GENETIC DIVERSITY, NUTRITIONAL AND PHYTOCHEMICAL COMPOSITION, AND MICROPROPAGATION OF ENSET (*Ensete ventricosum* (Welw.) Cheesman) LANDRACES USED IN TRADITIONAL MEDICINE

BY

GIZACHEW WOLDESENBET NURAGA

A THESIS SUBMITTEDTOINSTITUTE OF BIOTECHNOLOGY, ADDIS ABABA UNIVERSITYIN PARTIAL FULFILLMENT OF THE REQUIREMENTS FORTHE DEGREE OF DOCTOR OF PHILOSOPHY IN BIOTECHNOLOGY (AGRICULTURAL BIOTECHNOLOGY)

ADDIS ABABA

JULY 2020

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JULY 2020

DEDICATION

This thesis is dedicated to my family: My parents, who are the foundation for my success, and to my late eldest brother, Tenkir Woldesenbet, who was the pillar of the family. I also dedicate the thesis to my lovely wife Aster Tiruha for her unreserved support and inspiration throughout the study period.

ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
INSTITUTE OF BIOTECHNOLOGY

Genetic Diversity, Nutritional and Phytochemical composition, and Micropropagation of Enset (Ensete ventricosum (Welw.) Cheesman) landraces used in traditional medicine

By

Gizachew Woldesenbet

A Thesis Presented to the School of Graduate Studies of the Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Biotechnology

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I. Gizachew Woldesenbet Nuraga, Tileye Feyissa, Kassahun Tesfaye, Sebsebe Demissew and Zerihun Tadele (2019). Phenotypic diversity of enset (*Ensete ventricosum* (Welw.) Cheesman) landraces used in traditional medicine. *Genetic Resources and Crop Evolution*, 66 (8): 1761–1772.

II. Gizachew Woldesenbet Nuraga, Tileye Feyissa, Sebsebe Demissew, Kassahun Tesfaye and Ashagrie Zewdu Woldegiorgis (2019). Comparison of proximate, mineral and phytochemical composition of enset (*Ensete ventricosum* (Welw.) Cheesman) landraces used for a different purpose. *African Journal of Agricultural Research* 14(30):1326-1334.

ACRONYMS AND ABBREVIATIONS

AFLP: Amplified Fragment Length Polymorphism;

AMoVA: Analysis of Molecular Variance;

ANoVA: Analysis of Variance;

BAP: 6-benzyl amino-purine

CRD: Completely randomized design

CV: Coefficient of Variation;

CTAB: Cetyltrimethylammonium bromide;

DAP: Diammonium phosphate;

EIAR: Ethiopian Institute of Agricultural Research;

GD: Genetic Distance;

IBA: Indole-3-butyric acid;

ISSR: Inter Simple Sequence Repeats;

MS: Mean Square;

NJ: Neighbor-Joining;

PCA: Principal Component Analysis;

PCoA: Principal Coordinate Analysis;

PGRs: Plant Growth Regulators;

PIC: Polymorphic Information Content;

RAPD: Random Amplified Polymorphic DNA;

RFLP: Restricted Fragment Length Polymorphism;

SAS: Statistical Analysis System;

SNNP: Southern Nations Nationalities and Peoples Regional State;

SSR: Simple Sequence Repeats;

TDCP: Total Digestible Crude Protein;

TDM: Total Dry Matter;

TME: Total Metabolizable Energy;

UPGMA: Unweighted pair-group method with arithmetic average

Abstract

Enset (*Ensete ventricosum* (Welw.) Cheesman) is a multipurpose food crop extensively cultivated in southern and southwestern parts of Ethiopia. In addition to its food and feed value, some enset landraces are also used in traditional medicine in some parts of the country. The current study was conducted to investigate the genetic diversity, nutritional and phytochemical composition of enset landraces used in traditional medicine, and also to develop micropropagation protocol. Forty enset landraces used in traditional medicine were identified from four Administrative Zones and one special District in the Southern Nations, Nationalities and Peoples Region of Ethiopia. The genetic diversity of these landraces was assessed using 14 qualitative traits and 15 simple sequences repeats (SSR) markers. Proximate, mineral and phytochemical compositions of corms of three of the landraces (*astara*, *kibnar* and *guarye*) used in traditional medicine were analysed by following standard methods, and compared with two landraces (*amerat* and *yeshiraqinke*) that have other use-values. Micropropagation of two most important landraces (*guarye* and *kibnar*) using shoot tip explants was conducted on Murashige and Skoog medium containing different concentrations of plant growth regulators, 6-benzyl amino-purine (BAP) and Indole-3-butyric acid (IBA), and cefotaxime antibiotics. Although there is a slight variation in grouping pattern, both the principal component and cluster analysis of the qualitative data clustered the landraces in to 4, irrespective of their origin. Both analyzes showed that, the landraces with different vernacular names have distinct character. The principal component analysis also showed that all the 14 qualitative traits contributed for the variation among the landraces. In the analysis of proximate, mineral and phytochemical composition, the three landraces used in traditional medicine, generally showed higher protein, phosphorus, zinc and tannin contents than other landraces. This could be the reason for selective use of these landraces in traditional medicine, as the compounds are reported to have different health benefit. In the genetic diversity study using SSR markers, a total of 38 alleles, were detected. Similar to qualitative data, cluster analysis of SSR data grouped the landraces in to 4, but in different clustering pattern. Except two (*bisha-eset* and *mekelwesa)*, all the landraces with different vernacular names were genetically diverse, confirming that vernacular names are good indicators of genetic distinctiveness. At the initiation stage of micropropagation, significantly highest contamination (17.67%) was recorded from an MS medium without cefotaxime than remaining two treatments (250 mg l^{-1} and 500 mg l^{-1}). The highest mean shoot number (8.8±1.40) per shoot tip was regenerated on MS medium containing 5.0 mgl^{-1} BAP + 1.0 mg l^{-1} IBA, and from the two landraces, higher mean shoot number (6.88) per shoot tip was obtained from *guarye*. The highest root number (3.0) per shoot was obtained from both full and half-strength MS medium containing 3.0 mgl^{-1} IBA and 2.0 mgl^{-1} IBA, respectively. The result showed that almost all landraces with differnet vernacular name were genetically distinct and shall be conserved. Landraces used in traditional medicine are, generally higher in protein, phosphorus, zink and tannin content than other landraces, and the selective use of these landraces in the treatment of bone fracture, traditionally, is reasonable. Enset landraces, *guarye* and *kibnar*, can be successfully propagated *in vitro*, and the protocol developed will be used for rapid multiplication the landraces, and it can be applied for related genotypes.

Keywords: *Ensete ventricosum;* genetic diversity; micropropagation; nutritional composition; phenotypic traits; SSR markers; phytochemical

Chapter 1.

INTRODUCTION

1.1. Background and Justification

The genus *Ensete* falls within the family *Musaceae*, which includes the genus *Musa*, comprising the edible bananas and plantains (Borell *et al*., 2019). According to Kress (1990), seven enset species which are distributed in Africa, India and China are recognized. *Ensete ventricosum* is herbaceous, monocarpic and monocotyledonous large banana-like evergreen plant that grows 4-8m (sometimes even up to 11m) in height. Since edible parts of enset plant are the pseudostem and corm instead of the fruit as in banana, it is often called "false banana". It is one of the oldest cultivated plants in Ethiopia, and its domestication is arguably as early as 10000 years ago (Brandt *et al.* 1997). Since it can tolerate prolonged drought and flooding, it is considered as a priority crop in Ethiopia, where it makes a major contribution to the food security of the country. Regions growing enset as staple food are usually less affected by the recurrent drought periods that occurred in Ethiopia.

Enset crop is known to adapt a wide range of altitudes from 1200 to 3100 meters above sea level. Its productivity per land use is high, and the areas where it is used as a staple food are characterized by a high density of human population, which hardly can be supported with any other type of land use. Enset has several food and non-food uses, and is highly integrated in the economic, social and cultural life of the highlands of southern, southwestern and central Ethiopia (Admasu Tsegaye and Struik, 2001). Enset can be harvested at any time of the year during shortage of food and when other crops fail due to different factors, and the products are

easily stored in a pit for many years without a need for modern storage facility. According to Borrell *et al*. (2019), enset provides a staple food source for approximately 20 milion people in major enset growing region of Ethiopia.

Kocho and bulla are the main food products of enset, which are mainly obtained by fermenting a mixture of scraped pulp of pseudostem and pulverized corm. Many different dishes are prepared from these products, of which, the most common are pancake-like bread and porridge. Moreover, the fresh corm can be cooked and consumed in a similar way as potato, cassava, and other root and tuber crops, especially during shortage of food. The crop also serves as a source of cash and, the fiber and the midrib are used for the production of ropes and making mats. The leaves are used for animal feed, baking bread, wrapping, and lining the *kocho* fermentation pit.

In addition to the wide use of enset as food, animal feed and production of household materials, some enset landraces play essential roles in traditional medicine. Corms of a number of enset landraces are reported to have medicinal importance in the treatment of bone fracture or other bone related human ailments, and to cure similar disorders in domestic animals (Yemane Tsehaye and Facil Kebebew, 2006). It can be hypothesized that this could be attributed to high calcium or phosphorus contents of certain landraces to help fast recovery of bone related disorders and/or some of the landraces contain phytochemicals that have some benefits in healing of wounds. This is supported by a report from Hölscher and Schneider (1998), who identified a novel phenylphenalenone in enset, that have anti-bacterial, anti-cancer, and nematicidic property. The othe medicinal use of enset is some to facilitate placental discharge

after birth or miscarriage both in humans and livestock (Yemane Tsehaye and Facil Kebebew, 2006; Temesgen Magule *et al*., 2014).

Since some of enset landraces used in traditional medicine; such as *astara*, *guarye* and *kibnar* are the sweet types; they are highly preferred and selectively attacked by wild animals attacking the crop, like porcupine and wild pig (Personal observation; Feta Negash 2007). Moreover, in Gurage area, farmers that have 'medicinal' enset landraces in their farms are obliged to give freely or with low price for any sick person requesting, and those who refuse are considered as evil persons. Hence most farmers are showing less interest to cultivate these landraces, and the production of these specific groups of enset landraces is considerably reduced in recent years (personal observation). Unless some measure is taken, this condition may lead to the loss of some important landraces. Seifu Gebre-Mariam (1996) and Almaz Negash *et al*. (2002) also reported the loss of some valuable enset genotypes due to various human and environmental factors.

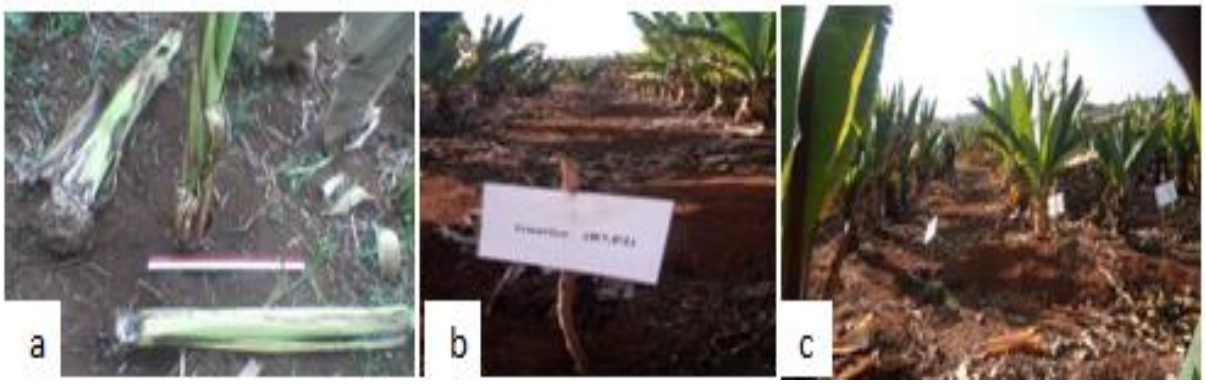

Figure 1.1 Selective attack of 'medicinal' enset landraces by rodents (porcupine and wild pig) a) in a farmer home garden (Feta Negash, 2007), b) and c) rows of 'medicinal' landraces totally abandoned, at research field of Wolkite University (photo by: Gizachew Woldesenbet)

In many enset growing areas, the major uses of enset in the traditional medicine is to feed a corm in repairing bone fracture. Bone is mainly composed of calcium and phosphorus (Veis and Sabsay, 1987) and an immediate increase in metabolic demand during bone fracture, and the high energy requirement of the patient for early healing was reported (Smith, 1987). According to Heaney (2004) adequate phosphorus intake is important for bone building. Hence, enset landraces used in treatment of bone fractures are expected to be rich in calcium (Ca), phosphorus (P) and/or caloric content as compared to other types.

Ayalew Debebe (2006) reported that generally, enset is rich in Ca, magnesium (Mg), potassium (K) and iron (Fe). According to Minaleshewa Atlabachew, and Chandravanshi (2008), enset products, *kocho* and *bulla*, from unknown landraces were reported to have the highest Ca and zink (Zn) contents as compared to other similar foodstuffs. Ajebu Nurfeta *et al.* (2008) reported the elemental content of ten enset landraces, in which highest calcium content was recorded from *astara*, 'medicinally' used landrace. Since the information in this regard is generally very scant, the comparative analysis of proximate, mineral and phytochemical composition of the two groups of enset landrace is important in the identification of the sources of the health benefits of enset products.

The loss of valuable genetic resources due to exploitation of natural populations, natural hazards, cultural, political and economic issues, is a global concern. For that reason, gene banks are established in many countries to conserve plant genetic resources to guarantee adequate food supplies and other benefits, like medicinal uses for future human generations (Rao, 2004). The most common methods of conserving the genetic resources of vegetatively propagated

plants, like enset are in a field gene bank, which is very costly in terms of requirements for land, maintenance, and labor. In such cases, a clear understanding of the extent of genetic diversity in a species is essential to reduce unnecessary duplication of germplasm (Rao and Hodgkin, 2002).

Diversity study using phenotypic traits is inexpensive and can be useful for preliminary assessment of genetic diversity among phenotypically distinguishable cultivars. Farmers use phenotypically visible traits to precisely distinguish between cultivars. Chiwona-Karltun *et al.* (1998) reported that farmers distinguished genotypes of cassava better than learned investigators using a standard botanical key. It is evident that phenotypic characterization of enset has contributed considerably to the bulk of knowledge to the present day enset.

In the genetic diversity of enset using morphological or phenotypic traits, only few studies were reported. Yemane Tsehaye and Facil Kebebew (2006) characterized forty two enset landraces growing in Kaffa zone using morphological traits and reported the distinctiveness of the landrace. Cluster analysis of 279 enset accessions conserved in a field gene bank at Areka research center was carried out using qualitative and quantitative phenotypic traits, and the existence of variability based on some of traits was reported (Mikias Yeshitila, 2014). Similarly, Zerihun Yemataw *et al.* (2014, 2018) assessed the diversity of enset landraces conserved at Areka research center using morphological and use value related traits, and reported the existence of considerable variability among the landraces. In these studies, many of landraces used in traditional medicines were not included. Therefore, further exhaustive identification and characterization of the landraces used in traditional medicines is required.

Due to the influence of environment, molecular markers can be used to validate phenotypic study. Molecular markers are powerful tools in the assessment of genetic variation, in the clarification of genetic relationships, and assist in managing of plant genetic resources (Virk *et al*., 2000; Teixeira da Silva, 2005). Simple sequence repeat (SSR) is an efficient molecular marker due to its simplicity, rich polymorphism and stability, and it is usually applied in genetic diversity analysis (Zhang *et al*., 2007).

Almaz Negash *et al*. (2002) and Genet Birmeta *et al*. (2002) analyzed the genetic diversity of enset using amplified fragment length polymorphism (AFLP) and random amplification of polymorphic DNA (RAPD), respectively. These two marker types are dominant, and are not efficient as the co-dominant marker such as the SSRs (Powell *et al*., 1996). Analysis of genetic diversity among cultivated enset populations from two location of Southwestern Ethiopia, using inter simple sequence repeats (ISSRs) marker was also reported (Dagmawit Chombe and Endashaw Bekele, 2011). Selamawit Getachew *et al*. (2014) analyzed the genetic diversity of enset accessions collected from nine zones of Southern Nations, Nationalities and Peoples (SNNP) region using 12 transferable SSR markers of banana. As far as we know, Temesgen Magule *et al*. (2015) and Feta Negash *et al*. (2019) are the only report available regarding the use of SSR markers developed for enset. However, in these studies, many of enset landraces used in traditional medicine were not included. Therefore, a study of genetic diversity and relationship among landraces used in traditional medicine, using effective molecular markers like, SSR is important for effective conservation and utilization.

Enset is commonly propagated vegetatively although it can also be propagated by seeds. Propagation by seed is not a common practice, because harvesting is usually done before the maturity of the crop, and some of the landraces rarely produce seed. Recalcitrant nature of the seed is also another obstacle. Although cultivated enset resembles banana morphologically, mostly it doesn't produce suckers naturally, instead it must be induced artificially by removing the apical meristem of a 2 to 4-year-old suckers. This conventional method of propagation requires large area of land, and results in poor propagation rates (Mulugeta Diro and Endale Tabogie, 1994; Abrham Shumbulo *et al.*, 2012). Moreover, this method is vulnerable to transmission of devastating diseases like bacterial wilt. It is reported that the productivity and area coverage of the crop is declining due to various biotic and abiotic factors (SARI, 2014). In this regard, micropropagation techniques can have a huge potential in overcoming some of the limiting factors, especialy for multiplication of improved varieties and the vulnerable medicinal landraces.

Afza *et al.* (1996) reported the induction of adventitious buds and somatic embryos from corm tissues of *Ensete ventricosum*, unmentioned landrace. Similarly, Mathew and Philip (1996) reported the induction of a proliferating embryogenic callus from the corm tissue of *in vitro* regenerated plants for *E. superbum*, while the attempt of shoot multiplication from unknown species of the genus *Ensete* failed due to high oxidation of phenols (Tilahun Zeweldu and Lüdders, 1998). Almaz Negsh *et al.* (2000) investigated the use of zygotic embryos, corm and leaf tissue explants for initiating cultures of three enset landraces. Mulugeta Diro (2003) also reported *in vitro* regeneration and micropropgation of three *Ensete ventricosum* landraces: *keberia, mazia* and *oniya*. In these investigations, only partial success and/or low rate of

multiplication were achieved. Moreover, since indirect regeneration of adventitious shoots from a callus may result in genetic variability (Krikorian, 1989). Such methods are not applicable for mass propagation. Although high contamination and loss of many explants during culture initiation was recorded, efficient micropropagation of seven enset landraces from shoot-tip explants was reported (Genet Birmeta and Welander, 2004). Indirect *in vitro* regeneration of *bedadeti* enset landrace, from corm discs containing intercalary meristem was also reported (Tripathi *et al*., 2017).

Although enset is a diverse crop, with more than 387 landraces (Zerihun Yemataw *et al*., 2018), micropropagation of only few enset landraces were reported. Previous studies (Genene Gezahegn and Frew Mekbib, 2016, on enset; Asmare Dagnew, 2012, on banana; Bhatia *et al*., 2005 and Tileye Feyyisa, 2005on tomato and *Hagenia abyssinica*, respectively) showed that, growth media requirement during micropropagation is genotype dependent. Hence, developing a protocol for micropropagation of those vulnerable enset landraces used in traditional medicine is very important to facilitate large-scale production of the landraces to fill the gaps of planting material need of the society, and to conserve the existing diversity of enset germplasm.

1.2. Objectives of the Study

General objective:

The general objective of the study was to determine the level of genetic diversity, proximate, mineral and phytochemical composition of enset landraces used in traditional medicine, and to develop a micropropagation protocols for some of the landraces using shoot-tip culture for sustainable production and conservation

Specific objectives:

- To detrmine the extent of phenotypic diversity among enset landraces used in traditional medicine using qualitative traits,
- To determine the proximate, mineral and phytochemical composition of enset landraces used in traditional medicine and to compare with other landraces,
- To determine the extent of genetic diversity among enset landraces used in traditional medicine using SSR markers,
- To develop micropropagation protocol for enset landraces (*guarye* and *kibnar*) used in traditional medicine.

Chapter 2.

LITERATURE REVIEW

2.1. Taxonomy of Enset

The genus *Ensete* falls within the botanical order *Zingiberales*, family *Musaceae*, which also includes the genus *Musa*, comprising the edible bananas and plantains. Unlike banana, its genome is diploid with haploid chromosome number n= 9 (Cheesman, 1947). *Musella*, which is currently recognized as a separate genus with a single species (Li, 1978; Wu and Kress, 2001) was originally included in the genus *Ensete* (Cheesman, 1947; Simmonds, 1960). A Scottish traveler and writer Bruce, was the first person to describe and figure enset under its native name Ensete, and it was referred to the Linnaean genus *Musa* by Gmelin (1791), in the 13th edition of *Systema Naturae*, as cited by Cheesman (1947). According to him, "*Ensete*" was used as the specific name.

Horaninow (1862) was the first to note a noticeable difference of enset from *Musa* and considered as a separate genus *Ensete,* in which he created a single species, *Ensete edule*. However, general recognition was given to the genus only after Cheesman (1947). The assignment of number of species to the genus reported considerably in different ways. Cheesman (1947) characterized the genus *Ensete* Horan., as single-stemmed monocarpic waxy herbs, with pseudostem dilated at the base, persistent green bracts, large seeds, irregularly globose and smooth, and shown that it is different from *Musa* L. According to the author, 25 species were recognized, of which twenty were of African origin and five described as extra-African species. Baker and Simmonds (1953) reduced them to 8 distinct species largely by

including a number of species under *E. ventricosum*. Later on, Simmonds (1960) classified them to 6 species, with one undescribed species, while Kress (1990) listed seven species.

The currently known species includes *E. gilleti* (De Wild.) Cheesman, which is native to West Africa, from Sierra Leone to Angola, *E. glaucum* (Roxb.) Cheesman s distributed over a wide area from Burma to the Philippines and Indonesia, *E. homblei* (Bequaert. ex De Wild.) Cheesman, distributed in the Congo and Zambia, *E. perrieri* (Claverie) Cheesman, endemic to Madagascar, *E. superbum* (Roxb.) Cheesman, native to India, *E. ventricosum* (Welw.) Cheesman, distributed in the central Africa, from Cameroon to Ethiopia and South Africa, and *E.wilsoni*. (Tutcher) Cheesman (Endashaw Bekele, 2019).

2.2. Origin and Distribution of *Ensete ventricosum*

Centre of origin means a geographical area where a plant species (domesticated or wild) first developed its distinctive properties, whereas center of crop diversity means a geographic area containing a high level of genetic diversity in *in situ* conditions (FAO, 2009). Although, the origin of enset is not fully resolved, Vavilov (1951) mentioned that Ethiopia is an independent primary center of origin of enset *(Ensete ventricosum),* which he called Abyssinian banana, and other crop species like, *Eragrostis tef* and *Coffea arabica*. According to Vavilov (1926), as cited by Harlan (1992), a geographic region with a greatest genetic diversity, where wild forms, representing the natural relatives of domesticated crop plants are found, is also the region of origin. Therefore, it is reasonable that Ethiopia is not only primary center of origin, but also center of diversity for *Ensete ventricosum*.

Ensete ventricosum is economically important and domesticated species as food and fiber in Ethiopia, while its wild form occurs in central and South Africa (Endashaw Bekele, 2018). According to the author, in Ethiopia, the wild form of *E. ventricosum* is found at lower altitudes, in a relatively drier area than the cultivated form. The domestication of enset in Ethiopia is argued to be around 10,000 years ago (Brandt *et al.*, 1997).

2.3. Morphological Description of Enset

Enset, which is also called false banana, is a close relative of banana (*Musa* spp.), and morphologically, they are similar. Both are large herbaceous and monocarpic plants that flower only once and then die. Enset is thicker and taller than banana plant, and its fruits are not edible (Admasu Tsegaye and Westphal, 1992). According to the author, enset is a perennial plant, that grows up to 4-11 m height and its root system is adventitious. The large overlapping leaf sheaths, which emerge from the basal portion of the plant forms the above ground stem (pseudostem of the plant, and the underground and basal swollen parts of the leaf sheaths form a corm, which is 0.7-1.8 m long and 1.5-2.5 m in diameter at maturity.

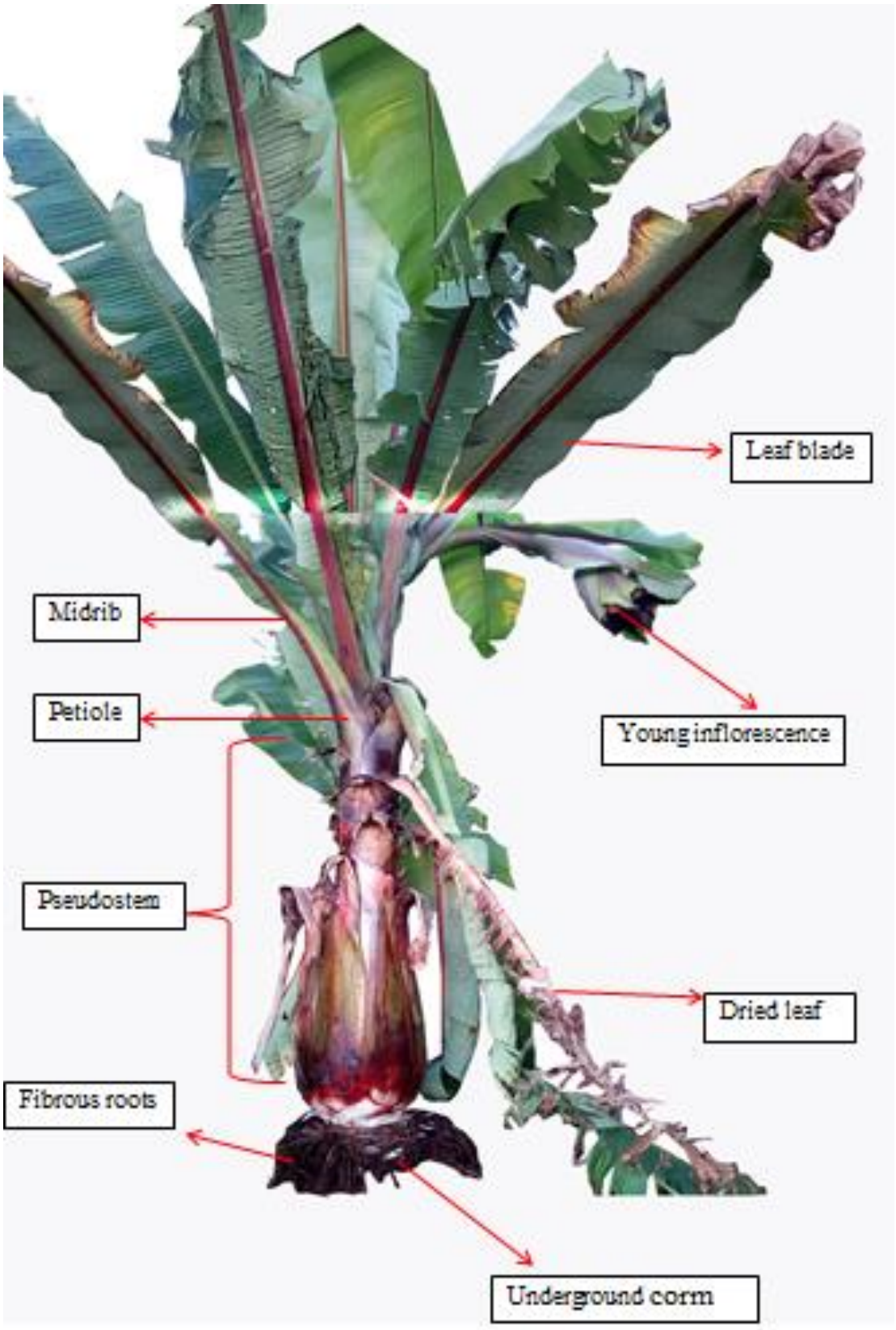

Figure 2.1 Matured *Ensete ventricosum at* early flowering stage

The leaves, that emerge from the apex of the corm are spirally arranged, and have entire leaf blades (5 m long x 1.5 m broad), and strongly channeled midrib with numerous lateral veins (Admasu Tsegaye and Westphal, 1992). According to the authors, the inflorescence grows at the apex and hangs down from the center of the pseudostem, and flowers are unisexual, arranged in two rows within each bract. The female flowers develop at the proximal end while, the male flowers develop at the distal end of the inflorescence. The dry and fibrous fruits attain orange color when they mature, and contain large seeds that have very strong dark seed coat.

2.4. Importance of Enset

2.4.1. Food value

Enset is a multipurpose crop, with all parts of the plant, except the fibrous roots having some importance. However, it is mainly cultivated for a starchy food and contributing to food security and rural livelihoods of around 20 milion people in Ethiopia (Borell *et al.*, 2019). The pseudostem and the corm are the most important source of food, the major foods obtained from enset being *kocho*, *bulla* and *amicho*, and they are given different names by different ethnic groups. According to Mesfin Sahle (2018), *kocho* is a staple food for 97.5% of the households in the Wabe River catchment of Gurage Mountains.

Kocho is obtained by fermenting a mixture of the scraped pulp of pseudostem (leaf sheath) pulverized corm, stalk of inflorescence and internal soft tissue of petiole. It can be prepared in

different forms and mostly consumed with animal products, like milk, cheese, and yogurt, or vegetables, like cabbage, and also with pulses. The quality of *kocho* depends on the type of landrace, age of the harvested plant, and the harvesting season. It is also influenced by the part of leaf sheath processed, the innermost parts of the pseudostem and the corm produce good quality (white in color), while the outer layers and top part of pseudostem produce poor grade, gray color *kocho*.

Bulla is obtained from the pulps of scraped pseudostem, decorticated corm and peduncle which are squeezed in to a pit lined with enset leaves, and the resulting white granule starch is allowed to precipitate. It can be prepared as a pancake, porridge, or other forms of food. *Amicho* is the boiled enset corm, usually from younger plants of selected landrace (Pijls *et al.*, 1995; Personal observation). Depending on the area, different forms of foods or dinks are produced from enset products. For example, in Ari, South Omo Zone, there is a special kind of traditional beer called *agemi gola,* occasionally brewed from the young flowering stems of enset (Masayoshi Shigeta, 1991).

2.4.2. Animal feed and other house hold uses

Enset is an important source of animal feed, especially during dry period, where other feeds sources are scarce. According to Desta and Oba (2004), about 85% of the farmers in the Bale highlands of Ethiopia provide enset leaves and other by-products to their livestock during the dry season. Masayoshi Shigeta (1991) also reported that enset occasionally fulfill for the daily required animals feed in the dry season, when the pasture grass is scarce. According to Dirsha Demam (2019), enset leaf and leaf midribs contributes 50.77% total drymater (TDM), 70.86%

total digestible crude protein (TDCP) and 54.81% total metabolizable energy (TME) from the total available cattle feeding in Gurage Zone, and it is crucial in safeguarding the life of animals especially during drought season.

2.4.3. The nutritional composition of enset products

Only few reports on the nutritional composition of enset products; *kocho*, *bulla* and corm/*amicho/*, are documented. Agren and Gibbson (1968) reported high energy value of *kocho* (1410 to 1950 kJg^{-2}) and *bulla* (1580 to 1850 kJ g^{-2}) in dry matter basis. According to the authors, the protein, fat, carbohydrate, fiber, ash, calcium, phosphorus and iron contents of *kocho* per 100 g dry matter was 1.1–2.8 g, 0.2–0.5 g, 95–98 g, 2.3–6.2 g, 1.7 g, 60 mg, 68 mg and 7 mg respectively. Whereas, 100 g *bulla* (in dry weight basis) contains 0.4–0.8 g protein, 0.2–0.4 g fat, 93–98 g carbohydrates, 0.6–0.8 g fiber, 0.2 g ash, 91 mg calcium, 44 mg phosphorus and 0.8 mg iron. According to Mohammed *et al.* (2013), enset corm contains 17 of the 20 amino acids at concentration level similar or higher than potato. Admasu Tsegaye (2015) analyzed the nutritional components of corms of enset landraces suitable for corm production, and reported that, there is a noticeable variability in nutritional composition of the landraces.

Ajebu Nurfeta *et al.* (2008), who evaluated the macro and trace mineral contents of ten enset varieties, reported the varietal differences in the mineral contents of various enset fractions (leaf lamina, midrib, pseudostem and corm) except phosphorus. According to the authors, most of the fractions were rich in phosphorus (P), potassium (K), calcium (Ca) (except corm) and

magnesium (Mg), while sodium (Na) content was very low. Most fractions were also rich in iron (Fe) and manganese (Mn), but deficient in copper (Cu), except leaf lamina, while zinc (Zn) content was high in corm, but low in other fractions. Minaleshewa Atlabachew and Chandravanshi (2008) reported that, *kocho* and *bulla* are generally, rich in Ca and Zn, compared to other similar food stuffs, whereas it contains equivalent concentration of Cu, Fe and Mn. On the other hand, Mohammed Feyisso and Yohannes Addisu (2018) reported the prevalence of anemia in Southern Ethiopia, where enset is used as a staple food, due to the shortage of Fe.

Melese Temesgen (2013) reported that fermentation time, enset landrace and processing methods affected the chemical composition of *kocho*. As the author pointed out, traditional *kocho* processing methods showed the higher crude protein and carbohydrate content than the modified processing method, which was prepared by adding minimally boiled pulp of chopped pseudo stem in to traditional processing method. On the other hand, higher crude protein, crude fat and carbohydrate contents were recorded from *kinnare* landrace, while *astara* showed higher crude fiber, total ash and moisture contents. When the *kocho* from cultivated and wild enset was compared, the one from wild enset showed higher concentration of crude fat, crude fiber, ash potassium, calcium, magnesium, copper and sodium than the cultivated one (Meseret Haile, 2015).

2.4.4. Enset as traditional medicine

In different enset growing societies, peoples have a belief that some enset landraces have medicinal property for various diseases including repairing of bone fractures, broken bones, assisting the discharge of placenta after birth, etc. Yemane Tsehay and Fassil Kebebew (2006) reported that boiled corms and starchy powder, *bulla,* of *toyo* enset landrace is eaten with milk to treat ailments such as joint displacement and swelling, broken bone or fractures in Bonga area, and it is also used to cure similar disorders in domestic animals. The authors also revealed that, corm's of the landrace *choro*, which has a deep red pseudostem and midrib color, is eaten with specially prepared cheese with butter and milk for the discharge of a placenta after birth. Similarly, it is used for dairy cows by feeding the corm mixed with salt. This landrace is also believed to safe guard against devils' and all evil spirit attacks, and for that reason it is usually planted at the front, where it is visible to all.

According to Tadessa Daba and Masayoshi Shigeta (2016), enset landrace called *sweete* is a strongly recommended in treatment of bone related problems around Areka area. Kaya *et al*. The widely use of some enset landraces in traditional treatment bone related illness could be due to their high calcium and phosphorous content, as these minerals are crucial for bone growth and healing (2015) and Penido and Alon (2012). Feeding *bulla* to mothers who have just delivered a baby is a common practice even in the central highlands of Ethiopia (where enset is not so commonly a staple food), as it is believed it will help them recover fast from the suffering they endured during pregnancy and labor.

In some parts of Gurage community, the medicinal use of enset landraces *astara, kibnar* and *guarye* for bone related illness, *bush-wyse* for discharge of a delayed placenta after birth,

mymote for treating wound and hemorrhoid disease and *lemmate* landrace for the development of strong bones was reported (Kedir Abdella, 2016). Ashenafi Ayenew *et al.* (2016) reported 38 enset landraces to have medicinal value in Kembata-Tembaro localities, of which gishera, tesa, welagela, and cherqewa have major importance to cure broken bones and bone fracture, discharge of delayed placenta, skin scabies and for expulsion of spines buried in the body tissues. According to Temesgen Magule *et al.* (2014), enset landraces, *gefetanuwa* and *agino* in the form of food, are used in healing bone fractures and to strengthen women that gave birth respectively. *Halla* and *meziya* landraces are used as remedy for stomach cramps, while *lochingia* landrace to facilitate placental discharge in cows around Wolaita.

2.4.5. Potentials of enset for industrial uses

In addition to its multipurpose uses at homestead level, the potential of enset for industrial application was reported. Abrham Wondimu *et al.* (2014) reviewed that enset starch used for numerous pharmaceutical applications, including binder, disintegrant, super disintegrant, and gelling agent. The authors also revealed that, the cross-linked and acetylated form of enset starch showed a novel drug delivery system; hence enset can be used as alternative sources of starch in pharmaceutical industries. The modified enset starch also showed improved flowability, compressibility, and enhanced swelling capacity than the native starch (Tsige Gebre-Mariam, 2016). On the other hand, enset starch, *bulla*, is reported to be a comparable gelling agent as industrial product, agar, for *in vitro* propagation of pineapple, and it could save up to 76 % of gelling cost (Biruk Ayenew *et al.*, 2012). Even a better performance of enset starch as a gelling agent was reported in micropropagation of *Vanilla planifolia* (Ayelign Mengesha *et al.*, 2012).

Enset fiber is another product which can have various promising industrial uses. Teli, *et al.* (2017) reported that enset fiber is comparable with the well-known world class natural fibers like; abaca, flax, sisal, hemp, jute, kenaf and ramie in its cellulose composition, tensile strength and elongation at break, and thermal behavior. Brandt *et al.* (1997) also reported about the excellent structure and strength of enset fiber which is equivalent to the fiber of abaca. Therefore, apart from the existing traditional utilization, this fiber has a potential to be used as a textile material at industrial level. According to Amare Gessese (2016), the high quality of enset fiber can be used for the production of specialty papers, such as currency notes, tea bags, etc. that require the use of long and high strength fibers and also for the production of high quality papers and packaging, or to supplement short and inferior fibers in the pulp and paper industries.

2.5. Enset Production and Constraints

2.5.1. Environmental adaptation and production trend

Domesticated enset is growing at altitudes ranging from 1500 to 3100 meters above sea level and, most enset-growing areas have annual rainfall of about 1100 to 1500 mm, which falls mainly between March and September (Admasu Tsegaye, 2002). According to the author, the average temperature of enset growing area falls between 10 and 21 °C, while the relative humidity is 63 to 80 %. Enset is growing in a moderately acidic to alkaline (pH 5.6 to 7.3) soil, with 2 to 3% organic matter (Taye Bezuneh and Asrat Feleke, 1966).

Borell *et al.*, (2019) analyzed a data from Ethiopia's Central Statistics Agency (CSA, 1995–2017) and reported that the area of land under enset production has increased approximately 46% over the past twenty years, whilst yield has been reported to increase twelve-fold, implying substantial productivity improvements. However, the authors also reviewed that, there are studies reporting that farmers perceive enset production is declining (Admasu Tsegaye, 2002; SARI, 2014; Zerihun Yemataw *et al.*, 2017), which makes the validity of the data debatable.

2.5.2. Propagation and other management practices

Conventionally enset is propagated vegetatively, although it can be propagated by seeds. Propagation by seed is not a common practice because, harvesting of the crop is usually done before maturity, and some landraces rearely flower. Recalcitrant nature of the seed could also be another reason. Although enset plant look likes banana, morphologically, it doesn't produce suckers naturally, instead it must be induced artificially by cutting down the immature, about 3 to 4-year-old plant at around 10 to 15cm above the junction between pseudostem and the corm and then removing the apical meristem. When apical meristem is not damaged, only one shoot is produced (Mulugeta Diro and Endale Tabogie, 1994; Taye Buke *et al.*, 2016). The suckers that emerge after 2 to 3 months are transplanted after remaining on site for at least a year. Regarding the number of suckers produced, various results were reported. Mulugeta Diro and Endale Tabogie (1994) reported the production of not more than 10 or 15 suckers per corm, while Abrham Shumbulo *et al.* (2012) reported 30 to 50 suckers. According to Brand *et al.* (1997), it is common for a farmer to have 5 to 15 mother corm pieces each year, and usually 20 to 100 suckers are produced per corm piece.

In the traditional cropping system, it is a common practice to transplant suckers three to four times in nursery beds before harvesting, which varies place to place and the transplants at each stage have different name. Although there is a great variation from place to place, the suckers stay at each nursery for one year and in the last stage for two years. However, direct transplanting to the permanent field at 1.5m x 3m spacing significantly increased the *kocho* yield of enset (Mulugeta Diro and Endale Tabogie, 1994). The use of cattle manure for fertilization of enset is a common practice before any other crop (Brand *et al*., 1997).

2.5.3. Harvesting

Under the traditional production system, 5-10 years are required for maturation of enset, which varies depending on the altitude where it grows, and management practices (Admasu, 2002). The harvesting age of enset varies from place to place; for example, the Gurage people harvest most of the plants nearly at maturity, while the Sheko people tend to harvest many young plants for *corm*. According to Brand *et al*. (1997), farmers tend to believe that harvesting at or near plant maturity is better, and harvesting younger plants indicates the shortage of food supply or poverty. For *amicho* /corm/ and animal feed, harvesting of enset at any younger stage is a common practice. Harvesting a mixture of landraces at a time, which is guided by tradition of multi-enset food recipes, was reported by Temesgen Magule *et al*. (2014).

The yield of enset varies with the type of landraces, growing area, harvesting stage, and management practices. According to Endale Tabogie (1997), the yield of unsqueezed *kocho* in Gurage, Kenbata and Sidama areas ranged from 8 to 70 kg/plant with an average of 30.3

kg/plant, while Abrham Shumbulo *et al.* (2012), reported 15 to 61 kg/plant in the Wolaita zone. Fiber yield ranging from 0.38 to 0.59 $tonha^{-1}$ yr^{-1}, which varies among enset landraces, was reported by Atnafua Bekele *et al.* (2008), as cited by Taye Bezuneh (2012). According to Ethiopian Central Statistics Agency (CSA, 2017), annual number of enset plants to be harvested was estimated to be 127,235,588 and, the total produce in the form of *kocho*, *bula* and *amicho* was, 34,782.94 tons, 1,017.82 tons and 29,307.64 tons, respectively.

2.5.4. Production constraints

The productivity and area coverage of enset is declining due to various biotic and abiotic factors, of which, bacterial wilt which is caused by *Xanthomonas campestris* pv. *musacearum* is the major threat (SARI, 2014). If bacterial wilt, or any other factor damage enset plant in the later stage of its life cycle, after long year investment, the loss is very significant (Brand *et al.*, 1997). In some areas, this situation has caused farmers to abandon their enset farming, and shift to annual crop production.

There are some attempts to devise control methods against the disease, and to develop resistant enset landraces with desirable agronomic traits. On the evaluation of landraces against bacterial wilt disease resistance, complete resistance was not observed, however, some landraces including; *meziya*, *hineba*, *bedadet* and *warke-dima* showed better tolerance, among all *meziya* was found to have the lowest of disease prevalence (Hunduma *et al.*, 2015). The better tolerance of *meziya* landrace to bacterial wilt disease was also reported by Weldemichael *et al.* (2008). Since these landraces often fail to combine disease tolerance with quality and desirable

agronomic traits (SARI, 2014), cultural disease management is still the most important control method.

Pests, like porcupine, wild pig, mealy bugs, leaf hoper and mole rat are the other enset production challenges. They usually damage the plant by feeding on the corm and pseudostem. Among the pests, the mole rat ranks first (Brandt *et al.*, 1997). The major controlling mechanisms employed by the farmers are constructing woven fences and ditches around enset fields and trapping the animals. Shortage of land, periodic drought, lack of improved variety, lack of improved processing, and unimproved agronomic practices are also reported to be challenges of enset production (Abrham Shumbulo *et al.*, 2012).

2.6. Genetic Diversity Analysis of Enset

The four components of genetic diversity that can be distinguished are; the number of alleles found in different populations, their distribution, the effect they have on performance, and the overall distinctness among different populations (Rao and Hodgkin, 2002). Genetic diversity studies make direct contributions in selecting what to conserve, and ensuring that the resources are well managed and used. By measuring the range of the existing genetic diversity within and among populations of any species, collection, evaluation and breeding strategies can be adjusted to attain maximum variation (Morikawa and Leggett, 1990).

Maintenance of field gene-banks is costly in terms of requirements for land, supplies, and labor. A high rate of loss of accessions usually occurs due to biotic and abiotic factors. For these reasons, there is obviously a need to limit the number of accessions that can be conserved

in field gene-banks (Rao and Hodgkin, 2002), which should be carried out rationally and based on sound scientific principles. The major criterion is avoiding duplicate during collection, which can reduce the size of field genebanks in many cases. Grouping of accessions based on their morphological similarity is the first and most important step in identifying duplicates, which is usually, verified using molecular markers (Connolly *et al.*, 1994).

2.6.1. Genetic diversity analysis of enset using phenotypic markers

Conventionally, diversity within and among populations was determined using morphological markers, which are visually accessible traits such as flower and seed color, shape of seed and fruit, growth habits, and etc. The method does not need costly technology, but large area of land often required for field experiments makes it possibly more expensive than molecular techniques in developed countries, and equally expensive in Asian and Middle East countries due to cost and availability of labor (Govindaraj *et al.*, 2015). According to the authors, morphological markers are limited in number and, mostly they are either dominant or recessive. Moreover, these marker traits are usually influenced by environment and susceptible to phenotypic plasticity; on the contrary, this allows assessing diversity in the existence of environmental variation, which cannot be ignored from the genotypic variation. These markers have still an advantage and mandatory to differentiate the adult plants from genetic mix-up in the field.

In areas where enset is growing as a staple food, producing diverse types of enset landraces, which are used for different purposes, is common practice and, farmers have indigenous knowledge in characterization and identification of the landraces using phenotypic traits. Many of enset diversity studies were not focusing on measuring or assessing the phenotypic traits to

characterize the landraces, rather they targeted on the identification of number of enset landraces with different vernacular names produced in different enset growing community (Abrham *et al*., 2012; Awol Zeberga *et al*., 2014; Temesgen Magule *et al*., 2014; Zerihun Yemataw *et al*., 2014b; Amare Seifu and Daniel Fitamo, 2016). Most of the authors reported that farmers in each specific study area have indigenous knowledge in characterizing, identifying and naming of enset landraces.

Ari people recognize the differences among cultivated enset landraces and designate their own name one by one and classify according to their characteristics (Masayoshi Shigeta, 1991). According to the author, Ari people identify individual enset plant in their field as if they remember the individual person by his/her name. Due to an intimate relationship with enset, people can remember many landrace names and it is reasonable to assume that the rare landrace names are preserved through this manner. As they revealed, pigmentation on the plant bodies is always among the first that Ari people recognize as an important characteristic for the identification of enset landrace name, while other characters came after them.

Yemane Tsehaye and Facil Kebebew (2006) reported the existence of different enset landraces, which are identified, named and categorized by the farmers in Bonga area based on pseudostem, petiole and midrib color, size, and various end-use and disease tolerance characteristics. It was revealed, there was a reasonable consistency among farmers in naming the varieties. According to the authors, diversity within and among enset landraces was high, and the significant perceptual distinctiveness in the recognition of variation played a role in selection and maintenance of the diversity.

Abrham Shumbulo *et al*. (2012) reported mixture of different purposes enset landraces ranging from 2 to more than 50 are produced in Offa district, Wolaita zone. According to the author, the farmers differentiate one landrace from the other phenotypically by looking at the color of petiole, mid-rib, leaf sheath, angle of leaf orientation, size and color of leaves and circumference & length of pseudostem. Temesgen Magule *et al*. (2014) reported 67 different enset landrace vernacular names, which are under cultivation similarly in Wolaita zone. According to the author, farmers in the area have indigenous knowledge to identify the landraces using descriptors related to; morphological traits (pseudostem color, color of midrib, petiole patches/strips colors), agronomic characteristics (drought resistance, disease and pests resistance, time of maturity), use-values (uses for food, fiber, fodder, medicinal) and culinary quality. They also explained that, the farmers use combinations of descriptors for identification of landraces, although the morphological characters are referred as the first key identification characteristics.

Mikias Yeshitila (2014) assessed the diversity of 279 enset accessions using morphological descriptors from six qualitative and 22 quantitative traits, and yield and yield related traits. According to the author, cluster analysis of the qualitative characters grouped the accessions in to six clusters, and existence of variability was observed in their leaf, midrib and petiole colors. The author also reported that, analysis of variance for quantitative characters indicated significant variation among the accessions in all the 22 yield and yield components of an enset plant. It was also indicated that, cluster analysis based on quantitative characters indicated the

formation of six clusters, and the distance between most of the clusters was highly significant ($P < 0.01$).

Zerihun Yemataw *et al.* (2014a), who characterized 165 enset landraces using 17 morphological and use value quantitative traits, reported the presence of substantial genetic variability among the landraces. Zerihun Yemataw *et al.* (2018) studied morphological variation among 387 enset accessions collected from nine major enset growing regions of Ethiopia using 15 quantitative traits, and the existence of high genetic diversity was reported. As the authors pointed out, cluster analysis grouped the accessions in to five distinct classes regardless of their origin.

2.6.2. Genetic diversity analyses of enset using molecular markers

Molecular marker can be defined as a genomic locus, detected through probe or primer which, in virtue of its presence, differentiates clearly the chromosomic trait which it characterizes, as well as the flanking regions at the 3′ and 5′ extremity (Barcaccia *et al.*, 2000). These markers can detect the variation that arises from deletion, duplication, inversion, and/or insertion in the chromosomes (Zhu *et al.*, 2008). The markers are inherited both in dominant and co-dominant patterns, and do not affect the phenotype of the traits of interest because they are located only near or linked to genes controlling the traits (Rabiei, 2010). Molecular markers have a number of advantages over the conventional, phenotype-based alternatives because they are stable and detectable in all tissues at any growth and development stage, or defense status of the cell. Moreover, they are not affected by environmental and epistatic effects (Winter and Kahl, 1995; Govindaraj *et al.*, 2015).

A few enset diversity studies were carried out using molecular techniques including; Amplified Fragment Length Polymorphism (AFLP) (Almaz Negash *et al.*, 2002), Random Amplification of Polymorphic DNA (RAPD) (Genet Birmeta *et al.*, 2002), Inter Simple Sequence Repeat (ISSR) (Dagmawit, and Endashaw, 2011) and Simple Sequence Repeat (SSR) (Selamawit Getachew *et al.*, 2014; Temesgen Magule *et al.*, 2015).

Almaz Negash *et al.* (2002) analyzed the genetic diversity among146 enset clones from five different regions of southern and south western Ethiopia using AFLP markers. As the authors revealed, a total of 180 bands were scored; of which 104 (58%) were polymorphic and a wide variations were observed among the accessions. However, 21 groups of duplication that consist 58 clones were identified and the possible cause was suggested to be related with different use value of enset clones and changing of vernacular names after exchange of planting materials. They also reported that, only 4.8% of the genetic variation was found among regions, while 95.2% was within regions, which was explained by the existence of a regular large distance exchange of clones among enset growing regions.

In the genetic diversity evaluation of 111 clones of *Ensete ventricosum* from 9 sites in southwestern Ethiopia, using 12 RAPD markers, 97 reproducible polymorphic bands were generated and each clone proved to have a unique DNA profile, except 8 duplications among the vernacular names (Genet Birmeta *et al.*, 2002). According to the authors, genetic variation within collection sites was relatively high, with Shannon-Weaver diversity index values ranging from 0.44 to 0.55. Partitioning of the total variability also showed that only 14% was due to differences among collection sites while 86% variation within the sites. It was also

indicated that, estimates of genetic distances did not correspond with geographical distances between collection sites.

Dagmawit Chombe and Endashaw Bekele (2011) evaluated the genetic diversity of 71 enset clonal varieties from two cultivated enset population, Essera and Kefficho, using two ISSR markers. According to the author, cluster analysis grouped the landraces in to two separate clusters which were based on the origin of populations, with a few mixing. This grouping was also observed in the principal coordinated (PCo) analysis. The authors also revealed high genetic diversity in the two enset populations, and clones from Kefficho were more diverse than Essera the majority of the variation was within populations than between the two populations.

Selamawit Getachew *et al.* (2014) evaluated transferability of 71 SSR markers from banana *(Musa accuminata)* to enset and assessed the genetic diversity of enset accessions, representing large numbers of populations and areas of Ethiopia. Among the twelve transferable markers, eleven were used to examine the diversity of the 220 enset accessions collected from eight different zones. The authors reported that, the markers were less informative and the alleles observed per locus were ranged from 2 to 10, with a total of 61 alleles. The expected heterozygosity was ranged from 0.2 to 0.74 with a mean value of 0.55, and a mean observed heterozygosity was 0.67, ranged from 0.22 to 0. 95. In the study, the 220 accessions were categorized into 3 major groups.

Temesgen Magule *et al*. (2015) developed the first set of enset microsatellite markers, and they used 34 polymorphic SSR markers to study 70 enset accessions, both cultivated (collected from Ari, Gamo-Gofa, Sidama and Wolaita zones) and wild (collected from Dawro zone) and 18 *Mussa* accessions for cross-transferability evaluation. The authors revealed that, most of the markers were highly informative with average polymorphic information content (PIC) values of > 0.5. In the study, a total of 202 alleles, allelic richness, ranging from 2-12 alleles, with average of 5.94 alleles per SSR was detected, and the overall genetic diversity level was moderate to high. According to the authors, all enset accessions were clustered separately from the *Musa* and, within enset accessions, wild ensets were distinctly grouped from cultivated landraces, while the cultivated landraces were grouped in to four clusters in the dendrogram, irrespective of their geographic origin.

Feta Negash (2019) evaluated the genetic diversity and population structure of 79 cultivated landraces collected from Gurage zone and Yem special district, and 4 wild type of enset collected from Dawro and Gurage zones using twelve polymorphic enset SSR markers and reported a total of 77 alleles and PIC of 0.62 to 0.77 with a mean value of 0.69. According to the authors, the average observed heterozygosity ranged from 0.51 to 0.67, and gene diversity was in range of 0.55 to 0.62. They also revealed that variations within population contributed more to the genetic diversity than the variations among population, and the 83 enset germplasms were grouped into three major clusters, where the wild individuals clustered distinctly.

2.7. Micropropagation

Micropropagation is the true-to-type propagation of a selected genotype using *in vitro* culture techniques (Debergh and Zimmerman, 1991). It is one of the most widely used technique of tissue culture which is used to multiply novel plants, such as those that have been genetically modified or breed through conventional plant breeding methods. It offers the potential to produce thousands, or even millions, of plants per annum but is usually limited by the numbers that can be handled (Smith and Drew, 1990). Micro propagation is also used to provide a sufficient number of plantlets for planting from a stock plant which does not produce seeds, or does not respond well to vegetative reproduction (Sharma, 2016). Three stages (I to III) protocol for micropropagation was proposed by Murashige (1974). Later on Debergh and Maene (1981) proposed additional stage, Stage-0, and as a result of increased information, five main stages (0–IV) were described for successful micropropagation (Debergh and Read, 1991).

2.7.1. Factors affecting micropropagation

2.7.1.1. Genotype of source plant

It is well documented that genotype plays a major role in different phases of micropropagation (Bhatia *et al.*, 2005; Tileye Feyissa, 2005; Gomes *et al.*, 2010). Bhatia *et al.* (2005) reported that genotypic variation in terms of shoot regeneration response, number of shoots, and shoot height was apparent in micropropagation of tomato and, Tileye Feyissa *et al.* (2005) reported significant differences both in percentage of regeneration and the number of shoots per explant among the genotypes of *Hagenia abyssinica*, at different concentrations of growth regulators. Gomes *et al.* (2010) also reported the effect of genotype of the donor plants on multiplication

of strawberry tree. According to Ono *et al*. (1994), as well, genotype of rapeseed strongly influenced shoot regeneration response with a range of variation from 97% to 0%.

Kumar and Reddy (2011) also reviewed the effect of genotype on shoot regeneration and elongation in many species. The authors explained that the difference could be partly due to the levels of endogenous hormones, particularly cytokinin levels during the induction period, although the precise mechanism remains unclear. Generally it was reported that, dicots regenerate more easily than monocots (Yildiz, 2012) and, herbaceous plants regenerate more easily than woody plants (Pierik, 1987).

2.7.1.2. Type and source of explants

The correct choice of explant material can have an important effect on the success of micropropagation. Christopher and Rajam (1996), who evaluated the effect three types of explants (hypocotyl, cotyledon and leaf) of seven red pepper genotypes, reported that shoot regeneration was significantly affected by types of explants. According to the authors, leaf explants consistently regenerated more shoots than hypocotyls or cotyledons in all the genotypes. Similarly, variation in regeneration percentage and shoot number per explant, among five types of explants (complete leaf, complete petiole, leaf segment, petiole segment and leaflet) in five *Hagenia abyssinica* genotypes, was reported (Tileye Feyissa *et al*., 2005). According to Gubiš *et al*. (2003), who compared the regeneration capacity of six types of explants (segments from hypocotyl, cotyledons, epicotyl, leaf, internodes and petiole) in 13 cultivars of tomato, a significant influence of explant type was observed. Yogesh *et al*. (2016) compared three explant types (Shoot tips, young leaf and nodal explants) of *Glycyrrhiza*

glabra, and reported different response towards establishment and shoot proliferation, with the highest value recorded from the nodal explant.

Yildiz, (2012), who compared hypocotyl explants of flax isolated from *in vitro* and greenhouse-grown seedlings, reported the highest shoot regeneration percentage, shoot number per explant and total number of shoots per Petri dish in explants isolated from *in vitro*-grown seedlings of all cultivars. According to Kumar and Reddy (2011), the variation in the performance of explants from different sources could be related to the level of endogenous hormones they contain. Martini *et al.* (2013) reported that cultures derived from juvenile tissues (seedlings, sprouts, micropropagated plantlets) were established more easily, and the microshoots rooted at higher percentages compared with cultures from buds of adult plants in a tree species. The authors also stated that explants from adult plants released phenolic compounds into the medium at a higher percentage than juvenile explants.

2.7.1.3. Growth media

Significant effect of media has been observed on plant regeneration from different parts of a plant (Sharswat and Chand, 2004). Various basal media like, B5 medium and Gamborg have been employed for micropropagation (Khan *et al.*, 1988; Prakash and Gurumurtthi, 2005), but most widely used culture medium is Murashige and Skoog (1962) because most of the plants respond favorably to MS medium. Selection, strength and combination of media are also important parameters for optimizing the regeneration protocol (Khan *et al.*, 1988; Prakash and Gurumurtthi, 2005).

2.7.1.4. Plant growth regulators

Plant growth regulators are organic compounds naturally synthesized in higher plants that have an influence on growth and development. In addition to the natural compounds, chemicals with similar physiological activities have been developed synthetically (Pierik, 1997). Some of the classes of plant growth regulators include; cytokinins, auxins and abscisic acid. The interaction and balance between the growth regulators supplied in the medium, and the growth substances produced endogenously regulate the growth and morphogenesis *in vitro* (George, 1993). A balance between auxins and cytokinin is most often required for the formation of adventitious shoots and roots. High levels of auxins relative to cytokinins stimulated the formation of roots, whereas high levels of cytokinins relative to auxins led to the formation of shoots (Taiz and Zeiger, 1991).

The balance of growth regulators during micropropagation depends on the phase considered (initiation, multiplication or rooting). In the multiplication phase, the level of cytokinins should be normally higher than of auxins, while in the rooting phase, higher levels of auxins, or in some cases without cytokinins can be supplemented to the culture medium (Torres, 1989). The most common cytokinins used in culture medium are kinetin, BA (6-benzyladenine) and 2iP (Pierik, 1997) and the commonly used auxins are IAA (indole-3-acetic acid), IBA (indole-3-butyric acid), NAA (naphthaleneacetic acid) or 2, 4-D (2, 4 dichlorophenoxyacetic acid) (George, 1993).

Cytokinins stimulate plant cells to divide, and they were shown to affect many other physiological and developmental process. These effects include the delay of senescence in

detached organs, the mobilization of nutrients, chloroplast maturation, and the control of morphogenesis (Taiz and Zeiger, 1991). These compounds overcome apical dominance and release lateral buds from dormancy (George, 1993). Auxins are involved in the regulation of several physiological processes, like apical dominance and formation of lateral and adventitious roots as well as the inhibition of adventitious and axillary shoot formation (Pierik, 1997).

2.7.2. Micropropagation of *Ensete ventricosum*

A few numbers of investigations on micropropagation of *Ensete ventricosum* from different types of explants, type and level of growth regulators, with various levels of successes and challenges were reported. Almaz Negash *et al.* (2000) investigated *in vitro* regeneration and micropropagation potential of three enset landraces *nobo*, *ketano* and *choro* on MS media containing two levels of BAP (10 and 20 μ M) from corm and leaf tissues of greenhouse-grown plants as well as *in vitro* germinated zygotic embryos. In their investigation, significant differences between the landraces and BAP concentrations were observed on shoot formation, but the most influential factor was the BAP concentration. The higher concentration of BAP (20 μ M) produced a higher total number of shoots after 16 weeks. However, tissue browning was more pronounced in this medium, ultimately leading to loss of plantlets.

In the comparison of splitted and intact shoot tip explants, during micropropagation of *oniya* enset landrace, a higher number of shoots (3.6) from the latter explant, while only one shoot from the former was reported (Mulugeta Diro and Van Staden, 2005). According to the authors, increase in the concentration of cytokinin could not release the lateral buds from apical

dominance, and at the higher concentration of cytokinin, the regenerated buds were small and hyperhydric and they didn't grew into normal shoots. The author also reported that explants obtained from *in vitro* regenerated seedling showed a better performance in producing higher number of shoots and less blackening, as compared to greenhouse raised sucker.

Genet Birmeta and Welander (2004) reported *in vitro* propagation of *Ensete ventricosum* from shoot tips (5–8 mm) explants with subtending corm tissues, obtained from glasshouse grown sprouts. During the bud proliferation stage, modified MS media with a combination of cytokinins were used and the formation of multiple buds on the corm tissue was induced only if the meristem region was wounded before transfering to bud proliferation medium. Based on their report, up to 75 healthy shoots per explant were produced, whereas unwounded explants produced only 1to 2 shoots per explant. Genene Gezahegn and Frew Mekbib (2016) also reported a maximum mean number of shoots (23) from shoot tip explant (10mm) of *mazia enset* landrace on MS media containing 4.5 mg/l BAP and 1.5 mg/l NAA in one sub-culture.

Rooting of *in vitro* regenerated *Ensete ventricosum* shoots took place within fourteen days of transfer in half strength MS medium supplemented with 5 μM IBA and 1 μM BAP (Almaz Negash *et al.*, 2000). According to the author, the primary root developed approximately in ten days, followed by the development of secondary roots within the next four days. Genet Birmeta and Welander (2004) reported development of branched roots in different modified MS media, with the best root growth achieved in 1/2 MS medium containing 1.6 mM NAA, 4.4 mM BAP. On the other hand, the minimum number of days (10.5) to root induction for *mazia* enset landrace on a media with 1.5mg/l IBA and the maximum root number (3.77) was recorded at

IBA concentration level of 2mg/l (Genene Gezahegn and Frew Mekbib, 2016), while Tilahun Zeweldu and Ladders (1998) reported high rooting capacity of *Ensete ventricosum* on hormone-free MS medium.

Browning and eventual death of the tissue due to the excessive production of polyphenols is a frequently encountered problem during initial stages of enset *in vitro* culture (Afza *et al*., 1996; Tilahun Zeweldu and Ladders, 1997 and Mulugeta Diro and Van Staden, 2005). The process is initiated by browning of the surface of plant tissues due to the oxidation of phenolic compounds resulting in the formation of quinones which are highly reactive and toxic to plant tissue (Taji and Williams, 1996). Lower BAP concentration and ascorbic acid (0.57 mM) together with increased frequency of sub culturing reduced the blackening of enset cultures (Almaz Negash *et al*., 2000). According to Genet Birmeta and Welander (2004), the addition of 0.5–1% (w/v) activated charcoal to a medium was essential for prevention of phenol exudation.

The other challenge in micropropagation of enset is contamination by endophytes. Bacterial contaminants found during explants initiation, present in explants and resistant to surface disinfection, are likely to be endophytic (Reed *et al*., 1995). They may survive in the plant material for numerous subculture cycles and over long periods of time without expressing signs in the medium or in the tissue (Van den Houwe and Swennen, 2000). The presence of these bacteria in the culture is very detrimental due to adverse effects on growth and the reproducibility of tissue-culture protocols (Thomas, 2004), and potential threat to *in vitro* gene banks (Van den Houwe and Swennen, 2000). On the other hand, endophytes are known to

release various effective secondary metabolites that can minimize the potential consequences of pathogenic attack (Gao *et al.*, 2010).

Very high (50–60%) internal contamination and loss of explants during culture initiation of *Ensete ventricosum* was reported (Genet Birmeta and Welander, 2004). According to the authors, level of contamination varied with the type of clones. Similarly, contamination due to endogenous bacteria and loss of large number of explants in banana was reported (Hadiuzzaman, 2001).

Incorporation of antibiotics into the growth media of plant cultures has been reported to eliminate the contaminants (Reed *et al.*, 1995).Treatment with antibiotics and antifungal agents may be used to reduce the contamination; however, its effects differ according to plant species, type of explant and the concentration. Nakano and Mii (1993) reported that, carbenicillin at a concentration of 500 mg L^{-1} efficiently induced somatic embryo formation of *Dianthus* cultivars. According to Manchanda *et al.* (2011), maximum shoot multiplication and elongation of two banana genotypes was obtained with the use of cefotaxime at a rate of 400 mgl^{-1} and 500 mgl^{-1}. Although its effect was not clearly indicated, the inclusion of 250 mgl^{-1} cefotaxime, in micropropagation of *Ensete ventricosum* was reported (Almaz Negash *et al.*, 2000).

Chapter 3.

MATERIALS AND METHODS

3.1. Genetic Diversity of Enset (*Ensete ventricosum* (Welw.) Cheesman) Landraces Used in Traditional Medicine Using Phenotypic Traits

3.7.1. Description of the study area

The field study was conducted in four administrative zones (Gurage, Hadya, Kembata-Tembaro and Dawro) and one special district (Yem) of Southern Nations, Nationalities and Peoples Region (SNNPR), which is the major enset growing region of Ethiopia. The geographical coordinates of the study areas ranged from 7°3'36"N to 8° 4'48" N and from 37°9'36"E to 38° 1'48" E, as well as altitudes ranging from 1976 to 2834 meter above sea level. The map of the study area is shown **in Figure 3.1**.

3.7.2. Sampling

A field survey was conducted during November 2016 to March 2017 to study diversity among enset landraces used in traditional medicine. Based on the previous study on richness of enset diversity (Zerihun Yemataw *et al*., 2014b; 2016), four Administrative Zones (namely, Dawro, Gurage, Hadya and Kembata-Tembaro) and one special District (Yem) in SNNPR were selected purposively to carry out the present study. From each zone, two districts and from each district two peasant associations (PAs: the lowest tier of civil administration unit) were selected based on enset diversity and distribution data obtained from Agriculture offices. Hence, a total of 18 PAs from 8 districts and 1 special district were included in the study.

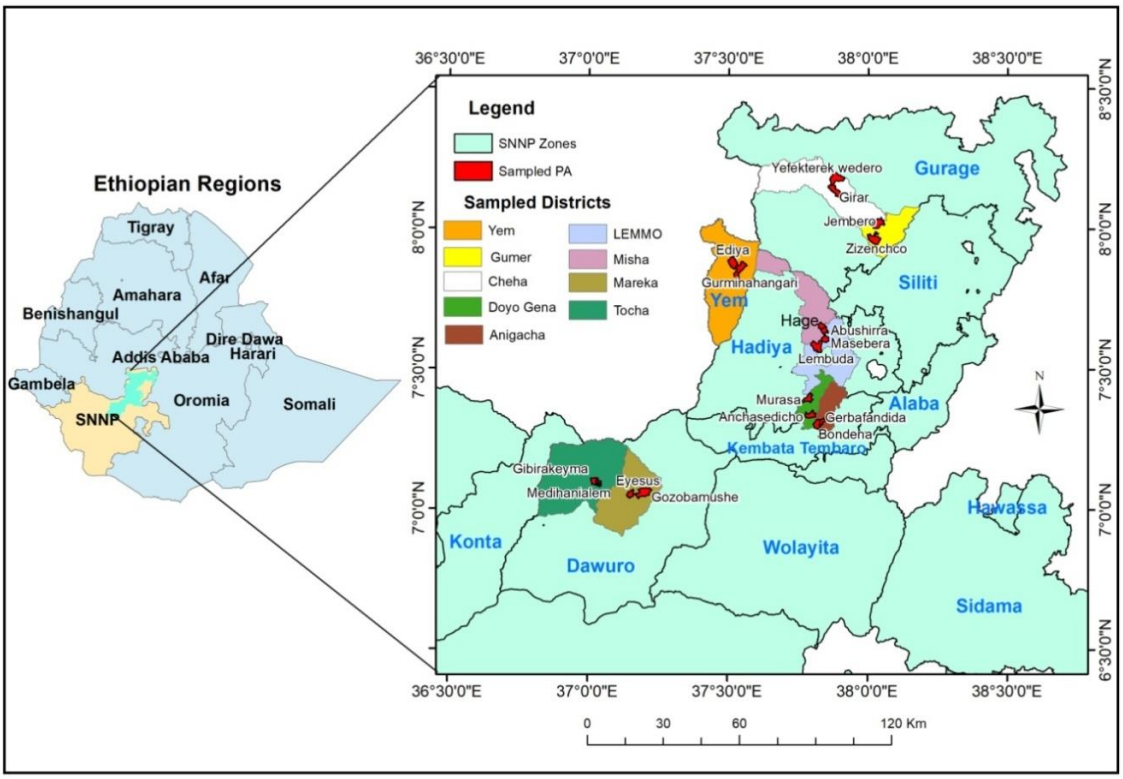

Figure 3.1 Map of Ethiopia showing its Federal Regions (left) and enset sample collection sites that represent the differently colred 9 studied districts, that contain red colored 18 PA, within 4 zones and 1 special district of SNNP Region. The map was constructed using geographic coordinates and elevation data collected from each sites using global positioning system (GPS). PA= Peasant association (Kebele)

3.7.3. Data collection

For identification and characterization of enset landraces used in traditional medicine, key informants (3–5 in number) were selected at each PA, based on their experience and knowledge in production and medicinal use of enset landraces, by the help of agricultural development agents and PA administrations. During the identification of landraces, slight dialect variations within the same ethnic group were not considered. Whereas, landraces having identical names, but originated from different administrative zones (ethnic groups) were considered as different, and included as a new landrace, as the existence of homonyms, giving the same name for

different enset genotypes at different localities, was reported (Temesgen Magule *et al*., 2015; Endale, 1997). A total of 40 enset landraces used in traditional medicine were identified from the 18 PAs (Table 3.1, Appendix Table 2).

Table 3.1 List of 40 enset landraces used as traditional medicine included in the phenotypic diversity study and sites of collection

Local name of the landraces	**Number of landraces**	**Site of collection**	
		Zone	**Altitude range**
Astara, atshakit, bisha-eset, denkinet chehuyet, dere, guarye, kibnar, sinwot and terye	10	Gurage	1976-2834
Agede, astar, haywona, kiniwara gishra bedededa, kombotra, mekelwesa and oniya	9	Hadya	2324-2520
Tesa, kiklekey, kiklenech, agene, sebera, mentiwea, cherkiwa, oniya2, astar2, gishra2 and beleka	11	Kembata-Tembaro	2356-2558
Asu, kinkisir, gariye, deya, anchiro and karona	6	Yem*	2541-2586
Lochingia, arke, tsela and botsameze	4	Dawro	2327-2702

* Special district

The data were collected from five to six-year-old plants which were close to maturity or fully matured, and with no disease symptoms. As the landraces grew at different environmental conditions, only qualitative traits were used, as they are less affected by the environment than quantitative traits. Since no standardized descriptor was developed for enset, fourteen qualitative traits, as shown in **Table 3.2** and **Appendix Table 3**, were used based on farmers' indigenous knowledge and previous reports (Mikias Yeshitila, 2014; Zerihun Yemataw *et al*., 2014a) with some modifications.

Table 3.2 Ttraits and number of trait categories used in phenotypic diversity of enset landraces used in traditional medicine

Trait Name	Acronyms	No of trait categories
Pseudo stem Color	PSSC	14
Color of petiole ventral surface	CPVS	5
Color of petiole dorsal surface	CPDS	8
Color of midrib ventral surface	CMVS	5
Color of midrib dorsal surface	CMDS	8
Color of leaf upper surface	CLUS	5
Color of leaf edge and tip	CLET	4
Leaf growth habit	LGRH	3
Corm quality	COQU	4
Kocho quality	KOQU	4
Bulla quality	BUQU	4
Fiber quality	FOQU	4
Bacterial wilt tolerance	BWDT	3
Drought tolerance	DROT	3

Color traits are among the first descriptors that farmers use in the identification of enset landraces (Masayoshi Shigeta, 1991). The data on color descriptors of pseudostem color (PSSC), color of petiole ventral surface (CPVS), color of petiole dorsal surface (CPDS), color of midrib ventral surface (CMVS), color of leaf upper surface (CLUS), color of leaf dorsal surface (CLDS) and color of leaf edge and tip (CLET) were collected directly through field observation and by comparing with color chart (Munsell, 1970). To reduce environmental effects, all the data were collected from three to four individual plants of each landrace that grew at different environmental conditions. Moreover, during sampling, informants with wide

experience in the enset culture and indigenous knowledge assisted the sampling, so that a representative sample of each landrace was included in the study. The picture of each landrace was also taken for further verification and documentation.

The data on leaf growth habit (LGRH), corm quality (COQU), taste, quality of *kocho* (KOQU), taste, quality of *bulla* (BUQU), taste, quality of fiber (FIQU), strength, bacterial wilt disease tolerance (BWDT) and drought tolerance (DROT), in comparison with non-medicinal landraces, were collected through focus group discussions. Focus group discussions were held at each peasant association by involving elder farmers and agricultural development agents, which have rich knowledge and experience in enset production, processing and consumption.

3.7.4. Data scoring and analysis

The data collected on the fourteen qualitative traits was scored numerically as 1, 2, 3…etc, based on the number of trait categories, and the landraces vs trait data matrics was used for analysis.

Frequency distribution

The frequency distribution of each of the descriptors for the fourteen qualitative traits was analyzed using SAS statistical software version 9.2 (SAS, 2002).

Principal component analysis (PCA)

To investigate patterns of correlations among observed variables (phenotypic trats) and the contributions of individual traits to the observed diversity, PCA was performed using the SAS software version 9.2 (SAS, 2002) and MINITAB software version 14 (MINITAB 2003). Eigen values, the percentage of the variation accumulated by the PCs and the load coefficient values between the original traits and respective principal components were quantified. The first two principal components which accounted for the highest variation were used to plot a two-dimensional dispersion or scatter diagram of the landraces, and to identify the overall diversity of enset landraces.

Cluster analysis

For cluster analysis, the landrace vs qualitative data matrix was used to generate hierarchical dendrogram through an unweighted pair-group method with arithmetic average (UPGMA) based on the average linkage method with Euclidean distance using SAS statistical software version 9.2 (SAS, 2002).

3.2. Comparison of Proximate, Mineral and Phytochemical Composition of Enset (*Ensete ventricosum* (Welw.) Cheesman) Landraces Used in Traditional Medicine and Other Landraces

3.2.1. Plant material and treatments

The experiment was carried out on three enset landraces known by avernacular names: *astara, guarye* and *kibnar* which are popularly used in treatment of bone fractures, traditionally, and two other landraces (*amerat* and *yeshiraqinqe*) that have other use values (Table 3.3). Since the mainly used part of the plant used in treatment of bone fracture is the corm, the corm samples were collected from the five enset landraces growing in farmers' field at Gurage zone, SNNP Region of Ethiopia, in August 2016. To reduce soil nutrient effect, collection was made from a single farm (when possible) or from two border farms, and the age of the sampled plant was restricted to four to five years, the commonly used age for traditional medicine. The treatments were replicated three times, and to represent the different enset growing agro-ecology, the replicate samples were collected from three different altitudes (1900, 2100 and 2300 meter above sea level). Each collected samples were put in a labeled zip locked plastic bag and preserved in ice box during transportation for analysis.

Table 3.3 Enset (*Ensete ventricosum*) landraces collected from Gurage zone, SNNPR for proximate, mineral and phytochemical composition analysis of the corm

S. N	Name enset landrace	Major uses	Predominant character
1	*Amerat*	food	good *kocho* quality
2	*Astara*	food and traditional medicine	sweet corm
3	*Guarye*	food and traditional medicine	sweet corm
4	*Kibnar*	food and traditional medicine	sweet corm
5	*yeshiraqinqe*	Food	high yielding and disease tolerance

3.2.2. Sample preparation

The collected enset corm samples were washed, sliced into pieces (ca.5 mm) separately, weighed and dried in an oven at 70 ^{o}C for 42 hours, until constant weight was attained. The dried samples were then finely ground using Laboratory Miller as shown in **Figure 3.2**. The samples were sealed in airtight plastic cups, and stored in desiccators for further analysis of different parameters, at Ethiopian Public Health Institute and Addis Ababa University, Centre of Food Science and Nutrition laboratories.

3.2.3. Proximate analysis

Moisture content

Moisture contents of enset corm samples were determined by the method of the Association of Official Analytical Chemists' (AOAC, 2005); the Official Method 930.15.

Figure 3.2. Sample preparation for proximate, mineral and phytochemical analysis of enset corm; A/ slicing the the corm, B/ weighing the raw sample, and C/grinding the dried sample

After the cleaned and dried crucibles were placed in desiccators and weighed (W_1), about 40 g sample was accurately weighed (W_2) in the previously weighed crucible, and put in desiccators. Then the crucible with its content was put into an oven (Binder GMBH, Model; M-115, Germany) and dried at 70 ˚C for 42 h until constant mass was attained, and weighted

(W_3) after cooling in desiccators to room temperature. The moisture content was then determined using the equation as follows:

$$\text{Moisture content in percent (\%)} = \left(\frac{W_2 - W_3}{W_2 - W_1}\right) \times 100 \quad (1)$$

Crude fiber content

Two-gram of enset corm sample was transferred to 600 ml beaker and boiled with 200 ml 1.25% sulfuric acid for 30 min. After it was digested by 20 ml 28% NaOH for 30 min, the mixtures were filtered through a crucible filled with a layer of sea sand using a vacuum pump and the residues were washed with hot distilled water several times. The left residue was washed three times under vacuum, each time with 30 ml of 1% sulfuric acid solution and then distilled water, 1% sodium hydroxide solution and then distilled water and acetone, and dried by suction. It was then dried at 130˚C for 2 h, cooled in desiccators, and weighed (W1). After incinerating the samples in a muffle furnace at 550 ˚C for 2 h, the samples were cooled in desiccators and weighed again (W2). The total crude fiber was expressed in percentage as:

$$\text{Crude fiber} = \left(\frac{W_1 - W_2}{W_3}\right) \times 100 \quad (2)$$

Where; W_3 is the weight of samples

Ash content

Ash was determined by the method of the Association of Official Analytical Chemists' (AOAC, 2016), using the Official Method 923.03. Clean crucibles, dried at 100 ˚C in an oven were cooled in desiccators and weighed (W_1). Then 3 g sample was weighed into a previously

weighed crucible (W_2). Then crucibles with their contents were burned in a Muffle furnace (Stuart SF, U.K) set at 550 ˚C for 2 h until light gray ash was resulted, and then weighed (W3) after cooling. The weight of the ash was expressed as a percentage of the initial weight of the sample as follows:

$$\text{Total Ash (\%)} = \left(\frac{W_2 - W_3}{W_2 - W_1} \right) \times 100 \qquad (3)$$

Crude protein content

Crude protein was determined using the Kjeldahl method (AOAC, 2016), official method 2001.11. One gram of the corm sample was weighed into a digestion flask and 12 ml of concentrated H_2SO_4 was carefully added to each flask and 3 ml of 30% hydrogen peroxide was added step by step after mixed carefully. As soon as a violent reaction ceased the tubes were shaken and 3 g catalyst, a mixture of copper sulfate with potassium sulfate, was added and digested by heating at 420 ˚C. After the digestion had been completed, the digestion flasks were connected to a receiving flask containing2% boric acid and indicators methyl red and bromocresol green. The solution in the digestion flask was then made alkaline by addition of 40% NaOH to liberate ammonia gas into receiving flask, where it converts the boric acid to the borate ion. Finally, the distillate was titrated with standard 0.1 M HCl and the percent total nitrogen and crude protein were calculated according to AOAC (2016), using the equation below:

$$\text{Nitrogen (\%)} = \frac{(V2 - V1) \times N \times 14.007 \times 100}{W_o}$$

Where: V2 = Volume in ml of standard sulfuric acid solution used in the titration for the test material.

V1 = Volume in ml of standard sulfuric acid solution used in the titration for the blank determination.

N = Normality of standard sulfuric acid (0.1N).

W_o = sample weight on dry matter basis and 14.007 is the molecular weight of nitrogen.

The crude protein content was the determined using conversion factor; Crude protein content percent per weight = total nitrogen * 6.25 (James, 1995).

Crude fat content

Crude fat was determined based on the Soxhlet extraction method (AOAC, 2003), using the official method 2003.06. After aluminum cups with boiling chips were dried in drying oven at102 $\pm$ 2 ˚C for at least 30 min, they were cooled in the desiccators to room temperature and weighed (W1). Five gram of sample was weighed into thimbles and extracted with 70 ml diethyl ether. After finishing the extraction process, the cups were removed from the extractor and dried in 102 $\pm$ 2 ˚C oven for 30 min to evaporate the solvent. The extraction cups were cooled in desiccators for 30 min and weighed (W2) immediately after they are taken out from the desiccators. The obtained fat was expressed as a percentage of the initial weight of the sample using the formula:

$$\text{Crude fat } (\%) = \left(\frac{W2 - W1}{SW} \right) \times 100 \qquad (4)$$

Where, SW is the weight of the sample

Total carbohydrate content

The percentage of total carbohydrate was computed by difference, which involves adding the total values of crude protein, crude fat, moisture and ash constituents of the sample and subtracting it from 100%. It is determined as follows:

$$Total\%Carbohydrate = 100 - (\%M + \%P + \%F + \%A) \quad (5)$$

Where, M = moisture, P = protein, F = fat and A= ash contents Mineral analysis

3.2.4. Mineral analysis

Approximately 3 g of each powdered enset corm sample was ignited to ash at 550 ˚C in a muffle furnace and dissolved in 20% HCl and boiled to bring the ash into solution form. The solution was cooled and filtered through a filter paper (42mm Whatman) into 100 ml acid washed volumetric flask. The residue was dissolved and transferred to the volumetric flask and the volume was adjusted with distilled, deionized water. Blank solution was prepared in a similar way and the minerals calcium, magnesium, iron, zinc, manganese and copper were determined using atomic absorption spectrometer (Shimadzu, model AA-6800, Tokyo, Japan). Phosphorus was determined by using UV-VIS spectrophotometer (Thermoscientific model; evolution 220, USA) based on AOAC (1990), while, sodium and potassium contents were determined by employing flame photometry (Jenway model; pfp7, UK) as per Osborne and Voogt (1978).

3.2.5. Phytochemical analysis

Laboratory procedure for the determination of phytate was as outlined by Latta and Eskin (1980) and later modified by Vaintraub and Lapteva (1988). Dried sample of 0.5gm was extracted with 10ml of 0.2N HCl for 1hr at an ambient temperature and centrifuged at 3000rpm for 30min. Then 2 ml of wade reagent was added to 3ml of the supernatant sample solution and centrifuged at 3000rpm for 10minut after homogenized. The absorbance at 500nm was measured using UV-Vis spectrophotometer (Thermoscientific model; evolution 220, USA). The phytate concentration was calculated from the difference between the absorbance of the blank (3ml of 0.2N HCl + 2ml of Wade reagent (a mixture equal volume of 0.03% Sulfosalcilic acid and 0.03% $FeCl_3.6H_2O$) and that of examined sample. Then the amount of phytic acid was calculated using phytic acid standard curve and the result was expressed as phytic acid in percent.

For analysis of tannin concentration, One gram of each sample was weighed in a screw cap test tube, and 10ml of 1%HCl in methanol was added to the tube containing sample. The tube was put on mechanical shaker for 24 hr at room temperature, and centrifuged at 1000rpm for 5minute. One milliliter of supernatant was taken and mixed with 5ml of vanillin-HCl reagent in another test tube, and then after 20 minute the absorbance at 500nm was read in a spectrophotometer, based on Burns (1971) principle, as modified by Maxson and Rooney (1972).

3.2.6. Statistical analysis

The data collected on proximate, mineral and phytochemical composition of the five enset landraces, with three replications, determined were subjected to analysis of variance using SAS

statistical software, version 9.2 (SAS, 2002). When a significant difference existed among the landraces, comparisons of means were made using Duncan's Multiple Range test at 5% probability levels.

3.3. Genetic Diversity of Enset (*Ensete ventricosum*) Landraces Used in Traditional Medicine Using SSR Markers

3.3.1. Plant material and genomic DNA extraction

Thirty eight cultivated and named *Ensete ventricosum* landraces used in the treatment of seven different diseases were identified with the help of knowledgeable village elders from 5 locations (Gurage, Hadya, Kembata-Tembaro, Dawro and Yem) of north eastern parts of SNNP regional state of Ethiopia. For comparison, 13 enset landraces that have other non-medicinal use values were also included (principally used for human food), which brought the total number of landraces to 51. The samples were collected from individual farmers' fields, located at 18 Kebele (the lowest tier of civil administration unit) from across the enset distribution. Since different landraces may have been given the same vernacular name at different locations (Temesgen Magule *et al*., 2015), landraces having identical names, but originated from different locations, were labeled by including the first letter of names of location after a vernacular name. To test the consistency of naming of landraces within each location, up to 4 duplicate samples (based on their availability) were collected for some landraces; so that a total of 92 plant samples were collected. The detail of the samples is shown in **Apendix Table 4.**

Healthy young cigar leaf (a recently emerged leaf still rolled as a cylinder) tissue samples were collected from the 92 individual plants (one to three plants per landrace) from November to March 2017 and they were stored in a zip locked plastic bag containing silica gel and preserved until extraction of genomic DNA. The genomic DNA was extracted following modified CTAB (cetyltrimethylammonium bromide) extraction protocol (Borsch *et al.*, 2003) with some minor modifications, as described in below.

About 150mg of dried leaf samples were ground with grinder beads and each pulverized leaf sample was put into a 2ml autoclaved and labeled Eppendrof tubes. Then after a CTAB solution (1000 µl per sample) which was mixed with 0.2 vol % Mercapto-ethanol and warmed in water bath up to 65°, was pored into each Eppendrof tubes containing the leaf sample to each tube to break down cells and cellular contents. Next, the contents in each tube were mixed well, and kept in water bath for 30 minute at 65 ^{0}C, and then centrifuged at 13,000 rpm for 7 minutes. Afterwards, the supernatant solution was transferred into new Eppendrof tubes using blue pipette tips which are cut. Chloroform (600 µl) was added to the caps with supernatant immediately and shaked carefully for approximately 5 minutes and then centrifuged for 7 minutes at 13000 rpm. The supernatant was transferred to a new Eppendorf-cap, and the step cleaning with chloroform was repeated make sure that all impurities are removed. Cooled Isopropanol (4°C), approximately $^{2}/_{3}$ of the solution volume was added to the harvested supernatant solution and shaked carefully by inversing the Eppendorf cap. At this point, the samples were incubated at -20ºC for 2 hours.

Subsequently, the samples were removed from the freezer and centrifuged at 13,000 rpm for 15 minutes and then, the liquid part is aspirate using yellow tips, without touching pellet. Then, 200 µl of 70 % ethanol was added to the pellet and centrifuged for 15 min at 13000 rpm, after rinsing inner surface by turning the cap. Following that, the ethanol was aspirated using yellow tips and the DNA-pellet was air-dried by placing the tubes upside down on a paper towel, for 15 minutes at room temperature. The pellet was then dissolved in 100 µl TE and stored at 4° for overnight.

In the following day, cooled 7.5 M NH_4Ac-solution (half of the solution volume) was added to each tube and mixed carefully. Then after cooled 100 % ethanol (double of the solution volume) was added to each tube and after carefully mixed, they were freezed for more than 2hr at -20°C. Subsequently, they were centrifuge 35 min at 13000 rpm and the fluid was aspirated carefully. Then, 200 µl ethanol (70%) was added and, after rinse the inner cap surface by turning the cap, they were centrifuge 10 min. at 15000 rpm. The liquid was aspirated, and the pellet was dried at room temperature, the the pellet was dissolve in 100 µl TE. After repeating the cleaning step, with 3 M NaAC-solution and 100% ethanol, air-dried DNA pellets were dissolved in 100 µl TE, and stored at 4°C.The quality of DNA was then determined by using 1% agarose gel, while NanoDrop (Thermo Scientific NanoDrop 2000/2000c Spectrophotometer**,** NanoDropTechnologies, Wilmington, DE) was used to determine the concentration of DNA. The DNA was dissolved in 100 µl TE buffer and stored at -20 ˚C until used for PCR.

3.3.2. PCR amplification and electrophoresis

Twenty one enset SSRs primer-pairs (14 from Temesgen Magule *et al.*, 2015 and 7 from Biswas *et al.*, unpublished /http://enset-project.org/EnMom@base.html) were initially screened for good amplification, polymorphism and specificity to their target loci using 15 DNA samples, representing the 4 locations of *Ensete ventricosum*. This led to the selection of 15 primer-pairs to genotype the landraces. The detail of the 21 SSR primers is presented in **Table 3.4**.

Table 3.4. Description and source of the 15 SSR primers used in genetic diversity of 92 enset landraces and 7 other primers that donot amplify or are not polymorphic

Marker name	Forward primer sequence (5'–3')	Reverse primer sequence (5'–3')	Repeat motif	Size (bp)	Reference	PCR Result	Ta** (°C)
Evg-01	AGTCATTGTGCGCAGTTTCC	CGGAGGACTCCATGTGGATGAG	(CTT)8	100–120	TM* *et al*., 2015	++	60
Evg-02	GGAGAAGCATTTGAAGGTTCTTG	TTCGCATTTATCCCTGGCAC	(AG)12	118–153	TM* *et al*., 2015	++	62
Evg-03	ACAGCATAAGCGAAATAGCAG	ACAGCATAAGCGAAATAGCAG	(AG)12	_	TM* *et al*., 2015	_	60
Evg-04	GCCATCGAGAGCTAAGGGG	GGCAAGGCCGTAAGATCAAC	(AG)21	113–147	TM* *et al*., 2015	++	60
Evg-05	AGTTGTCACCAATTGCACCG	CCATCCTCCACACATGCC	(GA)22	103–141	TM* *et al*., 2015	++	62
Evg-06	CCGAAGTGCAACACCAGAG	TCGCTTTGCTCAACATCACC	(GAA)9	202–211	TM* *et al*., 2015	++	62
Evg-07	GGTTGTCCTCAAGAACGTGG	TGATGCCTAATGCCTCTCCC	(GTG)9	_	TM* *et al*., 2015	_	60
Evg-08	CCATCGACGCCTTAACAGAG	TGAACCTCGGGAGTGACATAAG	(GA)21	164–190	TM* *et al*., 2015	++	60
Evg-09	GCCTTTCGTATGCTTGGTGG	ACGTTGTTGCCGACATTCTG	(GA)13	141–175	TM* *et al*., 2015	++	60
Evg-10	CAGCCTGTGCAGCTAATCAC	CAGCAGTTGCAGATCGTGTC	(AG)21	191–210	TM* *et al*., 2015	++	60
Evg-11	GGCCTAGTGACATGATGGTG	TGATGCTAGATTCAAAGTCAAGG	(AC)13	135–160	TM* *et al*., 2015	++	62
Evg-13	CTTGAAAGCATTGCATGTGGC	TCACCACTGTAGACCTCAGC	(CA)14	189–229	TM* *et al*., 2015	++	62
Evg-14	AACCAATCTGCCTGCATGTG	GCCAGTGATTGTTGAGGTGG	(TGA)8	153–159	TM* *et al*., 2015	++	62
Evg-15	TCCTTTAGGTTATTTGGTTGCC	CCTTGGACATGCCTCACATC	(AG)15	110–134	TM* *et al*., 2015	Not poly	60
EnOnj	ATCTGCATGCACCCTAGCTT	AAACCCTAACGTCCCTCCTC	(GT)10	189	Biswas et al., unpublished	++	62
EnBed	ATCAAGGTCATGTGCTGTGC	ATCAAGGTCATGTGCTGTGC	(CT)11	116	Biswas et al., unpublished	++	62
EnM0001	GATCTGATCCACCTCCTCGT	CGACAAGGATCAAAATGGCT	(AGG)5	277	Biswas et al., unpublished	++	64
EnM0002	TTCTCTTGCTGCACACACC	TCATGATCCCTGTCCTCCTC	(GA)9	313	Biswas et al., unpublished	++	64
EnJun018	CGATGTCCACGTTTAGCACA	TGCACAAAGCTACGAACCAC	(AG)13	122	Biswas et al., unpublished	Not poly	59
EnJun007	TTGGCAACAATGGGCTTAAT	CGTGTGTGTGTGTGTGTGTG	(TC)13	81	Biswas et al., unpublished	Not poly	57
EnDer 039	AGTTGGGATGAGGTGTGGAG	ATTATGGACATGGGAACGGA	(AG)11	186	Biswas et al., unpublished	Not poly	60

TM* = Temesgen Magule, Ta**= annealing temperature, EnOnj= EnOnjSSR049028, EnBed= EnBedSSR020585, EnM0001= EnM00011571 and EnM0002 =EnM00025665, Enjun018 = EnJunSSR01801, EnJun007= EnJunSSR007429, EnDer 039 = EnDerSSR039568, ++ = polymorphic, - = not amplified and Not poly = not polymorphic

PCR amplification was carried out in a 20 µl reaction volume containing 1.5 µl standardized (100 mM) template DNA, 11.5 µl molecular reagent water, W 4502 (Sigma, St. Louis USA) 0.75 µl dNTPs (10 mM), (Bio line, London) 2.5 µl Taq buffer (10 × Thermopol reaction buffer), 1.25 µl $MgCl_2$ (50 mM), 1 µl forward and reverse primers (10 mM) and 0.5 µl (5 U/µl) BioTaq DNA polymerase (Bioline, London) and amplified using PCR thermal cycler (BiometraTOne, Terra Universal, Germany). A three step amplification program consisted of: initial (1) denaturation for 2 min at 95°C, (2) 35 cycles of denaturation at 95°C for 1min, annealing at a temperature specific to each primer set, as presented in **Table 3.4**, for 1 min, extension at 72°C for 1min and (3) final extension at 72°C for 10 min. The PCR products were stored at 4°C until electrophoresis was carried out.

Separation of the amplified product was accomplished in a 4% (w/v) agarose (Molecular grade, Bioline, London) gel in 1% (w/v) TAE (Tris-acetate-EDTA) buffer containing ethidium bromide, and electrophoresed at 80 V for 3 h. A standard DNA ladder of 100 bp (Q step 2, Yorkshire Bioscience Ltd Biocenter, UK) was loaded together with the samples to estimate molecular weight. The band pattern was visualized using gel documentation system (NuGenius, SYNGENE, Cambridge, UK), and the pictures were documented for scoring.

3.3.3. Data scoring and analysis

The sizes of clearly amplified fragments were estimated by comparing the migration distance of bands relative to the molecular weight of known size markers, 100 base pairs (bp) DNA ladder. The gels pictures were aligned horizontally using the standard marker,

and the DNA bands that migrated to the same position, for each locus, were ascribed a number (1, 2, 3...) across all the 92 samples. The data was then organized as in excel sheet for analysis.

Number of different alleles (N_a), the effective number of alleles (N_e), Shannon's information index (I), observed heterozygosity (H_o), expected heterozygosity (H_e), un-biased expected heterozygosity (uH_e), for each locus were computed using GENALEX ver. 6.503 (Peakall R and Smouse PE 2012). The N_a, N_e, I, H_o, H_e (gene diversity,), uHe and percent polymorphism (PPL) for each location was estimated using GENALEX ver. 6.503.

Analyses of molecular variation (AMOVA), which was performed to evaluate the relative level of genetic variations among populations and individuals within populations, was computed using GENALEX 6.503 software. The neighbor-joining (NJ) tree was constructed using the software DARwin (Perrier and Jacquemoud-Collet, 2006) based on Nei's genetic distance (Nei, 1972) to reveal the genetic relationships among the location and individual landraces. The resulting tree was displayed using Fig Tree ver.1.4.3 (Andrew, 2016). Principal coordinates analysis (PCoA) was carried out to evaluate the genetic similarity between the landraces used in traditional medicine and those with other use values, using GENALEX 6.503.

3.4. Micropropagation of Enset (*Ensete ventricosum* (Welw.) Cheesman) Landraces Used in Traditional Medicine Using Shoot Tip Explants

3.4.1. Establishing Stock Plants

Two types of enset (*Ensete ventricosum*) landraces (*guarye* and *kibnar*), which are important in traditional treatment of bone fracture and other diseases were used in this experiment. Three to four-years-old plants, from the two landraces (*guarye* and *kibnar*) obtained from farmers' backyard at Cheha district, Gurage zone, were multiplied using traditional system to be used as stock plants. Multiplication was employed by cutting down the plant at around 10 to 15 cm above the junction between pseudostem and the corm and to prevent apical dominance and to induce sprouting of the lateral buds, the meristem region was removed with a knife. The corms with a portion of pseudostem were then splitted into two and each part was planted in a 60 cm diameter plastic pot containing forest soil and sand mixture (2:1 ratio) and grown in a greenhouse at Wolkite University. The sprouts were emerged after 2 months, and the suckers were separated and transplanted individually in to 16 cm diameter polyethylene bags 2 months after emergence as shown in **Figure 3.3**. The suckers grown for 2 months after transplanting were used as starting material in the micropropagation experiment.

3.4.2. Preparation of stock solution and growth media

Murashige and Skoog (MS) (Murashige and Skoog, 1962 media was the basal media used throughout this study and, full strength stock solution of macronutrients, micronutrients, Iron-EDTA and vitamins were prepared separatel, and stored at -20°C until used. The macro nutrients were 10 times concentrated, so that 100 ml per liter was used during media preparation, while micro nutrients, iron-EDTA and vitamins were 100 times concentrated, and from each 10 ml was used for preparation of one liter of media.

Plant growth regulators used in all parts of this study were 6-benzyl amino-purine (BAP), and Indole-3-butyric acid (IBA). In preparing stock solutions, powders of each growth

regulators were dissolved in 3-4 drops of 1 Normal NaOH/HCl acid and then distilled water was added to adjust the required volume in 1mg/ml concentration.

From MS stock solution prepared, working solution was by suplimenting 30g sucrose, 1.5g activated charcoal and 1.5 mg BAP per liter and solidified with 8g l^{-1} agar. In all cases the pH of the media was adjusted to 5.8 before autoclaving at a temperature of 121°C and, pressure of 105 kPa for 15 minutes. In order to study the effect of antibiotic in controlling the endogenous bacterial contamination, two different concentrations (250 and 500 mg l^{-1}) of cefotaxime, sterilized using syringe-driven filter with a pore size of 0.22 µm, were incorporated to the autoclaved MS medium after cooling in the laminar air-flow hood, while the control treatments were devoid of the cefotaxime.

3.4.3. Sterilization and culture initiation

From the suckers of 6 months old greenhouse grown *guarye* and *kibnar* enset landraces, shoots with 1.5 cm of corms 4 to 5cm in length and 2 to 3cm in diameter were excised using a stainless steel knife and placed into labeled beakers. After cleaning the shoots from dirt and phenol exudates using running tap water and detergents for around fifteen minutes, they were trimmed to a length of 2.5 to 3cm, and around 1.5 cm in diameter, and rinsed three times with sterile distilled water.

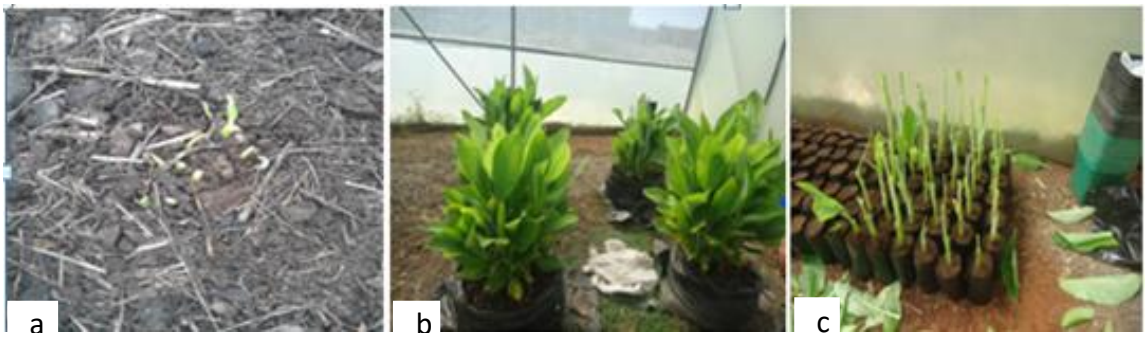

Figure 3.3 *Ex vitro* multiplication of *guarye* and *kibnar* enset landraces in a greenhouse as explant source for micropropagation: a) emerging shoots after 2 months of planting, b) suckers 2 months after emergence and c) sucker transplanted in to a pot individually

The shoots were the surface sterilized by immersing and shaking in a 70% (v/v) ethanol for 10 minutes. After rinsing three times with sterile distilled water, further sterilization was carried out using 2 % (v/v) sodium hypochlorite (NaOCl) containing three drops of Tween-20 for 20 min, and then rinsed three times again with sterile distilled water.

After the final sterilization, the outher leaf sheaths were removed and the height of shoots and corms were trimmed to a final size of 1.0 cm height and diameter shoot tip explants with subtended corm. The explants were then splitted in to half longitudinally, and each halve of the shoot tips was cultured into glass jars containing 60 ml MS growth medium. The cultures were maintained in dark, at room temperature for 15 days, until the shoots start to grow, then after, placed in a growth shelf under a 16/8-h (day/night) photoperiod under light intensity of 22 µmol $m^{-2}s^{-1}$ provided by cool-white fluorescent lamps. They were sub-cultured in 23-25 days in to the same medium compositions so that the corms at the base swollen well and ready for multiplication experiment. The two types of enset landraces and three levels of cefotaxime formed 6 (2x3) treatments were replicated 20 times and arranged in a complete randomized design (CRD).

3.4.4. Shoot multiplication

The initiated shoots were cultured for 15 days on the same fresh medium except antibiotics, to avoid carryover effect of cefotaxime. After 15 days, multiplication was carried out on MS medium supplemented with 10 different concentrations of BAP or in combination with IBA in mg l^{-1} (2.5 BAP, 5.0 BAP, 7.5BAP, 2.5 BAP + 0.5 IBA, 5.0 BAP + 0.5 I BA, 5.0 BAP +1.0 IBA, 5.0 BAP + 1.5 IBA, 7.5 BAP + 1.5 IBA and 7.5 BAP + 2.0 IBA), 30g l^{-1} sucrose, 1.5g l^{-1} activated charcoal and 8 g l^{-1}agar. During culturing, the external dark/brown portions of corms of initiated shoots were trimmed with a sterilized blade. The cultures were then maintained at room temperature in the dark for 15 days, until multiple buds start to regenerate, and then maintained at at room temperature under 16/8-h (day/night) photoperiod and 22 µmol $m^{-2}s^{-1}$ light intensity provided by cool-white fluorescent lamps. The two enset landraces and 10 concentrations of PGRs formed 20 (2x10) treatments and replicated 5 times, and arranged in CRD. After 23 to 25 days, 60% of the cultures were used for elongation and rooting experiment, while the remaining were successively sub-cultured on the best performed bud proliferation medium (MS medium supplemented with 5.0 mg l^{-1} BAP + 1.0 mg l^{-1} IBA) for further multiplication of shoots and to look at the performance in the successive multiplication.

3.4.5. Shoot Elongation and Rooting

After multiple shoots were developed in the bud proliferation /multiplication/ medium, shoot clusters were sub-cultured in the same fresh multiplication medium for better elongation before rooting experiment. The cultures were maintained at room temperature under a 16/8-h (day/night) and 22 µmol $m^{-2}s^{-1}$ light intensity provided by cool-white fluorescent lamps for 23 to 25 days, until shoots were well grown. In order to avoid

carryover effects of PGRs during rooting experiment , the shoots were cultured on PGRs free MS medium containing 30 g l^{-1} sucrose, 1.5 g l^{-1} activated charcoal and 8 g l^{-1} agar for 15 days.

For root induction, the well grown individual shoots were separated and transferred to MS and 1/2 MS media each containing (1, 2, 3 and 4 mgl^{-1} IBA). The two enset landraces, the 10 MS/half-MS media + IBA concentrations combinations formed 20 (2x10) treatments, which were replicated 5 times and arranged in CRD. The pH of the medium and the growth condition were as indicated in shoot proliferation stage.

3.4.6. Acclimatization

Plantlets were removed from the culture vessel after 25days and, roots were washed gently under running tap water followed by planting on perforated seed trays containing forest soil and sand in a 2:1 ratio. The trays were labeled and covered with polyethylene bags and kept moist under shade in a greenhouse, to ensure high humidity and to reduce the direct sun light effect. The humidity was gradually reduced by reducing watering frequency and by reducing the shade. Then the shade was totally removed after 21 days so that plantlets were kept in an open space, in the greenhouse, and they were transferred to polythene bags filled with forest soil and sand in a 2:1 ratio.

3.4.7. Data collection and statistical analysis

The data on number of contaminanted explants due to endogenous microorganisms (those occurred at the base of the explants in the culture, after 7^{th} day), number of shoots per shoot tip, height of shoots, number of roots per shoot, length of roots, and performance of

plantlets during acclimatization in the greenhouse were recorded. The number of shoots regenerated from half shoots was multiplied by two in order to indicate the rate of multiplication per shoot tip. In all cases the data were then subjected to analysis of variance using SAS statistical software, version 9.2 (SAS, 2002), and significant treatment means were separated using Fisher's least significant difference (LSD) test at a 5% probability.

Chapter 4.

RESULTS

4.1. Genetic Diversity of Enset (Ensete ventricosum (Welw.) Cheesman) Landraces Used in Traditional Medicine Using Phenotypic Traits

4.1.1. Frequency distribution

The frequency distribution of 14 qualitative trait categories of the forty enset landraces used in traditional medicine is as shown in **Table 4.1**. The predominant pseudostem colors of enset samples were green-yellow with black patches/strip/spot (20%) and light to medium green with black patch and spots (15%), which were followed by both green yellow and black with red purple patches/spots and red purple with black or brown patches/strips (10%). The variability in color of pseudostem, petiole and midrib are shown for selected enset landraces (Figure 4.1). Regarding the color of petiole, the predominant colors of the ventral and dorsal surface were light to medium green with black spots/strip/ patches (40%) and red-purple with light to medium green at both sides (37.5%). Rarely observed colors at the ventral and dorsal surface of the petiole were red-purple and red-pink (2.5% each) and red purple with black and green strips at both sides (2.5%). On the other hand, the highest proportions of midrib ventral and dorsal surface colors were light to medium green (52.5%) and red-purple with light to medium green at both sides (37.5%).

Almost half (52.5%) of the landraces were characterized by light to medium green leaf color on the ventral surface, while only 2.5% of the landraces possess the red-pink with green strip color. The variability in color of the edge and tip of the leaf was low, with

Table 4.1 Frequency distribution for 14 qualitative traits of enset landraces used in traditional medicine

No	Traits	Description of trait categories	Frequency	
			Number	%
1	Pseudostem color (PSSC)	light to medium green with black patch and spots	6	15.0
		light to medium green with black and red purple strips/patches	2	5.0
		light to medium green mixed with light pink	1	2.5
		light to medium green mixed with black	2	5.0
		green yellow with black patches/strip/spot	8	20.0
		green yellow with red purple base	2	5.0
		green yellow and black with red purple patches/ spots	4	10.0
		yellow red with black and red purple patches/spots	2	5.0
		red purple with green patches/strips	3	7.5
		red purple with yellow patches/spot	1	2.5
		red purple with black or brown patches/strips	4	10.0
		red purple	2	5.0
		dark red purple	2	5.0
		red pink	1	2.5
2	Color of petiole ventral surface (CPVS)	light to medium green	13	32.5
		light to medium green with black spot/ strip/ patches	16	40.0
		red purple with light to medium green at both side	6	15
		red purple	1	2.5
		dark red purple	3	7.5
		red pink	1	2.5
3	Color of petiole dorsal surface (CPDS)	light to medium green with black spots/strip/ patches	21	52.5
		red purple with light green side parts and black spots/ patches	9	22.5
		red purple with light yellow side parts	2	5.0
		red purple with black and green strips at both side	1	2.5
		red purple with green strips	3	7.5
		red pink	1	2.5
		black with green strips and patches	3	7.5
4	Color of midrib ventral surface (CMVS)	light to medium green	21	52.5
		light to medium green with black spot	2	5.0
		red purple with light to medium green at both side	10	25.0
		red purple	3	7.5
		dark red purple	3	7.5
		red pink	1	2.5

5	Color Leaf upper surface (CLUS)	light to medium green	21	52.5
		green to deep green	14	35.0
		light green with red purple strips	1	2.5
		red purple with green strips	3	7.5
		red pink with green strip	1	2.5
6	Color of midrib dorsal surface(CMDS	light to medium green	8	20.0
		light to medium green with black spots/ strips/patches	7	17.5
		light to medium green with red purple strips	2	5.0
		light yellow	2	5.0
		red purple with light to medium green at both side	15	37.5
		red purple	3	7.5
		dark red purple	2	5.0
		red pink	1	2.5
7	Color leaf-edge and tip (CLET)	Brown	35	87.5
		black brown	2	5.0
		red purple	2	5.0
		red pink	1	2.5
8	Leaf growth habit (LGRH)	Erect	21	52.5
		Intermediate	14	35.0
		Drooping	5	12.5
9	Corm quality (COQU)	Low	7	17.5
		Intermediate	13	32.5
		High	19	47.5
		Other	1	2.5
10	*Kocho* quality (KOQU)	Low	8	20.0
		Intermediate	8	20.0
		High	23	57.5
		Other	1	2.5
11	*Bulla* quality (BUQU)	Low	9	22.5
		Intermediate	11	27.5
		High	18	45.0
		Other	2	5.0
12	Fiber quality (FIQU)	Low	19	47.5
		Intermediate	13	32.5
		High	6	15.0
		Other	2	5.0
13	Bacterial wilt tolerance (BWDT)	Low	32	80.0
		Intermediate	7	17.5
		High	1	2.5
14	Drought tolerance (DROT)	Low	15	37.5
		Intermediate	18	45.0
		High	7	17.5

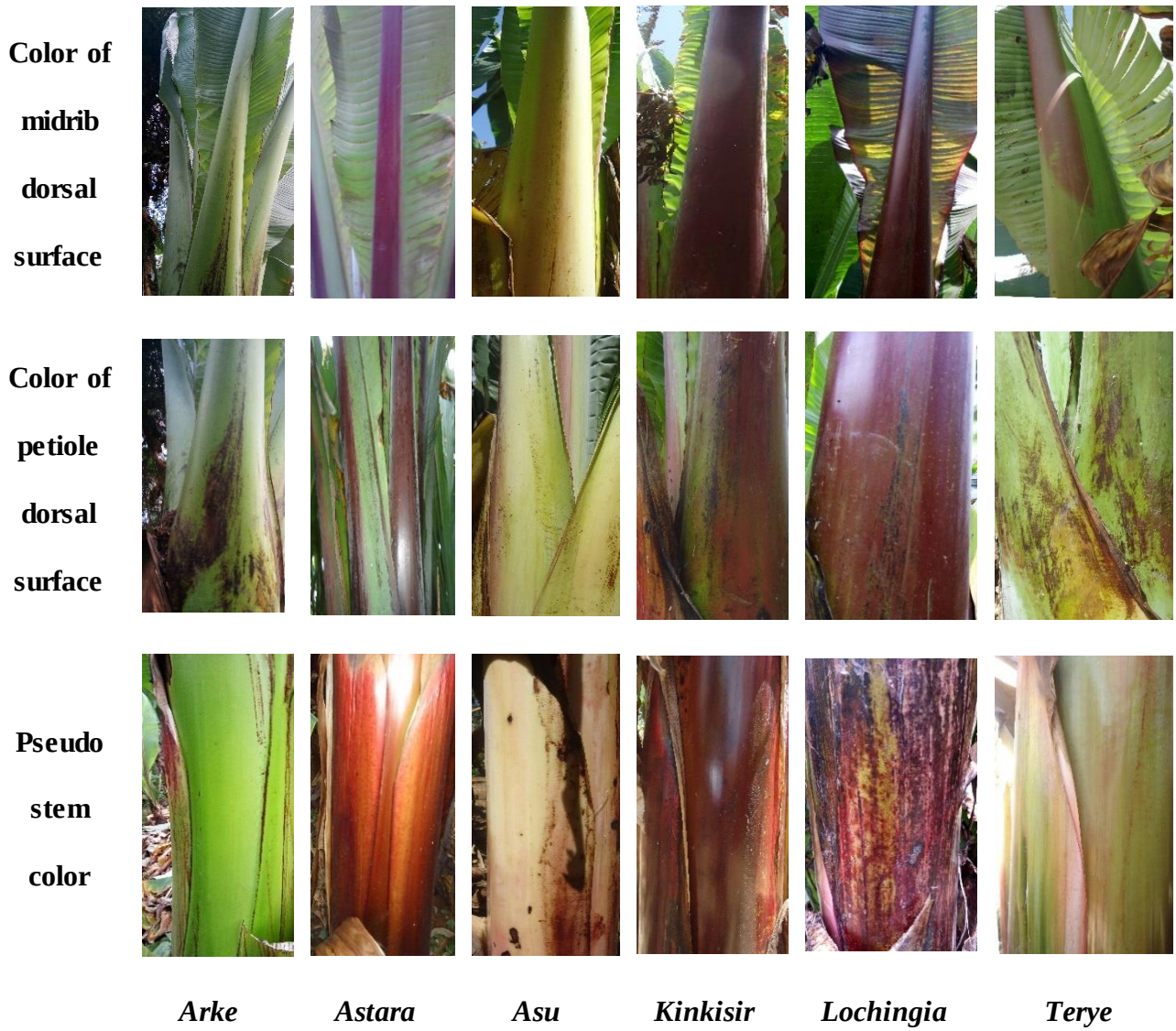

Figure 4.1 Variability in the color of midrib, petiole and pseudostem of six enset landraces used for traditional medicine

87.5% of the genotypes had brown color while the remaining 12.5% were black-brown, red-purple or red-pink. Regarding the growth habit, half of the landraces had erect leaf growth, while the remaining had either intermediate or intermediate drooping growth habit.

The proportions of landraces with high quality of corm, *kocho a*nd *bulla*, in terms of taste were 47.5% 57.5 % and 45% respectively, while the proportions landraces with low quality were 17.5%, 20%, and 22.5% respectively for corm, *kocho* and *bulla* respectively.

Landraces with high fiber quality (in terms of strength) were only 15% whereas, 47.5% were characterized by low fiber quality. With regard to bacterial wilt tolerance, only 2.5% of the landraces were highly tolerant, while 80% were low in tolerance. Similarly only small proportions (17.5%) of landraces are high drought tolerance, the remaining 72.5% had intermediate or low level of tolerance.

4.1.2. Principal component analysis

The principal component analysis (PCA) was carried out to investigate the overall diversity and the traits that have major contributions to the observed phenotypic diversity among the enset landraces. The first four principal components (PCs) with coefficient values greater than 1.0 together explained 74.1% of the total variance among all the studied landraces (Table 4.2). Based on eigen vectors with values greater than or equal to 0.50, traits such as pseudostem color, color of petiole ventral and dorsal surface, color of midrib ventral and dorsal surface, color of leaf upper surface, and color of leaf edge and tip are the major discriminatory characters associated with first principal component (PC-1) which explained 34.4% of the total variations.

The second principal component (PC-2) that explained 18.1% of the total variations was highly associated with corm quality, *bulla* quality and *kocho* quality. The predominant traits in the third component (PC-3) which explained for 13.5% of the variations were fiber quality, drought tolerance and bacterial wilt tolerance, while leaf growth habit was the dominant trait in the fourth component (PC-4), which accounted for 8% of the total variations. In the two dimensional scatter plot generated from the first two most important

principal components (PC1 and PC2), the fourteen phenotypic traits classified the 40 enset landraces into four groups, except one outlier landrace (*bisha-eset*) as shown in **Figure 4.2**.

Table 4.2 The first four principal components (PC) generated from 14 phenotypic traits of the 40 enset landraces used in traditional medicine

Eigen vectors Characters	**PC1**	**PC2**	**PC3**	**PC4**
Pseudostem color	**0.726**	0.186	0.201	0.199
Color of petiole ventral surface	**0.857**	-0.051	0.006	-0.048
Color of petiole dorsal surface	**0.680**	-0.037	-0.231	-0.03
Color of midrib ventral surface	**0.862**	0.075	0.012	0.172
Color of midrib dorsal surface	**0.825**	0.120	0.010	-0.128
Color of leaf upper surface	**0.875**	-0.079	0.071	0.067
Color of leaf-edge and tip	**0.795**	-0.144	-0.128	0.153
Leaf growth habit	-0.333	-0.169	0.037	**0.817**
Corm quality	-0.111	**0.884**	0.070	0.020
Kocho quality	-0.131	**0.864**	0.278	0.127
Bula quality	0.276	**0.715**	0.465	0.128
Fiber quality	0.164	-0.200	**0.685**	0.449
Bacterial wilt tolerance	0.127	-0.217	**0.84**	0.071
Drought tolerance	0.05	-0.542	**0.547**	0.308
Eigen values	4.81	2.54	1.89	1.12
Variance (%)	34.39	18.13	13.52	8.01
Cumulative (%)	34.39	52.52	66.05	74.06

Values in bold indicate the most important traits (>0.50) that have large contributions to the total variance of a particular principal component

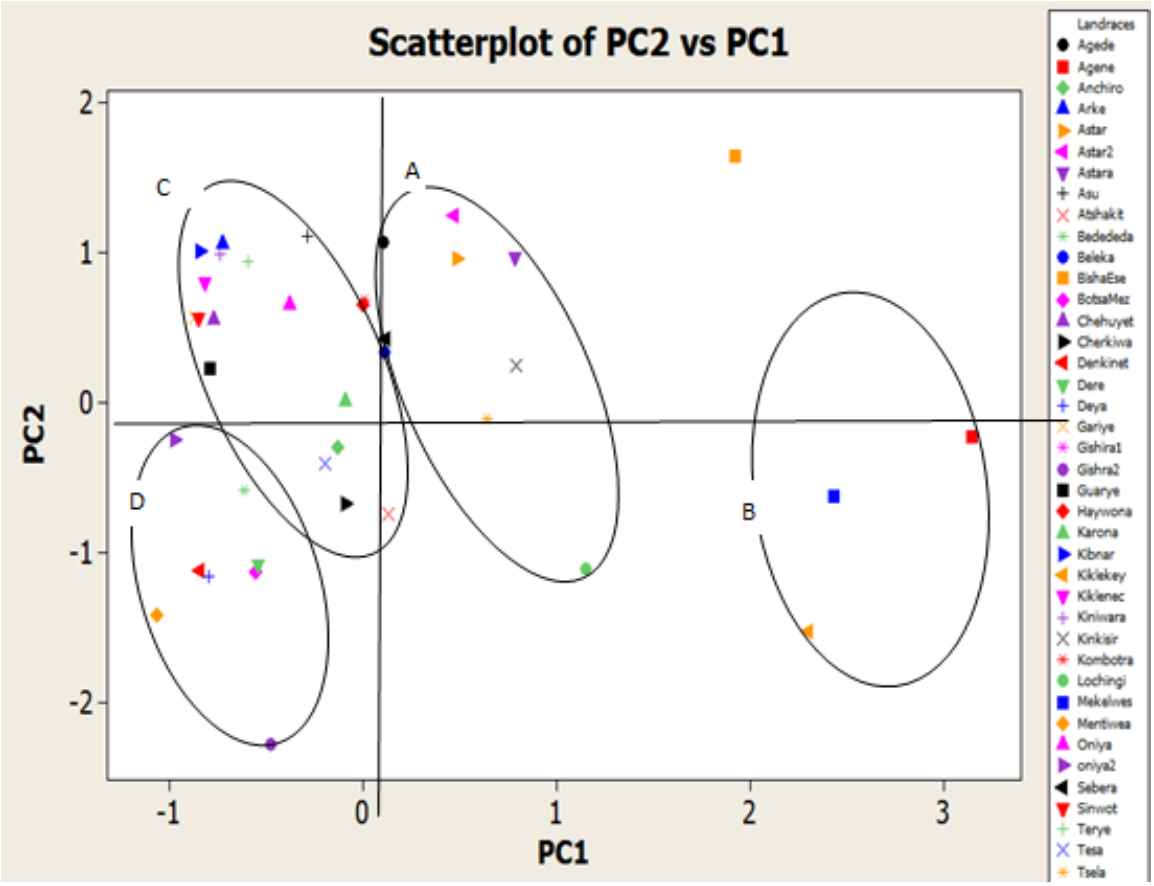

Figure 4.2 Scatter plot of 40 enset landraces based on the first two principal components (PC1 and PC2)

4.1.3. Cluster analysis

In the cluster analysis based on unweighted pair group method with arithmetic mean (UPGMA), the 14 qualitative traits grouped the 40 landraces into four major clusters (namely, A, B, C and D) as shown in **Figure 4.3**. Cluster C with 15 landraces, (37.5% of all landraces), was the largest and constitutes genotypes with pseudostem color of green-yellow or yellow-red and petiole dorsal surface color of red-purple. On the other hand, cluster B contained the smallest number of landraces (four in number) with above ground plant color of dark red-purple or red-purple.

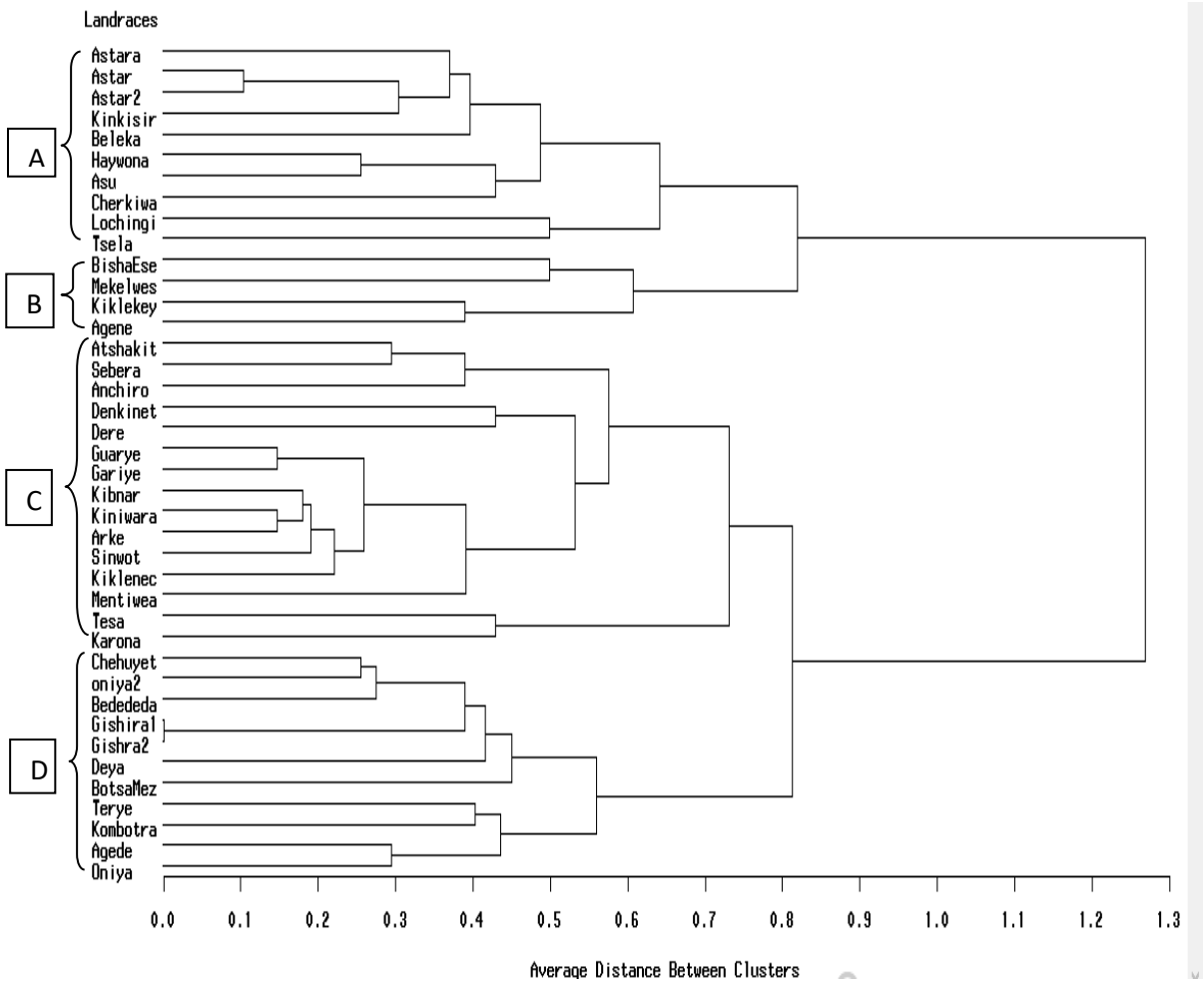

Figure 4.3 Dendrogram resulting from average linkage clustering using the unweighted pair-group method with arithmetic average (UPGMA) for 40 enset landraces based on 14 qualitative traits

In cluster A, nine landraces were included, and they were characterized by red-purple pseudostem and petiole dorsal surface color with different pigmentations. Cluster D was the second largest group, which included eleven landraces and they were basically characterized by light to medium green pseudostem, and petiole dorsal surface color with various pigmentations. It was observed that each cluster was further divided into a sub and sub-sub clusters at different dissimilarity coefficients based on their relation, while at 0.1 level of dissimilarity, almost all the 40 landraces were distinct from one another.

4.2. Proximate, mineral and phytochemical composition of enset (*Ensete ventricosum* (Welw.) Cheesman) landraces used in traditional medicine

In order to investigate the medicinal property of enset landraces, the proximate, mineral and phytochemical composition, corms of three enset landraces commonly used in the treatment of bone fracture were analysed and compared with two landraces used for other puposes.

4.2.1. Proximate composition

There was variation in crude fiber and crude protein contents only among the corms of enset landraces (Table 4.3), on a dry weight basis (ANOVA, $p < 0.05$).

The moisture content of corm samples analyzed varied from 65.3–71.94%, while the crude fiber content ranged from 2.38 to 4.43%, highly significant value (4.43% at $p < 0.05$) value (4.43%) was obtained from *amerat* enset landrace (Table 4.3). The ash and crude fat contents of the landraces ranged from 2.22–3.22% and 0.56–0.64% respectively (Table 4.4). Highly Significant ($p < 0.05$) crude protein contents (4.74 and 4.06%), which are statistically at par, were obtained from the two enset landraces *kibnar* and *guarye* respectively, whereas, the lowest (2.42) from *yeshiraqinqe*. No significant difference was observed in the total carbohydrate content of the landraces (Table 4.3).

4.2.2. Mineral composition

Among the five enset landraces analyzed, there was a significant difference (ANOVA, $P < 0.05$) in phosphorus, potassium and zinc contents. However, there was no significant

difference in calcium, magnesium, sodium, iron, copper and manganese contents (Table 4.4).

Table 4.3 The mean proximate composition (on dry matter basis) of corms of 3 enset landraces used in traditional medicine and 2 other landraces

Enset landraces	Proximate composition (%)					
	Moisture content	Crude fiber	Ash	Crude Fat	Crude protein	Total carbohydrate
Astara	68.57[a]	2.99[b]	2.97[a]	0.62[a]	3.36[b]	77.62[a]
Guarye	65.30[a]	2.93[b]	2.64[a]	0.62[a]	4.06[ab]	79.85[a]
Kibnar	67.06[a]	2.38[b]	2.88[a]	0.52[a]	4.74[a]	75.84[a]
Amerat	69.06[a]	4.43[a]	2.22[a]	0.64[a]	3.37[b]	79.39[a]
Yeshiraqinqe	71.94[a]	2.87b	3.22[a]	0.56[a]	2.42[c]	77.49[a]

Value with different superscript in the same column, are statistically different at probability $p < 0.05$

Table 4.4 The mean mineral composition of corms of three enset landraces used for traditional medicine and two other landraces that have other use values

Enset Landraces	Mineral Composition (mg g^{-2})								
	Ca	Mg	P	K	Na	Fe	Zn	Cu	Mn
Astara	114.27[a]	14.31[a]	127.41[a]	1440.9[ab]	21.06[a]	2.83[a]	8.52[a]	0.80[a]	0.92[a]
Guarye	99.1[a]	13.69[a]	116.38[ab]	1160.17[c]	19.16[a]	2.66[a]	4.84[b]	0.60[a]	0.72[a]
Kibnar	97.27[a]	14.12[a]	99.97[dc]	1261[bc]	14.58[a]	2.05[a]	5.06[b]	0.53[a]	0.62[a]
YQ*	101[a]	12.99[a]	111.31[bc]	790.8[d]	17.86[a]	2.5[a]	4.49[b]	0.56[a]	1.22[a]
Amerat	114.1[a]	15.52[a]	94.76[d]	1654.2[a]	22.5[a]	2.81[a]	5.22[b]	0.71[a]	1.08[a]

Value with different superscript in the same column are statistically different at probability $p < 0.05$, *YQ =*yeshiraqinqe* landrace

4.2.3. Comparison of proximate and mineral composition of enset corm with other root and tuber crops

Some of the proximate and mineral compositions of enset corm obtained in the present study were compared with commonly used root and tuber crops: sweet potato, taro, yam, cassava and Irish potato (Table 4.5). The protein contents of enset (2.4–4.7%) were slightly lower than yam (3.1–5.4%) and Irish potato (4.8%), but it is better than sweet potato (2.1–2.8%), cassava (1.2–1.8%) and taro (0.9–1.7%). Total carbohydrate (75.8–79.9%) was comparable to Irish potato (73.8%) and cassava (80.1–86.3%), while it was slightly lower than taro (97.6–98%) and sweet potato (90–91.5%). The fat content of enset corm was lower than sweet potato, Irish potato and yam, while it was better than taro and cassava.

The calcium composition of enset in mg g^{-2} obtained in the present study (97.3–114.3) was superior to sweet potato (20.7–25.1), taro (55), yam (31–118.8) and cassava (32–44), as reported by different authors (Table 4.4). The phosphorus content (94.8–127.4) of enset was also the highest of all root and tuber crops compared, while the zinc content (4.5–8.5) of enset corm was extremely superior to all root and tuber crops. On the other hand, the enset corm showed inferiority in iron content as compared to all root and tuber crops (Table 4.5).

4.2.4. Phytochemical composition

The phytate contents of the five enset landraces corm ranged from 149.33–195.15 mg g^{-2} but there was no significant difference among the landraces. Among the five enset landraces analyzed, there was a significant difference (ANOVA, $P < 0.05$) in tannin content (Table 4.6). The highest tannin content (153.94 mg g^{-2}) was observed in *kibnar* landrace,

and it was followed by *amerat*. The remaining three enset landraces did not show a significant difference.

Table 4.5 Comparison of some of proximate and mineral composition of enset with other root and tuber crops

Nd*= not determined

	Proximate (%) and minerals (mg g $^{-2}$)							
Type of food	Protein	Fat	Tot CHO	Calcium	Phosphorus	Iron	Zink	Reference
Enset Corm	2.4–4.7	0.5–0.6	75.8–79.9	97.3–114.3	94.8–127.4	2.1–2.8	4.5–8.5	Present study
Sweet potato	2.1–2.8	1.3–1.5	90–91.5	20.7–25.1	5.1–5.5	5.1–10.2	2.2–3.2	Demelash Hailu and Tilahun Abera, 2016.
Taro	0.9–1.7	0.1–0.2	97.6–98	55	Nd*	2.95	1.67	Alcantara *et al.*, 2013 and Tattiyakul *et al.*, 2006.
Yam	3.1–5.4	0.3–1.2	Nd*	31–118.8	15.1–56.5	20.3–69.7	0.5–0.8	Atnafua Bekele and Endashaw Bekele, 2018.
Cassava	1.2–1.8	0.1–0.8	80.1–86.3	32–44	98–118	0.6–7.8	0.7–2.0	Rojas et al., 2007 and Charles *et al.*, 2005.
Irish potato	4.8	1.6	73.8	Nd*	14.4–35.8	1.7–16.4	0.8–2	Tesfaye Abebe et *al.*, 2012 and Adegunloye and Oparinde, 2017.

Table 4.6 The mean phytate and tannin composition of corms of three enset landraces used for traditional medicine and two other landraces

	Phytochemical composition (%)	
Enset landraces	Phytate	Tannin
Astara	172.06[a]	65.23[bc]
Guarye	149.33[a]	50.48[c]
Kibnar	166.04[a]	153.94[a]
Amerat	152.12[a]	103.56[b]
Yeshiraqinqe	195.15[a]	68.50[bc]

Values with different superscript in the same column are statistically different at probability $p < 0.5$

4.3. Genetic Diversity of Enset (*Ensete ventricosum*) Landraces Used in Traditional Medicine Using SSR Markers

Fifteen SSR markers that produced clear and scorable bands were analyzed to evaluate genetic diversity of 92 *Ensete ventricosum* samples collected from different geographic areas.

4.3.1. SSR markers polymorphism and genetic diversity

The results confirmed that the SSR markers were highly polymorphic as shown in **figure 4.4.** A total of 38 alleles were detected across 15 SSR loci in 92 individual samples **(**Table 4.7**)**. The number of alleles generated per locus ranged from 2 to 3, with an average of 2.53 alleles. The observed heterozygosity (H_o) and expected heterozygosity (H_e) ranged from 0 to 0.65 and

0.16 to 0.63 respectively, while Shannon's information index (I) ranged from 0.29 to 1.04 (Table 4.7).

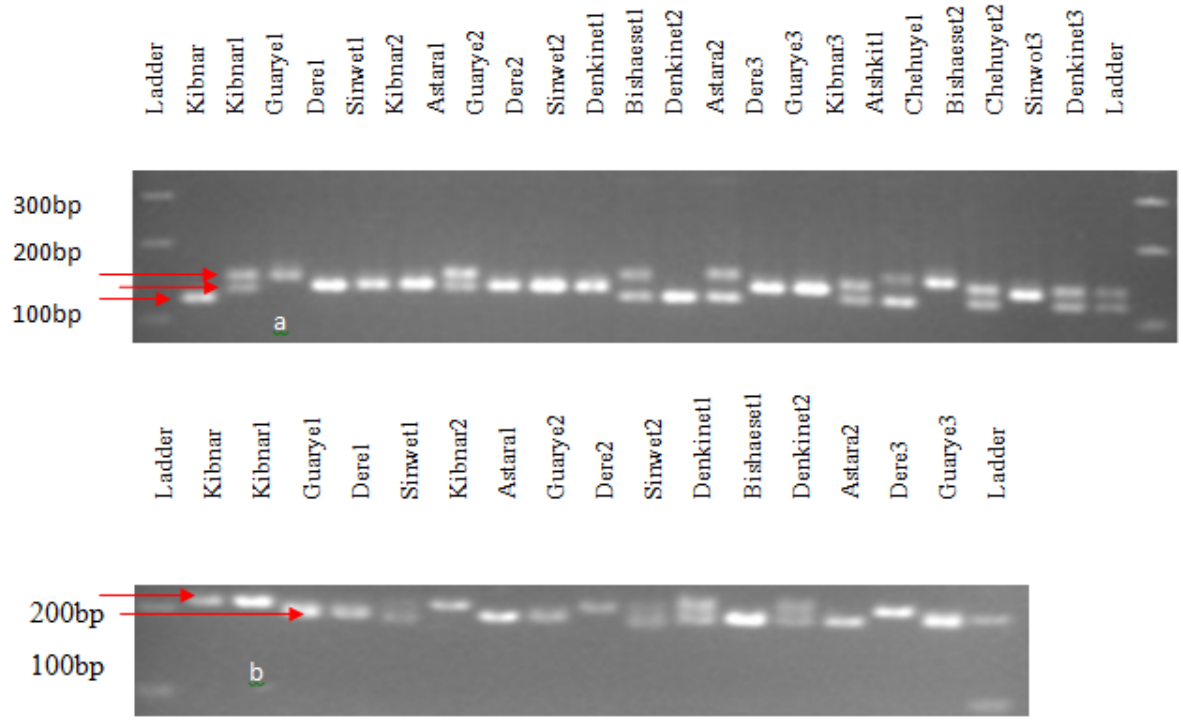

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Figure 4.4 DNA fragments amplified in selected enset landraces by SSR primer; a. Evg2 (22 samples and 2 ladders, 100base pair) and b. EnM00011571 (16 samples and 2 ladders, 100base pair) resolved in agarose gel electrophoresis

A summary of genetic diversity estimate in the four locations is presented in **Table 4.8.** The number of different allele (N_a) ranged from 2.33 for Dawro, to 2.53 both Gurage and Hadya locations, respectively. Shannon's information index in the four locations varied from 0.72 (Dawro) to 0.77 (Kembata-Tembaro). The observed heterozygosity values were in a range of 0.36 (Gurage) to 0.52 (Dawro) and H_e varied from 0.44 (Dawro) to 0.49 (Kembata-Tembaro).

Table 4.7 Levels of diversity indices of the SSR loci across the 4 locations

SSR Loci	N_a	N_e	I	H_o	H_e	uH_e
Evg1	3.00	2.24	0.88	0.41	0.54	0.55
Evg2	3.00	2.38	0.95	0.45	0.57	0.59
Evg4	3.00	2.20	0.87	0.65	0.54	0.56
Evg5	2.00	1.84	0.65	0.56	0.45	0.47
Evg6	2.00	1.48	0.44	0.00	0.29	0.30
Evg8	3.00	2.21	0.86	0.49	0.54	0.56
Evg9	3.00	2.36	0.95	0.48	0.56	0.58
Evg10	2.00	1.57	0.50	0.00	0.33	0.34
Evg11	3.00	1.83	0.71	0.43	0.44	0.46
Evg13	3.00	2.11	0.82	0.58	0.52	0.54
Evg14	2.00	1.89	0.66	0.65	0.47	0.49
EnO28	3.00	2.71	1.04	0.58	0.63	0.65
EnB85	2.00	1.21	0.29	0.19	0.16	0.17
EnM71	2.00	1.93	0.67	0.56	0.48	0.50
EnM65	2.00	1.60	0.56	0.41	0.3	0.39
Mean	2.53	1.97	0.72	0.43	0.46	0.48

N_a number of different alleles, Ne number of effective alleles, I Shannon's information index, H_o observed heterozygosity, H_e expected heterozygosity, uH_e unbiased expected heterozygosity, EnO28EnOnjSSR049028, EnB85 EnBedSSR020585,EnM71 EnM00011571, EnM65 EnM00025665,ns not significant

Table 4.8 Summary of different locations diversity indices over the 15 loci

Location	N	N_a	N_e	I	H_o	H_e	uH_e
Gurage	34	2.53	1.91	0.72	0.36	0.46	0.46
Hadya	17	2.53	1.95	0.73	0.38	0.46	0.48
Kembata-Tembaro	21	2.47	2.06	0.77	0.43	0.49	0.50
Dawro	12	2.33	2.00	0.69	0.52	0.44	0.48
Yem	8	2.40	1.92	0.71	0.46	0.45	0.48

N number of samples, N_a number of different alleles, N_e number of effective alleles, I Shannon's Information Index, H_o observed heterozygosity, H_e expected heterozygosity, uH_e unbiased expected heterozygosity

4.3.2. Genetic relationships

The unrooted neighbor joining tree cluster analysis performed using Nei's genetic distance grouped the 92 enset genotypes into 4 main clusters, Cluster A–D (Figure 4.5). Cluster A was the largest in containing 27 landraces; 12 from Gurage, 9 from Kembata-Tembaro, 4 from Yem and 2 from Hadya. The second large cluster, cluster C, comprised 23 landraces; 6 from Dawro, 3 from Yem, 8 from Gurage, 3 from Kembata-Tembaro and 3 from Hadya. Cluster B was the smallest in containing 20 landraces; 8 from Kembata-Tembaro, 5 from Hadya, 4 from Dawro, and 3 from Gurage locations.

A principal coordinate analysis (PCoA) bi-plot of the first two axes ($PCoA_1$ and $PCoA_2$), that accounted for 33.3 % of the total genetic variability, showed a wide dispersion of landraces along the four quadrants (Figure 4.6). The two groups of enset landrace, medicinal and non-medicinal, didn't form a distinct cluster in the plot.

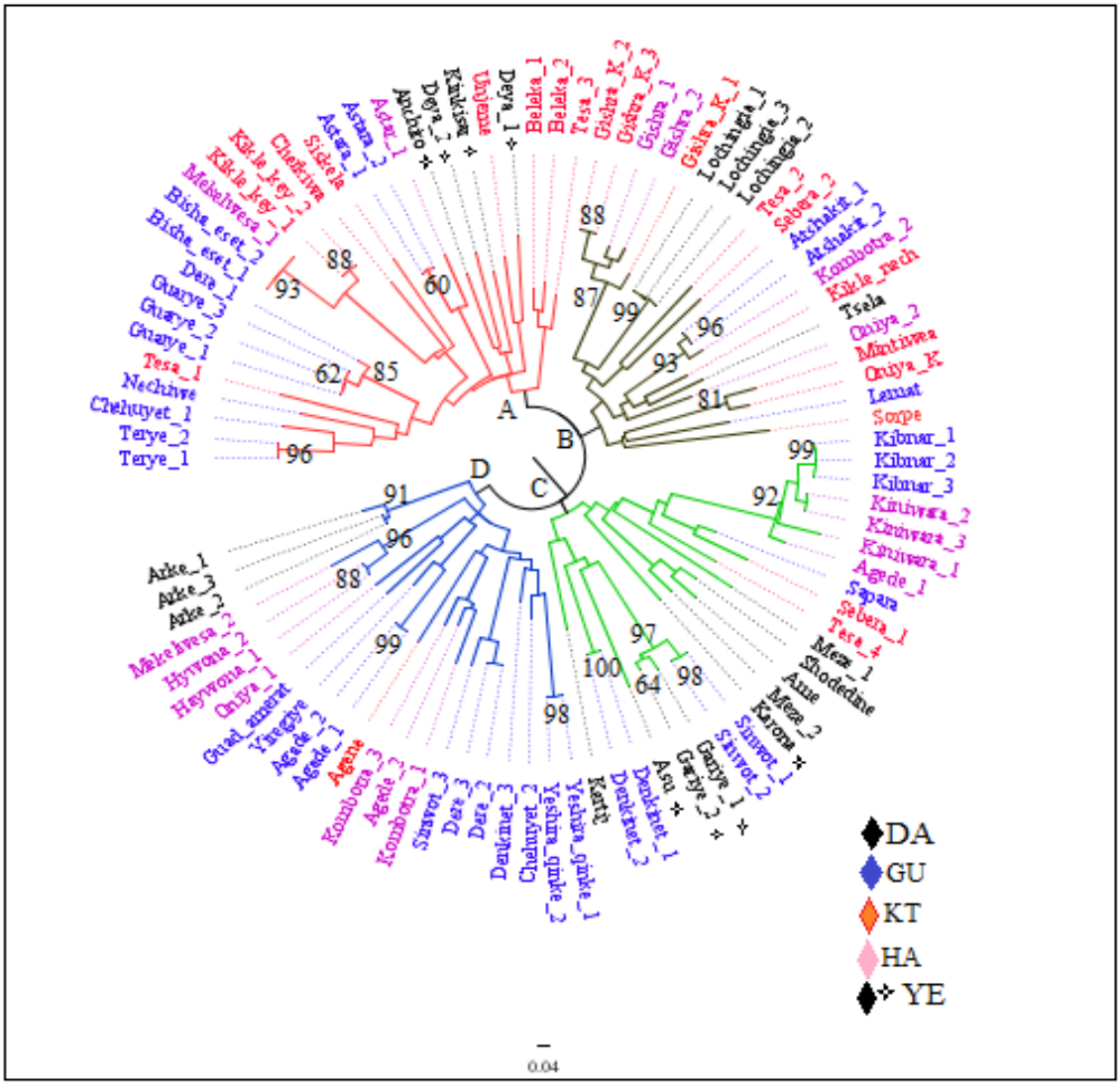


Figure 4. 5 Unrooted neighbor-joining tree generated based on simple matching dissimilarity coefficients over 1000 replicates for 92 *Ensete ventricosum* landraces (including duplicates) using 15 SSR markers. Each node label is color-coded according to location of origin; DA Dawro, GU Gurage, KT Kembata Tembaro, HA Haya and YE Yem. Numbers at the nodes are bootstrap values ≥0.60.

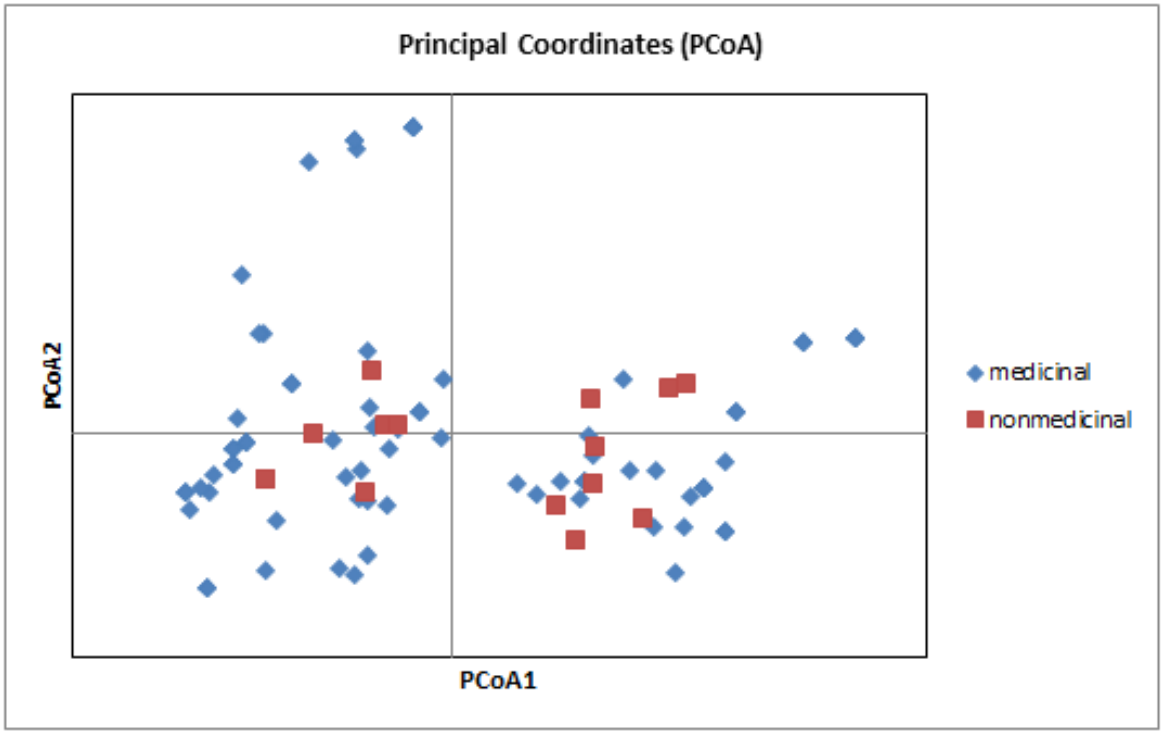


Figure 4.6 Principal coordinate analysis (PCoA) bi-plot showing the clustering of 92 enset landraces (including duplicates) based on 15 microsatellite data, labeled based on the use-values of the landraces

4.4. Micropropagation of Enset (*Ensete ventricosum* (Welw.) Cheesman) Landraces Used in Traditional Medicine Using Shoot Tip Explants

4.4.1. Culture initiation

4.4.1.1. Surface sterilization

Surface sterilization of shoot tip explants using 70% (v/v) ethanol for 10 min, and 2% sodium hypochlorite (NaOCl) for 25 min, resulted in only 6% contaminated explants in the first 7 days, a period at which exogenous contamination usually appears, while 96% of the explants successfully established. On the other hand, the inclusion of 1.5g l^{-1} activated charcoal in the

culture medium was found to be very effective in controlling blackening of explants during culture initiation. Death of explants due to blackening was almost none, and no adverse effect was visible by using the activated charcoal.

4.4.1.2. Effect of cefotaxime on microbial contamination

Analysis of variance showed that endogenous microbial contamination was significantly different with the application of antibiotics, cefotaxime, on MS medium, whereas, type of landrace and the interaction of cefotaxime with the landraces didn't affect the percent of contamination (Table 4.9). The contamination level (17.67%), is significantly higher ($p<0.05$) on MS medium that do not contain cefotaxime, while the remaining two treatments ($250mgl^{-1}$ and $500mgl^{-1}$) didn't show a significant difference (Table 4.10; Figure 4.7). Contamination level of *kibnar* enset landrace (13.56%) was statistically similar to *guarye* (12.78%). It was also recognized that there was no phytotoxicity (necrotic) effect due to the application of cefotaxime during the initiation stage.

Table 4.9 Statistical significance of the effect of cefotaxime and type of landrace on percent of contamination during *in vitro* initiation of enset from shoot tips

Treatment	F-probability level
	shoot number
Cefotaxime level	0.024^{*}
Landrace types	$<0.70^{ns}$
Cefotaxime X Landrace types	0.86^{ns}

*= significant treatment effect, ns= non-significant treatment effect

Table 4.10 Effect of cefotaxime level and type of landrace on percent contamination at initiation stage of enset on MS medium

	Treatments	Percent of contamination Mean ±SD
Cefotaxime concentration (mg/l)	0	17.67±4.13[a]
	250	11.50±3.83[b]
	500	10.33± 3.61[b]
Type of landrace	*Kibnar*	13.56±4.90[a]
	guarye	12.78±5.19[a]

SD = standard deviation. Means followed by the same letter are not significantly different at 5% probability level

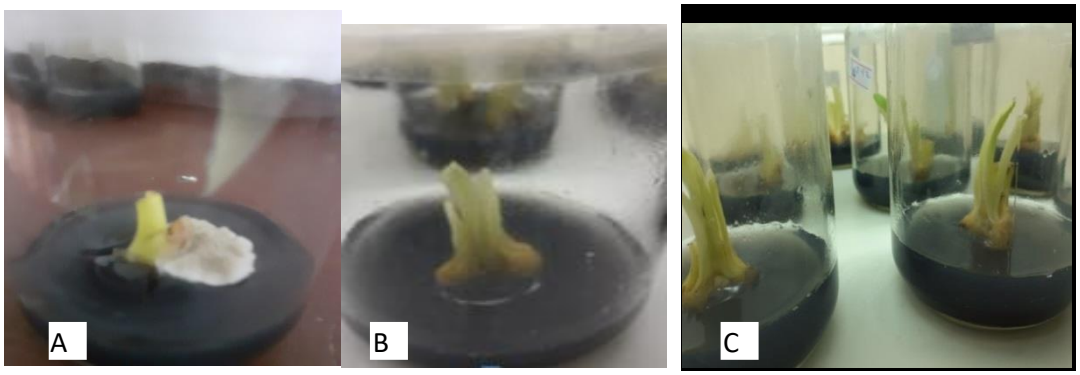


Figure 4.7 Shoot tip explants of enset at initiation stage; A) contaminated explant on MS medium without cefotaxime, B) contaminated explant on MS medium containing 250mgl^{-1} cefotaxime, C) contamination free explants on MS medium containing 500mgl^{-1} cefotaxime

4.4.2. The effect of plant growth regulators and type of enset landrace on number and height of *in vitro* regenerated enset shoots

The number and height of shoots regenerated from shoot tip responded significantly to different concentrations of BAP in combination with IBA on MS medium, and types of landraces, but the interaction effect was not significant as shown in **Table 4.11**. Significantly ($p< 0.05$) highest mean shoots number (8.8±1.40) per shoot tip was regenerated from MS medium supplemented with 5 mg/l BAP + 1.0 mg/l IBA, while the lowest mean shoot number (3.4±0.97) was recorded from MS medium without plant growth regulators (Table 4.12; Figure 4.8). It was preceded by the medium containing 2.5 mg/l BAP + 0 IBA and 2.5 mg/l BAP + 0.5 mg/l IBA, which are statistically similar. From all the remaining combinations of PGRs, there was no statistically significant difference in the average numbers of shoots regenerated. From the two types of enset landraces, significantly highest mean shoot number (6.88±2.18) per shoot tip were regenerated from g*uarye*, while 6.08±1.66 shoots per shoot tip were regenerated from *kibnar* landrace. Moreover, from two successive sub-culturing of shoots on the best performing media (MS medium supplemented with 5.0 BAP + 1.0 IBA), an average of 8 new shoots per shoot tip could be regenerated per month.

Table 4.11 Statistical significance of the effect of plant growth regulators and type of landrace on number and height of enset shoots regenerated *in vitro* from shoot tips

Treatment	F-probability level	
	shoot number	shoot height
PGRs	0.0036^{*}	0.0348^{*}
Landrace types	$<0.0001^{*}$	0.3204^{ns}
PGRs X Landrace types	0.9318^{ns}	0.4501^{ns}

*= significant treatment effect, ns= non-significant treatment effect

Table 4.12 Effect of plant growth regulators and type of landrace on number and height of *in vitro* regenerated shoots of enset on MS medium

BAP(mgl^{-1})	IBA(mgl^{-1})	Shoot number	Shoot height (cm)
		Mean ±SD	Mean ±SD
5.0	1.0	8.8±1.40a	6.25±1.62 [a]
5.0	0.5	7.6±1.26b	6.31±1.55[a]
5.0	1.5	7.2± 1.93b	6.44±1.73 [a]
5.0	0.0	7±1.70b	6.50±1.21 [a]
7.5	1.5	7±1.41b	6.31±0.98 [a]
7.5	0.0	7±1.05b	6.47±1.82 [a]
7.5	2.0	6.8±1.03b	6.16±0.89 [a]
2.5	0.0	5.2±1.40c	6.56±0.81 [a]
2.5	0.5	4.8±1.03c	6.53±0.99 [a]
0.0	0.0	3.4±0.97d	5.13±0.7[b]
Guarye		6.88±2.18[a]	6.10±1.32[a]
Kibnar		6.08±1.66[b]	6.43±1.30[a]

SD = standard deviation. Means followed by the same letter within the same column are not significantly different at 5% probability level

On the other hand, all levels of plant growth regulator combinations resulted in statistically similar mean shoot height, ranging from 6.10 cm to 6.56 cm, while growth regulators free medium produced the lowest mean shoot height (Table 4.12). The two types of enset landraces didn't show significant difference in shoot height.

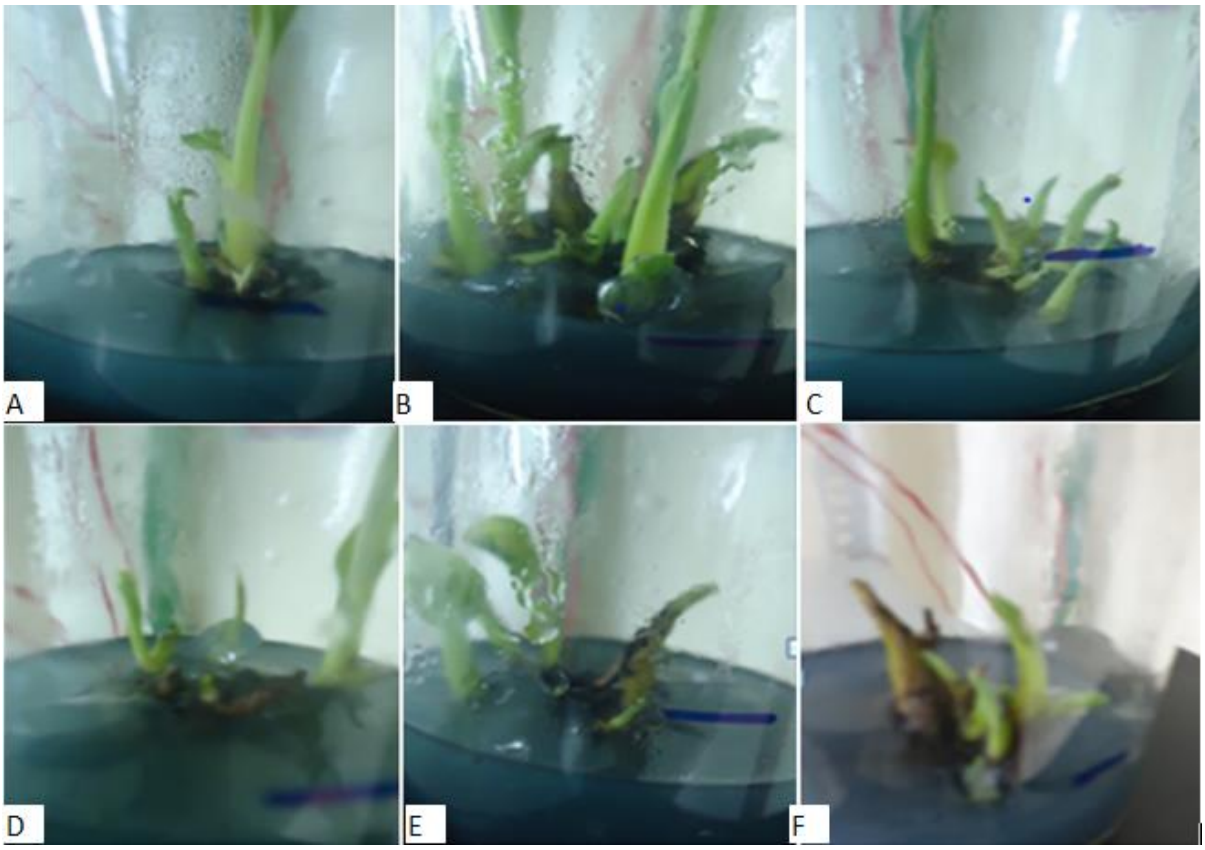


Figure 4.8 Multiple shoots regenerated from half shoot tips of *guarye* enset landrace in MS medium containing different concentration of BAP and IBA: A) Growth regulators free medium, B) 5 BAP+0.5 IBA, C) 5.0 mgl^{-1} BAP+1.0 mgl^{-1}IBA, D) 5.0 mgl^{-1} BAP+1.5 mgl^{-1} IBA, E) 7.5 mgl^{-1} BAP, F) 7.5 mgl^{-1} BAP+1.5 mgl^{-1}IBA, Bars =1cm

4.4.3. Rooting of microshoots

Rooting of the microshoot was significantly affected by the different concentrations of IBA on MS or half- strength MS medium, whereas, the effect of type of landrace and its interaction with IBA were not significant, as shown in **Table 4.13**. Significantly ($p< 0.05$) highest mean root numbers (3.00 ± 0.67 and 3.00 ± 0.47 mgl^{-1}) per shoot were regenerated from MS and 1/2MS medium containing 3 mg l^{-1} and 2 mg l^{-1} IBA respectively, and they were followed by MS medium containing 2 and 4mg l^{-1}, and half-strength MS with 3 mg l^{-1} IBA

(Table 4.14; Figure 4.9). The lowest mean root number (0.80±0.42 and 1.20±0.63), which are statistically similar, were recorded from ½ and full-strength MS medium devoid of plant growth regulators, respectively. On the other hand, statistically similar mean roots number per shoot (2.00±1.01 and 1.86±0.81) were regenerated from the two types of enset landraces, g*uarye* and *kibnar*, respectively.

Table 4.13 Statistical significance of the effect of plant growth media composition and type of landrace on number and length of roots regenerated *in vitro*

Treatment	F-probability level	
	Root number	Root length
Media	<0.0001*	<0.0001*
Landrace types	0.58ns	0.62ns
Media X Landrace types	0.52ns	0.41ns

*= significant treatment effect, ns= non-significant treatment effect

Significantly the highest ($p < 0.05$) mean root lengths (2.50±0.18cm and 2.46±0.10cm) were regenerated from shoots cultured on MS medium supplemented with 3 mgl^{-1} and 4mgl^{-1} IBA respectively, which were followed by MS medium containing 1 mgl^{-1} or 2 mgl^{-1} IBA, or half-strength MS medium fortified with 3 mgl^{-1} IBA, that produced statistically equal mean root lengths (Table 4.14). Statistically similar mean lowest root lengths (1.77±0.17 cm, 1.77±0.24 cm, 1.92±0.16 cm) were recorded from both full MS and half-strength MS medium without PGR, and from half-strength MS medium supplemented with 4 mgl^{-1} IBA respectively. However, statistically similar mean root length (2.08±0.39 and 2.10±1.37) was observed from the two enset landraces, *guarye* and *kinbar*, respectively.

Table 4.14 Effect of plant growth regulators (PGRs) and type of landrace on number and height of enset roots regenerated *in vitro*

Medium strength	IBA (mg l^{-1}) treatments	Root number Mean ±SD	Root length (cm) Mean ±SD
Full	0.0	1.20±0.63^{cd}	1.77±0.17^{d}
Full	1.0	1.60±0.70^{c}	2.25±0.18^{b}
Full	2.0	2.10± 0.57^{b}	2.26±0.23^{b}
Full	3.0	3.00±0.67^{a}	2.50±0.18^{a}
Full	4.0	2.30±0.48^{b}	2.46±0.10^{a}
Half	0.0	0.80±0.42^{d}	1.77±0.24^{d}
Half	1.0	1.50±0.53^{c}	2.00±0.41^{c}
Half	2.0	3.00±0.47^{a}	2.01±0.17^{c}
Half	3.0	2.20±0.42^{b}	2.26±0.12^{b}
Half	4.0	1.60±0.52^{c}	1.92±0.16^{cd}
Type of landrace	*Guarye*	2.00±1.01^{a}	2.08±0.39^{a}
	Kibnar	1.86±0.81^{a}	2.10±1.37^{a}

SD = standard deviation. Means followed by the same letter within the same column are not significantly different at 5% probability level

Figure 4.9 *In vitro* rooting of guarye enset landrace: A) plantlets in a rooting medium, B) Plants rooted on MS medium devoid of IBA, C) On MS medium containing 3 mgl-1 IBA, and D) On 1/2 MS medium supplemented with 2 mgl-1 IBA

4.4.4. Acclimatization of microshoots

Among the plantlets transferred to polyethylene bag (with forest soil and sand at 2:1 ratio), 75% were successfully acclimatized in a greenhouse. The plants were well established, and no phenotypic variation was observed (Fig 4.10).

Figure 4.10 Acclimatization of *in vitro* rooted microshoots of *guarye* enset landrace. (A) Plantlets in the 1st week of acclimatized (B) Acclimatized plantlets in the 3rd week (C) Established plants in the 5th week.

Chapter 5.

DISCUSSIONS

5.1. Genetic Diversity of Enset (*Ensete ventricosum* (Welw.) Cheesman) Landraces Used in Traditional Medicine Using Phenotypic Traits

5.1.1. Frequency distribution

The study on 40 enset landraces used in traditional medicine showed a wide range of diversity in trait categories of the 14 phenotypic traits. From the fourteen pseudostem colors categories, the green-yellow with black patches/strip/spot and light to medium green with black patch and spots were found to be dominant. The predominant colors of the petiole ventral and dorsal surface were light to medium green with black spots/strip/ patches and red-purple with light to medium green at both sides. Similarly, the highest proportions of midrib ventral and dorsal surface colors were light to medium green and red-purple with light to medium green at both sides respectively. Comparable finding was reported by Mikias Yeshitila (2014) in evaluation of enset phenotypic diversity at Areka condition.

In agreement with Mikias Yeshitila (2014) and Zerihun Yemataw *et al.* (2016), who studied enset population as a whole, the majority of the landraces were characterized by light to medium green leaf color on the ventral surface, while red-pink with green strip color occurred rarely. The variability in color of leaf edge and tip was relatively low, with the majority of landraces having brown color. Mikias Yeshitila (2014) also reported comparable similar result.

Regarding the growth habit, half of the landraces had erect leaf growth, while the remaining had either intermediate or drooping growth habit. Traits related to color of different plant parts, are the major descriptors used by the farmers in the identification of enset landraces, and the expertise of farmers to enset plants in their farm is very amazing. According to Masayoshi Shigeta (1991) some farmers in Ari, South western Ethiopia, identify individual enset plant as if we know people by their face.

The quality parameters were the other traits used in characterization of the landraces. Around half of the landraces studied were characterized by high corm (47.5%), *kocho* (57.5 %) and *bulla (*45%) quality in terms of taste. Although the landraces used in this study are specifically those having medicinal importance, they are also characterized by high food quality, according to the perception of the the society. In the general enset population study (Zerihun Yemataw *et al*., 2016) 88% and 58% of enset population studied were poor in bulla and corm quality respectively, indicating that more proportion of landraces used in traditional medicine have high food quality than other landraces. Unfortunately, around half of the medicinal landraces were characterized by low fiber quality, in terms of strength, which may be due to food quality is negatively associated with fiber quality. The majorities of enset landraces under investigation were also susceptible or intermediate to bacterial wilt, while relatively lower proportion (88%) susceptibility was reported (Zerihun Yemataw *et al*., 2016). The majority of the landraces were also susceptible to drought. The result indicates the vulnerability of enset landraces used in traditional medicine.

5.1.2. Principal component analysis

The PCA analysis showed that, three-quarters of the total variance among landraces was explained by the first four PCAs. All the fourteen phenotypic traits showed variability and contributed for discrimination of the landraces. Earlier findings also revealed the variability on morphological traits, such as the ventral and dorsal colors of the midrib the the petiole, color of leaf, as well as the color of the leaf tip and edge of enset landraces (Mikias Yeshitila, 2014). Similarly, the existence of considerable variability, due to color of midrib, color of petiole and color leaf tip and edge, as well as quality of enset fiber was reported (Zerihun Yemataw *et al*., 2014a).

The two-dimensional scatter plot generated from the first two principal components classified the landraces into four groups except for a landrace called *bisha-eset*. This particular landrace is characterized by red-purple color of the whole above ground part and is visually very similar with landraces such as *kiklekey*, *mekelwesa* and *lochingia*. It was also observed that no landraces with different vernacular name was found to be identical. This implies the existence of diversity among the landraces. Two landraces that have similar vernacular name (*gishra*1 and *gishra* 2) which are originated from different locations, were overlapped each other in the scator plot, that implies the existence of planting material among locations.

5.1.3. Cluster analysis

The dendrogram of the hierarchical cluster analysis showed four major clusters. Although there is a slight variation in grouping pattern, it exhibited a consistent results with that of the PCA from the scatter plot. It was observed that clustering was not based on the geographic origin of the landraces; instead landraces from the same origin categorized into different groups and

those from different origins were grouped under the same cluster. For example, cluster A contained landraces from Gurage (1), Hadya (2), Kembata-Tembaro (3), Yem (2) and Dawro(2). Similar clustering pattern was earlier reported which was not related to geographical origin of the genotypes (Temesgen Magule *et al*., 2015). Our finding is also in agreement with that of Atnafu Bekele *et al*. (2013) which indicated that genetic diversity is not apparently related to geographic diversity in enset.

The landraces from the five different localities were distributed to all the four or at least to three of the clusters, showing the existence of genetic diversity among the landraces, within location. Yemane Tsehaye and Facil Kebebew (2006) also reported high diversity among enset landraces growing in Bonga *in situ* conservation site. According to earlier reports, clonality tends to increase genetic variation within populations, while it has the opposite effect on genetic differentiation among populations (Balloux *et al*., 2003; Halkett *et al*., 2005). This means, in strictly clonal organisms, the alleles at one locus evolve independently and accumulate diverse mutations over time (Halkett *et al.*, 2005). This accumulation of mutations in the absence of sex promotes the divergence between alleles at a single locus within individuals (Balloux *et al*., 2003; Meloni *et al*., 2013). Hence clonality appears to positively affect the genetic diversity by increasing allelic diversity, polymorphism, and heterozygosity. Similarly, in strict clonally propagated crops, frequent somatic mutations provide genetic variations and contribute to adaptive evolution (McKey *et al*., 2010).

Since enset is a clonally propagated crop, the variation occurred among the landraces in the current study could be related to the aforementioned reasons. Moreover, the sampling method

that involved the selection of landraces with distinct vernacular names, which could be corresponding to the genotype (Tostain *et al*., 2006), could be a source of diversity. The tendency of the farmers to maintain enset landraces having different use values could also be another reason for the higher genetic diversity.

From the landraces having identical or similar names but originated from different administrative zones, some were found placed together or distantly placed in the dendrogram, while *gishra* landraces (*gishra*1 and *gishra*2*)* originated from Hadya and Kembata-Tembaro Zones were found to be identical, showing the existence of some level of planting materials exchange and consistency of naming between the neighboring ethnic groups or zones. On the other hand, *oniya* landraces (*oniya* and *oniya*2) from Hadya and from Kembata-Tembaro were placed far apart in the cluster, which might be due to giving the same name for different enset landraces at different localities (Temesgen Magule *et al*., 2015; Endale, 1997). In the dendrogram, no landrace with different vernacular name was identical, showing that vernaculars names are good indicators of phenotypic distinctiveness. However, Endale Tabogie (1997) and Zerihun Yemataw *et al*. (2014b) reported naming of identical enset landraces differently /duplication of enset vernacular names/. The difference in the results could be related to the fact that the samples collected in the current study are only small groups of targeted landraces which are used intraditional medicine.

5.2. Comparison of proximate, mineral and phytochemical composition of enset (*Ensete ventricosum* (Welw.) Cheesman) landraces used in traditional medicine and other landraces

5.2.1. Proximate composition

The moisture contents obtained (65.3-71.9%) were by far lower than the value (85.92%) reported for unspecified enset landrace having an age of about 6 to 7 years, obtained from Hawassa (Mohammed *et al*., 2013). The variation in the results could be due to landrace, age of sampled plant or growing environmental difference. Harvesting period of the sample cn also be another factor for the variation (Alphonse *et al*., 2018). The crude fiber contents obtained in the current study (2.38%-4.43%) were lower than the value (5.65%) reported by Mohammed *et al*. (2013) for unspecified enset landrace, but quite lower than the value (17.4%) reported by Tadesse Daba and Masayoshi Shigeta (2016) for *naqaqa* enset landrace of unspecified age. The wider variations could be related to harvesting age, genotype, growing environment or harvesting period differences. Similarly, wider variation of crude fiber contents due to watering level and genotype difference was reported (Solomon Zewdie *et al*., 2008). The ash content was similar to the value (3.2%) reported by Tadesse Daba and Masayoshi Shigeta (2016) for *Naqaqa* enset landrace. Whereas, this finding was quite lower than the value (5.2%) reported by Sirawdink Fikreyesus *et al*. (2013) for the *Nobo* enset landrace of unspecified age obtained from Sidama. The variation could be due to genotype, growing environment, age or harvesting period differences (Alphonse *et al*., 2018).

The current result on crude fat content (0.52%-0.64%) was comparable to the value (0.6%) reported by Tadesse Daba and Masayoshi Shigeta (2016) for *Naqaqa* enset landrace, whereas, Mohammed *et al*. (2013) reported 0.4%. On the other hand, relatively higher fat content (1.24%) of the *Nobo* enset landrace was reported by Sirawdink Fikreyesus *et al*. (2013). The wider variation could be related to the difference in genotype, age, growing environment or

harvesting period (Alphonse *et al.*, 2018). The highest protein content was recorded from *kibnar* and *guarye* enset landraces, which are used for traditional medicine. Probably this could be the reason for selective use of these landraces for the treatment of bone fracture and breakage, traditionally. Crude protein content (3.33%) reported from unspecified enset landrace corm (Mohammed *et al.*, 2013) was comparable to the present finding (2.42%-4.74%). However, larger crude protein contents (8.3%) from *Naqaqa* and (8.23%) from *Nobo* landraces were reported by Tadesse Daba and Masayoshi Shigeta (2016) and Sirawdink Fikreyesus *et al.* (2013) respectively. In terms of total carbohydrate content, the current finding (75.84% -79.85%) was superior to the value (64.8%) reported by Tadesse Daba and Masayoshi Shigeta (2016) for *Naqaqa* enset landrace. The variation could be related to genotype, environmental or harvesting period difference (Alphonse *et al.*, 2018).

5.2.2. Mineral composition

As opposed to the hypothesis, no significant difference in calcium content was observed among enset landraces used for traditional medicine and other purpose landraces. The calcium contents (99.7 mg g^{-2} from *neqaqa* and 100 mg g^{-2} from *Nobo*) enset landraces reported by Tadesse Daba and Masayoshi Shigeta (2016) and Sirawdink Fikreyesus *et al.* (2013) respectively, were comparable to the current finding (97.27 mg g^{-2}-114.27 mg g^{-2}). On the other hand, Ajebu Nurfeta *et al.* (2008) reported calcium contents of ten enset landraces ranging from 50–200 mg g^{-2} with an average calcium content of 130 mg g^{-2}. The variation could be related to the genotype or growing environment difference (Ajebu Nurfeta *et al.*, 2008; Wekesa *et al.*, 2014). As compared to the current finding (12.99mg g^{-2}-15.52mg g^{-2}), larger magnesium contents (63.2 mg g^{-2}) from *Naqaqa* and (60–260 mg g^{-2}) from ten different

landraces were reported by Tadesse Daba and Masayoshi Shigeta (2016) and Ajebu Nurfeta *et al*. (2008) respectively. The variations could be related to genotype or environmental difference (Ajebu Nurfeta *et al*., 2008; Wekesa *et al*., 2014).

The highest phosphorus contents were obtained from *astara* and *guarye* landraces, which are used in the traditional treatment of bone fracture and breakage. This could be the reason for the selective use of the landraces for the mentioned traditional medicine, as the presence of phosphate is crucial for bone growth and mineralization (Penido and Alon, 2012). The current finding was in line with different findings. Sirawdink Fikreyesus *et al*. (2013) reported 90 mg g^{-2} from *Nobo* landrace and Mohammed *et al*. (2013) reported 110 mg g^{-2} phosphorus content of unspecified enset landrace. Whereas, Ajebu Nurfeta *et al*. (2008) reported in a range of 80–140 mg g^{-2} and average value of 120 mg g^{-2} phosphorus content from ten enset landraces. According to Ajebu Nurfeta *et al*. (2008), the phosphorus content of corms from *astara* landrace was 80 mg g^{-2}, which is quite lower than the current finding (127.41 mg g^{-2}), the variation could be due to the difference in the growing environment (Wekesa *et al*., 2014) or it may be related to giving the same vernacular name for different enset landraces (Endale, 1997).

Similar to the current finding (790.8 mg g^{-2}-1654.2 mg g^{-2}), a wider variation in potassium content (860–3050 mg g^{-2}) of enset landraces was reported (Ajebu Nurfeta *et al*., 2008). Sirawdink Fikreyesus *et al*. (2013) also reported high (2180 mg g^{-2}) potassium content of enset corm. In all cases, it was shown that enset corm is very rich in potassium content. The sodium content (14.58mg g^{-2}-22.5mg g^{-2}) was in the same range with the values (4-23mg g^{-2} and 15

mg g^{-2}) reported by Ajebu Nurfeta *et al.* (2008) for ten enset landraces and Sirawdink Fikreyesus *et al.* (2013) for *Nobo* landrace respectively. On the other hand, smaller values (5.2 mg g^{-2}) for enset corms of *neqaqa* and 3 mg g^{-2} for unspecified enset landraces were reported by Tadesse Daba and Masayoshi Shigeta (2016) and Mohammed *et al.* (2013) respectively. These reported variations could be related to the difference in landrace, growing environment or age of sampled plants (Ajebu Nurfeta *et al.*, 2008; Wekesa *et al.*, 2014). The iron content of enset corm (2.05mg g^{-2}-2.83mg g^{-2}) was found to be small in general, but it was higher than the value (0.7 mg g^{-2}) reported by Abebe Yewelsew *et al.* (2007) in boiled unspecified enset landrace. However, it was smaller than the value (12.3 mg g^{-2}) reported by Tadesse Daba and Masayoshi Shigeta (2016) for *Naqaqa* landrace. This variation could be either due to the genotype or growing environment difference (Ajebu Nurfeta *et al.*, 2008; Wekesa *et al.*, 2014) or boiling of the corm may result in the reduction of iron content (Bethke and Jansky, 2008).

Astara enset landrace performed better in zinc content than other landraces. The selective use of this landrace in traditional treatment of bone fracture or breakage, probably related to its richness in Zn content, as scientific findings on the synergetic stimulatory effect of zinc on the healing of bone, was reported (Kaya *et al.*, 2015). Lin *et al.* (2018) also reviewed that zinc plays a major role in regulating every phase of the wound healing process. The zinc contents (12.3mg $g^{-2}$and 7.3mg g^{-2}–22.6mg g^{-2}) of different landraces reported by Tadesse Daba and Masayoshi Shigeta (2016) and Ajebu Nurfeta *et al.* (2008) respectively were higher than the current finding (4.49mg g^{-2}-8.52mg g^{-2}), while smaller value (1.33 mg g^{-2}) was reported by Abebe Yewelsew *et al.* (2007). The variation could be related to the difference in genotype

(Ajebu Nurfeta *et al.*, 2008), the environment (Wekesa *et al.*, 2014) or processing form (Bethke and Jansky, 2008).

The copper content (0.53mg g^{-2}-0.80mg g^{-2}) was within a range of the findings (0.28mg g^{-2}–1.17 mg g^{-2}) reported by Ajebu Nurfeta *et al.* (2008) on ten enset landraces, and it was comparable to the values (0.7 mg g^{-2}) from *Naqaqa* and (0.52) from *Nobo* landraces reported by Tadesse Daba and Masayoshi Shigeta (2016) and Sirawdink Fikreyesus *et al.* (2013) respectively. The current finding in manganese content (0.2mg g^{-2}-1.22mg g^{-2}) was quite lower than the values (4.33 and 5.84 mg g^{-2}) reported by Mohammed *et al.* (2013) and Tadesse Daba and Masayoshi Shigeta (2016) respectively, the variation of the results could be related to genotype or environmental difference (Ajebu Nurfeta *et al.*, 2008; Wekesa *et al.*, 2014).

5.2.3. Comparison of proximate and mineral composition of enset corm with other root and tuber crops

As compared to other root and tuber crops, enset corm was found to be lower in protein, while comparable in total carbohydrate content (Table 5). Traditional healers usually recommend the inclusion of protein rich animal products, like milk, yogurt and meat together withenset corm for treating bone breakage, probably they have indigenous knowledge about the protein deficiency of enset products. It was also observed that enset corm showed better performance in calcium content than most of the root and tuber crops and the highest of all root and tuber crops compared in phosphorus contents. It was also extremely superior to all root and tuber crops in zinc content, while it showed inferiority in iron content (Table 5). The zinc content of enset corm is even much higher than *teff*, which is known to have good zinc content (Tadesse

Daba and Masayoshi Shigeta, 2016). In areas where enset serves as a staple food, therefore, strategic supplementation of foods rich in protein, fat and iron is required to alleviate the deficiencies.

5.2.4. Phytochemical composition

Studies on phytate and tannin contents of enset products are very scanty. Hiwot Bekele (2015) reported lower phytate content (84.25mg g^{-2}–112.56 mg g^{-2}) of one of enset product, *bulla* than the current finding (149.3mg g^{-2}–195.15mg g^{-2}). Enset corm showed relatively higher phytate content than the value (115.43 mg g^{-2}) reported on taro (Gulelat Desse *et al.*, 2013). This highest phytate content of enset corm may have an implication on human health. Despite several studies described that phytate has negative effect on the availability of minerals (Weaver and Kannan, 2002; Masum *et al.*, 2011), a number of dietary components that can neutralize its inhibitory effect were also reviewed (Schlemmer, 2009). The author also reviewed that phytate has a number of health benefits including; inhibition of calcification, anticancer and antioxidative activities.

In recent times, food phenolic and tannins are getting great attention due to their antioxidant capacity and their promising beneficial effect in human health. The antimicrobial activity of tannin was also documented (de Sousa *et al.*, 2015; Nouioua *et al.*, 2016; Tomiyama*et al.*, 2016). The traditional healers usually recommend consuming corms of *kibnar* enset landrace at the first stage of bone illness, probably due to its highest tannin content, which would be important to protect infection. Haslam (1996) also reported that plants rich in tannins are used

in traditional medicine as drugs for the treatment of various diseases, including the healing process of wounds, burns, and inflammation.

5.3. Genetic Diversity of Enset (*Ensete ventricosum*) Landraces Used in Traditional Medicine Using SSR Markers

5.3.1. Genetic diversity

SSR markers were used to assess the genetic diversity of 38 cultivated enset landraces (77 individual, including the duplicates) used in traditional medicine and 15 landraces that have non-medicinal values. According to our results, moderate level of genetic diversity (H_e =0.46) was detected. A relatively higher H_e values (0.55 and 0.59) of enset were reported (Selamawit Getachew *et al.*, 2014) and (both Temesgen Magule *et al.*, 2015 and Feta Negash *et al.*, 2019) respectively. The value of I (0.72) in the current study was also lower as compared to the earlier report (1.08) (Feta Negash *et al.*, 2019). The variation is probably due to the fact that the lower resolution power of agarose gel used in the current study. The sampling (inclusion of only specific group of landraces, those used in traditional medicine) could also be another factor for the lower. However, due to the difference in number and type of the markers and the landraces used, making direct comparison among the studies is difficult. On the other hand, lower genetic diversity estimates were reported in the diversity study using ISSR (Dagmawit Chombe and Endashaw Bekele, 2011) and RAPD (Genet Birmeta *et al.*, 2002) markers. Comparisons of detailed diversity estimates from marker systems with different properties and origins of variation do not allow useful conclusions.

Although there was only slight difference in the diversity, as estimated by H_e and I, among the locations, Yem-Dawro and Kembata-Tembaro are relatively more diverse. The occurrence of wild form of *Ensete ventricosum* in Dawro area (Feta Negash *et al*., 2019), which is one the indicator of center of diversity, supports the higher genetic diversity of enset in the area. The result is in agreement with Zerihun Yemataw *et al*. (2016), who reported the highest diversity index for Dawro than other 7 ethnic groups.

5.3.2. Genetic relationship

In the neighbor joining tree, the landraces clustered irrespective of their location of origin, indicating the existence of large number of shared alleles among enset landraces collected from different locations. This could be partly due to exchange of planting material, which is a common practice among enset growing communities (Admasu Tsegaye, 2002; Bizuayehu Tesfaye and Lüdders, 2003; Temesgen Magule*et al*., 2014; Zerihun Yemataw *et al*., 2016).

From the landraces that have similar vernacular names (replicate samples), and originated from the same location, the majority were genetically identical, while some others, showed a slight difference, placed in proximity, in the neighbor joining tree. This indicates that farmers have rich indigenous knowledge in identifying and naming enset landraces based on phenotypic traits. However, some other replicates of landraces were placed in different cluster, indicating that genetically different landraces were given the same vernacular name. It is expected that perfect identification of genotypes using morphological traits is hardly possible due to resemblance of some landraces. Similarly, the existence of a homonym, giving the same

vernacular name for genetically different landrace was reported (Temesgen Magule *et al.*, 2015).

Except two, all landraces with distinct vernacular names were different, showing that vernacular names are good indicator of genetic distinctiveness in these specific group of landraces used in traditional medicine. Whereas, the existence of 37 and 8 duplicates of landrace in diversity analysis of enset using 4 AFLP (Almaz Negash *et al.*, 2002) and 12 RAPD (Genet Birmeta *et al.*, 2002) markers respectively, was reported. Feta Negash *et al.* (2019) who studied comparable number (83) of enset genotypes using 12 SSR markers also reported 10 duplicate landraces. Although full identity among the landraces can only be determined when the entire genomes are compared, it is expected that the larger number of SSR markers used in the current study could sufficiently discriminate the landrace than the studies that reported higher number of duplicates. The variation of the results therefore, could be due to the sample collection method followed in the current study, which involved only specific types of landraces which are used in traditional medicine.

The PCoA analysis based on the genetic distance matrix resulted in dispersion of the landraces along the four quadrants, which clarify the genetic variability of the landraces. Similar to neighbor joining tree, grouping of the landraces was not correlated with their geographic location. The 15 landraces that are not used in the traditional medicine also didn't form a separate cluster or sub cluster, instead they were mixed with the other landraces, showing that medicinal properties do not appear to be monophyletic. The naturally out crossing nature of enset (Brandt *et al.*, 1997) together with the practice of growing the two groups of landrace

together may contributed to the lower genetic differentiation. However, individual landraces with distinct vernacular names didn't overlap, except *Mekelwesa1* with *Bisha-eset*1and 2, which were originated from Gurage and Hadya, respectively.

5.4. Micropropagation of Enset (*Ensete ventricosum* (Welw.) Cheesman) Landraces Used in Traditional Medicine Using Shoot Tip Explants

5.4.1. Culture initiation

Although the plant materials were disinfected and all vessels and media sterilized properly, microbial contamination in tissue culture, especially bacteria were reported (Leifert and Cassells, 2001; Mona *et al*., 2019). Such contaminants resistant to surface disinfestations are likely to be endophytic (Reed *et al*., 1995), and they can be controlled by antibiotics Gubišová and Gubiš, 2019). The role of cefotaxime antibiotics in controlling bacterial contamination of enset shoot tip culture was investigated on two landraces, *guarye* and *kibnar*. The result showed that, the inclusion of two concentrations of cefotaxime (250 mg l^{-1} and 500 mg l^{-1}) in MS medium resulted in less bacterial contamination than the control (the medium devoid of cefotaxime). Similarly, Kumari *et al*. (2015), who compared the effect of different antibiotics on micropropagation of sugarcane, reported that cefotaxime at a concentration of 250 mg l^{-1} and 500 mg l^{-1} was the most effective in reducing bacterial contamination than other antibiotics.

Cefotaxime is usually considered to be non-toxic to plant cells (Ogawa and Mii, 2004). No toxicity effect was also visible in the current study. The average level of contamination during culture initiation of the current study (13.17) was much lower than the loss of explants (50-

60%) reported due to internal contamination of enset shoot tip culture without the use of antibiotics (Genet and Welander, 2004), which indicates that the use of cefotaxime in the growth media is advantageous for efficient micropropagation of enset.

5.4.2. Shoot multiplication

The most important part of the success obtained in the micropropagation a plant has been often based on the successful adjustment of type and combination of plant growth regulators. The highest mean shoot number (8.8) per shoot tip regenerated on a MS cultured medium supplemented with 5 mgl^{-1} BAP + 1.0 mgl^{-1} IBA, shows that this level of plant growth regulator is appropriate for multiplication of enset landraces *guarye* and *kibnar*. The role of BAP to havein stimulating axillary shoot formation and proliferation reported (Soelaiman and Ernawati, 2013; Taiz and Zeiger, 1991). Although, availability of IBA in the media does not have a significant effect on shoot induction, it possibly assists the performance of BAP in the elongation of stem cells (Sofian *et al*., 2018).

The amount of plant growth regulators required for micropropagation usually depends on the genotype used. In the current study however, the interaction effect of PGRs and genotype was not significant. Similarly, the highest shoot proliferation rate of seven enset landraces was recorded from a single type of medium formulated (Genet Birmeta and Welander, 2004). The mean highest shoot number obtained was much higher than the shoot number (3.6) reported by Mulugeta Diro *et al*. (2003) from splitted shoot tips of *oniya* enset landrace on MS medium containing 2.5 mgl^{-1} BA (Benzyl adenine) + 1mgl^{-1} IAA (Indole-3-acetic acid) and 2.5 mg^{-1}

BA+ 1 mgl^{-1} NAA (α-Naphthalene acetic acid). Almaz Negash *et al*. (2000) also reported lower shoot number (2.5, 2.6 and 2.3) from three enset landraces (c*horo*, *nobo* and *ketano*), respectively on MS medium containing 20mgl^{-1} BAP.

On the other hand, the mean shoot number recorded in the current study was much lower than the values (23 and 16) from shoot tip explants of *mazia* and *digomerza* enset landraces respectively, but comparable to the shoot number (7) from *arkiya* landrace on MS medium containing 4.5 mgl^{-1} BAP and 1.5 mgl^{-1} NAA (Genene Gezahegn and Frew Mekbib, 2016). Regeneration of very high number of shoots from shoot tip culture on formulated macronutrients medium with double-standard concentration of MS micronutrients, with a wide range of variation among the landraces (14.7 to 74.7 shoots) was also reported (Genet Birmeta and Welander, 2004). Since the authors used different basal media composition, and data recording was made lately, 9 to 10 weeks after initiation, making direct comparison is difficult. However, the wider variations even in the same experiments reported indicates, much of the variation among the results seems to be due to the differences in the types of enset landraces used. The variation in multiplication responses among enset landraces was also observed in the current study. From the two enset landraces, significantly higher number of shoots wereregenerated from *guarye*.

The increase in concentration of BAP from 0.0 to 5.0 mg l^{-1} significantly increased the number of shoots regenerated per shoot tip. However, the increased concentration of BAP from 5.0 mg l^{-1} to 7.5 mg l^{-1} didn't result in significant increase in shoot numbers, which could be probably due to the blackening effect at the higher BAP concentration. Mulugeta *et al*. (2003) also

reported that, the higher concentration of BA didn't show a significant increase in the number of shoots per shoot tip both with intact and halved shoot tips. From the successive culturing of shoots on the best performed media (MS medium supplemented with 5 BAP + 1 IBA), an average 8 new shoots per shoot tip could be regenerated in one month.

5.4.3. Rooting of microshoots

Rooting of micro-shoots was primarily initiated in full and half-strength MS medium containing different concentrations of IBA, after 12 days of culturing. Similarly, Almaz Negash *et al.* (2000) reported the development of primary root in approximately ten days and the occurrence of secondary roots within the next four days. However, some of the explants showed the tendency of rooting while they were in multiplication and elongation medium, with or without PGRs. Genet and Welander (2004) also reported the development of roots in the elongation medium without a need of rooting stage. The highest mean number of roots per shoot (3.0) regenerated from both full and half-strength MS medium supplemented with 3.0 and 2.0 mgl^{-1} IBA, respectively, was slightly lower than the root number (3.77) reported by Genene Gezahegn and Frew Mekbib (2016) on MS medium supplemented with 2 mgl^{-1} IBA, but higher than the values (0 to 1.5) reported by Mulugeta Diro and van Staden (2004) on MS medium supplemented with 12 different concentrations of auxins and cytokinin. The highest mean root lengths recorded was also by far lower than the value (5.96 cm) reported by Genene Gezahegn and Frew Mekbib (2016). The variation of the results probably could be due to the difference in the types of landrace used.

The results also indicated that there was an increasing trend of root number per shoot and average root length with increased concentration of IBA up to 3 mgl^{-1} on MS and, up to 2 mgl^{-1} and 3 mgl^{-1} on half-strength MS medium, respectively. On the other hand, the highest root length was recorded from the lowest concentration of IBA (Genene Gezahegn and Frew Mekbib, 2016).

During elongation and rooting of shoots, some level of shoot-tip necrosis was observed, of which, some were able to survive, and few others led to the total death of the plants. Similarly, Mathew and Philip (1996) reported that, shoot-tip necrosis is one of the constraints during *in vitro* regeneration of *Ensete superbum*. According to the authors, the cause for that was explained to be shortage of calcium in the growth medium. Genet Birmeta and Welander (2004) could minimize enset shoot-tip necrosis by replacing calcium chloride with calcium gluconate monohydrate in the growth medium. On the other hand, Martin *et al.* (2007) reported that, shoot-tip necrosis in micropropagation of banana was influenced by cultivar type, and from several methods tested to reduce the necrosis, only increasing concentration of calcium chloride was effective.

5.4.4. Acclimatization

During acclimatization of plantlets in greenhouse, which is not automated, 75% survival rate was recorded for both *guarye* and *kibnar* after 21 days. A comparable survival rate (80 to 90%) of *in vitro* regenerated enset after 15 days of acclimatization was reported (Genene Gezahegn and Frew Mekbib, 2016). Genet Birmeta and Welander (2004) also reported (100%) success in acclimatization of *in vitro* regenerated enset plantlets in a glass house. The type of green house used in the current study, a simple plastic house with no contoling capcity to growth

factors such as; temperature and humidity, may contributed for the lower survival rate. The difference in type of landrace, and time acclimatization (very hot period, March) probably contributed for the variation.

Chapter 6.

CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

There are a number of enset (*Ensete ventricosum*) landraces that are grown for one or more trait/s of interest. Some of the landraces are basically grown for traditional medicine although they are good source of food. These landraces are vulnerable to various constraints. The genetic diversity, nutritional and phytochemical composition, and micropropagation of these landraces were investigated in the current study for effective conservation and utilization.

The diversity study based on 14 qualitative traits revealed that there is a wide range of variability for most traits. Around half of the landraces studied were characterized by high corm, *kocho*, and *bulla* quality in terms of taste. The majorities of the landraces investigated were susceptible to bacterial wilt, and have low drought tolerance level. According to principal component analysis, the first four principal components together explained 74.1% of the total variance and all the fourteen phenotypic traits were found to be important in discriminating and classifying the 40 landraces into four groups. All the 14 qualitative traits were found to have large contribution to variation among the landraces. Although the grouping pattern varied slightly, cluster analysis also grouped the landraces in to four clusters irrespective of their origin. In both cases of the multivariate analyses, no overlapping or duplication of the landraces with different vernacular name was observed, indicating the existence of diversity among the landraces.

In the evaluation and comparison of nutritional and phytochemical composition, it was found out that the corms of three landraces used in traditional medicine (*astara, guarye and kibnar*) were significantly different from two landraces (*guarye* and *kibnar*) that have non-medicinal uses, in protein, phosphorus, zinc and tannin contents in general, and *astara* landrace was superior in most of the proximate and mineral contents. As opposed to the hypothesis, no significant difference in calcium content was observed among the two groups of enset landraces. However, enset corm, in general, showed the highest calcium, phosphorus and zinc content and comparable in protein and carbohydrate composition as compared to some of root and tuber crops such as, Irish potato, sweet potato and taro, while its iron and fat content was quite low.

The genetic diversity analysis of enset landraces used in traditional medicine showed the existence of moderate level of genetic diversity. Neighbor joining tree grouped the landraces in to four clusters, irrespective of their origin, indicating the higher genetic variation among landraces with in location. The result also revealed that naming of enset landraces in each locatio is more or less consistet, indicating that farmers have rich indigenous knowledge in identification and naming enset landraces based on phenotypic traits. However, some other replicates of landraces were placed in different cluster in the neighbor joining tree, showing that genetically different landraces were given the same vernacular name. This is expected that perfect identification of genotypes using morphological traits is hardly possible. On the other hand, almost all the landraces with distinct vernacular names were different, which implies that vernacular names are good indicator of genetic distinctiveness in these specific groups of landraces.

MS medium supplemented with 5 mgl^{-1} BAP + 1.0 mgl^{-1} IBA was suitable for shoot multiplication of *Ensete ventricosum* landraces; *guarye and kibnar* , from which and a mean shoot number (8.8) per shoot tip was regenerated. The shoots were better rooted on full and half strength-MS media containing 3.0 and 2.0 mgl^{-1} IBA respectively, and they were acclimatized in a greenhouse with 75% survival rate. Although it has poor efficiency, enset can be propagated through conventional vegetative methods. However, micropropagation will have a great importance in multiplication of the vulnerable medicinal enset landraces, so that producers will have access to disease free planting materials, and the loss of such important landraces can be reduced. The data generated in the present study will be useful for conservation and micropropagation of enset landraces used in traditional medicine, and it can also provide baseline information to promote further studies in exploiting the medicinal value of the landraces.

6.2. Recommendations

Based on the results of the present study, it can be recommended that the selective use of some enset landraces by traditional healers, in the treatment of bone fracture, is reasonable. As these landraces are vulnerable to environmental and human related factors, those found to be distinct shall be conserved. The micropropagation protocol developed shall be used for rapid multiplication of *guarye* and *kibnar* enset landraces, and it can be applied for related genotypes to support sustainable production and conservation of the landraces. A more extensive collection to include enset landraces from areas not covered in the present study will be important to exploit the possible genetic diversity and to reverse the current genetic erosion of the crop through effective conservation. In addition, nutritional and phytochemical

composition should be carried out on a greater number of landraces to represent most of the enset population.

REFERENCES

Abebe Yewelsew, Bogale Alemtsehay, Hambidge, K.M., Stoecker, B.J., Bailey, K. and Gibson, R.S. (2007). Phytate, zinc, iron and calcium content of selected raw and prepared foods consumed in rural Sidama, southern Ethiopia, and implications for bioavailability. J. FOOD COMPOS. ANAL. 20: 161–168.

Abrham Shumbulo, Yishak Gecho and Melese Tora (2012). Diversity, Challenges and Potentials of Enset (Ensete ventricosum) Production: In Case of Offa Woreda, Wolaita Zone, Southern Ethiopia. Food Science and Quality Management 7:24-31.

Abrham Wondimu, Fantahun Molla, Dinda S. C., Naod Gebre-Samuel, Ebisa Tadese, (2014). Literature Review on Enset Starch: Physico-Chemical Properties and Pharmaceutical Applications. Journal of Drug Delivery and Therapeutics 4(3): 1-6.

Adegunloye, D.V. and Oparinde, T.C. (2017). Effects of fermentation on the proximate composition of Irish (*Solanum tuberosum*) and sweet potato (*Ipomoea batatas*) peels. Adv. Microbiol., 7:565–574.

Admasu Tsegaye (2002). On indigenous production, genetic diversity and crop ecology of enset (*Ensete ventricosum* (Welw.) Cheesman). PhD Thesis, Wageningen University, Netherlands, 198Pp.

Admasu Tsegaye (2015). Identification and analysis of nutritional components of enset *(Ensete ventricosum)* landraces suitable for corm production in Southern Ethiopia. Ethiopian Journal of Development Research, 37 (2):1-27.

Admasu Tsegaye and Struik P.C. (2001). Enset (*Ensete ventricosum* (Welw.) Cheesman) *kocho* yield under different crop establishment methods as compared to yields of other carbohydrate rich foods. Neth. J. Agri. Sci., 49:81–94.

Admasu Tsegaye and Westphal, E. (1992). *Ensete ventricosum* (Welw.) Cheesman. Record from PROTA4U. In: PROTA (Plant Resources of Tropical Africa) (Oyen, L.P.A.; Lemmens, R.H.M.J., ed.). Wageningen, Netherlands.

Afza, R., van Dure, M. and Morpurgo, R. (1996). Regeneration of *Ensete ventricosum* through somatic embryogenesis and adventitious buds. Plant Cell Reports 15: 445-448.

Ahloowalia, B.S., Prakash, J., Savangikar, V.A., and Savangikar, C. (2004). Plant tissue culture. In: Proceedings of FAO and international atomic energy agency (IAEA) technical meeting on low cost options for tissue culture technology in developing countries, Pp. 26–30 August, 2002. IAEA, Vienna, Austria.

Ahmadi, A., Azadfar, D., Mofidabadi, A.J. (2010). Study of inter-generic hybridization possibility between *Salix aegyptiaca* and *Populus caspica* to achieve new hybrids. Int. J. Plant Prod. 4(2):143-147.

Ajebu Nurfeta, Tolera Adugna, Eik, L.O. and Sundstøl, F. (2008). Yield and mineral content of ten *enset* (*Ensete ventricosum*) varieties. Tropical Animal Health and Production40:299–309.

Akin-Idowu, P.E, Ibitoye, D.O, Ademoyegun, O.T. (2009).Tissue culture as a plant production technique for horticultural crops. Afr. J. Biotechnol. 8(16): 3782-3788.

Alcantara, R.M., Wilma A.H. and Erlinda I.D. (2013). The nutritional value and phytochemical components of taro [*Colocasia esculenta* (L.) Schott] powder and its Selected processed foods. J. Nutr. Food Sci., **3**:207.

Almaz Negash, Admasu Tsegaye, Van, T.R. and Visser, B. (2002). AFLP analysis of enset clonal diversity in south and southwestern Ethiopia for conservation. Crop Science 42: 1105 –1111.

Almaz Negash, Puite, K., Schaart, J., Visser, B. and Krens, F. (2000). *In vitro* regeneration and micropropagation of enset from southwestern Ethiopia. Plant Cell Organ Cult 62:153–158.

Alphonse, L, Benoît B.K., Habiba, K. and Elias, N.N. (2018). Effect of harvest period on the proximate composition, functional and sensory properties of gari produced from local and improved cassava (*Manihot esculenta*) varieties. INT. J. FOOD SCI. TECH., https://doi.org/10.1155/2018/6241035.

Amare Gessese (2016). Industrial uses of enset: application in the food, animal feed, paper, textile, and brewery industries. In: International Workshop on enset (*Ensete ventricosum*) for Sustainable Development, Oct. 17-18, 2016, Addis Ababa University, Addis Ababa, Ethiopia.

Amare Seifu and Daniel Fitamo, (2016). Diversity of Enset Landraces (*Ensete ventricosum* (Welw) Cheesman) in Aleta Chuko District, Sidama Zone, South Nation Nationality People and Regional State, Ethiopia. Journal of Plant Sciences 4 (1):1-7.

Andrew R. (2016) FigTree: Tree figure drawing tool, Version 1.4.3. Institute of Evolutionary Biology, University of Edinburgh, United Kingdom.

AOAC (1990). Official methods of analysis of AOAC International, 15th ed.AOAC International, Washington (DC).

AOAC (2005). Official Methods of Analysis of AOAC International, 18th ed. AOAC International, Maryland.

AOAC (2016). Official Methods of Analysis of AOAC International, 20th ed. AOAC International, USA.

AOAC (2003). Official methods of analysis of AOAC International, 17th ed. 2nd rev. AOAC International, Gaithersburg (MD).

Ashenafi Ayenew, Abiyselassie Mulatu, Bruk Lemma, Demissie Girma (2016). An Ethnobotanical Study of Enset (*Ensete ventricosum* (Welw) Cheesman) in Angacha Woreda, Kembata-Tembaro Zone, South Region, Ethiopia. American Journal of Life Sciences 4 (6):195-204.

Atnafu Bekele, Mulugeta Diro and Mikias Yeshitila (2013). The Diversity and Associated Yield Components of Enset (*Ensete ventricosum*) based on its agro-morphological traits from southern Ethiopia. *SINET*: Ethiopian Journal of Science 36:49–54.

Atnafua Bekele and Endashaw Bekele (2018). Proximate and mineral composition variability in Ethiopian yam (*Dioscorea spp*). Food Nutr. Sci., 6: 12–17.

Awol Zeberga, Zerihun Yemataw, Woldeyesus Sinebo., Sadik Musemil and Daniel Ambachew. (2014). On Farm Cultivar Diversity of Enset *(Ensete Ventricosum* W.) in Southern Ethiopian Journal of Advanced Dielectrics 4(1):62-85.

Ayalew Debebe (2006). Studies of enset (*Ensete ventricosum*) for major, minor and trace elements. M.Sc. Thesis, Addis Ababa University, Addis Ababa, Ethiopia, Pp. 1–47

Ayelign Mengesha, Biruk Ayenew, Elias Gebre-Mariam and Tewodros Tadesse. (2012). Micro-Propagation of *Vanilla lanifolia* Using Enset (*Ensete ventricosum* (Welw, Cheesman)) Starch as a Gelling Agent. In Current Research Journal of Biological Sciences 4(4): 519-525.

Baker, R.E.D. and Simmonds, N.W. (1953).The genus *Ensete* in Africa. Imperial College of Tropical Agriculture, Trinidad, *Kew Bulletin* 8:405–416

Balloux, .F, Lehmann, L. and de Meeûs, T. (2003). The Population Genetics of Clonal and Partially Clonal Diploids. Genetics 164:1635–1644.

Barcaccia, G. Albertini, E. Rosellini, D. Tavoletti, S. and Veronesi, F. (2000). Inheritance and mapping of 2n-egg production in diploid alfalfa, Genome 43 (3): 528–537.

Belhu Terefe and Endale Tabogie (1989). A review of the available research recommendations and future strategies on enset. In Proceedings of 19th National Crop Improvements Conference, April 1987, Pp. 337–342. Institute of Agricultural Research, Addis Ababa.

Bethke, P.C., Jansky, S.H. (2008). The effects of Boiling and Leaching on the Content of Potassium and other minerals in potatoes. J. Food Sci. 75: 80–85.

Bhatia, P., Ashwath, N. and Midmore, D.J. (2005). Effects of genotype, explant orientation, and wounding on shoot regeneration in tomato. *In vitro* Cell. Dev. Biol. - Plant, 41: 457–464.

Biruk Ayenew, Ayelign Mengesha, Tewodros Tadesse and Elias Gebre-Mariam (2012).*Ensete ventricosum* (Welw.) Cheesman: a Cheap and Alternative Gelling Agent for Pineapple (*Ananas comosus* Var. Smooth Cayenne) *In vitro* Propagation. Journal of Microbiology, Biotechnology and Food Sciences 2 (2): 640-652

Bizuayehu Tesfaye and Lüdders P., (2003). Diversity and distribution patterns of enset landraces in Sidama, Southern Ethiopia. Genetic Resources and Crop Evolution. 50:359–71

Borrell, J.S., Biswas, M.K., Goodwin, M., Blomme, G., Schwarzacher, T., Heslop-Harrison, J.S, Wendawek, A.M., Berhanu, A., Kallow, S., Janssens, S., Molla, E.L., Davis, A.P. Woldeyes, F., Willis, K., Demissew, S. and Wilkin, P. (2019). Enset in Ethiopia: a poorly characterized but resilient starch staple. Annals of Botany **123**: 747-766.

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

Brandt S.A., Spring A., Hiebsch, C., McCabe, J.T., Endale Tabogie, Mulugeta Diro, Gizachew Weldemichael, Gebre Yntiso, Masayoshi Shigeta and Shiferaw Tesfaye (1997). The "Tree Against Hunger": Enset- Based Agricultural Systems in Ethiopia. American Association for the Advancement of Science, Washington.

Brandt, A.S. (1996). A model for the origins and evolution of enset food production. In: Enset-Based Sustainable Agriculture in Ethiopia, Pp. 172-187 (Tsedeke Abate, Clifton H., Steven B.A. and Seifu Gebre-Mariam eds.). Proceedings from international workshop on enset, Institute of Agricultural Research, Addis Ababa.

Brar, D.S. and Uchimiya, H. (1990). Genetic Engineering of Crop plants. In: Plant Tissue Culture: Applications and Limitations Pp. 301–315 (Bhojwani, S.S., ed.); Elsevier, Amsterdam, Netherland.

Bridgen, M.P. (1994). A Review of Plant Embryo Culture. HORTSCIENCE, 29(11):1243-1246.

Brown, D.C.W. and Thorpe, T.A. (1995). Crop improvement through tissue culture. World J. Microbiol & Biotechnol. 11: 409-415.

Burns, R.B. (1971). Methods of estimation of tannin in the grain sorghum. Agron J. 63: 511–512.

Central Statistics Agency (2017). Central Statistics Agency-Agricultural Sample Survey (Belg and Meher seasons). Retrieved from http://www.csa.gov.et/survey-report/category/58-meher-main-season-agricultur al-sample-survey.

Chandana, B.C., Nagaveni, H.C., Kumari, L.D., Shashikala S.K. and Heena M.S. (2018). Role of plant tissue culture in micropropagation, secondary metabolites production and conservation of some endangered medicinal crops. Journal of Pharmacognosy and Phytochemistry 7(3S): 246-251.

Chandra, S., Bandopadhyay, R. Kumar, V. and Chandra, R. (2010). Acclimatization of tissue cultured plantlets: from laboratory to land. Biotechnol Lett 32:1199–1205.

Charles, A.L., Sriroth, K. and Huang, T. (2005). Proximate composition, mineral contents, hydrogen cyanide and phytic acid of 5 cassava genotypes. Food Chemistry 92:615–620.

Cheesman, E. E. (1947). Classification of the Bananas: The Genus Ensete Horan. Kew Bulletin 2(2):97-106.

Chiwona-Karltun, L., Mkumbira, J., Saka, J., Bovin, M., Mahungu, N.M., Rosling, H. (1998).The importance of being bitter- a qualitative study on cultivar preference in Malawi. Ecology of Food and Nutrition 37:219-245.

Cholastova, T. and Knotova, D.(2012) .Using Morphological and Microsatellite (SSR) Markers to Assess the Genetic Diversity in Alfalfa (*Medicago sativa* L.). Internatio nal Journal of Agricultural and Biosystems Engineering 6 (9):781-788

Christopher, T. and Rajam, M.V. (1996). Effect of genotype, explant and medium on *in vitro* regeneration of red pepper. Plant Cell Tiss Organ Cult 46: 245–250.

Conner, A.J. and Thomas, M.B. (1982). Re-establishing plantlets from tissue culture: a review. The International Plant Propagators' Society Combined Proceedings, 31, 1981. Pp. 342–35).

Connolly, A.G., Godwin, I., Cooper, M. and DeLacy, I.N.D. (1994). Interpretation of randomly amplified polymorphic DNA marker data for fingerprinting sweet potato (*Ipomoea batatas* L.) genotypes. Theor. Appl. Genet. 88(3–7): 332–336.

Dagmawit Chombe Tobiaw and Enashaw Bekele (2011). Analysis of genetic diversity among cultivated enset (*Ensete ventricosum*) populations from Essera and Kefficho, southwestern part of Ethiopia using inter simple sequence repeats (ISSRs) marker. African Journal of Biotechnology 10(70): 15697–15709.

de Sousa Leal, A., de Carvaiho Leal, .L.H, da Silva, D., Nunes, L.C.C., Lopes, J.A.D. (2015). Incorporation of tannic acid in formulations for topical use in wound healing: A technological prospecting. Afr. J Pharm. Pharmacol., 9: 662–674.

Debergh, P.C. and Read, P.E. (1991). Micropropagation. In: Debergh and Zimmerman (eds.) Micropropagation: Technology and Application. Kluwer Academic Publishers; Dordrecht*I* Boston*I* London.

Debergh, P.C., Maene, L.J. (1981). A scheme for commercial propagation of ornamental plants by tissue culture, Scientia Hort 14: 335-345.

Demelash Hailu and Tilahun Abera (2017). Nutrient and antinutrient composition of improved sweet potato (*Ipomea batatas* (L) Lam) varieties grown in eastern Ethiopia. J. Nutr. Food Sci., 47: 369–380.

Desta, H.Z. and Oba, G. (2004). Feed scarcity and livestock mortality in enset farming systems in the Bale highlands of southern Ethiopia. Outlook on Agriculture, 33(4): 277–280.

Devos, K.M., Atkinson, M.D., Chinoy, C.N. (1993). Chromosomal rearrangements in the rye genome relative to that of wheat. Theoretical and Applied Genetics, 85 (6-7): 673–680.

Diana, K.J. and George, K.V. (2013). Urinary stone formation: Efficacy of seed extract of *Ensete superbum* (Roxb.) Cheesman on growth inhibition calcium hydrogen phosphate dihydrate crystals. J. Cryst. Growth363: 164–170.

Dirsha Demam (2019). Analysis of enset (*Ensete ventricosum*) based cattle production system; availability and quality of common feed resources in Gurage zone, southern Ethiopia. PhD dissertation submitted to Department of Animal Production, Addis Ababa University Ethiopia.

Endale Tabogie (1997). Morphological characterization of Enset (*Ensete ventricosum* (Welw.) Cheesman) clones and association of yield with different traits. M.Sc. Thesis. Alemaya University of Agriculture, Alemaya, Ethiopia

Endashaw Bekele (2018). The center of origin and domestication of *Ensete ventricosum* (welw.) Cheesman and its phylogenetic relationship to some *Musa* species; Ethiop. J. Biol. Sci. 17(Suppl.): 37–49.

FAO (2009). International Treaty on Plant Genetic Resources for Food and Agriculture, Rome, Italy.

Feta Negash (2007). Diversity and indigenous management of enset (*Ensete ventricosum* (Welw.) Cheesman) landraces in Gurage zone, Southern Ethiopia. MSc thesis, Hawasa University, Hawasa, Ethiopia.

Feta Negash, Meressa, B.H., Martina, K., Tesfaye, A., Temesgen Magule and Nasser, Y. (2019). Genetic diversity and population structure of enset (*Ensete ventricosum* Welw Cheesman) landraces of Gurage zone, Ethiopia. Genet Resour Crop Evol https://doi.org/10.1007/s10722-019-00825-2

Fujita, Y. (1990). The Production of Industrial Compounds. In: *Plant Tissue Culture: Applications and Limitations* Pp. 259–275 (Bhojwani, S.S., ed.): Elsevier; Amsterdam, Netherland.

Gao, F.k, Dai, C.C. and Liu, X.Z. (2010). Mechanisms of fungal endophytes in plant protection against pathogens. *Afr J Microbiol Res*. 4:1346–1351.

Garcia-Gonzales, R., Quiroz, K., Carrasco, B., Caligari, P. (2010). Plant tissue culture: Current status, opportunities and challenges. Cien. Inv. Agr. 37(3): 5-30.

Genene Gezahegn and Firew Mekbib (2016). *In vitro* regeneration of disease free enset (*Ensete ventricosum* (Welw) Cheesman) planting materials from bacterial wilt diseased plants using shoot tip culture. African Journal of Biotechnology 15(40): 2192-2201.

Genet Birmeta and Welander, M. (2004b). Efficient micropropagation of *Ensete ventricosum* applying meristem wounding: a three-step protocol. Plant Cell Rep 23:277–283.

Genet Birmeta, Nybom, H. and Endashaw Bekele (2004a). Distinction between wild and cultivated Enset *(Ensete ventricosum*) gene pools in Ethiopia using RAPD markers. Hereditas 140: 139–148.

Genet Birmeta, Nybom, H., and Endashaw Bekele (2002). RAPD analysis of genetic diversity among clones of the Ethiopian crop plant *Ensete ventricosum*. Euphytica 124(3): 315-25.

George, E.F. (1993). Plant Propagation by Tissue Culture. Part 1, the Technology. Exegetics; Edington, UK, 7p.

Gomes, F., Simões M, Lopes M.L., Canhoto JM. (2010). Effect of plant growth regulators and genotype on the micropropagation of adult trees of *Arbutus unedo* L. (strawberry tree). N Biotechnol 27(6):882-92.

Govindaraj, M., Vetriventhan, M., and Srinivasan, M. (2015). Importance of Genetic Diversity Assessment in Crop Plants and Its Recent Advances: An Overview of Its Analytical Perspectives. Genetics Research International, Volume 2015, Article ID 431487.

Gubiš, J., Lajchová, Z., Faragó, J. and Jureková, Z. (2003). Effect of Genotype and Explant Type on Shoot Regeneration in Tomato (*Lycopersicon esculentum* Mill.) *in vitro*. Czech J. Genet. Plant Breed. 39 (1): 9–14.

Gubišová, M. and Gubiš, J. (2019). Growths of potato shoot culture on media with antibiotics for elimination of bacterial contamination. Agriculture (Pol'nohospodárstvo) 65 (3): 99 – 106.

Gulelat Desse, Adane Tilahun, Shimelis Admasu, Negussie, Retta and Birhanu Tilahun (2013). Effect of processing method on the Proximate Composition, Mineral Content and Antinutritional Factors of Taro (*Colocasia esculenta*, L.) Grown in Ethiopia. Afr. J. Food Agric. Nutr. Dev., 13: 7383–7398.

Hadiuzzaman, S., Habiba, U., Reza, S., Saha, M.L. and Khan, M.R. (2001). Development of a sustainable protocol for contamination free culture of table bananas and identification of associated endogenous bacteria. In: 4th International Plant Tissue Culture Conf., Pp. 24, Abstracts. 1st - 3rd November, 2001. Dhaka, Bangladesh.

Halkett, F., Simon, J.C. and Balloux, F. (2005). Tackling the population genetics of clonal and partially clonal organisms. Trends in Ecology and Evolution 20:194–201.

Harlan, J.R. (1992). Crops and man. American Society of Agronomy & Crop Science Society of America, Madison.284 Pp. doi:10.2135/1992.cropsandman.frontmatter

Haslam, E. (1996). Natural polyphones (vegetable tannins) as drugs and medicines: possible modes of action. Natural Product 59:205–215.

He, G., Prakash, C.S. and Jarret, R.L. (1995). Analysis of genetic diversity in a sweet potato (Ipomoea batatas) germplasm collection using DNA amplification fingerprinting. Genome 38: 938–945.

Heaney, R.P. (2004). Phosphorus nutrition and the treatment of osteoporosis. Mayo Clin Proc, 79:91–7.

Hiwot Bekele (2015). Effect of *Enset* (*Ensete ventricosum* (Welw*)* Cheesman) Variety and Fermentation on Nutritional Composition, Anti-nutritional Factors, Physicochemical Characteristics and Functional property of *Bulla*. MSc Thesis, Addis Ababa University. Addis Ababa.

Hölscher, D. and Schneider, B. (1998). Phenylphenalenones from *Ensete ventricosum*. Phytochemistry 49:2155–2157.

Hunduma, T., Sadessa, K., Hilu, E., Oli, M. (2015). Evaluation of Enset Clones Resistance against Enset Bacterial Wilt Disease (*Xanthomonas campestris pv*. *musacearum*). J Veterinar Sci Technol 6:232. doi:10.4172/2157-7579.1000232

Hussain A., Qarshi A. I., Nazir H. and Ullah I. (2012). Plant Tissue Culture: Current Status and Opportunities.https://www.researchgate.net/publication/297777765_Plant_Tissue_Culture_Curr ent_Status_and_Opportunities

Joshi, S.P., Ranjanekar, P.K. and Gupta, V.S. (1999).Molecular markers in plant genome analysis. Curr. Sci.77: 230–240.

Karaagac, E., Yilma, S., Cuesta-Marcos, A. and Vales, M.I. (2014). Molecular analysis of potatoes from the Pacific Northwest tri-state variety development program and selection of markers for practical DNA fingerprinting applications. Am J Potato Res **91**(2): 195-203.

Kaya, A., Aktas, A., Akdemir, M. F., Kaya, B. and Deveci, E. (2015). The effect of dietary supplements on healing of alloplastic bone grafted defects in rat tibia. Int. J. Morphol. 33(2): 725-731.

Kedir Abdella (2016). Traditional Medicinal Use of *Ensete ventricosum* (Welw.) Cheesman in Gedebano Gutazer Welene District, Gurage Zone, SNNP Region, Ethiopia. MSc Thesis Addis Ababa University, Addis Ababa, Ethiopia.

Kress, W.J. (1990). The phylogeny and classification of the Zingiberales. Annals of Missouri Botanical Garden 77: 698-721.

Krikorian, A.D. (1989). *In vitro* culture of bananas and plantains, back ground, update, and call for information. Tropical Agriculture (Trinidad) 66: 194–200 Mathew and Philip (1996)

Kumar N. and Reddy M. P. (2011). *In vitro* Plant Propagation: A Review. Journal of Forest Science 27(2):61-72.

Kumari, K., Saxena, S., Anand, A. and Lal, M. (2015). Use of antibiotics to control bacterial contamination during *in vitro* micropropagation of sugarcane. Agrica 4:41-44.

Latta, M. and Eskin, M. (1980). A simple and rapid colorimetric method for phytate evaluation. Agric. Food Chem., 28: 1313–1315.

Leifert, C. and Cassells, A.C. (2001). Microbial hazards in plant tissue and cell cultures. *In vitro* Cell Dev. Biol. Plant, 37(2): 133-138.

Li, X.W. (1978). The Musaceae of Yunnan.Acta Phytotax. Sin. 16(3): 54–64

Lin, P.H, Sermersheim M., Li, H., Lee, P.H.U., Steinberg, S.M. and Ma, J. (2018). Zinc in wound healing modulation. Nutrients, 10: 1–20.

Maene, L.M. and Debergh, P.C. (1983). Rooting Of Tissue Cultured Plants Under In Vivo Conditions. Acta Hortic.131:201-208 DOI:10.17660/ActaHortic.1983.131.22.

Manchanda, P., Kaur A. and Gosal, S.S. (2011).Impact of cefotaxime on *in vitro* shoot elongation and regeneration in banana (*Musa acuminata*).Journal of Applied Horticulture 13(1):52-55.

Marks, J., Edward, S., Debnam, E.S. and Unwi, R.J. (2010). Phosphate homeostasis and the renal-gastrointestinal axis. Am. J. Physiol. Renal Physiol. 299:285–296.

Martin, K. P., Zhang C., Slater, A. and Madassery, J. (2007). Control of shoot necrosis and plant death during micropropagation of banana and plantains (*Musa spp.)*. Plant Cell Tiss Organ Cult, 88:51–59.

Martini, A.N., Papafotiou, M. and Vemmos, S.N. (2013). Season and Explant Origin Affect Phenolic Content, Browning of Explants, and Micropropagation of *Malosorbus florentina* (Zucc.) Browicz. HortScience 48 (1): 102-107.

Masayoshi Shigeta (1991). The ethnobotanical study of enset (*Ensete ventricosum*) in the southwestern Ethiopia. A PhD dissertation, University of Kyoto, Japan.

Masum, A.A.S.M.G., Crawford, H., Berthold, J., Talukder, Z.I. and Hossain, K. (2011). Minerals (Zn, Fe, Ca and Mg) and Antinutrient (Phytic Acid) Constituents in Common Bean. Am J Food Technol., 6(3):235–243.

Mathew, M.M., Philip, V.J. (1996). Clonal propagation of Enset (*Ensete superbum* (Roxb.). Cheesman) through shoot tip cultures. Plant Cell Rep 16:232–234.

Maxson, E.D. and Rooney, L.W. (1972). Evaluation of methods for tannin analysis in sorghum grain, ed. AACC, 49: 719–728.

McKey, D., Elias, M., Pujo, B. and Duputié, A. (2010). The evolutionary ecology of clonally propagated domesticated plants. New phytologist 182(2): 318–332.

Melese Temesgen Yirmaga (2013). Improving the Indigenous Processing of Kocho, an Ethiopian Traditional Fermented Food. J Nutr Food Sci 3:182. Doi: 10.4172/2155-9600.1000182

Meloni, M., Reid, A., Caujapé-Castells Marrero, J.Á., Fernández-Palacios, J.M., Mesa-Coelo, R.A. and Conti, E. (2013). Effects of clonality on the genetic variability of rare, insular species: the case of *Ruta microcarpa* from the Canary Islands. Ecology and Evolution 3(6): 1569–1579.

Meseret Haile (2015). Comparison of the nutritional and microbial composition of *kocho* from wild and cultivated enset from Bonga, Ethiopia. MSc thesis submitted to Centre of Food Science and Nutrition, Addis Ababa University.

Mesfin Sahle, Kumlachew Yeshitela and Saito, O. (2018). Mapping the supply and demand of Enset crop to improve food security in Southern Ethiopia. Agronomy for Sustainable Development, 38:7 https://doi.org/10.1007/s13593-017-0484-0

Mikias Yeshitila (2014). Cluster analysis for evaluation of genetic diversity in enset *(Ensete ventricosum* (Welw.) Cheesman) clones at Areka condition. Journal of Plant Sciences2:55–69.

Miller, C., Skoog, F., Von Saltza, M.H., and Strong, F.M. (1955). Kinetin, a cell division factor from desoxyribonucleic acid.J. Am. Chem. Soc. **77,** 1392.

Minaleshewa Atlabachew and Chandravanshi, B.S. (2008). Levels of major, minor and trace elements in commercially available enset (*Ensete ventricosum* (Welw.), Cheesman) food products (*Kocho* and *Bulla*) in Ethiopia. Journal of Food Composition and Analysis 21: 545-55.

MINITAB (2003). MINITAB software, version 14. Minitab Inc, USA

Mohammed Feyisso Shaka and Yohannes Addisu Wondimagegne (2018). Anemia, a moderate public health concern among adolescents in South Ethiopia. PLoS ONE 13(7): e0191467.

Mohammed, B., Martin, G. and Laila, M.K. (2013). Nutritive values of the drought tolerant food and fodder crop enset. African Journal of Agricultural Research 8(20): 2326–2333.

Mona, Q., Anna, M. P., Mysore, V.T., Traud, W., Melanie, B. (2014). Endophytic bacteria in plant tissue culture: differences between easy- and difficult-to-propagate *Prunus avium* genotypes, Tree Physiology, 34(5): 524–533.

Mullis, K.B., Faloona, F.A., Scharf, S.J., Saiki, S.K., Horn, G.T. and Erlich, H.A. (1986). Specific enzymatic amplification of DNA *in vitro*: the polymerase chain reaction. Cold Spring Harbor. Symp. Quant. Biol. (51): 263-273.

Mulugeta Diro (2003). *In vitro* Propagation of Enset *(Ensete ventricosum* (Welw.) Cheesman). PhD dissertation submitted to School of Botany and Zoology University of Natal Pietermaritzburg Republic of South Africa.

Mulugeta Diro and Endale Tabogie (1994). Preliminary investigation on enset culture. In: Proceedings of the second national horticultural workshop of Ethiopia. 1-3 Dec 1992

(Herath, E. and Lemma Dessalegn, eds.), Pp. 120–131. Institute of Agricultural Research, Addis Ababa.

Mulugeta Diro and van Staden, J. (2004). Propagation of Ensete *in vitro*: a review. South African Journal of Botany, 70(4): 497–501.

Mulugeta Diro and van Staden, J. (2005). The type of explant plays a determining role in the micropropagation of *Ensete ventricosum*. South African Journal of Botany 71(2): 154–159.

Mulugeta Diro, Bezuayehu Haile and Endale Tabogie (1996). Enset propagation research: review. In: Enset-based Sustainable Agriculture in Ethiopia, Pp. 242–250 (Tsedeke Abate, Hiebsch, C., Brandt, S.A. and Seifu Gebre-Mariam, eds.). Proceedings from the International Workshop on Enset held 13–20 December 1993, Institute of Agricultural Research, Addis Ababa, Ethiopia.

Munsell (1970). Munsell book of color. Munsell color company, Baltimore, Maryland, USA.

Murashige, T. (1977). Plant cell and organ cultures as horticultural practices. In: Proceedings of the Symposium on Tissue Culture for Horticultural Purposes, Ghent, Belgium.

Murashige, T. and Skoog, F. (1962).A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant 15**:** 473–497.

Nakano, M. and Mii, M. (1993). Antibiotics stimulate somatic embryogenesis without plant growth regulators in several *Dianthus* cultivars. Plant Physiology, Hanover 141: 721-725.

Nei, M. (1972). Genetic distance between populations. Amer. Naturalist **106**: 283-292

Neumann K.H., Kumar A. and Imani J. (2009). Plant Cell and Tissue Culture - A Tool in Biotechnology: Basics and Application. Springer; Verlag, Berlin Heidelberg.

Nouioua, W., Sofiane, G. and Mouhamed, K. (2016). The antioxidant and antimicrobial activities of flavonoids and tannins extracted from *Phlomis bovei* De Noé. Euro. J. Exp. Bio., 6:55–61.

Ogawa, Y. and Mii, M. (2004). Screening for highly active β-lactam antibiotics against *Agrobacterium tumefaciens*. Archives of Microbiology 181:331-336.

Oggem, J.N., Kinyua, M.G., Ouma, J.P. and Owuoche, J.O. (2007). Agronomic performance of locally adapted sweet potato (*Ipomoea batatas* (L) Lam) cultivars derived from tissue culture regenerated plants. African Journal of Biotechnology 6 (12): 1418–1425.

Ono, Y., Takahata, Y. and Kaizum, N. (1994). Effect of genotype on shoot regeneration from cotyledonary explants of rapeseed (*Brassica napus* L.). Plant Cell Reports 14 (1): 13–17.

Osborne, D.R. and Voogt, P. (1978). The Analysis of nutrients in foods. Academic Press, New York: London.

Peakall R, Smouse PE. (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetics software for teaching and research–an update. Bioinformatics 28:2537–9.

Penido, M.G., Alon, U.S. (2012). Phosphate homeostasis and its role in bone health. PEDIATR.NEPHROL. 27:2039–2048.

Perrier, X., Jacquemoud-Collet JP.DARwin software (2006). http://darwin.cirad. fr/. Accessed 22 Jul 2019.

Pierik, R.L.M. (1987). *In vitro* Culture of Higher Plants. Martinus Nijhoff Publishers, Dordrecht.

Pierik, R.L.M. (1997). *In vitro* culture of higher plants. Kluwer Academic Publishers; Wageningen, Netherlands

Pijls, L.T.J., Timmer, A.A.M., Wolde-Gebriel, Z., and West, C.E. (1995). Cultivation, Preparation and consumption of Enset in Ethiopia. Journal of the Science of Food and Agriculture, 67: 1–11.

Powell, W., Morgante, M., Andre, C., Hanafey, M., Vogel, J. and Tingey, S. (1996). The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Mol Breed 2: 225-38.

Prashant, Y.M.V. and Bhadane, V. (2008). Some rare plants of ethnomedicinal properties from Jalgaon district of Maharashtra. Int. j. green pharm., 2: 76–78.

Rabiei B. (2010). Linkage map of SSR markers and QTLs detection for heading date of Iranian rice cultivars. Journal of Agricultural Science and Technology, 9:235-42.

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

Rao, N.K. (2004). Plant genetic resources: Advancing conservation and use through biotechnology. African Journal of Biotechnology 3 (2): 136-145.

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

Reed, B.M., Buckley, P.M. and Dewilde, T.N. (1995). Detection and eradication of endophytic bacteria from micro propagated mint plants. *In vitro* Cellular and Developmental Biology - Plant 31(1): 53 - 57.

Rojas, C.C., Nair, B., Herbas, A. and Bergenståhl, B. (2007). Proximal composition and mineral contents of six varieties of cassava (*Mannihot esculenta*, Crantz), from Bolivia. Bolivian journal of chemistry 24:1.

SAS (2002). Statistical analysis systems SAS, Version 9.2.SAS Institute Inc., Cary, NC, USA.

Schlemmer, U., Frølich, W., Prieto, R.M. and Grases, F. (2009). Phytate in foods and significance for humans: Food sources, intake, processing, bioavailability, protective role and analysis. MOL. NUTR. FOOD RES., 53: 330–375.

Seifu Gebre-Mariam (1996). Enset research in Ethiopia: 1976–1984. In: Enset based sustainable agriculture in Ethiopia, Pp. 204–220 (Tsedeke Abate, Hiebish C, Brandt, S.A., Seifu Gebre-Mariam, eds.). Institute of Agricultural Research, Addis Ababa, Ethiopia.

Selamawit Getachew, Firew Mekbib, Belayneh Admassu, Segenet Kelemu, Sisay Kidane, Kefyalew Negisho, Djikeng A. and Nzuk I. (2014). A Look into genetic diversity of Enset (Ensete ventricosum (Welw.) Cheesman) using transferable microsatellite sequences of banana in Ethiopia. Journal of Crop Improvement 28: 159-183.

Semal, J. and Lepoivre, P. (1990). Application of Tissue Culture Variability to Crop Improvement, In: Plant Tissue Culture: Applications and Limitations Pp. 346–365 (Bhojwani, S.S., ed.); Elsevier; Amsterdam, Netherland.

Sengar, R.S., Chaudhary, R., Tyagi, S.K. (2010). Present status and scope of floriculture developed through different biological tools. Res J. of Agri. Sci. 1(4): 306-314.

Simmonds, N.W. (1953). Segregations in some diploid bananas. Journal of Genetics 51: 458–469. http://dx.doi. org/10.1007/BF02982938

Simmonds, N.W. (1958). Enset cultivation in the Southern highlands of Ethiopia: A review. Tropical Agriculture 35:302- 307.

Simmonds, N.W. (1960). Notes on banana taxonomy. Kew Bulletin 14: 198–212. http://dx.doi.org/10.2307/41147781.

Simmonds, N.W. and Shepherd, K. (1955). The taxonomy and origins of the cultivated bananas. J Linn Soc (Bot) 55:302–312.

Singh, R.B. (1992). Current status and future prospects of plant biotechnologies in developing countries in Asia. In: Plant Biotechnologies for Developing Countries, 141-162 (Sasson, A, Costarini, ed.), London.

Sirawdink Fikreyesus Forsido, Vasantha Rupasinghe, H.P.V. and Astatkie, (2013). Antioxidant capacity, total phenolics and nutritional content in selected Ethiopian staple food ingredients. INT. J. FOOD SCI. NUTR. 64: 915–920.

Skoog, F. and Miller, C.O. (1957). Chemical regulation of growth and organ formation in plant tissue cultures *in vitro*. Symp. Soc. Exp. Biol. 11**:** 118–131.

Smith, M.K. and Drew, R.A. (1990). Current Applications of Tissue Culture in Plant Propagation and Improvement. Aust. J. Plant Physiol. 17: 267-89.

Solomon Zedie, Olsson, M. and Masresha Fetene (2008). Effect of Drought/irrigation on proximate composition and carbohydrate content of two enset [*Ensete ventricosum* (Welw.) Cheesman] clones. SINET: Ethiop.J.Sci. 31(2):81-88.

Southern Agricultural Research Institute **(**SARI**),** Ethiopia (2014)**.** The McKnight Foundation Collaborative Crop Research project "Integrated Management of Bacterial Wilt of Enset (*Ensete ventricosum* (Welw.) Cheesman) caused by *Xanthomonas campestris* pv. *musacearum* in Ethiopia" year three report.

Tadessa Daba and Masayoshi Shigeta (2016). Enset (*Ensete ventricosum*) production in Ethiopia: Its Nutritional and Socio-Cultural Values. Agriculture and Food Sciences Research 3: 66–74.

Taji, A.M. and R.R. Williams, (1996). Overview of Plant Tissue Culture. In: Tissue Culture of Australian Plants: Past, Present and Future, Pp.: 1-15 (Taji, A.M. and Williams, R.R. eds.); Armidale, Australia, University of New England Press.

Tattiyakul, J., Asavasaksakul, S. and Pradipasena, P. (2006). Chemical and physical properties of flour extracted from taro *Colocasia esculenta* (L.) schott grown in different regions of Thailand. Science Asia, 32: 279–284.

Taye Bezuneh (2012). Technological Challenges of enset farming system for production of Food/Fiber and industrial outputs. In: Enset Research and Development Experiences in Ethiopia (Mohammed, Y. and Tariku, H., eds.). Proceedings of enset national workshop, 19-20 August, 2010, Wolkite, Ethiopia.

Taye Bezuneh and Asrat Feleke (1966). The production and utilization of the genus *Ensete* in Ethiopia. Economic Botany 20 (1): 65-70.

Taye Buke, Bizuayehu Tesfaye and Demelash Kefale (2016). Studies on Conventional Vegetative Propagation of Enset (*Ensete ventricosum* (Welw.) Cheesman). International Journal of Research and Innovations in Earth Science, 3(2): 2394-1375.

Teixeira da Silva, J.A. (2005). Molecular markers for phylogeny, breeding and ecology in agriculture. In: Genetic Resources and Biotechnology (Vol. III), Pp.: 221-256

Teli, M.D. and Jelalu, M.T. (2017).Chemical, Physical and Thermal Characterization of *Ensete ventricosum* plant fiber. International Research Journal of Engineering and Technology, 04: 12

Temesgen Magule, Bizuayehu Tesfaye, Mario, A.P., Mario, E.P. and Marcello, C. (2015). Development of SSR markers and genetic diversity analysis in enset (*Ensete*

ventricosum (Welw.) Cheesman), an orphan food security crop from Southern Ethiopia. BMC Genetics 16:98. DOI: 10.1186/s12863-015-0250-8

Temesgen Magule, Bizuayehu, T., Catellani, M. and Pè, M.E. (2014). Indigenous knowledge, use and on-farm management of enset (*Ensete ventricosum* (Welw.) Cheesman) diversity in Wolaita, Southern Ethiopia. Journal of Ethnobiology and Ethnomedicine 10:41.

Tesfaye Abebe, Wongchaochant, S., Taychasinpitak, T. and Leelapon, O. (2012). Variation of mineral concentrations among different potato varieties grown at two distinct locations in Ethiopia. Kasetsart journal-Natural science 46: 837–850.

Tesfaye B (2008). The enset (*Ensete ventricosum*) gardens of Sidama: composition, structure and dynamics of a traditional poly-variety system. *Genet. Resour. Crop Evol.* 55:1347-1358.

Thomas, P. (2004). *In vitro* decline in plant cultures: detection of a legion of covert bacteria as the cause for degeneration of long-term micropropagated triploid watermelon cultures. Plant Cell, Tissue and Organ Culture 77(2): 173-9.

Thorpe, T. (2007). History of plant tissue culture. J. Mol. Microbial Biotechnol. 37: 169-180.

Thorpe, T.A. (1990). The current status of plant tissue culture, In: Plant Tissue Culture: Applications and Limitations Pp. 1–33 (Bhojwani, S.S., ed.); Elsevier, Amsterdam, Netherland.

Tilahun Zeweldu, Ladders, P. (1998). Preliminary tissue culture investigation in Ensete (*Ensete spp.*). Angew Bot 72:25–27.

Tileye Feyissa, Welander, M., Negash, L. (2005). *In vitro* regeneration of *Hagenia abyssinica* (Bruce) J.F. Gmel. (*Rosaceae*) from leaf explants. Plant Cell Rep 24:392-400.

Tomiyama, K., Mukai, Y., Saito, M., Watanabe, K, Kumada, H., Nihei, T., Nobushiro, H. and Teranaka, T. (2016). Antibacterial Action of a Condensed Tannin Extracted from Astringent Persimmon as a Component of Food Addictive Pancil PS-M on Oral Polymicrobial Biofilms. BioMed Research International, doi /10.1155/2016/5730748.

Torres K.C., (1989). Tissue Culture Techniques for Horticultural Crops. Van Nostrand Reinhold; New York.

Tostain, S., Agbangla, C., Scarcelli, N., Mariac, C., Dai¨nou, O., Berthaud, J. and Pham, J.L. (2006). Genetic diversity analysis of yam cultivars (*Dioscorea rotundata* Poir.) in Benin using simple sequence repeat (SSR) markers. Plant Genetic Resources: Characterization and Utilization 5(2): 71–81.

Tripathi J., Matheka J., Merga I., Endale Gebre and Tripathi L. (2017). Efficient regeneration system for rapid multiplication of clean planting material of *Ensete ventricosum* (Welw.) Cheesman. *In vitro* Cell Dev Biol Plant 53(6): 624–630.

Tripathi, J., Matheka, J., Merga, I., Endale, G. and Tripathi, L. (2017). Efficient regeneration system for rapid multiplication of clean planting material of *Ensete ventricosum* (Welw.) Cheesman. *In vitro* Cell.Dev.Biol. –Plant 53:624–630.

Tsige Gebre-Mariam (2016). Industrial applications of enset starch and its modifications: from native starch to nanoparticles. In: International Workshop on enset (*Ensete ventricosum*) for Sustainable Development, Oct. 17-18, 2016, Addis Ababa University, Addis Ababa, Ethiopia

Tyagi, R.K., Agrawal, A., Mahalakshmi, C., Hussain, Z., Tyagi, H. (2007). Low-cost media for *in vitro* conservation of turmeric (*Curcuma longa* L.) and genetic stability assessment using RAPD markers. *In vitro* Cell. Develop. Biol. Plant. 43: 51-58.

Vaintraub, I.A., Lapteva, N.A. (1988).Colorimetric determination of phytate in unpurified extracts of seed and the products of their processing. Anal. Biochem., 175: 227–30.

Van den Houwe, I. and R. Swennen (2000). Characterization and control of bacterial contaminants *in vitro* cultures of banana (*Musa spp*.). Acta Horticulturae 532: 69-79

Vavilov, N.I. (1951). The origin, variation, immunity and breeding of cultivated plants. Chronica bot. 13 (1-6): 1-364.

Veis, A. and Sabsay, B. (1987). The collagen of mineralized matrices. In: Bone and Mineral Research, Vol 5, Pp. 1- 63 (Peck, W.A., ed.), New York; Elsevier.

Virk, P.S., Newbury, J.H., Bryan, G.J., Jackson, M.T., Ford-Lloyd, B.V. (2000). Are mapped or anonymous markers more useful for assessing genetic diversity? Theor. Appl. Genet. 100: 607-613.

Weaver, C.M. and Kannan, S. (2002). Phytate and mineral bioavailability. In: Food Phytate, Pp. 211–223 (Reddy, N.R., and Sathe, S.K., eds.). CRC Press, Boca Raton.

Wekesa, M.N. Okoth, M.W., Abong, G.O., Muthoni, J. and Kabira, J.N. (2014). Effect of soil characteristics on potato tuber minerals composition of selected Kenyan varieties. J. AGR. SCI. 6: 163–171.

Weldemichael, G., Bobosha, K., Blomme, G., Addis, T., Mengesha, T. and Mekonnen, S. (2008). Evaluation of enset clones against enset bacterial wilt. African Crop Science Journal 16 (1): 89 - 95.

Westphal, E. (1975). Agricultural systems in Ethiopia. Centre for Agricultural Publication and Documentation. Wageningen, The Netherlands. Pp.278.

White, P.R. (1934). Potentially unlimited growth of excised tomato root tips in a liquid medium. Plant Physiol. 9:585–600.

Winter, P. and Kahl, G. (1995). Molecular marker technologies for plant improvement. World Journal of Microbiology and Biotechnology 11(4):438-48.

Wu, T.L. and Kress W.J. (2001). Musaceae. In: Flora of China Vol 24, Pp. 314–318 (Wu C. Y., Raven P.H., eds.); Science Press, Beijing.

Amare Seifu Assefa and Daniel Fitamo. (2016). Diversity of Enset Landraces (*Ensete ventricosum* (Welw) Cheesman) in Aleta Chuko District, Sidama Zone, South Nation Nationality People and Regional State, Ethiopia. Journal of Plant Sciences, 4(1), 1–7.

James, C.S. (1995). Experimental methods in analytical chemistry of foods. New York: Chapman and Hall.

Asmare Dagnew, Surafel Shibru, Abel Debebe, Alemshet Lemma, Lemma Dessalegn, Behailu Berhanu, Worku Beyene and Yulien Miguelez Sierra (2012). Micropropagation of Banana Varieties (*Musa spp.)* using shoot-tip culture. Ethiop. J. Agric. Sci. 22:14-25.

Soelaiman, V. and Ernawati, A. (2013). Growth and De-velopment of *In Vitro* Curly Pepper (Capsicum annuum L) in some Cencentration BAP and IAA. Bulletin Agrohorti. 1(1), 62-66.

Taiz, L. and Zeiger, E. (1991) Plant physiology: Mineral nutrition. The Benjamin Cummings Publishing Co., Inc. Redwood City, 100-119.

Sofian, A.A., Prihastanti, E., and Suedy, S.W.A. (2018). Effect of IBA and BAP on Shoot Growth of Tawangmangu Tangerine (*Citrus reticulate*) by *In vitro*. Biosainti-fika: Journal of Biology and Biology Education, 10(2), 379-387.

Yemane Tsehaye and Facil Kebebew (2006). Diversity and cultural use of enset (Enset ventricosum Welw.) Cheesman) in Bonga in situ conservation site, Ethiopia. Ethnobotany research and applications 4:147–157.

Yeung, E.C., Thorpe, T.A. and Jensen, C.J. (1981). *In vitro* fertilization and embryo culture. In: Plant tissue culture: Methods and applications in agriculture, Pp. 253–271 (Thorpe, T.A., ed.); Academic, New York.

Yildiz, M. (2012). The Prerequisite of the Success in Plant Tissue Culture: High Frequency Shoot Regeneration. Intech Open. https://www.researchgate.net/publication/300239624

Yogesh, B., Yadav, A.S. and Bajaj, A. (2016). Effect of explant sources and different concentrations of plant growth regulators on *in vitro* micropropagation of *Glycyrrhiza glabra* L. Indo American Journal of Pharmaceutical Research 6(06):5830-5840.

Zerihun Yemataw, Hussein M, Mulugeta Diro, Temesgen, A. and Guy, B. (2014a). Enset (*Ensete ventricosum*) Clone Selection by Farmers and their Cultural Practices in Southern Ethiopia. Genetic Resource and Crop Evolution 6:1091–1104.

Zerihun Yemataw, Hussein M., Mulugeta Diro, Temesgen, A. and Guy, B. (2014b). Ethnic based diversity and distribution of enset (*Ensete ventricosum*) clones in Southern Ethiopia. Journal of Ecology and Natural Environment 6:244–251.

Zerihun Yemataw, Kassahun Tesfaye, Awol Zeberga and Blomme G. (2016). Exploiting indigenous knowledge of subsistence farmers for the management and conservation of Enset (*Ensete ventricosum* (Welw.) Cheesman) (*Musaceae* family) diversity on-farm. Journal of Ethnobiology and Ethnomedicine 12(1): 34.

Zerihun Yemataw, Tesfaye K, Grant M, Studholme D.J. and Chala, A. (2018). Multivariate analysis of morphological variation in enset (*Ensete ventricosum* (Welw.) Cheesman) reveals regional and clonal variation in germplasm from south and south western Ethiopia. Australian journal of crop science 12(12):1849-1858.

Zhang, S.B., Zhu, Z., Zhao, L., Zhang, Y.D., Chen, T, Lin, J., Wang, C.L. (2007). Identification of SSR markers closely linked to eui gene in rice. Yi Chuan (Hereditas-Beijing), 29(3): 365-370.

Zhong, J.J. (2001). Biochemical engineering of the production of plant-specific secondary metabolites by cell suspension cultures. Adv Biochem Eng Biotechnol 72:26.

Zhu, C., Gore, M., Buckler, E.S. and Yu, J. (2008) Status and prospects of association mapping in plants. The plant genome 1(1):5-20.

APPENDICES

Appendix Table 1 Questionnaire used for collection of phenotypic data to study the genetic diversity of enset landraces used intraditional medicine

Study area: Zone_____ District_____ Kebele (PA) _____ Village _____

Altitude_____ latitude_____ longitude_____

S.N.	Local name of the landraces /clones/	Pseudo-stem color (PSC)	Color Petiole ventral side (CPVS)	Color Petiole dorsal side (CPDS)	Color Midrib ventral side (CMVS)	Color midrib dorsal side (CMDS)	Color of leaf upper surface (CLUS)	Color leaf edge and tip (CLET)
1								
2								
3								
4								
5								
6								
8								
9								
10								

Appendix Table 1. Cont...

S.N.	Local name of the landraces /clone/	Color leaf edge and tip (CLET)	Leaf growth habit (LGRH)	Corm quality (COQU)	Kocho quality (KOQU)	Bulla quality (BUQU)	Fiber quality (FIQU)	Bacterial wilt tolerance (BWDT)	Drought tolerance (DROT)
1									
2									
3									
4									
5									
6									
8									
9									
10									

Appendix Table 2. List of 40 enset landraces used in traditional medicine included in the diversity study using qualitative traits, and sites of collections

No.	Local name	Site of collection			
		Zone/Special destrict	Districts	Peasant associations	Altitude
1	Astara	Gurage	Cheha & Gumer	Girar, Jemboro & Zizencho	2252-2834
2	Atshakit	Gurage	Gumer	Jemboro & Zizencho	2756-2834
3	Bisha-eset	Gurage	Cheha and Gumer	Girar, Jemboro & Zizencho	2252-2834
4	Chehuyet	Gurage	Gumer	Jemboro & Zizencho	2756-2834
5	Denkinet	Gurage	Cheha and Gumer	Girar, Jemboro & Zizencho	2252-2834
6	Dere	Gurage	Cheha and Gumer	Yefekterek, Girar & Zizencho	1976-2835
7	Guarye	Gurage	Cheha and Gumer	Yefekterek, Girar & Jemboro	1976-2756
8	Kibnar	Gurage	Cheha and Gumer	Yefekterek, Girar & Jemboro	1976-2756
9	Sinwot	Gurage	Cheha	Yefekterek & Girar	1976-2252
10	Terye	Gurage	Gumer	Jemboro & Zizencho	2756-2834
11	Agede	Hadya	Misha and Lemo	Masbera, Lembuda, Hage & Abushira	2324-2520
12	Astar	Hadya	Misha and Lemo	Masbera, Lembuda, & Abushira	2324-2520
13	Haywona	Hadya	Misha and Lemo	Masbera, Lembuda, Hage & Abushira	2324-2520
14	Bedededa	Hadya	Misha	Hage & Abushira	2376-2520
15	Gishra	Hadya	Misha and Lemo	Masbera, Lembuda, Hage & Abushira	2324-2520
16	Kiniwara	Hadya	Misha and Lemo	Hage & Lembuda	2376-2395
17	Kombotra	Hadya	Lemo	Masbera & Lembuda	2324-2395
18	Mekelwes	Hadya	Lemo	Masbera & Lembuda	2324-2395
19	Oniya	Hadya	Lemo	Masbera & Lembuda	2324-2395
20	Tesa	Kembata-Tembaro	Angacha & Doyogena	Bondena, Gerbafendida & Murasa	2356-2558

21	Kiklekey	Kembata-Tembaro	Angacha & Doyogena	Bondena, Gerbafendida &	2416-2558
22	Kiklenech	Kembata-Tembaro	Angacha	Bondena & Gerbafendida	2416-2519
23	Agene	Kembata-Tembaro	Angacha	Bondena & Gerbafendida	2416-2519
24	Sebera	Kembata-Tembaro	Angacha & Doyogena	Bondena, Gerbafendida & Anchasedich	2416-2558
25	Mentiwea	Kembata-Tembaro	Angacha	Bondena & Gerbafendida	2416-2519
26	Cherkiwa	Kembata-Tembaro	Angacha	Bondena & Gerbafendida	2416-2519
27	Oniya2	Kembata-Tembaro	Angacha & Doyogena	Bondena & Murasa	2356-2416
28	Astar2	Kembata-Tembaro	Angacha & Doyogena	Bondena, Gerbafendida & Murasa	2356-2519
29	Beleka	Kembata-Tembaro	Doyogena	An & Murasa	2356-2558
30	Gishra2	Kembata-Tembaro	Angacha & Doyogena	Bondena & Gerbafendida	2356-2519
31	Asu	Yem*	Yem	Gurminahangary	2586
32	Kinkisir	Yem	Yem	Gurminahangary	2586
33	Gariye	Yem	Yem	Gurminahangary	2586
34	Deya	Yem	Yem	Ediya & Gurminahangary	2541-2586
35	Anchiro	Yem	Yem	Ediya	2541
36	Karona	Yem	Yem	Ediya	2541
37	Lochingia	Dawro	Mareka & Tocha	Eyesus, Medhanialem & Gibrakeyma	2361-2702
38	Arke	Dawro	Tocha	Medhanialem & Gibrakeyma	2683-2702
39	Tsela	Dawro	Tocha	Medhanialem & Gibrakeyma	2683-2702
40	Botsamez	Dawro	Mareka & Tocha	Gozabamushe, Eyesus & Medhanialem	2327-2683

Appendix Table 3. Description of traits and trait categories used in phenotypic diversity of enset landraces used in traditional medicine

Qulitative trait	Acro-nym	No of trait categories	Description of trait categories adopted and index (in parenthesis)
Pseudo stem Color	PSSC	14	light to medium green with black spot/strip/patches (1); light to medium green with black and red purple strips/patches (2); Light to medium green mixed with light pink (3); light to medium green mixed with black and with black patches and strip(4); Green yellow with black spots/strips/patches (5); Green yellow with red purple base (6); Green yellow and black with red purple patches and black spot (7); Yellow red with black and red purple patches and spot (8); red purple with green strips/patches (9); Red purple with yellow patches and spots (10); Red purple with black or brown strip/ patches(11); Red purple (12); Dark red purple (13); and Red pink (14)
Color of petiole ventral surface	CPVS	5	Light to medium green (1); Light to medium green with black spot /strip / patches (2); Red purple with light to medium green at both sides (3); Red purple (4); and dark red purple (5)
Color of petiole dorsal surface	CPDS	8	Light to medium green with black spots/strip/patch (1); Light red purple with green side parts and with black patches (2); Red purple with light green at both sides and black spots /patches (3); Red purple with light yellow at both sides and black patches (4); Red purple with black and green strips at both sides (5); Red purple with green strips (6); Dark red purple with green strip (7); and Black with green strip and patches (8)

Color of midrib ventral surface	CMVS	5	Light to medium green (1); Light to medium green with spot (2); Red purple with light to medium green at both sides (3); Red purple (4); and Dark red purple (5)
Color of midrib dorsal surface	CMDS	8	Light to medium green (1); Light to medium green with black spots / strip/patches (2); Light to medium green with red purple strip (3); Light yellow (4); Red purple with light to medium green at both sides (5); Red purple (6); Dark red purple (7); and Red pink (8)
Color of leaf upper surface	CLUS	5	Light to medium green (1); Green to deep green (2); Light green with red purple strip (3); Red purple with green strips (4); and Red pink with green strip (5)
Color of leaf edge and tip	CLET	4	Brown (1); Black brown (2); Red purple (3); and Red pink (4)
Leaf growth habit	LGRH	3	Erect (1); intermediate (2); and drooping (3)
Corm quality	COQU	4	Low (1); intermediate (2); high (3); and other (4)
Kocho quality	KOQU	4	Low (1); intermediate (2); high (3); and other (4)
Bulla quality	BUQU	4	Low (1); intermediate (2); high (3); and other (4)
Fiber quality	FOQU	4	Low (1); intermediate (2); high (3); and other (4)
Bacterial wilt tolerance	BWDT	3	Low (1); intermediate (2); and high (3)
Drought tolerance	DROT	3	Low (1); intermediate (2); and high (3)

Appendix Table 4. Description of plant materials used in genetic diversity study of enset (Ensete ventricosum) using SSR markers

S.N	Landrace Vernacular name	Major use value	Origin of collection					
			Zone/ Special district	District	Peasant association	Altitude	Latitude	Longitude
1	kibnar1	TM*	Gurage	Cheha	YK****	1976	9° 2' 24" N	37° 39' 36" E
2	guarye1	TM*	Gurage	Cheha	YK****	1976	9° 2' 24" N	37° 39' 36" E
3	dere1	TM*	Gurage	Cheha	YK****	1976	9° 2' 24" N	37° 39' 36" E
4	sinwot1	TM*	Gurage	Cheha	YK****	1976	9° 2' 24" N	37° 39' 36" E
5	kibnar2	TM*	Gurage	Cheha	YK****	1976	9° 2' 24" N	37° 39' 36" E
6	astara1	TM*	Gurage	Cheha	Girar	2252	8° 5' 24" N	37° 58' 12" E
7	guarye2	TM*	Gurage	Cheha	Girar	2252	8° 5' 24" N	37° 58' 12" E
8	dere2	TM*	Gurage	Cheha	Girar	2252	8° 5' 24" N	37° 58' 12" E
9	sinwot2	TM*	Gurage	Cheha	Girar	2252	8° 5' 24" N	37° 58' 12" E
10	denkinet1	TM*	Gurage	Cheha	Girar	2252	8° 5' 24" N	37° 58' 12" E
11	bishaeset1	TM*	Gurage	Cheha	Girar	2252	8° 5' 24" N	37° 58' 12" E
12	denkinet2	TM*	Gurage	Cheha	Girar	2252	8° 5' 24" N	37° 58' 12" E
13	astara2	TM*	Gurage	Gumer	Zizencho	2834	7° 57' 36" N	38° 1' 12" E
14	dere3	TM*	Gurage	Gumer	Zizencho	2834	7° 57' 36" N	38° 1' 12" E
15	guarye3	TM*	Gurage	Gumer	Zizencho	2834	7° 57' 36" N	38° 1' 12" E
16	kibnar3	TM*	Gurage	Gumer	Zizencho	2834	7° 57' 36" N	38° 1' 12" E
17	atshakit1		Gurage	Gumer	Jemboro	2756	8° 1' 12" N	38° 2' 24" E
18	chehuyet1	TM*	Gurage	Gumer	Zizencho	2834	7° 57' 36" N	38° 1' 12" E
19	bishaeset2	TM*	Gurage	Gumer	Zizencho	2834	7° 57' 36" N	38° 1' 12" E
20	chehuyet2	TM*	Gurage	Gumer	Jemboro	2756	8° 1' 12" N	38° 2' 24" E

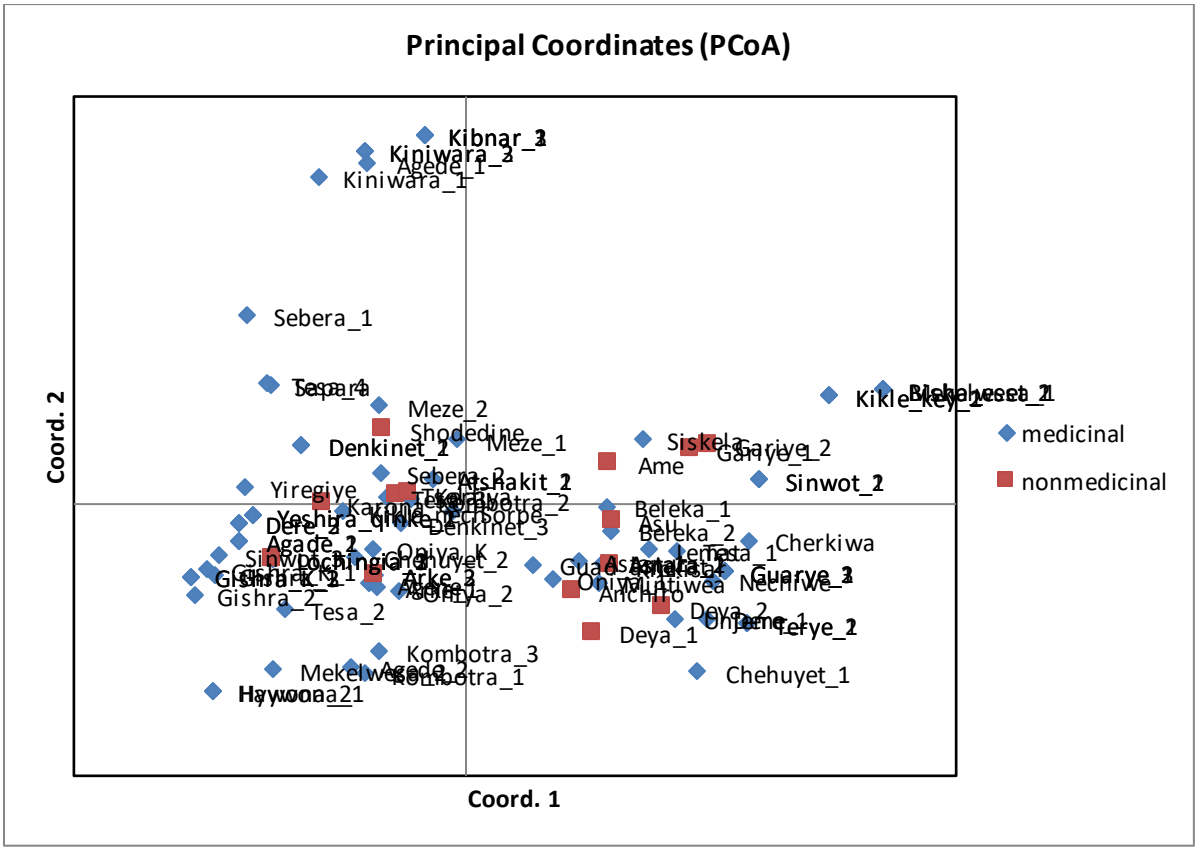
21	sinwot3	TM*	Gurage	Gumer	Jemboro	2756	8° 1' 12" N	38° 2' 24" E
22	denkinet3	TM*	Gurage	Gumer	Jemboro	2756	8° 1' 12" N	38° 2' 24" E
23	atshakit2	TM*	Gurage	Gumer	Jemboro	2756	8° 1' 12" N	38° 2' 24" E
24	terye1	TM*	Gurage	Gumer	Jemboro	2756	8° 1' 12" N	38° 2' 24" E
25	terye2	TM*	Gurage	Gumer	Jemboro	2756	8° 1' 12" N	38° 2' 24" E
26	Guadamerat	FO*	Gurage	Cheha	Girar	2252	8° 5' 24" N	37° 58' 12" E
27	agade1	FO*	Gurage	Cheha	Girar	2252	8° 5' 24" N	37° 58' 12" E
28	Sapara	FO*	Gurage	Cheha	Girar	2252	8° 5' 24" N	37° 58' 12" E
29	Yiregiye	FO*	Gurage	Cheha	Girar	2252	8° 5' 24" N	37° 58' 12" E
30	yeshiraqinke1	FO*	Gurage	Cheha	Girar	2252	8° 5' 24" N	37° 58' 12" E
31	Nechiwe	FO*	Gurage	Cheha	Girar	2252	8° 5' 24" N	37° 58' 12" E
32	Lemat	FO*	Gurage	Cheha	Girar	2252	8° 5' 24" N	37° 58' 12" E
33	agade2	FO*	Gurage	Cheha	Girar	2252	8° 5' 24" N	37° 58' 12" E
34	yeshiraqinqe2	FO*	Gurage	Cheha	Girar	2252	8° 5' 24" N	37° 58' 12" E
35	oniya1	TM*	Hadya	Lemo	Lembuda	2395	7° 37' 12" N	37° 49' 48" E
36	kiniwara1	TM*	Hadya	Lemo	Lembuda	2395	7° 37' 12" N	37° 49' 48" E
37	mekelwesa1	TM*	Hadya	Lemo	Lembuda	2395	7° 37' 12" N	37° 49' 48" E
38	agede1	TM*	Hadya	Lemo	Lembuda	2395	7° 37' 12" N	37° 49' 48" E
39	gishra1	TM*	Hadya	Lemo	Lembuda	2395	7° 37' 12" N	37° 49' 48" E
40	mekelwesa2	TM*	Hadya	Lemo	Masbera	2324	7° 36' 36" N	37° 51' 0" E
41	oniya2	TM*	Hadya	Lemo	Lembuda	2395	7° 37' 12" N	37° 49' 48" E
42	gishra2	TM*	Hadya	Lemo	Lembuda	2395	7° 37' 12" N	37° 49' 48" E
43	haywona1	TM*	Hadya	Lemo	Lembuda	2395	7° 37' 12" N	37° 49' 48" E
44	kiniwara2	TM*	Hadya	Lemo	Lembuda	2395	7° 37' 12" N	37° 49' 48" E

45	kombotra1	TM*	Hadya	Lemo	Lembuda	2395	7° 37' 12" N	37° 49' 48" E
46	agede2	TM*	Hadya	Lemo	Lembuda	2395	7° 37' 12" N	37° 49' 48" E
47	kombotra2	TM*	Hadya	Lemo	Lembuda	2395	7° 37' 12" N	37° 49' 48" E
48	kiniwara3	TM*	Hadya	Misha	Abushira	2520	7° 39' 36" N	37° 50' 24" E
49	astar1	TM*	Hadya	Misha	Abushira	2520	7° 39' 36" N	37° 50' 24" E
50	hywona2	TM*	Hadya	Misha	Abushira	2520	7° 39' 36" N	37° 50' 24" E
51	kombotra3	TM*	Hadya	Lemo	Lembuda	2395	7° 37' 12" N	37° 49' 48" E
52	gishra_k1	TM*	KT***	Angacha	Gerbafandida	2519	7° 18' 36" N	37° 49' 12" E
53	tesa1	TM*	KT***	Angacha	Gerbafandida	2519	7° 18' 36" N	37° 49' 12" E
54	Kiklenech	TM*	KT***	Angacha	Gerbafandida	2519	7° 18' 36" N	37° 49' 12" E
55	cherkiwa	TM*	KT***	Angacha	Bondena	2416	7° 19' 12" N	37° 47' 60" E
56	tesa2	TM*	KT***	Angacha	Bondena	2416	7° 19' 12" N	37° 47' 60" E
57	kiklekey1	TM*	KT***	Angacha	Bondena	2416	7° 19' 12" N	37° 47' 60" E
58	gishra_k2	TM*	KT***	Angacha	Bondena	2416	7° 19' 12" N	37° 47' 60" E
59	sebera1	TM*	KT***	Angacha	Bondena	2416	7° 19' 12" N	37° 47' 60" E
60	agene	TM*	KT***	Angacha	Bondena	2416	7° 19' 12" N	37° 47' 60" E
61	Mintiwea	TM*	KT***	Angacha	Bondena	2416	7° 19' 12" N	37° 47' 60" E
62	oniya_k	TM*	KT***	Angacha	Bondena	2416	7° 19' 12" N	37° 47' 60" E
63	gishra_k3	TM*	KT***	Doyogena	Murasa	2356	7° 19' 12" N	37° 47' 60" E
64	beleka1	TM*	KT***	Doyogena	Anchasedicho	2558	7° 20' 24" N	37° 47' 24" E
65	beleka2	TM*	KT***	Doyogena	Anchasedicho	2558	7° 20' 24" N	37° 47' 24" E
66	tesa3	TM*	KT***	Doyogena	Anchasedicho	2558	7° 20' 24" N	37° 47' 24" E
67	tesa4	TM*	KT***	Doyogena	Anchasedicho	2558	7° 20' 24" N	37° 47' 24" E
68	kiklekey2	TM*	KT***	Doyogena	Anchasedicho	2558	7° 20' 24" N	37° 47' 24" E
69	sebera2	TM*	KT***	Doyogena	Anchasedicho	2558	7° 20' 24" N	37° 47' 24" E

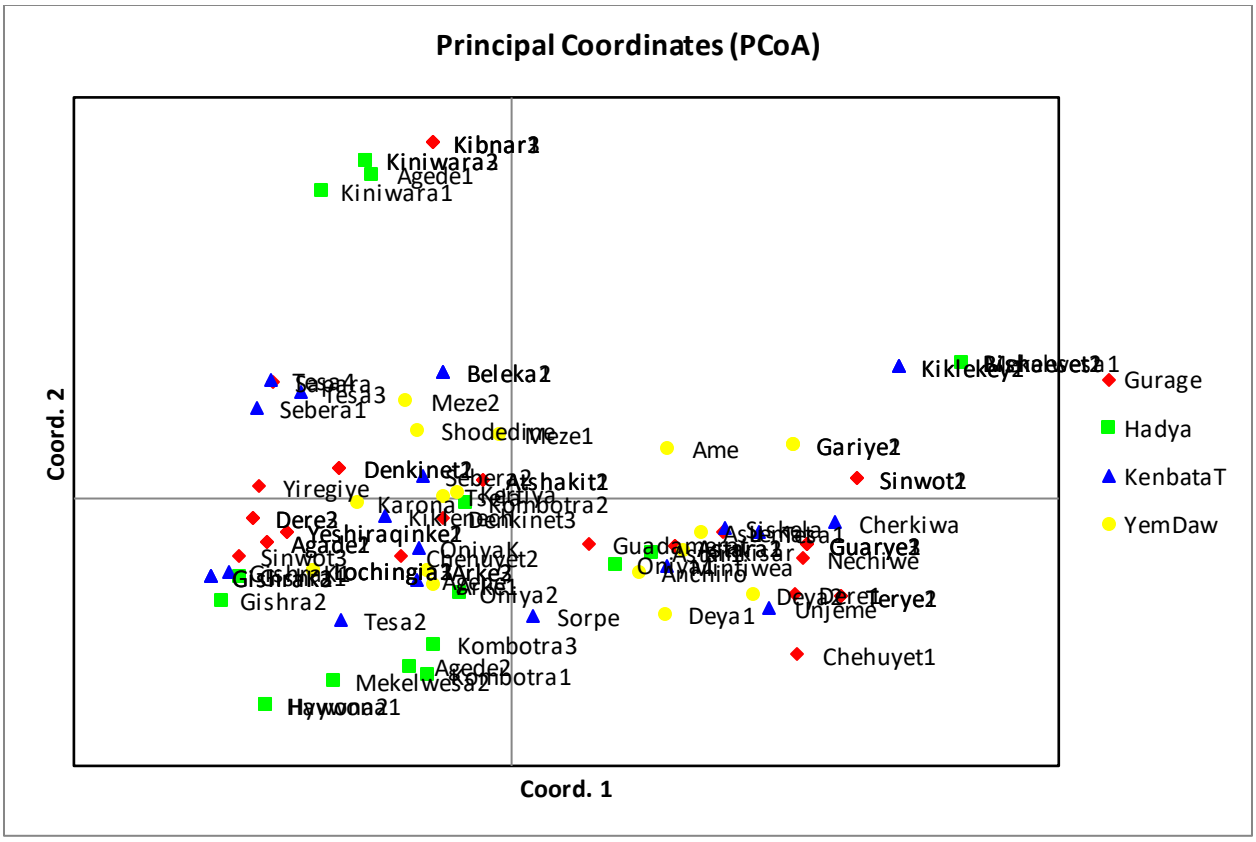
70	Unjeme	FO*	KT***	Doyogena	Murasa	2356	7° 19' 12" N	37° 47' 60" E
71	Sorpe	FO*	KT***	Angacha	Bondena	2416	7° 19' 12" N	37° 47' 60" E
72	Siskela	FO*	KT***	Angacha	Bondena	2416	7° 19' 12" N	37° 47' 60" E
73	arke1	TM*	Dawro	Tocha	Medahnialem	2683	7° 5' 24" N	37° 2' 24" E
74	arke2	TM*	Dawro	Tocha	Medahnialem	2683	7° 5' 24" N	37° 2' 24" E
75	lochingia1	TM*	Dawro	Tocha	Medahnialem	2683	7° 5' 24" N	37° 2' 24" E
76	meze1	TM*	Dawro	Tocha	Medahnialem	2683	7° 5' 24" N	37° 2' 24" E
77	meze2	TM*	Dawro	Mareka	Gozabamushe	2327	7° 3' 36" N	37° 11' 24" E
78	lochingia2	TM*	Dawro	Mareka	Eyesus	2361	7° 3' 36" N	37° 9' 36" E
79	arke3	TM*	Dawro	Tocha	Gibrakeyma	2702	7° 7' 12" N	37° 1' 12" E
80	lochingia3	TM*	Dawro	Tocha	Gibrakeyma	2702	7° 7' 12" N	37° 1' 12" E
81	Tsela	TM*	Dawro	Tocha	Gibrakeyma	2702	7° 7' 12" N	37° 1' 12" E
82	kinkisar	TM*	Yem	Yem	Gurmina-hangeri	2586	7° 52' 12" N	37° 31' 48" E
83	asu	TM*	Yem	Yem	Gurmina-hangeri	2586	7° 52' 12" N	37° 31' 48" E
84	gariye1	TM*	Yem	Yem	Gurmina-hangeri	2586	7° 52' 12" N	37° 31' 48" E
85	gariye2	TM*	Yem	Yem	Gurmina-hangeri	2586	7° 52' 12" N	37° 31' 48" E
86	deya1	TM*	Yem	Yem	Gurmina-hangeri	2586	7° 52' 12" N	37° 31' 48" E
87	anchiro	TM*	Yem	Yem	Ediya	2541	7° 52' 48" N	37° 31' 48" E
88	deya2	TM*	Yem	Yem	Ediya	2541	7° 52' 48" N	37° 31' 48" E
89	karona	TM*	Yem	Yem	Ediya	2541	7° 52' 48" N	37° 31' 48" E
90	Shodedine	FO**	Dawro	Mareka	Eyesus	2361	7° 3' 36" N	37° 9' 36" E
91	Kertiya	FO**	Dawro	Mareka	Eyesus	2361	7° 3' 36" N	37° 9' 36" E
92	Ame	FO**	Dawro	Mareka	Eyesus	2361	7° 3' 36" N	37° 9' 36" E

*= traditional medicine, ** food and other, KT***= Kembata-Tembaro, and YK****=Yefekterek-Wedro

Appendix Figure 1.Focus group discussion with key informants during phenotypic data collection at SNNP region; a) Dawro Zone, b) Kembata-Tembaro, c) Yem special district



Appendix Figure 2. Principal coordinate analysis (PCoA) bi-plot showing the clustering of 92 enset landraces based on 15 microsatellite data, labeled based on their use value



Appendix Figure 3. Appendix figure 3. Principal coordinate analysis (PCoA) bi-plot showing the clustering of 92 enset landraces based on 15 microsatellite data, labeled based on their origin of location