo,Ge,Ge)
	C5b	3	(54, 56, 57)	*D. rotundata*	(Wo,Wo,Wo)

Note: - Ji-Jima,Ga-Gamogofa, , Da-Dauro, Wo- Wolayita, Si-Sidama, Ar-Areka, Km-Kembata, Ge-Gedio, Wg-Wolega, Kf-Kefa

4.2.1. Principal component analysis

The PCA results revealed that the first axis largely accounted for the variation among yam accessions (24.87%) followed by the second axis (14.96%) and third axis (11.63%). The first five axes with eighteen values greater than unity accounted for the total variations among 33 characters describing 69.31% and the first three axes accounted for 51.48 % (Table 1 3 and Table 14).

The hierarchal clustering patterns in the dendrogram supports the grouping pattern observed in the scatter diagrams (Figures 9 and 10). Traits with comparatively greater weight in PC1 and PC3 were young petiole color. Correspondingly greater weight in PC2 was color of tuber after cooking. Similarly, flesh color at the lower part of tuber, and flavor of cooked tuber resulted in larger weight in the first three principal components while tendency of tuber to branch gave greater weight to the first two principal components.

Table 13: Eigen values, variability percentage and accumulated variation with respect to five character in 60 yam accessions

SN	Plant Characters	Eigen values	Variation of each component (%)	Accumulated variation (%)
1	leaf color	3.42	24.88	24.88
2	leaf margin color	2.05	14.97	39.84
3	vein color	1.59	11.64	51.48
4	young petiole color	1.27	9.23	60.71
5	young petiole wing color	1.18	8.60	69.31

Note: - SN= Serial number

Table 14: First 3 principal components (PCs) scores of 33 morphological traits across yam genotypes collected from 11 major growing areas of Ethiopia

SN	**Traits**	**PC1**	**PC2**	**PC3**
1	leaf color	0.0813	0.0763	0.1160
2	leaf margin color	0.0664	0.2335	0.0434
3	vein color	0.1859	0.0522	0.1136
4	young petiole color	**-0.9765**	-0.1410	**-0.6175**
5	young petiole wing color	0.0836	0.0784	-0.0530
6	stem color after emergency	0.0148	0.1391	0.1029
7	Stem spines	-0.0201	-0.0488	0.0241
8	Mature petiole color	0.1051	0.0811	0.0075
9	Leaf density	0.1531	0.1683	0.1752
10	Leathernes	-0.0991	-0.0644	0.0008
11	Waxiness upper surface	-0.0555	-0.1583	0.1090
12	Distance between lobes	0.1234	-0.0665	0.0231
13	upward folding of leaf along main vein	0.2860	-0.2163	-0.1774
14	Dawn ward folding of leaf along main vein	-0.0211	-0.1028	-0.0398
15	twining direction	0.0155	-0.0532	-0.0265
16	Stem color	0.1884	0.1268	0.0464
17	corm size	-0.0454	0.1533	0.0638
18	Root spines	0.2627	-0.0496	-0.2855
19	over all acceptability	0.1587	0.0000	0.0854
20	Tendency of tuber to branch	**-0.5125**	**0.3635**	-0.2231
21	Absence and presence cracks on tuber surface	-0.0467	0.0503	0.0445
22	Roots on tuber surface	0.1763	0.0309	-0.2152
23	Place of roots on the tuber	0.2880	0.1962	-0.2392
24	Stickiness of cooked tuber	0.1267	0.1009	-0.0341
25	Flavor of cooked tuber	**-0.4546**	**-0.5504**	**0.6893**
26	Absence or presence of moisture on cooked tuber	-0.0348	0.0077	-0.0067
27	Bitterness of cooked tuber	-0.0591	0.0177	-0.0221
28	After cooking appearance	-0.0625	-0.3185	0.2617
29	color of tuber after cooking	0.1375	**0.8728**	0.2634
30	Over all acceptability	-0.0259	**-0.3934**	0.1703
31	Flesh color lower part tuber	**0.9137**	**0.5159**	**-0.4399**
32	Uniformity of flesh color	-0.1156	0.0159	0.0792
33	Maturity after emergency	-0.0833	0.0328	0.0605

Note:- Note: - SN= Serial number; *Bolded values revealed highly correlated morphological traits to respective principal components

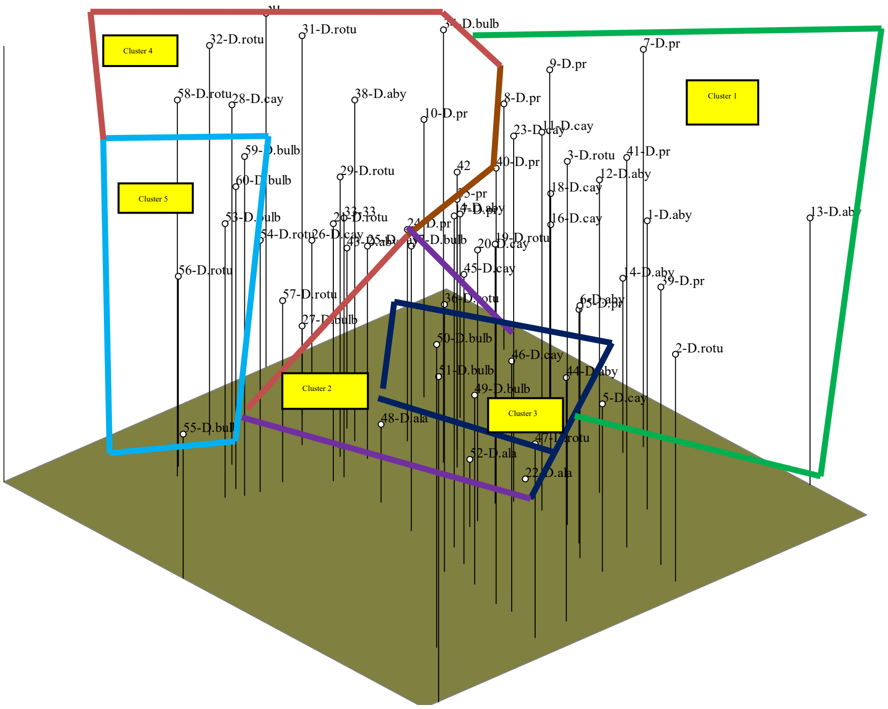


Figure 9. A 3-diamentional scatter plot of yam (*Dioscorea* species) from ten major growing areas of Ethiopia based on the scores of the first three principal components

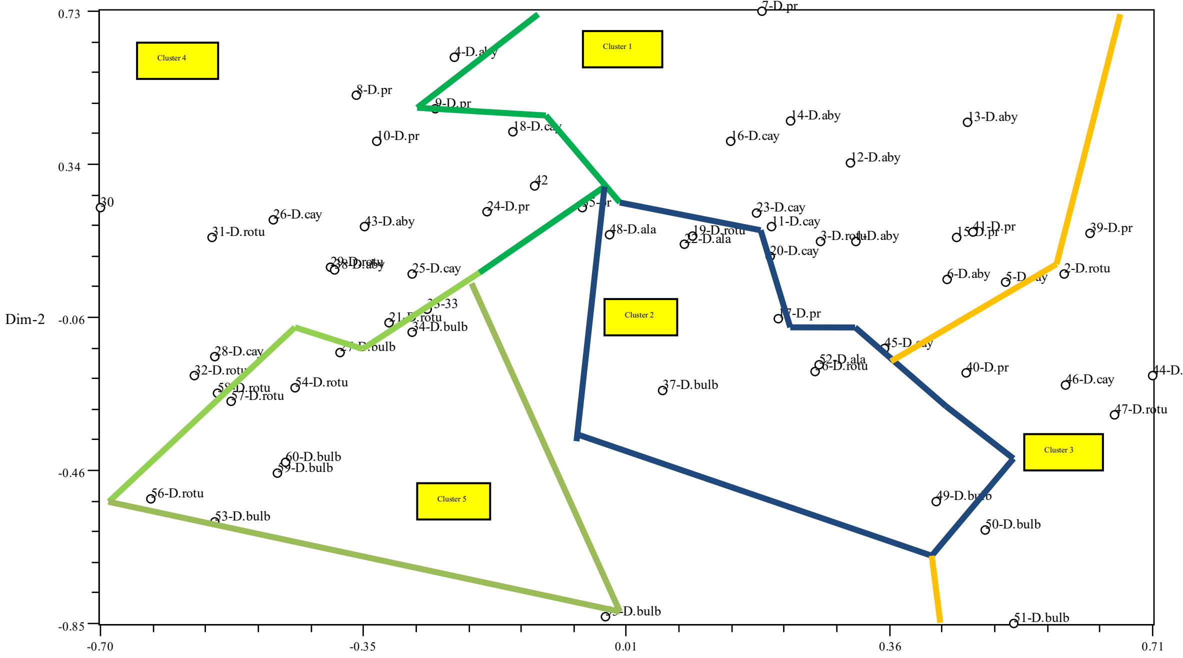


Figure 10. Two dimensional plot of the first two principal components (PC-1 and PC-2) accessions that are enclosed lines and marked as cluster 1, 2, 3, 4 and 5 corresponding groups in the cluster analysis

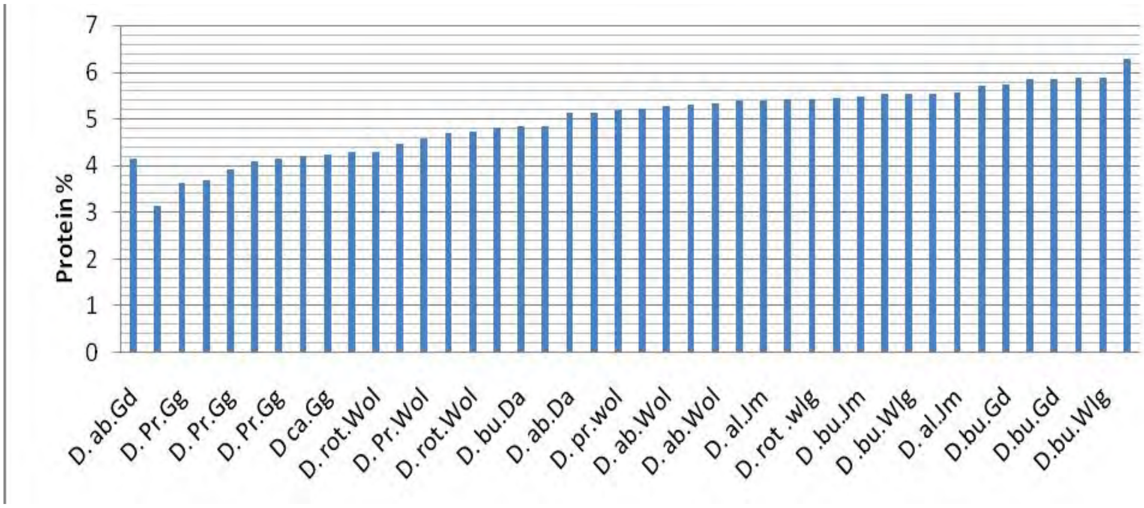
4.3. Proximate and mineral composition of Ethiopian yam (*Dioscorea* spp)

4.3.1. Nutritional value variations among yam (*Dioscorea* Species) of Ethiopia

4.3.1.1. Protein content (%)

Average performance for protein was higer in *D.alata* and *D. bulbifera* (Fig. 11). The range of protein content for all the fresh tubers observed in the study was between 3.13% to 6.29 % .*D. bulbifera* species from Wolega region recorded the highest protien content (6.29 %) compared to all other yam species and *D.abyssinica* from Kembata. revealed minimum value (3.13%).

The range was also greater among *D.abyssinica* species (3.13%-5.38%) in comparison to *D.praehensilis* (3.26%-5.2%), *D. cayenensis* (4.24%-4.27%), *D. rotundata* (4.2%-5.4%) and *D.alata* (5.3%-5.7%) (Fig. 11 and Table 16).



***Dioscorea* species with respective locations**

Figure 11. Protein (%) Content of yam (*Dioscorea* species) tuber from Ethiopia

Notes: - D.ab = *D. abyssinica,* **D.bu**= *D. bulbifera* , **D.ca**=*D. cayenensis*, **D.pr** = *D.preahensilis,* **D.rot**= *D. rotundata,* **D.al**=*D.alata***Wol**= Wolayita, **Wlg**= Wolega, **Da**=Dauro , **Jm**= Jima, **Ga**= Gamogofa

4.3.1.2. Fat content (%)

D. cayenensis from Gamogofa origin recorded highest fat content (7.86%) followed by *D. preahensilis* of same orign (7.83%) compared to all other species (Fig. 12). The lowest fat content percentage also resulted from *D. preahensilis* from the same location. The range for fat content was also greater among *D. preahensilis* (0.26%-7.83%) and *D. cayenensis* (0.59-7.86%) (Fig. 12 and Table, 17).

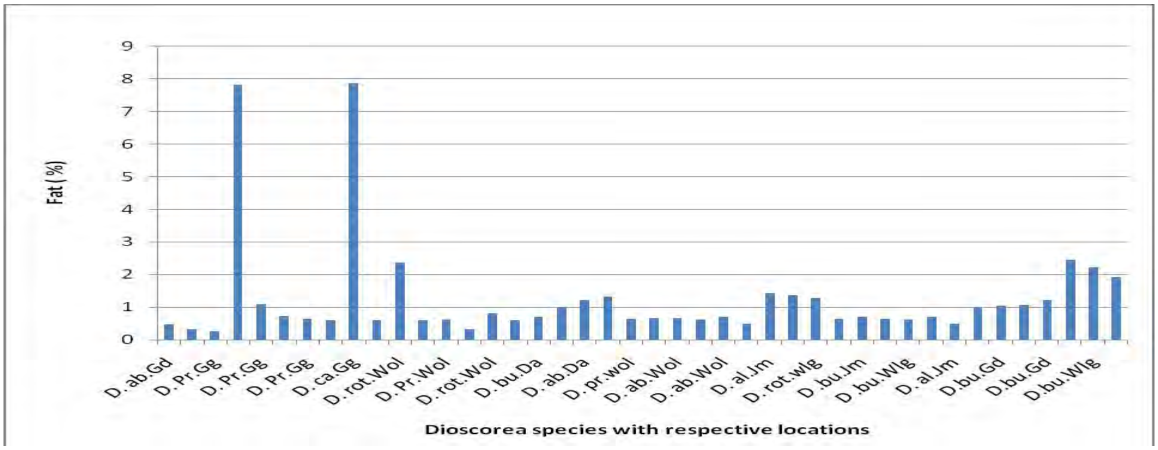


Figure 12. Fat (%) content of yam (*Dioscorea* species) from Ethiopia

Notes: - D.ab = *D. abyssinica,* **D.bu**= *D. bulbifera,* **D.ca**=*D. cayenensis*, **D.pr** = *D.preahensilis,* **D.rot**= *D. rotundata*, **D.al**=*D. alata* **Wol**= Wolayita, **Wlg**= Wolega, **Da**=Dauro , **Jm**= Jima, **Ga**= Gamogofa

4.3.1.3. Fiber (%) content

Average fiber content (%) was higher for *D. alata* species followed by *D.bulbifera*. The range of fiber content was (1.82%-6.36%) for all samples considered in the study. The lowest fiber content was recorded from *D. bulbifera* of Wolayita orign . The highest range of fiber content was observed from *D. bulbifera* (1.82%-5.16%) and *D. alata* (3.65%-6.36%) and *D. abyssinica* (1.94%-2.97%) (Fig. 13 and Table 16).

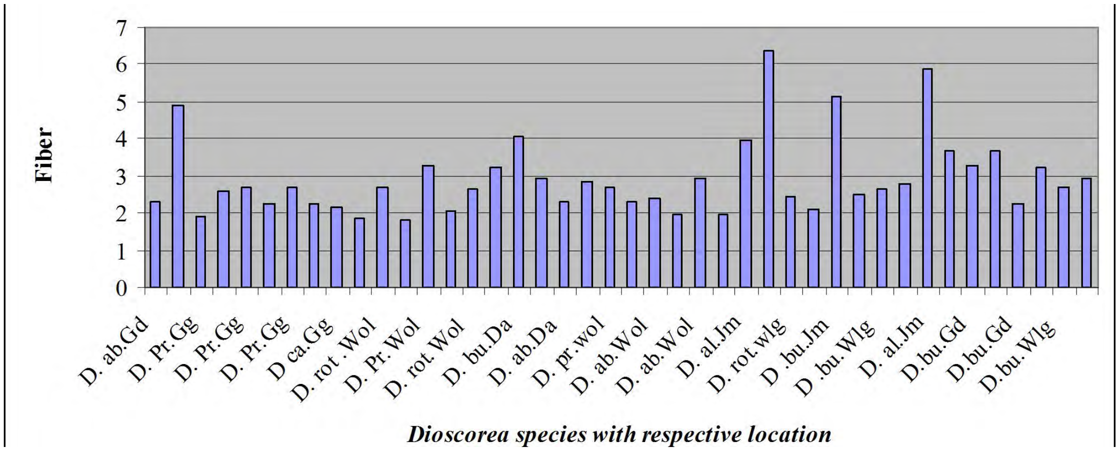


Figure 13. Fiber (%) content of yam (*Dioscorea* species) from Ethiopia

Notes: - D.ab = *D. abyssinica,* **D.bu**= *D. bulbifera* , **D.ca**=*D. cayenensis*, **D.pr** = *D.preahensilis,* **D.rot**= *D. rotundata,* **D.al**=*D.alata***Wol**= Wolayita, **Wlg**= Wolega, **Da**=Dauro , **Jm**= Jima, **Ga**= Gamogofa

4.3.1.4. Moisture (%) content

The range for moisture content was between (4.67%-13.57) for all samples studied (Fig. 14 and Table 16). *D.alata* from Jima region and *D.bulbifera* from Wolega region scored the highest and lowest moisture content respectively (Fig. 14) . *D.bulbifera* had the highest range (4.67%-12.41%) followed by *D. rotundata* (8.08%-12.17%)and *D. preahensilis* (8.37%-11.85%) for this trait.

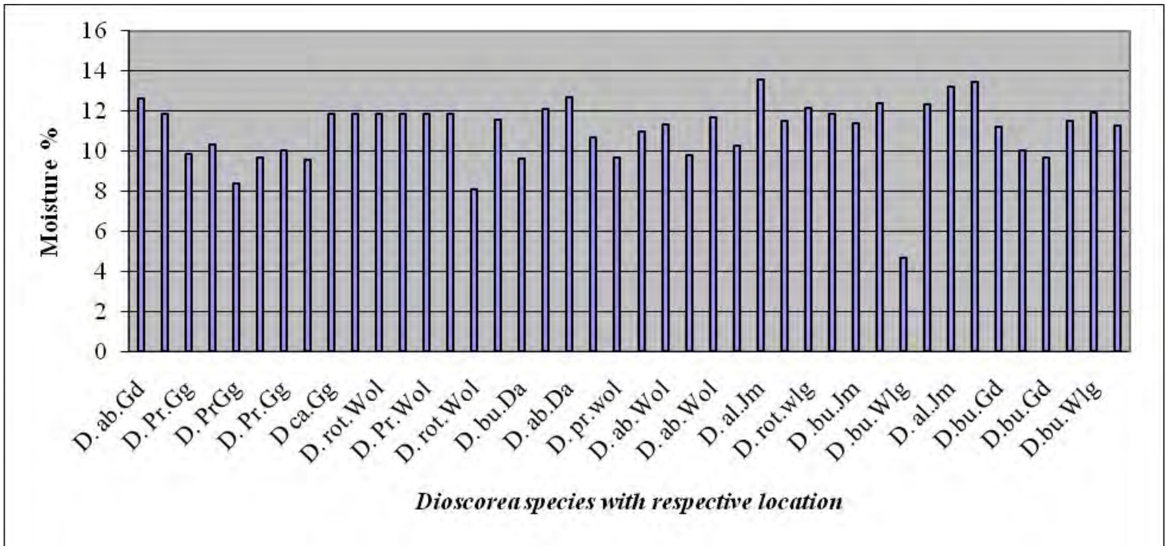


Figure 14. Moisture (%) content of yam (*Dioscorea* species) from Ethiopia

Notes:- D.ab = *D. abyssinica,* **D.bu**= *D. bulbifera* , **D.ca**=*D. cayenensis*, **D.pr** = *D.preahensilis,* **D.rot**= *D. rotundata,* **D.al**=*D. alata***Wol**= Wolayita, **Wlg**= Wolega, **Da**=Dauro , **Jm**= Jima, **Ga**= Gamogofa

4.3.1.5. Iron Content (Mg/100g)

The range of iron content revealed from all samples was (17.21 mg/100g to 90.85 mg/100g) (Fig. 15 and Table 16). The highest iron content recorded from species *D. bulbifera* from Jima region (90.85 mg/100g) and the lowest was from *D. cayenensis* from Gamogofa (17.21mg/100g) (Fig. 15).

The highest range was observed among species *D. bulbifera* (20.96 mg/100g -90.85 mg/100g) followed by *D. rotundata* (17.75 mg/100g -69.73 mg/100g) and *D. preahensilis* for (18.36 mg/100g -76.36 mg/100g).

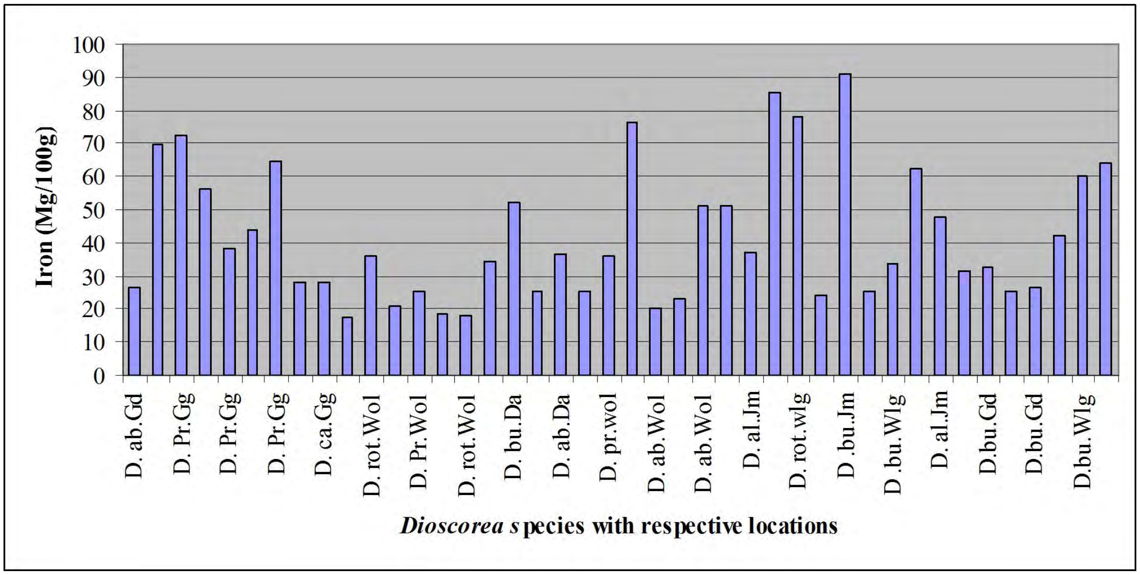


Figure 15. Iron (mg/100g) content of yam (*Dioscorea* species) from Ethiopia

Notes:- D.ab = *D. abyssinica,* **D.bu**= *D. bulbifera* , **D.ca**=*D. cayenensis*, **D.pr** = *D.preahensilis,* **D.rot**= *D. rotundata*, **D.al**=*D. alata***Wol**= Wolayita, **Wlg**= Wolega, **Da**=Dauro , **Jm**= Jima, **Ga**= Gamogofa

4.3.1.6. Ash (%) Content

The highest value (4.41%) and lowest value (1.68 %) of ash content was recorded from *D. bulbifera* species from Wolega and Gedio regions respectively (Fig. 16 and Table 16). Average ash content was higher for *D. cayenensis* (3.34%) followed by *D.alata* (3.16%) and *D. bulbifera* (3.16%). The lowest mean value was obtained from *D. rotundata* (2.5%) (Fig.16).

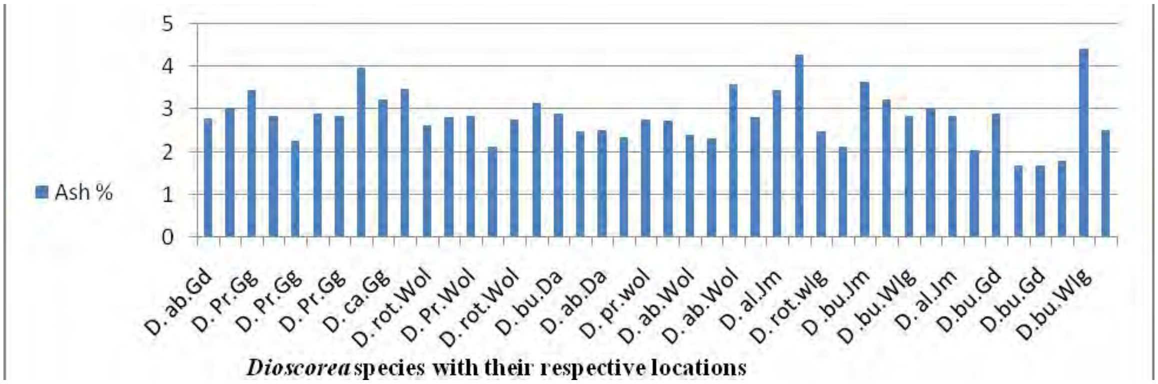


Figure 16. Ash (%) content of yam (*Dioscorea* species) from Ethiopia

Notes:- **D.ab** = *D. abyssinica,* **D.bu**= *D. bulbifera* , **D.ca**=*D. cayenensis*, **D.pr** = *D.preahensilis,* **D.rot**= *D. rotundata*, **D.al**=*D. alata*Wol= Wolayita, **Wlg**= Wolega, **Da**=Dauro , **Jm**= Jima, **Ga**= Gamogofa

4.3.1.7. Zink content (g/100mg)

The species *D. bulbifera* of Wolayita origin recorded greater zink content (8.33 g/100mg) in comparison to *D. rotundata* species from the same locality (.35g/100mg) (Fig. 17). The range for zink content was also greater among *D. bulbifera* species (.4 g/100mg-8.33 g/100mg) compared to *D.alata* (.38 g/100mg-1.18 g/100mg), (.35 g/100mg-1.02 g/100mg) *D. rotundata*, (.4 g/100mg-1.09 g/100mg) *D. preahensilis* and (.49 g/100mg - .77 g/100mg) *D. abyssinica* (Fig. 17 and Table 16).

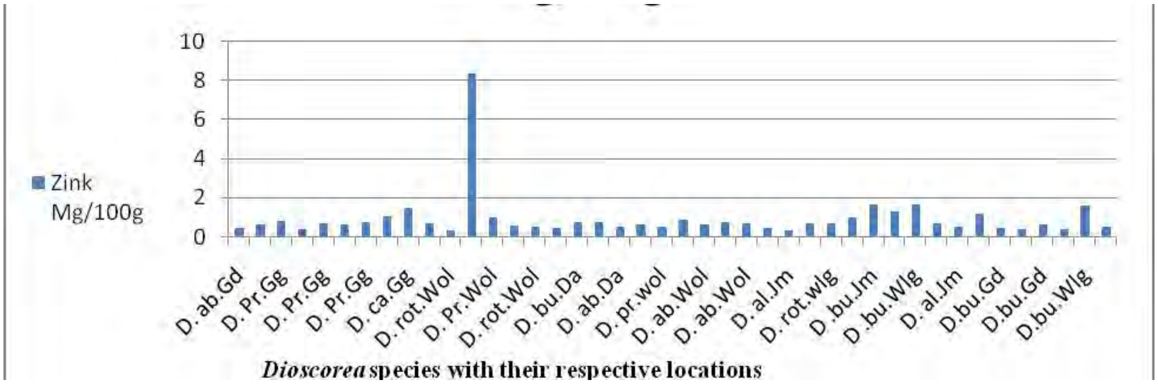


Figure 17. Zink (mg/100g) content of yam (*Dioscorea* species) from Ethiopia
Notes:- **D.ab** = *D. abyssinica,* **D.bu**= *D. bulbifera* , **D.ca**=*D. cayenensis*, **D.pr** = *D.preahensilis,* **D.rot**= *D. rotundata,* **D.al**=*D. alata,* **Wol**= Wolayita, **Wlg**= Wolega, **Da**=Dauro , **Jm**= Jima, **Ga**= Gamogofa

4.3.1.8. Calcium content (g/100mg)

The range for calcium content varied between (31.02 mg/100g -118.81 mg/100g) , (13.12 mg/100g -105.08 mg/100g), (6.3 mg/100g-27.46 mg/100g) (22.77 mg/100g-114.37), (15.74 mg/100g-121.26 mg/100g), (112.36 mg/100g -130.94 mg/100g) for species *D. abyssinica*, *D. preahensilis*, *D. cayenensis*, *D. rotundata* and *D. alata* respectively (Fig. 18 and Table 16). The highest calcium content was obtained from *D. bulbifera* from Jima region (121.26 mg/100g) whereas the lowest was from *D.cayenensis* of Gamogofa region (6.3 mg/100g) (Fig. 18).

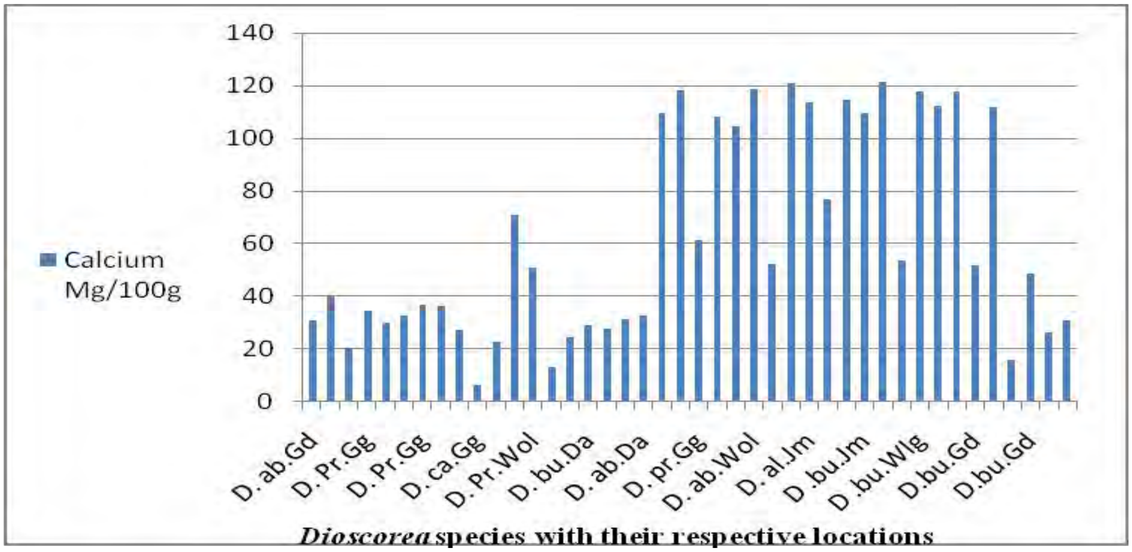


Figure 18. Calcium (mg/100g) content of yam (*Dioscorea* species) from Ethiopia
Notes:- D.ab = *D. abyssinica,* **D.bu**= *D. bulbifera* , **D.ca**=*D. cayenensis*, **D.pr** = *D.preahensilis,* **D.rot**= *D. rotundata,* **D.al**=*D. alata*Wol= Wolayita, **Wlg**= Wolega, **Da**=Dauro , **Jm**= Jima, **Ga**= Gamogofa

4.3.1.9. Phosphorous content (g/100mg)

The highest phosporous content was recorded from *D. preahensilis* of Gamogofa region (20.92 mg/100g) in contrast to the lowest value recorded from *D. bulbifera* (8.72 mg/100g) of Gedio orign (Fig. 19 and Table 16). Average phosphorous content was high for *D. abyssinica* (32.34 mg/100g) followed by *D. rotundata* (30.78 mg/100g) and *D. preahensilis* (29.48 mg/100g) .

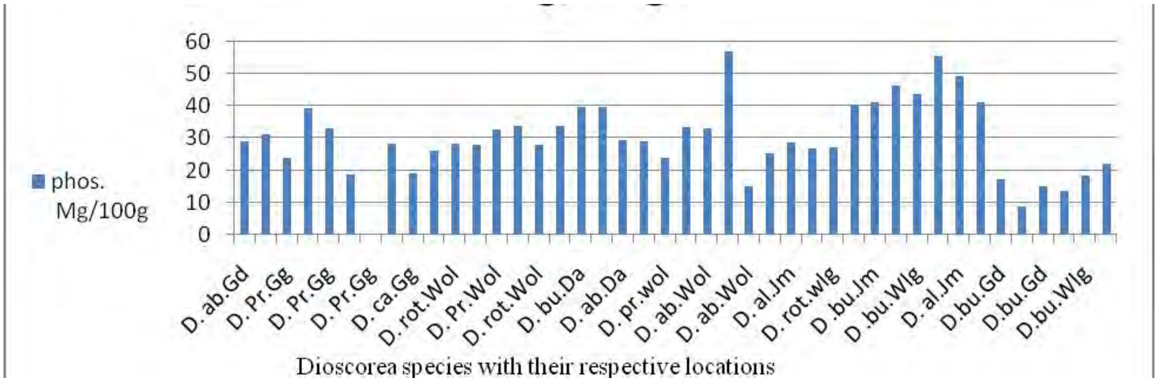


Figure 19. Phosphorous (mg/100g) content of yam (*Dioscorea* species) from Ethiopia
Notes:- D.ab = *D. abyssinica,* **D.bu**= *D. bulbifera* , **D.ca**=*D. cayenensis*, **D.pr** = *D.preahensilis,* **D.rot**= *D. rotundata,* **D.al**=*D. alata*Wol= Wolayita, **Wlg**= Wolega, **Da**=Dauro , **Jm**= Jima, **Ga**= Gamogofa

Yam species showed variation with respect to proximate composition and mineral content. Generaly moisture content was highest and they had high nutritional content with respect to protein and fiber and relatively low fat content (Fig. 20 and Table 16). Similarly high calcium and iron content was recorded but comparatively the phosphorous and zink content was low (Fig. 21).

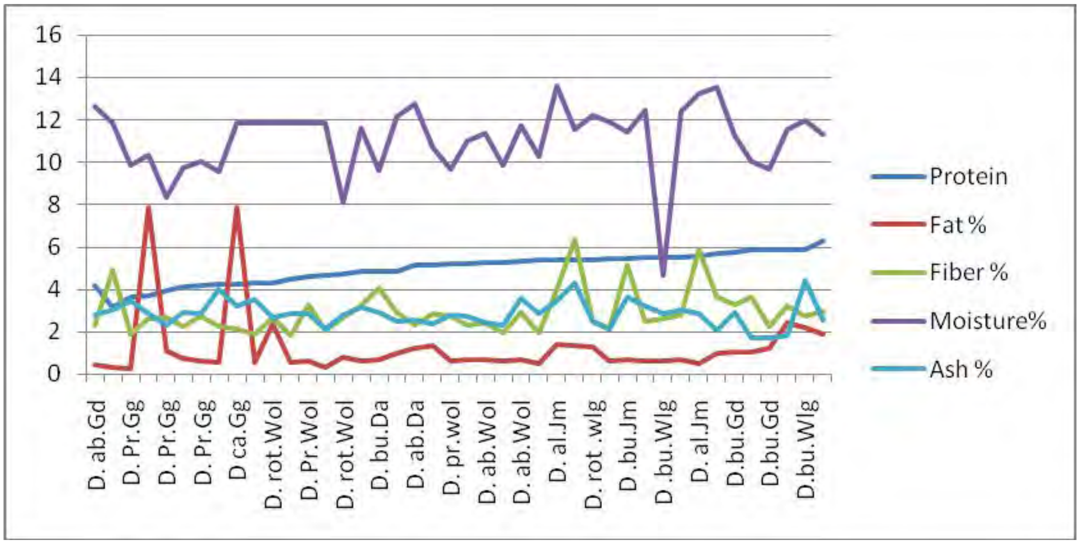


Figure 20. Comparison in Proximate composition of 100 g. edible portion of yam (*Dioscorea* species) from Ethiopia

Notes:- **D.ab** = *D. abyssinica,* **D.bu**= *D. bulbifera* , **D.ca**=*D. cayenensis*, **D.pr** = *D.preahensilis,* **D.rot**= *D. rotundata,* **D.al**=*D. alata***Wol**= Wolayita, **Wlg**= Wolega, **Da**=Dauro , **Jm**= Jima, **Ga**= Gamogofa

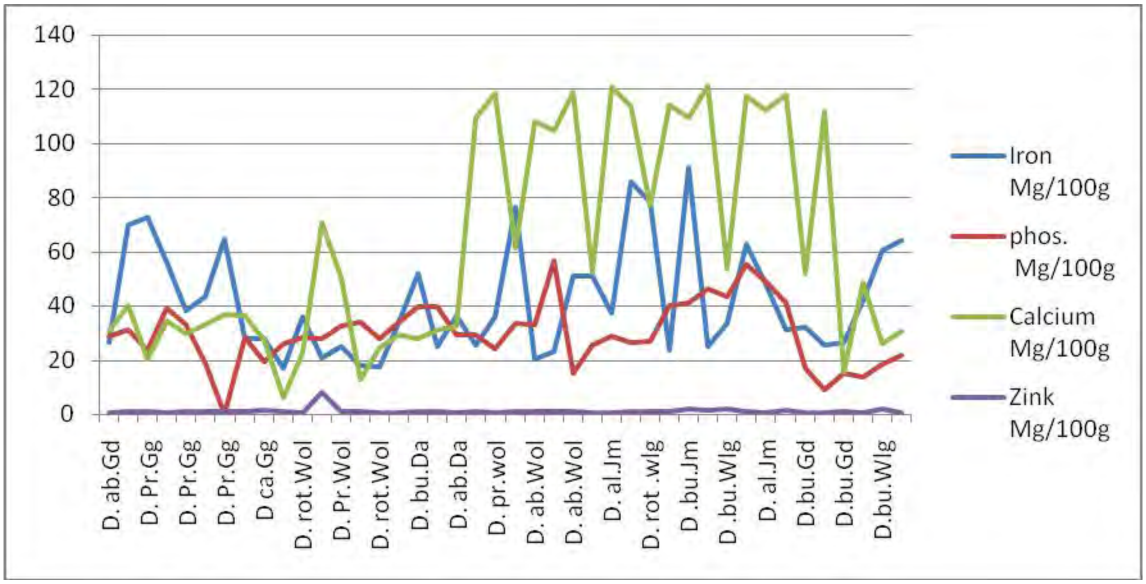


Figure 21. Comparison of minerals contents in 100 g. edible portion of yam (*Dioscorea* species) in Ethiopia

Notes:- D.ab = *D. abyssinica,* **D.bu**= *D. bulbifera* , **D.ca**=*D. cayenensis*, **D.pr** = *D.preahensilis,* **D.rot**= *D. rotundata*, **D.al**=*D. alata***Wol**= Wolayita, **Wlg**= Wolega, **Da**=Dauro , **Jm**= Jima, **Ga**= Gamogofa

4.3.2. Correlation coefficients among the yield, nutritional values and some quantitative traits in yam *(Dioscorea species)* of Ethiopia

The correlation coefficients (r) among the traits are given in (Table 15). Significant and positive correlation was found between fiber content and calcium content (r=0.373*), iron content and ash content (r=0.413**), iron content and fiber content (0.453**), phosphorous content and average stem height (r=0.368*), average tuber yield per plot and calcium content (r=0.586 **), fiber content (r=0.491**) and moisture content (r=0.431 **). Nevertheless there were no significant relationships between zink content, protein content, fat content, and number of leaves at 30 days after emergence as well as for to the rest of the traits studied

Table 15: Correlation coefficients among yield, nutritional values and some quantitative traits in yam (*Dioscorea* species)

	1	2	3	4	5	6	7	8	9	10	11
1. Av Stem height											
2. Ash (%)	0.01										
3. Calcium content	0.18	-0.003									
4.Fiber content	0.034	0.256	0.373*								
5.Iron content	0.059	0.413**	0.085	0.453**							
6. Moisture (%)	0.065	0.094	0.26	0.227	0.057						
7.phosphorus (%	0.368*	0.153	-0.061	-0.111	0.228	-0.092					
8.Zink content	-0.101	0.112	0.062	-0.164	-0.118	0.033	-0.006				
9. Fat (%)	-0.286	0.043	-0.197	-0.082	0.035	0.057	-0.143	-0.043			
10. Protein (%)	-0.002	0.209	-0.057	-0.06	0.139	-0.155	0.31	0.254	0.004		
11. Av tuber yield	0.105	-0.04	0.586**	0.491**	0.169	0.431**	0.121	-0.039	0.214	0.020	
12.LN at3o days	-0.284	0.256	0.292	0.17	0.274	0.039	0.044	-0.006	-0.034	-0.156	0.175

Note:- * Significant at the 0.05 level ** significant at the 0.01 level Av=Average LN=Leaf number at 30 days of emergence

Table 16: Range of proximate composition and minerals content of six yam (*Dioscorea* species) from Ethiopia

	yam (*Dioscorea*) Species					
Nutrient (%)	***D.alata***	***D.rotundata***	***D.bulbifera***	***D.cayenensis***	***D.abyssinica***	***D.praeihe-nsilis***
Proteins	5.39-5.70	4.29-5.43	4.47-6.29	4.24-4.27	3.13-5.37	3.64-5.22
Fat	0.5-1.42	0.63-2.37	0.59-2.46	0.59-7.86	0.31-1.22	0.26-7.83
Fiber	3.65-6.36	2.12-2.19	1.82-5.16	1.87-2.133	1.94-4.91	1.91-3.21
Moisture	9.83-12.71	8.08-12.7	4.67-12.4	11.85	9.83-12.71	8.37-11.85
Ash	2.05-4.29	2.13-2.76	0.68-4.41	3.22-3.47	2.31-3.58	2.13-3.97
	yam (*Dioscorea*) Species					
Minerals (mg/100g)	***D.alata***	***D.rotundata***	***D.bulbifera***	***D.cayene-nsis***	***D.abyssinica***	***D.praeihe-nsilis***
Iron	17.75-51.1	17.75-78.29	20.26-90.85	17.21-27.95	20.34-69.73	18.31-76.36
Zink	0.38-1.18	0.35-1.02	0.4-8.33	0.74-0.75	0.48-0.77	0.4-1.09
Calcium	112.36-120.94	22.77-114.37	15.74-121.26	0.3-27.6	31.02-118.81	13.12-118.2
Phosphorous	26.59-49.12	26.96-40.21	8.72-55.26	19.15-26.12	15.11-56.53	20.92-39.03

4.4 Proximate composition and mineral content diversity in Ethiopian yam determined by cluster and principal content analysis

The relationship among forty two yams (*Dioscorea* spp) studied with respect to proximate compositions and mineral contents were observed using cluster and principal component analysis (PCA). Based on the relative magnitude of Euclidian distance matrix and dendrogram using Shan through an un-weighted pair-group method average (UPGMA) cluster analysis the forty two yams (*Dioscorea* species) were grouped into five clusters (fig. 22).

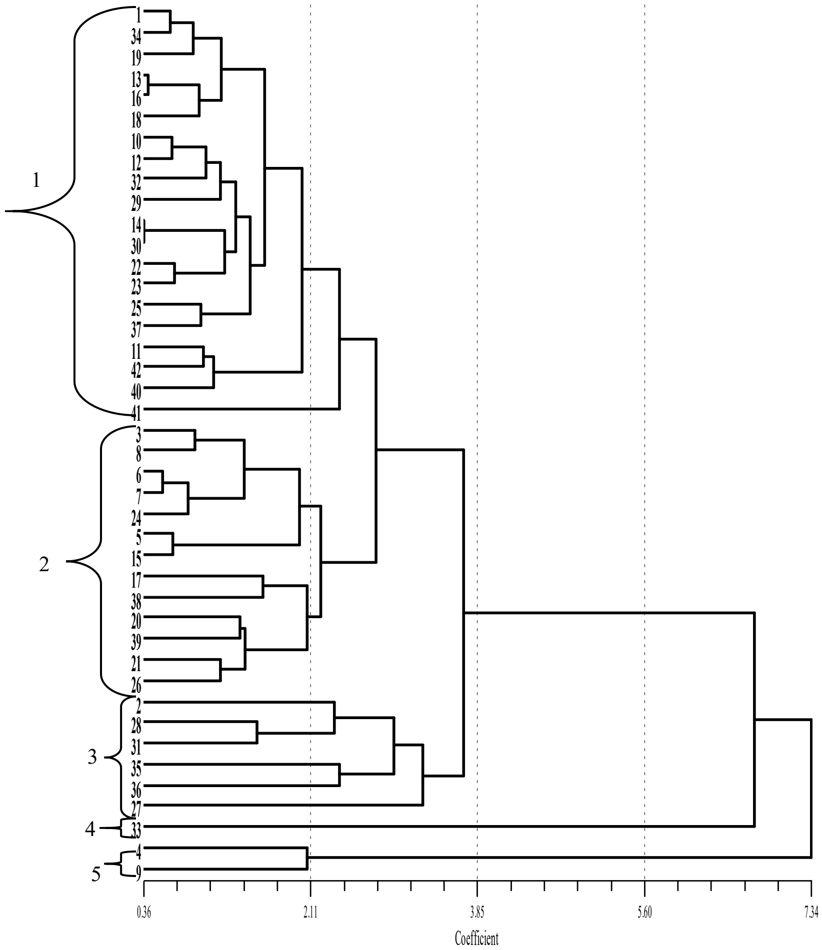


Figure 22. Complete linkage hierarchical (Euclidean distance) dendrogram based on nine proximate and mineral content for 42 yam (*Dioscorea* species) germplasms from Southern Eastern and Southern parts of Ethiopia

Table 17: Clustering pattern of 42 yam (*Dioscorea* species) germplasms derived from the Euclidian similarity matrix based on their nutritional content

Clusters	Serial of Accessions	Vernacular name	Yam Species	Origin
C1	1	-	*D. abyssinica*	Gedio
	34	Dhoknuma	*D .bulbifera*	Wolega
	19	Gebiche-2	*D. abyssinica*	Dauro
	13	Arkiya	*D. praeihensilis*	Wolayita
	16	Dorsita	*D. bulbifera*	Dauro
	18	Gebiche-1	*D. abyssinica*	Dauro
	10	Bune-3	*D. cayenensis*	Gamogofa
	12	Oha	*D. rotundata*	Wolayita
	32	-	*D .bulbifera*	Jima
	29	Lalo-1	*D. rotundata*	Wolega
	14	Arkiya	*D. Praeihensilis*	Wolayita
	30	Lalo-2	*D. rotundata*	Wolrga
	22	Bonke	*D. praeihensilis*	Gamogofa
	23	Wiyacha	*D. abyssinica*	Wolayita
	25	Gudina-1	*D. abyssinica*	Wolayita
	37	Genticho-1	*D. alata*	Jima
	11	Oha	*D. rotundata*	Wolayita
	42	Roba	*D.bulbifera*	Wolega
	40	Nifo	*D.bulbiferaa*	Gedio
	41	Roba-1	*D.bulbiferaa*	Wolega
C2	3	Tola	*D. abyssinica*	Gamogofa
	8	Bune-1	*D. abyssinica*	Gamogofa
	6	Bune-2	*D. abyssinica*	Gamogofa
	7	Bune-3	*D. abyssinica*	Gamogofa
	24	Wiyacha-2	*D. abyssinica*	Wolayita
	5	Bune-1	*D. abyssinica*	Gamogofa
	15	Gassa	*D. rotundata*	Wolayita
	17	Dorsita-2	*D. bulbifera*	Dauro
	38	Ganticho-2	*D.bulbifera*	Gedio
	39	Nifo-1	*D.bulbiferaa*	Gedio
	21	Gafela-2	*D. praeihensilis*	Wolayita
	26	Gudina-2	*D. abyssinica*	Wolayita
	20	Gafela-1	*D. praeihensilis*	Wolayita
C3	2	Boyebene	*D. abyssinica*	Kembata
	28,35,36,27	-	*D. alata*	Jima
	31	-	*D .bulbifera*	Jima
C4	33	Dhoknuma-1	*D .bulbifera*	Wolega
C5	4	Tola-2	*D. abyssinica*	Gamogofa
	9	Bune-5	*D. cayenensis*	Gamogofa

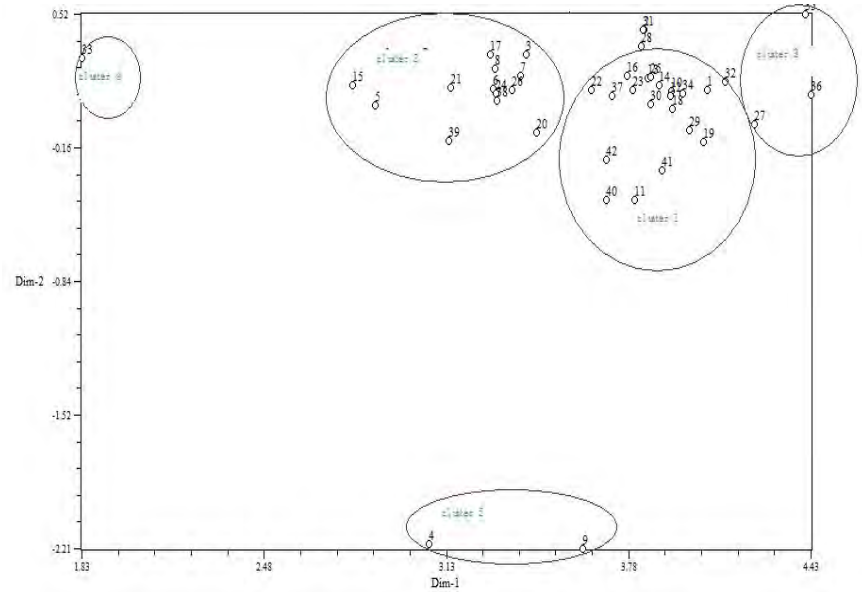


Figure 23. Plot of the first two principal components based on nine proximate mineral composition of 42 yam (*Dioscorea* species) germplasms from South eastern and southern parts of Ethiopia

Differences were observed in cluster means for almost all nutritional characters in the 42 genotypes studied (Table 18). Cluster one and cluster two consisted of maximum number of yam land races which were 20 and 13 respectively whereas cluster four was made of only one accession from East Wolega zone with highest mean protein content (5.52 mg/100g). Cluster 1 incorporated yam land races from Gamogofa, Gedio, Wolayita, Dauro and East Wolega and Jima (Table 17).

Cluster 1 exhibited the highest mean calcium content (58.24 mg/100g) followed by cluster 2 (55.47 mg/100g) and cluster 4 (53.6 mg/100g). It also showed medium values of zink (1.12 mg/100g) and phosphorous content (30.01mg/100g). Cluster one also had the second highest mean values of protein and zink content (5.14 mg/100g) and (1.12 mg/100g) respectively (Table 17).

The results of the dendrogram and scatter diagram revealed that cluster five was far apart from other groups. Cluster 5 exhibited highest means for fat (7.85%) and iron content (41.94 mg/100g) which the second highest after cluster 3. Hence, the two accessions of cluster V from Gamogofa origin have the potential to be used as parents for improvement of yam nutritive value through breeding. In addition, collections from Wolayita, Dauro Wolega and Jima regions were distributed in two different clusters and were shown to have had comparatively more diversity. Thus, using yam germplasms from these regions it could be possible to enhance nutritional content through selection.

Table 18: Cluster means and Grand means ± standard deviation for nine different Nutritional characters in 42 yam germplasms of Ethiopia

Nutrients (%)	**C1**	**C2**	**C3**	**C4**	**C5**	**Grand Mean±St dv**
Protein	5.14	4.79	5.11	5.52	3.97	4.91±0.73
Fat	1.03	0.78	0.88	0.61	7.85	2.23±1.58
Fiber	2.61	2.61	4.99	2.62	2.37	3.04±1.04
Moisture	11.85	9.65	12.51	4.67	11.09	9.95±1.63
Ash	2.80	2.67	3.22	2.84	3.04	2.91±0.62
Minerals (mg/100g)	**C1**	**C2**	**C3**	**C4**	**C5**	**Grand Mean ±St dv**
Iron	38.82	38.80	60.46	33.56	41.94	42.72±20.25
Zink	1.12	0.68	0.86	1.65	0.95	1.05±1.21
Calcium	58.24	55.47	102.43	53.67	30.94	60.15±39.64
Phosphorous	30.01	27.44	36.19	43.36	29.09	33.22±33.63

Cluster four had one accession from East Wolega and was superior for mean values of proteins (5.52 mg/100g) and Zink contents (1.65 mg/100g). Cluster 3 consists of five yam land races from Jima zone except for one accession from Kembata origin (Table 17).

The present results indicated that cluster 3 exhibited highest means for majority of observed nutritional content parametrs. It recorded highest mean value of fiber (4.99%), moisture (12.51%), ash (3.22%), phosphorous (36.19 mg/100g) and iron (60.46 mg/100g) content (Table 18).

4.4.1. The divergence of yam genotypes based on nutritional value

The Principal component analysis (PCA) revealed from that the first two principal components accounted for 76.49 % of the total variation (Table 19) out of this 45.32% was contributed by the first principal component. The PCA observed that in Vector 1 the important characters accounting for divergence in the major axis of differentiation were zink and phosphorous content whereas, in vector II, which was the second axis of differentiation were all characters except protein and fat content.

The phosphorous and zink contents were positive for both vectors across the two axes. This indicated those were the important components of divergence in these germplasms. These characters contributed to the total divergence in 42 yam genotypes. Plotting of the first and second coordinates clearly separated five different clusters which was also highly consistent with the results of the cluster analysis (Fig. 17).

Table 19*:* Eigen values, percent of variation, cumulative percentage and latent vectors for nine nutritional characters in 42 Ethiopian yam species

Latent roots/ Eigen values	0.16	0.12
Percentage of Variance	45.32	31.17
Cumulative percentage	45.32	76.49
	Vector 1	**Vector 2**
Protein	-0.07358	-0.03809
Fat	-0.37395	-0.92118
Fibre	-0.17930	0.18466
Moisture	-0.90550	0.34017
Iron	-0.00141	0.00054
Ash	-0.05139	0.00982
Zink	0.00001	0.00002
Calcium	-0.00480	0.00807
Phosphorous	0.00304	0.00175

4.5. Genetic characterization of six Ethiopian yam (*Dioscorea* spp) with Simple Sequence Repeat

4.5.1 Genetic variation in the Ethiopian yam (*Dioscorea* species)

Average polymorphism was 92% in six yams (*Diosocrea* spp). This indicates presence of high genetic diversity in the Ethiopian yam (*Diosorea* spp). Allelic diversity at SSR loci was high. The allelic frequency of polymorphic locus in six yams (*Dioscorea* species) presented in Table 22. The Analysis of Molecular Variance indicated a highly significant difference both among and within each of the six yam *Dioscorea* species (*P* >= 0.001; Table 20).

Table 20: Analysis of molecular variance (AMOVA) by grouping 210 yam accessions at to six *Dioscorea* species using Simple Sequence Repeat

Source	Df	SS	MS	Est. Var.	%	Value	P
Among *D. rotundata*	3	35.809	11.936	0.643	12%	0.125	0.001
Within *D. rotundata*	52	234.012	4.500	4.500	88%		
Total	55	269.821		5.109	100%	0.497	0.001
Among *D. alata*	2	21.007	10.503	0.480	8%	0.082	0.013
Within *D. alata*	33	177.688	5.384	5.384	92%		
Total	35	198.694		5.864	100%		
Among *D. abyssinica*	4	45.350	11.338	0.571	10%	0.102	0.001
Within *D. abyssinica*	61	307.316	5.038	5.038	90%		
Total	65	352.667		5.609	100%		
Among *D. cayenensis*	3	22.333	7.444	0.250	5%	0.053	0.002
Within *D. cayenensis*	44	197.333	4.485	4.485	95%		
Total	47	219.667		4.735	100%		

Among *D. praehensilis*	4	49.008	12.252	0.317	6%	0.055	0.001
Within *D. praehensilis*	113	613.915	5.433	5.433	94%		
Total	117	662.924		5.750	100%		
Among *D. bulbifera*	6	48.719	8.120	0.360	7%	0.070	0.001
Within *D. bulbifera*	61	289.546	4.747	4.747	93%		
Total	67	338.265		5.107	100%		

Notes:- * df= degrees of freedom; SS =sum of square and MS = mean of squares

4.5.2. Jaccard coefficient of similarity among Ethiopian yam (*Dioscorea* spp)

Jaccard coefficient of similarity was used to estimate the gene relationship within and among yam (*Dioscorea* species). The pairwise population comparisons revealed highest value of genetic similarity were observed between accessions from *D. rotundata* and *D. abyssinica* (0.98); *D. rotundata* and *D. praehensilis* (0.97); *abyssinica* and *D. praehensilis* (0.96) wherea*s* generally *D. alata* revealed the lowest value of genetic similarity to all studied *Dioscorea* species (Table 21).

Table 21: Jacquard coefficient of similarity in six populations of Ethiopian yam (*Dioscore*a species) collected from ten different locations of the Country

	D. rotundata	*D.alata*	*D.abyssinica*	*D. cayenensis*	*D. praehensilis*
D. rotundata					
D.alata	0.82				
D.abyssinica	0.98	0.83			
D. cayenensis	0.94	0.78	0.92		
D. praehensilis	0.97	0.79	0.96	0.91	
D.bulbifera	0.92	0.89	0.91	0.89	0.89

4.5.3. The genetic structure and allelic variation at microsatellite loci

Sixty eight different alleles were recorded for the 12 loci studied. The mean number of alleles per locus was 5.7. The mean number of effective number of alleles for *D. rotundata, D.alata, D.abyssinica, D. cayenensis, D. praehensilis* and *D.bulbifera* was 1.46, 1.65, 1.55, 1.49, 1.49 and 1.53 respectively.The range of allelic frequency in the studied *Dioscorea* species was between 0.07 (at locus Da1fo8) and 0.47 (at two loci D55 and Da1Do8) (Table 22).

Across all the six *Dioscorea* species studied, the range of effective number of alleles per locus was from 1.46 (*D. rotundata*) to 1.55 (*D.abyssinica*) Table (23). Among the studied *Dioscorea* species, each population *D. rotundata, D. cayenensis* and *D.bulbifera* at D.83, *D.alata* and *D.bulbifera* at Dpr3Fo4 and *D.bulbifera* at Da1ao1 has one monomorphic locus.

Table 22: Total number of observed alleles (Na) per locus, mean effective number of alleles in brackets and mean allele frequencies per *Dioscorea* species under each population of the 12 loci

		Dioscorea species (mean effective number of alleles) mean allele frequencies					
Locus	Na	*D. rotundata* (1.46)	*D.alata* (1.65)	*D.abyssinica* (1.55)	*D. cayenensis* (1.49)	*D. praehensilis* (1.49)	*D.bulbifera* (1.53)
Dalc12	5	0.22	0.26	0.19	0.32	0.39	0.24
Ym30	4	0.18	0.28	0.28	0.21	0.13	0.28
Da1Do8	4	0.26	0.47	0.36	0.24	0.23	0.30
Dpr3fo4	10	0.23	0.31	0.24	0.15	0.15	0.19
D55	2	0.27	0.47	0.29	0.42	0.32	0.36
D83	8	0.14	0.40	0.29	0.21	0.22	0.23
Da1ao1	6	0.15	0.14	0.22	0.18	0.25	0.19
dab2c12	4	0.16	0.25	0.26	0.14	0.13	0.16
Da1fo8	6	0.13	0.17	0.23	0.10	0.07	0.16
YM13	5	0.27	0.44	0.15	0.35	0.34	0.32
Dpr3D06	6	0.22	0.28	0.22	0.25	0.26	0.29
Dpr3B12	8	0.22	0.14	0.16	0.15	0.16	0.17

4.5.4. Genetic diversity within Ethiopian yam (*Dioscorea* spp)

Shanon Weaver diversity index, Polymorphism percentage, Neis' Gene diversity and Jaccard's similarity coefficient were used to estimate genetic diversity within each Ethiopian yam (*Dioscorea* species) (Table 24). The mean percentage of polymorphism in yam (*Dioscorea* species) was 92 %. The highest percent polymorphism among species was recorded for *D. abyssinica* (97.06), *D. praehensilis* and *D. rotundata* (95.59) with a mean value of 91.67. The range of mean Jaccard's similarity coefficient for comparisons among accessions within six *Dioscorea* species was from *D. abyssinica* (0.94) to *D.alata* (0.82). The gene diversity recorded for *D.alata, D.abyssinica* and *D. praehensilis* were 0.377, 0.333 and 0.327 respectively. Similarly the highest Shanon information index was observed from *D. abyssinica* (0.467) followed by *D. praehensilis* (0.457) (Table 23).

Table 23: Summary of gene diversity parameters within six Ethiopian yam (*Dioscorea* species*)*

***Dioscorea* species**	**No of entries**	**P%**	**Jaccard's Coefficient**	**Nei's gene Diversity (h)**	**Shannon's Information Index (*I*)**
D. rotundata	37	95.59	0.93	0.292	0.448
D.alata	17	80.88	0.82	0.377	0.450
D.abyssinica	36	97.06	0.94	0.333	0.467
D.cayenensis	31	89.71	0.90	0.304	0.421
D.bulbifera	33	91.18	0.91	0.294	0.437
D. praehensilis	56	95.59	0.90	0.327	0.457
Mean		92	0.90	0.318	0.487
Total Number Polymorphic Loci	209				
Total % of Polymorphic Loci	99.5%				

4.5.5. Cluster analysis

When we treated the entire species sample together without considering species differentiation in each accession the genetic distance based cluster analysis grouped six *Dioscorea* species of 210 yam accessions into eight different cluster groups (Figure 24). These cluster groups were supported by partitioning of genetic variation by AMOVA (Table 20) which revealed significant genetic variations in both among and within each *Dioscorea* species. The cluster groups five and six were composed each of only one species *D. abyssinica* (13 accessions) and *D. cayenensi* (33 accessions). Similarly, cluster three and cluster seven were each of only one species *D.bulbifera* with fifteen and nine number of accessions respectively. Cluster one is the largest group comprising 106 yam genotypes that were sub clustered in to 3 sub groups. The sub group 1a consisted of a total of 42 accessions of two different species namely *D. praehensilis* (23) and *D. abyssinica* (15). The other two sub clusters (1b and 1c) are composed of 64 yam land races and all six species were overlapped in this group.

Cluster two consisted of 24 yam landraces obtained from accessions of *D. rotundata*, (20) *D. abyssinica* (2) and *D. praehensilis*. (2). Cluster four and cluster eight were comprised of two species namely *D. praehensilis* and *D. rotundata*.

The relationships among the pooled *Dioscorea* species was further worked out by the results of principal coordinate analysis. The principal component scores obtained in the analysis were used to plot the *Dioscorea* species using PCO1 and PCO2 which cumulatively explained 89.85% of the variability. The first principal coordinate axis

(PCO1) and the second axis accounted for 74.74% and 15.11% respectively. These two axes revealed that four *Dioscorea* species namely *D. abyssinica*, *D. cayenensis, D. praehensilis and D. rotundata* were more closer to each than to *D.alata* (Fig. 25).

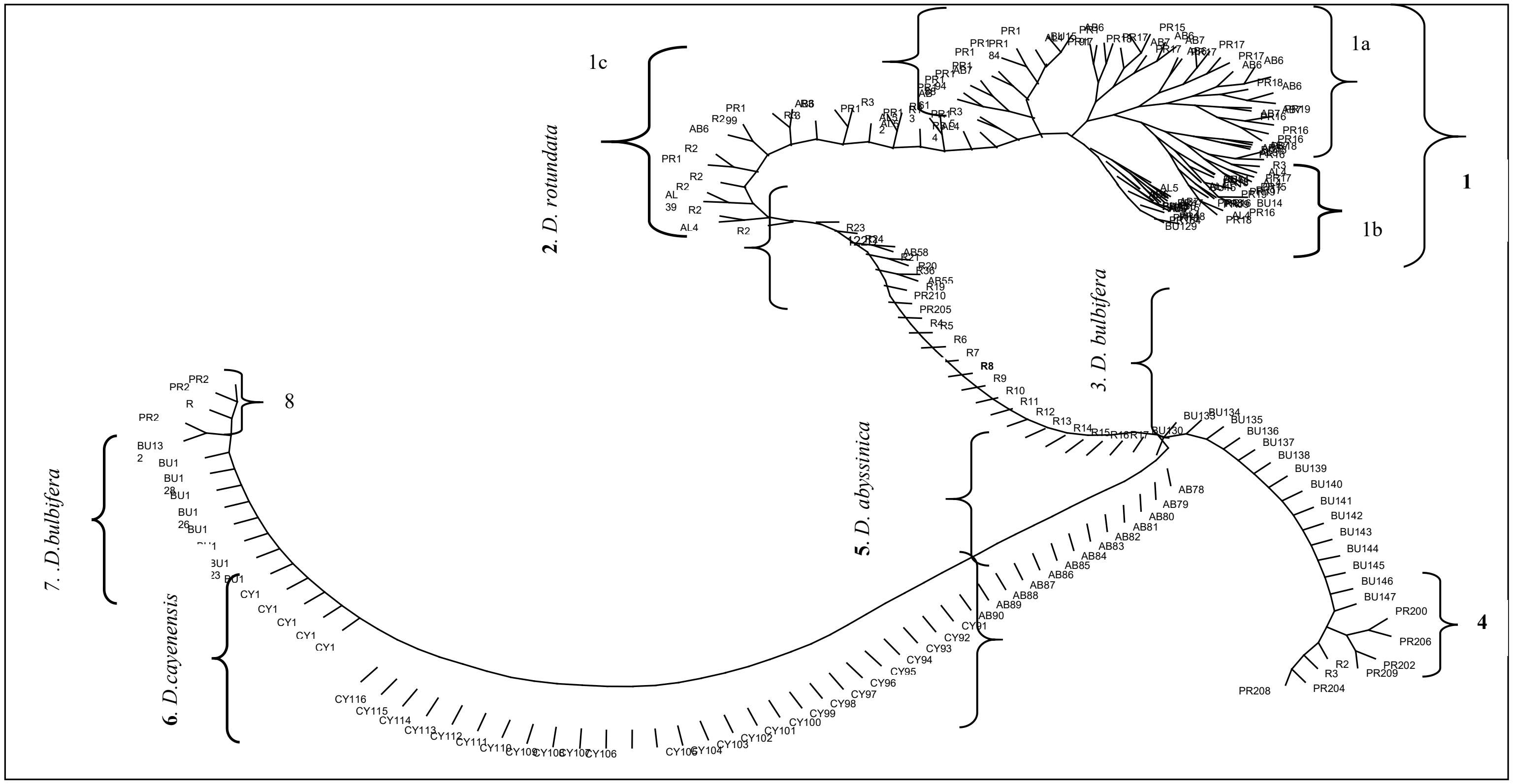


Figure 24: Distance matrix derived un-rooted Phylogram of 210 accessions of six *Dioscorea* species from major growing areas of Ethiopia using Simple Sequence Repeat

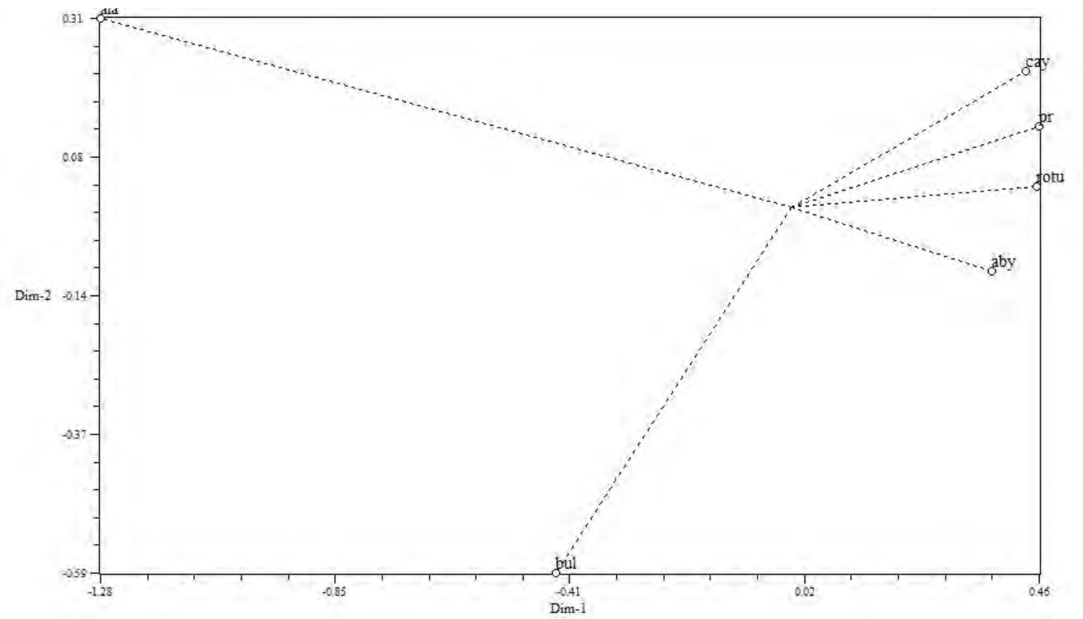


Figure 25: Scatter plot showing the first and second axis of principal coordinate analysis for 210 individuals of six *Dioscorea* species from major growing areas of Ethiopia using Simple Sequence Repeat

4.6. Genetic diversity analysis of Ethiopian yam from different geographic origin using Simple Sequence Repeat

The present study tried to cover more geographic regions of major yam growing areas of the country than any previous studies (Appendix 1). It evaluates the genetic diversity and relationships of 210 yam accessions across ten geographic area of Ethiopia using Simple Sequence Repeat Poly Acrylamaide Gel Electrophoresis based analysis. The findings from this study will have an important contribution to germplasm conservation activities on Ethiopian yam germplasms.

4.6.1. Yam (*Dioscorea* species) diversity among and within geographical locations and allelic variation at microsatellite loci

Analysis of Molecular Variance of all samples of the species of *Dioscorea* pooled revealed a significant difference among and within yam accessions based on their collection sites ($P >=0.001$; Table 24 and 26). The cluster analysis revealed geographic pattern of diversity within yam accessions except for cluster group 2 which did not show clear separation of yam accessions based on their collection site. This cluster grouping is supported by genetic variations revealed by AMOVA which had significance difference of yam accessions in both among and within each location sites except for Dauro zone which did not reveal any genetic variation among yam accessions from this zone.

This significant genetic variation of yam accessions within each collection site indicated availability of more genetic variation in yam accessions within each geographical location. Therefore, large amount of yam genetic variation can be obtained by considering only a small number of geographical sites.

Genetic diversity was estimated within location using Jaccard‘s similarity coefficient, Neis' Gene diversity, Polymorphism percentage and Shanon Weaver diversity index (Table 25). The total percentage of polymorphic loci ranged from 98.53 % (Wolayita) to 94.14 % (Gamogofa). Gene diversity was highest (0.38) within accessions collected from Gedio followed by (0.35) within accessions from Jima and Gamogofa. Accessions from Wolega and Kembata with (0.29) revealed lower Gene diversity. Shannon's information index (I) also showed the existence of high genetic variation within the accessions from

Gedio, Wolayita and Gamogofa. The mean of Jacard's similarity coefficient for comparisons among accessions within locations was 0.18 (Table 25).The mean allelic frequency ranged from .001 to 1.00 at locus Dpr3fo4 (Kembata) and at locus Da1DO8 (Kembata) respectively. For all the populations studied the smallest effective number of alleles per locus was 1.45 (Sidama) and the largest was 1.64 (Gedio) (Table 27).

Table 24: Analysis of Molecular Variance (AMOVA) showing the genetic variation of 210 yam accessions within and among ten collection sites

Source	**Df**	**SS**	**MS**	**Est. Var.**	**%**	**Value**	**P**
Among CS	9	79.033	8.781	0.087	2%	0.016	0.001
Within CS	410	2185.925	5.332	5.332	98%		
Total	419	2264.957		5.419	100%		

Notes:- * df= degrees of freedom; SS =um of square, MS = mean of squares and CS= collection site

Table 25: Summary of gene diversity parameters with in different geographical locations of 210 yam (*Dioscorea* species) landraces from Ethiopia

Zone	No of entries	P%	Jaccard's Coefficient	Nei's gene Diversity (h)	Shannon's Information Index (*I*)
Sidama	31	89.71	0.14	0.28	0.427
Wolayita	53	98.53	0.20	0.30	0.458
Wolega	12	82.35	0.17	0.29	0.399
ARC	34	92.65	0.19	0.32	0.459
Gamogofa	36	94.12	0.24	0.35	0.481
Gedio	12	82.35	0.20	0.38	0.457
Kefa	6	75.00	0.09	0.34	0.417
Kembata	6	47.06	0.21	0.29	0.295
Jima	11	80.88	0.17	0.35	0.444
Dauro	9	79.41	0.16	0.31	0.414
Mean		82.21	0.18	0.32	0.414
Total Number Polymorphic Loci	208				
Total % of Polymorphic Loci	99.05				

Table 26: Analysis of molecular variance (AMOVA) by grouping yam (*Dioscorea* spp) in to ten geographical collection sites

Source	Df	SS	MS	Est. Var.	%	Value	P
Among Sidama	3	33.243	11.081	0.423	8%	0.082	0.001
Within Sidama	58	272.628	4.700	4.700	92%		
Total	61	305.871		5.123	100%		
Among Wolayita	5	63.282	12.656	0.464	9%	0.087	0.001
Within Wolyita	102	494.274	4.846	4.846	91%		
Total	107	557.556		5.310	100%		
Among East Wolega	2	17.750	8.875	0.596	12%	0.117	0.007
Within East Wolega	21	94.583	4.504	4.504	88%		
Total	23	112.333		5.100	100%		
Among ARC	4	34.690	8.672	0.272	5%	0.050	0.004
Within ARC	63	324.795	5.155	5.155	95%		
Total	67	359.485		5.428	100%		
Among Gamo-	3	39.334	13.111	0.620	10%	0.103	0.001
Within Gamo-gofa	70	378.220	5.403	5.403	90%		
Total	73	417.554		6.023	100%		
Among Gedio	1	13.208	13.208	0.847	13%	0.132	0.009
Within Gedio	22	122.833	5.583	5.583	87%		
Total	23	136.042		6.431	100%		
Among Jima	2	30.938	15.469	1.528	25%	0.247	0.001
Within Jima	23	107.063	4.655	4.655	75%		
Total	25	138.000		6.183	100%		
Among Dauro	1	4.169	4.169	0.000			
Within Dauro	16	83.275	5.205	5.205	100%		
Total	17	87.444		5.205	100%		

Notes:- *df= degree of freedom: SS= sum of square; MS=mean of squares

Table 27: Total number of observed alleles (Na) per locus, mean effective number of alleles (in brackets) and mean allele frequencies per ten geographic origin population under each region of the twelve loci studied

Locus	Na	Sidama (1.45)	Wolayita (1.48)	Wolega (1.47)	ARC (1.53)	Gamogofa (1.57)	Gedio (1.64)	Kefa (1.57)	Kembata (1.46)	Jima (1.58)	Dauro (1.51)
Dalc12	5	0.23	0.29	0.26	0.26	0.24	0.51	0.17	0.17	0.23	0.17
Ym30	4	0.20	0.18	0.21	0.24	0.29	0.25	0.38	0.38	0.19	0.19
Da1Do8	4	0.42	0.59	0.46	0.65	0.58	0.92	0.67	1.00	0.73	0.44
Dpr3fo4	10	0.16	0.21	0.17	0.19	0.22	0.22	0.27	0.01	0.28	0.29
D55	2	0.50	0.25	0.33	0.40	0.25	0.42	0.33	0.50	0.38	0.33
D83	8	0.18	0.21	0.18	0.27	0.28	0.22	0.23	0.28	0.30	0.21
Da1ao1	6	0.17	0.19	0.15	0.16	0.22	0.24	0.39	0.21	0.10	0.22
dab2c12	4	0.17	0.16	0.19	0.18	0.19	0.19	0.29	0.19	0.15	0.11
Da1fo8	6	0.08	0.19	0.08	0.13	0.16	0.21	0.17	0.08	0.14	0.09
YM13	5	0.27	0.22	0.35	0.41	0.28	0.44	0.21	0.31	0.42	0.17
Dpr3D06	6	0.24	0.19	0.31	0.28	0.30	0.19	0.28	0.17	0.31	0.28
Dpr3b12	8	0.19	0.13	0.15	0.14	0.21	0.24	0.06	0.13	0.19	0.26

4.6.2. Yam (*Dioscorea* species) diversity among geographical locations

The Jaccards similarity was computed based on 10 yam geographical location where the germplasms was originally collected. The highest genetic similarity encountered was between Gamogofa and Wolayita (0.95), followed by Wolayita and Sidama (0.94) and Gamogofa and Sidama (0.96). Generally lowest genetic similarities were observed between Kembata and all other locations. Similarly, lower genetic similarities were revealed between Kefa, Wolega, and Dauro and all other localities (Table 28).

Table 28: Jaccards similarity coefficient between Ethiopian undifferentiated yam (*Dioscorea* species) on the basis of ten geographical sites

	Sidama	Wolayita	Wolega	ARC	Gamogofa	Gedio	kefa	Kembata	Jima
Sidama									
Wolayita	0.94								
Wolega	0.76	0.77							
ARC	0.92	0.94	0.73						
Gamogofa	0.92	0.95	0.77	0.92					
Gedio	0.84	0.83	0.75	0.82	0.84				
Kefa	0.70	0.71	0.66	0.68	0.75	0.69			
Kembata	0.52	0.50	0.56	0.52	0.53	0.56	0.55		
Jima	0.91	0.89	0.73	0.88	0.88	0.83	0.70	0.53	
Dauro	0.79	0.76	0.72	0.77	0.79	0.79	0.76	0.56	0.77

4.6. 3. Cluster analysis

A dendrogram was constructed with a UPGMA cluster analysis based on DICE coefficient of similarity among 10 sites. The dendrogram based on SSR markers distinguished 210 yam landraces into nine distinct cluster groups (Fig. 26). Comparison of yam species aggregated land races within the same geographical zone revealed that most of yam populations from the same origin falls in one or two cluster groups and had the range of similarity coefficient from 0.09 to 0. 24 (Table 25).This indicates the presence of associations between geographical locations and genetic distance.

The biggest cluster group (Cluster 2) consisted of 40, 10,7,6,4, 6 and 11 yam land races from Gamogofa, Gedio, Jima, Kefa, Kembata, Wolayita zones and Areka Research Center respectively. Group 1 is the smallest whereas cluster 6 is the largest group and consists of 1 and 52 yam landraces respectively from Wolayita zone. Groups 3 and 5 are composed of 14 and 10 yam land races from Sidama Zone. Group 8 is composed of 18 yam accessions from Areka Research Center and Group 9 is a smaller group next to cluster 1 which is composed of 5 yam accessions where 2 and 1 accessions from Jima, Dauro and Sidama Zones are represented respectively.

A three dimensional PCO was carried out using Nei's distances among 10 geographical sites (Fig. 27). The first three principal coordinates accounted for 29% of the total variation among geographical sites where PCO-1 accounts for 14%, PCO-2 for 8 % and PCO-3 for 7%. As it is observed from the 3D graph (Fig. 27) yam germplasms from Gamogofa zone are isolated from most of the yam germplasm of other localities.

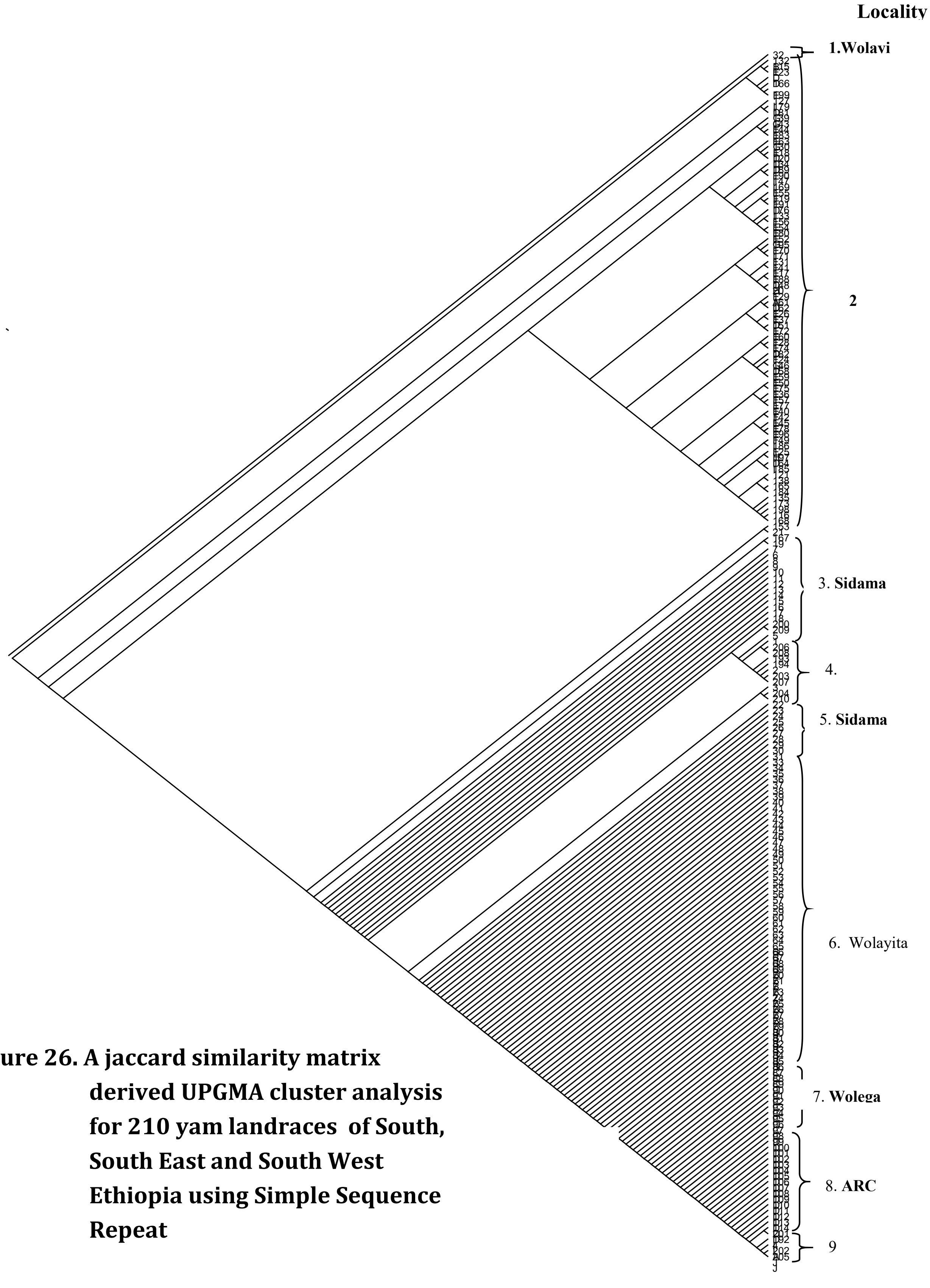


Figure 26. A jaccard similarity matrix derived UPGMA cluster analysis for 210 yam landraces of South, South East and South West Ethiopia using Simple Sequence Repeat

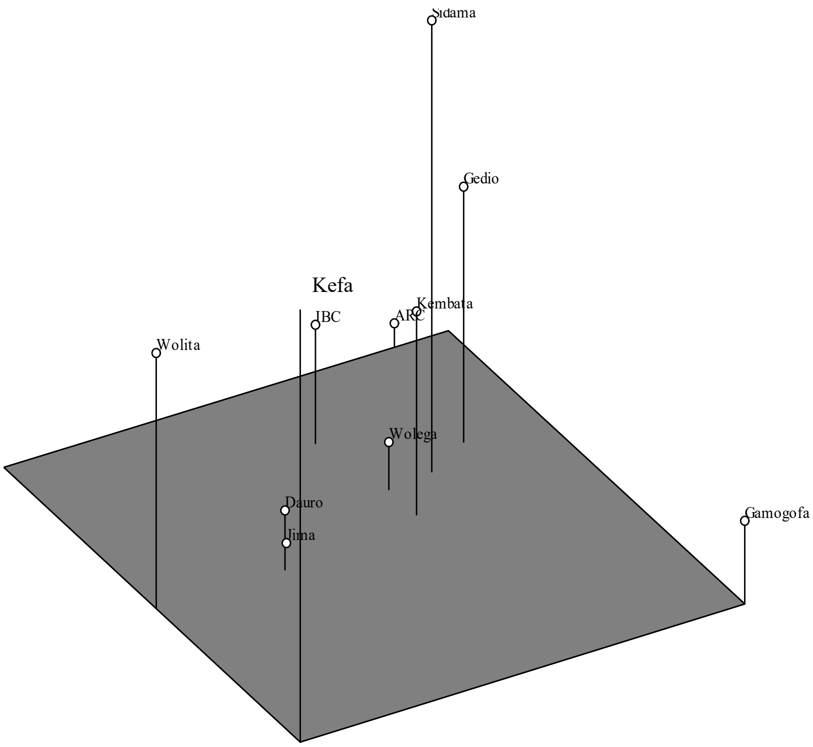


Figure 27. Three dimentional plot for 210 yam landraces from 10 major growing areas of South, South East and South West Ethiopia using Simple Sequence Repeat

4.7. Genetic diversity of Ethiopian yam (*Dioscorea* spp) reviewed through combined data analysis

4.7.1. Combined analysis of nutritional, morphological and molecular data

4.7.2. Analysis of Molecular Variance (AMOVA)

Species aggregated analysis of Molecular Variance (AMOVA) indicated 29 % and 71% of the molecular variation in yam (*Dioscorea* spp) existed among and within populations respectively. Permutation tests (based on 999 permutations) suggest that the overall ΦPT was significant (ΦPT=0.229, P=0.001; Table 31), which indicates the differences among *Dioscorea* species are significant.

Analysis of Molecular Variance (AMOVA) showed presence of high value of genetic variation among and within *Dioscorea* species (Table 29 and 30) compared to AMOVA values indicated in Table 20. This may be explained by the fact that combined data analysis was computed on yam (*Dioscorea* spp) from six geographical locations among which most of these sites (four sites) had high values of genetic variation and genetic diversity as shown in Table 25 and 26.

The highest genetic variation was observed among *D.bulbifera* followed by *D.abyssinica and D. praehensilis* (Table 30). This genetic variation result was in line with cluster analysis as presented in figure 28 and Table 32 where *Dioscorea* species *D.bulbifera, D.abyssinica and D. praehensilis* were grouped separetly in cluster groups (G &I); F and H respectively.

Table 29: Analysis of molecular variance (AMOVA) for 42 individuals of aggregated species of yam (*Dioscorea* spp) populations using twelve DNA microsatellite markers

Source of variation	**df**	**SS**	**MS**	**Est. Var.**	**%Variation**	**Value**	**P**
Among DS	5	130.348	26.070	2.966	29%	0. 229	.001
Within DS	36	255.200	7.089	7.089	71%		
Total	41	385.548		10.055	100%		

Notes:-* df= degrees of freedom; SS =um of square ; MS = mean of squares and DS= *Dioscorea* species

Table 30: AMOVA of 42 yam accessions considered in combining data set analysis by grouping the accessions based on their species group

Source	**Df**	**SS**	**MS**	**Est. Var.**	**%**	**Value**	**P**
Among *D. rotundata*	1	5.933	5.933	0.299	8	0.084	0.105
Within *D. rotundata*	16	52.400	3.275	3.275	92		
Total	17	58.333		3.574	100		
Among *D. abyssinica*	3	47.571	15.857	2.250	43	0.429	0.001
Within *D. abyssinica*	24	72.000	3.000	3.000	57		
Total	27	119.571		5.250	100		
Among *D. praehensilis*	1	15.167	15.167	2.216	40	0.398	0.003
Within *D. praehensilis*	10	33.500	3.350	3.350	60		
Total	11	48.667		5.566	100		
Among *D. bulbifera*	3	46.792	15.597	2.228	47	0.474	0.001
Within *D. bulbifera*	20	49.500	2.475	2.475	53		
Total	23	96.292		4.703	100		

Notes:- * df= degrees of freedom; SS =sum of square , MS = mean of squares

4.7.3. Jaccard coefficient of similarity among 42 Ethiopian yam (*Dioscorea* spp) observed in combined data set analysis using Simple Sequence Repeat

The genetic similarity was highest between *D.abyssinica* and *D. rotundata* (0.55); *D. praehensilis and D.abyssinica* (0.50) whereas the lowest genetic similarity was revealed between *D. cayenensis and D.alata (*0.07). The lower genetic similarity of *D.alata* to other *Dioscorea* species was expected however lower genetic similarity of *D. cayenensis* to other studied *Dioscorea* species was mainly due to the fact that particularly in combined data set analysis observed samples of this species were small (Table 31).

Table 31: Jaccard similarity coefficient in 42 Ethiopian yam (*Dioscorea* species) observed in combined data set analysis using Simple Sequence Repeat

	D. rotundata	*D.alata*	*D.abyssinica*	*D. cayenensis*	*D. praehensilis*
D. rotundata					
D.alata	0.24				
D.abyssinica	0.55	0.32			
D. cayenensis	0.23	0.07	0.18		
D. praehensilis	0.44	0.28	0.50	0.15	
D.bulbifera	0.40	0.28	0.44	0.24	0.40

4.7.4. Dendrogram based on combined analysis of morphological, nutritional and molecular data set

Four yams (*Dioscorea* spp) from different geographic origins were grouped separately within five cluster groups (Fig. 28). These were cluster C (*D.alata*) (Jima), cluster F (*D. abyssinica*) (Wolayita origin), cluster G (*D. bulbifera*) (Wolega and Jima origin) cluster H (*D. preahensilis*) (Gamogofa origin) and cluster I (*D. bulbifera*) (Gedio origin).

The differences in the cluster groups differences were also expressed in phenotypic variations among studied yam genotypes as indicated in Table 32.This indicated that these morphological variations are heritable.

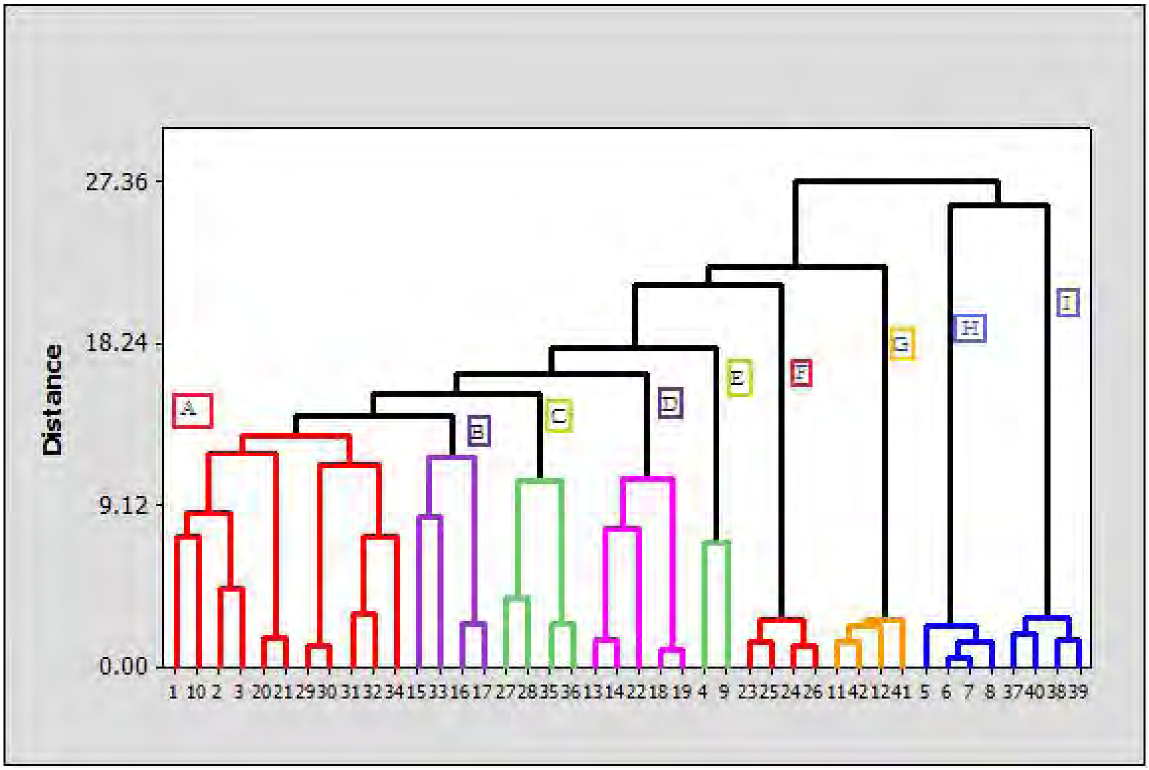


Figure 28. Dendrogram using Ward's method derived from a dissimilarity matrix of 42 yam (*Dioscorea* spp) landraces of Ethiopia based on combined data set analysis

Table 32: Clustering groups of six different Ethiopian *Dioscorea* species based on combined analysis of nutritional content, morphological and molecular markers

Cluster name	Number of accessions	Species name and their frequencies		Qualitative morphological traits and/or geographic origins
A	11	*D. abyssinica*	2	Green mature petiole colour with purple leaf junction and intermediate distance between leaf lobes
		D.preahensilis	3	
		D. bulbifera	3	
		D. cayenensis	1	
		Un known	2	
B	4	*D. rotundata*	1	intermediate distance between leaf lobes, intermediate corm size, presence of cracks on tuber surface and low overall farmer assessment of cooked tuber
		D. bulbifera	3	
C	4	*D.alata*	4	Green mature petiole color with purple base, late maturity group and low over all farmer assessment of cooked tuber
D	5	*D. preahensilis*	1	High leaf density, intermediate distance between lobes and intermediate place of roots on tuber
		D. abyssinica	1	
		D. bulbifera	2	
		Un known		
E	2	*D.preahensilis*	1	Pale leaf color, high leaf density and purplish green mature stem color
		D. cayenensis	1	
F	4	*D. abyssinica*	4	Wolayita origin
G	4	*D. bulbifera*	4	Wolega and Jima origin
H	4	*D. preahensilis*	4	Gamogofa origin
I	4	*D. bulbifera*	4	Gedio origin

5. Discussion

5.1. Morphological characterization

Morphological characterization is highly recommended as a first step that should be made before the more in depth biochemical or molecular studies are attempted (Otoo *et al.*, 2009).

In the present study, tuber flesh colour and tuber shape revealed differences among different species (Figures 6 and 7). This is in support of Coursey, 1967; Onwueme, 1978; Martin and Ruberte, 1976 who stated that tubers vary greatly in size, colour and shape depending on species, cultivar and environment. In this study, yam tubers of *D. praehensilis*, *D.bulbifera* and *D. alata* had finger like appearance while tubers of *D. rotundata, D. cayenensis* and *D. abyssinica* were long wih oval shape (Fig.7). Lebot (1998) also reported that the tuber shape of *D.alata* is often irregular which increases harvest time and thus is labour intensive.

In the present study pink and red purplish colors were observed in *D. abyssinica* and *D. Praehensilis*.Whereas, *D. cayenensis* and *D. alata* revealed yellow and white tuber flesh colors respectively. Martin and Ruberte (1976) indicated that anthocyanins and carotenoids are pigments found in yams (*Dioscorea* spp) which give them distinctive tuber flesh colors. Indeed, B-carotene and Xantophyll esters are responsible for the yellow flesh color of *D. cayenensis*. Rasper and Coursey (1967) also reported that pink or red purplish color in *D. trifida* is due to presence of anthocyanin pigment in its tuber.

Morphological characterization of Ethiopian yam genotypes from ten geographical locations indicated that most accessions from southern regions are closely related despite of their geographic location being wide spread as presented in (Table 11 and Table 12). Morphological markers had only one cluster which grouped yam accessions based on their geographic origin where most accessions of Welega origin were in cluster 3 (Figures 8, 9 and 10) (Table 12). Such a cluster is supported by Singh (1990) and he recommended that geographic diversity may serve as an indicator of genetic diversity in parental selection. Yam genotypes of Wolayita origin showed more morphological diversity and were distributed in all clustering groups (Table 12). The presence of more morphological diversity in Wolayita yam cultivars was indicated by Muluneh Tamru (2006). Species of *D. abyssinica*, *D. rotundata*, *D. cayenensis* and *D. praehensilis* revealed relatedness. This relationship could be due to the likelihood of *D. praehensilis* and *D. abyssinica* being one of the wild parents of several cultivars of *D. cayenensis* and *D. rotundata,* Miege J. & Sebsebe Demisew (1997).

The efficiency of different cluster algorithms can be compared through estimation of the ‘cophenetic correlation coefficient. It is a product moment correlation coefficient measuring agreement between the dissimilarity and similarity indicated by the dendrogram which is output of the dissimilarity and similarity matrix (Rolf, 1998). In this analysis the cophenetic correlation coefficient r=80 revealed the efficiency of the dendrogram.

In this study, morphological traits that best discriminate between the landraces were young petiole color, color of tuber after cooking, flesh color at lower part of tuber, and flavor of cooked tuber and tendency of tuber to branch. This result was in agreement with the result obtained by Sayed *et als.*, (2008) who reported from his studies of *D. alata* that morphological variability scores on the first principal component (PC-1) were highly correlated with the characters related to the tuber flesh colours and petiole colour. Similarly, Mwirigi *et al.,* (2009) in his studies of morphological variability of Kenyan yam reported that, Pc3 was mainly correlated to characters related to the tuber flesh colour. It is also indicated that the most effectively used characters in classification of *D. alata* are tuber, leaf and growth characteristics (Martin and Rhodes, 1977) quoted in Sayed *et al.*, (2008).

5.2. Nutritional composition analysis

Proximate and mineral composition study indicated that Ethiopian yams (*Dioscorea* species) are nutritionally rich. Generally, Ethiopian yam genotypes have high nutritional content of protein, fiber , phosporous. ash , and iron. Though comparatively, they have low fat and zink content (Table 16). They gave highest nutritional content compared to earlier reports of West Africa varieties by Osagie (1992) with respect to protein, iron, calcium, fat and fiber content. Opara (1999) reports on nutritional content of yam also magnify superiority of Ethiopian yam with iron, calcium, fat and fibber contents.

It was also observed that there was high moisture content in yam germplasms studied. The range for moisture content was between (4.67%-13.57 %) for all samples studied.

Similar result was reported by Osagie and Eka, (1998), who indicated that the presence of high content of moisture in yam indicates a good source of minerals. In this study, calcium and iron content in yam species was highest whereas phosphorous and zinc content was comparatively low

Similar low zinc content in yam was reported by Esayas (2009) and high calcium content was reported by Wansudera& Ravindran, (1993). Contrary to these observations Bradbury and Hollowy (1988) as quoted by Joyce (2010) reported that yam has low calcium. However, our study revealed that Ethiopian yam species have high calcium content. High protein content, high amount of fiber and ash content was obtained but fat content was relatively low as indicated in Figure 20. Onwume (1978) reported higher protein content in yam tubers compared to other tropical roots. Zinash (2008) also reported that yam is an excellent source of protein and minerals.

High variability was observed among and within yam species due to their proximate composition and mineral content. The maximum nutritional variation was observed in overall range, within same and different species. *D. alata* and *D.bulbifera*, revealed highest mean value of protein and fiber content compared to the rest of yam species. Similarly, the range was superior among germplasms of *D.bulbifera*, in moisture and zink content. *D.bulbifera* of Jima origin revealed the highest calcium content. Earlier studies by Hahn *et al*., (1987) quoted by Muluneh Tamru (2006) stated that mean protein content of aerial yam is higher than the protein value of sweet potato, potato, cassava, taro and plantain.

The highest fat content range was recorded from *D. praehensilis* and *D.cayenensis and* the highest range of protein and calcium content was from *D. abyssinica*. This result confirms that genetic base difference with respect to species and population will also lead to nutritional content variation in yam (*Dioscorea* species).

The nutritional composition study revealed that yam genotypes from Wolega distributed over four different clusters and additionally landraces of Wolayita and Jima distributed over three different clusters which indicated that presence of more diversity in this region with respect to nutritional content compared to other regions. Similarly, collections from Gedio and Gamogofa regions were distributed in two different clusters and had comparatively lower diversity compared to the other two regions (Fig 22). Hence, it is necessary to consider the germplasms of these regions for selecting better cultivars for improvement of nutritional aspect of yam.

In the present experiment, highly significant and positive correlation was observed between phosphorous content and average stem height; Average tuber yield per plot and Calcium content; iron content and fibber content; fibber content and moisture content. Positively significant correlation was found between fiber content and calcium content; and iron content and ash content. However, there was no significant correlation between zinc content, protein content, and fat content, number of leaves at 30 days after emergence and the rest of the traits studied.

In the present study, absence of correlation of fat content to the rest of the traits studied may be due to the fact that lipids that consist of fats and their derivatives which are often unrelated in physiological and chemical process (Pandey and Sinha, 1999).

The present study also indicated positive correlation between calcium content and tuber yield. This result is due to the fact that root growth is particularly affected by calcium deficiency which will lead to premature inactivity of the vine tips consequently negatively affecting the growing tip of the tuber (Jane, 2010).

5.3. Microsatellite Markers

In this study, SSR analysis among and within Ethiopian yam (*Dioscorea* spp) revealed a higher level of genetic variation among individuals yam accessions within population compared to variation among populations.

The UPGMA dendrogram and the scatter diagram indicated the separation of *D. cayenensis, D. abyssinica* and *D. bulbifera* by grouping them in cluster 6, cluster 5 and clusters (3 and 1) respectively.The cluster dendrogram as it is observed in sub cluster 1a separated *Dioscorea* species (*D. praehensilis* and *D. abyssinica*) from other *Dioscorea* species. On the other hand, cluster 4 and cluster 8 contained only *D. praehensilis and D.rotundata.* Similarly cluster 2 was consisted of *D. abyssinica*, *D. praehensilis and D.rotundata* (Figure 24 and 25). These cluster groups were supported by partitioning of genetic variation by AMOVA. There were significant genetic variations in both among and within *Dioscorea* species as indicated in Table 20.

In the present study, Jaccard coefficient of similarity was used to estimate the gene relationship within and among yam (*Dioscorea* species). When the pairwise population comparisons were made, the highest values of Genetic similarity (0.99) were observed to be between accessions from *D. rotundata* and *D. abyssinica* . The similarities between *D. praehensilis* and *D. rotundata*, *D. praehensilis* and *D. abyssinica* were also high as observed in Table 22. Dumont *et al*, (2006), based on different molecular markers explained that the two species *D. abyssinica* and *D. praehensilis* are possible ancestors of the cultivated Guinea yams and are considered to be the major source of variability. Danis *et al*., (2001), also indicate that a variety domesticated from *D. praehensilis* genetically resembled *D. abyssinica*.

The present study showed presence of the lowest genetic similarity among *D. alata* to all other studied *Dioscorea* species (Table 22). Though, *D. alata* was closer to *D.bulbifera* than to other species. This result is in line to Muluneh Tamru (2006) result whom stated that some cultivar of *D. alata* produce areal tubers lend support to its closeness to *D.bulbifera*.

In this study species of *D. rotundata* and *D. cayenensis* were grouped in different cluster groups. Minoguna *et al*., (1998), stated that the Guinea yams are distinct but related species and the varietals groups of *D. cayenensis* were genetically distant from those of *D. rotundata.* Similarly Muluneh Tamru (2006) indicated that these two species are distinct groups. However, in contrast Wendawek Abebe *et al*., (2012) reported that AFLP data failed to produce any clear species boundaries between the Guinea yam accessions of Sheko origin and their wild relatives.

This study gave only two sub clusters (1b and 1c) composed of 64 yam accessions which overlapped five species together excluding *D. cayenensis*. This specific grouping is in line with the results Wendawek Abebe *et al.*, (2012) yam diversity study using AFLP data.

In this study the highest polymorphic percentages were recorded within *D. abyssinica* followed by *D. praehensilis and D. rotundata.* Similarly high genetic diversity was recorded within *D. abyssinica* species (Table 24). According to Coursey, (1967), Ethiopia is considered the center of origin for *D. abyssinica*, which is found widely distributed in the savanna region of West Africa. Miege'e and Sebsebe Demissew, (1997), indicated that *D. abyssinica* is widely distributed in the southern, western and northern part of the country in woodlands or wooded grasslands between 1000m and 1800m above sea level.

Wendawek Abebe (2008 and 2012) studied 58 accessions of wild (21) and cultivated (37) yams (*D. abyssinica*, *D. praehensilis* and *D. cayenensis*/*D.rotundata*) diversity collected from Sheko, Sidama, Gedio, Wolaita, Kefa and West Wolega using seven microsatellite loci among these four loci Da1Do8, Da1Fo8, Dpr3Do6 and Dpr3Fo4 were also incorporated in our study. In this study, Microsatellite locus Da1Fo8 gave the smallest allelic frequency. Wendawek Abebe (2008) also reported the same result.

The present study showed high polymorphism among Ethiopian yam landraces. The polymorphism percentage among populations of different sites ranged from 98.53 % (Wolayita) to Kembata (47.06%) with a mean value of 82.21%. This high level of polymorphism obtained using SSR markers indicated that this molecular marker is very useful and powerful for yam characterization.

Microsatellite markers used in this study indicated presence of a geographical pattern of diversity within Ethiopian yam landraces except for cluster group 2 which had similar pattern to phenotypic characterization (Fig. 27). Most yam land races from different localities were observed in each cluster group. This indicates that the existence of a geographical pattern of diversity within Ethiopian yam land races with exception of cluster group 2 whose diversity does not relate to geographic region. Genetic polymorphism was highest within yam (*Dioscorea* spp) of Wolayita origin. The gene diversity was highest within yam accessions of Gedio followed by Jima and Gamogofa zones. As indicated in Analysis of Molecular Variance (AMOVA) (Table 26), yam accessions genetic variations had considerably high values within geographical sites. It is therefore likely that specific regions may hold a considerable amount of yam genetic variability.

In the present study, as indicated in Table 1 and Figure 2 collection areas of yam germplasms covered more diverse geographic areas than many previous studies. In addition to this two species *D. bulbifera* and *D. alata* are included, these two species which were not included in the previous studies using codominant markers. Hence, this coverage of more diverse yam growing areas and more species may help to better elucidate gentoyping with regard to their *Dioscorea* species, geographic origin and distances.

5.4 Combined data analysis

The combined analysis of mineral content and proximate composition to morphological and SSR markers discriminated four yam (*Dioscorea* species) namely *D.alata*, *D.abyssinica, D.bulbifera and D. praehensilis* separately into different cluster groups. Hence, observing proximate and mineral composition variability of yam is vital to indicate the extent of diversity present in yam (*Dioscorea* spp) in addition to its nutritional content improvement. Generally, it is important to study both single and combined effects of different markers for effective conservation and improvement efforts of yam (*Dioscorea* spp) germplasms in order to enhance its' productivity in diverse aspects.

6. Conclusion and Recommendations

In the present study 33 morphological traits revealed morphological variability. This indicates the presence of a large amount of polymorphism in yam (*Dioscorea)* species of Ethiopia. Hence, it is possible to obtain important trait combination using the germplasms found in the country to achieve the goals of genetic improvement of the crop.

Clustering the accessions helped in identifying parents with diverse characters for future improvement programs. Most of the morphological variations among the yam genotypes were contributed by young petiole color, tendency of tuber to branch, color of tuber after cooking, flavor of cooked tuber and flesh color at lower part of tuber. Hence, these morphological traits could be taken as useful markers for identification of cultivars and species of yam crops in the country for selection and conservation puroposes. In future studies, it is also important to examine these phenotypic traits to construct a genetic linkage map for Ethiopian yam breeding programs in yam genetic improvement programs.

Morphological characterization and genetic diversity assessment of Ethiopian yam accessions using molecular markers (SSR) showed that Wolayita regions had wide genetic diversity. while, most yam landraces from Wolega revealed different genetic base. Therefore, it is important to consider yam germplasms from Wolega and Wolayita regions during breeding and improvement activities of the crop.

The results of the present study revealed that yam is nutritionally rich. Generally, they have highest nutritional content with respect to protein, fiber, calcium and iron content but comparatively low fat, ash, phosphorous and zink content.

In present study, highly significant and positive association was observed between phosphorous content and average stem height; average tuber yield per plot and calcium content; iron content and fiber content; fiber content and moisture content. Positively significant correlations were found between fibber content and calcium content; and iron content and ash content. Nevertheless there were no significant relationships between zinc content, protein content, and fat content, number of leaf at 30 days after emergence and also for the rest of the traits studied. This result indicates that two nutrients namely phosphorous and calcium are vital for growth and tuber yield of yam tuber respectively.

High variability was observed among and within yam species in their proximate composition and mineral content. The maximum nutritional variation was observed in overall range, within the same and different species. *D. alata* and *D.bulbifera*, revealed the highest mean value of protein and fiber content compared to the other yam species. The highest range of fiber content was observed from *D.bulbifera* (1.82%-5.16%) and *D.alata* (3.65%-6.36%) and *D. abyssinica* (1.94%-2.97%).

Similarly, the range was superior among germplasms of *D.bulbifera*, in the content of moisture (4.67%-12.41%), iron (20.96% mg/100g-90.85mg/100gm) and zink (0.4g/100mg-8.33g/mg). The genotypes in *D. abyssinica* gave the highest range of protein (3.13%- 5.38%) and calcium content (31.02mg/100g- 118.81mg/100g). The highest fat content range was recorded from *D. praehensilis* (0.26%-7.83%) and *D.cayenensis* (0.59-7.86%).

The present result contributes to the data available to researcher and crop breeders for the improvement of nutritional quality of yams through breeding and selection to assure the food security of the country. In Ethiopia during yam planting farmers use animal manure rather than inorganic fertilizers. Therefore, the comparative role of mineral nutrition (soil nutrient) and yam (*Dioscorea* spp) landraces to the observed range of mineral contents requires further investigation.

Even though the orange-fleshed sweet potato is rich in vitamin A, farmers in most of southern regions of Ethiopia do not prefer to consume the food rather they give it to their infants mainly because of its watery texture.

Hence, the yellow yam may be a better choice for adults and will help to improve the balanced intake of food in subsistence communities. It is vital to study the variability of yam germplasms in forms of their vitamin content and whether there is association between vitamin content and tuber flesh colour.

The dendrogram and scatter diagrams constructed using yam nutritional composition revealed that cluster V is distantly related to other groups. Hence, the two accessions of cluster V from Gamogofa origin can be used as parents for improvement of yam nutritive value through breeding.

Yam landraces from Jima origin gave the highest mean values for most of proximate and mineral contents. Accordingly, it is vital to consider these yam accessions during yam improvement for better nutritive value.

In addition, collections from Wolayita, Dauro Wolega and Jima regions were distributed in two different clusters and were shown to have comparatively higher diversity followed by yam accessions of Gamogofa origin. This indicates that using yam germplasms from these regions could be used to boost nutritional content through selection.

Yams can play an important role in attaining food security in the country by helping the subsistence farming communities to get a balanced diet. In this study the yam genotypes showed high mean value for calcium, protein and iron content. Generally, a significant variation was observed among most of Ethiopian yam species with respect to their nutritional content. Hence, there is a high potential for selecting cultivars on the basis of nutritive value. However, the genotype and environmental interaction and the contribution of both genetic and environmental factors as well as the heritability of these traits need to be determined across diverse environmental factors.

The existence of genetic variability is the basis of any plant breeding and improvement activities. The present study showed highest mean percentage of polymorphism (92%) in

Ethiopian yam *Dioscorea* species. The high level of polymorphism observed with SSR techniques indicates that this molecular marker is a powerful tool for yam molecular characterization studies.

The cluster dendrogram constructed using microsatellite markers grouped *D. rotundata* and *D. cayenensis* in different clusters. Generally, the present study indicated presence of the lowest genetic similarity among *D. alata* to all other studied *Dioscorea* species. While *D.alata* was comparatively closer to *D. bulbifera* than to other studied *Dioscorea* species.

This study indicated presence of high genetic diversity in Ethiopian yam landraces. There was clear relationship between genetic divergence and geographical distance in most of clustering groups. Hence, it is advisable to use the yam landraces from different localities as a source of parental material in yam breeding program.

The landraces from Kembata exhibit the highest genetic distance from the majority of yam land races of other localities. Similarly yam germplasms from Kefa, Wolega and Dauro showed higher genetic distance than those from other locations. It will therefore be important to consider these distantly related populations of Kembata, Kefa, Wolega and Dauro for future improvement of this crop through breeding. Although, yam landraces of Gambella regions were not included in this and previous studies. Hence, it will be important to consider these regions in the future researches.

High genetic diversity was obtained within Gedio, Jima and Gamogofa yam landraces. Accordingly, yam landraces of these three regions will have an important role to play in improving productivity of the crop through selection. These results indeed indicate that the importance of giving emphasis to Ethiopian yam conservation activities in order to maintain and protect its diversity from extinction.

This study indicates that microsatellite markers are useful in assessing genetic difference and association among yam populations. The high level of genetic variation in Ethiopian yam populations points towards that the importance of each population as a valuable genetic resource for future yam improvement programs. Thus, prudent use of the genetic diversity present in Ethiopian yam (*Dioscorea* spp) is of crucial importance.

The present study indicated that in comparison to morphological, proximate and mineral content diversity, Microsatelite markers were effective molecular marker for separating different yam (*Dioscorea* species) to their own population group. However, the other two schemes have an important role in revealing divesrsity within yam genotypes. As shown in the results of the present study, both morphological markers and nutritional content diversity gave results which were in agreement with results of SSR marker . Thus, similar emphasis should be given to different kinds of schems during Yam (*Dioscorea species*) diversity analysis rather than depending on only on one marker type. This will help to minimize costs and lessen the time taken for effective conservation activities towards ensuring food security in the Country.

Domestication of wild species as crops is ongoing and will continue for a long time in the future. Wild species that have little importance today may have great importance in the future. As the present study has revealed there is presence of high genetic diversity in Ethiopian yam landraces. There is therefore a great possibility for improving productivity in this important crop through selection. Both *in situ* and e*x situ* yam germplasm conservation efforts also require to be accorded keen attaintion both at the federal and regional levels in order to keep the wild relatives of yam from extinction.

7. References

Alexander, J. & Coursey, D. (1969). The origins of yam cultivation. In: *The Domestication and Exploitation of Plants and Animals*, 405-425 (Eds Ucko P. J. and Dimbleby), London.

Arora, R. and Anjula, P. (1996). Wild edible plants of India, Diversity, Conservation and Use. National Bureau of Plant genetic resources, New Delhi.

Asiedu, R.., Wanyera, N.M., Ng, S.Y. and Ng, N.Q. (1997). Yams. ***In***: *Biodiversity in Trust: Conservation and Use of Plant Genetic Resources in CGIAR Centers* (Fuccillo, D. editor). Cambridge Univ. Press, Cambridge, UK, pp. 57-66

Asiedu, R. and Sarite, A. (2010). Crops that feed the World 1. Yams. Yams for income and food security. *Food Sec 2:305–315.*

Asha, K. and Nair, M. (2002). Ethnic knowledge system on wild *Dioscorea* (yams) by the Kanikkars of Southern Western Ghats, Kerala. *Indian J. Plant Genet. Res*. 2002;15: 146–149.

Ayensu, E.S. and Coursey, D.G. (1972). Guinea yams: the botany, ethnobotany, use and possible future of yams in West Africa. *Economic Botany* 26:301-318.

Borsch, T., Hilu, K.W., Quandt, D., Wilde,V., Neinhuis,C. and Barthlott,W.(2003). Noncoding plastid trnT-trnF sequences reveal a well resolved phylogeny of basal angiosperms.*J. Evol. Biol.* **16:** 558-576.

Brucher, H. (1989). Useful plants of Neotropical origin and their wild relatives. Springer-Verlang, Berlin, Germany.

Bradbury, J.,and Holloway, W. (1988). Chemistry of Tropical Root Crops: Significance for Nutrition and Agriculture in the Pacific. ACIAR Monograph, Australian Centre for International Agricultural Research.

Brunnschweiler, J., Luethi, D., Escher, F. and Conde Petit, B. (2004). Isolation and characterization of yam starch (*Dioscorea alata* and *Dioscorea cayenensis*) from the Ivory Coast in comparison to other tuber starches.

Bhandari, M.R., Kasai, T. and Kawabata, J. (2003). Nutritional evaluation of wild yam (*Dioscorea* spp.) tubers of Nepal. Food Chemistry 82:619-623.

Brücher, H. (1989). Tubers / corms / rhizomes. In: *Usefull plants of neotropical origin and their wild relatives*. Springer verlag, 4-53 Berlin.

Burkill, I. (1960). Organomongraphy and evolution of *Dioscoreaceae*, the family of yams. *J. Linn. Soc*. (*Bot*), 56: 319-412.

Brucher, H. (1968). Biogeography and Ecology in South America. 1: 251-300.

Chukwu, G. And lkewelle (2000). Yam: Threats to it sustainability in Nigeria. Palawija New, 17:1. The CGPRT Centre Newletter.

Coursey, D. G.(1967). Yams. An account of the nature, origins, cultivation and utilization of the useful members of the Dioscoreacea. London.

Coursey, D.G. (1976). The origins and domestication of yams in Africa. Quoted *In*: Muluneh Tamru. (2006). Assessing Diversity in Yams (*Dioscorea spp.*) from Ethiopia based on, Morphology, AFLP Markers and Tuber Quality, and Farmers Management of Landraces.

Coursey, D. (1983). Yams. Handbook of Tropical Foods (Chan, H. C. (Jr.), ed.), pp. 555- 601. New York.

Craufurd, Q., Summerfield, J. and Asiedu, R. (2006). Dormancy in yams. *Expl Agric*. (2001), 37: 141-181.

Danis, A., Migonouna, H., Pillay, M., and Zok, S. (2001). Ploidy variation in the cultivated yams (*Dioscorea cayenensis complex*) from Cameroon as determined by Flow cytometry. *Euphytic* 119:301-307.

Dansi, A. , Dantsey, B. H., Dossou, A., N'Kpenu, E. K., Agré, A. P., Sunu Y. D., Kombaté, K., Loko, Y. L., Dansi, M., Assogba, P. and Vodouhè, R. (2013). Varietal diversity and genetic erosion of cultivated yams (*Dioscorea cayenensis* Poir - *D. rotundata* Lam complex and *D. alata* L.) in Togo. Vol.5(4), pp223-239.

De Candolle, A. (1886). Origin of cultivated plants. 2nd ed. Reprinted by Hafner, New York

Dumont, R., Danisi, A., venier, P. and Zoundjihe'kpon, J. (2006). Biodiversity and Domestication of yams in West Africa: Traditional practices leading to *Dioscorea rotundata* poir. CIRAD, ISBN 2-87614-632-0.

Edossa Etissa (1996). Root and tuber crops potential as food crops in the humid areas of Ethiopia. *Newsletter of Agricultural Research* 11:9-11.

Edossa Etissa (1998). Yams: exploration, collection and evaluation. Newsletter of Ethiopia Agricultural Research Organization. Vol.13, No.2

Edison, S. M., Unnikrishnan, B., Santha, V., Pillai, M.N., Sheela, M.T., Sreekumari S. and Abraham, K. (2006) . Biodiversity of Tropical Tuber Crops in India. NBA Scientific Bulletin Number 7, National Biodiversity Authority, Chennai, TamilNadu, India.

Elisabeth, A., H. (2003). Motives and opportunities for domestication: an ethnoarchaeological study in southwest Ethiopia.*Journal of Anthropological Archaeology* 22 (2003) 358–375.

Esayas Ayele (2009). Effect of Boiling Temperatures on Mineral Content and Anti nutritional Factors of Yam and Taro Grown in Southern Ethiopia. A thesis submitted to the School of Graduate Studies of Addis Ababa University in partial fulfillment of the requirement for the Degree of Master of Science in Food Science and Nutrition

Excoffier, L., Smouse, P. and Quattro, J. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics* 131: 479-491.

FAO, (2003). Food energy methods of analysis and conversion factors. Report of technical workshop. Rome, Italy.

FAO, (2005). Food and Agricultural Organization Statistics. Rome, Italy.

FAO, (2006). FAOSTAT Agriculture database; agricultural production, crops primary. Yams. Food and Agriculture Organization, Rome, Italy.

FAO, (1991). *Food outlook, FAO, Rome.*

FAO, (1999) FAO's Position paper. Food and Agriculture Organization of the United Nations, Rome, Italy.

FAO, (2012). Foreign Agricultural Investment Country Profile. Nigeria. Rome, Italy.

FAOSTAT, (2013). Quoted in 2010-2013 Factfish, Germany.

Franklin, W. (1976). Tropical Yams and Their Potential Part 3. *Dioscorea alata,* Washington, D.C.

Franklin, W. and Lucien, D. (1978). Tropical Yams and Their Potential Part 6. Minor Cultivated *Dioscorea* Species, Washington, D.C.

Franklin, W. and Lucien, D. (1978). Tropical Yams and their Potential. Part 5. *Dioscorea trífida,* Washington, D.C.

Govaerts, R. and Wilkin, P. (2007). World Checklist of *Dioscoreaceae.* The Board of Trustees of the Royal Botanic Gardens.

García, R. and Báez, A.(2012). Atomic Absorption Spectrometry (AAS), Atomic Absorption Spectroscopy, Dr.Muhammad Akhyar Farrukh (Ed.), ISBN: 978-953-307-817-5

Hahn , S.(1995). Yams. In: *Evolution of crop plants, Smartt J and Simmonds NW (eds.) second edition*, Longman Group Limited, UK.

Hair, J., Anderson, R., Tatum, R. and Black, W. (1992). Multivariate Data Analysis. 3rd edn. New York.

Hair, J. Anderson, R., Tatham, T. and Black, W. (1995). Multivariate data analysis with readings. Quoted in Mohammadi S. and Prasanna , B., (2003). Analysis of Genetic Diversity in Crop Plants Statistical Tools and Considerations Crop science 4th edition, Prentice-Hall

Hodeba, D. Mathew, M. and Robert, A. (2008). Genomics of yams, a common source of food and medicine in the tropics. In: *Genomics of Tropical Plants*, pp. 549-570.

Hahn, S., Osiru, D**.,** Akoroda, M. & Otoo, J. (1987). Yam production and its future prospects. *Outlook in Agriculture* 16:105-110.

Hildebrand, E. (2003). Motives and opportunities for domestication: anthropological study in southwest Ethiopia. *Journal of Anthropological Archeology 22:358-*

375.

IBC, (2008). Ethiopia; Second Country Report on the State of PGRFA to FAO, Addis Ababa, Ethiopia.

IPGRI/IITA, (1997) Description for yam (*Dioscorea* spp.). International Institute for tropical Agriculture, Ibadan, Nigeria/ International Plant Genetic Resource Institute, Rome, Italy.

IITA, (2010). Global initiative underway to preserve yam biodiversity. Ibadan, Nigeria.

Jane, N.O'. (2010). Yam Nutrition, nutrient disorders and soil fertility management. Australian Center for International Agricultural Research.

Jones, D.B. **(**1941). Factors for converting percentages of nitrogen in foods and feeds into percentages of proteins**.** Quoted in FAO. (2003). Food energy methods of analysis and conversion factors. Report of technical workshop, Rome, Italy.

Jayasurya, A. (1984). Systematic arrangement of the genus *Dioscorea* (*Dioscoreaceae*) in Indian Sub-continent, Revised hand book to the Flora of Ceylon IX. Royal Botanic Gardens, Kew Richmond, UK.

Kambaska, K., Santilata, S. and Aratibala, P. (2008). Effect of plant growth regulator on in vitro micro propagation of 'Bitter yam' (*Dioscorea hispada Dennst*). *IJIB* 4(1) 50-54.

Kambaska, K. and Trinanth, M. (2009). Biochemical Quantification of protein, Fat, Starch, Crude fibre, Ash and Dry matter content in different Collection of Greater Yam (*Dioscorea alata* L.) found in Orissa. *Nature and Science*, ISSN 1545-0740.

Kay, D. (1987). Root Crops Tropical Development and Research Institute. London. Lape, I.M. and Treche, S. (1994). Nutritional quality of yam (*Dioscorea dumetorum* and *D. rotundata*) flours for growing rats. *J. Sci. Food Agric*., 66(4): 447-455.

Lebot, V. Genetic improvement of the Pacific Island root crops to increase their nutritional values, 98825 New Caledonia, CIRAD, Pouembout

Lebot, V., Trilles, B., Noyer J., and Modesto, J. (1998). Genetic relationship between *Dioscorea alata* L. cultivars. *Genet. Resour. Crop Evol*. 45(6): 499– 509.

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

Leon, J., (1976). Agronomic aspects of yam root and tuber crops. Prof. 4th symp. Int. Soc. Trop. production in the common wealth Caribbean. In: *Root Crops*, pp: 20-36.

Lebot , V. (2009). *Tropical root and tuber crops Cassava, sweet potato, yams and aroids*. Publ. CABI. 413p

Malaurie, B., Trouslot, M., Engelmann, F. & Chabrillange, N. (1998). Effect of pretreatment conditions on the cryopreservation of in vitro cultured yams (*Dioscorea alata* 'Brazo Fuerte' and D. bulbifera 'Noumea Imboro') shoot Apices by encapsulation dehydration. Cryoletters 19:15–26.

Malapa, R.., Arnau, G., Noyer, J..L. and Lebot, V. (2005). Genetic diversity of the greater yam (*Dioscorea alata*) and relatedness to *D. nummularia* Lam. and *D. transversa* Br. as revealed with ALFP markers. *Genetic Resources and Crop Evolution* **52**:919-929.

Melaku, W., Awegechew, T. and Tesfaye, T. (2000). Participatory approaches linking farmers' access to gene banks: Ethiopia. In : *The Participatory Approaches to the Conservation and Use of Plant Genetic Resourcs*, Pp 56-68. (Fris-H., Esbern and Bhuwon S. eds.) IPGRI, Rome, Italy.

Mignouna, H. and Dansia, (2003). Yam (*Dioscorea* ssp.) Domestication by the Nago and   Fon ethnic groups in Benin. *Genetic Resources and Crop Evolution*

50: 519-528.

Muzac T. I., Asemota, H., Ahmad, M.(1993). Biochemical composition and storage of Jamaican yams (*Dioscorea* spp). *J. Sci.Food Agric., 62(3):219- 224.*

Mie'ge, J. (1952). Quoted by Wendawek Abebe (2008). Morphological and Molecular Characterization of Cultivated Guinea Yam Accessions and their Wild Relatives (*Dioscorea cayenensis* Lam. Complex) from South and Southwest Ethiopia. A Thesis Submitted to the School of Graduate studies of Addis Ababa University in partial fulfilment of the requirement for the Degree of Docto of Philosophy in Biology.

Miège, J., and sebsebe Demissew (1997). *Dioscoreaceae. In: Flora of Ethiopia and Eriterea (*Edwards, S., Sebsebe Demissew and Hedberg, I., eds.). A joint publication of the National Herbarium, Biology Department, AAU, Ethiopia and the Department of Systematic Botany, Uppsala University, Sweden.

Mignouna, H., Ellis, N. , Knox, M., Asiedu, R. and Quat, N. (1998). Analysis of genetic diversity in Guinea yams (*Dioscorea* spp) Using AFLP Fingerprinting. Trop Agric. (Trinidad) Vol. 75. No.2

Mignouna, H., Abang, M. and Asiedu, R.. (2004). Harnessing Modern Biotechnology for Tropical tuber crop improvement: yam (*Dioscorea* spp) breeding. *African journal of biotechnology.* Vol. 2(12) pp478-485.

Mie'ge, J. (1968). *Dioscoreacea* ***In****: Flora of tropical West Africa* (eds. Hutchison J Dalizel, M. and Hepper, F. N.) Crown Agents, London, UK V-3, pp 144-154

Mie'ge, j. (1982). Notes species *Dioscorea cayenesis* Lamk. Et, *Dioscorea rotundata* Poir. In: *Yams* (Eds, Miege, J & Lyonga, S.) Oxford University Press, Oxford UK, pp. 367-375

Muluneh Tamru. (2006). Assessing Diversity in Yams (*Dioscorea spp*.) from Ethiopia based on, Morphology, AFLP Markers and Tuber Quality, and Farmers Management of Landraces. A dissertation submitted for the degree of doctor of Agricultural Sciences, George-Augest University, Germany, Culvillier Verlag, Göttingen.

Mohammadi, S. and Prasanna , B., (2003). Analysis of Genetic Diversity in Crop Plants Statistical Tools and Considerations. Crop science 4th edition, Prentice Hall.

Martin, F., and Rhodes, A., (1977). Intra specific classification of *Dioscorea alata*. Quoted in Sayed, M., Andrew, A., Ngadin, L., Ramisah, M., Shah1., and Norizan, M.,(2008) Morphological variability of greater yam (*Dioscorea alata* L.) in Malaysia. Faculty of Agro technology and Food Science and Faculty of Science and Technology, Malaysia

Mwirigi, P., Kahangi, E., Nyende, A. and Mamati, E. (2009). Morphological variability within the Kenyan yam (*Dioscorea* spp.). Journal of Applied Biosciences 16: 894 – 901. ISSN 1997–5902.

Martin, F. and Ruberté, R. (1976). The polyphenol of *Dioscorea alata* (yam) tubers associated with oxidative browning. *J. Agric. Food Chem*. 24 (1): 67-70.

Nei's, M. (1978). Estimation of average heterozygosis and genetic distance from a small number of individuals. *Genetics*, 3 (89). 583–590.

Nweke, F., Ugwu, B., Asadu, C. (1991). Production costs in the yam-based cropping systems of South western Nigeria. Resource and Crop Management Division Research Monograph No. 6. IITA, Ibadan, Nigeria.

Orkwor, G., Asadu, C. (1998). Agronomy In: *Food yams. Advances in research.* (Eds Orkwor, G., Asiedu. R., and Ekanayake,) IITA NRCRI. pp. 105-141.

Otoo, E., Akromah, R., Kolesnik, A. and Astedu, R. (2009). Ethno-botany Morphological characterization of the yam pona complex in Ghana . *African Crop Science Conference Proceedings*, 9: 407 – 414 Uganda.

Onwueme, I. (1978). The Tropical Tuber Crops: Yam, Cassava, Sweet Potato and Cocoyams. John Wiley & Sons LtD., Chichester, USA, pp 234.

Onwueme, I. C. and Charles, W.B. (1994). Tropical root and tuber crops production, prospective and future prospects. FAO Plant Production and Protection Paper, Rome, Italy.

Okaka, J., Okorie, P. and Ozon, O., (1991). Quality Evaluation of sun dried yam chips. *Tropical Science* 30: 365-275.

Orkwor, G., (1998). The importance of yams. In: *Food yams. Advances in research.* Orkwor, G.C., Asiedu, R. and Ekanayake, I..J.editors. International Institute of Tropical Agriculture, ibadan, Nigeria and National root Crops Research Institute, Umudike, Nigeria.

Opara, L. (1999). Yam storage. In: *CIGR Handbook of Agricultural Engineering Volume IV Agro Processing*. The American Society of Agricultural Engineers, St. Joseph, MI.

Osagie, A.(1992). The yam tuber in storage. Post Harvest Research Unit, University of Benin, Nigeria .

Osagie, A. and Eka, O. (1998). Chemical Composition of Root and Tuber crops. In: *Nutritional quality of plant foods.* Department of biochemistry, University of Benin, Nigeria.

Paul, G. (2004). Crop Domestication as a Long term Selection Experiment. Department of Agronomy and Range Science University of California, USA.

Peakall, R., and Smouse, P. (2006). GenAlEx 6: Genetic analysis in Excel Population Genetic software. Mol. Ecol. Notes 6, 288-295.

Pavlicek, A., Hrda, S. and Flegr, J. (1999). Free tree free ware program for construction of phylogenetic trees on the basis of distance data and bootstrap/Jack Knife analysis of the tree robustness. Application in the RAPD analysis of genus *Frenkelia*. *Folia. Biologica.* **45**:97-99.

Rasper, V. and Coursey, D. G. (1967). Properties of starches of some West African yams. *J. Sci. Food Agric*. **18**: 240-244.

Rolf, F. (1998). NTSYSpc Numerical Taxonomy and Multivariate Analysis System, Exeter software, New York, USA.

Rous, K. (1995).Optimization and troubleshooting in PCR, *in PCR primer.* Diefferbach, C.W. and Dveksler, G..S. editors. CSH press, New York.

SAS (1999). *SAS User's guide*. Released 8.2 edition. SAS Institute Inc., Cary, North Carolina.

Sayed, M., Andrew, A., Ngadin, L., Ramisah, M., Shah1., and Norizan, M.,(2008) Morphological variability of greater yam (*Dioscorea alata* L.) in Malaysia. Faculty of Agro technology and Food Science and Faculty of Science and Technology, Malaysia.

Rodgers, C.P., Smith, D.W, and Bosland, P.W. (1997). A novel statistical approach to analyse genetic resource evaluations using capsicum as an example. *Crop Science* 37:1000-1002.

Sebsebe Demissew, Nordal, I. and Stabbetorp, P.O. (2003). Flowers of Ethiopia and Eritrea: Aloes and other Lilies. 1st edition. Shama Books, Addis Ababa Ethiopia.

Sebsebe Demissew and Ingel, N. (2010). Aloes and Lilies of Ethiopia and Eritrea. Oslo, Norway.

Sen, H. and Das, P. (1991). Relative performance of newly introduced white yams (*Dioscorea rotundata*) in Gangetic plains West Bengal. *Journal of Root Crops* 17(2):150-151

Singh, B.D. (1990). Plant Breeding: Principles and Methods. Kalyani Publishers, New Delhi Ludhiana.

Sneath, P. and Sokal, R. (1973) . Principles of Numerical Taxonomy.Freeman. San Francisco.

Smartt, J. and Simmonds, N.W. (1995) quoted in Paul, G. (2004). Crop Domestication as a Long term Selection Experiment. Department of Agronomy and Range Science University of California, USA.

Ugwu, F. (2009) .The potentials of roots and tubers as weaning food. *Pakistan Journal of Nutrition*. 8: 1701-1705.

Vavilov, N. (1951). Quoted in Muluneh Tamru. (2006). Assessing Diversity in Yams (*Dioscorea spp*.) from Ethiopia based on, Morphology, AFLP Markers and Tuber Quality, and Farmers Management of Landraces.

Wanasundera, J. and Ravindran, G. (1994). Nutritional assessment of yam (*Dioscorea alata*) tubers. Plant Foods for Human Nutrition 46(1), 33–39. Quoted in Jane N. (2010). Yam Nutrition, nutrient disorders and soil fertility management. Australian Center for International Agricultural Research.

Wang, S., and Liu, H.(2008). Characterisation and preliminary lipid-lowering evaluation of starch from chinese yam. *Food Chemistry*, 108: 176 – 81

Wilkin, P. (2001). *Dioscoreaceae* of South and Central Africa, *Kew Bulletin*. 56:361-404.

Wilkin, P., (1998). A morphometric study of *Dioscorea quartiniana*. A. Rich (*Dioscoreaceae*). *Kew Bulletin* 54:1–18

Westpal, E. (1975). Agricultural system in Ethiopia. Center for Agricultural Publishing and Documentation, Wageningen, The Netherlands, PP 278.

Wendawek Abebe (2008). Morphological and Molecular Characterization of Cultivated Guinea Yam Accessions and their Wild Relatives (*Dioscorea cayenensis*) from South and South West Ethiopia. A Thesis Submitted to the School of Graduate Studies of Addis Ababa University in partial fulfilment of the requirement for the Degree of Doctor of Philosophy in Biology.

Wendawek Abebe, Sebsebe Demissew, Fay M.F., Smith, R.J., Nordal, I. and Wilkin, P. (2012). Genetic diversity and species delimitation in the cultivated and wild Guinea yams (*Dioscorea* spp.) from Southwest Ethiopia as determined by AFLP (amplified fragment length polymorphism) markers. *Genet Resour Crop Evol* DOI 10.1007/s10722-012-9925-4.

Wendawek Abebe, Sebsebe Demissew , Fay M.F., Smith, R.J., Nordal, I. and Wilkin, P. (2012). Genetic diversity and population structure of Guinea yams and their wild relatives in South and South West Ethiopia as revealed by microsatellite markers. *Genet Resour Crop Evol* DOI 10.1007/s10722-012-9856-0.

Yang, D.J., Lu, T.J. and Huang, L.S. (2009). Effect of endogenous glycosidase on stability of steroidal saponins in Taiwanese yam (*Dioscorea pseudojaponica)* during drying processes. *Food Chemical*, 113: 155-159.

Yeh, F. And Boyle, T.(1997). Population genetic analysis of co-dominant markers and quantitative traits. Belgian Journal of Botany, 129: 157.

Zinash , D. (2008). Minimizing Postharvest Losses in Yam(*Dioscorea spp.*): Department of Agricultural and Bioresources Engineering,Federal University of Technology, Minna, Nigeria In: *Using Food Science and Technology to Improve Nutrition and Promote National Development*, Robertson, G.L. & Lupien, J.R. (Eds), International Union of Food Science & Technology.

8. Appendices

Apendix 1. List of Collection localities of the 210 accessions of Ethiopian yam (*Dioscorea* spp) studied for genetic diversity using SSR based on ten geographical sites

SN	Acc.	Zone	Location	Species	Altitude (Local name)
1	Si1	Gamogofa	Kucha	*D. rotundata*	1500 (Bunne3)
2	Si2	Sidama	Dalle	*D. rotundata*	1940 (Gellawcho-1)
3	Si3	Sidama	Dalle	*D. rotundata*	1940 (Gelawcho-2)
4	Si4	Sidama	Dalle	*D. rotundata*	1940 (Midasho-1)
5	Si5	Sidama	Dalle	*D. rotundata*	1940 (Midasho-2)
6	Si6	Sidama	Dalle	*D.bulbifera*	1940 (Ouwisho-1)
7	Si7	Sidama	Dalle	*D.bulbifera*	1940 (Ouwisho-2)
8	Si8	Sidama	Dalle	*D.bulbifera*	1940 (Ouwisho-3)
9	Si9	Sidama	Dalle	*D.bulbifera*	1940 (Wendu-1)
10	Si10	Sidama	Dalle	*D. rotundata*	1940 (Adameado-1)
11	Si11	Sidama	Dalle	*D. rotundata*	1940 (Adameado-2)
12	Si12	Sidama	Dalle	*D. rotundata*	1940 (Wendu-2)
13	Si13	Sidama	Dalle	*D.bulbifera*	1940 (Wendu-3)
14	Si14	Sidama	Dalle	*D.bulbifera*	1940 (Wendu-4)
15	Si15	Sidama	Dalle	*D. rotundata*	1940 (Genticha-1)
16	Si16	Sidama	Dalle	*D. rotundata*	1940
17	Si17	Sidama	Dalle	*D. cayenensis*	1940 (Genticha-2)

18	Si18	Sidama	Dalle	*D. cayenensis*	1940 (Genticha-3)
19	Si19	Sidama	Dalle	*D. cayenensis*	1940 (Genticha-4)
20	Si20	Sidama	Dalle	*D. cayenensis*	1940
21	Si21	Sidama	Dalle	*D. praehensilis*	1940 (Gellawcho-1)
22	Si22	Sidama	Dalle	*D. praehensilis*	1940
23	Si23	Sidama	Dalle	*D. rotundata*	1940 (Adameado)
24	Si24	Sidama	Dalle	*D. rotundata*	1940 (Gellawcho-2)
25	Si25	Sidama	Dalle	*D. cayenensis*	1940 (Genticha-5)
26	Si26	Sidama	Dalle	*D. cayenensis*	1940 (Genticha-6)
27	Si27	Sidama	Dalle	*D. rotundata*	1940 (Midasho)
28	Si28	Sidama	Dalle	*D. cayenensis*	1940 (Genticha-7)
29	Si29	Sidama	Dalle	*D. praehensilis*	1940 (Wendu-5)
30	Si30	Sidama	Dalle	*D. praehensilis*	1940 (Wendu-6)
31	Si31	Sidama		*D.bulbifera*	
32	Wo1	Wolayita	Kedida Gamela	*D.bulbifera*	1970
33	Wo2	Wolayita	Kedida Gamela	*D.bulbifera*	1970
34	Wo3	Wolayita	Damot woyde	*D.bulbifera*	1780 (Oha-1)
35	Wo4	Wolayita	-	*D. rotundata*	
36	Wo5	Wolayita	-	*D. rotundata*	
37	Wo6	Wolayita	-	*D. rotundata*	
38	Wo7	Wolayita	-	*D. rotundata*	
39	Wo8	Wolayita	-	*D. rotundata*	

40	Wo9	Wolayita	-	*D. rotundata*	
41	Wo10	Wolayita	-	*D. rotundata*	
42	Wo11	Wolayita	Damotgalle	*D. rotundata*	1950 (Gassa)
43	Wo12	Wolayita	Sodo zuria	*D. rotundata*	1780 (Oha 2)
44	Wo13	Wolayita	Sodo zuria	*D. rotundata*	1780 (Oha 3)
45	Wo14	Wolayita	Sodo zuria	*D. rotundata*	1780 (Oha 4)
46	Wo15	Wolayita	Sodo zuria	*D. rotundata*	1850 (Chocha-1)
47	Wo16	Wolayita	Sodo zuria	*D. rotundata*	1850 (Chocha-2)
48	Wo17	Wolayita	-	*D. abyssinica*	
49	Wo18	Wolayita	-	*D. abyssinica*	
50	Wo19	Wolayita	Kedida Gamela	*D. abyssinica*	1970
51	Wo20	Wolayita	Kedida Gamela	*D. abyssinica*	1970
52	Wo21	Wolayita	Kedida Gamela	*D. abyssinica*	1970
53	Wo22	Wolayita	Alabana tembar	*D. abyssinica*	1630
54	Wo23	Wolayita	Alabana tembar	*D. abyssinica*	1630
55	Wo24	Wolayita	Alabana tembar	*D. abyssinica*	1630
56	Wo25	Wolayita	-	*D. abyssinica*	
57	Wo26	Wolayita	-	*D. abyssinica*	
58	Wo27	Wolayita	Damot woyde	*D. abyssinica*	1780 (Wiyacha-1)
59	Wo28	Wolayita	Damot woyde	*D. abyssinica*	1780 (Wiyacha-2)
60	Wo29	Wolayita	-	*D. abyssinica*	
61	Wo30	Wolayita	Damotwoyde	*D.bulbifera,*	1780 (Oha-5)

62	Wo31	Wolayita	Damotwoyde	*D.bulbifera,*	1780 (Oha-6)
63	Wo32	Wolayita	-	*D.bulbifera,*	
64	Wo33	Wolayita	Kokate	*D.bulbifera,*	Chaye Woncharo
65	Wo34	Wolayita	Damotgalle	*D. praehensilis*	2220 (Arkiya)
66	Wo35	Wolayita	Areka	*D. praehensilis*	1870 (Gaffella-1)
67	Wo36	Wolayita	Areka	*D. praehensilis*	1870 (Gaffella-2)
68	Wo37	Wolayita	Areka	*D. praehensilis*	1870 (Gaffella-3)
69	Wo38	Wolayita	-	*D. praehensilis*	
70	Wo39	Wolayita	-	*D. praehensilis*	
71	Wo40	Wolayita	Areka	*D. praehensilis*	1870 (Gaffela-4)
72	Wo41	Wolayita	Areka	*D. praehensilis*	1870 (Gaffela-5)
73	Wo42	Wolayita	-	*D.bulbifera*	
74	Wo43	Wolayita	-	*D. cayenensis*	(Wancharo-1)
75	Wo44	Wolayita	-	*D. cayenensis*	(Wancharo-2)
76	Wo45	Wolayita	-	*D. cayenensis*	(Wancharo-3)
77	Wo46	Wolayita	-	*D. cayenensis*	-
78	Wo47	Wolayita	Damotgalle	*D. praehensilis*	2220 (Arkiya-1)
79	Wo48	Wolayita	Damotgalle	*D. praehensilis*	2220 (Arkiya-2)
80	Wo49	Wolayita	-	*D.alata*	-
81	Wo50	Wolayita	Damotwoyde	*D. abyssinica*	2220 (Wiyacha-1)
82	Wo51	Wolayita	Damotwoyde	*D. abyssinica*	2220 (Wiyacha-2)
83	Wo52	Wolayita	Damotwoyde	*D. abyssinica*	2220 (Wiyacha-3)

84	Wo53	Wolayita	-	*D. abyssinica*	-
85	Eg1	East Wolega	Diga	*D. rotundata*	1650 (Lalo-1)
86	Eg2	East Wolega	Diga	*D. rotundata*	1650 (Lalo-2)
87	Eg3	East Wolega	Diga	*D. rotundata*	1650 (Lalo-3)
88	Eg4	East Wolega	Diga	*D. cayenensis*	1650 (Msreta-1)
89	Eg5	East Wolega	Diga	*D. cayenensis*	1650 (Msreta-2)
90	Eg6	East Wolega	Diga	*D. cayenensis*	1650 (Msereta-3)
91	Eg7	East Wolega	Sasiga	*D. cayenensis*	1750 (Haro-1)
92	Eg8	East Wolega	Sasiga	*D. cayenensis*	1750 (Haro-2)
93	Eg9	East Wolega	Sasiga	*D. cayenensis*	1750 (Haro-3)
94	Eg10	East Wolega	Diga	*D.bulbifera,*	1650 (Dhoknuma-1)
95	Eg11	East Wolega	Diga	*D.bulbifera,*	1650 (Dhoknuma-2)
96	Eg12	East Wolega	Diga	*D. rotundata*	1650 (Dhokuma-3)
97	Ar1	ARC	ARC	*D.bulbifera*	1780
98	Ar2	ARC	ARC	*D .alata*	1780
99	Ar3	ARC	ARC	*D.bulbifera*	1780
100	Ar4	ARC	ARC	*D. cayenensis*	1780
101	Ar5	ARC	ARC	*D. cayenensis*	1780
102	Ar6	ARC	ARC	*D. cayenensis*	1780
103	Ar7	ARC	ARC	*D. cayenensis*	1780
104	Ar8	ARC	ARC	*D. cayenensis*	1780
105	Ar9	ARC	ARC	*D. cayenensis*	1780

106	Ar10	ARC	ARC	*D. cayenensis*	1780
107	Ar11	ARC	ARC	*D. cayenensis*	1780
108	Ar12	ARC	ARC	*D. praehensilis*	1780
109	Ar13	ARC	ARC	*D. praehensilis*	1780
110	Ar14	ARC	ARC	*D. praehensilis*	1780
111	Ar15	ARC	ARC	*D. praehensilis*	1780
112	Ar16	ARC	ARC	*D. praehensilis*	1780
113	Ar17	ARC	ARC	*D. rotundata*	1780
114	Ar18	ARC	ARC	*D.alata*	1780
115	Ar19	ARC	ARC	*D.alata*	1780
116	Ar20	ARC	ARC	*D.alata*	1780
117	Ar21	ARC	ARC	*D. rotundata*	1780
118	Ar22	ARC	ARC	*D.alata*	1780
119	Ar23	ARC	ARC	*D.alata*	1780
120	Ar24	ARC	ARC	*D.alata*	1780
121	Ar25	ARC	ARC	*D.bulbifera*	1780
122	Ar26	ARC	ARC	*D.bulbifera*	1780
123	Ar27	ARC	ARC	*D.bulbifera*	1780
124	Ar28	ARC	ARC	*D. praehensilis*	1780
125	Ar29	ARC	ARC	*D. praehensilis*	1780
126	Ar30	ARC	ARC	*D. praehensilis*	1780
127	Ar31	ARC	ARC	*D. praehensilis*	1780

128	Ar32	ARC	ARC	*D. praehensilis*	1780
129	Ar33	ARC	ARC	*D. praehensilis*	1780
130	Ar34	ARC	ARC	*D.alata*	1780
131	Ga1	Gamo Gofa	Gofa	*D. praehensilis*	1340
132	Ga2	Gamo Gofa	Kucha	*D. rotundata*	1500 (Bunne-3)
133	Ga3	Gamo Gofa	Bonke	*D. abyssinica*	2070 (Kemba-1)
134	Ga4	Gamo Gofa	Bonke	*D. praehensilis*	2070 (Kemba-2)
135	Ga5	Gamo Gofa	Bonke	*D. praehensilis*	2070 (Kemba-3)
136	Ga6	Gamo Gofa	Arbaminch	*D. praehensilis*	1140 (Hatiya-2)
137	Ga7	Gamo Gofa	Breda	*D. praehensilis*	1655 (Bunne-2)
138	Ga8	Gamo Gofa	Gofa	*D. praehensilis*	1340 (Tolla-1)
139	Ga9	Gamo Gofa	Gofa	*D. praehensilis*	1340 (Tolla-2)
140	Ga10	Gamo Gofa	Gofa	*D. praehensilis*	1340 (Tolla-3)
141	Ga11	Gamo Gofa	Gofa	*D. praehensilis*	1340 (Tolla-3)
142	Ga12	Gamo Gofa	Gofa	*D. praehensilis*	1340 (Bunne-1)
143	Ga13	Gamo Gofa	Gofa	*D. praehensilis*	1340 (Bunne-2)
144	Ga14	Gamo Gofa	Gofa	*D. praehensilis*	1340
145	Ga15	Gamo Gofa	Gofa	*D. praehensilis*	1340
146	Ga16	Gamo Gofa	Gofa	*D. praehensilis*	1340
147	Ga17	Gamo Gofa	Gofa	*D. praehensilis*	1340
148	Ga18	Gamo Gofa	Gofa	*D. praehensilis*	1340
149	Ga19	Gamo Gofa	Gofa	*D. praehensilis*	1340

150	Ga20	Gamo Gofa	Gofa	*D. praehensilis*	1340
151	Ga21	Gamo Gofa	Bonke	*D. cayenensis*	1540 (Arfa-1)
152	Ga22	Gamo Gofa	Bonke	*D. cayenensis*	1540 (Arfa-2)
153	Ga23	Gamo Gofa	Bonke	*D. cayenensis*	1540 (Arfa-3)
154	Ga24	Gamo Gofa	Bonke	*D. cayenensis*	1540 (Arfa-4)
155	Ga25	Gamo Gofa	Gofa	*D. cayenensis*	1340
156	Ga26	Gamo Gofa	Gofa	*D. praehensilis*	1340
157	Ga27	Gamo Gofa	Kucha	*D. praehensilis*	1500 (Bunne-3)
158	Ga28	Gamo Gofa	Gofa	*D. praehensilis*	1340 (Tolla)
159	Ga29	Gamo Gofa	Arba minch	*D. praehensilis*	1140 (Bunne-3)
160	Ga30	Gamo Gofa	Arba minch	*D. praehensilis*	1140 (Bunne-4)
161	Ga31	Gamo Gofa	Arba minch	*D. praehensilis*	1140 (Bunne-5)
162	Ga32	Gamo Gofa	Arba minch	*D. praehensilis*	1140 (Bunne-6)
163	Ga33	Gamo Gofa	Bonke	*D. abyssinica*	1540 (Kemba-1)
164	Ga34	Gamo Gofa	Bonke	*D. abyssinica*	1540 (Kemba-2)
165	Ga35	Gamo Gofa	Bonke	*D. abyssinica*	1540 (Kemba-3)
166	Ga36	Gamo Gofa	Gofa	*D. abyssinica*	1340
167	Gd1	Gedio	Gedio	*D. praehensilis*	1590
168	Gd2	Gedio	Gedio	*D. praehensilis*	1590
169	Gd3	Gedio	Gedio	*D. praehensilis*	1590
170	Gd4	Gedio	Gedio	*D.bulbifera*	1590
171	Gd5	Gedio	Gedio	*D.bulbifera*	1590

172	Gd6	Gedio	Gedio	*D.bulbifera*	1770 (Ganticho1)
173	Gd7	Gedio	Gedio	*D. abyssinica*	1770 (Ganticho2)
174	Gd8	Gedio	Wonago	*D. cayenensis*	1770
175	Gd9	Gedio	Wonago	*D. cayenensis*	1770
176	Gd10	Gedio	Wonago	*D. cayenensis*	1770
177	Gd11	Gedio	Wonago	*D. rotundata*	1770
178	Gd12	Gedio	Wonago	*D. rotundata*	1770
179	Ib1	Kefa	Kefa	*D. abyssinica*	1600
180	Ib2	Kefa	Kefa	*D. abyssinica*	1600
181	Ib3	Kefa	Kefa	*D. abyssinica*	1600
182	Ib4	Kefa	Kefa	*D. abyssinica*	1600
183	Ib5	Kefa	Kefa	*D. abyssinica*	1600
184	Ib6	Kefa	Kefa	*D. abyssinica*	1600
185	K1	Kembata	Hadero	*D. abyssinica*	1140
186	K2	Kembata	Hadero	*D. praehensilis*	1140 (Makawa-1
187	K3	Kembata	Hadero	*D. praehensilis*	1140 (Makawa-2)
188	J1	Jima	Jima	*D. praehensilis*	1780
189	J2	Jima	Jima	*D. rotundata*	1780
190	J3	Jima	Jima	*D. rotundata*	1780
191	J4	Jima	Jima	*D.alata*	1780
192	J5	Jima	Jima	*D.alata*	1780
193	J6	Jima	Jima	*D.alata*	1780

194	J7	Jima	Jima	*D.alata*	1780
195	J8	Jima	Jima	*D.bulbifera*	1780
196	J9	Jima	Jima	*D.alata*	1780
197	J10	Jima	Jima	*D.alata*	1780
198	J11	Jima	Jima	*D.alata*	1780
199	J12	Jima	Jima	*D.bulbifera*	1780
200	J13	Jima	Jima	*D.bulbifera*	1780
201	J14	Jima	Jima	*D.alata*	1780
202	Da1	Dauro	Konta	*D. abyssinica*	1900 (Gebiche-1)
203	Da2	Dauro	Konta	*D. abyssinica*	1900 (Gebiche-2)
204	Da3	Dauro	Konta	*D. abyssinica*	1900 (Gebiche-3)
205	Da4	Dauro	Konta	*D. abyssinica*	1900 (Gebiche-4)
206	Da5	Dauro	Mareka	*D.bulbifera*	1580 (Dorsita-1)
207	Da6	Dauro	Mareka	*D.bulbifera*	1580 (Dorsita-2)
208	Da7	Dauro	Mareka	*D.bulbifera*	1580 (Dorsita-3)
209	Da8	Dauro	Mareka	*D.bulbifera*	1580 (Dorsita-4)
210	Da9	Dauro	Mareka	*D.bulbifera*	1580 (Dorsita-5)

Notes: - Acc.= accessions