ADDIS ABABA UNIVERSITY

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCE

DEPARTMENT OF MICROBIAL, CELLULAR AND MOLECULAR BIOLOGY

Ethno-botany, Genetic Diversity, Micro-propagation and Nutritional Profiling of Enset [*Ensete ventricosum* (Welw.) Cheesman] Landraces from Central Ethiopia

By

Tesfaye Dilebo Jabir

Addis Ababa, Ethiopia

June, 2024

Ethno-botany, Genetic Diversity, Micro-propagation and Nutritional Profiling of Enset [*Ensete ventricosum* (Welw.) Cheesman] Landraces from Central Ethiopia

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

By

Tesfaye Dilebo Jabir

DECLARATION

I declare and confirm that this dissertation submitted by me herewith for the degree of Doctor of Philosophy (Ph.D) is my own original work and has never been submitted before, either by me or anyone else, to another institution regarding the granting of any type of certificate, diploma, or degree in academics. The sources of all the materials utilized in the dissertation have been properly credited.

Name: Tesfaye Dilebo Jabir Signature:…… Date:……

DEDICATION

I dedicate this dissertation to my parents, my wife, and my children for their continuous encouragement and for being so patient with me during my post-graduate journey.

ACKNOWLEDGMENTS

First of all, I am greatly indebted to convey my deepest appreciation to my main supervisor, Prof. Tileye Feyissa, for his valuable suggestions and guidance, encouragement, friendly and brotherly approach, and assistance during the entire process of conducting the research and the final write-up of this dissertation. He has also offered me the freedom to include new topics of my interest.

I am deeply thankful to my co-supervisor, Prof. Zemede Asfaw, for his professional guidance, follow-up, critical reading and suggestions, immediate and insightful feedback, and support as this dissertation takes on its final form. I also want to express my gratitude to my other co-supervisor, Dr. Ashagire Zewdu, for his constructive ideas, advice, and positive interaction.

I express my deepest gratitude to Prof. Kassahun Tesfaye for allowing me to plant genetic research labroatory to conduct some parts of molecular research activities. I would like to thank the academic and administrative staff of the Addis Ababa University Department of Microbial, Cellular, and Molecular Biology, particularly (Applied Genetics group) Prof. Gurja Belay, Dr. Dereje Beyene, Dr. Adey Feleke, and Dr. Helen Nigusse for their thoughtful support and for allowing me the chance to work on this crop for my Ph.D and financial support for my research and studies. I also want to express my appreciation to Dr. Muluken Birara, Dr. Tilahun Mekonen, Dr. Tilahun Hailu, Dr. Tsegaye Getahun, Mr. Temesgen Dagne, and Mr. Yosef Yambo (a Ph.D candidate) for their assistance and support a lot during my molecular laboratory work. Furthermore, I would like to thank Dr. Fekadu Gadissa for his support during molecular data analysis. I am grateful to Dr. Eshetu Fantau, Dr. Samson Tilahun, Mr. Ayele Guche, and Mr. Agegnew Wase for sharing their knowledge and supporting me for the completion of this study.

Many other friends and colleagues have also been involved in accomplishing this study, either directly or indirectly. I sincerely thank all of them for being such an important part of complting my Ph.D study, since it would not have been possible without their support.

My warmest thanks and appreciation also go to the authority of Hossana College of Education for giving me the chance to attend this Ph.D study, all other staff members of the College, and staff members of the Biology Departments for their motivation and encouragement. In particular, I deeply appreciate Dr. Tsedeke Abate, Dr. Ageze Abza, and Mr. Teke Moche for their ideas and support, and guidinance through my study. I would like to thank the Center of Food Science and Nutrition, the Department of Chemistry at Addis Ababa University, and the Ethiopian Public Health Institute for their support during laboratory analysis. I also thank and greatly appreciate the Areka Agricultural Research Center, the zonal and *woreda* agricultural office experts, agricultural extension workers, and enset-producing farmers for their kind support during sample collection and willingness to share all necessary information and knowledge on the different aspects of the enset crop.

I would like to deeply thank my father, Ato Dilebo Jabir, and my mother, W/o Lendo Binchamo, for the costs they endured to ensure my success and endeavors. I appreciate and thank my brothers, Jemal, Hassen, Mohammed, Kedir, and my sister, Kedija Dilebo, for their continuous support and for playing crucial roles in progressing me to this stage.

Finally, my deepest gratitude goes to my wife, Seada Sadik, and my children, Selima, Ekram, Firdous, and Emiran Tesfaye, for their patience and for providing continuous moral support and being with me throughout the entire study period.

Nothing is insurmountable for the Almighty God!

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF PUBLICATIONS

This dissertation is based on the work contained in the following, published papers as indicated below:

I. Tesfaye Dilebo, Tileye Feyissa, Zemede Asfaw and Ashagire Zewdu (2023). On-farm diversity, use pattern, and conservation of enset (*Ensete ventricosum*) genetic resources in southern Ethiopia. (***Journal of Ethnobiology and Ethnomedicine*, 19(1): 2**. https://doi.org /10.1186 /s13002-022-00569-x.)

II. Tesfaye Dilebo, Tileye Feyissa, Zemede Asfaw (2023). Farmers' local knowledge on classification, utilization, and on‑farm management of enset (*Ensete ventricosum* [Welw.] Cheesman) landraces diversity in Hadiya, southern Ethiopia. (***Genetic Resources and Crop Evolution*, 70: 1-29**. https://doi.org/10.1007/s10722-023-01714-5.)

III. Tesfaye Dilebo, Tileye Feyissa, Zemede Asfaw, Fekadu Gadissa (2024). Exploring the extents of genetic diversity and population structure of enset [*Ensete ventricosum* (Welw.) Cheesman] from southern Ethiopia using simple sequence repeat markers: Implications for Crop Improvement and Conservation. (***Ecological Genetics and Genomics*, 30**: **100218.** https://doi.org/10.1016/j.egg.2024.100218.)

IV. Tesfaye Dilebo, Tileye Feyissa, Zemede Asfaw (2023). *In vitro* propagation of multi-use enset [*Ensete ventricosum* (Welw.) Cheesman] landraces using *bulla* as gelling agents. (***Plant Cell, Tissue and Organ Culture (PCTOC)*, 155: 693-708**. https://doi.org /10.1007 /s11240-023-02590-8.)

V. Tesfaye Dilebo, Tileye Feyissa, Zemede Asfaw, Ashagire Zewdu (2023). Analysis of proximate composition, mineral contents, and anti-nutritional factors of enset (*Ensete ventricosum*) landraces commonly used for *amicho* preparation in Hadiya Zone, Southern Ethiopia: Implications for food security and mineral bioavailability. **(*Journal of Agriculture and Food Research*, 14: 100771**. https://doi.org/10.1016 /j.jafr.2023.100771.)

LIST OF ACRONYMS AND ABBREVIATIONS

AARC	Areka Agricultural Research Center
AC	Activated charcoal
AFLP	Amplified fragment length polymorphism
AMOVA	Analysis of molecular variance
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
BAP	6-Benzylaminopurine
CRD	Completely randomized design
CTAB	Cetyltrimethylammonium bromide
CV	Coefficient of variation
DMRT	Duncan's multiple range test
FAO	Food and Agricultural Organization
Fst	Genetic differentiation among populations
H'	Shannon Weaver diversity Index
He	Expected heterozygosity
Ho	Observed heterozygosity
I	Shanon information index
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
ISSR	Inter simple sequence repeat
MAS	Marker-assisted selection
MOA	Ministry of Agriculture
Na	Number of alleles
NAA	α-Naphthalene acetic acid
NaOCl	Sodium hypochlorite
Ne	Number of effective alleles
PCoA	Principal coordinate analysis
PGR	Plant growth regulator
PIC	Polymorphic information content
RAPD	Random amplified Polymorphic DNA
SAS	Statistical analysis system
SNNPR	Southern Nation Nationalities Peoples Region
SNPs	Single Nucleotide polymorphisms
SPSS	Statistical Package for Social Science
SSR	Simple sequence repeats
UPGMA	Unweighted pair-group method with arithmetic mean

ABSTRACT

Enset [Ensete ventricosum (Welw.) Cheesman] is a perennial, multipurpose crop that serves as a staple or co-staple food for about 25 million people in Ethiopia. Despite its significant importance and the existing knowledge about diverse enset landraces and their external morphology and complex internal anatomical features, there are limited documented studies on this crop. Furthermore, the ethnobotany of ethnolinguistic communities in the country, culturally linked to the use and management of enset, is a complex and under-researched area. Therefore,this study was focused on the wealth of farmers' indigenous knowledge on farm-level enset diversity, distribution, and selection patterns, the local landrace identification criteria, and nomenclatural system with the evaluation of the extent of genetic diversity and population structure, further developing an efficient micro-propagation protocol and identifying the nutritional and anti-nutritional contents of selected landraces. The study and sample collections were performed in the Hadiya, Kembata, Gurage, and Silte administrative zones of Ethiopia. A total of 240 enset farmers were surveyed using semi-structured interviews for ethnobotanical research and documentation of indigenous knowledge. The Shannon-Weaver, Simpson, Pielou, and Sorenson's similarity indices were also used to evaluate the diversity and similarity of the enset landraces. To evaluate the extent of genetic diversity and population structure, a total of 147 individual leaf samples were collected from the Hadiya, Kembata, Gurage, and Silte zones and the Areka Agricultural Research Center, and the analysis was computed by using 12 simple sequence repeat (SSR) markers. For the in vitro propagation approach, about 1.0 cm long shoot tips were cultured on MS medium gelled with 5%–10% bulla and supplemented with 1–6 mg/l BAP, either separately or in combination with IAA. Regarding the proximate, the Association of Official Analytical Chemists (AOAC) standard methods were applied. Minerals, phytate, tannin and Oxalate contents were determined using the different models of the spectrophotometer methods and the standard procedures. A total of 282 farmer-named enset landraces have been identified, ranging from 2 to 32 on individual homegardens. The Hadiya Zone had the highest number of enset landraces (86), while the Silte Zone had the lowest number (57). The results of the Shannon diversity index and Simpson's 1-D diversity index indicated the presence of high enset diversity in the study zones. The Sorenson's similarity index ranged from 0.24 to 0.73, sharing 16–47 landraces in common. Of the 282 landraces, 210 (74.5%) were recorded in more than one zone, whereas 72 (25.5%) had a narrow distribution being recorded in a single zone. The local names of some enset landraces indicate their uniqueness in morphological traits, place of origin, agronomic features, and quality attributes of their end products. The 12 SSR markers result shows a total of 289 alleles, ranging from 12 to 41 alleles per locus. The polymorphism information content (PIC) for each locus varied from 0.86 to 0.95. The number of effective alleles ranged from 5.13 to 11.79. The expected and observed heterozygosity showed average values of 0.85 and 0.84, respectively. Among the six populations, the wild-growing population had the highest percentage of polymorphic loci (100%). AMOVA attributed 89% of the genetic variation to intra-population and only 11% to among populations. The UPGMA and principal coordinates indicate three major groups. The 8% (w/v) of enset bulla was ideal and provided significant figures in the number and length of shoots and roots per shoot when compared with 0.6% (w/v) agar-gelled MS media. MS medium containing 2.0–3.0 mg/l BAP was the appropriate concentration for in vitro shoot induction and growth. The 4.0 mg/l BAP alone and 5.0 mg/l BAP in combination with 1.0 mg/l IAA were suitable for multiple shoot induction, whereas 2.0 mg/l IBA and 1.0 mg/NAA separately were found to be the optimum concentrations

for root induction and development. The proximate composition (%) ranged in moisture content from 68.2–79.4, crude protein (2.43–11.90), crude fat (0.61–0.89), crude fiber (2.42–4.11), and total ash (2.01–4.60), while the total carbohydrates came to 80.89–89.92, and gross energy was 369.96–385.12 kcal/100 g. The mineral concentrations (mg/100 g) ranged from 22.46–49.74 for calcium, 28.51–86.56 for potassium, and lower for magnesium, phosphorus, sodium, iron, and zinc on a dry weight basis. The anti-nutritional contents (mg/100 g) for phytate, tannin, and oxalate ranged from 221.75–276.12, 27.97–113.74, and 5.69–9.10, respectively. Except for phytate×calcium to zinc, and oxalate to calcium, the molar ratios were above the standard values. Overall, the information gained from this study would be useful for improving programs and conservation strategies for the enset crop, which enhances Ethiopia's sustained food security.

Key words: Corm, Enset ethnobotany, Gene flow, Local taxonomic system, Micro-propagation, Nutritional profile, Polymorphism, Solidifying agent

Chapter One
General Introduction

1.1 Background

Enset (*Ensete ventricosum*) is a staple or co-staple food security crop for over 25 million Ethiopians (Borrell *et al*., 2020). Ethiopia is one of the eight agricultural gene centers (Vavilov, 1951; Harlan, 1969) and the primary center of origin and diversity for many plant species, including enset (Harlan, 1969; Purseglove, 1976; Engels and Hawkes, 1991). The enset crop domestication, perhaps as far back as 10,000 years ago (Brandt, 1996) but its cultivation and utilization as human food are restricted to Ethiopia (Brandt *et al*., 1997). In addition to human food, it is employed for many other purposes, such as in traditional medicine, animal feed, fiber production, and construction materials (Almaz Negash, 2001).

Enset is a crop with a wide range of altitudinal adaptability and agroecological distribution (Abate Senbeta *et al*., 2022; Chase *et al*., 2022). Nonetheless, it grows opulently between 2000 and 2750 meters above sea level (Admasu Tsegaye and Struik, 2002). The plant needs a mean temperature of 16–20°C and 1100–1500 mm of rainfall annually for optimal growth (Tesfaye Abebe *et al.*, 2005). The optimal soils have 2–3% organic matter and are mildly acidic to alkaline (pH = 5.6–7.3) (Uloro *et al*., 1996). Enset can be harvested at any time of the year, from 3 to 12 years; however, it is often done 4–6 years after transplantation (Spring *et al*., 1996; Admasu Tsegaye and Struik, 2002; Borrell *et al*., 2020). Enset requires considerably lower inputs and management than cereals and provides the highest materials/products per hectare (Pijls *et al*., 1995; Taye Bezuneh and Asrat Feleke, 1996; Bewuketu Haile *et al*., 2022). Thus it offers farmers' and their families' food security. This was made clear by the severe famine that devastated Ethiopia between 1888 and 1892 and the 1980s (Pankhurst, 1996; Genet Birmeta *et*

al., 2004), though it is obvious that most of the edible portion is derived from seedlings planted during previous years, harvesting the mature enset from the same farm stretches over several years, giving not only a staggered harvesting option but also the possibility for many years of storage of *qocho* in underground pits. Which is the reason why enset is known as "The Tree Against Hunger" (Brandt *et al*., 1997). The edible portions of the enset are the corm (underground stem) and pseudostem after being pulverized and fermented into *kocho* (*qocho)* and *bulla*, and fresh or unfermented *amicho* (cooked corm) foods (Tadessa Daba and Shigeta, 2016). *Kocho* keeps well in storage for extended periods (Brandt *et al*., 1997; Abraham Bosha, 2018). The corm of the enset is usually harvested at the young stages of the crop, and it can be prepared and consumed similarly to other roots and tubers (Admasu Tsegaye and Struik, 2002; Mohammed *et al*., 2013; Tesfaye Dilebo *et al*., 2023b).

Farmers' practices are thought to have a major impact on shaping the genetic structure and evolutionary history of crops in traditional farming systems such as the enset-planting complex (Bewuketu Haile *et al*., 2022), and they have maintained the diversity of enset landraces for many generations with little outside influences on their homegardens, almost purely based on indigenous knowledge and skills. Some previous studies have shown that Ethiopia is home to a vast diversity of enset landraces (Spring *et al*., 1996; Almaz Negash, 2001; Admasu Tsegaye and Struik, 2002; Genet Birmeta *et al*., 2004; Awol Zeberga *et al*., 2014; Zerihun Yemataw *et al*., 2016). Farmers conserve enset diversity on-farm by sharing, exchanging, and purchasing suckers for cultivation, thereby continuing to diversify it (Tesfaye Dilebo *et al*., 2023 a, b). According to Maxted *et al*. (2002), on-farm conservation practice has been described as a one of the reliable approach for maintaining crop genetic resources. Thus, on-farm conservation balances *ex-situ* preservation, and it supports and encourages continuing crop evolution while sustaining ongoing

indigenous knowledge schemes related to genetic resources (Parra *et al*., 2010). Moreover, on-farm conservation allows the sustainable preservation of landraces to grow under natural circumstances (Bewuketu Haile *et al*., 2022).

Farmers have developed a plethora of indigenous knowledge about enset production in their communities through informal experiments and the accumulation of experience over generations (Temesgen Olango *et al*., 2014; Tesfaye Dilebo *et al*., 2023b). The enset-based farming approach is an indigenous and sustainable farming system in Ethiopia, defined by the production of enset as the primary staple or as a co-staple with other crops (Westphal, 1975), and more than 45 ethnic groups in Ethiopia, practice and use it for food and other household purposes (Brandt *et al*., 1997; Almaz Negash, 2001; Zemede Asfaw, 2018). Thus, for these communities, enset is valued not only as a source of food, feed, and cash income but also considered as part of the people's social, and cultural heritages and symbolizes nature's benefits to communities that care for it (Shigeta, 1992; Bizuayehu Tesfaye and Ludder, 2003; Temesgen Olango *et al*., 2014; Zerihun Yemataw *et al*., 2016; Bewuketu Haile *et al*., 2022; Tesfaye Dilebo *et al*., 2023b) and it is a unifying crop (Kefale Alemu and Sanford, 1996).

Various prior studies have indicated that many enset landraces are cultivated in the homegardens of different enset growing areas (Almaz Negash, 2001; Admasu Tsegaye and Struik, 2002; Zerihun Yemataw *et al*., 2014). Since no single landrace can satisfy every requirement (Admasu Tsegaye, 2002), local farmers are able to identify their landraces with clarity based on physical and internal features as well as use values. Thus, each enset landrace has its own local name that is evenly distributed throughout the area where the same language is spoken (Bizuayehu Tesfaye, 2008). The local names for plants and animals are the source of indigenous biodiversity knowledge, which also helps to distinguish between different plant species (Nedelcheva and

Dogan, 2009). It is widely accepted that for millennia, traditional farmers have used their indigenous knowledge to classify, name, and group their crop kinds in order to preserve the genetic variety of those crops on the farm (Bizuayehu Tesfaye, 2008; Temesgen Olango *et al*., 2014; Bewuketu Haile *et al*., 2022).

Farmers who cultivate enset have preserved and enhanced the crop's diversity by employing their accumulated indigenous knowledge through identification and selection (Almaz Negash, 2001; Olango *et al*., 2014), and according to them, the use of enset diversity is closely related to the several ways of generating its end products that are applied for both food and non-food purposes (Zerihun Yemataw *et al*., 2016). This implies that farmers are the principal conservationists of the enset diversity. Enset exhibits discernible differences in its morphological, agronomic, and use values (Almaz Negash, 2001; Admasu Tsegaye, 2002; Bizuayehu Tesfaye and Ludder, 2003; Temesgen Olango *et al*., 2014; Bewuketu Haile *et al*., 2022; Tesfaye Dilebo *et al*., 2023b). Enset landraces have usually been described phenotypically; however, the expense, time, and area needed to conduct visual evaluations and measurements restrict the scope of phenotypic description (Hinze *et al*., 2017). Furthermore, these characteristics could be altered as a result of ecological factors (Nkhata *et al*., 2020). Given this, their usage as markers is insufficient for identifying the diversity that already exists in the enset landraces. The study of molecular markers is a helpful method for examining the evolutionary connections and genetic diversity within and among enset landraces. Molecular markers are present throughout the entire genome and are independent of plant age or parts, the environment, or management techniques. SSRs are one of the locus-specific molecular markers that reveal variations in PCR product length from different genotypes within or between individuals in a population (Amiteye, 2021). This makes them ideal for crops like enset (Temesgen Olango *et al*., 2015; Fetta Gerura *et al*., 2019).

Concerns about food security systems and other issues must be addressed by conserving plant diversity and using it responsibly (Chappell and LaValle, 2011; Delfini *et al*., 2021). According to Gupta *et al.* (2016), the protection of plant diversity is crucial for safeguarding crops in the future since biotic and abiotic stressors are becoming more and more problematic. Within this framework, *in vitro* propagation methods facilitate the improvement of multiple aspects pertaining to plant development and productivity, which can subsequently be applied to *ex-situ* preservation (Lavanya *et al*., 2014). There are several advantages to using tissue culture technology in comparison to conventional plant propagation techniques (Chandran *et al*., 2020) since several pathogen-free plantlets can be produced from tiny plant fragments in a short period throughout the year (Mattick, 2018). Furthermore, the tissue culture approach can be applied to maintaining plants in their vegetative state (Lambardi *et al*., 2008), like enset, which is a livelihood security crop (Brandt *et al*., 1997).

There are areas of Ethiopia that experience droughts and other natural disasters in different years, which contributes to a growing number of undernourished people and food insecurity (Borrell *et al.*, 2019). Nonetheless, the area where enset is the primary crop is characterized by a low frequency of droughts and a high population density per hectare (Brandt *et al*., 1997; Borrell *et al*., 2019). Enset food products are rich in carbohydrates and mineral sources (Pijls *et al*., 1995; Abraham Bosha *et al*., 2016; Tadessa Daba and Shigeta, 2016); however, their nutrient contents differ among the landraces (Mohammed *et al*., 2013; Tadessa Daba and Shigeta, 2016).

1.2 Some of the existing research gaps in enset crop

It is obvious that farmers cultivate enset plants mainly as staple or co-staple crops; thus, it ensures food security for many millions of people, particularly those inhabiting the central,

south, and southwest parts of Ethiopia. Also, it serves several virtues, which include animal fodder, traditional medicine for humans and cattle, socio-economic importance, and cultural values. Enset diversity offers food security and resilience in the face of unfavorable environmental circumstances. The key actors in conserving its genetic resources are small-scale farmers as an important part of their cultural heritages, through their indigenous knowledge and accumulated experiences over the years and many generations.

Although enset is a multi-use crop for local farmers in the major areas where enset is cultivated, inadequate research with coverage has been employed to enhance its cultivation and production and also to conserve its genetic resources. The major early research interventions focused on its agronomy, the general importance of some unspecified landraces, and the listing of landraces from limited areas or numbers. Therefore, knowledge and research gaps that need to be addressed for sustainable utilization and the continued maintenance of its genetic resources include the following:

1. Understanding the sociocultural values and ethnobotanical knowledge of enset cultivating communities, along with different ethnolinguistics, is crucial to recognizing the farmers' traditional practices on selection criteria, on-farm maintaining practices, and different patterns of uses of enset landrace diversity in different communities,

2. The different attributes and relevant information on indigenous knowledge of enset are still not fully understood and examined by researchers. Since enset is a crop that has coexisted with the community for millennia and has been used historically, as well as the accumulation of well-established ethnobotanical knowledge, related attributes need further investigation.

3. Considering the extent of genetic diversity of the existing enset is vital to select the desirable traits, along with resistance or tolerance to environmental and abiotic stresses through the improvement program to increase food security. Thus, collecting, identifying, and screening available enset populations through applying different molecular markers are essential to establish the groundwork for the enset crop's eventual enhancement and long-term preservation.

4. Nowadays, enset farmers face some challenges due to some biotic stress; therefore, an appropriate biotechnological approach (e.g., tissue culture technology and the application of a mechanized production system) is needed to obtain a healthy planting material that helps as a backup approach for maintaining the present diversity of enset germplasm and to alleviate the hardships faced during harvesting and processing.

5. Enset landraces are more diverse in nature and use patterns; thus, it is imperative to pursue adequate information on the nutritional and other chemical constituents of their fermented and unfermented products for nutritive and sustainable consumption.

1.3 Statements of the Problem

Given that enset could substantially enhance food security for the people who lived mainly in the central, south, and southwest parts (regions) of Ethiopia, and considering the possibility of extending production and consumption to other regions of the country, it has attracted more attention recently. The enset-based farming method appears to be becoming more and more understood; though it still has a lot of challenges. The main challenges originate from a lack of understanding regarding the diverse indigenous farming practices, on-farm diversity, crop pathogens, and the crop's long growth cycle. Hence, understanding the sociocultural aspects, ethnobotanical knowledge, farmers' selection criteria, and preservation practices of enset

landrace diversity in various ethnolinguistic communities is essential for the sustainable utilization and on-farm conservation of its genetic resources as well as future crop improvement. Moreover, an appropriate backup strategy of enset germplasm and searching for the nutritional profile of such diverse and multipurpose landraces is therefore essential for local and regional consumption, as it will support both food security and the long-term use of enset.

1.4 Research questions

In this dissertation, the following key research questions were used to investigate the enset crop's landrace diversity, indigenous knowledge, genetic diversity, propagation, and nutritional aspects:

1. What is the extent of the enset landraces diversity and its distribution on-farm (homegardens), and what are the farmers' specific preferences criteria?

2. How do farmers characterize their enset landraces using their indigenous knowledge for identification, naming, classification, and other requirements?

3. What is the level of genetic diversity of enset landraces within and among growing zones or regions in southern Ethiopia?

4. Can an efficient micro-propagation protocol be developed by using agar and *bulla* as gelling agents separately for enset landraces?

5. What degree of proximate, minerals, anti-nutritional compositions, and molar ratios are found in cultivated enset landraces?

1.5 Objectives of the Study

The general objective of this study was to investigate the genetic diversity, micro-propagation, and nutritional content of enset landraces. The specific objectives of this study were:

- To investigate and document farmers' ecological knowledge, tradition, and practices regarding the diversity and distribution of enset landraces at the farm level;
- To search for and document indigenous knowledge of farmers' in enset-related ethnobotanical lore and the traditional practices relevant to the maintenance of on-farm enset diversity;
- To evaluate the level of genetic diversity and population structure of enset by using SSR molecular markers;
- To develop and optimize an efficient protocol for the shoot tips of selected enset landraces by using *bulla* and agar-gelled media;
- To identify and compare the proximate composition, mineral content, anti-nutritional factors, mineral bioavailability, and physicochemical properties of some widely consumed and locally favored corms of enset landraces.

1.6 The structure of the dissertation

Overall, this dissertation is arranged into eight chapters as follows: Chapter One deals with the general introduction parts, which contain the background of the study, a statement of the problem and the objectives of the dissertation. Chapter Two offers a review of the literature on enset origin, taxonomy, distribution, botanical description, in situ/on-farm diversity, farmers' indigenous knowledge, genetic diversity, micro-propagation, and nutritional contents of enset. Chapter Three contains a brief description of the on-farm diversity, use pattern, and conservation

of enset (*Ensete ventricosum*) genetic resources from Hadiya, Kembata, Gurage, and Silte Zones of Ethiopia. Chapter Four deals with the farmers' local knowledge of enset cultivation, the local landrace taxonomic system (identification, naming, categorization, or classification), and the traditional use and management of the landraces. This is linked to generating reliable data focused on farmers' enset-related ethnobotanical lore and the traditional practices relevant to the maintenance of on-farm enset landrace diversity in the Hadiya Zone of southern Ethiopia. Chapter five presents the studies on the genetic diversity, differentiation, and population structure of cultivated landraces, released, and wild-growing types and cultivated landraces of the enset species from the four (Hadiya, Kembata, Gurage, and Silte) administrative zones and the Areka Agricultural Research Center using simple sequence repeats (SSRs). Chapter six provides the protocol for an *in vitro* propagation approach using enset *bulla* and agar separately as gelling agents with different concentrations of cytokinin and auxin for the shoot tips of farmers' selected enset landraces. Chapter seven gives a brief analysis of the proximate composition, mineral content, anti-nutritional factors, mineral bioavailability, and physicochemical properties of the widely consumed and locally favored corms of enset landraces, representing the sweet, moderate, and bitter but traditionally medicinal corm types of the cultivated enset landraces. The dissertation concludes with Chapter eight, which deals with the general discussions, conclusions and recommendations.

Chapter Two
Literature Review

2.1 Origin, Taxonomy and Distribution of enset

The enset plant was initially reported by James Bruce while he was searching for the Nile's source in the years 1768–1773. He noted that enset grows to a high degree of perfection at Gonder, west of the Nile, and close to Lake Tana. He also emphasized the distinction between *Ensete* and the *Musa* (Baker and Simmonds, 1953). Since enset was formerly thought to be a species belonging to the genus *Musa* (Baker and Simmonds, 1953; Simmonds, 1960). Cheesman (1947) conducted genetic and taxonomy research for the genus *Ensete* formally stated by Horaninow in 1862, and he re-established the genus by relating it to other 25 African and a few Asian *Musa* species. Later, Simmonds (1960) evaluated the findings of Cheesman and listed the crucial taxonomic traits of *Ensete*.

The *Ensete* is a monophyletic genus belonging to the Musaceae family within the order Zingiberales, currently containing seven distinct species in Africa (four species) and Asia (three species) (Borrell *et al*., 2019). Among these, four species, such as *E. ventricosum*, *E. livingstonianum* or *E. gilletii*, *E. homblei*, and *E. perrieri* (Madagascar banana) are native to Africa, and *E. superbum* (Cliff banana, endemic to India), *E. glaucum* (Snow banana), and *E. lecongkietii* (endemic to Vietnam) are native to Asia (Simmonds, 1962; Borrell *et al*., 2019).

E. ventricosum has been widely distributed in a wild state in Africa, from central to eastern Africa and South Africa, and domesticated for food and non-food in Ethiopia (Purseglove, 1976; Shank, 1994). Vavilov (1951) and Harlan (1969) reported that its primary center of origin and diversity is Ethiopia. *E. ventricosum* is also called false banana, Ethiopian banana, or Abyssinian

banana due to its morphological similarity to a banana crop (Genet Birmeta, 2004). It is also recognized by its vernacular or local names, such as *enset* (Amharic as well as English), *asat* (Guragegn), *wessa* (Hadiyyisa), and *wassa* (Sidamign). Due to this, we also refer to *E. ventricosum* as "enset" in the same way in all contexts in this study. Enset has become of special importance for Ethiopian farming systems in the central, southern, and southwestern regions.

According to Westphal (1975) and Admasu Tsegaye and Struik (2002), there are four major farming systems in Ethiopia: the seed-farming complex, shifting cultivation, pastoralism, and the enset-planting complex. The enset-cultivating system is the indigenous production system, which sustains a more densely populated area of the country than other farming system (Brandt *et al*., 1997; Bloome *et al*., 2018). In enset-based farming, enset has historically and traditionally been placed as a primary food crop, and it is an essential staple food. As a result, enset can accommodate a higher population density per unit of land than places that mostly grow grains (Teshome Yirgu, 2016).

2.2 Botanical description of enset

E. ventricosum is a monocarpic, large perennial herbaceous plant and 4-11 meters tall (Brandt *et al*., 1997; Genet Birmeta *et al*., 2004), depends on climatic factors, soil fertility, and management practice (Tsegaye and Struik, 2002). The plant consists of an inflorescence, leaves, a pseudostem that is noticeably dilated at the base, overlapping leaf sheaths that emerge from the base of the plant, an underground stem structure called a corm, and an adventitious root system (Brandt *et al*., 1997; Borrell *et al*., 2019). The pseudostem can reach a height of about 5 m and has a variety of colors, including green, red, purple, brown, or a combination of green and red

(Almaz Negash, 2001; Admasu Tsegaye and Struik, 2002). The paddle-shaped leaves are up to 7 meters long and 1 meter wide, oblong to oblanceolate, bright to dark green, with their conspicuous midribs, petioles, and margins (Edwards and Laye, 1997; Borrell *et al*., 2019). *E*. *ventricosum* differs from banana in that it does not have stooling, has a monocarpic habit, has an expanded basal region of the pseudostem, and has smooth, big seeds that can reach a diameter of up to 18 mm as opposed to 10 mm, the relatively erect leaves, and an adaptation to cooler to drier environments than most *Musa* species (Baker and Simmonds, 1953), and a chromosome number of 2n=18 (Simmonds, 1960).

2.3 On-farm diversity of enset

Conservation and maintenance of landraces is an essential element of the socio-cultural heritage of a region or country (Laishram *et al*., 2020). Understanding the worth of a landrace is essential prior to settling on any conservation approach, and its use values play a crucial part in promoting its on-farm conservation (Loko *et al*., 2018). On-farm maintenance of plant diversity can reduce risk, stabilize yields, encourage dietary diversity, and maximize profits by employing low levels of technology and scarce resources (Abeli *et al*., 2020). Diversity in plants provides an opportunity for plant breeders to develop new and improved varieties with desired characteristics, including traits favored by farmers (potential for yields) and breeders (resistance to pests and diseases) (Govindaraj *et al*., 2015). Biodiversity offers ways of coping with the uncertainty of highly changing environments (Enjalbert *et al*., 2011). Thus, the diversity of different species is also maintained by local farmers for cultural, socioeconomic, and aesthetic values (Bernues *et al*., 2014).

Farmers are the primary custodians of enset landraces diversity in Ethiopia. Previous studies have shown that farmers-maintained hundreds of different enset varieties in various growing areas or regions (Shigeta, 1990; Kefale Alemu and Sandford, 1991; Almaz Negash, 2001; Admasu Tsegaye and Struik, 2002; Yemane Tsehay and Fassil Kebebew, 2006; Zerihun Yemataw *et al*., 2014; Awol Zeberga *et al*., 2014; Temesgen Olango *et al*., 2014; Zerihun Yemataw *et al*., 2016; Ambachew Zerfu *et al*., 2018; Bewuketu Haile *et al*., 2022 Table 2.1). However, it must be noted that the criteria used in enset landrace identification and research intensity or area and culture covered may vary from researcher to researcher.

Table 2. 1 Major enset growing areas and number of documented landraces

Study areas (zone, *woreda*)	**Number of Enset landrace**	**References**
Ari (South Omo)	76	Shigeta, 1990
Wolaita, Gamo, Dawro, and Goffa	158	Kefale Alemu and Sanford,1991
Keffa and Sheka	146	Almaz Negash, 2001
Sidama, Hadiya, and Wolaita	166	Admasu Tsegaye and Struik, 2002
Sidama	79	Bizuayehu Tesfaye, 2003
Keffa	42	Yemane Tsehay and Fassil Kebebaw, 2006
Sidama, Dawro, Gamo, Gurage, Goffa, Hadiya, Kembata-Tembaro, and Wolaita	218	Zerihun Yemataw *et al*., 2014
Silte, Sidama, Dawro, Gamo, Gurage, Goffa, Hadiya, Kembata-Tembaro, and Wolaita	218	Awol Zeberge *et al*., 2014
Wolaita	67	Temesgen Olango *et al*., 2014
Kembata-Tembaro	111	Melese Maryo *et al*., 2014
Sidama, Dawro, Silte, Gedeo, Gurage, Hadiya, Kembata-Tembaro, and Wolaita	312	Zerihun Yemataw *et al*.,2016
Yem	93	Ambachew Zerfu *et al*., 2018
Sheka	91	Bewuketu Haile *et al.,* 2022

The on-farm diversity of enset landraces is not distributed evenly, with a small proportion of highly abundant landraces cultivated over wider areas than the majority of moderately frequent

or rare landraces (Zerihun Yemataw *et al*., 2014). Enset landraces, particularly those with characteristics of better quality and quantity of end products, are widespread both within and among zones (Zerihun Yemataw *et al.*, 2016). Furthermore, some enset landraces are maintained more for their traditional medicinal purposes than for food or other purposes (Yemane Tsehay and Fassil Kebebew, 2006; Gizachew Nuraga *et al*., 2019a).

2.4 Farmers indigenous knowledge and Folk Taxonomy in Maintaining enset Genetic resources

Indigenous knowledge is the experience developed over years through practices over generations and is the common resource of the indigenous communities, which is transferred verbally as well as through practices (Villa *et al*., 2005). Over time, each individual in the community contributes to this indigenous knowledge. Farmers have a great deal of experience in assessing, maintaining, and managing their important crops in the traditional agricultural system (Sthapit, 1996), and they are also linked with these long-term practices for various economic, social, cultural, and religious events (Ulian *et al*., 2020).

Local farmers' contributions to genetic resource conservation are crucial since they help to ensure food security, livelihoods, and agricultural diversity (Nakabonge *et al*., 2018). Farmers' extensive knowledge of enset, gathered over many years, is important for characterizing and maintaining the existing genetic variety of this crop (Zerihun Yemataw *et al*., 2018). In an indigenous agricultural system like the enset cultivation complex, farmers' experiences are believed to play an important role in modeling the genetic structure of enset crops and their evolution (Bewuketu Haile *et al*., 2022), in which local farmers had constrained access to farming inputs such as pesticides and fertilizers and relied on a wide range of infra-specific

diversity to mitigate the risk of crop failure caused by climate whims, diseases, pests, and low soil fertility (Fahad and Wang, 2018). Local farmers possess the ability to discern their enset landraces with clarity via agromorphological features, phenological properties, post-harvest features, and varying adaptation performances under biotic and abiotic stresses (Brush and Meng, 1998; Loko *et al*., 2018). Genet Birmeta (2004), Admasu Tsegaye and Struik (2002), and Zerihun Yemataw *et al*. (2016) found genetic diversity in enset that has been cultivated in a particular area, which appears to be related to the amount of enset cultivation, the culture, and the management patterns of the various ethnic groups. They also identified a large number of local farmers' landraces of enset from major growing regions. Furthermore, they have names for them, and it is believed that various landraces have distinct adaptations to various soil types, cultivating times, maturation dates, plant heights, nutritional values, uses, and other characteristics (Sthapit *et al*., 1996).

Farmers give local names to plants according to their opinions through the ethnobotanical naming system, also known as folklore nomenclature. These names are a result of people's willingness to communicate verbally clearly and to discern between various plant species (Nedelcheva and Dogan, 2009). Given that communities and groups regularly build ethnobotanical names and classification systems in response to their surroundings, these systems are continuously passed down from generation to generation (Bewuketu Haile *et al*., 2022). The local names for plants and animals are the source of indigenous biodiversity knowledge (Nedelcheva and Dogan, 2009).

Different communities may have various folk classification systems. They classify plants mainly based on local language, production practices, social customs, legends, economic

utilization, morphological characteristics, and growth habits, which have very important economic and functional values (Kanglin *et al*., 2000).

2.5 Genetic Diversity of enset

Knowing and understanding the genetic structure and level of diversity within and among individuals, populations, species, and gene pools is vital for conserving, managing, and utilizing plant genetic resources effectively (Amom and Nongdam, 2017). Genetic diversity offers opportunities to produce new, more productive plants that are resilient to diseases and pests and flexible to changing environmental conditions (Rao, 2004). Detection of genetic variation provides incredibly valuable essential information in selecting genotypes with desirable traits for a breeding program (Ozkan *et al.,* 2022), and enhancement for sustainable agricultural growth and food security (Pawlak and Kołodziejczak, 2020). Measuring and characterizing genetic diversity have always placed a high priority in population genetic studies (Agarwal *et al*., 2008). Consequently, characterizing the germplasm is necessary for genetic resource conservation efforts (Karp *et al*., 1997).

Diversity levels and patterns have been traditionally employed with the aid of morphological characters and ethnological and cytological parameters; however, these characters alone only make up a minor percentage of the plant genome (Collard *et al*., 2005). Since then, genetic markers have progressed from morphological markers through cytological and biochemical markers to DNA markers (Rieger *et al*., 2012). DNA markers represent the change in DNA sequences within the individuals of a species, and they are stable and unaffected by environmental factors, pleiotropic effects, and epistatic effects (Govindaraj *et al*., 2015). The establishment of molecular markers, which are based on DNA polymorphism, has tremendously

aided study in a number of fields, including taxonomy, phylogeny, ecology, genetics, and plant breeding (Amiteye, 2021). It has been demonstrated that molecular markers are an effective tool for determining the genetic diversity of plants and for examining the genetic variables influencing quantitatively inherited features (Nadeem *et al*., 2018). For the purpose of evaluating the genetic diversity of plant germplasm, several DNA marker systems have been created and are being used (Collard *et al*., 2005).

Among DNA markers, simple sequence repeats (SSRs) are particularly pertinent to evaluating genetic diversity (Kiran *et al*., 2015). SSRs are simple sequences that combine in tandem repeats of mono-, di-, tri-, tetra-, penta-, and hexa-nucleotide motifs (Nadeem *et al*., 2018) that are highly abundant, of co-dominant nature, highly polymorphic and uniformly distributed in the nuclear, mitochondrial, and chloroplast genomes of many plant species, and highly reproducible for other research laboratories (Oliveira *et al*., 2006; Kalia *et al*., 2011). SSRs have also been proven to exist in protein-coding genes and ESTs (Yan *et al*., 2017). The most commonly prevailing motifs are mono-nucleotides, di-nucleotides, tri-nucleotides, and tetra-nucleotides, typically 2–6 bp in length (Kalia *et al*., 2011; Amiteye, 2021).

The SSR polymorphism is revealed by variations in the length of the PCR product from various genotypes or repeats of the SSR motifs within or between individuals in a population (Amiteye, 2021). The length variation is attributed to the difference in a specific locus's repeat units that may be the result of slippage during DNA replication (Tomar *et al*., 2010). The separation of SSR PCR fragments is carried out using agarose or polyacrylamide gel electrophoresis (Collard *et al*., 2005). SSRs are locus-specific markers that offer a significant advantage for effective plant genome mapping, population genetic studies, variety identification, and marker-assisted selection (Kumar *et al*., 2022; Padmakar *et al*., 2015). SSRs provide a wealth of information, but

the marker discovery or development process, which involves DNA sequencing, can be rather expensive (Grover and Sharma, 2016).

The study of molecular markers is a helpful method for examining the evolutionary connections and genetic diversity within and among enset landraces. Molecular markers are present throughout the entire genome and are independent of plant age or parts, the environment, or management techniques. This makes them ideal for crops like enset (Almaz Negash *et al*., 2001; Temesgen Olango *et al*., 2015; Fetta Gerura *et al*., 2019). Molecular genetic marker techniques such as Amplified Fragment Length Polymorphism (AFLP), Random Amplified Polymorphic DNA (RAPD), Inter Simple Sequence Repeats (ISSR), and Simple Sequence Repeat (SSR) have been used to evaluate the germplasm of cultivated enset from several enset-growing regions of Ethiopia (Almaz Negash, 2001; Genet Birmeta *et al*., 2002, 2004; Dagmawit Tobiaw and Endashaw Bekele, 2011; Temesgen Olango *et al*., 2015; Fetta Gerura *et al*., 2019; Gizachew Nuraga *et al*., 2022) (Table 2.2).

Table 2. 2 Enset genotypes collection sites, numbers reported and DNA markers used

Enset genotypes collectionzone/district/areas	**Number of genotypes**	**DNA marker used**	**References**
Keffa-Sheka, Sidama, Hadiya & Wolaita	146 cultivated	AFLP	Almaz Negash *et al*., 2001
Wolkite, Setunae, Seltae, Bonga, Shonae, Worka, Answae, Wondo, & Chencha	111 cultivated	RAPD	Genet Birmeta *et al*., 2002
Keffa	48 wild & nine cultivated	RAPD	Genet Birmeta *et al*., 2004
Keffa and Essera	71 cultivated	ISSR	Dagmawit Tobiaw and Endashaw Bekele, 2011
Dawro, Keffa-Sheka, Sidama,Gedio, East Shewa, West Shewa, South west Shewa, Jimma,& Yem	220 cultivated & six released varieties	SSR developed for/from banana	Selamawit Getachew *et al*., 2014

Table 2.2 Cont'

Maintained in AARC (originally collected from Ari, Gamo-Goffa, Sidama &Wolaita	66 cultivated & six wild	SSR	Temesgen Olango *et al*., 2015
Maintained in AARC	458	SNP	Zerihun Yemataw 2018
Gurage,Yem, and AARC	83 (79 cultivated & four wild	SSR	Fetta Gerura *et al*., 2019
Gurage, Hadiya, Kembata, Dawro, & Yem	52 (38 traditional medicinal)	SSR	Gizachew Nuraga *et al*., 2022

AARC, Areka Agricultural Research Center

2.6 Micro-propagation

Plant tissue culture is a method in which fragments of plant tissues are cultured *in vitro* on an artificial medium under controlled and aseptic conditions from selected plants to generate a great deal of plantlets (Hussain *et al*., 2012). The controlled situations include the proper amount of nutrients, energy, growth regulators, pH of the medium, optimum temperature, and light intensity (Phillips and Garda, 2019). Plant tissue culture entails culturing explants, such as shoot tips, seeds, embryos, pollen grains, ovules, or even a single cell, that have been separated from the mother plant on a sterile nutritional medium, which promotes cell division, growth, and plant regeneration (Vidyagina *et al*., 2021). Plant tissue culture has been widely employed to gain insight into plant conservation, pathology, physiology, and metabolite studies (Taalat *et al*., 2021; Vidyagina *et al*., 2021). The conservation of plant diversity is essential for future crop protection due to growing problems of biotic and abiotic stresses (Alexandratos and Bruinsma, 2012; Gupta *et al*., 2016). In this context, *in vitro* approaches allow for the enhancement of various features related to plant development and yield that may then be applied to *ex-situ* conservation (Lavanya *et al*., 2014).

The use of tissue culture technology has a number of benefits over natural plant propagation methods (Chandran *et al.,* 2020). It is a quick process because thousands of seedlings can be developed from tiny plant fragments in a short period of time in comparison to conventionally grown flora (Mattick, 2018). This aids in hastening the production of new crop varieties with superior features, as tissue culture studies take up less time and space than *in vivo* plant growth (Krasteva *et al*., 2020). Additionally, it aids in the production of pathogen-free micro-plants that are protected from a number of diseases, and new plants developed through tissue culture in aseptic conditions are likewise sterile (Bayoudh *et al*., 2015; Tegen and Mohammed, 2016). Furthermore, some plant species produce seeds that cannot be stored for long periods of time. In these cases, tissue culture can be used for conserving plants in their vegetative state, usually under slow growth conditions (Lambardi *et al*., 2008), or for cryopreservation (Garca-Gonzales *et al*., 2010).

Shoot tip culture is produced from detached shoot tips or buds from the terminal section of a shoot, including the meristem composed of primordial and producing leaves and nearby stem tissue (Sandhu *et al*., 2018). Consequently, the shoot tip has multiple primordial leaves and is frequently cultivated so that each one produces numerous shoots (Espinosa-Leal *et al*., 2018). The genetic stability built with this method is one of its benefits. Direct shoot development from the meristems prevents callus formation and accidental organogenesis, hence reducing genetic instability and/or somaclonal diversity (Hasnain *et al*., 2022).

Enset can be propagated sexually by seed and asexually by vegetative propagation techniques. However, the cultivated enset crops are commonly propagated by vegetative means using the

corm of a three- or four-year-old plant after cutting away the pseudostem at about 10 cm above the ground (Admasu Tsegaye and Struik, 2002). New suckers will emerge after two to three months (Blomme *et al*., 2018). As a result, farmers typically do not wait to harvest until the seeds are mature; hence, the production of seeds is not a common activity (Admasu Tsegaye, 2002). Moreover, as the seeds mature, the enset dries up and completely loses its food value (Almaz Negash *et al*., 2000). Furthermore, the hard, asymmetrical-shaped seeds are so challenging to germinate. Due to this, there is a low (12%) germination rate and a long germination period (Karlsson *et al*., 2015). The vegetative method reduces the juvenile period of the stand and makes it possible to propagate true-to-type plants of a desired genotype **(**Justine *et al*., 2022).

Enset cultivation mainly benefits the rural communities of the enset-growing regions of Ethiopia as food and non-food materials. Hence, recognizing the related challenges involved in producing it and developing effective methods for addressing these issues are paramount and crucial. According to our survey, there has been considerable interest among farmers in cultivating healthy genotypes of enset crops. This indicates that its production is at risk due to various pathogenic organisms. Thus, to sustain enset production, the development and preparation of disease-free enset genotypes for its plantations are very reasonable. Therefore, micro-propagation methods have been applied to grow healthy, disease-free enset plants year-round that also perform better in the field. There are only a few reports available on the micro-propagation of different genotypes of enset (Table 2.3).

Table 2. 3 Enset genotypes used, applied hormones and solidifying agents, and numbers of produced plantlets by different authors

Genotype employed and its collection area	Applied solidifying agents, PGRs, and others	Maximum numbers of micro-shoots & roots	Reference
Choro, *Nobo*, & *Ketano* from Keffa-Sheka	Agar 5g/l+Gelrite 1g/L 2.25 mg/L BAP + 0.2 mg/LIAA and 5 mg/L IBA+1 mg/L IAA +1 mg/L BAP	2-3 shoots	Almaz Negesh *et al*., 2000
Mariya & *Oniya* from AARC (originally from Kembata)	Agar 11 g/L, 2.5 mg/L BAP,	3.7 shoots	Mulugeta Diro & Van Staden, 2003
Adoo, *Bofaro*, *Botae*, *Feresae*, *Gwarae*, *Kantcho*, & *Swetae* from AARC	1.6 μM NAA+4.4 μM BAP+23.2 μM Kinetin + 22.6 μM + 2-ip N^6	75 shoots	Genet Birmeta & Welander, 2004
Arkiya & *Mazia* from Dawuro, *Digeomerza* from Kembata	Agar 6 g/L, 4.5 mg/L BAP +1.5 mg/L NAA, and 2 mg/L IBA	23 shoots and 3.8 roots	Genene Gezahegn & Firew Mekbib, 2016

The most widely employed medium in plant tissue culture is Murashige and Skoog's (MS) basal medium (Murashige and Skoog, 1962). One of the most expensive media parts is the gelling agent (Ebile *et al*., 2022). About three-fourths of the total cost of the media is spent on gelling agents per unit of the media, which may vary slightly depending on the recipe's specifics and the items' local prices (Teixeira Da Silva, 2019). Agar is frequently employed in tissue culture as a gelling agent due to its appropriate gelling features (Babbar *et al*., 2005; Jain-Raina and Babbar, 2011). However, the cost of agar restricts its use, particularly in developing countries (Pati *et al*., 2011; Sanchez-Cardozo *et al*., 2019). As a result, research has been done to identify less expensive alternatives to agar (Daud *et al*., 2011; Jain-Raina and Babbar, 2011). Among them are potato starch, rice flour, cassava flour, and corn flour (Kuria *et al*., 2008); these have been

applied either alone or in combination with others, with different levels of success (Ebile *et al*., 2022).

One of the primary starchy products of enset is called *bulla*; it is made by squeezing a mixture of the unfermented decorticated pseudostem and the ground-up corm, decanting the liquid, and then air-drying the resultant material (Tadessa Daba and Shigeta, 2016). It is used mostly as porridge, gruel, and a crumbled form and is thought to be the best quality enset meal (Pijls *et al*., 1995; Temesgen Olango *et al*., 2014). It is also possible to bind and dissolve compressed tablets using enset *bulla* (Tsige Gebre-Mariam and Nikolayev, 1993; Tsige Gebre-Mariam and Schmidt, 1996). In addition, *bulla* has been used as a gelling agent in *in vitro* propagation media of vanilla (Ayelign Mengesha *et al*., 2012), pineapple (Biruk Ayenew *et al*., 2012), and cassava (Manaye Ayalew *et al*., 2017) in order to reduce the cost of the culture media's production. The utility of enset starch for both industrial and food uses was reported by Hirose *et al*. (2010), who also revealed that it had good gelatinization capabilities.

2.7 Nutritional contents of enset

Food and nutrition insecurity has been a serious problem in sub-Saharan Africa due to climatic extremes and other factors (Abebe Yimer *et al*., 2023), and periodic droughts are not a recent occurrence in the Horn of Africa (Ashenafi Kassaye *et al*., 2021). Therefore, it has been suggested that, in order to combat food insecurity and malnutrition, diet diversification and the search for alternate food sources be undertaken (Abebe Yimer *et al*., 2023). In many regions of the world, especially in developing nations, plant-based products serve as the primary source of food for humans (Castro-Alba *et al*., 2019**)**. Enset in central, south, and southwest parts of Ethiopia is an indigenous agri-system that may have developed characteristics that offer

resilience techniques to handle environmental change and mitigate food insecurity (Tafesse Matewos, 2019; Chase *et al*., 2022). Hence, enset-based agriculture is a good illustration of a local agricultural system that has reportedly been adapted to withstand climate change (Westphal, 1975; Pijls *et al*., 1995; Brandt *et al.*, 1997).

Enset farming is a direct strategy for assisting individuals in achieving independent livelihood security (Abate Senbeta *et al*., 2022), and for the more than 20 million residents in the central, southern, and southwestern mid and highlands of Ethiopia, it is a major staple crop (Zerihun Yemataw *et al*., 2016; Borrell *et al*., 2020). Due to its role in food security, enset is known as 'the tree against hunger' by its growers and consumers (Brandt *et al*., 1997). Many people are living in the area where enset is the primary food source, which cannot be supported by any other type of land use in Ethiopia (Admasu Tsegaye and Struik, 2002; Zemede Asfaw, 2018). In the same manner, Pijls *et al.* (1995), and Admasu Tsegaye and Struik (2001) indicated that enset has a relatively high yield when compared to the yields of other food crops.

Enset may be harvested all year round, thus ensuring a constant supply of nourishment. The major food types from enset are *qocho* (prepared through fermentation), *bulla* (produced through extraction), and *amicho* (boiled corm) (Brandt *et al*., 1997; Admasu Tsegaye and Struik, 2002). Its abundant, relatively cheap, and long-term storable carbohydrates are important for Ethiopia, where 25–35% of the population is undernourished (Abraham Bosha *et al*., 2016). Studies revealed that different community groups in their regions or administrative zones in Ethiopia have maintained and utilized many enset landraces (Admasu Tsegaye and Struik, 2002; Zerihun Yemataw *et al.* 2016; Bloome *et al*., 2018; Bewuketu Haile *et al*., 2022). For particular uses, specific landraces are recommended by enset growers.

The majority of enset landraces are grown primarily for their *qocho*, or *bulla*, which can be processed through fermentation, but certain others are grown mainly for their *amicho,* or cooked corm (Admasu Tsegaye and Struik, 2002; Tadessa Daba and Shigeta, 2016; Zerihun Yemataw *et al.*, 2016)**.** *Amicho* is a fresh or unfermented type of enset product that is prepared from the young or immature enset plant corm and is cooked before eating like many other root and tuber crops (Admasu Tsegaye and Struik, 2002; Borrell *et al*., 2020). It can be uprooted and utilized as food at any point in the plant's growth, making it a reliable supply (Mohammed *et al*., 2013; Admasu Tsegaye, 2015). In addition, in the central, southern, and southwestern regions of Ethiopia, corms of certain enset landraces are regarded as having traditional medicinal benefits for a variety of ailments in both people and cattle (Almaz Negash, 2001; Yemane Tsehay and Fassil Kebebew, 2006; Gizachew Nuraga *et al*., 2019 a).

Previous studies have suggested that enset products are a good source of minerals and carbohydrates. However, the production and nutrient content of enset vary according to the variety of landraces, age of the plants, management system, and environmental conditions (Abraham Bosha *et al.,* 2016; Tadessa Daba and Shigeta, 2016).

Table 2. 4 The proximate composition (%) of corms of various enset landraces by different authors

Landrace	Moisture	Protein	Fat	Fiber	Ash	CHO	Source	References
Nobo	-	8.2	1.24	-	5.12	-	Jimma Zone	Sirawdink Forsido *et al*., 2013
-	-	3.33	0.41	5.65	8.57	82	Sidama region	Mohammed *et al*., 2013
Askala,Gossalo,Made etc.,	-	0.9-2.4		0.8-1.1	-	27-39	Sidama region	Admasu Tsegaye, 2015
Neqaqa	-	8.3	0.6	17.4	3.2	64.8	AARC	Tadessa Daba and Shigeta, 2016
*Amerat Astare Guarye Kibnare Yeshi**	65-72	2.4-4.1	0.5-0.6	2.4-4.4	2.2-3.2	76-80	Gurage Zone	Gizachew Nuraga *et al*., 2019b

Note: CHO= Carbohydrate, *Yeshi**= Yeshirakinqe, AARC= Areka Agricultural Research Center, - = Undefined or not indicated.

Table 2. 5 The mineral contents (mg/100g) of corms of different enset landraces as reported by different authors

Landrace	Ca	K	Mg	P	Na	Fe	Zn	Source	References
-	25	-	-	-	-	0.7	1.33	Sidama region	Yewelsew Abebe *et al*., 2007
Nobo	100	-	80	90	15	-	-	Jimma Zone	Sirawdink Forsido *et al*., 2013
Askala, Gossalo, Made,etc.,	-	-	-	-	-	1.1-4.3	6-16	Sidama region	Admasu Tsegaye, 2015
Neqaqa	99.7	1.24	59.6	80.4	5.2	12.3	22	AARC	Tadessa Daba and Shigeta, 2016

Chapter Three

On-farm Diversity, Use Pattern and Conservation of Enset (*Ensete ventricosum*) Genetic Resources in Southern Ethiopia

Abstract

Enset is an important source of food and is consumed by about 25 million people as a staple or co-staple food crop mainly in southern parts of Ethiopia. Large numbers of enset landraces exist in different administrative zones of Ethiopia with a wide range of altitudes and agroclimatic zones. However, limited information is available on the diversity, distribution, and utilization pattern corresponding to the diverse ethnolinguistic as well as socio-cultural communities of the country. Hence, this study was devised to explore and document the richness of farmers' tradition and practice on the diversity and distribution of enset landraces on the farm level and selection pattern for different purposes regarding the production, utilization, and conservation of enset genetic resources. The study was conducted in four major enset growing administrative zones of Ethiopia namely, Hadiya, Kembata-Tembaro, Gurage, and Silte. A total of 240 farm households were surveyed using individual interviews, 18 key informant interviews, 36 focus group discussions with 5 participants, and direct on-farm field observations for data collection. Considering that enset has a rich cultural background and indigenous knowledge ethnobotanical research approach was applied to data collection and analysis. The Shannon - Weaver, Simpson, Pielou, and Sorenson's similarity indices were used to evaluate the diversity and similarity of the landraces as well as using descriptive statistics in SPSS Ver. 24. Preference in direct matrix ranking was also used to compute and rank the enset landraces most preferred by the people in the context of specific use value in the study area. A total of 282 farmer-named enset landraces have been identified, with a range from 2 to 32 on individual homegardens. The largest number of landraces was found in the Hadiya Zone (86); while the lowest was scored in the Silte Zone (57). The Shannon diversity index (H') ranged from 3.73 (Silte) to 3.96 (Hadiya). Similarly, landraces revealed a very narrow range of variances in Simpson's 1-D diversity index, and it ranged from 0.963 (Silte) to 0.978 (Hadiya). Likewise, the similarity index ranged from 0.24 to 0.73 sharing 16-47 landraces in common. Of the 282 landraces, 210 (74.5%) were recorded in more than one zones; whereas, 72 (25.5%) had narrow distribution being restricted to a single zone. Farmers have established longterm practices and experiences in cultivation, utilization, and conservation of a diverse group of enset landraces to fill their domestic and market purposes in each zone. The variation is likely to be related to agroclimatic differences, ethnicity factors, food cultures and historical backgrounds. Therefore, to facilitate on-farm conservation as well as sustainable utilization of the enset genetic resources, farmers need to be supported by different stakeholders for all their worth, and also in crop improvement programs.

Keywords: Abundance, Farmer-named landraces, Inter-specific diversity, Landrace richness On-farm management

3.1 Introduction

Enset [*Ensete ventricosum* (Welw.) Cheesman] is a large perennial monocarpic herbaceous plant, similar to the banana in form, in the family Musaceae within the monocot order of Zingiberales (Borrell *et al*., 2019). *Ensete ventricosum* is domesticated and the corm (short underground stem) and pseudostem (thick and soft midrib) are processed and consumed as a staple and co-staple food in the south and southwestern parts of Ethiopia (Brandt *et al*., 1997). Enset is distributed at altitudes between 1100 and 3500 masl and it is chiefly propagated vegetatively (Tesfaye Abebe, 2005). It is noted for its tolerance to environmental fluctuations, storability, and for its multiple uses that play a pivotal role in preventing famine (Westphal, 1975; Borrell *et al*., 2020). Moreover, enset in Ethiopia is arguably a very important crop contributing to food security and rural livelihoods for about 25% of the country's population (Stanley, 1996; Brandt *et al*., 1997; Bloom *et al*., 2018) with diverse ethnic and cultural backgrounds. Ethiopia is both the center of origin and center of diversity for enset and many other crops (Vavilov, 1951). This diversity is maintained on-farm by farmers who also continue to diversify it through exchanging, sharing, and purchasing seedlings for cultivation. Genetic diversity for farmers means varietal diversity, which they can differentiate on the basis of agromorphological traits, phenological attributes, product quality, post-harvest characteristics, and differential adaptive performance under abiotic and biotic stresses (Sthapit *et al*., 1996; Brush and Meng, 1998; Timu *et al*., 2014).

Farmers have managed the diversity of enset landraces for centuries with limited or no research influences (Temesgen Olango *et al*., 2014; Zerihun Yemataw *et al*., 2016) being managed almost purely by indigenous knowledge and skills. Numerous landraces are grown for different uses and for the cultural requirements of the people at different sites of cultivation (Zippel, 2005;

Bizuayehu Tesfaye, 2008). Some prior studies indicate that numerous enset cultivars were identified in south and southwest part of Ethiopia and the observed genetic diversity in cultivated enset in a particular area appears to be related to the agroclimatic variation, extent of enset cultivation and the culture and distribution pattern of the different ethnic groups including the Gurage, Hadiya, Kembata, Silte, Wolaita, Dawuro, Ari, Kefa, Sheko and many others (Almaz Negash, 2001; Admasu Tsegaye, 2002; Genet Birmeta, 2004; Awol Zeberga *et al*., 2014; Zerihun Yemataw *et al*., 2016). Farmers select enset landraces based on the quality and quantity of food products (the fermented scrapings known as *qocho*, the juice from the scrapings known as *bulla*, and the boiled corm known as *amicho*), rate of maturation, disease and drought tolerance, forage quality, medicinal value, ease of scraping, quality of corm and productivity (Alemu and Sandford, 1996; Admasu Tsegaye, 2002; Zerihun Yemataw *et al*., 2018).

Understanding the diversity and distribution of enset is crucial for sustainably managing genetic resources and crop improvement efforts. Zerihun Yemataw and co-workers (2016) reported that the abundance and distribution of enset landraces in Gurage, Hadiya, Kembata, Silte, Wolaita, Dawuro, Gedo and Sidama zones exhibited substantial variances based on their use value and local naming and classification system. Some landraces, especially those with attributes of better quantity and quality of products, have a wider distribution both within and between zones.

Shigeta (1990) described that different enset landraces are recognized in different growing areas of Ethiopia, the only country where it is grown as a food crop, and are being grown in mixtures. Each enset landrace as identified by farmers has its name that is commonly used across the areas inhabited by people that speak the same language (with possible dialects/cognate names within some languages) but is sometimes shared by adjacent ethnic groups (Endale Tabogie, 1997; Almaz Negash, 2001; Admasu Tsegaye, 2002). Farmers discern one landrace from the other

phenotypically by looking at the color of the petiole, midrib, leaf sheath, angle of leaf orientation, size, and color of leaves, and circumference and length of pseudostem (Almaz Negash, 2001; Admasu Tsegaye, 2002; Zerihun Yemataw *et al.*, 2014; Gizachew Nuraga *et al*., 2019). Hence, vernacular names are often descriptive and reflect variations of landraces in places of origin, morphology, as well as agronomic and cooking characteristics (Temesgen Olango *et al.*, 2014; Hapsari, 2017). However, in some cases there are similar landraces known by different vernaculars and there are also different landraces known by similar vernaculars and with similar phenotypic appearance (Endale Tabogie, 1997; Glato, 2017).

The high genetic diversity of enset warrants conservation, as it provides resilience to the enset farming system and thus food security for farming communities (Almaz Negash, 2001; Genet Birmeta, 2004; Zerihun Yemataw *et al*., 2016). Enset plays a crucial economic role, providing a higher production under low input conditions compared to other crops in Ethiopia (Shank and Chernet Ertiro, 1996; Asnaketch Woldetensaye, 1997; Admasu Tsegaye and Struik, 2002). It is a multipurpose crop and nearly every part of the plant has some sort of uses as food and non-food (Brandt *et al*., 1997; Tadessa Daba and Shigeta, 2016). Farmers often say that enset is their food, their cloth, their house, their bed, their cattle feed, and their plate (Brandt *et al*., 1997). The major food types obtained from enset are *qocho*, *bulla* and *amicho*. Furthermore, some enset varieties are used traditionally to cure bone fractures, birth problems, and diarrhea in humans (Almaz Negash, 2001; Yemane Tsehay and Fassil Kebebew, 2006; Gizachew Nuraga *et al*., 2019).

Enset landraces are grown in homegardens with different local names and often with wide distribution and varietal diversity with implications to genetic diversity. For the sustainable utilization and on-farm conservation of its genetic resources as well as future improvement of the crop, understanding the socio-cultural, ethnobotanical knowledge, farmers' selection criteria, and

retention practices of enset landrace diversity in different ethnolinguistic communities is vital. However, limited documentation (Asnaketch Woldetensaye, 1997; Almaz Negash, 2001; Admasu Tsegaye, 2002) is available concerning the on-farm varietal diversity, its distribution, and the pattern of uses in different zones or ethnic groups. Due to this, the present study helps to fill the knowledge gap concerning farmers' traditional practice on enset cultivation and utilization in Hadiya, Kembata-Tembaro, Gurage, and Silte zones, the major enset production areas in central Ethiopia. Therefore, this study aimed at documenting the richness of farmers'ecological knowledge, tradition, and practices regarding the diversity and distribution of enset landraces on the farm level and the naming and selection criteria for different purposes concerning the production, utilization, and conservation of genetic resources.

3.2 Materials and Methods

3.2.1 The study area and site selection

The study was conducted in four enset-growing administrative zones, namely, Hadiya, Kembata-Tembaro, Gurage, and Silte of southern Ethiopia (Figure 3.1). The zones are basically distinguished by distinct languages, cultural background, and farming systems and also named based on the name of the majority ethnic group for that administrative location. The Hadiya and Kembata-Tembaro peoples speak a Cushitic language family, while the Gurage and Silte peoples belong to groups speaking the Semitic language family. Generally, the study zones are located between the great Ethiopian Rift Valley and Gibe-Omo River system, and are bordered by the Oromia region to the north and east, and with Wolaita zone in the south. The zones are structured into different *woredas*, which are further organized into *kebeles* (the lowest administrative units in Ethiopia). The study *woredas* and *kebeles* were selected from each administrative zone based

on enset diversity where prior information was obtained from the departments of agriculture of the respective zones and *woredas* (Table 3.1).

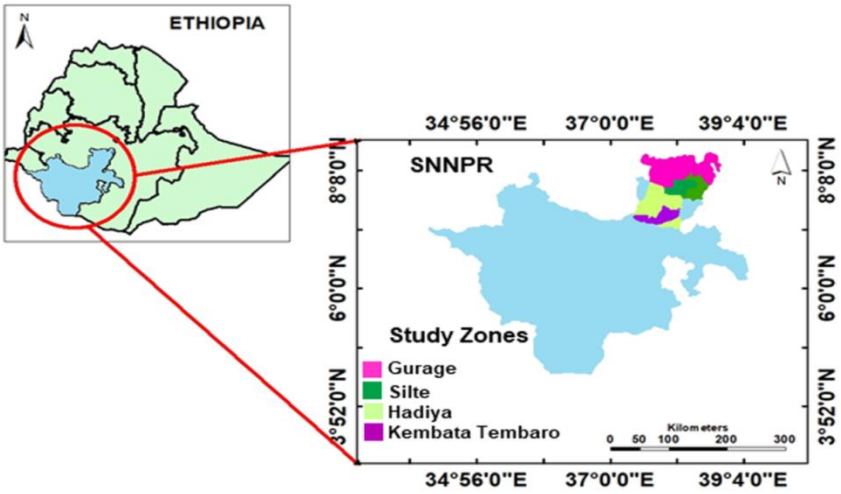

Figure 3. 1 Location of the Southern Nations Nationalities and Peoples' Region (SNNPR) in the map of Ethiopia (left) and the four study zones in the SNNPR (right)

3.2.2 Sampling technique and sample size

For this study, a multistage sampling method was performed for the selection of individual enset-grower farmers in each zone. Of the four administrative zones, 12 *woredas* (three from each zone) were selected purposefully based on enset frequency of occurrence and production level. From each *woreda*, three *kebeles* were also chosen purposefully according to the information obtained from the agricultural office of each *woreda*. Therefore, a total of 36 *kebeles* were selected for data collection. From each *kebele*, six to seven individual farmers were selected randomly that make a total of 240 households (60 household heads from each zone) in the whole study sites.

3.2.3 Ethical consideration

The Microbial, Cellular, and Molecular Biology Department, College of Natural and Computational Science, Addis Ababa University initially reviewed the study proposal. Following the approval, a supporting letter was written to the zonal administrative offices of the study area adhering to the existing national guidelines. As a result, each district/*woreda* official was informed of the study's objectives and wrote supporting letters to notify their respective *kebele* administrative offices. After obtaining the *kebele* leaders' permission, the investigator, local elders, and the agricultural extension workers of each *kebele* had a comprehensive discussion about the study's objectives and a schedule for the fieldwork and interview sessions. Following verbal informed consent of each informant, interviews and discussions were conducted to gather indigenous knowledge (non-clinical sample study) held by knowledgeable informed volunteers and participants about the on-farm diversity, use patterns, and traditional management practice of the enset crop.

3.2.4 Data collection

Both primary and secondary data collection methods were conducted to assess and document farmers' local knowledge regarding on-farm diversity, distribution, and utilization of enset crops in the study area. Two rounds of data collection and field observation were conducted. The first round was from June to September 2019 for preliminary observation and conducting the majority of interviews, and the second round was from January to March 2020 for direct observation of field activities like planting and transplanting.

To develop semi-structured interviews, different kinds of discussion were conducted initially with three to four elder enset farmers in each zone to generate needed information to be collected in the study area. In-depth individual interviews were conducted together with trained

agricultural extension workers, who are working closely with the communities in the respective selected *kebeles* in local languages (Hadiya, Kembata, Gurage, and Silte languages using a translator) and in the Amharic official language. The principal investigator can also communicate and understand three of the above-listed local languages; hence this made our work easier and the communication very smooth.

Table 3. 1 Description of the studied areas, number of respondents and altitude ranges

Administrative zone (Major language family spoken)	**Study woredas**	**Sampled kebeles**	**Number of respondents per kebeles**	**Altitude ranges (m)**
Kembata-T* (Cushitic)	Doyogena	Murasa	7	2145 – 2255
		Hawora-Arara	6	2335 – 2565
		Serera	7	2650 – 2822
	Angacha	Wasera	7	2550 – 2675
		Qerekecho	7	2225 – 2360
		Funamura	6	2150 – 2220
	Damboya	Dato	6	2680 – 2760
		Kazala	7	2250 – 2470
		Bonga	7	2300 – 2435
Hadiya (Cushitic)	Misha	Tulla	7	2675 – 2915
		Dengawora-S*	7	2567 – 2784
		Semenwasgebeta	6	2250 – 2465
	Lemmo	Shurmo-Dacho	7	2200 – 2350
		Dijo-Demala	7	2201 – 2418
		Lisana	6	2145 – 2250
	Dunna	Somicho	7	2475 – 2676
		Woramera	6	2300 – 2565
		Qenqicho	7	2435 – 2550
Gurage (Semitic)	Endegegn	Shewora	7	2210 – 2345
		Wolecho	7	2165 – 2455
		Zigez	6	2275 – 2335
	Gumer	Esen -Adengez	6	2635 – 2785
		Gura-Fezer	7	2695 – 2750
		Qebul	7	2570 – 2790
	Enamor-Ener	Agata	7	2235 – 2560
		Kochira	7	2195 – 2275
		Jatu	6	1850 – 2230

Table 3.1 Cont

Silte (Semitic)	Mirab A*	Willo	6	2485 – 2560
		Woger-gunjubul	7	2445 – 2585
		Mugo	7	2850 – 3195
	Misraq A*	Awerad	7	2265 – 2310
		Semerdin-D*	6	2220 – 2385
		Gomoro - Bucha	7	2315 – 2425
	Alicho W*	Abzena hulat	6	2775 – 3170
		Kutere	7	2450 – 2575
		Bune saqemo	7	2550 – 2680

Note: A*=Azernet, D*= Derawote, S*=Segamo, T*=Tembaro, W*= Weriro.

The farmers were also asked about their perception of names and naming systems. To obtain the detailed local knowledge of farmers in each zone, 4-5 key informants were also selected based on prior information obtained from *woreda* agricultural experts, agricultural extension workers of *kebeles*, elderly farmers, and local leaders. For the focus group discussions, from each of the selected *woredas,* about five participants were involved together with the members of the local administration, community elders, agricultural extension workers, and other members of participating communities. Additional data was also collected through, preference in direct matrix ranking by involving 12 key informants (three from each zone). Secondary data were also reviewed from the reports of the agricultural office of each zone, different books, research articles, and journals.

3.2.5 Data analysis

All listed landraces throughout the collection sites were checked for known synonyms or local names that refer to the same or different landraces in each study zone and *woreda* (district) with the help of key informants and other knowledgeable senior farmers. Moreover, some minor dialect variations in naming landraces within the same ethnic group were not considered

different and were disregarded in landrace authentication. However, landraces having the same names, but originating from different ethnic group or zones, were documented as its. The collected survey data were analyzed using descriptive statistics (frequency, percentages, and average) in SPSS Ver. 24. The landrace richness, distribution, and abundance per homegarden were also calculated using Microsoft Excel 2010. Richness was computed to show the total number of landraces per homegarden based on data recorded in each administrative zone as this is a simple applicable biodiversity index to use and compare diversity in enset landraces. Abundance was determined as the total number of individual enset plants of each landrace per homegarden. Preference in direct matrix ranking was conducted to analyze the most preferred enset landraces, in the context of the four specific use values for the seven enset landraces. Twelve key informants participated in the arrangement of the values by giving the most favored enset landraces a score of 10, the least preferred enset a score of 1, 0 for the uses not known, and the others a score that fell somewhere in between. Based on the total scores obtained for each landrace, these values were then summed for all respondents and ranked.

Diversity and similarity indices of species can be quantified in different ways. In this study, the diversity indices were calculated from the number of landraces existing in 60 farmers' homegardens within each zone. The Shannon and Weaver (1949) and Simpson Index (1949) was used to evaluat the landraces diversity. Both of them are widely used tools as a measure of heterogeneity (Magurran, 1988), and these were calculated for all sample zones to explore enset diversity. Shannon - Weaver diversity index is the most popular measure of species diversity because it accounts both for species richness (numbers) and evenness, and it is not affected by sample size (Krebs, 1999). The resulting index is high when the relative abundance of the different species or landraces in the sample is even and is low when a few species or landraces

are more abundant than the others. It was calculated using the formula: $H' = -\Sigma\ pi\ ln\ pi$ (Magurran, 1988). Where pi is the proportional abundance of the i^{th} landrace.

Even though Shannon's index takes into account the evenness of the abundance of landraces, evenness (equitability) can also be computed separately. It is a measure of the proportion of the observed diversity for the maximum diversity expected and was calculated through the Pielou index (1975) as the ratio, $E = H'/H'max = H'/lnS$, where: E is the evenness (equity) index; H' = diversity; H'max is a maximum diversity; lnS**,** in which S refers to the number of landraces in each zone. The higher the value of E, the more even the species is in their distribution within the community or the plots. Similarly, the higher the value of H', the more diverse the community or the plot is. A high evenness, resulting from all cultivars (landraces) having an equal abundance, is normally equivalent to high diversity (Magurran, 1988).

Simpson's diversity index (**D**) is a measure of diversity. It measures the probability that two individuals randomly selected from an area will belong to the same species (Simpson 1949) and hence, as D increases, diversity decreases. The index was, therefore, transformed as 1-D so that greater diversity corresponds to higher values: The formula for calculating D is presented as:

$D = \frac{\sum n_i(n_i - 1)}{N(N-1)}$ **,** or $(1\text{-}D) = 1\text{-}\{\Sigma n\ (n-1)/N\ (N-1)\}$ where n_i =the number of i^{th} individuals of enset landraces, N= the total numbers of enset landraces in the studied zones. The value of this index ranges between 0 and 1; the greater the value, the greater the diversity, 1 represents infinite diversity and 0, no diversity. The index was computed for all study zones.

Sorenson similarity index was employed to assess differentiation or beta (*b*) diversity (Magurran, 1988), and it compares the similarity of species (landrace) diversity among the study zones. The

expected variation in landrace composition that exists between the study zones was computed using Sorenson's similarity coefficient (Cs) (Sorensen 1948).

$$Cs=2J/a+b$$

Where: *a* is the number of landraces at zone A, *b* is the number of landraces at zone B, and *J* is the number of landraces common to both locations. Sorenson's similarity coefficient ranges in value from zero (no similarity) to one (complete similarity).

3.3 Results and Discussion

3.3.1 Socio-economic characteristics of respondent households

A sample of respondents on socioeconomic characteristics has been described in Table 3.2. Among the respondents, 82.1% of families were male-headed households, while 17.9% were female-headed households. About 50.4% of the heads of households were between the ages of 45 and 65, while 25.4% of the respondents were over 65. Approximately 41% of respondents were illiterate, whereas 28.8% had informal education and could read and write. However, 51% of the respondents overall in the studied administrative zones who participated in the interview were female. They are knowledgeable enset cultivators who have a great deal of knowledge about planting, managing in the field, harvesting, and using enset products. They rely on enset products for the most of their food needs, medical requirements, needs for fodder, and environments. They also gain benefits from the rich ecosystem of goods and services created by the enset agrosystem.

Table 3. 2 Socio-economic characteristics of respondent households

Variable	Category	Zone								
		K-T		Had		Gur		Sil		
		N	%	N	%	N	%	N	%	Mean%
Sex of HH	Male	49	81.67	51	85	47	78.33	50	83.33	82.08
	Female	11	18.33	9	15	13	21.67	10	16.67	17.92
Age of HH	<45	16	26.67	16	26.67	14	23.33	12	20	24.17
	45-65	30	50	28	46.67	33	55	30	50	50.42
	>65	14	23.33	16	26.67	13	21.67	18	30	25.42
Education level	Illiterate	23	38.33	24	40	25	41.67	26	43.33	40.83
	Read and write	16	26.67	15	25	19	31.67	19	31.67	28.75
	Primary	13	21.67	13	21.67	12	20	11	18.33	20.42
	Secondary	8	13.33	9	15	4	6.67	4	6.67	10.42

Note: HH=household, K-T= Kembata-Tembaro, Had= Hadiya, Gur= Gurage, Sil= Silte, N= number of respondents

3.3.2 Extent of richness and diversity of enset landraces

In this study, we identified and recorded 282 locally named enset landraces in the Hadiya, Kembata-Tembaro, Gurage, and Silte zones of southern Ethiopia. Enset growers can easily distinguish one enset landrace from the other by observing the external (leaf structure, size, orientation, midrib color, and other) and internal features (leaf and midrib anatomy and fiber structure) of the enset plants, and they give distinct vernacular names for each landrace. Each local farmer in the studied area was observed cultivating diverse enset landraces in his or her homegarden, which shows a considerable variation in the number of enset landraces on

individual homegardens. It ranges from 2 to 32 in this study (Table 3.3). According to farmers' knowledge of local names: 86 enset landraces from Hadiya, 73 from Kembata-Tembaro, 66 from Gurage, and 57 from Silte were recorded. The highest and lowest number of landraces per homegarden was documented in Hadiya and Silte zones, respectively (Table 3.3). In comparison to earlier reports, a relatively larger number of landraces have been identified in this study. The literature shows that Admasu Tsegaye (2002) recorded 146 different enset landraces including 59 from Hadiya, 55 from Wolaita, and 52 from Sidama while Almaz Negash (2001) reported the same total number including 65 from Kefa-Sheka, 30 from Sidama, 45 from Hadiya and 6 from Wolaita. Likewise, Genet Birmeta (2004) described 111 enset landraces from nine enset growing localities of Ethiopia that contrasted with the findings of the present study as in some other previous studies. For instance, Zerihun Yemataw *et al*. (2016) and Awol Zeberga *et al.* (2014) described the same numbers of (312) different enset landraces from eight ethnic groups, out of these 69 from Silte, 66 from Kembata-Tembaro, 63 from Gurage and 51 from Hadiya. Furthermore, Zerihun Yemataw *et al.* (2014a), who described 218 different enset landraces from seven zones, came up with 59 landraces from Hadiya, 43 from Kembata, 41 from Dawuro, 39 from Wolaita, 34 from Gamo Goffa, 31 from Gurage and 30 from Sidama. Some of these values are slightly comparable to the findings of the present study but such records are impossible to make a direct comparison of the number of enset landrace diversity with results of the current study due to variation in the method and size of the sampling area. However, in most cases, the richness of enset landraces recorded in the current study is far higher than the reports of the previous studies which is likely to be related to the rigor and intensity (including the sampling frame) as well as the knowledge of the men and women informants that participated in the present study. The number of enset landraces in the present study could be attributed to the

technique of sampling, the area the study covered, and the nature of the agroecological condition of the study area, which embraces midland and highland that is suitable for enset cultivation. Moreover, the study zones like Hadiya are bordered by all the other study zones, so the exchange and earning of suckers are common traditions among farmers. In the same manner, Admasu Tsegaye (2002) and Zerihun Yemataw *et al.* (2014) stated that the exchange of enset landraces from the neighboring ethnic groups perhaps contributed to the richness of enset landrace diversity in Ethiopia.

Table 3. 3 Enset landrace diversity in the four administrative zones, richness, Simpson (1-D), Shannon (H') diversity indices and Evenness (E)

Zone	Richness (%)	Min[a]	Max[b]	Mean[c]	Unique[d]	1- D	H'	E
Hadiya	86 (30.5)	3	32	10.23	22	0.978	3.96	0.89
K-T*	73 (25.9)	3	19	8.71	26	0.976	3.88	0.90
Gurage	66 (23.4)	4	24	9.52	14	0.975	3.83	0.91
Silte	57 (20.2)	2	22	8.24	10	0.963	3.73	0.92

Note: *= Kembata-Tembaro, a= Minimum richness, b= Maximum richness, c= Mean richness / homegarden, d= Number of unique landraces

The Shannon diversity index (H') ranged from 3.73 (Silte) to 3.96 (Hadiya), this signifies the existence of a high richness of enset landraces in the study zones. Even though zones varied in richness, they revealed a very narrow range of variances in Simpson's 1-D and evenness indices. The Simpson's 1-D ranged from 0.963 (Silte) to 0.978 (Hadiya) and evenness indices ranged from 0.89 to 0.92. All these results specify the presence of high enset landraces diversity in these four zones (Table 3.3). This finding is in line with earlier reports (Awol Zeberga, 2014; Zerihun Yemataw, 2016). According to Rosenzweig (1995), the value of a diversity index increases when both richness and evenness increase and is maximized when all species are nearly equally abundant. In biodiversity studies, Shannon diversity indices (H') typical values range between 1.5 and 3.5 and the index is rarely greater than 4 (Magurran, 2004). The higher the value of H',

the more diverse the communities and the Shannon index increases as both the richness and evenness of the communities increase.

3.3.3 Similarities and differences of enset landraces diversity among zones

The similarity among pairs of zones (taking two zones at a time) concerning farmers named landraces was evaluated using Sorenson's similarity index (Table 3.4). Generally, the similarity index ranged from 0.24 to 0.73, and the number of commonly shared landraces varied from 16 to 47. Hadiya and Kembata-Tembaro were the most similar zones based on commonly shared enset landraces, followed by Gurage and Silte (Table 3.4). Hadiya also shared 38 and 35 enset landraces with Gurage and Silte zones, respectively. This high sharing of enset landraces among zones may be due to sociocultural and linguistic similarities, and geographical locations. For instance, Hadiya is bordered by the all study zones, so the informal exchange of enset suckers from the adjacent zones possibly contributed to the high similarity of enset landrace diversity among zones in the present study. This agrees with the report of Awol Zeberga *et al*. (2014) and Zerihun Yemataw *et al*. (2014), who reported the existence of a high amount of sharing similar enset landraces among Hadiya and Kembata, Gurage and Silte, and Wolaita and Dawuro zones of Ethiopia. On the other hand, pairs of zones with relatively least similarity were Kembata-Tembaro and Silte, and Gurage and Kembata-Tembaro 0.24 and 0.25 for each pair, respectively. This may be due to geographical distance between the two zones and also variation in sociocultural factors.

Table 3. 4 Enset landraces shared (bold) and Sorensen similarity indices between pairs of zones

Zone	Hadiya	Kembata	Gurage	Silte
Hadiya		**47**	**38**	**35**
K-T*	0.59		**17**	**16**
Gurage	0.50	0.24		**45**
Silte	0.49	0.25	0.73	

3.3.4 Distribution and abundances of enset landraces

Out of 282 enset landraces recorded, 15 (5.3%) were widely distributed in all four zones. These were *Agade*, *Astara*, *Bededete*/ *Badade*, *Gimbo*/*Gimbuwa*, *Heniwa*/ *Hiniba*/ *Enba*, *Kasete*, *Manduluqa*/ *Mande*, *Mariye*, *Merza*, *Mesmesia*, *Moche*, *Separa*/*Sebera*, *Torora*, *Weshemeja* and *Zobira* (Table 3.5). Similarly, 33 (11.7%) farmers' named enset landraces were commonly cultivated and found in three (Hadiya, Gurage, and Silte) out of four zones (Table 3.5). Likewise, 72 (25.5%) of the enset landraces had a narrow distribution and were specific to a single zone (Table 3.5). But the remaining 210 (74.5%) were recorded in more than one administrative zone. The finding of this study was in line with the previous study from the same or different zones in Ethiopia (Admasu Tsegaye, 2002; Awol Zeberga *et al*., 2014; Zerihun Yemataw *et al*., 2014).

The abundance of enset landraces also differed among the study zones in addition to their distribution. Few enset landraces such as *Gimbo*, *Hiniba*, and *Separa* were relatively high in abundance at all four study zones. *Agade*, *Bedededa*, and *Zobira* were also other most frequent enset landraces in three out of the four zones (Table 3.5). Some landraces were well encountered in two zones but virtually absent from the other study zones. For example, *Sisqella* and *Gishira* were the most abundant landraces of the enset homegardens visited in Hadiya and Kembata-Tembaro zones but were almost absent or rare in other zones. Moreover, some landraces such as *Abate-Merza*, *Dego-Merza*, *Dirbo* and *Unjame* in Kembata-Tembaro, *Amerate* and *Lemat* in

Gurage, *Shewrad* in Silte, *Disho,* and *Bequcho* in Hadiya zones were dominant but outside these zones, they were found with a low abundance. A similar observation was reported by Zerihun Yemataw *et al*. (2016) and Awol Zeberga *et al*. (2014): landrace *Agade* in Silte, *Amerate* in Gurage, *Shododenia* in Dawro, and *Addo* and *Genticha* in Sidama encountered a high local abundance at each studied zones. This may be due to the environmental adaptability of the landraces or/and different attributes of farmers. Almaz Negash (2001) and AdmasuTsegaye (2002) also reported that enset landrace diversity and distribution were influenced by factors such as household resources, cultural background, population pressure, and agroecology. Enset landraces namely: *Manduluqa*, *Mariye*, *Mesmesia*, *Moche,* and *Torora* described in this study were found in a limited number of homegardens but widely spread in each zone. In the same manner, Zerihun Yemataw *et al*. (2016) and Awol Zeberga *et al*. (2014) indicated that household features, the distance between locations, and ethnic preference contribute to the landrace diversity and abundance.

Table 3. 5 List of farmers- named landraces and its richness in the four administrative zones

No	Hadiya	N	K-T*	N	Gurage	N	Silte	N
1	*Addo*	2	*Abatmerza*	55	*Agade*	51	*Agade*	59
2	*Agade*	38	*Agade*	6	*Agoregure*	11	*Agermir*	12
3	*Alabite*	3	*Aganche*	8	*Ahiro*	18	*Ahiro*	31
4	*Anchire*	5	*Arke*	4	*Amerate*	49	*Ameret*	6
5	*Arke*	2	*Ashure*	26	*Ankufuye*	28	*Ankufaye*	8
6	*Astara*	21	*Astara*	8	*Ashaqit*	4	*Ashaqit*	6
7	*Awunada*	12	*Ayase*	15	*Astara*	42	*Astara*	28
8	*Banko*	2	*Bededed*	9	*Awunad*	6	*Awunade*	7
9	*Bedededa*	32	*Banko*	12	*Aywogna*	5	*Aywongna*	29
10	*Beneje*	18	*Cherquwa*	11	*Bededet*	37	*Bededet*	36
11	*Bequcho*	6	*Danxia*	7	*Benezhe*	32	*Manduluqe*	3
12	*Beshiqiye*	3	*Degomerza*	39	*Bezeria*	23	*Beneje*	30
13	*Bezeriya*	4	*Dereqeta*	8	*Bitena*	3	*Bezeria*	4
14	*Birwesa*	3	*Derga*	6	*Bossora*	21	*Bossora*	16
15	*Boicho*	12	*Dirbo*	12	*Chehoyet*	8	*Bushawesse*	4
16	*Boshosha*	2	*Dirbo-qey*	38	*Dare*	26	*Dem-worad*	11

Table 3.5 Cont'

17	*Danxia*	6	*Disho*	21	*Demyetrnech*	7	*Deriye*	12
18	*Dego*	31	*Uskuruz*	14	*Demyetrqey*	4	*Ferezeye*	6
19	*Dirbo*	21	*Etene*	29	*Egendye*	26	*Fenqo*	3
20	*Disho*	39	*Fechache*	6	*Enba*	38	*Fugnaqir*	2
21	*Egandiya*	6	*Felegede*	4	*Fenqo*	4	*Garado*	6
22	*Etine*	11	*Fello*	3	*Ferezeya*	17	*Guariye*	31
23	*Fechecha*	4	*Ferchase*	9	*Gazner*	8	*Gefate*	3
24	*Fello*	2	*Gagabo*	6	*Gegered*	11	*Gimbo*	41
25	*Feraziya*	3	*Gimbuwa*	39	*Gimbuwa*	28	*Gudero*	6
26	*Gagabo*	2	*Ginawa*	11	*Ginad*	6	*Hanzana*	5
27	*Gariya*	25	*Ginjona*	13	*Gozoda*	12	*Hiniba*	39
28	*Gimbo*	57	*Gishira*	29	*Guarye*	24	*Kaset*	11
29	*Ginjowona*	2	*Guderete*	3	*Gumbura*	3	*Kembat*	12
30	*Gishira*	38	*Gomorsa*	6	*Hanzana*	12	*Kemele*	2
31	*Gomorsa*	5	*Gunze*	3	*Kanchewa*	8	*Kombotir*	4
32	*Gozoda*	4	*Hargema*	5	*Kaset*	9	*Megribe*	3
33	*Gudere*	8	*Hella*	22	*Kebere*	3	*Mariye*	6
34	*Hanazana*	7	*Heniwa*	29	*Kembat*	11	*Merza*	3
35	*Haqucho*	3	*Keset*	4	*Kemele*	4	*Mesmesia*	2
36	*Hayiwona*	29	*Ketane*	2	*Kemota*	2	*Moche*	8
37	*Hella*	24	*Korbo*	2	*Kona*	5	*Nechewo*	5
38	*Hiniba*	41	*Lenbona*	3	*Lemat*	22	*Orad*	6
39	*Hyro*	8	*Leqeqa*	28	*Manduluqe*	2	*Qeshqeshe*	4
40	*Jegirada*	7	*Lokande*	5	*Mariye*	5	*Qiniware*	26
41	*Kaseta*	12	*Manduluqa*	12	*Merza*	4	*Separa*	38
42	*Kekera*	9	*Mariye*	18	*Mesmesia*	7	*Sherafire*	12
43	*Kerqere*	2	*Mesmesa*	15	*Mishirad*	3	*Shewrad*	15
44	*Korina*	8	*Moche*	9	*Moche*	6	*Shigez*	4
45	*Lechebo*	5	*Morala*	3	*Nechewa*	21	*Shireteye*	31
46	*Lendwese*	3	*Mutite*	3	*Oniya*	8	*Sino*	12
47	*Leqeqa*	13	*Nejawro*	2	*Oret*	24	*Sisqella*	2
48	*Lokanda*	6	*Oniya*	21	*Qeshqeshe*	6	*Tegeded*	6
49	*Manduluqa*	3	*Qeqile-ne*	12	*Qibnare*	39	*Tem-wese*	3
50	*Mariye*	11	*Qeqile-qe*	16	*Separa*	42	*Torora*	5
51	*Meqelwesa*	18	*Qerqere*	5	*Shewatia*	6	*Wonade*	9
52	*Merza*	34	*Qorate*	2	*Shewora*	5	*Woshemaja*	6
53	*Mesmesia*	18	*Quina*	22	*Shireteye*	29	*Yekechere*	2
54	*Moche*	25	*Sebera*	37	*Sisasir*	3	*Yetibare*	2
55	*Mutite*	3	*Shate*	2	*Tegeded*	18	*Zegizik*	2
56	*Nechewo*	7	*Shelleqe*	16	*Tereye*	8	*Zerbededet*	9
57	*Oniya*	22	*Sinera*	4	*Torora*	7	*Zobir*	28
58	*Orada*	11	*Sisqella ne*	44	*Wonadia*	11		
59	*Ossosa*	4	*Sisqella tik*	12	*Woshemadia*	6		
60	*Qebere*	7	*Sorpie*	8	*Yeqesewa*	18		

Table 3.5 Cont'

61	*Qenchewa*	2	*Unjame*	41	*Yeshirafire*	12
62	*Qeshqeshe*	6	*W'ea*	12	*Yeshiraqinqe*	15
63	*Qeteqeta*	2	*Wachiso*	7	*Zegirad*	9
64	*Qiniwara*	26	*Wellanche*	5	*Zerbededet*	12
65	*Qombotira*	15	*Weshemeja*	2	*Zobir nech*	3
66	*Quiena*	9	*Woio woe*	3	*Zobir qey*	27
67	*Separa*	43	*Wolegella*	8		
68	*Shate*	29	*Wongorate*	3		
69	*Shelleqe*	3	*Xebare*	22		
70	*Shereqa*	2	*Xessa*	29		
71	*shewora*	7	*Xorore*	27		
72	*Shirafire*	14	*Zinke*	4		
73	*Sinera*	3	*Zobira*	6		
74	*Sisqella*	53				
75	*Soqido*	18				
76	*Suwandiya*	2				
77	*Tegeded*	6				
78	*Unjame*	19				
79	*Uskurusa*	5				
80	*Wea*	3				
81	*Wonade*	6				
82	*Woshamaja*	7				
83	*Xessa*	13				
84	*Xiggo*	9				
85	*Xorore*	27				
86	*Zobira*	39				

Note: N=Number of respondents who are growing above listed landraces, K-T*= Kembata Tembaro

3.3.5 Diverse local names of the enset landraces among zones

The local names of enset (*Ensete ventricosum*) and its different growth stages vary from one ethnic group to another. Enset is called *wessa* in Hadiya and Kembata-Tembaro, *wesse* in Silte, and a*set* in Gurage. Moreover, each growth (transplanting) stage has a distinct name by which it is identified. The Hadiya and Kembata-Tembaro farmers share almost the same local names for all sucker stages. These are known as *dubbo*, *simma*, *ero*/*kiniba*, and *balwesa*, but in Silte one and two years old suckers are called *bosho* and *daporo*, respectively, and the other two stages are

nearly similar to the Hadiya and Kembata-Tembaro zones (Figure 3.2a-d). In Gurage, one-year-old sucker is *fonfo* but the second and third stages are named the same as other studied zones.

According to the interviewed farmers, the same enset landraces are sometimes known by different names in different administrative zones (Table 3.6). In this study, 11 farmer-named landraces identified with the help of key informants and knowledgeable farmers in each zone indicated that the same enset landraces were known by different names in the other studied zones (Table 3.6). The role of knowledgeable men and women enset farmers was so critical in this research since they are experts in the landrace identification and description of ethnobotanical methodology. The landrace names given by enset farmers mostly reveal distinct morphological appearances or other culinary characteristics such as taste or use values (data not shown). Each ethnic group has its series of local names for enset landraces. For example, the landrace *Shate* in Hadiya, and *Shirteye* in Silte and Gurage is the same landrace with different local names often representing the bitter- tasting characteristics of all its parts. Enset landrace *Xiggo* in Hadiya, called *Qeqile-nech* in Kembata-Tembaro, is well known to the enset farmers as its bleeding (red sap) when parts are cut. The origin of certainly cultivated enset is evident from the name. One such example in this study is *Kembat* which may be originated from Kembata, however, its name in Kembata-Tembaro and Hadiya is called *Disho* (Table 3.6).

In addition, according to farmers, some landraces were named based on the color of pseudostem and leaf (*Bushawese* in Silte meaning red enset), but this landrace in Hadiya is given the name *Meqelwesa*, meaning placental enset, which is related to use characters. Similarly, landraces *Soqido* meaning salt (taste of boiled corm or *amicho*) in Hadiya whilst in Silte and Gurage it is *Kemele* meaning Ape (maybe the color of the pseudostem or petiole) (Table 3.6). In general, this is observed due to the use of various local names in the different communities of the study area,

having their specific characters and method of perceiving by the local farmers. Based on key informants' responses and focus group discussion, some cultivated enset landraces were named with minor or slight dialect differences in the local names among study zones. Those include landraces: *Gimbo*/*Gimbuwa*, *Hiniba*/*Heniwa*/*Enba*, *Jegirada*/*Z'girad*, *Hyro*/*Ahiro*, *Qibnare*/ *Qinare*/*Qiniwara,* and *Guary*/ *Gariya*. This reveals that sometimes the same landraces are often known by different names in different or the same regions. The method of the naming of landraces as indicated by farmers in our study is also similar to what has been reported in other enset growing zones. For instance, Temesgen Olango *et al*. (2014), Bizuayehu Tesfaye (2008), and Shigeta (1990) reported that the naming criteria of some enset landraces in the Wolaita, Sidama, and Ari respectively, are mostly based on morphological and agronomic traits, place of origin, various uses, and culinary attributes. In the study areas, farmers use their local language in everyday speech and communication in each zone. There are numerous enset landrace names and synonyms in these different languages and dialects were recorded throughout the study zones (Table 3.5). For instance, in this study 15 identically named enset landraces were identified from all four studied zones. In the same manner, three zones (Hadiya, Silte, and Gurage) commonly share 33 of the same named enset landraces in the present study. A similar observation was reported by Temesgen Olango *et al*. (2014), Zerihun Yemataw *et al*. (2016), and Bizuayehu Tesfaye (2008). These authors also described the existence of identically named enset landraces in more than one ethnolinguistic community. This may occur due to getting the enset planting materials and a long-lasting practice of farmers in sharing with their respective landrace names from adjacent administrative zones. Similarly, Temesgen Olango *et al*. (2014) stated the presence of 'borrowed' landrace names between ethnolinguistic groups. Similar trends were also observed in different traditional crops such as sorghum (Timu *et al*., 2014), banana (Hapsari *et*

al., 2017), sweet potato (Glato *et al*., 2017), cassava (Nakabonge *et al*., 2018), and common bean (Tura Bareke *et al*., 2018; Betelhem Abera *et al*., 2020). Our study has also shown that enset growers sometimes give various names for the same landrace within the zones. For instance, the landrace named *Ayase* is known as *Hella* in Hadiya Duna *woreda*, *Qombotira* is called *Asheqit* in Silte, and also *Gegard* is known as *Heniwa* in Endegegn *woreda* of Gurage zone. Bareke *et al*. (2018) and Betelhem Abera *et al*. (2020) also reported similar results from Ethiopia for common beans. Likewise, the different names for the same enset landrace also exist among zones (e.g. *Disho* in Hadiya and Kembata-Tembaro is known as *Kembat* in Silte and Gurage, *Tem-wese* in Silte is also called *Xebere* in Kembata-Tembaro or *Qebere* in Hadiya) (Table 3.6). Moreover, the names of some enset landraces have the same meaning but it was locally known with different folk names throughout study zones. For instance, *Xiggo* in the Hadiya, *Dem-wored* in the Silte and *Dem-yeteret* in the Gurage refer to bleeding because of exuding red fluid when any part of the enset is cut. This is similar to the findings of Betelhem Abera *et al*. (2020) who found that common bean producers provided different names in terms of seed color in two areas but the names have the same meaning.

Table 3. 6 Different local (vernacular) names for the same enset plants within or among zones

No	Hadiya	Kembata-T	Silte	Gurage
1	*Shate/Shatedegn*	*Shate*	*Shireteye*	*Shireteye*
2	*Disho*	*Disho*	*Kembat*	*Kembat/ Hambediya*
3	*Xiggo*	*Qeqile-Nech*	*Dem-worad*	*Demyertete nech*
4	*Meqelwesa*	*Qeqile- Qey*	*Bushawese*	*Demyertete qey*
5	*Bequcho*	-	-	*Sisasir*
6	*Shereqa*	-	*Megrib*	*Yeqisew/Qesew*
7	*Soqido/Soqe*	-	*Kemele*	*Kemele*
8	*Qombotira*	-	*Ashaqit/Kombotir*	*Ashaqit*
9	*Dego*	*Degomerza*	-	-
10	*Merza*	*Abatemerza*	*Merza*	*Merza*
11	*Boshosha/Qebere*	*Xebere*	*Tem-wese*	-

3.3.6 Pattern of use and management practices undertaken by farmers

Traditionally, farmers in the study area were familiar with the utilization and management of enset from earlier generations to meet their food, medicinal and other requirements. In the study area, all enset landraces were primarily cultivated for food and feed use, except landrace *Meqelwesa* or *Qeqile-qey* which was rarely used as food. This landrace is one of the most traditionally preferred medicinal enset landraces recommended for human and cattle ailments (Table 3.9). Based on the information we acquired during the individual interview and focus group discussion, enset farmers preferred landraces with early maturity and vigorous growth, easily harvestable, early fermenting, high *qocho* and *bulla* yielding, and good cooking qualities. In addition, in all four zones, generally, multi-use enset landraces were highly chosen and more cultivated than specific-use landraces. However, in some situations, there was regional or ethnic preference across the study zones.

According to a result of the key informants ranking from the five commonly shared and the other two, *Gimbo* became the first, *Separa* the second, and *Agade* the third most preferred enset landraces for their *qocho* and *bulla* quality. *Astara* and *Agade* scored the highest points for both their *amicho* (cooked corm) taste and medicinal value, and *Sisqella*, *Bedededе*, and *Gimbo* stood first to third, respectively for their fiber quality (Table 3.10). For instance, extracting *bulla* from other harvested masses of enset (Figure 3. 2e) in Gumer *woreda* of Gurage zone by women is not common practice, unlike other *woredas* and zones. But they purchase it from other adjacent *woreda* markets for different purposes. In the same pattern, the use and production of fiber, which is another enset product obtained from the decorticating of petiole and pseudostem are decreasing in most of the studied zones. Because it employs a traditional production method that requires more time and labor. In addition, nowadays most of the traditional fiber-made products

are replaced by other plastic materials. However, some enset farmers in Hadiya and Kembata-Tembaro prefer more drought tolerant and high fiber quantity and quality in addition to *qocho* and *bulla* yield while those in Gurage and Silte favored easy harvesting and processing, early fermenting, and less fibrous landraces (Table 3.7). The present study also indicated that there were slight differences in terms of perceiving enset end-users across the study zones.

Table 3. 7 Enset landraces selected by farmers for *Amicho*

No	In Hadiya zone		In Kembata-T		In Silte zone		In Gurage zone	
	Landrace	**N=60**	**Landrace**	**N=60**	**Landrace**	**N=60**	**Landrace**	**N=60**
1	*Soqido*	52	*Leqeqa*	58	*Qinare*	60	*Qinare*	60
2	*Qiniwara*	51	*Xebere*	51	*Astare*	60	*Astare*	60
3	*Astara*	51	*Quena*	50	*Gariye*	57	*Guarye*	58
4	*Gariya*	47	*Xorore*	50	*Ashaqit*	50	*Kemele*	43
5	*Leqeqa*	39	*Astara*	46	*Agade*	48	*Ginad*	35
6	*Xorore*	38	*Sebara*	36	*Oret*	36	*Oret*	36
7	*Quena*	37	*Etene*	35	*Torore*	35	*Torore*	37
8	*Qombotira*	37					*Qesew*	39
9	*Qebere*	36					*Ashaqit*	37
10	*Orada*	35					*Bezeria*	36

Moreover, interviewed farmers in Kembata-Tembaro grouped enset landraces into two major sex categories: female enset and male enset. The division of male and female is not linked to biological reproduction but it is based on perceived features of the landraces. The female groups are known for ease of decorticating, early fermentation, corm palatability, more susceptibility to different diseases, and low strength of fiber whereas the male groups contrast to these characteristics. Similar observations were reported from Kaffa-Shaka by Almaz Negash (2001) and Wolaita by Temesgen Olango *et al*. (2014) zones. In contrast, farmers in Hadiya, Gurage, and Silte did not tend to classify enset plants into sex designation. Admasu Tsegaye (2002) also reported the relationship to the difference in food culture, socio-cultural preferences for different enset products, and farming systems of the regions. Similarly, Kujawska *et al*. (2017) described

the influence of cultural background on plant species diversity and the uses of plant species for different purposes. Enset landrace diversity within the same and different cultural groups nicely demonstrates that cultural needs and requirements are key factors in the diversification of crop varieties. In particular, the unique landraces recorded in the different ethnic communities indicate the origin and maintenance of those landraces by specific ethnic groups because they need them for their food, medicine, and other uses.

According to the farmers' report, we identified a total of 32 landraces which were applied in different proportions by each ethnic group: 10 in Hadiya, 9 in Silte, 7 in Kembata-Tembaro and 6 enset landraces in Gurage as traditionally medicinal use to treat various health problems in human and cattle (Table 3.9). Out of the total listed, 12 medicinally used enset landraces shared the identical name in at least two zones, so the total number decreased to 20. Landrace like *Astara* mentioned by the farmers is an example of enset that has multiple uses of traditional medicinal purposes in the all study areas. Furthermore, landraces such as *Qinare*/*Qiniwara*, *Gishira*, *Guary*, *Xessa*, *Hayiwona*, and *Agade* were also the most frequently used medicinal enset present in homegardens of two or more ethnic communities (Table 3.9). On the other hand, some medicinal landraces (*Cherquwa* and *Wolegella*) were identified as having narrow distribution in the study zones. However, in some cases the same kinds of enset those known with alternative local names used as medicines for different problems among the study communities. For instance, landrace *Xiggo* in Hadiya is mainly traditionally used to treat kidney and liver problems, whereas the same variety with different names (*Qeqile-nech* in Kembata and *Dem-word* in Silte) quoted by many farmers to remove the delayed placenta and for aborification (used to cause/facilitate abortion) purposes (Table 3.9).

Table 3. 8 Enset landraces selected for strong and long fiber

Hadiya zone landraces	N =60	Kembata-T zone Landraces	N =60	Silte zone landraces	N =60	Gurage zone Landraces	N =60
Sisqella	60	*Sisqella*	60	*Kembat*	52	*Kembat*	53
Disho	56	*Gishira*	57	*Bededet*	50	*Yeshirenqinke*	49
Unjame	54	*Unjame*	55	*Gimbo*	41	*Bededet*	48
Gishira	55	*Disho*	48	*Separa*	40	*Gimbuwa*	40
Dirbo	42	*Dirbo*	41	*Agade*	35	*Sebara*	38
Dego	40	*Shelleqe*	39				
Bequcho	39	*Hella*	38				
Bedededa	36	*Degomerza*	37				

Table 3. 9 Enset landraces selected for medicinal purposes

Admin. Zone	Landraces	N=60	Product uses to treat ailment
Hadiya	*Agade*	38	*Amicho* with yoghurt to cure bone fracture.
	Astara	48	*Amicho* with milk to cure bone and muscle problems in human.
	Bedededa	35	*Amicho* to initiate milk production in cattle.
	Gishira	60	*Amicho* and roasted *bulla* with milk to treat bone fracture, in humans and corm to cure broken bone in cattle.
	Hayiwona	45	*Amicho* with yoghurt to remove spines and swells with pus from the human body, and to initiate milk production in human and cattle.
	Meqelwesa	60	*Amicho* for human, leaf and pseudostem for cattle to discharge delayed placenta after birth.
	Qiniwara	50	*Amicho* with dairy products to cure bone problems in human.
	Qombotira	32	*Amicho* with yoghurt to treat muscular cramps and waist problem in human.
	Xessa	42	*Amicho* with milk is eaten to relief broken bone in human.
	Xiggo	48	*Amicho* to cure kidney problems and hepatitis.
K- T*	*Astara*	38	*Amicho* to treat bone problems in human.
	Cherquwa	56	*Amicho* with dairy products to remove spines and swells from human body.
	Gishira	58	*Amicho* and roasted *bulla* with dairy products to treat bone problem in human and raw corm to heal broken bone in cattle.
	Qeqile-nech	46	*Amicho* for aborification purposes and to treat kidney problem.
	Qeqile-qey	60	*Amicho* to remove delayed placenta after birth in human, and pseudostem and leaf for the same purpose in cattle.
	Wolagella	36	Water squeezed from pseudostem to treat skin problem in human.
	Xessa	58	*Amicho* with dairy products to cure broken bone in human.

Table 3.9 Cont'

Gurage	*Astare*	60	*Amicho* with milk to treat bone and muscle problems, and for the initiation milk production in human after delivery.
	Dare	41	*Amicho* to cure damaged parts of the human body.
	Dem-yeter	45	*Amicho* with milk to remove delayed placenta in human.
	Guary	56	*Amicho* with milk to heal bone fracture in human.
	Oret	39	*Amicho* with dairy products to expel swells from human body.
	Qibnare	60	*Amicho* with cheese or yoghurt to treat broken bone and lung diseases in human.
Silte	*Agade*	47	*Amicho* with milk to cure bone problems of human and cattle.
	Ashaqite	38	*Amicho* with yoghurt to treat waist problem in human.
	Astare	60	*Amicho* with dairy products to repair broken bone, muscles, and to initiating milk production in human.
	Demworad	55	*Amicho* with milk to remove delayed placenta, to cure kidney and liver problem in human.
	Deriye	43	*Amicho* to heal damaged parts of the human body.
	Guary	56	*Amicho* with milk to cure bone fracture.
	Hayiwogn	48	*Amicho* with yoghurt to expel swells and any spiny materials from human body.
	Qiniware	60	*Amicho* with dairy products to treat broken bones, muscle and lunge problems in human.
	Sino	42	*Amicho* with dairy products to expel swells from human body.

Note: K-T*= Kembata Tembaro

This may be due to each ethnic community having its ways, practice, and beliefs to utilize enset plants. All of the traditionally medicinal enset landraces were also selected for sweet *amicho* (cooked corm) production except landraces *Gishira, Dare, and Bedededa.* In the same manner, the most chosen part of enset for medicinal use was corm but the landrace *Meqelwesa* (in Hadiya) or *Qeqile-qey* (in Kembata) were all part used as traditional medicine. In some cases, farmers also used cooked *qocho* or porridge prepared from *bulla* to treat different health problems in the study zones (Figure 3. 2f and g). In terms of connection to the ailments shared by the farmers and the medicinal enset landraces used in their treatment, we observed that bone fracture, swelling of the pus and to expel the delayed placenta from humans and cattle were the most shared health problems in the study area and among the communities. We observed that

some ethnic groups (e.g Silte and Gurage, Hadiya and Kembata-Tembaro) share more medicinal enset landraces and show greater similarity in patterns of using enset crop (Table 3.9). In the same manner, Kujawska *et al*. (2017) stated that intercultural sharing may be explained by the pharmacological effectiveness of shared medicinal plants among ethnic groups.

Most enset growing farmers in the study area are familiar with maintaining and use of their different preferred landraces to stabilize many situations over a long period without external support and inputs of planting materials. Farmers in the study zones frequently produce their planting materials or suckers from homegardens but few farmers obtain them freely from neighbors, family, and friends as a gift or by purchasing from other farmers. This was in line with the reports of Zerihun Yemataw *et al.* (2016) and Blomme *et al*. (2018).

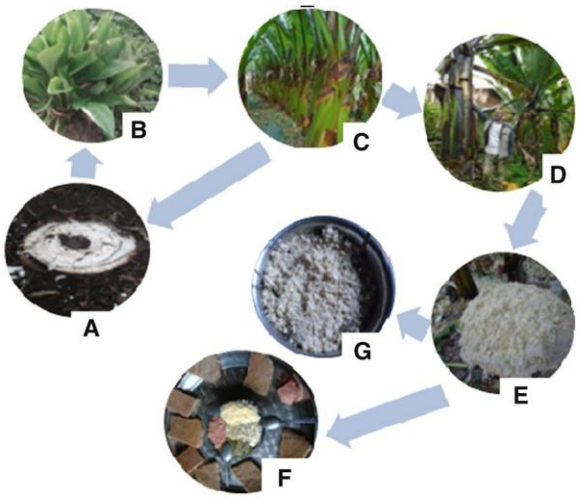

Figure 3. 2 Examples of the main enset propagation stages and its end products: a) mother corm; b) multiple suckers from mother corm; c) young enset ready to give mother corm and transplanted to permanent field; d) matured enset; e) mass of processed product; f and g) example of dishes prepared from the primary products

During our discussions with farmers and field observation, we observed that in two local markets: Alicho in Silte and Gumer in Gurage zones, enset suckers were purchased from January to April. These two sites are situated at a higher altitude than other studied *woredas* (Table 3.1). Moreover, some elder farmers from the Alicho and Gumer woredas mentioned that enset cultivation practice and its distribution into their *woreda* and villages were relatively late compared to others. They said that "We haven't been familiar with enset production and managing before 65 years ago." To some extent, this verifies that enset farming systems in the studied area are not equally and uniformly experienced within and among communities. Enset cultivation and use culture has been gradually and slowly moving to the peripheral areas from region to region, from zone to zone, and from district to district due to farmer "experimentation" and horizontal transfer of indigenous knowledge. In a similar vein, Brandt *et al*.(1997), Genet Birmeta (2004), and Borrell *et al*. (2020) indicated that the distribution of cultivated enset in Ethiopia appears to be expanding, especially after periods of devastating famines of the 1980s, when people in other regions learn about the benefits of this crop and attempt to incorporate it into their farming system. They have also shown that enset moves some distances into the Oromia region and this is observed in southwest Shewa and southeast Arsi and may be in the western part of Bale. Almaz Negash (2001) also noted that during the drought period, many farmers migrated from their villages to as far away in search of food, and there they learned about enset production. When they came back to their homesteads, they introduced enset. Furthermore, Satori *et al*. (2022) and Chase *et al*. (2022) have shown that smallholder farmers expand production area of the perennial crop enset as a climate coping strategy in a drought-prone indigenous agrisystem.

Table 3. 10 Preference in direct matrix ranking of five commonly shared and other two localized enset landraces cultivated in southern Ethiopia

Use value	landrace	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	Total	Rank
Qocho & *B** quality	*Agade*	7	8	7	6	7	6	8	8	7	9	8	9	90	3
	Astara	8	7	7	6	6	7	7	8	7	8	7	7	85	4
	Bededed	5	6	7	5	4	6	6	7	8	7	8	7	76	5
	Gimbo	10	9	10	9	8	10	9	8	7	8	7	8	103	1
	Gishira	4	3	3	4	5	4	0	0	0	0	0	0	23	7
	Separa	8	9	8	9	9	7	8	8	9	9	8	8	98	2
	Sisqella	6	5	6	7	6	7	6	0	0	5	4	0	52	6
Amicho tasty	*Agade*	7	8	8	6	7	7	8	9	8	9	7	9	93	2
	Astara	10	10	10	10	10	10	10	10	10	10	10	10	120	1
	Bededed	1	1	1	1	1	1	1	1	1	1	1	1	12	5
	Gimbo	6	7	6	7	5	6	6	5	6	7	5	6	72	4
	Gishira	1	1	1	1	1	1	0	0	0	0	0	0	6	7
	Separa	7	6	7	7	7	6	7	7	6	7	7	7	81	3
	Sisqella	1	1	1	1	1	1	1	0	0	1	1	0	9	6
Fiber quality	*Agade*	5	5	4	3	6	4	6	5	7	5	5	6	61	5
	Astara	4	5	4	3	4	3	5	6	3	6	4	3	50	6
	Bededed	7	6	7	6	6	7	7	7	6	8	6	7	80	2
	Gimbo	5	7	7	4	7	6	7	7	6	7	7	6	76	3
	Gishira	8	9	8	8	7	9	0	0	0	0	0	0	49	7
	Separa	5	6	6	7	5	4	7	4	5	6	6	4	65	4
	Sisqella	10	10	10	10	10	10	10	0	0	10	10	0	90	1
Medicinal use	*Agade*	7	6	8	4	5	3	8	7	9	6	7	7	77	2
	Astara	10	10	8	7	8	9	10	10	10	10	10	10	112	1
	Bededed	8	8	6	6	7	6	6	7	6	6	4	6	76	3
	Gimbo	6	3	2	4	3	1	3	1	3	1	2	3	32	6
	Gishira	10	10	10	10	10	10	0	0	0	0	0	0	60	4
	Separa	5	4	4	6	5	4	3	2	1	4	2	1	41	5
	Sisqella	3	2	1	2	1	1	1	0	0	1	0	0	12	7

Note: R for respondents, 10 for most valuable, 1 for least valuable, and 0 for the uses not known R1-R3 from Hadiya, R4-R6 from Kembata-Tembaro, R7-R9 from Gurage and R10-R12 from Silte zones; B* *Bulla*.

Farmers in the study area, maintain great enset landraces diversity within traditional cultivation and production systems insight towards meeting domestic subsistence requirements. Zerihun

Yematawe *et al*. (2016) also described that farmers observe and select the landraces based on their planting intentions for the coming year than the proportion to the quantity they have. The on-farm maintenance of biodiversity requires understanding by the farmer of how specific varieties should be grown, stored, and maintained to maximally realize the characteristics these farmers value (Edilegnaw Wale *et al*., 2011).

3.4 Conclusion

This study provides information on enset landraces existing in four major enset-producing administrative zones of Ethiopia based on local farmers-named landraces in each of the zones. The results obtained from this study indicate that farmers have developed diverse practices and experiences over time to cultivate, utilize and conserve a great extent of enset landraces in each zone. In addition, they understand the need to grow a mixture of enset landraces as this can have roles in the socio-economic and cultural life of communities. Our results have revealed that out of the cultivated enset landraces, a small proportion of landraces were widely distributed and abundant throughout the study zones. However, a larger number of landraces were highly localized in one or two studied zones and less distributed in other zones. Our study also confirms that farmers can distinguish their enset landraces by using their different local names. In this context, some enset landraces were commonly known and referred to by the same local names in all studied zones by different farmers. In contrast, enset of the same landraces were named differently by different farmers within and among studied zones. Moreover, results from this study also show that enset farmers have developed their way of selecting and characterization of landraces with some slight differences among them in terms of use patterns based upon their traditions and cultures in the study areas. Based upon the results of this study, the on-farm diversity existing in these landraces needs to be studied in detail (e.g molecular

characterization) for duplicates identification and clarification of synonymies, and to facilitate their on-farm conservation as well as sustainable utilization of enset farming communities and also in its improvement programs. A new study shows that frequent severe drought events led to an increase in enset production areas in Ethiopia. Indigenous staples are "saviors" during difficult times. This is why national investment in their conservation, improvement, and value addition is necessary for a changing climate.

Chapter Four

Farmers' local knowledge on classification, utilization, and on-farm management of enset (*Ensete ventricosum*) landrace diversity in Hadiya, central Ethiopia

Abstract

*Enset [Ensete ventricosum (Welw.)Cheesman] is a multipurpose perennial crop widely grown and consumed in Ethiopia. Although it is a high-value crop for local farmers, essential information on indigenous knowledge of its farming system, cultural practices, and traditional utilization is still an under-researched domain. This study was designed to assess and record the wealth of indigenous knowledge on enset, covering the local identification, categorization, and nomenclatural system in tandem with traditional uses of the enset crop. A random sample of 240 enset farmers from seven districts and 12 subdistricts of the Hadiya Zone were interviewed, along with four to six key informants selected from each subdistrict. D*irect *on-farm observation, 12 focus group discussions, and secondary data were considered in the documentation of indigenous and local knowledge associated with the enset farming system and use culture. We identified with the farmers 99 local farmer-named enset landraces under cultivation. Farmers identify and categorize enset landraces informally using morphological features as the main criterion. The local names of some landraces indicate their uniqueness in morphological* traits, place of origin, agronomic features, and quality attributes of their products. *Based on farmers' perception, cultivated enset landraces are grouped into* 'soft' (*qechalwesa*) and '*hardy'* (*qoxalwesa), considering characteristics regarding the strength of harvesting and processing, rate of fermentation,* and *quality of the end products. The indigenous knowledge about the cultural, social, and economic values of enset and its production system practiced by local farmers is crucial for the availability of the present landrace diversity. To sustainably use and conserve enset genetic resources, it is necessary to integrate indigenous knowledge with modern formal science, including in a field gene bank in Hadiya and other key enset growing areas in Ethiopia as a climate-smart crop.*

Key words/phrases: Ethnobotany, Farmer-named landraces, Enset landrace diversity**,** Local taxonomic system

4.1 Introduction

Traditional crops are those grown by local farmers over a long period and that are well adapted to their local conditions and also well suited to local socio-cultural needs (Villa *et al*., 2005; Ulian *et al*., 2020). Farmers have a wealth of indigenous knowledge on crop production in their communities acquired through accumulation of experience and informal experiments that could be useful for a robust crop breeding program, improving grain quality and storage equipment, designing weed and pest control strategies, organic seed conservation, food preparation, and environmental conservation (Tella, 2007). On-farm conservation involves farmers' continued cultivation and management of a diverse set of crop populations and accompanied taxa in the agro-ecosystem where the crop evolved, or in secondary centers of diversity (Alvarez *et al.*, 2005), where the landraces attained their characteristic features.

According to Vavilov (1951), the Ethiopian highlands are both the center of origin and diversity for many crops, including enset. Enset is believed to have been domesticated in Ethiopia by ancient people who migrated out of the lowlands and grasslands due to an extended dry period and settled in the highlands of southwestern Ethiopia and found enset that they probably started consuming and eventually cultivating (Brandt, 1996). Enset [*Ensete ventricosum* (Welw.) Cheesman] is a monocarpic, tree-sized perennial herb that belongs to the order Zingiberales and the family Musaceae. This family is subdivided into the genera *Musa* and *Ensete* (Simmonds, 1966). The wild form of *E. ventricosum* is common and widespread in tropical Africa, from Ethiopia to Kenya and Uganda, south to Mozambique, and west to the Democratic Republic of the Congo and Cameroon (Simmonds, 1958). *E. ventricosum* grows within altitude ranges of 1500–3100 m above sea level, but scattered plants can also be found at lower altitudes (Tesfaye

Abebe, 2005), and some scattered cultivation exists at higher altitudes (3350 m). For optimum growth, the plant requires an average rainfall of 1100–1500 mm per year and a mean temperature of 16–20°C. Enset grows well in drained and fertile soil types (Taye Bezuneh and Asrat Feleke, 1966), and it takes 8–16 years to reach full maturity (Shank and Cherent Ertiro, 1996), depending on environmental conditions, management practices, and varietal types.

Ensete ventricosum is an important crop contributing to food security and rural livelihoods for about a quarter of the people of Ethiopia, distributed in the densely populated southern and southwestern parts of Ethiopia (Brandt *et al*., 1997; Borrell *et al*., 2020). The enset-based farming system, which is characterized by the cultivation of enset as the main staple or as a co-staple with other crops, is an indigenous and sustainable agricultural system in Ethiopia (Westphal, 1975), and it is practiced by over 45 ethnic groups for food and other household needs (Brandt *et al*., 1997; Almaz Negash, 2001; Zemede Asfaw, 2018). It also guarantees food security and stability for the household economy because processed products can be stored for a long time. In addition, the live plant can be maintained on-farm and harvested any time when the need arises (Almaz Negash, 2001; Borrell *et al*., 2020). Farmers in enset-growing areas are often heard affirming that "enset is the enemy of hunger, both human and livestock life is impossible without it" (Admasu Tsegaye, 2002).

The primary food products obtained from enset are *qocho*, *bulla*, and *amicho*. *Qocho* is fermented starch obtained from scraped pseudostems and pulverized corms. *Bulla* is another enset product prepared by squeezing out the liquid containing starch from scraped pseudostem and pulverized corm and allowing the resultant starch to concentrate into a white

powder. *Amicho* is an inner, fresh corm used as cooked potato-like product of enset corm. This product is mainly obtained from relatively younger enset plants that are prepared and consumed in a similar way to other root and tuber crops (Brandt *et al*., 1997).

The social values of enset to the people in the southern and southwestern parts of the country not only depend on its being the source of human food and cash income but also consider it part of their cultural heritage and symbol, and it is a unifying crop for the highland areas of the southern parts of the country (Shigeta, 1990; Kefale Alemu and Sanford, 1996). Moreover, enset has ecologically important functions in producing organic matter, creating a nutrient reservoir in the soil, controlling erosion, and contributing to the stability and continuation of the farming system (Asnaketch Woldetensaye, 1997). Enset plant architecture makes it suitable for rainwater harvesting and storage. Furthermore, the fibrous roots of enset plants form a mat-like structure holding the soil intact, which, on decay, improves the soil. This water harvesting capacity in enset enables farmers to intercrop it with various crops (Tadessa Kanshie, 2002), but this is mostly the case in the early stage of the crop, as observed in the field as the later stages fill up the spaces when the plants expand and produce larger leaves.

The ethnobotanical naming system (sometimes called folkloric nomenclature) involves people giving names to plants based on their viewpoints. The people create these names in their willingness to distinguish the different types of plants from one another and to be precise in their verbal communication (Nedelcheva and Dogan, 2009). Indigenous (traditional) biodiversity knowledge has its roots in the local names of plants and animals. Since ethnobotanical systems of naming and classification are constantly transmitted from generation to generation and recreated by communities and groups in response to their environment (Khasbagan and Soyolt,

2008). Genet Birmeta (2004) identified numerous local farmers' varieties of enset from major growing regions and also observed genetic diversity in cultivated enset in a particular area, which appears to be related to the extent of enset cultivation, the culture, and the settlement patterns of the different ethnic groups. Farmers can clearly distinguish their varieties based on agromorphological traits, phenological attributes, post-harvest characteristics, and different adaptive performances under abiotic and biotic stresses (Brush and Meng, 1998; Loko *et al*., 2018). Moreover, they have names for them, and different landraces are understood to differ in their adaptation to soil type, time of seeding, date of maturity, plant height, nutritive value, use, and other properties (Sthapit *et al*., 2010).

Local farmers grow enset plants for several merits, which are mainly attributed to the food, socioeconomic, and cultural benefits. Although enset is a vital crop of high value for local farmers, necessary information on indigenous and local knowledge of its classification, management, and traditional utilization practices in different parts of the country is insufficiently documented and reported. Various authors (Bizuayehu Tesfaye, 2008; Melesse Maryo *et al*., 2014; Temesgen Olango *et al*., 2014) reported that the distribution of some indigenous crops (e.g., enset) at the local level in Ethiopia remains limited to the traditional areas of cultivation, though enset has gone into an expansion phase in its recent history. Previous studies in Hadiya enset farming systems have emphasized only the recording of cultivating and management practices as well as a listing of landrace names from the limited districts (Admasu Tsegaye and Struik, 2002; Zerihun Yemataw *et al*., 2016). The attributes of enset are still insufficiently known and not fully investigated by researchers and the scientific community. While enset is a crop that has lived with the Hadiya people for millennia with rich cultivation and use history and the

accumulation of time-honored ethnobotanical knowledge. The limited prior studies were focused on agronomy and the listing of landraces, while our study applied state-of-the-art ethnobotanical methods and showed the depth of the ethnobotanical knowledge of the Hadiya people, which is critical for improving enset research and for the development of enset genetic resources conservation strategies and sustainable uses.

Therefore, the objectives of this study were designed to search for and document the wealth of indigenous and local knowledge of enset cultivation, the local landrace taxonomic system (identification, naming, categorization, or classification), and traditional use and management of the species and its landraces. A related objective is to investigate and generate reliable data focused on farmers' enset-related ethnobotanical lore and the traditional practices relevant to the maintenance of on-farm enset landrace diversity in the Hadiya Zone of southen Ethiopia.

4.2 Materials and Methods

4.2.1 Description of the study area

The study was conducted in the Hadiya Zone of southern Ethiopia, located between $7^0$07'–$7^0$92'N and $37^0$29'–$38^0$13'E. This zone topographically lies within an elevation ranging from 501 to 3100 m above sea level, and its administrative center is Hossana town, located 232 km southwest of Addis Ababa, the capital city of Ethiopia. The zone is bordered on the south by the Kembata-Tembaro Zone, on the southwest by the Dawro Zone, on the west by the Omo River, which separates it from Oromia Region and the Yem special *Woreda (* special district*)*, on the north by the Gurage Zone, on the northeast by the Silte Zone, and on the east by the Alaba Zone; the *woredas* of Siraro, Mirab, and Misraq Badawacho form an exclave separated from the rest of

the zone and bordered in the north by Kembata-Tembaro, in the south by Wolaita, and in the southeast by Oromia Region (Figure 4.1). Based on the simplified agroclimatic classification of Ethiopia (MOA 2000), the zone has three agroecological zones, namely *Kola, or* semi-desert (lowland <1500 m), covering about 12.9% of the land area; *Woina Dega,* or cool semi-arid (mid-altitude 1500–2500 m), about 69.1%; and *Dega,* or cool and humid (highland > 2500 m), about 18%. Most of the area lies within the mid-altitude zone. For administrative purposes, the zone is divided into 13 districts, or *woredas,* which are further divided and organized into *kebeles* (subdistricts). Inhabitants (Dwellers) of the Hadiya Zone are mainly the Hadiya communities speaking the Cushitic language group, Hadiyyisa. For the midland and highland inhabitants of the Hadiya, enset cultivation and utilization make the main cropping system, a pillar of the farming system in the area. Enset is the key food security and livelihood crop for the humans and domestic animals of the study area.

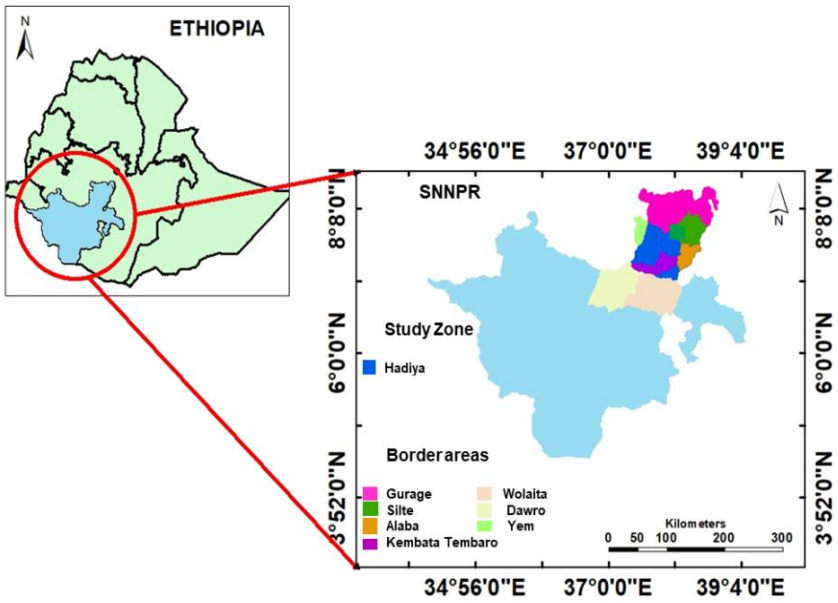

Figure 4. 1 Map of the Southern Nations Nationalities and Peoples' Region (SNNPR) in Ethiopia (left) and location of the Hadiya Zone with the zones bordering it (right)

4.2.2 Study site selection

The study was conducted in seven *woredas* or districts (Misha, Lemmo, Anlemmo, Dunna, Sorro, Gibe, and Mirab-bedawacho) in the Hadiya Zone, southern Ethiopia (Table 4.1). The sites were selected based on the potential and importance of enset cultivation, diversity, and distribution, as determined by the information obtained from the Agriculture offices of the zone and districts. Hadiya Zone is one of the major enset-growing areas in the country. Hence, the crop is the main food security and livelihood source, especially for the mid and highland inhabitants. Therefore, the study sites range in altitude from 1858 m.a.s.l. (Halelicho-Bero) to 2918 m.a.s.l. (Tulla) subdistrict (*kebele),* which cover nearly the entire ecological range of enset in the Hadiya Zone (Table 4.1). Since the local people of the selected study sites have managed and used enset, they also have adequate information about enset ethnobotanical lore encoded in its farming system, genetic resources, and uses.

4.2.3 Sampling

A combination of purposeful and random sampling techniques were employed for the selection of sample *woredas* from the Hadiya Zone since enset is primarily cultivated in the midland and highland zones of the study area. Of the twelve *kebeles*, five *kebeles* from the highland and seven *kebeles* from the midland found in the seven *woredas* of the zone were selected based on consultation with agricultural officers of the Hadiya Zone and the respective *woredas*. A total of 240 households (20 from each *kebele*) were selected randomly (Table 4.1). Among these, 143 (59.58%) men and 97 (40.42%) women participated in the individual interviews, with an average age of 47 years (lowest 33 years; highest 91 years). Four to six key informants were also chosen based on prior information obtained from agricultural extension workers, elders, and local leaders in each *kebele*.

Table 4. 1 Sampled *woredas*, *kebeles*, elevation range, and number of households interviewed

Administrative *Woredas*	Administrative *Kebeles*	Altitude (m)	No of households
Misha	Dangawora-Sagamo	2465–2784	20
	Shiro	2547–2730	20
	Tulla	2520–2918	20
Lemmo	Dijo-Demala	2245–2418	20
	Shurmo-Dacho	2218–2349	20
An-Lemmo	Bendelicho	2115–2263	20
	Layign-Fonko	2241–2485	20
Dunna	Qenqicho	2430–2568	20
	Somicho	2455–2676	20
Sorro	Qosha	2210–2402	20
Gibe	Halelicho-Bero	1858–2017	20
Mirab-Bedewacho	Danama	1875–1930	20
Total			240

4.2.5 Data collection

A combination of data collection methods was applied to synthesize the diverse features and collect indigenous and local knowledge associated with the enset plant in this study. Primary data collection was employed with the help of individual interviews, direct on-farm observation, key informant interviews, and focus group discussions. Before the actual study, a preliminary observation was arranged to pre-test the semi-structured interview questions to obtain a better understanding of the study area, the people, their culture and traditions, and the farming systems

with three elder enset-grower farmers per district (*woreda*). The in-depth individual interview was conducted by the investigator and agricultural extension workers in the local language, Hadiyyisa, using a pre-prepared semi-structured interview schedule with simultaneous translation from the pre-printed English version (Appendix 1). The agricultural extension agents who collaborated with the principal investigator were well known to the informants since they were working closely with the farmers in each farming activity. The interview questions and free-listing methods (Quinlan, 2005; Albuquerque *et al*., 2014) commonly employed in ethnobotanical research assisted in generating detailed and specific information on the enset farming system by specifically assessing farmers' views of landrace diversity, local naming, and classification, the meaning of names, traditional utilization approaches, cultural practices of cultivation, and the management system in the center of diversity. To obtain the detailed local knowledge of farmers in the study area, four to six key informant interviews were conducted with individuals who were selected for having good knowledge of the enset crop, culture, cultivation system, characteristics of landraces, and other related topics. Focus group discussions (one per kebele, each with six discussants) were also carried out to examine farmers' indigenous knowledge in joint or group interview sessions. Several issues were taken up for discussion, such as perceptions, farmers' identification and classification criteria of landraces, landrace names and naming systems, socio-economic values, cultural events, and so forth, and their insights regarding the contribution of enset agriculture to the local community. These techniques greatly facilitated gaining sufficient information in line with the objectives of implementing this study. Secondary data were also used to generate information that provided the background context of the enset farming system and cultural links to the farming communities of Hadiya from different published and unpublished sources and reports. Furthermore, on-farm characterizations of

landraces were recorded carefully for the identification of each enset landrace at maturity after examining it with individual farmers and key informants.

4.2.6 Data analysis

After recurrent discussions with key informants and knowledgeable senior farmers, we verified some variations and also validated basic information collected from individual interviews. The names of all listed enset landraces were also cross-checked for minor dialect (known as cognates in ethnobotany) variations after arranging in the group for known synonyms throughout the study *woredas*. The collected data were analyzed by applying descriptive statistics such as frequencies, percentages, and averages using Microsoft Excel 2010 and SPSS Version 24. Landrace richness was calculated as the total number of all individual enset landraces per farm named by all farmers dwelling in the studied administrative zone. Frequency was estimated as the number of individuals of a landrace with respect to the total number of landraces composing the enset farm.

4.3 Results

4.3.1 Status of enset cultivation in Hadiya

The cultivation of enset is closely associated with the daily lives of the Hadiya farmers, primarily in midland and highland areas. In the study area, farmers indicated that enset is a multipurpose crop available all year round, and throughout the various growth stages; the leaves and pseudostem in fresh and dried form, as well as corms, are used for several purposes. Thus, every farming household cultivates, manages, and utilizes diverse enset landraces. Farmers revealed that enset is the most important crop for livelihoods and food security in the major growing areas of the zone. Therefore, enset has been chosen as a classic multi-use crop since, except for its

biological roots, the whole part is used for diverse purposes such as food, feed, traditional medicine, construction material, cultural values, and ornamental uses.

During our discussion, many farmers have observed clearly, that most annual crops are severely disrupted by changing weather conditions such as prolonged drought, too little or heavy rain, and frost. This results in recurrent crop failures, leading to poor yields and food insecurity. However, different enset landraces can survive such unpredictable and harsh climate. Therefore, farmers generally approve that enset is a food and feed security crop that cannot be substituted by other crops for the enset farming communities in the studied area.

4.3.2 The extent of on-farm diversity and richness of enset

Hadiya farmers cultivate and maintain an impressive level of enset diversity in their homegardens. In this study, 99 vernacular names of enset landraces growing on farmers' fields were documented (Table 4.2). Based on the farmers' report and field observation, the number of enset landraces recorded per household ranged from 3 to 35. About half of the farmers (50.41%) cultivated 7–12 enset landraces, followed by 24.16% who cultivated 13–18; 17.5% of the farmers cultivated less than seven landraces, and only 3.3% cultivated 25–35 enset landraces per homegarden. The cultivation ranges of enset landraces maintained by farmers in the study area were expressed in percentages in Figure 4. 2.

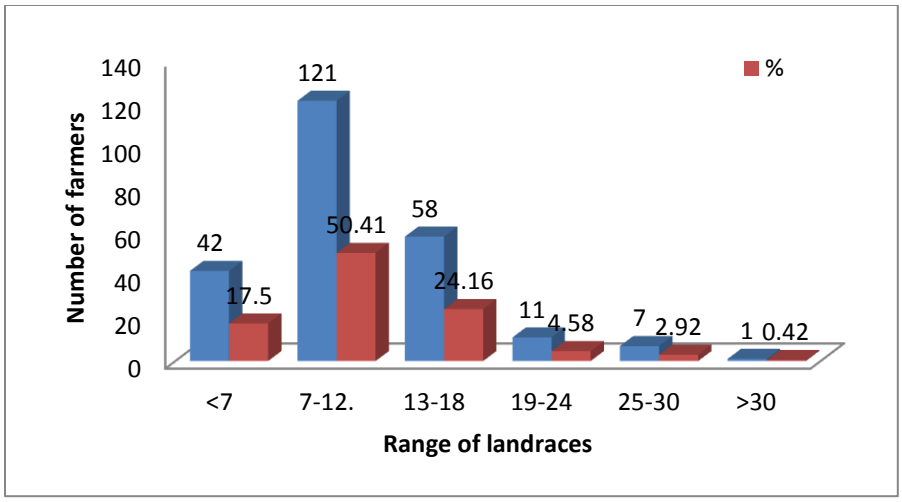

Figure 4. 2 The cultivation range of the enset landraces recorded in the homegardens of the Hadiya Zone, Ethiopia

In the present study, there was a remarkable variation among the enset landraces concerning their distribution. Of the recorded enset landraces, 21% were most frequently mentioned by local farmers and belonged to widely distributed enset types. According to farmers, these were multi-use types. The 15% were the least mentioned and unique types of enset landraces, rarely recorded at a few farmers' homegardens in the studied *kebeles* (Table 4.2).

Table 4. 2 List of enset landrace names known to the Hadiya community maintained on the farmers' homegardens and their distribution status

No	Status of enset landraces in Hadiya Zone			
	Common	**Moderate**	**Rare**	**Unique**
1	*Agade*	*Agandiya*	*Anchire*	*Ado*
2	*Bedededa*	*Alabite*	*Bequcho*	*Arke*
3	*Beneja*	*Astara*	*Beshiqiya*	*Ashame*
4	*Dirbo*	*Awuneda*	*Bezeria*	*Benqo*
5	*Disho*	*Ayase*	*Boshosha*	*Chalqo*
6	*Gimbo*	*Boicho*	*Bossora*	*Gasupa*
7	*Gishira*	*Dego*	*Butto*	*Gulfea*
8	*Hayiwona*	*Etine*	*Danxicho*	*Kerqerea*

		Table 4.2	**Cont'**	
9	*Hiniba*	*Gariya*	*Feraziya*	*Laddare*
10	*Merza*	*Hanzena*	*Gagabo*	*Ladore*
11	*Moche*	*Jegireda*	*Gemmera*	*Lokanda*
12	*Oniya*	*Kaseta*	*Geremeda*	*Mazawora*
13	*Separa*	*Kekera*	*Ginawe*	*Qeteqeta*
14	*Shate*	*Korina*	*Gomersa*	*Wee'a*
15	*Sherafire*	*Lechebo*	*Gozoda*	*Xiggo*
16	*Shewora*	*Manduluqa*	*Gudere*	
17	*Sisqella*	*Mariye*	*Haqucho*	
18	*Unjame*	*Mesmesia*	*Hyrio*	
19	*Uzguruza*	*Mutite*	*Lendwesa*	
20	*Xorora*	*Necho*	*Leqeqa*	
21	*Zobira*	*Orada*	*Meqelwesa*	
22		*Qiniwara*	*Michorera*	
23		*Qombotira*	*Ososa*	
24		*Quina*	*Qargae*	
25		*Shellege*	*Qebere*	
26		*Soqido*	*Qenchowa*	
27		*Tegadeda*	*Qeshqashe*	
28		*Wonade*	*Qitira*	
29		*Woshamaja*	*Sheraqa*	
30		*Xessa*	*Sinera*	
31			*Suwandiya*	
32			*Wocherda*	
33			*Wohee*	
Total	21	30	33	15
%	21.2	30.3	33.3	15.2
Grand total				99

Common Enset landraces are found in all studied *kebeles*, *Moderate* Enset landraces occur in more than four *kebeles*, *Rare* Enset landraces are recorded in two to four *kebeles*, and *Unique* Enset landraces recorded in only one *kebele* of the studied area.

During the discussions and interviews with farmers, it was also found that the local people in the study area cultivated different enset landraces based on several selection criteria, such as end-product quality and quantity, fermentation and maturity rate, medicinal values, disease resistance, and drought tolerance, and that they often mixed landraces with these attributes in each garden.

4.3.3 Farmers' traditional categorization of enset landrace

In Hadiya Zone, farmers regularly identify and classify numerous enset landraces based on different criteria. Some of the criteria that farmers frequently use to distinguish one enset type from others are: color of pseudostem, petiole, and midrib; size (width and length); angle of leaf orientation; various end uses; disease and drought tolerance characteristics. Generally, the local farmers follow three main traditional categorization steps in authenticating their enset landraces now under cultivation. First, they identify the landraces; second, they give them different local names; and finally, they classify them into various groups based on common characteristics.

4.3.3.1 The Indigenous practice of enset identification

All the informants in the study area affirmed that they could distinguish all of their enset landraces growing in their respective homegardens based on combinations of some common identification features. They commonly used three identification characteristics: (i) four morphological characters, which include the color of pseudostem, petiole, midrib, and leaf blade (Figure 4.3); (ii) agronomic characters (reaction to drought and disease, susceptibility to pests, maturity time, and vigorousness); (iii) end-use value (*qocho* and *bulla* yield and quality; *amicho* use and taste; fiber quality; and medicinal value). In some cases, farmers also use limited characters such as fluid color and angle of leaf orientation for identification of their enset

landraces. During our discussions with local farmers about key selection criteria, the morphological characteristics of a landrace were identified as the major and first ones (Figure 4. 3). However, the agronomic and use-value characteristics identification criteria came only after morphological characteristics. Identification criteria such as fluid color were used for the identification of landrace *Xiggo*, which means 'the bleeding type', referring to the red sap color oozing out upon cutting or wounding parts of the leaves and/or pseudostem as compared to the watery fluid of most of the other enset landraces. Similarly, the angle of leaf orientation as a descriptor was used for some landraces

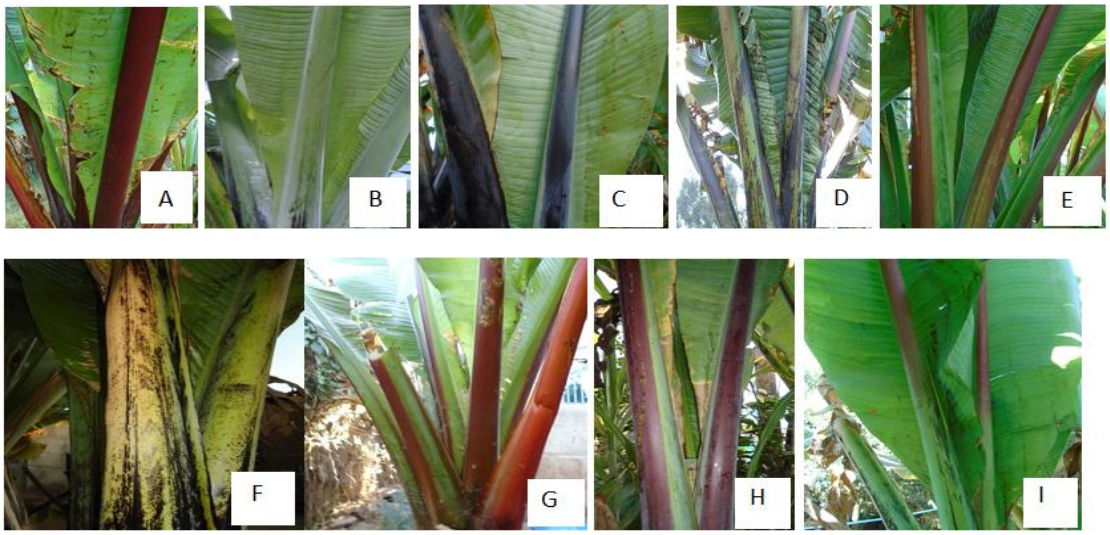

Figure 4. 3 Representative morphological variation in midrib and petiole color farmers used for identification in Hadiya: (A) *Korina*; (B) *Boicho*; (C) *Qebere*; (D) *Separa*; (E) *Qombotra*; (F) *Hella*; (G) *Etine*; (H) *Astara*; (I) *Kaseta*

(e.g., landraces *Hiniba* and *Beneja*) known for their narrow-leaf orientation or erect leaf arrangement, whereas landraces *Oniya* and *Sisqella* were identified by most farmers as having a

more bent or wide type of leaf orientation as compared to the other enset landraces (Figure 4. 4A).

Figure 4. 4 Variation in the angle of leaf orientation or bending and color of lamina for farmers' identification as descriptors: (A) from left to right, *Sisqella* (wide-dropping), *Hiniba* (narrow-erect), and *Separa* (intermediate); (B) color of leaf lamina, red for only landraces *Meqelwesa*, green for all others

Pseudostem color, petiole strip color, and midrib color were the most frequently used characteristics (Figures 4.3 and 4.5), while the leaf lamina color, fluid color, and angle of leaf orientation were descriptors mentioned less frequently for the identification of enset landraces by the farmers in the study area.

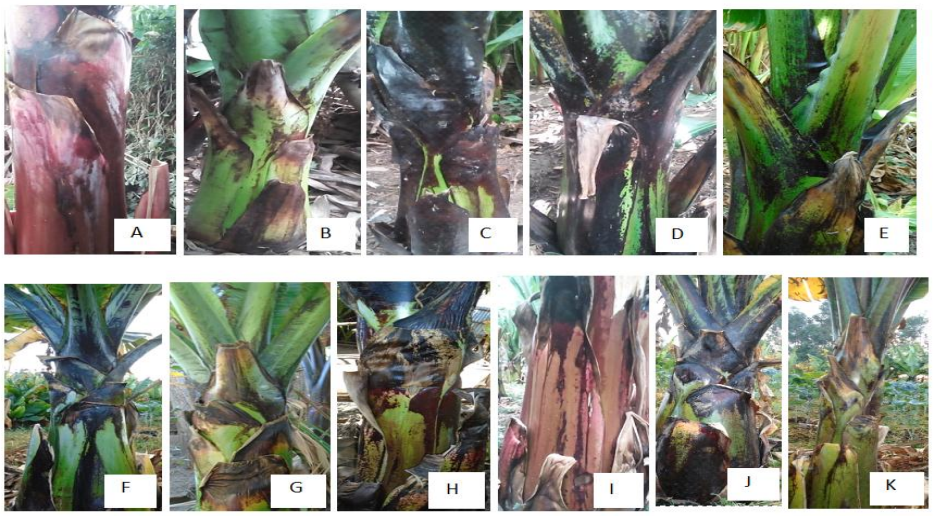

Figure 4. 5 Examples of pseudostem variation in color and in end-use value: (A-E) landraces with sweet *amicho*, *Astara*, *Leqeqa*, *Qebere*, *Xessa*, and *Orada*; (F-H) landraces with non-edible and hard *amicho*, *Sisqella*, *Gishira*, and *Hella* from left to right; (I-K) landraces considered best in fermented products (*qocho* and *bulla*), *Gimbo*, *Separa*, and *Hiniba* from left to right

Almost all recorded enset landraces (Table 4.3) had green leaves except one enset landrace named *Meqelwesa* with a deep red leaf lamina (Figure 4.4B). For the color of the midrib, the dominant colors were: 35% red; 33% green; 26% white-yellow pigmented; and the black and dark-red pigments accounted for only 4% and 2% of the landraces, respectively. For petiole, the green color was in 37% of the landraces, red in 29%, brown in 19%, grey in 9%, and black in 6% of the landraces. Green and white-yellow were the major pseudostem colors in 31% and 28%, respectively, whereas grey (6%), black (4%), and dark-red (2%) were the least occurring pigments in landraces. With regard to leaf shape and pattern, the intermediate leaf orientation was dominant, accounting for 91%, while narrow-erect and wide-dropping patterns each accounted for 4% (Figure 4.4A). With sap or fluid color, only one enset landrace, *Xiggo*, was recorded as having reddish fluid.

4.3.3.2 Farmers' local names and naming of enset landraces

In Hadiya, after identification, enset growers give distinct vernacular names for each landrace based on several attributes, which can either be external appearance or internal quality. Farmers can easily distinguish each landrace from others based on their local names. The names of landraces were often consistent and shared among the farmers, and each name usually reflecte a clear variation of each landrace from the others. However, in this study, we observed that some enset landraces have alternative local names given by farmers; e.g., *Ayase*, *Butto*, and *Gulfea* are also known as *Hella*, *Birwesa*, and *Fecheche*, respectively.

Table 4. 3 The four common frequently used morphological characteristics (leaf, midrib, petiole, and pseudostem colors) and the other two descriptors

Identification criteria (Enset morphology)	**Color (descriptor state)**	**Examples of representative landraces**	**Percentages of color (frequencies)%**
Leaf blade color	Green	*Gimbo, Agade, Merza, Sisqella*	99
	Red	*Meqelwesa*	1
Midrib dorsal color	Dark-red	*Meqelwesa, Korina*	2
	Red	*Gimbo, Astara, Wonade, Etine*	35
	White- yellow	*Moche, Anchire, Sinera, Gudere*	26
	Green	*Hiniba, Shewora, Kaseta, Agade*	33
	Black	*Qebere, Boshosha*	4
Petiole color	Green	*Quina, Shewora, Shate, Agade*	37
	Red	*Gimbo, Wonade, Etine, Jegirada*	29
	Brown	*Hella, Gozoda, Merza*	19
	Black	*Lokanda , Qebere, Boshosha, Soqido*	6
	Grey	*Hayiwona, Sherafire, Awunada*	9
Pseudostem color	Dark-red	*Korina, Meqelwesa*	2
	Green	*Hiniba, Agade, Kaseta, Xorora*	31
	Red	*Gimbo, Astara, Wonade, Etine*	19
	White-yellow	*Moche, Anchire, Sinera, Gariya*	28
	Brown	*Separa, Gozoda, Merza, Unjame*	10
	Black	*Boshosha, Qebere, Soqido, Lokanda*	4
	Grey	*Hayiwona, Awunada, Ososa*	6
Pattern of leaf growth	Narrow-erect	*Hiniba, Hyrio, Beneja*	4
	Intermediate	*Gimbo, Wonade, Boicho, Separa*	92
	Wide-dropping	*Oniya, Qebere, Sisqella*	4
Fluid (sap) color	Red fluid	*Xiggo*	1
	Watery fluid	All other landraces	99

Farmers always refer to the local names of enset landraces when propagating, planting, managing, harvesting, utilizing, and exchanging enset planting material in the study area. A general vernacular term used as a local name for enset in Hadiya is *Wesho* (singular), and *Wessa* is the plural form of the name for the enset plant. The local names given to some enset landraces are usually indicators of variation and reveal the uniqueness of landraces in morphological traits, places of origin, agronomic features, and quality attributes (taste and color) of end products (Table 4.4). For instance, the local names reflect a broad spectrum of information and may include indications of observed phenomena (e.g., *Benqo*, the Thunder), names of places, landrace names (e.g., *Gudere*, the name of the local river), and animals (e.g., *Bequcho*, mule, and *Moche*, wild) (Table 4.4). Local farmers also used different criteria to distinguish their enset landraces in the homegarden such as literally using words in the local language to describe the specific morphological characters (e.g., *Jegirade*, tall; *Ado*, milk); growth attributes (e.g., *Gimbo*, gaint); and cooking quality attributes of specific landraces (e.g., *Soqido*, salt, referring to the taste of the boiled corm or *amicho*). Sometimes, the local names are given based on the functional attributes (e.g., *Meqelwesa*, placental enset; *Sisqella*, repels or breaks the scraping bamboo; and *Unjame*, gets less attention from growers and users) and other peculiar characters (e.g., *Xiggo*, bleeding; red fluid from its parts; and *Lendwesa*, girl's enset) (Table 4.4).

Table 4. 4 Examples of local enset landrace names with their meanings, descriptions, and identification criteria for the naming in Hadiya, southern Ethiopia

Examples of local landrace names with their meaning	**Description of the landrace name**	**Local naming of landraces after the morphological, agronomic and other features**
Ado (milk)	Referring to the white or creamy color of the pseudostem and midrib	typical morphological trait
Xiggo (bleeding)	Referring to the red fluid from the midrib	
Butto (mixture of black and white)	Indicating the spotted petiole and pseudostem	
Chalqo (weak, unable to stand)	Leaf and pseudostem strength	
Qeshqeshe (fragile)	Implying pseudostem and corm strength	
Qombotira (fragile)		
Necho (thin)	Referring to the size of a leaf	
Gimbo (huge), *Jegirada* (as tall as pillars), *Benqo* (the thunder)	Implying size and length	
Gagabo (fast)	Indicate the early maturity of the landrace	typical agronomic feature
Lendwesa (girl's enset)	Easily harvestable	
Unjame (gets less attention)	Implying fewer managements and care to cultivate it.	
Qebere (wide, bended)	Implying the pattern of growth	
Bequcho (mule),	Indicating the strength during harvesting	name of animals, person, local river, and origin of the place or source
Moche (wild)		
Gudere (name of local river)	Referring to the place where landrace was obtained	
Mesmesicho (Clan group in Hadiya)		
Lechebo (name of the people)	Implying the name of the	

Gemera (name of the person)	person who introduced landrace to the area	
Soqido (salt), *Shate* (bitter)	Implying the taste of *amicho* and other parts	cooking or other qualities of landraces
Sisqella (break bamboo, the screptors)	Implying the strength during harvesting	traditional harvesting tools
Meqelwesa (placental enset)	Implies its importance to expel the placenta	typical traditional use of landrace
*Bezeria** (gust)	Indicating the suitability of the corm to feed the visitors	landraces may be borrowed with their names from the neighboring ethnic group
*Feraziya** (horse) *Wonade** (mare)	Referring to the size of the enset	
*Hyiro*** (the sun)	Reflects the color of the landrace	

* and ** Landraces may have been borrowed with their names from the neighboring zones Gurage and Silte, respectively.

The majority of enset local names are single expressions, semantically unitary. However, a limited number of the identified landrace names are organized into 'secondary' names by adding modifiers that further describe the landrace. For instance, landrace local names *Qedel-sisqella*, *Hemach-sisqella*, *Buch-xorora*, *Buch-gariya*, *Kesher-bedadeda,* and *Hemach-bedadeda* are derived from 'primary' landrace names *Sisqella*, *Xorora*, *Gariya*, and *Bedadeda,* respectively; the additional modifiers describe the color of the landraces. In general, the local names regularly describe some salient features and information about the enset landraces or their parts, in which the local farmers are interested, and it makes communication easier. However, the majority of enset landrace names do not refer to anything obvious; they are simply names for differentiating one enset type from others, or else the linkage is no longer traceable to the original name due to modification over a long period.

4.3.3.3 Farmers grouping systems of enset landraces

In Hadiya, enset growers, after identification and naming, classify their enset landraces according to their cultural competence, experience, and interest. Local farmers in the study area use different traditional classification systems for their enset landraces. Almost all of the respondents agree that enset landraces are mostly grown for food, fodder, fiber, and medicinal purposes. However, landrace *Meqelwesa* is planted only for its traditional medicinal values. Generally, all other enset landraces were cultivated for both food and non-food uses.

Based on focus group discussion and key informants, cultivated enset landraces are classified into two general groups according to their use or characteristics regarding the strength of harvesting and processing, rate of fermentation, and quality of end value (product and usage). These were grouped as 'soft' (*qechalwesa*) and 'hardy' (*qoxalwesa*) enset landraces**.** According to them, 'soft' enset (*qechalwesa*) landraces are easily harvestable and involve simple processing, early fermentation, less fibrous, and more preferable products. In addition, this group includes the majority of medicinally important landraces. Regarding corm cooking quality or palatability (*amicho* taste), farmers also classify 'soft' enset landraces into three subgroups: sweet, medium, and inedible types. Farmers in Hadiya listed out over 31 enset landraces with sweet *amicho* (tender corm). Some examples of those landraces were *Soqido, Leqeqa, Xorora, Astara, Qiniwara, Orada, Gariya, Qebere, Quina, Qombotira, Qorate, Mazawora, Ososa, Arke, Qeshiqeshe, Korina, Agandiya, Boshosha, Boicho, Gozoda, Xessa,* and *Shereqa*. The enset landraces have edible corm (*amicho*), although they were ranked in the second position (*amicho* with a medium taste), which includes *Agade, Separa, Tegededa, Mutite, Ancheqera, Hayiwona, Hiniba, Shewora, Gimbo, Kekera, Etine,* and *Zobira.* The 'soft' enset landraces with inedible

corm types include *Shate*, *Woshemaja*, *Necho*, *Menduluke*, *Moche*, *Oniya*, and *Hanzana*. The landrace *Shate* is known by most farmers for the bitter taste of all its parts, and it is considered to have better resistance to some enset diseases, including the disease locally known as *alloya*, which may be a kind of bacterial wilt that commonly infects enset crops.

According to the local farmers' characterization, 'hardy' enset (*qoxalwesa*) landraces also have specific characteristics that differ from those of the 'soft' enset group, such as difficulty in harvesting and processing, late fermentation, more fibrous, unpalatable corms, being less attractive to mammalian pests, and being more tolerant to drought. These landraces include *Sisqella, Disho, Unjame, Bequcho, Gishira, Anchire, Shelleqe, Beshiqiya, Dirbo, Qitira, Lendwesa, Sisasira, Dego, Hella,* and *Lokanda*. Some are early maturing (e.g., *Sisqella* and *Disho*), while landraces such as *Anchire* and *Shelleqe* are late maturing. *Sisqella*, *Disho*, and *Gishira* were more common landraces, whereas *Bequcho*, *Lendwesa*, and *Anchire* were rare in all the study sites. Farmers in the study area select *Sisqella*, *Unjame*, and *Disho* landraces to be the best not only in terms of yield but also in terms of quality such as strength, length, and durability of fiber production. These enset landraces are among the most commonly used types for livestock fodder during the scarcity of grasses. Out of the described 'hardy' enset (*qoxalwesa*) landraces, only *Gishira* was perceived as medicinal for humans and livestock, but most of the other medicinal enset landraces belong to the 'soft' enset landrace category.

In the study area, very few farmers used other classification criteria to classify enset landraces based on their adaptability to different ecosystems at higher elevations. They grouped *Hansawa wesa* (high altitude) and *Kala wesa* (low altitude) enset landraces. However, while the majority

of the landraces can be adapted to both highlands and lowlands, the farmers claim that some 'soft' enset landraces are specifically adapted to *Hansawa* ecosystems.

4.3.4 Indigenous use of enset

4.3.4.1 The traditional food of enset

In Hadiya, every household cultivates enset in their homegarden as the main staple or co-staple food crop, and different dishes are derived from three primary enset products (*wassa* or *qocho,* buoo or *bulla*, and *hamicho or amicho*). In Hadiya, the term '*wassa'* represents both steam-baked flat bread and the fermented mixture of the decorticated pseudostem, grated corm, and *gammama* (starter). The *gammama* (starter*)* is prepared in the central part of the corm, while in the ground, by pulverizing using the serrated end of a wooden tool locally known as *a Jango,* and this result in a bowl-shaped cavity at the center of the corm. Subsequently, the empty sides of the hollowed-out corm cavity are rubbed by the pre-fermented central pith of the pseudostem of the young enset, followed by adding the chopped pieces of the pith (the soft and central) from the center of the pseudostem. In addition, clean water is added and mixed within the cavity. The filled cavity of the corm is tightly wrapped with enset leaves and leaf sheaths, which are left attached at the base for the final package. A heavy load is placed on the pack and left until it is fermented and required for use. Of the primary enset products, *qocho* is the most commonly consumed and largely produced. According to the interviewed farmers, the favored *qocho* type is white, while the lowest grade is brown. They also identified that most enset landraces have good *qocho* quality when harvested immediately upon flower setting. Based upon the information obtained from the individual interviews, the key informant, and focus group discussions, Hadiya farmers distinguish three types of *qocho* (fermented enset products):

(i) *Tiqoota* is the *qocho* type prepared by mixing the decorticated psedostem and grated corm of enset with early fermenting landraces without the need for adding starter or *gammama*. The *gammama* is mostly prepared with *Gimbo*, *Shirafire*, *Uzguruza,* and others. This *qocho* can be ready for consumption within 8 to 10 days. It is considered a 'poor household's food, regularly eaten in seasons of food scarcity. The favored enset landraces for *tiqoota* are 'soft' enset types. (ii) *Buhesso* is also a type of *qocho* prepared from the early fermented enset groups but by adding the starters, or *gammama*. It is also available for consumption within 10–15 days. (iii) *Gojjo'o* is the *qocho* type prepared from the scraped psedostem and grated corm of any enset ('hard' and 'soft') types mixed with *gammama* (starter). It is usually available for feeding after a month; in addition, it can be stored for a long period. In the community, *gojjo'o* is considered the best type of *qocho* because of its longer fermentation period. People in the study area believe that the longer the fermentation period, the higher the *qocho* quality.

Bulla, locally called *buoo,* is obtained by squeezing a mixture of the unfermented decorticated pseudostem and pulverized or grated corm. It is considered the best-quality enset food and is obtained mainly from fully mature enset plants. The local farmers frequently mentioned that enset landraces such as *Gimbo*, *Separa*, *Hiniba*, *Hayiwona*, *Awunada*, *Beneja*, *Astara*, *and Etine* are known for the production of quality *bulla* as they give white and visually more attractive food products. The Hadiya community indicated that a variety of dishes prepared from *bulla* are traditionally incorporated into different cultural and religious events, such as births, male circumcision ceremonies, weddings, and festivals. During births, a postnatal mother eats *moqqa*, which is a special porridge prepared from *bulla* by mixing it with butter and spices. The traditional festival of the New Year in Hadiya, called Yaa-hode (in September, *Masqala*), is

celebrated by eating special enset foods. The main and most known of them is called *atakana*. *Atakana* is made mainly from dried, powdered, and roasted *bulla* on flat clay or iron material and then cooled. To make it suitable to be eaten with a spoon, it is spiced with various ingredients, such as milk (yogurt), cheese, and purified butter. *Atakana* is usually prepared for special guests (visitors) and some other special occasions; in addition, it is typically consumed on the eve of Yaa-hode and throughout the celebration weeks. Different *bulla dishes* also serve as traditional medicinal value to treat different health problems, including bone fractures and muscle cramps in humans.

Hamicho (*Amicho*) is the cooked enset corm, usually of a medium-aged enset or before the flowering of the plant, and eaten directly with no processing separately like other tubers or with other foods such as dairy products and vegetables. Therefore, it is commonly harvested for immediate consumption. In Hadiya, the enset growers distinguish and prefer some enset landraces for *hamicho* (cooking type). Landraces such as *Astara*, *Soqido*, *Qiniwara*, *Leqeqa*, *Gariya*, *Quina, Orada, Qebere, and Xorora* were highly recognized by the local people for their *amicho* consumption. These enset groups are conserved and managed with special care. For instance, before the harvesting stage, supplying wood ashes and other household wastes is preferable in the local communities rather than using animal manure for the *amicho* (cooking type) landraces. Farmers consider that frequent manuring will reduce the palatability of *amicho* (cooked corm). However, manuring these landraces at early stages is a common practice by local farmers, and it is also used to keep mammalian pests (e.g., porcupines) out of the enset field. In the study area, female farmers indicated that the amount and quality of the corms are among the

best components that affect the quality and yield of *qocho* and *bulla*. Generally, the corms of the enset plant have more useful values than their leaves and pseudostem.

4.3.4.2 Traditional medicinal use of enset

Many enset landraces have played a crucial role as a source of traditional medicine in the study area since time immemorial to combat different human and livestock ailments. Local farmers consider some of the enset landraces for medicinal purposes; among these, *Qiniwara*, *Astara*, *Gishira*, *Xessa*, *Gariya*, and *Agade* are commonly used by most of the local community members for problems related to bones and joints (Table 4.5). Enset landraces that are said to have medicinal values are also used for food and other purposes, indicating that enset is a nutraceutical plant. However, it needs to be noted that the landrace *Meqelwesa* has a deep red color in all its parts and was reported only for medicinal purposes, where the cooked corm is recommended for humans and all parts for livestock to discharge delayed placenta afterbirth and as an abortifacient. In the study area, local farmers indicated that the corm was the most frequently utilized part of the enset plant for purposes of traditional medicine. In a few cases, other plant materials, including inflorescence, leaves, and pseudostem, were mentioned as having medicinal value (Table 4.5).

Table 4.2 Enset landraces selected for medicinal purposes to treat various ailments

Enset landraces	**Traditional treatment of ailments**	**Enset part (product) used and additives recommended**
Qiniwara, *Xessa*, *Gishira*, *Gariya*, *Astara* , and *Agade*	Bone fractures and joint displacements	
Hayiwona and *Orada*	A painful swelling and other damaged parts of the human body	Cooked corm (*amicho*) with yoghurt
Qombotira	Muscular cramps and waist problems	
Xiggo	Kidney problems and hepatitis	
Meqelwesa	Delayed placenta	
Shate and *Moche*	Most skin infections or problems in humans	watery fluid squeezed from the pseudostem
Separa	Coughing in children	soup prepared from the inflorescence
Hayiwona and *Bedededa*	Dairy cows' failure to produce milk after giving delivery	the raw corm
Gimbo	Hepatitis	the prepared fresh *bulla* with milk
Most enset landraces	Dysentery	*qocho* prepared from a thoroughly fermented enset products.

4.3.4.3 The cultural and economic value of enset

Based on the respondent's information, cultivating and conserving a large number of enset plants around the homegardens has specific cultural value and meaning for the midland and highland dwellers of Hadiya communities. For the local people, enset is not only a food crop but also a multi-use crop and an expression of their identity. According to the informants from the study

area, enset farming is an age-old agricultural practice, which indicates that local farmers and enset have been highly intimate for many generations. In the study area, it is common to hear local community members saying, "Enset is life for us and our cattle." The cultivation of enset and the rearing of cattle are directly linked to the local people. In the study area, cultivation of enset is understood as a family inheritance and starts in childhood. However, the interviewed farmers affirmed that they independently initiated cultivating enset standing on the plot of land with various landraces they inherited from their parents in adulthood after getting married. In Hadiya, most households offer a plot of land, a cow, and an ox with diverse enset landraces to an adult married son as a longstanding tradition passed from generation to generation.

The respondents indicated that enset farming is crucial in cultural, economic, and family daily life. Farmers with a large number of more mature enset plants around their homegardens obtain local community appreciation and particular respectful local titles (names) such as *Asmache*, *Gerada*, *Berkafatta*, *Abaa-gadda*, and so on for men, and *Ajete* or *I'tee* for women. These farmers and their households are considered rich in the community, which reveals the cultural values of enset in determining the social position or recognition as a higher social class and its high intimacy with the community. As much as it places one in a higher social class, the extent of enset ownership also places one in a higher wealth status and hence at a higher economic level.

4.3.4.4 The gender roles in management and use of enset

According to informants' responses and our field observation, female farmers play key roles in different kinds of day-to-day activities with enset plants, such as manuring, landrace selection, harvesting, processing, storing, preparing different types of daily meals from the various

products of enset, and marketing. Local farmers in the study area noted that if women were not involved in harvesting and processing the enset crop, it would be very difficult to obtain *qocho* and *bulla* from it. Moreover, female farmers can easily recognize the different landraces, their detailed features and uses, and the quality and quantity of the product of each landrace. On the other hand, men are involved in preparing the land, propagating, planting, and transplanting activities.

The enset plant is symbolically linked to the female farmers' identity, where clear gender boundaries can be recognized. The farmers at the study sites also indicated that women have control over and entirely manage the enset field because it is an important source of daily income from the sale of products and materials extracted from it. They sell *qocho*, *bulla*, fiber, fresh leaves, and mattresses (local beds). In the study area, all activities related to enset cultivation can provide women with reliable and mutual support as well as a means for social independence and economic self-sufficiency.

4.3.5 Indigenous cultivation and management practice

In Hadiya Zone, farmers regularly cultivate mixtures of enset landraces to maintain on-farm landrace diversity and to compensate harvested enset for different consumption throughout the year. This traditional cultivation pattern involves three consecutive stages: vegetative propagation, transplanting, and harvesting.

4.3.5.1 Perpetual propagation

In the study area, enset propagation is a common practice conducted by all local farmers every year to regulate the enset cycle and maintain on-farm landrace diversity. Enset growers regularly propagate diverse landraces available in the homegarden. Farmers choose an appropriate place and a mixture of landraces for propagation. Based on our field observations and interviews, all farmers use vegetative propagation from the corm of a four-year-old enset plant, locally called *kiniba* (Figure 4.6A). They uproot it and cut it with a sharp knife or sickle at the junction of the pseudostem and corm. The center of the corm is removed, filled with soil to eliminate the central growing bud (apical meristem), and stored in a shaded area upside down for one or two days. The informants indicated that eliminating the central portion helps to produce more suckers; otherwise, only one large sucker, locally known as *morra*, may be formed. The newly developed suckers, locally called *dubo*, emerge mostly after three months and remain on the mother corm for a year. The propagation is usually carried out from November (mostly highland areas) to January (commonly midland areas) on the offset of the moon.

Based on their favorite types and availability, some landraces are more frequently propagated, almost every year. According to farmers' reports and the researcher's field observation, the number of suckers obtained from a single mother plant varied due to different factors such as the types and size of landraces, the season of propagation, management practices, and the amount of rainfall. Farmers in the study area reported that 30 to 150 suckers will be obtained from a single mother plant of four-years-old *kiniba* (Figure 4.6B). During the interviews, farmers asserted that they believe some enset landraces are mothers or ancestors for most of the phenotypically similar

and the same end-value landraces. For example, they said that *Gimbo* is considered a mother for most landraces of red (pseudostem, petiole, and midrib) color with edible *amicho* groups.

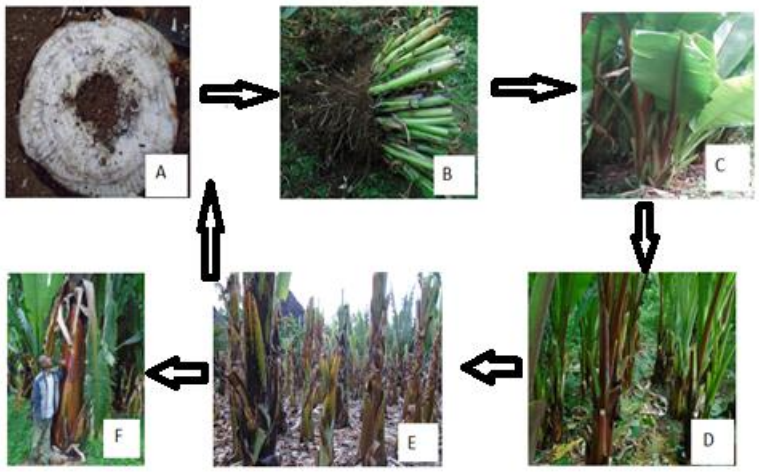

Figure 4. 6 Enset propagation and transplanting practices in Hadiya: (A) A mother corm (*Hamicho*) selected from *kiniba*; (B) Emerged multiple suckers (*dubo*) ready for transplanting; (C) *Simma,* the suckers detached from mother corm and planted into a single hole as groups of 4 to 6 in batches; (D) The *lammo* suckers are transplanted in groups of 2 or 3 in the middle rows of the larger suckers known as *ogojaa,* which are planted individually in a row; (E) The *kiniba* stage is ready to transplant in the permanent field and/or for the suckers (*dubo*) production; (F) *Ballwesa,* the last stage where the enset remains permanently until harvesting

4.3.5.2 Frequent transplanting

Enset farmers in Hadiya do not plant suckers directly in a permanent enset field as a first and last operation. Transplanting enset at different levels takes four or five growth stages before harvesting. Each stage of transplanting enset has different local names (*dubo*, *simma*, *lammo*, *ogojaa*, *erro* or *kiniba*, and *ballwesa*), as it shows the growth stage of enset (Figures 4. 6A–F). Farmers regularly cut all leaves, uprooting the suckers and removing roots to transplant at the newly prepared homegarden site. Transplantation is usually carried out at the onset of the rainy season (normally January and March). Farmers are not interested in planting in February.

They consider that enset planted in February becomes more rooted, and with the large corm at early stages, such a situation is not preferred by farmers since frequent transplanting at each stage requires more labor and time. Enset growers in the Hadiya Zone specified the following transplanting stages and names to indicate these stages:

i. The first sucker production stage is called *dubo*; it is the stage where suckers (new shoots) usually grow in mass (clumps) on the mother corm. It takes one year before detaching and planting in a nursery.

ii. The second stage is locally called *simma* (seedlings are split into individual plants), where the suckers are split from the mother corm and planted into a single hole as groups of four to six suckers in batches. Suckers (*simma*) at this stage remain for one year.

iii. In the third year, the *simma* is transplanted into groups of two or three, and this is called *lammo.* The *lammo* suckers are transplanted in the middle rows of *ogojaa*, and they also last for a year. *Ogojaa* suckers are the larger *lammo* suckers planted individually in a row; they remain in the same place, and they take the name *erro* after the third year.

iv. During the fourth year, the *erro* enset, which has spent two years in the same place, is ready to transplant to its permanent site, and it takes a local name called *kiniba*. Farmers use *kiniba* for the suckers (*dubo*) production and to plant in the permanent field.

v. The last stage is locally known as *ballwesa*; it is the stage where the enset plant remains permanently until harvesting and flowering. An inflorescence stalk is locally called *qelimma*. This stage is selected for harvest by most women for the best quality of *qocho* and *bulla*.

4.3.6 Indigenous management practice of enset diversity

Farmers in the study area maintained and managed diversified landraces of enset with limited inputs in their homegardens to meet their subsistence needs. Based on our field observation and

informants' reports, their landrace composition varies according to age (*dubo, simma, lammo, ogojaa, erro* or *kiniba,* and *ballwesa*) (Figure 4.6), fermentation and maturity rate (early and late), yield and qualities, taste attributes, medicinal requirements, and many other values and needs. Traditionally, farmer-to-farmer communications ensure the maintenance of landrace composition in homegardens. Most of the interviewed farmers (80.42%) use corms of the *kiniba* enset from their farms as sources of planting materials, but a few farmers indicated that they purchase from other farmers, receive gifts from neighbors and relatives, and exchange them for other landraces at the *kiniba* stage (Figure 4.7). In the study area, enset as a source of planting material in an open market at any stage is not a common tradition, except for its final products and other materials. However, gifts for the young farmers are common traditional practices to enrich and maintain their homegardens.

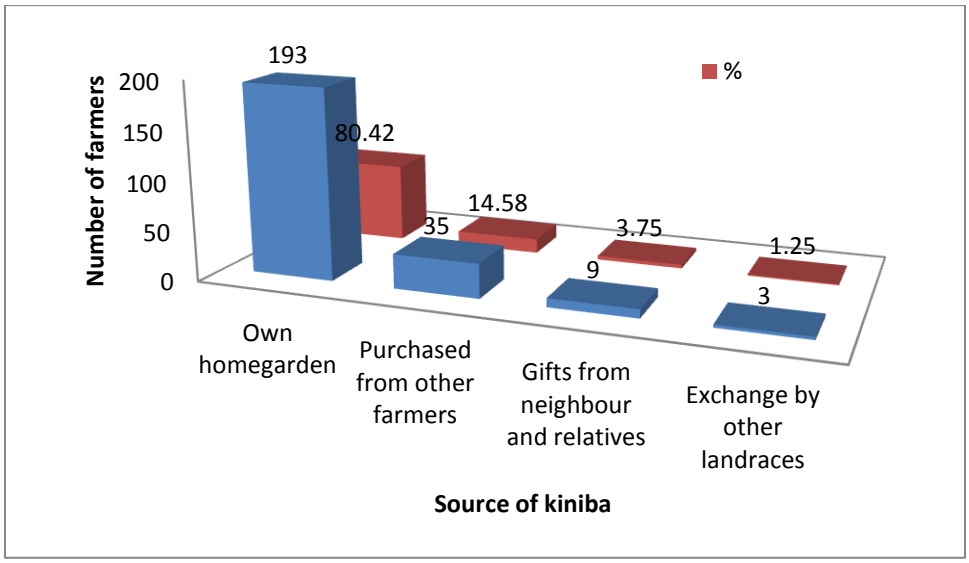

Figure 4. 7 Proportion of the source of planting material (*kiniba*) in the Hadiya zone

In Hadiya, the enset homegardens have specific arrangements and placements for each stage of landraces and a specific order for different purposes. In the study area, well-managed and

conserved enset landrace fields show attractive and respectful values for the household, which also encourages other farmers to maintain and cultivate more diverse enset landraces in their homegardens. The arrangements of enset in the homegarden around the living houses vary in composition and diversity based on age, end-use value, and preferences. On average, most of the studied enset crops on the farming sites have a typical planting pattern or arrangement. The patterns, taking the living house as a reference point, are as follows: The part in front of the house is for rearing livestock (Figure 4.8B) and other social purposes; one side of the homegarden is for the cultivation or growth and transplanting of different ages of enset suckers from *dubo* to *kiniba* (the closeness to the living house is decreasing from *dubo* to *kiniba*). The other side of the living house comprises mixtures of enset (*ballwesa*), including the flowering stages (*qellima*) (Figure 4.8A). The cultivation pattern also follows different arrangements on this site.

Figure 4. 8 A representative example of enset plantations and cattle feeding by enset products in a homegarden in the Hadiya Zone: (A) left side of the traditional house (*balwesa* stage) enset plantation until harvesting; the right side includes all other stages (*dubo* to *kiniba*); (B) a calf feeding on the corm of enset

The enset landraces that have been chosen for *amicho* based on taste and other qualities like medicinal value (such as *Astara*, *Leqeqa*, *Soqido*, *Qiniwara*, *Xiggo*, *Gariya*, *Orada*, *Xessa*,

Hayiwona, and *Xorora*) are planted in the homegarden very close to home or in hideous sites because they are also extensively selected by wild mammalian pests like porcupines. Therefore, local farmers usually give special care and more protection strategies to their favorite landraces. Other enset groups, such as those recognized for their enormous size, yield, and fermentation qualities (*Gimbo*, *Agade*, *Zobira*, *Merza*, *Hayiwona*, *Awunda*, *Boicho*, *Beneja*, etc.), are cultivated near the residential areas. But some enset landraces such as *Sisqella*, *Unjame*, *Disho*, *Dego*, *Lokanda*, *Gishira*, *Dirbo*, *Shelleqe*, and *Anchire* are planted at peripheral sites of enset homegardens, which is related to the fact that they do not attract and are not damaged by mammalian pests. Thus, these landraces are described as requiring only limited management and protection compared to other groups.

4.4 Discussion

4.4.1 Status of enset cultivation in Hadiya

Based on farmers, enset cultivation is the most important agricultural system for the rural community of Hadiya. Enset is the plant of choice and a vital food security source for humans and livestock in the midland and highland communities of the study area. Many local farmers consider that enset is less vulnerable to changes in agricultural systems and environmental conditions. This local farmer's perception agrees with other earlier studies on the importance of the enset crop (Kefale Alemu and Sanford, 1996; Temesgen Olango *et al*., 2014; Borrell *et al*., 2020). There is no part of the enset plant not used except the biological root (Brandt *et al*., 1997; Almaz Negash, 2001). Local farmers also confirm that the production of enset does not comprise the use of chemical inputs, and this practice is still active in the present generation. In the same manner, Brandt *et al*. (1997), Almaz Negash (2001), Admasu Tsegaye and Struik (2002),

Temesgen Olango *et al*. (2014), and Zerihun Yemataw *et al*. (2016) reported that the enset system is a well-established, sustainable, and environmentally resilient farming system that contributes to the food safety of farmers and, in particular, it serves as a food security crop in densely populated areas of the south and southwest parts of Ethiopia. The findings that showed more landraces in the rare category (33) and fewer in the unique category (15) may be related to declining phases or new introductions in the former case, and may be a case of highly localized knowledge in the latter. However, still, the common category is dominant and widely distributed in most of the farmer's homegardens. This finding agrees with Admasu Tsegaye and Struik (2002) and Zerihun Yemataw *et al*. (2016), who reported that multipurpose landraces have a wider distribution and abundance than uncommon landraces within and among regions.

4.4.2 Extent of on-farm landrace diversity and richness of enset

Our results from this study show that farmers maintain a higher diversity of landraces than any other crop in their homegardens. The 99 enset landraces recorded in the present study are much higher than the 59 enset landraces reported in a previous diversity study from two sites involving 105 households in the Hadiya Zone (Admasu Tsegaye, 2002). It is also higher than the 59 landraces reported by Zerihun Yemataw *et al*. (2014) from two *woredas* involving 40 respondents from the same zone. The earlier reports are far below the number of enset landraces reported in the present study, which managed to investigate a much wider area by interviewing 240 farmer informants and conducting a significant number of focus groups discussion. However, a direct comparison of the landrace richness documented in this study with prior reports is difficult because of differences in the number of farmers interviewed and visited, the number of sampled *woredas* and *kebeles*, and the approaches followed. Moreover, the study area is bordered by other major enset growing areas such as Kembata-Tambaro, Gurage, Silte,

Dawro, and Wolaita zones and Yem special *woreda,* where the suckers are easily exchanged, and all the study areas comprise midland and highland that are more suitable for enset cultivation.

In the same manner, different ethnolinguistic communities in southern Ethiopia maintain diverse enset landraces in their homegarden, which is similar to what we have recorded in this study. For instance, 78 landraces from Ari by Shigeta (1992), 65 from Kaffa-Shaka by Almaz Negash (2001), 79 from Sidama by Bizuayehu Tesfaye and Ludders (2003), 105 from Gamo Gofa by Shara and Mulugeta Diro (2012), 111 from Kembata-Tambaro by Melesse Maryo *et al*. (2014), 67 from Wolayita by Temesgen Olango *et al*. (2014), and 93 from Yem special *woreda* by Ambachew Zerfu *et al.* (2018). Admasu Tsegaye (2002) also stated that most farmers in the study area tend to grow and keep a diverse range of enset types on their farmlands because concentrating on a single variety with a particular trait cannot meet different needs and addresses.

4.4.3 Farmers' traditional categorization of enset landraces

The study revealed that the local farmers have amazing knowledge of the enset plant, which has been accumulated over many years and transmitted from generation to generation through oral tradition. It has played key roles in the classification, utilization, and maintenance of the available genetic diversity of enset.

In the study area, farmers, as the main growers and experts, have their own identification, local naming, and classification systems to distinguish one enset landrace from the other. The same trend was reported by Bizuayehu Tesfaye and Ludders (2003) and Temesgen Olango *et al*. (2014) from Sidama and Wolaita in using indigenous knowledge of biosystematics for enset

landraces, respectively. In the present study, traditional identification and naming of the recorded landraces were primarily based on morphological features, unique traits, and end-use value of the plants. This agrees with the descriptors reported from other enset producing regions, such as Ari, Kefa-Sheka, Sidama, and Wolaita in southern and southwestern Ethiopia (Shigeta, 1990; Almaz Negash, 2001; Bizuayehu Tesfaye, 2008; Temesgen Olango *et al*., 2014). As indicated by Zippel and Ludders (2002), Yemane Tsehay and Fassil Kebebew (2006), and Zerihun Yemataw *et al*. (2016), in the southern part of Ethiopia, enset-growing farmers usually distinguish and classify enset plants based on their morphological traits, such as leaf, midrib, petiole, and pseudostem color, length and width, types of use, quality (taste, palatability, color, etc.), and quantity of each distinctive product. These identification features are consistent with our results in this study.

In the studied areas, enset farmers give various local names for their landraces after identifying different features, such as morphology, its supposed origin or sources, cooking qualities, and different end uses. Nonetheless, the meanings of the names for most of the recorded landraces were unexplained by the enset farmers in the studied areas. Similar findings have also been reported that unexplained meanings of folk names were common in other enset cultural areas of the country (Bizuayehu Tesfaye and Ludders, 2003; Temesgen Olango *et al*., 2014; Zerihun Yemataw *et al*., 2016). The same pattern was also perceived in other crops such as sorghum in Ethiopia (Firew Mekbib, 2007), banana in Indonesia (Hapsari *et al*., 2017), and cassava in Uganda (Nakabonge *et al*., 2020). The unexplained meaning of the name could partly be related to the loss of local knowledge that may have been obvious to knowledgeable cultivators of enset in the past. Those names that cannot be easily associated with obvious things tend to be remembered by fewer members, and as time passes, this can be further compounded if the landrace does not have special features and uses that could be associated with the name. We can

probably explain it with the phenomenon known as language dynamics: some terms exist but their meanings are no longer known by members of the community. Some names may have been borrowed from other crops (e.g., *Chalqo* and *Sinera*, from the local names of barley landraces). However, before taking the absence of meaning for a landrace as a true statement, thorough surveys have to be made within the entire area where that language is spoken, targeting a search for the meaning of the name of a particular landrace. Some landrace names recorded in this study were also described from other neighboring ethnolinguistic communities (Spring *et al*., 1996; Melesse Maryo *et al*., 2014; Zerihun Yemataw *et al*., 2016). This might be due to farmer-to-farmer exchange of planting material among adjacent communities and could indicate such 'borrowed' landrace names between ethnolinguistic groups (Temesgen Olango *et al*., 2014; Nakabonge *et al*., 2020). Farmers always refer to the folk names when planting, transplanting, managing, utilizing, giving, and exchanging enset (Shigeta, 1990; Bizuayehu Tesfaye, 2008; Temesgen Olango *et al*., 2014; Zerihun Yemataw *et al*., 2014; Zerihun Yemataw *et al*., 2016).

In the study area, farmers maintain and categorize their enset landraces based on their external appearances and internal features. Moreover, they not only identify each landrace and give it a unique place in their homegardens but also elucidate its life history, quality of end products, and specific use values. According to the reports from Ari, Kefa-Sheka, Wolaita, and Kembata in southern Ethiopia, farmers categorize enset landraces as having male or female features (Shigeta, 1992; Kefale Alemu and Sanford, 1996; Almaz Negash, 2001; Yemane Tsehay and Fassil Kebebew, 2006; Melesse Maryo *et al*., 2014; Temesgen Olango *et al*., 2014). According to them, the male enset landraces are drought-tolerant. Female landraces are described by farmers as less vigorous, susceptible to disease, having a higher *qocho* quality, and producing edible and tasty *amicho*. In addition, they are early-maturing and have poor fiber strength. But Zippel and

Ludders (2002) reported three categories of enset local varieties: male, female, and intermediate categories from the North Omo of Ethiopia. However, in the present study, categorization of enset as male or female was not a commonly practiced grouping method; instead, the primary criteria were the stiffness of landraces during harvesting and processing, rate of fermentation, and quality of the end value (product and usage). Based on these, local farmers generally grouped their enset crops into 'soft' or 'hardy' landraces**.** These results are consistent with findings reported by Tadesse Kippie (2002) from Gedeo in southern Ethiopia. The parallelism in the grouping as male and female versus hardy and soft is likely grounded on similar thoughts and understanding the morphology and character of the enset crop.

4.4.4 Indigenous uses of enset

4.4.4.1 The traditional food

The results of this study indicated that enset is a multi-purpose crop that provides the local community with food and non-food materials. In the studied area, farmers cultivate enset in their homegardens as the main staple or co-staple food crop. The different dishes are derived from the three primary enset products, namely: *wassa*, *or qocho*; *buoo, or bulla*; and *hamicho, or amicho*. This agrees with previous studies conducted in other enset-growing regions of Ethiopia (Brandt *et al*., 1997; Almaz Negash, 2001; Bizuayehu Tesfaye and Ludder, 2003; Temesgen Olango *et al*., 2014; Taddesse Daba and Shigeta, 2016). Working with farmers, of the three types of *qocho* (fermented enset products), two of them (*tiqoota* and *buhesso)* were prepared from 'soft' enset types with or without additions of *gammama*. The other third *qocho, gojjoo,* is prepared from a mixture of both 'soft' and 'hardy' enset types with the addition of *gammama* (starter). In the study area, local communities consider the recipes from *tiqoota* poor food,

usually consumed in seasons of food shortages. Similar result was reported from Wolaita, southern Ethiopia (Temesgen Olango *et al*., 2014). Farmers asserted that *wassa* or *qocho* is produced in large quantities and consumed frequently, whereas *bulla* is considered the best and most favored enset food product in the community. The specialty of *bulla* food in the Hadiya community is seen in the different dishes prepared from it traditionally served for different medicinal values as well as cultural and religious events. On the other hand, *amicho* is harvested for its immediate use as food with no processing. Similar findings were reported by Brandt *et al*. (1997) and Admasu Tsegaye (2002) in their country-level studies.

4.4.4.2 Traditional medicinal use of enset

Farmers in the study area maintained several enset landraces for medicinal purposes with other landraces. According to their reports, cooked corm (*amicho*) with dairy products is often used to cure different ailments in humans. Out of the mentioned landraces, most are traditionally applied to treat bone and joint problems. Melesse Maryo *et al*. (2014), Ashenafi Ayenew *et al*. (2016) from the Kembata-Tembaro Zone, and Gizachew Nuraga *et al*. (2019) from five administrative zones and one *woreda* also described several of these landraces with similar folk names. Likewise, our results are consistent with the findings reported by Admasu Tsegaye and Struik (2002) from the same study area. In the study area, *Meqelwesa* is a landrace known for a deep red leaf, midrib, and pseudostem. The *amicho* (cooked corm) of this enset with milk stimulates delayed placental discharge after delivery in humans and cattle. In the same manner, some enset landraces were also reported from different study areas for similar treatment purposes, such as *Qeqille* by Melesse Maryo *et al.* (2014) from Kembata, *Asikala* by Amare Assefa and Daniel Fitamo (2016) from Sidama, and *Choro* by Yemane Tsehay and Fassil Kebebew (2006) from Bonga, southwestern Ethiopia. This may indicate either the independent development of similar

cultures, the diffusion of cultures, or both the utilization and management of crops over the years (Kujawska *et al*., 2017).

4.4.4.3 Cultural and Economic values of Enset

Enset has played a more significant role in meeting the cultural and economic needs of rural communities than any other crop in the study area. This study showed that farmers in Hadiya had classified as either poor or rich based on the number of mature enset crops and the numbers of livestock they owned. Enset and cattle are highly interrelated in the studied areas since different parts of enset are used as fodder for cattle, and their wastes are also used as organic fertilizers for enset crops. These imply that enset plants have a long history and strong bond with humans and animals. Teshome Sirany *et al.* (2022) also stated that enset crops and animals are closely linked to win-win strategies. Similarly, Kefale Alemu and Sandford (1996), Admasu Tsegaye and Struik (2002), and Tsedale Waktola (2009) described a similar situation in other enset-producing communities in Ethiopia. Moreover, farmers in Hadiya obtain many social appreciations, notable respectful titles or names, and ranks from the local community based on the number and size of their enset crops. Likewise, Teshome Sirany *et al.* (2022) indicated that enset plants have special cultural meaning and value as an expression of identity for enset producers in Ethiopia. Therefore, enset cultivation in the community has a major socio-cultural value. In the same manner, Zippel and Kefale Alemu (1995) and Temesgen Olango *et al.* (2014) reported that in Wolaita, a farmer with a large enset plantation and many mature (big) enset plants can immediately be recognized by outsiders as a rich and respected man. Enset is a versatile crop in the Hadiya community; besides its food, medicinal, and socio-cultural values, it also serves as a source of income for rural communities. Kefale Alemu and Sanford (1996) also showed that enset plants can be sold sometimes, and processed products like *qocho* and *bulla* are sold

anytime in rural markets and towns. Therefore, it is an immediate, year-round cash income source for a household to buy their regular items.

4.4.4.4 The gender roles in management and use of enset

According to Mukhopadhyay and Pieri (1999), most farming systems have a division of labor, which determines the different tasks for which men and women are responsible. Similarly, farmers in the study areas indicated that women manage most activities of the enset since they are exclusively accountable for harvesting, processing, storing, preparing, and marketing the products. In our study, farmers revealed that male farmers were regularly involved in the early stages of activities such as land preparation, planting, and transplanting. This corroborates with the reports of Admasu Tsegaye (2002), who described that according to the men, 'it is a shame for the male to process or watch his wife process or carry enset products to the market.' Almaz Negash (2001) also stated that men believe enset processing is purely a woman's task. This indicates that it would be difficult to find any fermented enset products without the participation of women.

Female farmers in the study area also regulate extracted products such as *qocho*, *bulla*, fiber, and other materials like fresh leaves and mattresses (local beds) as an important source of regular income, which provides female farmers with social independence and economic self-sufficiency. The same situation was also reported by Shigeta (1992), Tibebu Habtewold *et al.* (1996), Brandt *et al.* (1997), and Almaz Negash (2001), who described that the fermented products and also the fresh and dried parts of enset are the main source of income for women in other enset-growing regions of Ethiopia. Generally, female farmers in the Hadiya Zone, due to their high intimacy with the enset plant, have more knowledge and experience. These opportunities qualify

them easily to recognize the different landraces, their detailed features and life cycles, and the yield and quality of the harvested products of each landrace. This has significant implications for women's roles in enset diversity, management, utilization, and conservation of genetic resources.

4.4.5 Indigenous cultivation and management practice

In Hadiya Zone, farmers regularly cultivate a mixture of enset landraces to conserve on-farm landrace diversity and to balance harvested enset for different consumptions throughout the year. Traditionally, farmers follow three consecutive cultivation stages, which include vegetative propagation, transplanting, and harvesting. Our results indicated that enset propagation is a cultural practice carried out by all farmers every year to regulate the enset cycle and maintain on-farm landrace diversity. This agrees with the reports of earlier studies in different enset-growing areas of the country (Almaz Negash, 2001; Admasu Tsegaye, 2002; Karlsson *et al*., 2015; Zerihun Yemataw *et al*., 2016; Borrell *et al*., 2020). Based on our field observations and interviews, most farmers use vegetative propagation from the corm of a four-year-old enset plant, locally called *kiniba*. A similar observation was also reported from other enset growing areas (Admasu Tsegaye, 2002). The propagation is usually carried out from November to January, depending on the offset of the moon, which is probably related to traditional beliefs held by some farmers since they think that cultivating enset during the onset of the moon may contribute to the development of certain enset diseases and production losses. A similar traditional practice was also observed by Temesgen Olango *et al*. (2014) in Wolaita. According to farmers in the study area, multiuse landraces are more frequently propagated than functionally specific landraces. Similarly, Zerihun Yemataw *et al*. (2016) also indicated that multipurpose landraces are propagated almost every year. Based upon farmers' reports, the number of suckers found from a single mother enset varied due to different factors such as genotypes and size of

landraces, age of landraces, management practices, and amount of rainfall. A similar report was observed by other researchers (Karlsson *et al*., 2015; Abraham Bosha, 2018). Most farmers in the study area mentioned that 30 to 150 suckers are obtained from a single mother plant of four-year-old *kiniba*.

The results of our study revealed that transplanting enset at different levels takes four times or five growth stages before harvesting. Each stage of transplanting has various local names in the Hadiya community (*dubo*, *simma*, *lammo*, *ogojaa*, *erro*, or *kiniba*, and *ballwesa*). In the same manner, Almaz Negash (2001) and Admasu Tsegaye (2002) stated that different growth stages and transplanting steps of enset are known in different zones of Ethiopia. Transplantation of *kiniba* to *ballwesa* usually occurs during the dry season, especially in January. However, transplanting of other stages is regularly performed at the beginning of the rainy season (normally in March). Comparable reports were shown by Almaz Negash (2001), Admasu Tsegaye (2002), and Temesgen Olango *et al*. (2014) from Keffa-Shaka, Sidama, and Wolaita, respectively. Furthermore, all enset growers in the study area regularly prune most of the leaves at each stage, except *ballwesa*, particularly in June and the end of August. A similar observation was reported by Admasu Tsegaye and Struik (2002). Temesgen Olango *et al*. (2014) indicated that the third stage in the Wolaita area is used as both the source of mother corm for sucker multiplication and harvested for consumption when there is less food in the family stock. In the same way, *kiniba* or *erro* stages are also used as the source of mother plants for sucker production in the study area. However, harvesting for human consumption at any stage, except *ballwesa*, is not common practice in the Hadiya community.

4.5 Conclusions

The information obtained from this study indicates that local farmers have a great wealth of knowledge on the naming, classification, utilization, and management of enset landraces in the Hadiya Zone. In the study area, enset is a vital and multipurpose crop; throughout the growth stage, every part is used for food and non-food purposes by most farm households. Enset, a food and fodder security crop, could not be substituted by other crops for the enset farming communities in the study area. With their daily association and knowledge accumulated over the years, the Hadiya farmers have been cultivating and maintaining several enset landraces in their homegardens for many generations. In this study, 99 vernacular names of enset landraces growing on farmers' fields were documented. Local farmers in the study area use a combination of methods to identify, name, and classify enset landraces in their homegardens.

The morphological characteristics of a landrace are the major and key identification criteria used by local farmers. Farmers in the study area give distinct local names to each landrace they grow based on its identifying characteristics. Moreover, the local names of landraces are often consistent and shared among the people, and they also reflect variations among the landraces. Generally, landraces were named based on places of origin or sources, morphology, culinary features, maturity period, growth attribute, and use. However, for most enset landraces, farmers do not know the meaning of the names as recorded during the interviews. Local farmers also classify enset landraces into different groups using their long-term experiences and enriched traditional knowledge of the crop. Special conservation attention needs to be given to the rare and unique types of enset landraces.

The results from this study also provide information about the indigenous knowledge of local farmers regarding the cultural, social, and economic values of enset and its production system. Such knowledge is crucial for understanding how local community members classify, utilize, maintain, and manage the existing enset landrace diversity in their homegardens and is a vital resource for the improvement and development of the crop in combinations with modern science.

Chapter Five

Exploring the extents of genetic diversity and population structure of enset [*Ensete ventricosum* (Welw.) Cheesman] from southern Ethiopia using simple sequence repeat markers: Implications for crop improvement and conservation

Abstract

Enset [Ensete ventricosum (Welw.)Cheesman] is a multi-use perennial herbaceous crop used as a staple food for over 25 million people in Ethiopia. Despite its high use values, very few studies have been conducted to improve this crop, particularly using molecular marker systems. In this context, the study aimed at evaluating the magnitude of genetic diversity and population structure of enset germplasm collections from four major enset growing zones in southern Ethiopia using 12 simple sequence repeat (SSR) markers. A total of 147 individual leaf samples were collected from the entire enset populations and gave 289 alleles, ranging from 12 to 41 alleles per locus, with a mean of 24.5. The polymorphism information content for each locus varied from 0.86 to 0.95, with a mean of 0.91. The number of effective alleles ranged from 5.13 to 11.79 with a mean of 8.27. The expected and observed heterozygosity showed average values of 0.85 and 0.84, respectively. The greatest genetic distance (1.16) was between Gurage and wild populations, while the smallest (0.37) was between Gurage and Silte. Among the six populations, the wild had the highest percentage of polymorphic loci (100%). AMOVA attributed 89% of the genetic variation to intra-population and only 11% among populations. The whole set of germplasm indicates low genetic differentiation and high gene flow (Nm). The UPGMA and principal coordinates largely correspond to each other and indicate three major groups. Overall, the information gained from this study would be useful for enset improvements and conservation strategies.

Keywords: Conservation, Gene flow, Genetic variation, Heterozygosity, Polymorphism

5. 1. Introduction

Ethiopia is recognized as both the center of origin and the center of diversity for many crops, including enset (Engels and Hawkes, 1991; Harlan, 1996). Enset [*Ensete ventricosum* (Welw.) Cheesman] is a large herbaceous monocarpic perennial plant belonging to the order Zingiberales, family Musaceae, and genus *Ensete* (Tomilson, 1969). It is sometimes known as a false banana or Ethiopian banana because of its similar morphology to the banana (*Musa* x *paradisiaca*) (Borrell *et al*., 2019). The huge size, single pseudostem structure, dilated base, and upright leaves of enset, among other characteristics, set it apart from the banana (Tesfaye Abebe *et al*., 2010). Moreover, enset is a diploid plant with chromosome number of 2n = 18, whereas *Musa* species, including edible bananas, have different ploidy levels and chromosome numbers (diploid, triploid, or tetraploid), with 2n = 20 or 2n = 22 (Cheesman, 1947; Endashaw Bekele and Shigeta, 2011; Borrell *et al*., 2019).

Enset domestication, cultivation, and human consumption as food are restricted to Ethiopia (Engels and Hawkes, 1991; Borrell *et al*., 2019). In most of the areas where enset is used as human food, it is usually a staple or a co-staple crop. It is a multipurpose crop that is selected mainly for assurances of food security and stability for over 25 million of the Ethiopian population (Brandt *et al*., 1997; Borrell *et al*., 2020) and also produces fodder, fiber, and other materials. In addition, some enset landraces are used in human and livestock medicine (Yemane Tsehay and Fassil Kebebew, 2006; Gizachew Nuraga *et al*., 2019a; Tesfaye Dilebo *et al*., 2023a, b). Besides these products and services, enset plays important roles in the sociocultural and environmental relations of the central, southern, and southwestern administrative regions of Ethiopia (Zerihun Yemataw *et al*., 2016; Bewuketu Haile *et al*., 2022). Furthermore, it is

comparatively drought-resilient, as it can withstand a shortage of rainfall for a certain period and is highly productive with minimum labor input (Admasu Tsegaye and Struik, 2002; Chase *et al*., 2022).

Enset-producing farmers have maintained and enriched the diversity of the crop through identification and selection using their accumulated indigenous and local knowledge, and for them, the use of enset diversity is directly linked to the diverse methods of generating products that are used as food as well as non-food values (Almaz Negash, 2001; Zemede Asfaw, 2018). Furthermore, they are the primary guardians of enset diversity, and previous authors have documented hundreds of enset genotypes (Shigeta, 1990; Kefale Alemu and Sandford, 1991; Almaz Negash, 2001; Admasu Tsegaye and Struik, 2002; Genet Birmeta *et al*., 2004; Zerihun Yemataw *et al*., 2016; Tesfaye Dilebo *et al*., 2023a). Enset shows noticeable variations in terms of morphological characteristics (e.g., the color of pseudostem, petiole, and midrib, size (width and length), and angle of leaf orientation), agronomic features (maturity rate, reaction to different diseases and pests, and agro-ecological adaptability), use values (quantity and quality of *kocho* (*qocho)* and *bulla*, use of corms, and fiber quality), and other attributes (Almaz Negash, 2001; Admasu Tsegaye and Struik, 2002; Bizuayehu Tesfaye, 2008; Temesgen Olango *et al*., 2014; Zerihun Yemataw *et al*., 2016). However, such features may change under the influence of ecological conditions (Nkhata *et al*., 2020), and thus, their use as markers is not fully effective in determining the existing diversity, and which is crucial in looking for the relevant markers.

Maintaining plant diversity and using it sustainably is vital to addressing concerns related to food security systems as well as other issues created by human population growth (Chappell and LaValle, 2011; Delfini *et al*., 2021). Genetic diversity is the degree of genetic variability found among individuals of a variety or a population within a species (Salgotra and

Chauhan, 2023), and the existence of variation among or within groups is crucial to improving any plant species either by a classical or molecular approach (Tileye Feyissa, 2020). Genetic diversity has partly resulted from genetic recombination, mutations, gene flow, and genetic drift (Hedrick, 2007; Ortiz-Barrientos, 2016). In addition, migration, hybridization, and polyploidization are responsible for creating variation in plants (Vallejo- Marín and Hiscock, 2016). These result in variations in DNA sequence, protein structure or isozymes, physiological properties, and morphological traits (Kumar *et al*., 2009).

Sustainable utilization of the genetic diversity of the existing enset genotypes is expected to play an important role in selecting desirable genotypes with better yield and quality traits, along with resistance/tolerance to biotic and abiotic stresses through the breeding system to increase food security. So far, the extent of enset genetic diversity has been detected using varied molecular methods such as random amplified polymorphic DNA (RAPD) (Genet Birmeta *et al*., 2002), amplified fragment length polymorphism (AFLP) (Almaz Negash *et al*., 2001), inter simple sequence repeat (ISSR) (Dagmawit Tobiaw and Endashaw Bekele, 2011), and simple sequence repeats (SSRs) using banana primers (Selamawit Getachew *et al*., 2014). In this regard, the enset genotypes examined by SSR markers in earlier studies were collected either from a limited geographical and agroecological range and/or were very limited in number. For example, Temesgen Olango *et al*. (2015) worked only on 66 samples maintained at the Areka agricultural research center that had been originally collected from Wolaita, Gamo-Gofa, Dawro, and Ari areas; Fetta Gerura *et al*. (2019), used 83 samples from the Gurage Zone, and Gizachew Nuraga *et al*. (2022) used 38 medicinal and other 13 enset landraces from southern Ethiopia. A few other markers have also been conducted recently, such as those using AFLP and SNPs (single nucleotide polymorphisms) from Dawro, Gurage, Keffa, South Omo, Sheka, and Sidama (Kiflu

Tesfamicael *et al*., 2020) and SNPs from the Sidama region, Gurage, and South Omo Zones (Alye Haile *et al*., 2023), which studied the genetic diversity among and within wild and cultivated enset landraces of Ethiopia.

Thus, it is essential to assess the genetic diversity of available enset populations that were not included in the previous studies and that grow in the diverse environments and farming practices of the Hadiya, Kembata-Tembaro, Silte, and Gurage zones of southern Ethiopia. Therefore, the objectives of this study were to evaluate the level of genetic diversity and population structure in germplasm collections from the above-listed zones and the Areka agricultural research center by using the SSR molecular marker method, which would back-up the ethnobotanical and other studies by Tesfaye Dilebo *et al*. (2023a) to ultimately provide the foundation for improvement and sustained conservation of the enset crop.

5. 2 Materials and Methods

5. 2. 1 Plant Material and Sample Collection

For this study, a total of 147 individuals (Table 5.1) from newly emerged central cigar leaf samples (132 cultivated landraces, 6 released varieties, and 9 wild-growing types) were collected from the four administrative zones (Gurage (29), Silte (29), Hadiya (42) and Kembata-Tembaro (32) in the central Ethiopia region and the Areka Agricultural Research Center (AARC) enset germplasm conservation site, located in Wolaita Zone of southern Ethiopia. The cultivated landraces were known by farmers with different local names and were accessed from farmers` homegardens. The collected individual samples were grouped into six populations depending on the administrative zones of collection, each of which is largely inhabited by a unique ethnic

group with its own cultural practices. In addition, the wild and released samples of enset varieties were included to represent their own populations (Appendix 2).

Table 5. 1 List of enset populations used in this study and their altitudes, geographic positions and collection districts along with their sample sizes

Population name	Collection districts	Altitude ranges (m) of collection sites	Latitude ranges (N)	Longitude ranges (E)	Sample size
Gurage	Endegegn,Gumer, Enemore Ener	1850–2790	$7^0$76'–8^0 45'	$37^0$46'–38°71'	29
Silte	Mirab A, Misraq A, Alicho Weriro	2218–3195	$7^0$49'–8^0 01	$37^0$96'–38°17'	29
Kembata	Doyogena, Angacha, Damboya	2145–2822	$7^0$08'–$7^0$30'	$37^0$22'–$38^0$04'	32
Hadiya	Misha, Lemmo, Dunna	2132–2915	$7^0$07'–$7^0$92'	$37^0$29'–$38^0$13'	42
Released	AARC	1774	7^0 04'	$37^0$42'	6
Wild	AARC	1774	7^0 04'	$37^0$42'	9
Total					**147**

Note: A=Azernet; AARC= Areka Agricultural Research Center; Original provenance of the wild populations: Ari District, South Omo Zone (A1 and A2), Dawro Zone (W1–W4), and Keffa Zone (W5–W7)

Enset-producing farmers recognize each landrace by its unique local name, which refers to different features such as morphological, agronomic, or quality of end products (Tesfaye Dilebo *et al*., 2023 a, b). Therefore, each landrace was considered a separate sample, and the identification and description for sample collection were carefully applied with the help of senior knowledgeable enset farmers in each zone, and the collection was performed from farmers' homegardens with the informed consent/permission and support of each man or woman

knowledgeable farmer. Moreover, landraces having the same local names but originating from different zones were collected and labeled as separate samples (Appendix 2) because each farming community considered them distinct farmers' varieties or landraces. The collected clean inner cigar-shaped enset leaf tissue, cut into smaller pieces were kept in zip-locked plastic bags containing silica gel (JHD, Guangdong Guanghua Sci-Tech Co, Ltd.) until the extraction of genomic DNA, and to improve proper desiccation, the silica gel (weight ratio of silica gel to enset leaf tissue: 10:1) was changed twice. Prior to the excision of the leaf samples, ethanol (75%) was used for cleaning and to avoid cross-contamination within the samples. The samples were brought to the Plant Biotechnology Research Laboratory, Addis Ababa University, Ethiopia, and kept there at room temperature until DNA extraction was carried out.

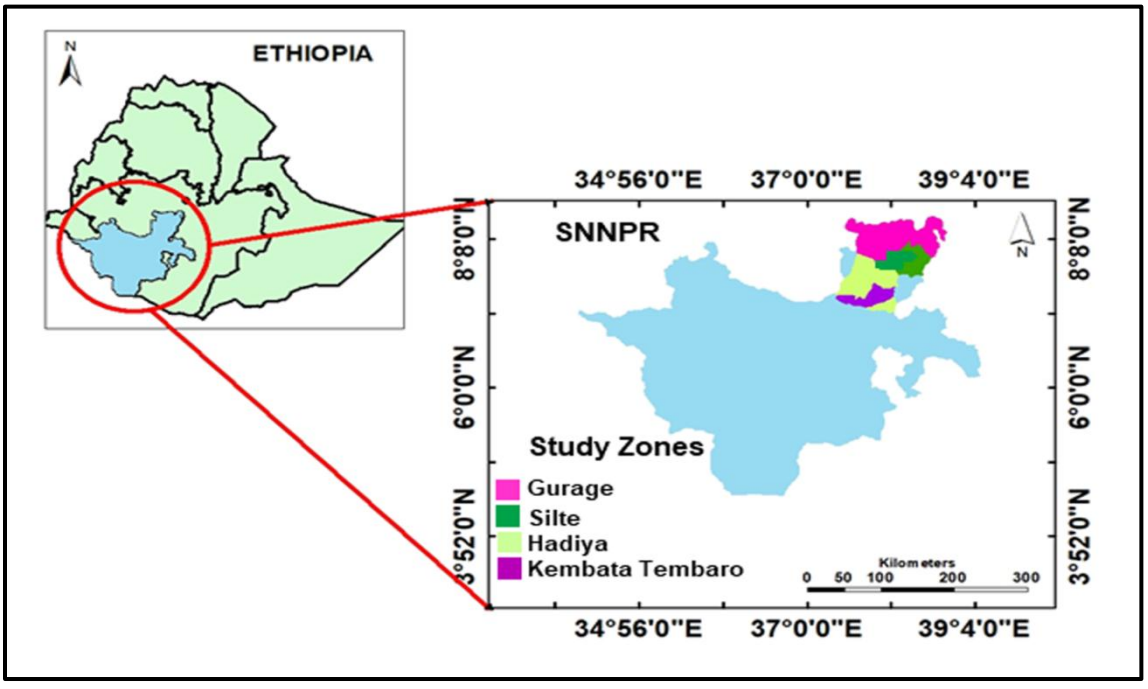

Figure 5. 1 Map of Ethiopia showing the location of the former Southern Nations, Nationalities and Peoples Region (SNNPR) and the four enset populations' collection zones

5.2.2 DNA Extraction

The silica gel-dried leaf samples of about 1g were ground using a Mixer and Miller (RETSCH Mixer - Mill MM400) for 5 minutes. The total genomic DNA was extracted from each powdered leaf sample following the mini-prep DNA double extraction protocol of Cetyl Trimethyl Ammonium Bromide (CTAB) with minor modification (Borsch *et al*., 2003). The quality of the extracted genomic DNA was checked using 1% agarose gel electrophoresis in 1 x TBE buffer, which was run for 1 hour at 100 volt. Similarly, the quantity (concentration) of the DNA was measured using a NanoDrop Spectrophotometer (ND-2000, USA). Finally, the DNA was adjusted to a concentration of 50 ng/µL using molecular-grade water and stored at -20 °C for further SSR analysis.

5.2.3 Primer Screening and PCR Amplifications

In the present study, a total of 15 SSR markers, 11 of which were developed by Temesgen Olango *et al*. (2015) and four from the Enset Molecular Data Base (Biswas *et al*., 2020) (Table 5.2), were effectively screened, optimized and used to reveal the extents of genetic diversity of the study populations.

The PCR amplifications were conducted in a 96-well thermo-cycler (Eppendorf AG Mastercycler 22331, Hamburg, Germany) using a total reaction volume of 15 µl containing 1.5 µl of genomic DNA (50 ng/ µl), 0.25 µl of each primer (10 pmol/ µl) (Sigma-Aldrich), 3 µl of 5xFIRPol@ Master Mix ready (5x reaction buffer B: 0.4 M Tris-Hcl, 0.1M (NH4)2, and 0.1% w/v Tween-20; 7.5 mM $MgCl_2$; 1mM dNTPs of each, blue and yellow dyes, Solis BioDyne, Tartu, Estonia), and 10.0 µl of molecular grade water. The amplification condition consisted of an initial denaturing step of 94 °C for 5 minutes, followed by 35 cycles of 94 °C

for 30 seconds, optimum annealing temperature (specific to each primer) for 1 minute, primer elongation at 72 °C for 1 minute, and a final elongation step of 72 °C for 15 minutes. The amplified products were separated by electrophoresis in a 3.5% (w/v) agarose gel (Sigma) in 1xTBE buffer containing ethidium bromide ($EtBr_2$) at 100V for 3 hours. The generated fragments were visualized using a gel documentation system (BIORAD Gel DocTM EZ System Imager) and a 100 base-pair DNA ladder (Thermo Fisher Scientific, Massachusetts, USA) was used to estimate the size of the bands.

Table 5. 2 List and characteristics of the 15 SSR markers used in the analyses of genetic diversity and population structure of Enset (*E. ventricosum*) populations in the present study

Marker code	Primer sequence of forward (F) and reverse (R) (5'–3')	Repeat motif	References	Size (bp)	Ta (^{0}C)
Evg-01	F: AGTCATTGTGCGCAGTTTCC R: CGGAGGACTCCATGTGGATGAG	(CTT)8	Temesgen Olango *et al*., 2015	103-135	56
Evg-02	F: GGAGAAGCATTTGAAGGTTCTTG R: TTCGCATTTATCCCTGGCAC	(AG)12	Temesgen Olango *et al*., 2015	108-158	56
Evg-03	F: ACAGCATAAGCGAAATAGCAG R: ACAGCATAAGCGAAATAGCAG	(AG)12	Temesgen Olango *et al*., 2015	109-125	58
Evg-04	F: GCCATCGAGAGCTAAGGGG R: GGCAAGGCCGTAAGATCAAC	(AG)21	Temesgen Olango *et al*., 2015	106-163	58
Evg-06	F: CCGAAGTGCAACACCAGAG R: TCGCTTTGCTCAACATCACC	(GAA)9	Temesgen Olango *et al*., 2015	200-219	57
Evg-08	F: CCATCGACGCCTTAACAGAG R: TGAACCTCGGGAGTGACATAAG	(GA)21	Temesgen Olango *et al*., 2015	152-196	58
Evg-09	F: GCCTTTCGTATGCTTGGTGG R: ACGTTGTTGCCGACATTCTG	(GA)13	Temesgen Olango *et al*., 2015	139-179	56
Evg-10	F: CAGCCTGTGCAGCTAATCAC R: CAGCAGTTGCAGATCGTGTC	(AG)21	Temesgen Olango *et al*., 2015	187-217	57

Table 5.2 Cont

Evg-11	F: GGCCTAGTGACATGATGGTG R: TGATGCTAGATTCAAAGTCAAGG	(AC)13	Temesgen Olango *et al.*, 2015	130-168	54
Evg-12	F: TGCAACCCTTTGCTGCATTC R: AGCATCATTCGCCATGGTTG	(TG)14	Temesgen Olango *et al.*, 2015	117-161	58
Evg-17	F: GCGTCTGGTATGCTCAACTG R: TCGGGAATGATACAGAGGCG	(TCA)8	Temesgen Olango *et al*., 2015	110-164	58
EnO-04	F: ATCTGCATGCACCCTAGCTT R: AAACCCTAACGTCCCTCCTC	(GT)10	Biswas *et al*., 2020	120-150	58
EnO-06	F: TGCCCAAAAACTTTGATGTG R; CCACACATCTCAGAGCCTCA	(AGA)11	Biswas *et al*., 2020	130-152	54
EnB-02	F: ATCAAGGTCATGTGCTGTGC R: ATCAAGGTCATGTGCTGTGC	(CT)11	Biswas *et al*., 2020	-	56
EnB-07	F: AGATCAACCGCATCCATCAT R: GCACGTGTGTCACATTGCAT	(GAA)15	Biswas *et al*., 2020	255-272	56

EnO-04=EnOnjSSR049028, EnO-06=EnOnjSSR061390, EnB-02=EnBedSSR020585, EnB-07= EnBedSSR071399

5.2.4 Data analysis

Genetic diversity indices such as the total number of alleles (Na), the number of effective alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He), unbiased expected heterozygosity (uHe), Shannon's Information Index (I), percentage of polymorphic loci (PPL), and Nei's gene diversity (GD) over the entire loci were computed using GenAlEx version 6.501 (Peakall and Smouse, 2012). Other locus-based genetic diversity parameters, including major allele frequency (MAF) and polymorphic information content (PIC) were estimated using PowerMarker version 3.25 (Liu and Muse, 2005).

To estimate the correlation between observed allelic diversity and sample size of populations, rarified allelic richness (Ar) and private rarified allelic richness (Ap) were computed using the rarefaction procedure applied in the HP-Rare 1.1 software (Kalinowski, 2005). An analysis of molecular variance (AMOVA) was computed to analyze the distribution pattern of genetic variances between groups and individuals within a group using Arlequin version 3.5.2.2 (Excoffier and Lischer, 2010). Wright's fixation index (F_{ST}) of the total populations and pairwise F_{ST} among all pairings of populations were computed using GenAlEx version 6.501 (Peakall and Smouse, 2012) to estimate population differentiation and the significance was evaluated using 1000 bootstraps. Gene flow (Nm), which measures the average number of individuals in each generation migrating among populations, was computed using the formula, Nm = 0.25 (1−Fst)/Fst (Slatkin and Barton,1989) Nei's standard genetic distance (DST, corrected) (Nei,1972) based on the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) (Sneath and Sokal,1973) tree was constructed using DARwin var. 6.0.14 (Perrier and Jacquemoud-Collet) and POPTREE2 (Takezaki *et al*., 2010), respectively, and significance was tested based on 1000 bootstraps (Felsenstein,1985). TreeView (Win32) 1.6.6 program (Page, 1996) and FigTree var. 1.4.3 (Andrew, 2016) were used to display the produced trees.

To assess the pattern of population structure of the studied enset populations and to detect the extents of admixture, a Bayesian model-based clustering algorithm was inferred using STRUCTURE version 2.3.4 (Pritchard *et al*., 2000; Falush *et al*., 2003). A burn-in period of 50,000 was employed in each run, and data were gathered over 500,000 Markov Chain Monte Carlo (MCMC) replications for K = 1 to K = 6 using 20 iterations for each K. This was done to estimate the most likely number of populations (K). The web-based STRUCTURE HARVESTER version. 0.6.92 (Earl and VonHoldt, 2012), was used to estimate the ideal K value

using the simulation method developed by Evanno *et al*. (2005). The bar plot for the most probable K was calculated by employing the Clumpak beta version (Kopelman *et al*., 2015).

5. 3 Results

To develop any conservation strategy for sustainable utilization and crop improvement programs, knowledge of the genetic diversity and population structure of any species is indispensable. In the present study, a total of 15 SSR markers have been used to estimate the extent of genetic diversity and population structure of enset populations from different administrative zones of the central Ethiopia. Out of which, twelve SSR markers (Table 5.2) exhibited reproducible polymorphic amplification during the screening process and were employed to analyze the 147 individual genotypes of the enset populations. The remaining two markers (Evg-03 and EnB-07) were observed to be monomorphic and hence, omitted from further analysis. The SSR fragment size ranged from 103 bp (Evg-01) to 217 bp (Evg-10) (Table 5.2).

5.3.1 Genetic Diversity

In the 147 enset genotypes considered, the selected 12 polymorphic SSR loci yielded a total of 289 alleles, with a mean of 24.5 alleles per locus. The number of alleles identified per locus ranged from 12 (in EnO-06) to 41 (in Evg-08) (Table 5.3). The major allele frequency (MAF) ranged from 0.09 (in Evg-12) to 0.25 (in Evg-10), with an average value of 0.154. The polymorphic information content (PIC) values ranged from 0.86 (in EnO-06) to 0.95 (in Evg-09), with an average of 0.91. Relatively, the highest effective number of alleles (Ne) (11.79), Nei's gene diversity (GD) (0.95), Shannon information index (I) (2.57), expected heterozygosity (He) (0.91), and unbiased expected heterozygosity (uHe) (0.94) were recorded for Evg-09, while the least parameters such as Ne, I, He, and uHe were observed in EnO-06. Similarly, Evg-09

revealed the highest value of gene flow (Nm = 7.02) and the lowest inbreeding coefficient based on genetic differentiation (Fst = 0.03) (Table 5.3).

Table 5. 3 Informativeness and levels of the different diversity indices of the SSR loci across the enset populations considered

Marker	MAF	NA	GD	PIC	Ne	I	Ho	He	uHe	Fst	Nm	PHWE
Evg-01	0.12	22.00	0.92	0.92	6.45	2.04	0.73	0.82	0.84	0.10	2.22	0.00**
Evg-02	0.12	26.00	0.93	0.93	9.39	2.37	0.88	0.87	0.90	0.08	2.91	0.00**
Evg-04	0.15	32.00	0.93	0.92	9.03	2.32	0.85	0.87	0.90	0.06	3.71	0.00**
Evg-06	0.19	18.00	0.87	0.86	6.03	1.95	0.77	0.82	0.85	0.06	4.10	0.00**
Evg-08	0.13	41.00	0.94	0.94	10.53	2.44	0.88	0.90	0.93	0.04	5.54	0.03*
Evg-09	0.10	31.00	0.95	0.95	11.79	2.57	0.86	0.91	0.94	0.03	7.02	0.03*
Evg-10	0.25	18.00	0.90	0.89	7.43	2.15	0.80	0.85	0.88	0.05	4.87	0.00**
Evg-11	0.11	21.00	0.93	0.93	9.05	2.23	0.92	0.85	0.88	0.07	3.19	0.21^{ns}
Evg-12	0.09	26.00	0.94	0.94	9.67	2.40	0.88	0.88	0.91	0.06	3.67	0.04*
Evg-17	0.18	27.00	0.92	0.92	8.25	2.17	0.85	0.85	0.87	0.09	2.41	0.04*
EnO-04	0.20	15.00	0.90	0.89	6.54	1.94	0.89	0.81	0.84	0.10	2.29	0.02*
EnO-06	0.21	12.00	0.87	0.86	5.13	1.78	0.79	0.78	0.80	0.10	2.30	0.00**

MAF=Major allele frequency; NA=Number of alleles; GD=Gene diversity; Ne=Effective number of alleles; PIC=Polymorphic information content; I=Shannon's Information Index; Ho=Observed heterozygosity; He=Expected heterozygosity; uHe=unbiased heterozygosity; Fst=inbreeding coefficient within subpopulations relative to total (genetic differentiation among subpopulations); Nm=gene flow estimated from Fst 0.25(1- Fst)/Fst; PHWE P-value for deviation from Hardy Weinberg equilibrium, ns not significant, * = $P < 0.05$, ** = $P < 0.0001$ and hence highly significant.

5.3.2 Extents of Genetic Diversity in the Populations Considered

Estimates of genetic diversity in the six enset populations considered are presented in Table 5.4. Accordingly, the Hadiya population showed the highest values with regard to the number of different alleles (Na), number of effective alleles (Ne), Shannon diversity index (I), and expected heterozygosity (He). However, the wild population showed the least in all these diversity indices. Similarly, the highest estimate of allelic richness (Ar) (7.80) was also recorded in the Hadiya

population, followed by the Kambata-Tembaro (7.73) population. On the other hand, the highest number of private allelic richness (Arp) (1.29) was observed in the wild population, whereas, released population contained lower number of private allelic richness (0.74). Relatively higher observed heterozygosity (Ho) was recorded in both the wild and released (Ho = 0.88) populations, while it was least in Gurage and Hadiya (I = 0.81) populations. The percentage polymorphic loci (PPL) ranged from 89% (in the Hadiya population) to 100% (in the wild population), with a mean value of 94.83%.

Table 5. 4 Summary of the different diversity indices scored for each population over the 12 loci considered

Population	Na	Ne	Ar	Arp	I	Ho	He	uHe	PPL%	F
Gurage	14.25	8.56	7.09	1.14	2.24	0.81	0.84	0.86	95.00	0.014
Silte	13.25	8.42	7.24	0.89	2.28	0.84	0.87	0.89	99.00	0.032
Kembata	16.00	9.76	7.73	0.91	2.44	0.83	0.88	0.90	96.00	0.060
Hadiya	17.25	10.64	7.80	1.09	2.50	0.81	0.89	0.90	89.00	0.085
Wild	6.83	5.44	6.83	1.29	1.72	0.88	0.78	0.85	100.00	-0.116
Released	8.50	6.83	7.06	0.74	1.98	0.88	0.84	0.89	90.00	-0.048
Mean	**12.68**	**8.27**	**7.29**	**1.01**	**2.20**	**0.84**	**0.85**	**0.88**	**94.83**	**0.005**

NA=Number of alleles; Ne=Effective number of alleles; Ar=Allelic richness; Arp=Private allelic richness; I=Shannon's Information Index; H_O=Observed heterozygosity; He=Expected heterozygosity; uHe=unbiased heterozygosity; PPL= percentage polymorphic loci; F=Fixation index

5.3.3 Population Genetic Differentiation and Gene Flow

Analysis of molecular variance (AMOVA) revealed that 89% of the total variation is accounted for the individuals within a group (population); whereas variation among the six populations and individual genotypes (samples) in each population contributed to only 5% and 6% of the total variation, respectively (Table 5.5). Thus, the heterozygosity of the individuals within each population accounts for most of the within-population variation. The overall genetic

differentiation among the six enset populations was limited and significantly low (F_{ST} = 0.05; p < 0.001). On the other hand, the average gene flow among the analyzed enset populations was high (Nm=5.14).

Table 5. 5 AMOVA among and within enset populations based on the 12 SSR markers

Source	Df	SS	MS	Est. Var.	%	Nm	F_{st}
Among Populations	5	88.36	17.67	0.26	5.00%		
Among Individuals	141	799.69	5.67	0.35	6.00%		
Within Individuals	147	731.00	4.97	4.97	89.00%		
Total	293	1619.05		5.58	100.00%	5.14	0.05

Df=Degrees of freedom, SS=Sum of squares, MS=mean squares, Est.Var=Estimated variation, Nm=gene flow

5.3.4 Extents of Genetic Distance between the Populations

The extent of pairwise genetic distances between the six enset populations is presented is Table 5.6. The highest genetic distance (1.16) was observed between populations from Gurage and wild, followed by populations from Silte and wild (1.02). Conversely, the lowest pairwise genetic distance was observed between populations from Gurage and Silte (0.37).

Table 5. 6 Pairwise Population Matrix of Nei's Genetic Distance Scored over the 12 SSR Markers

Populations	Gurage	Silte	Kembata	Hadiya	Wild	Released
Gurage	a					
Silte	0.37	a				
Kembata	0.79	0.59	a			
Hadiya	0.74	0.59	0.47	a		
Wild	1.16	1.02	1.01	0.71	a	
Released	0.83	0.63	0.73	0.47	0.74	a

a = Not applicable

5.3.5 Cluster Analysis

The Unweighted Pair-Group Method with Arithmetic Mean (UPGMA) based cluster analysis using the 12 polymorphic SSR markers grouped the 147 enset genotypes into three major clusters (I, II, and III) (Figure 5.2). Cluster I had the largest number of genotypes, which consisted of 81 (55.1%) of the total enset genotypes, that formed two separate sub-clusters (IA and IB). Among these sub-clusters, IA contained 16 landraces, which represent exclusively Kembata populations. On the other hand, sub-cluster IB consisted of 65 (44.2%) enset genotypes, of which 35 (53.85%) were from Hadiya, 13 (20.0%) from Kembata, and seven (10.77%) each from the Released and Wild populations. Cluster II comprised 60 genotypes, which were further divided into two distinct sub-clusters (IIA and IIB). Sub-cluster IIB contained 27 (75%) and 9 (25%) enset genotypes representing Gurage and Silte populations, respectively. In the same pattern, sub-cluster IIA counted 19 (79.17%) from the Silte population. Cluster III encompassed only six enset landraces, four from Hadiya and the rest two from Gurage populations. The grouping pattern revealed some degree of alignments with the geographic or administrative region of collections though intermixes from different geographic or administrative zones of collections have been observed (Figure 5.2).

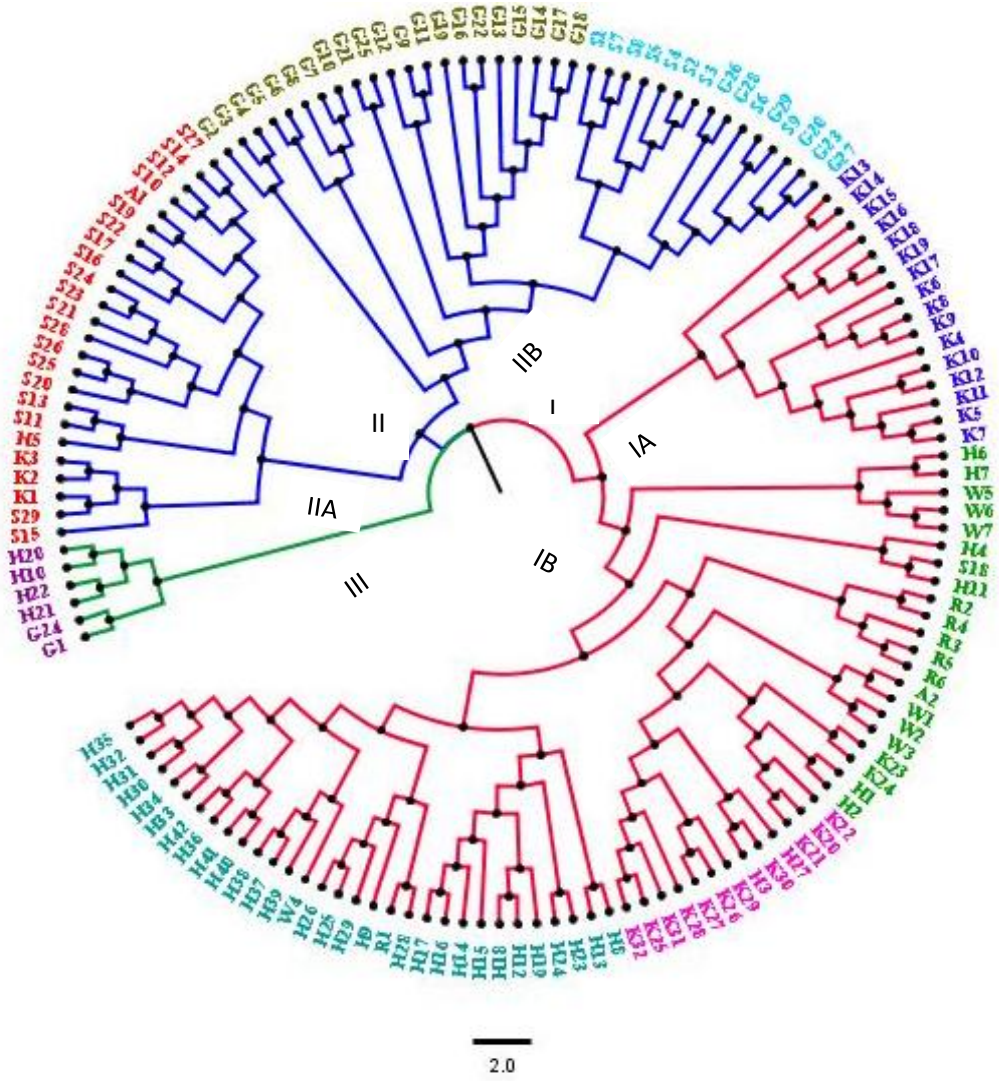

Figure 5. 2 A dendrogram based on the unweighted pair-group method with the arithmetic mean (UPGMA) of the 147 *E. ventricosum* individuals representing the six populations (Gurage, Silte, Kembata, Hadiya, Released, and Wild varieties) grouped in three major clusters (I, II, and III) and sub-clusters (IA, IB, IIA, and IIB).

5.3.6 Principal Coordinate Analysis

The principal coordinate analysis (PCoA) created using the first two components showed a similar pattern to UPGMA and followed a weak pattern of grouping following their geographic location of collections. Collections from different but adjacent collection zones were lumped

together with their distinctive small group. The majority of the enset samples collected from Gurage zone are grouped together on the upper left, and those from Silte administrative zone clustered at the center. The predominant enset population obtained from Kembata is clustered around the lower left and right sides of the axis (Figure 5.3). Enset populations collected from the Hadiya administrative zone are distributed on the upper right and lower right of the axis. The wild and released samples are more or less distributed on the upper right and upper left sides of Axis 1 (Figure 5.3).

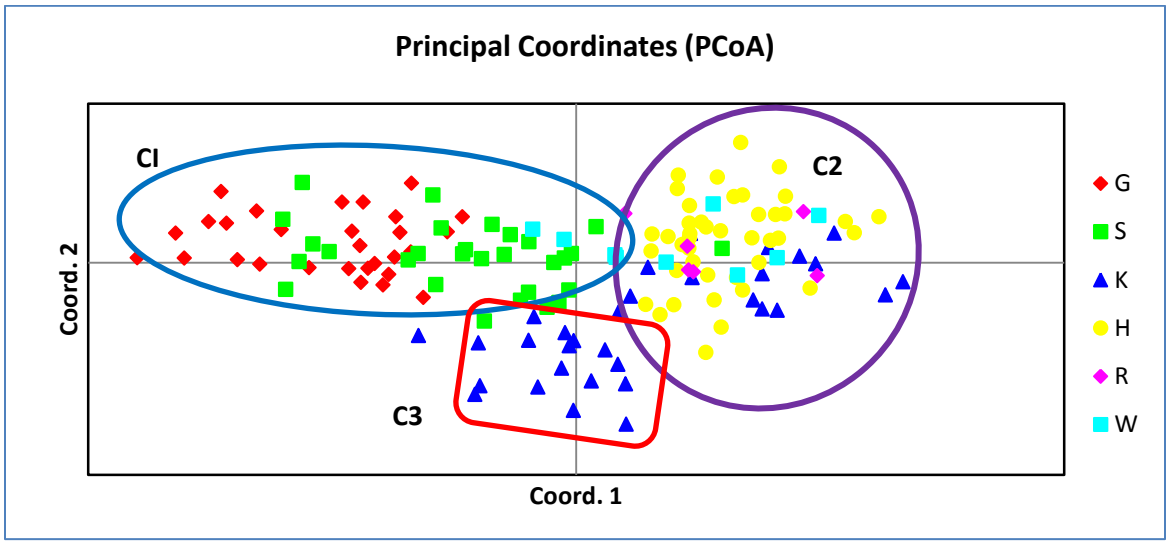

Figure 5. 3 Two-dimensional (2D) PCoA representation of the genetic relationships among the 147 individuals of *E. ventricosum* representing six populations (G, Gurage = red; S, Silte = deep green; K, Kembata = blue; H, Hadiya = yellow; R, released = pink; W, wild=light green) from Ethiopia.

5.3.7 Structure Analysis

To further comprehend the genetic structure of the entire 147 enset samples (genotypes), a Bayesian-based population structure following the Evanno *et al*. (2005) analysis was determined using STRUCTURE program outputs. Accordingly, the predicted highest K value was two (K=

2) (Figure 5. 4). Based on this value, the Clumpak result (bar plot) revealed a limited admixtures, and as a result, the populations were largely structured together according to the closeness of their collection sites or geographic origin.

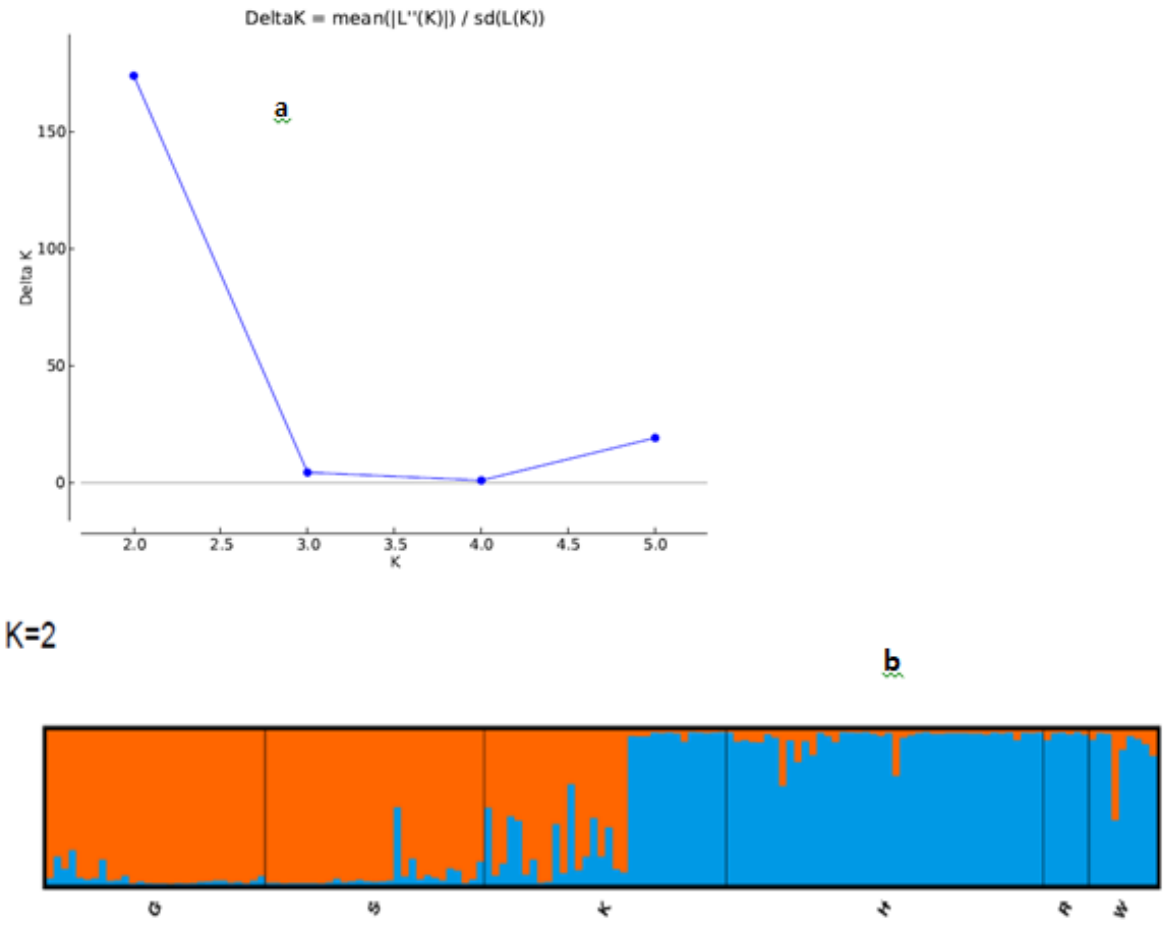

Figure 5. 4 Results of the population structure of the 147 *E*. *ventricosum* individuals in six pre-determined populations using Evano *et al*. (2005) method and Bayesian model-based estimation shows Delta K plot for maximum value at K =2 (a), CLUMPAK plot for K = 2 using the CLUMPP software, *G* Gurage, *S* Silte, *K* Kembata, *H* Hadiya, *R* Released, and *W* Wild (b).

5.4 Discussion

To further enhance enset crop conservation and improvement, the use of molecular markers, such as SSR markers, is a widely recognized approach. As enset is a diploid species (Cheesman, 1947; Endashaw Bekele and Shigeta, 2011; Borrell *et al*., 2019), an aggregate of two alleles per individual sample is anticipated at the single-copy microsatellite locus (Howells *et al*., 2016). The detection of two hundred and eighty-nine alleles (an average of 24.5 alleles per locus) using the twelve SSR markers, the present study is indicative of the presence of high genetic diversity in the populations considered. The number of alleles we detected is high compared to several of the reports so far. For example; Selemawit Getachew *et al*. (2014) reported 61 alleles from 220 enset genotypes by applying 11 banana SSR markers; Temesgen Olango *et al*. (2015) reported 202 alleles from 66 enset genotypes employing 34 enset SSR loci, Fetta Gerura *et al*. (2019) reported 77 alleles from 83 enset genotypes using 12 enset SSR microsatellites, and Gizachew Nuraga *et al*. (2022) detected 38 alleles from 52 genotypes using 15 SSR loci. Such a large number of alleles detected in the present study suggest that the microsatellite markers we selected are appropriate and provide an excellent chance to assess enset genetic resources for conservation and breeding.

5.4.1 Estimates of Genetic Diversity in the Populations

Genetic diversity offers opportunities to understand the genetic structure and level of diversity within and among genotypes, populations, and species (Delfini *et al*., 2022). In comparison to smaller, recently evolved populations, larger and older populations are predicted to have higher levels of accumulated and maintained genetic variation (Rampersad *et al*., 2013), which is crucial for enhancing fitness and consequently decreases the possibility of local extinction (Futuyma,

2008). In the present study, the polymorphic information content (PIC) of the 12 SSR markers applied showed larger values suggesting the high discriminatory power of the SSR markers employed following recommendations by Prabakaran *et al.* (2010) and Tiago *et al*. (2019) that suggested a PIC value of greater than 0.5 as very informative and helpful for determining the degree of polymorphism at a given locus. Similarly, Dutta *et al*. (2016) had also described a high PIC value as one of the most critical indications to effectively assess genetic diversity in a given species. Previous studies had shown slightly inconsistent PIC values using different numbers of SSR markers on enset. For instance, Temesgen Olango *et al*. (2015) observed a PIC value of 0.41–0.77 by evaluating the genetic diversity of 66 enset landrace collections; Fetta Gerura *et al*. (2019) described a PIC value of 0.62–0.77 in 83 enset landraces from the Gurage Zone using 12 SSR markers; and Gizachew Nuraga *et al*. (2022) obtained a lower PIC value ranging from 0.26–0.72 in 52 cultivated enset landraces using 15 SSR markers. The use of various types and numbers of genotypes and primers in each of those studies could be the reason for the disparity. It has further been explained by Biswas *et al*. (2020) who suggest that the number of genotypes and the genetic background of the genotypes have a significant impact on PIC values.

Of the total enset populations considered, the wild varieties retained the highest private allelic richness, which suggests it is a source of novel alleles for breeding and conservation. Such high private alleles could be attributed to a relatively high rate of mutation, particularly at SSR loci (Matus and Hayes, 2002). The result corroborates with the reports by Fetta Gerura *et al*. (2019).

The estimate of Shannon's information index (I) in this study also revealed high extent of genetic variation within the examined enset populations, which is an indication of the population diversity in a specific environment, according to Dido *et al*. (2022). Overall, the

values we detected were higher as compared to the previous findings by Temesgen Olango *et al.* (2015) (1.16), Fetta Gerura *et al*. (2019) (1.17), and Gizachew Nuraga *et al.* (2022) (0.74).

The expected (He) and observed (Ho) heterozygosities in the present study again revealed high extents of genetic diversity in the enset populations studied, with the observed and expected heterozygotes closely matching the same frequency (He = 0.85; Ho = 0.84). Likewise, the detected mean expected heterozygosity (He=0.85) is higher than the values reported by Temesgen Olango *et al.* (2015) (0.59), Fetta Gerura *et al*. (2019) (0.59), and Gizachew Nuraga *et al*. (2022) (0.47).

Jump *et al*. (2009) stated that heterozygosity is one of the key parameters to study genetic variation in natural populations in addition to revealing the structure and even history of a population. Similarly, the mean expected heterozygosity (He) within a population is the most reliable way to quantify genetic variation (Wendawek Abebe *et al*., 2013). In this regard, the present study indicated that the loci considered exhibited the existence of high heterozygosity that caused a considerable deviation from Hardy-Weinberg Equilibrium (HWE). Fekadu Gadissa *et al*. (2018) described high heterozygosity as being expected in historically outcrossing plant species that preserve their heterozygosity through vegetative propagation. This was expected considering that the majority of vegetatively propagated crops are highly heterozygous (Selemawit Getachew *et al*., 2014; Kiflu Tesfamicael *et al*., 2020; Alye Haile *et al*., 2023). Entirely all cultivated enset crops in general and the samples we collected from the farmers' homegardens are vegetatively propagating from young mother plants, but the wild relatives are originally regenerating through sexual reproduction and maintained by vegetative propagation for a number of years, *ex-situ* conservation sites for the enset crop. The same situations have

been noted in *Plectranthus edulis* by Fekadu Gadissa *et al*. (2018), cassava (*Manihot esculenta*) by Tiago *et al*. (2019), and banana by Biswas *et al*. (2020).

The enset populations we considered revealed a low fixation index (F = 0.005), suggesting accumulation and maintenance of the existing heterozygosity either through somatic mutation over time or through other mechanisms (McKey *et al.*, 2010; Alye Haile *et al*., 2023). On the other hand, the result suggests a minimized role of inbreeding that could facilitate homozygosity and hence a larger coefficient (Tiago *et al*., 2019; Zerihun Teshome *et al*., 2020).

The present study showed a relatively high extent of genetic differences within populations in terms of gene diversity, Shannon's information index, and heterozygosity. This may be attributed to the very diverse landrace resources cultivated and maintained for a long period by local farmers for their livelihood and other different socio-cultural purposes. Consequently, the regions could be viewed as genetic diversity hotspots and one of the potential in-situ conservation sites for the enset crop. This result also supports the existence of high on-farm or farmer diversity in the studied regions that have been sustainably conserved for generations (Awol Zeberga *et al*., 2014; Zerihun Yemataw *et al*., 2014; Tesfaye Dilebo *et al*., 2023a).

In general, the computed genetic diversity of enset in the present study is greater than that determined by earlier studies using different DNA markers such as RAPD (Genet Birmeta *et al.*, 2002), AFLP (Almaz Negash *et al*., 2002), and ISSR (Dagmawit Tobiaw and Endashaw Bekele, 2011) which is more likely as SSRs are more variable markers than RAPD, AFLP, and ISSR (Abdelhamid *et al*., 2014). Such a large allelic variability, as other SSR marker studies in enset may be attributed to the nature of SSR markers since they have a high mutation rate both forward and backward. However, drawing general conclusions from a direct comparison of

these studies is challenging due to the differences in the number as well as types of populations and DNA markers employed. In the same manner, Hamza *et al*. (2013) described that comparisons of comprehensive diversity estimates from marker systems with various properties and origins of difference do not permit relevant conclusions.

5.4.2 Implications of Population Genetic Differentiation and Gene Flow

The computed analysis of molecular variance (AMOVA) demonstrated a higher within-population variance than among the populations, suggesting less impact of the region or zone of origin. The result is in line with previous reports by Temesgen Olango *et al*. (2015), Fetta Gerura *et al*. (2019), and Gizachew Nuraga *et al*. (2022) using SSR marker systems. In the same manner, Almaz Negash *et al*. (2002) and Kiflu Tesfamicael *et al*. (2020), Genet Birmeta *et al*. (2002), and Dagmawit Tobiaw and Endashaw Bekele (2011) have reported that the among-population variance is lower than the within-population variance for enset genotypes using AFLP, RAPD, and ISSR markers, respectively. Overall, the result indicates the importance of within-population diversity to initiate conservation and utilization of the current enset diversity in the country at large.

Similarly, a low F_{ST} value (0.046) observed among the analyzed genotypes once again shows low genetic differentiation among the studied enset populations. Nkhata *et al.* (2020) indicated that genetic divergence among populations can be low if the value of F_{ST} is less than 0.05. This is most likely related to the high rate of gene flow between enset farming communities in different zones due to the close interaction between farming households and the deep-rooted tradition of sharing and exchanging enset germplasm by raising suckers of desired/preferred genotypes. Local cultures and relatedness of families in the enset-growing areas of Ethiopia as revealed in

their languages and practices, including collaborations in enset farming and harvesting activities of men and women. In general, such commonality in farming is attributed to the sociological characteristics and psychological make-up of the enset culture people of Ethiopia. A similar trend and observation have been documented by other researchers (Admasu Tsegaye and Struik, 2002; Zerihun Yemataw *et al*., 2016; Tesfaye Dilebo *et al*., 2023a,b). Such relatively high gene flow (mean Nm = 5.14) in the present study, contributes to the low level of population differentiation. In the same line, Slatkin and Barton (1989) had stated that if the average number of migrants (gene flow) per generation (Nm) is less than 1, then no random differentiation across populations may be anticipated. Wright (1951) had also observed an inverse correlation between gene flow (Nm) and population divergence (F_{ST}). Extensive vegetative propagation practices of enset by farmers may further restrict the extent of gene flow and consequently limit its population differentiation.

5.4.3 Patterns of Genetic Distance between the Populations

In terms of pair-wise population genetic distances, the present study showed a relatively higher Nei's standard genetic distance (1.16) between Gurage and wild populations. On the other hand, the lowest genetic distance (0.37) was observed between Silte and Gurage, followed by Kembata and Hadiya populations. The result suggests that the extent is parallel to the closeness of the collection administrative zones and vice versa, which could be attributed to the high sharing of enset genotypes as a planting source among the adjacent administrative or geographic regions. A similar pattern was reported in populations from Gamo Gofa *vs.* Wolaita and Wild *vs.* Ari (Temesgen Olango *et al*., 2015) and the enset populations from the Gurage zone of Ethiopia (Fetta Gerura *et al.*, 2019).

5.4.4 Patterns of Grouping and Population Structure

Understanding the genetic relationships and population structures between the diverse enset populations collected from different locations could provide a substantial advantage for improvement programs. In addition, it offers valuable knowledge regarding the extent of gene flow or evolutionary linkages in crop species at large. Accordingly, the UPGMA based cluster analysis confirmed the absence of pronounced intermixes of the enset samples from different collection sites or sources. Similar results have been reported by Temesgen Olango *et al*. (2015) and Fetta Gerura *et al*. (2019). Similarly, the principal coordinate analysis (PCoA), a multivariate dataset that allows us to explore the spatial distribution of the genetic distances between populations using the two-dimensional plot, according to Özkan *et al*. (2022), also revealed the same patterns of grouping, suggesting low genetic divergence among the populations. As reported by Kiflu Tesfamicael *et al*. (2020), PCoA employing AFLP markers revealed that wild and cultivated enset landraces formed clusters with a significant number of overlapping individuals from the two groups.

The population structure analysis also showed that the enset collections from the four collection zones and AARC were closely related to one another and overall, two inferred groups (K = 2) with little admixtures have been detected. It is remarkable to note that each enset genotype analyzed has alleles that come from the two groupings, the cultivated landraces, and wild collections. This signifies the existence of a higher level of admixture among the different populations, which confirms the high gene flow and eventually minimal population differentiation. This could result from the exchange of genetic materials between the local and regional enset farming communities. The result is in agreement with the previous reports by

Genet Birmeta *et al*. (2002), Temesgen Olango *et al*. (2015), Fetta Gerura *et al*. (2019), Kiflu Tesfamicael *et al*. (2020), Gizachew Nuraga *et al*. (2022), and Alye Haile *et al*. (2023).

5.4.5 SSR Markers for Conservation and Utilization of Enset Populations

One of the main uses of the SSR marker is offering a room for evaluation of genetic diversity within or among natural populations, which is essential for effective conservation and utilization of genetic resources (Biswas *et al*., 2020). Moreover, it is an essential tool in maintaining indigenous plant genetic resources for use in food security, a very important issue in reducing vulnerability since they have been adapted for centuries to the local climate, soil, landscape, and cultural heritage of each region (Berry *et al*., 2018) and thus, ensures the future needs of subsistence farmers, breeders, researchers, and the entire community (Khoury *et al*., 2010). The SSR marker system also generates baseline information on local crop diversity that could benefit farmers in terms of local adaptation to ecological diversity, pests, pathogens, risk management, rituals, and food culture (Rao and Sthapit, 2012). In this regard, the present study has established baseline information from the points of view of genetic diversity estimate in the enset population, one of the most economically important but neglected indigenous food crops, to help facilitate its conservation and utilization. In addition to the SSR markers, other more reliable and evenly distributed markers may be important, even if the allelic richness may be lower for inferring long-term evolutionary relationships or ancestry.

According to report, a significant number of the enset populations and their genetic diversity have decreased in different parts of the country over the past 50 years (Zippel and Ludders, 2005). As a result, maintenance of the remaining diversity is being undertaken by combining both *in situ* (on-farm) and *ex-situ* (*in vitro* or in-field gene banks) conservation initiatives. Almaz

Negash (2001) and Admasu Tsegaye and Struik (2002) suggested that on-farm conservation is an unquestionably important component to be supported along with the formal (*ex-situ*) conservation initiatives as it enables continued maintenance and evolution of landraces using conventional cultivating practices. To support community initiatives to conserve agro-biodiversity in many indigenous communities, strengthening local institutions and farmer leadership has been shown to be effective. Temesgen Olango *et al*. (2014) suggested that enset bio-cultural resource continuity must be based on participatory community approaches and the mobilization of both the young and the elder groups.

Nowadays, the Areka Agricultural Research Center (AARC) has maintained over 600 enset germplasm collections from 12 enset cultivating areas of Ethiopia in field gene banks. Temesgen Olango *et al*. (2014) reported that only 40% of the landraces that were known to the Wolaita agricultural community were represented by the AARC collection, indicating that the actual diversity is still not fully sampled. Similarly, Zerihun Yemataw (2018) asserted that the Areka collections have not undergone a thorough assessment. This indicated that there is a need for systematic collection from the major enset growing regions and other locations of interest, including wild populations, to apply appropriate conservation strategies both *in situ* (on-farm) and *ex-situ* (*in vitro*, or in-field gene banks), and to undertake full characterization of enset germplasm for its role in prospective breeding initiatives, and as a source of better-performing landraces. In this regard, the present study in general and the marker systems used in particular are essential and could offer tips to initiate and facilitate maintenance and enhancement of the crop.

5.5 Conclusion

Given the significance of the enset crop in the central, southern, and southwestern regions of the Ethiopian agriculture and food production system, the present study provides additional information on the extent of genetic diversity and population structure of enset genetic resources to help implement suitable conservation plans, crop improvement and breeding programs. The study revealed high genetic diversity among the enset samples with very low fixation indices among the populations. The computed AMOVA also showed the occurrence of high diversity among individuals within populations than among populations. This can be explained by a significant gene flow and maintained low genetic divergence among the populations. The patterns of genetic variability in cultivated enset landraces showed a weak link with cultivation regions. The findings of this study have demonstrated that the SSR marker system can be applied effectively to identify genetic diversity among enset genotypes and it also suggests including more germplasm collections from the remaining enset growing areas for conservation and to develop the best-performing enset varieties. Furthermore, this study inspires researchers to create genomic tools for this crucial crop, to enable faster delivery of improvements to Ethiopia's food and nutrition security through the use of modern breeding techniques like marker-assisted selection (MAS) and genomic selection (GS).

Chapter Six

In vitro propagation of multi-use enset [*Ensete ventricosum* (Welw.) Cheesman] landraces using *bulla* as gelling agent

Abstract

Enset is a perennial, multipurpose crop that is cultivated and consumed in Ethiopia. Nowadays, its traditional propagation systems face a challenge due to biotic and abiotic factors. Thus, shoot tip culture can be very advantageous for the quick multiplication of healthy plantlets to secure the conservation as well as propagation of the enset crop. Therefore, this study was designed to develop an efficient micro-propagation protocol for three popular multi-use enset genotypes by using locally available bulla and agar as gelling agents separately. The experiment was conducted in a completely randomized design with three replications in a factorial arrangement. About 1.0 cm long shoot tips were cultured on MS medium supplemented with 1 to 6 mg/l BAP separately or in combination with IAA. It was found that the 8% (w/v) enset bulla was ideal and provided significant figures in the number and length of shoots and roots per shoot and also early initiation of shoots and roots when compared with 0.6% (w/v) agar-gelled MS media. MS medium containing 2.0-3.0 mg/l BAP was the appropriate concentration for in vitro shoot induction and growth. The presence of 4.0 mg/l BAP alone, and 5.0 mg/l BAP in combination with 1.0 mg/l IAA was suitable for multiple shoot induction, whereas, 2.0 mg/l IBA and 1.0 mg/NAA separately were found to be the optimum concentration for root induction and development. Thus, bulla in addition to its alternative gelling potential with low cost has an essential role in the rapid production and conservation of enset with desirable traits and disease-free plantlets for farmers.

Keywords: Alternative solidifing agent, Conservation, Micro-propagation, Plant growth regulators, Shoot-tip, Tissue culture

6.1 Introduction

Enset (*Ensete ventricosum* (Welw.) Cheesman) is a giant perennial herbaceous monocarpic multipurpose crop. It belongs to the family Musaceae in the order Zingiberales. The Musaceae family comprises the genera *Musa* and *Ensete*, which are found in both Africa and Asia (Borrell *et al.*, 2019). *Ensete ventricosum* is likely the most widely distributed species within Musaceae, existing throughout much of central, southeast, and east Africa (Baker and Simmonds, 1953). However, its domestication, cultivation, and human consumption as food are limited to Ethiopia (Brandt *et al*., 1997). Enset is a major food crop for more than 25 million people living in central, south, and southwest Ethiopia (Zerihun Yemataw *et al*., 2016; Borrell *et al*., 2020). It is also used for animal feed, fuel wood, construction materials, and as a traditional herbal medicine for different human and livestock diseases (Brandt *et al.*, 1997).

Enset-based farming system is an indigenous and sustainable agricultural system that covers large areas of land in Ethiopia (Westphal, 1975; Borrell *et al*., 2020). Farmers in the enset growing regions have a wealth of knowledge in tackling variety selection and cultivations, as well as pest and disease management which are well adapted to their socioeconomic and environmental conditions (Zerihun Yemataw *et al*., 2016). Since enset is an indigenous crop, almost all production and processing practices are based on farmers' experiences (Abraham Bosha, 2018).

Cultivated enset is most commonly propagated by vegetative means using the corm of a three- or four-year-old plant after cutting away the pseudostem at about 10 cm above the ground, then exposed to sunlight for two days, and subsequently buried again in the soil after removing the

apical meristem area from the central part of the corm. After 2-3 months, new suckers will emerge (Admasu Tsegaye, 2002; Blomme *et al.*, 2018). This traditional method, however, is very tedious and laborious (Genet Birmeta and Welander, 2004) and also results in a poor propagation rate and diseased planting materials (Tripathi *et al*., 2017). Although enset can also be propagated by seeds, seed production is not a common practice, as farmers do not usually postpone harvesting until the maturity of the seeds (Almaz Negash, 2001; Tesfaye Abebe, 2005). During the maturation of the seeds, the enset becomes dried up, resulting in a total loss of its food value (Almaz Negash *et al.*, 2000). Moreover, the hard, irregularly shaped seeds are very difficult to germinate. This results in a low germination rate (12%) and a long germination time (Karlsson *et al*., 2015).

According to Westwood *et al*. (2021), more than 20% of plant species are threatened with extinction. Therefore, to establish a sustainable food system, it is essential to conserve plant species, especially food crops, and their regionally well-adapted cultivars (Bhat *et al*., 2022). Plant tissue culture is a technique that involves the growth and multiplication of totipotent cells, tissues, and organs of plants on defined solid or liquid media comprising nutrients under an aseptic and controlled environment (Thorpe, 2007; George *et al*., 2008) and will assist in conserving rare plants from extinction (Pegg, 2002). It is also used for cryopreservation, conservation of rare and highly endangered plants, and the production of secondary metabolites (Kaczmarczyk *et al*., 2011; Coste *et al*., 2012; Chandana *et al*., 2018). Micropropagation is one of the tissue culture techniques used for the production of ‘disease-free’, high-quality, and uniform planting material within a relatively short time for commercial purposes, independent of the season (Tileye Feyissa *et al.*, 2005; Garcia-Gonzales *et al*., 2010). However, the full

application of these techniques depends on the cost of their ingredients. Of the different gelling agents, agar is the most commonly used and expensive gelling agent for the preparation of solid and semi-solid media for plant culture as compared to other ingredients (Babbar *et al.,* 2005; Esekiel, 2010; Jain and Babbar, 2011), and it contributes about 70-75% of the total production cost (Deb and Pongener, 2010; Ebile *et al*., 2022). Subsequently, efforts have been taken to identify less expensive alternatives to agar as gelling agents to reduce medium costs without compromising the micropropagation rate or the quality of the plants produced. According to Nene and Sheila (1999), Raghu *et al*. (2007), and Daud *et al.* (2011), various alternative gelling agents such as potato, rice, barley, wheat, cassava, and corn starches have been used as sources, either singly or in combination, with varying degrees of success.

During traditional processing, enset crops produce three main starchy food products: *qocho*, *bulla*, and corm (*amicho*) (Admasu Tsegaye and Struik, 2002). *Bulla* is one of the starchy products of enset, which is obtained by squeezing a mixture of the unfermented decorticated pseudostem and pulverized corm and decanting the liquid, followed by air drying (Tadessa Daba and Shigeta, 2016). It is considered the best quality enset food (Pijls *et al*., 1995) and is consumed mainly as porridge, in gruel, and crumbled form (Temesgen Olango *et al*., 2014). According to ESTC (2003), starch produced from enset can be used for the paper, textile, and adhesive industries. Enset starch also has the potential to be used in binding and disintegrating compressed tablets (Tsige Gebre-Mariam and Nikolayev, 1993; Tsige Gebre-Mariam and Schmidt, 1996). Moreover, enset derivative flour or *bulla* in *in vitro* propagation media of pineapple (Biruk Ayenew *et al.,* 2012), vanilla (Ayelign Mengesha *et al*., 2012), and cassava (Manaye Ayalew *et al*., 2017) has been used as a gelling agent by substituting expensive

conventional agar and saving the production costs of the culture media. Hirose *et al*. (2010) verified that enset starch is used for both industrial and food purposes and found it to have high gelatinization properties.

The varietal diversity of the enset crop is relatively wide, and due to these features and others, the nutritional composition of the enset product *bulla* showed variability. The reports of Tadessa Daba and Shigeta (2016) and Ashenafi Tuffa (2019) indicated that the proximate values ranged from 0.45 to 1.0% (for protein, fat, fiber, and ash) and minerals content in mg/100g (calcium, potassium, magnesium, phosphorous, iron, and zinc were 11.4-58.7, 270-337, 5.2-11.9, 30.1-33.0, 2.5-7.0, and 0.2-4.5, respectively) for *bulla* from different enset landraces.

Despite the significance of enset as a food, feed, fiber, and medicinal crop, insufficient research has been conducted to improve its cultivation and production, and also to maintain its genetic resources. Moreover, nowadays many landraces are disappearing from farmers' home gardens due to biotic and abiotic factors, farmers' selection pressures, and changes in land use systems. Hence, micropropagation could be very advantageous for enset to produce healthy plantlets free from diseases that are easily transmitted when suckers are used as a source of new planting materials. Furthermore, micropropagation serves as a backup strategy for the conservation of the existing diversity of enset germplasm. Although there are a limited number of papers on the micropropagation of enset, as far as we know, no reports have been published in which *bulla* has been used as a gelling agent in *in- vitro* plant tissue culture on enset plants. Therefore, this study aims to develop and optimize an efficient protocol for the shoot tips of farmers' selected three multi-use enset landraces on *bulla* and agar-gelled media with different concentrations of

cytokinin and auxin. The research aimed at testing the hypothesis of whether or not *bulla* is an efficient gelling agent and whether using it would be more economical than using agar.

6.2 Materials and Methods

6.2.1 Plant materials

Four-month-old suckers of three enset landraces, namely *Astara*, *Gimbo,* and *Sisqella* were collected from a farmer's home garden in Hadiya zone, southern Ethiopia, and were planted subsequently in pots containing different soil mixtures in the greenhouse of Addis Ababa University until they resumed growth for three to five months before culturing, as shown in Figure 6.1A. These landraces are highly recognized by local people for their medicinal and edible sweet corm (*Astara*), high yield and quality of *qocho* and *bulla* (*Gimbo*), strong and durable fiber, and early maturing ability (*Sisqella*) (Tesfaye Dilebo *et al*., 2023b) and are also supported by previous works (Admasu Tsegaye and Struik, 2002; Zerihun Yemataw *et al*., 2014).

Figure 6. 1 Some mother enset suckers used as explant source, preparation for shoot initiation and shoot tip culture on different gelling agents: (A) Enset mother sucker used for explant source in the greenhouse; (B) Trimmed enset genotype *Astara* (left) and *Gimbo* (right); (C) Aseptically

prepared genotype *Sisqella* sucker in laminar flow hood cabinet ready for shoot tip inoculation; (D) Shoot tip culture on 0.6% agar gelled MS medium; (E) Shoot tip culture on 8% *bulla* gelled MS medium.

6.2.2 Extraction and analysis of some nutritional contents of *bulla*

The mass mixture from the scraped pseudostem and grated corms of two mature enset landraces of *Gimbo* were squeezed into the pit which was covered with enset leaves and a plastic sheet by using the bamboo-made sieve to collect the liquid. Then the resulting liquid was left overnight for sedimentation and the supernatant was discarded to obtain *bulla* (Figure 6.2). Thereafter, its surface was rinsed with clean water and dried in a moisture extraction oven. The dried *bulla* was milled into powder using a milling machine and weighed, packaged in zipped polythene bags to prevent rehydration, and stored in cool dry cardboard until required.

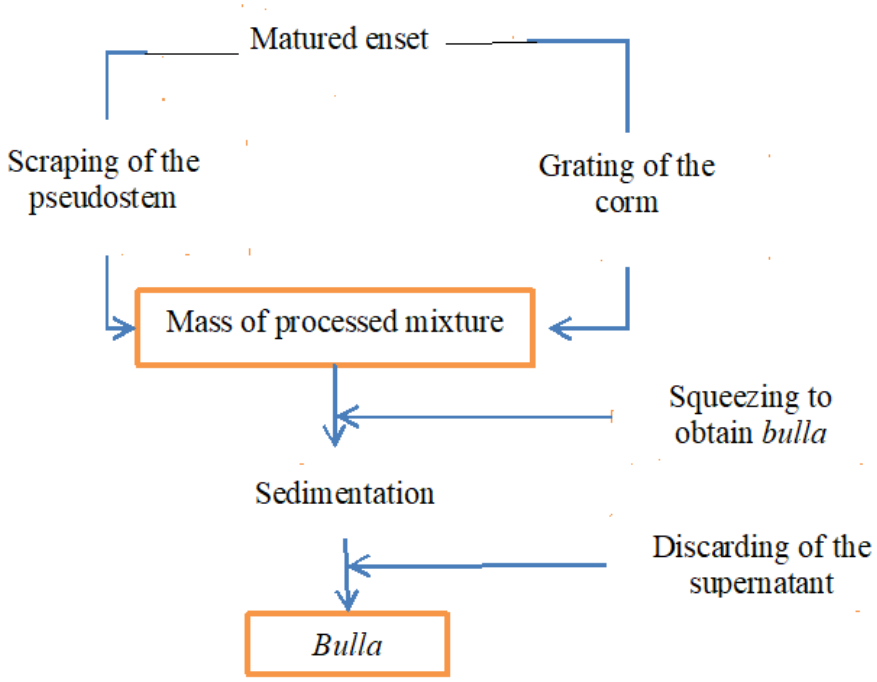

Figure 6. 2 Simplified flowchart that shows how *bulla* is produced

The extracted *bulla* flour was characterized for moisture content, crude protein, crude fat, crude fiber, and total ash using the methods developed by the Association of Official Analytical Chemists (AOAC, 2000). The procedures, respectively, were 925.09, 979.09, 920.39, 962.09, and 923.03 in which triplicate analysis was carried out in all cases. The total carbohydrate content was calculated by difference from other nutrients, using the formula as follows: Carbohydrate (%) = 100 – (% crude protein + % crude fiber + % total ash + % crude fat). The pH of *bulla* flour was determined from a 1/10 dilution of the sample. Minerals analyses were also determined according to the standard method of AOAC (2000) at the Center for Food Science and Nutrition, and in the Department of Chemistry Addis Ababa University, Ethiopia.

6.2.3 Gelling of culture medium using *bulla* flour

For this experiment, the MS culture medium was gelled with 5%, 6%, 7%, 8%, 9%, and 10% *bulla* flour, and 0.6% agar (Agar Plant Culture Test, Himedia Pvt. Ltd., Mumbai, India) was used as a control. The *bulla* flour was mixed with a portion of the cold culture medium in a 1-liter beaker separately, and then it was gently added to the remaining preheated medium at about 80^0C while being continuously stirred vigorously; otherwise, it settles at the bottom of the MS media jar. The pH of *bulla* flour was acidic (based on this study and other previous reports). Hence, it was required to adjust the pH to 5.75 just after the addition of *bulla* flour in the growth medium. The gelled culture medium was cooled down to about 60^0C, carefully dispensed into 50 ml glass jars or Magenta GA-7 culture vessels, and autoclaved at 120^0C for 20 min. Then it was allowed to cool in a laminar airflow hood before culture.

6.2.4 Preparation of stock solution and culture media

In this experiment, the MS (Murashige and Skoog, 1962) basal medium was used throughout each activity. The full strength (for initiation and multiplication) and the half strength (for rooting) of each stock solution were individually prepared and then stored at -20°C until the experiment was employed. In the same manner, the plant growth regulators (PGRs) used for this study were 6-benzyl aminopurine (BAP), α-naphthalene acetic acid (NAA), indol-3-butyric acid (IBA), and indol-acetic acid (IAA). Full-strength MS medium was prepared, and 3% sucrose was used as a carbon source, and 0.2% of activated charcoal (AC) was incorporated to prevent blackening due to polyphenol oxidation of explants (Mulugeta Diro *et al.*, 2004). Different concentrations of PGR were supplemented as given later, followed by adjusting the pH to 5.75, and subsequently, 6.0 g/l agar was added and melted on a stirring hot plate.

6.2.5 Culture initiation

Healthy suckers of explants were uprooted, and the leaves, pseudostem, and roots were carefully removed without affecting the shoot tips and corms. The explants were washed with household detergent and running tap water for 5 to 10 minutes. The outer layers, or leaf sheaths, of the explants were detached, and the shoots were trimmed to 3.0 cm length and 3.0 cm width (Figure 6.2B). Then, the explants were dipped in 70% (v/v) ethanol for 5 minutes followed by 4 times rinsing in sterile distilled water, and further sterilized with 20% sodium hypochlorite (NaOCl) for 20 and 10 minutes along with 3 drops of Tween-20, followed by 4 times rinsing with sterile distilled water. In each sterilization step, the outer tissue of the suckers that were exposed to disinfection solutions was removed, and the shoot tips were trimmed from all edges. Thereafter, the size of each explant shoot tip was further trimmed to 1.0 cm in length (Figure 6.2C). The

sterilized shoot tips of explants were cultured on a shoot initiation medium (Figures 6. 2D and E) and maintained in a relatively controlled culture room at 16 h photoperiod and 40±5µmolm^{-2}s^{-1} light intensity using cool white fluorescent light at 25 ± 2°C.

6.2.6 Shoot initiation

For shoot initiation, surface sterilized explants were cultured in culture jars containing 50 ml MS medium with 3% sucrose, 5-10% *bulla* powder, 0.2% activated charcoal (AC) and supplemented with different concentrations of BAP (0.0, 1.0, 2.0, 3.0, 4.0, 5.0, and 6.0 mg/l) in combination with IAA (1.0 and 2.0 mg/l). The experiment was carried out with three replications for each treatment level, with each Magenta GA-7 or culture jar containing one explant, for a total of 10 explants per treatment. Then, after culturing, the mouth of each culture vessel was appropriately closed with its cap and sealed with parafilm. The days for shoot initiation for each genotype and the shoot heights after a month were recorded.

6.2.7 Shoot multiplication

The initiated shoots were transferred to shoot multiplication MS medium supplemented with different concentrations of plant growth regulators as in shoot initiation. During transferring, the induced shoot tip was carefully removed near the corm without harming it by using a sterile surgical blade. After one month of culture in the growth room, well-developed plantlets were carefully separated from the explants and transferred to root induction media. The number of days for micro-shoot initiation, the number of induced shoots, and shoot height per explant were recorded.

6.2.8 Root induction

After 30 days of multiplication on MS media, shoots were separated and transferred to a half-strength MS basal medium containing the same concentrations of IBA and NAA 0.0, 1.0, 2.0, and 3.0 mg/l each separately. Growth regulators free MS medium was used as a control. Three replications with one explant per culture vessel were used. Finally, similar to the initiation and multiplication stages, cultures were placed in a controlled growth room and the number and length of roots were recorded after one month of culture.

6.2.9 Acclimatization

After four weeks on rooting medium, healthy plantlets with long roots and elongated shoots were carefully removed from the culture vessel, and the roots were washed thoroughly with tap water. The plantlets were transferred to pots containing a sterile soil mixture of red, compost, and sand in their respective ratios of 1:2:1. The plantlets were covered with a white, transparent polythene bag for five days and then placed in the greenhouse for acclimatization.

6.2.10 Experimental design and data analysis

The experiment was designed in a Completely Randomized Design (CRD) with factorial arrangements of nineteen treatments. Each treatment comprised ten explants in three replications, with one explant per culture vessel. The data for explants` responses was recorded for the number of days to shoot and root initiation, the number of shoots and roots per plant, and shoot and root length (cm). All the recorded data were subjected to statistical analyses using SAS statistical software version 9.4, and an ANOVA was constructed, followed by Duncan's multiple range test (DMRT) at a 0.05% probability level, and the results were presented as means of the

independent replications with standard error (SE±). The costs of *bulla* and plant propagation agar were computed using Microsoft Excel 2010. The photograph illustrations were taken with Sonny's 20.1 megapixel camera with 4X optical zoom.

6.3 Results

6.3.1 Applying *bulla* as gelling agent

It was observed that MS media supplemented with *bulla* at a concentration of 50 and 60 g/l didn't solidify. Our result also indicated that MS media containing 70 g/l *bulla* was semi-liquid. However, *bulla* at a concentration of 80 g/l was solidified. This exhibited that *bulla* at a concentration of 80 g/l has a similar nature of gelling and media stability to 6 g/l agar. However, 90 g/l and above became too hard to be used for culture. Based on our result, the mean values of the proximate compositions (moisture, protein, fiber, fat, and ash), total carbohydrate contents, pH, and some mineral values of *bulla* obtained from the enset *Gimbo* genotype are presented in Table 6.1.

Table 6. 1 Proximate, pH, and minerial values for *bulla* flour of *Gimbo* genotype

Parameter	**%**	**Parameter**	**mg/100g**
Moisture(wb)	49.4	Calcium	35.4
Crude protein (db)	0.7	Magnesium	14.7
Crude fiber (db)	0.86	Phosphorus	30.4
Crude fat (db)	0.52	Potassium	158.6
Total ash (db)	0.8	Sodium	3.2
Carbohydrate	47.72	Iron	2.8
pH	3.87	Nitrogen	0.11%

N.B: wb= wet basis, db= dry basis

6.3.2 Surface sterilization

In the present study, surface disinfection of shoot tip explants was effective by applying 70% v/v ethanol for 5 minutes followed by double sterilization with a 20% sodium hypochlorite solution containing 5% active chlorine first for 20 minutes and then for 10 minutes with three drops of Tween 20. Thus, this sterilization method for explants showed a considerable reduction in microbial contamination and a better survival rate of the shoot tips of explants in culture media. However, after 17 days of culture, some microbial growth was observed around the explants on the MS media and destroyed about 53%, 60%, and 71% of explants in the culture for *Sisqella*, *Gimbo*, and *Astara* genotypes, respectively. Most probably, the major source of contamination in this case, was observed to be endophytes. Therefore, to reduce or destroy these endophytic contaminants, 500 mg/l cefotaxime was included in the culture medium. Thus, the application of cefotaxime in the culture medium significantly reduced the loss of explants during this experiment (data not shown).

6.3.3 Shoot initiation

The shoot tips showed notable differences in days on shoot initiation among the three enset genotypes at 6 different levels of BAP with 2 levels of IAA combinations on the cultured media gelled with 80 g/l of the *bulla* (Tables 6.2 and 6.3). The results revealed that the relatively shortest period of days for shoot initiation was in genotype *Sisqella* at 2.0 mg/l BAP alone (4.0 days) and 3.0 mg/l BAP in combination with 1.0 mg/l IAA (4.25 days). In genotype *Gimbo*, single shoot initiation was shown after 5.0 days of culture on MS media with the same levels of BAP, and BAP in combination with 1.0 mg/l IAA as in *Sisqella*. On the contrary, the longest period (6.25 days) was recorded for *Astara*, with a similar concentration of BAP and IAA as in both *Sisqella* and *Gimbo*. However, the maximum period of days in shoot

induction was recorded for all genotypes on MS media gelled with 6.0 g/l of agar as compared to 80 g/l of *bulla* with the same concentrations of BAP and IAA (Tables 6.2 and 6.3). Based on this result, BAP concentrations at 5.0 and 6.0 mg/l separately or in combination with 2.0 mg/l IAA caused a long period of days in shoot initiation in all explants studied on both gelled media types. This indicated the different responses of genotypes to the growth regulators and gelling agents.

In this experiment, after 30 days of culture on the initiation medium, the length of initiated shoots showed significant variations among genotypes in both gelling agents with different concentrations of BAP alone and combined with two levels of IAA. The highest shoot length was obtained on MS medium gelled with *bulla* and supplemented with 2.0 mg/l BAP alone for *Sisqella* (9.5 cm), and 3.0 mg/l BAP in combination with 1.0 mg/l IAA for *Gimbo* (8.5 cm) and *Astara* (6.55 cm) (Table 6.2). In contrast, the highest shoot length for *Sisqella* (6.0 cm), *Gimbo* (5.0 cm), and *Astara* (4.25 cm) was observed on MS medium gelled with agar (Table 6.3). These values were almost non-comparable with the highest shoot length observed on MS media gelled with *bulla* and supplemented with the same levels of BAP and IAA (Tables 6.2 and 6.3). Media gelled with *bulla* and agar, and supplemented with 6 mg/l BAP and 2 mg/l IAA resulted in shorter shoot length followed by shoots from growth regulator-free MS medium (Tables 6. 2 and 6.3).

6.3.4 Shoot multiplication

Results of this study indicated that different multiplication rates were observed for shoot tips to induce multiple shoots among the studied genotypes after culturing on MS media gelled with *bulla* and agar individually, and supplemened with BAP and IAA at different

concentrations. Consequently, the results revealed that *Sisqella* produced multiple shoots in the fewest days, followed by *Gimbo* and *Astara* in the MS medium supplemented with 5.0 mg/l BAP and 1.0 mg/l IAA after the 13th, 19th, and 22nd days of culture in *bulla*-gelled media, respectively (Tables 6.2 and 6.3). However, on the same media with agar gelled more days to multiple shoot induction were recorded for *Sisqella* (22), *Gimbo* (25), and *Astara* (26) after culture. The second fewer number of days to multiple shoot induction was observed in the *bulla*-gelled media supplemented with 4.0 mg/l BAP alone for 17, 22, and 26 days to produce more normal shoots per shoot tip for *Sisqella*, *Gimbo,* and *Astara*, respectively. In this way, the *bulla*-gelled media used proved better for multiple shoot induction from the shoot tip of explants. Similarly, BAP without a combination with IAA is also suitable for multiple shoot induction.

The number of micro-shoots produced per explant also showed notable differences among genotypes, gelling agents, and growth regulators concentrations. The highest number of micro-shoots per explant was recorded for *Gimbo* (18) on MS medium supplemented with 5.0 mg/l BAP in combination with 1.0 mg/l IAA, followed by *Sisqella* (14) that contained 4.0 mg/l BAP on medium gelled with *bulla*. Whereas *Astara* resulted in less shoot proliferation (6) as compared to the other two genotypes on the same MS media (Table 6.2). However, on agar-gelled MS media *Gimbo*, *Sisqella,* and *Astara* produced 14, 11, and 4 shoots per explant, respectively, on the same concentrations of growth regulators. Explants cultured on growth regulators free MS medium produced the lowest number of shoots for all studied genotypes. Generally, this experiment revealed that medium gelled with *bulla* resulted in a higher number of micro-shoots per explant than medium gelled with agar (Tables 6.2 and 6.3).

Table 6. 2 *In vitro* shoot proliferation and growth of three enset genotypes on *bulla* -gelled MS media containing BAP (6-Benzylaminopurine) alone and BAP in combination with IAA (Indole -3-acetic acid) at different concentrations

		Enset genotypes											
mg/l		***Astara***				***Gimbo***				***Sisqella***			
BAP	**IAA**	**DS**	**DM**	**NS**	**LS**	**DS**	**DM**	**NS**	**LS**	**DS**	**DM**	**NS**	**LS**
0	0	9.55^{a}	37.25^{a}	1.75^{j}	1.82^{k}	8.35^{a}	32.17^{a}	3.37^{n}	1.75^{l}	7.33^{a}	27.50^{a}	2.50^{l}	2.00^{m}
1	0	7.25d^{e}	29.25^{b}	2.13^{i}	4.52^{e}	6.25^{gh}	25.05^{d}	4.50^{m}	5.33^{e}	5.25^{ef}	22.42^{b}	3.75^{k}	6.50^{d}
2	0	6.05^{j}	28.50^{c}	2.73^{g}	6.05^{b}	5.05^{j}	24.48^{f}	6.53^{k}	7.53^{b}	4.05^{j}	21.25^{c}	5.60^{ij}	9.50^{a}
3	0	6.50^{h}	27.50^{ef}	2.18^{i}	5.13^{c}	5.50^{i}	23.53^{i}	8.67^{i}	6.00^{d}	4.53^{h}	19.50^{e}	7.00^{h}	8.12^{c}
4	0	6.75^{f}	26.05^{j}	2.47^{h}	3.03^{h}	5.65^{i}	22.05^{l}	12.60^{e}	3.50^{h}	5.05^{f}	17.05^{i}	14.00^{a}	4.33^{gh}
5	0	7.06^{e}	26.53^{hi}	3.20^{f}	2.53^{i}	6.13^{h}	22.50^{k}	13.32^{d}	3.08^{i}	6.05^{d}	18.05^{h}	11.17^{cd}	3.65^{i}
6	0	7.25^{de}	27.75^{e}	3.67^{e}	2.12^{j}	7.18^{d}	23.75^{hi}	12.12^{f}	2.75^{j}	6.50^{b}	18.75^{fg}	12.11^{bc}	2.64^{kl}
1	1	7.05^{e}	28.75^{c}	1.35^{k}	3.50^{g}	6.50^{f}	24.13^{g}	3.18^{n}	4.20^{fg}	5.13^{f}	21.00^{c}	3.75^{k}	4.28^{h}
2	1	6.50^{h}	28.05^{d}	2.10^{i}	4.05^{f}	6.05^{h}	23.08^{j}	5.00^{l}	6.72^{c}	4.63^{gh}	20.08^{d}	8.25^{g}	6.53^{d}
3	1	6.25^{i}	27.42^{f}	3.52^{e}	6.55^{a}	5.05^{j}	23.50^{i}	11.18^{h}	8.50^{a}	4.25^{i}	19.07^{ef}	9.58^{ef}	8.57^{b}
4	1	7.42^{d}	26.70^{h}	4.50^{c}	3.30^{gh}	6.50^{f}	22.53^{k}	15.50^{b}	4.02^{g}	5.25^{ef}	19.50^{e}	10.50^{de}	4.52^{g}
5	1	8.05^{c}	21.87^{k}	6.12^{a}	2.52^{i}	6.75^{e}	19.05^{m}	18.02^{a}	2.72^{j}	5.42^{e}	13.05^{j}	12.00^{bc}	2.75^{k}
6	1	8.42^{b}	26.50^{hi}	5.23^{b}	2.03^{jk}	7.05^{d}	23.85^{h}	11.53^{g}	2.05^{k}	6.08^{d}	20.33^{d}	10.17^{de}	2.53^{kl}
1	2	7.25^{de}	26.82^{gh}	1.25^{k}	4.80^{d}	6.13^{h}	24.18^{g}	2.50^{o}	5.20^{e}	5.25^{ef}	21.50^{c}	3.50^{k}	5.43^{e}
2	2	6.53^{gh}	26.73^{h}	1.73^{j}	4.08^{f}	6.25^{gh}	24.50^{ef}	4.77^{lm}	4.43^{f}	4.75^{g}	21.08^{c}	5.00^{j}	5.03^{f}
3	2	6.72^{fg}	26.55^{hi}	2.10^{i}	3.15^{h}	6.35^{fg}	24.75^{e}	8.14^{j}	3.50^{h}	5.10^{f}	19.50^{e}	6.15^{hi}	3.77^{i}
4	2	7.13^{e}	26.27^{ij}	4.05^{d}	2.48^{i}	6.75^{e}	24.58^{ef}	13.34^{d}	2.60^{j}	5.25^{ef}	18.50^{gh}	9.17^{fg}	3.05^{j}
5	2	8.05^{c}	27.05^{g}	5.03^{b}	1.78^{k}	7.53^{c}	25.68^{c}	15.53^{b}	2.07^{k}	6.28^{c}	18.33^{gh}	11.13^{cd}	2.49^{l}
6	2	8.53^{b}	28.67^{c}	4.45^{c}	1.10^{l}	8.05^{b}	26.13^{b}	14.20^{c}	1.53^{l}	6.43^{bc}	22.50^{b}	12.25^{b}	1.53^{n}
SE±		0.11	0.18	0.14	0.16	0.12	0.15	0.20	0.16	0.11	0.29	0.56	0.14
CV		1.53	0.65	4.37	4.62	1.80	0.60	2.05	3.81	2.07	1.47	6.80	3.00

N.B: DS= No. of days for single shoot initiation, DM= No. of days for multiple shoot induction, NS= shoot number per explant, LS= Shoot length (cm) , SE= standard error, and CV= coefficient of variation.

Table 6. 3 *In vitro* shoot proliferation and growth of three enset genotypes on agar- gelled MS media containing BAP (6-Benzylaminopurine) alone and BAP in combination with IAA (Indole -3-acetic acid) at different concentrations

mg/l		Enset genotypes											
		Astara				***Gimbo***				***Sisqella***			
BAP	**IAA**	**DS**	**DM**	**NS**	**LS**	**DS**	**DM**	**NS**	**LS**	**DS**	**DM**	**NS**	**LS**
0	0	11.22^{a}	37.00^{a}	1.25^{i}	1.08^{j}	9.25^{a}	34.08^{a}	2.52^{m}	1.53^{k}	8.50^{a}	32.00^{a}	2.10^{o}	1.53^{k}
1	0	10.13^{b}	32.00^{b}	1.80^{g}	2.00^{f}	8.05^{d}	29.22^{b}	4.00^{k}	3.53^{cd}	7.05^{d}	27.10^{b}	3.25^{l}	4.10^{e}
2	0	8.53^{fg}	30.91^{c}	1.83^{g}	4.25^{a}	7.08^{g}	27.50^{c}	4.53^{j}	5.00^{a}	6.05^{g}	26.03^{c}	3.50^{k}	6.02^{a}
3	0	8.50^{fg}	28.13^{h}	2.03^{f}	3.03^{c}	7.65^{ef}	27.05^{d}	5.05^{i}	4.00^{b}	6.48^{f}	24.53^{f}	4.03^{j}	4.50^{d}
4	0	8.75^{ef}	27.13^{j}	4.05^{a}	2.53^{d}	7.75^{e}	26.10^{f}	14.07^{a}	2.70^{g}	6.75^{e}	24.08^{g}	11.10^{a}	3.02^{g}
5	0	9.05^{d}	27.25^{j}	2.33^{e}	2.00^{f}	8.25^{cd}	25.10^{h}	11.10^{b}	2.50^{h}	7.12^{d}	23.50^{h}	9.03^{c}	2.53^{h}
6	0	9.50^{c}	26.50^{k}	2.50^{e}	1.80^{g}	8.45^{bc}	26.05^{f}	9.08^{d}	2.03^{i}	7.65^{bc}	23.05^{i}	6.25^{f}	2.32^{i}
1	1	8.25^{hi}	28.50^{g}	1.00^{j}	2.25^{e}	7.50^{ef}	26.5^{e}	1.50^{o}	3.50^{d}	7.12d	26.10^{c}	2.50^{n}	3.04^{g}
2	1	7.85^{j}	28.75^{f}	1.50^{h}	3.15^{c}	7.00^{g}	26.05^{f}	3.03^{l}	3.70^{c}	6.37^{f}	25.00^{e}	5.03^{h}	5.03^{b}
3	1	8.15^{hi}	28.53^{fg}	2.03^{f}	3.03^{c}	7.42^{f}	25.75^{g}	7.05^{f}	3.50d	6.18^{g}	24.50^{f}	6.03^{g}	4.57^{d}
4	1	8.50^{fg}	28.12^{h}	3.03^{d}	2.60^{d}	7.75^{e}	25.50^{g}	10.58^{c}	3.03f	6.48^{f}	24.10^{g}	8.07^{d}	3.05^{g}
5	1	8.75^{ef}	26.00^{l}	4.00^{a}	2.03^{f}	7.65^{ef}	25.12^{h}	14.05^{a}	2.05i	7.53^{c}	22.05^{k}	9.50^{b}	2.50^{h}
6	1	9.25^{d}	27.50^{i}	3.50^{c}	1.27^{i}	8.53^{b}	27.05^{d}	11.06^{b}	1.53k	7.65^{bc}	24.18^{g}	6.25^{f}	1.53^{k}
1	2	8.50^{fg}	29.08^{e}	1.25^{i}	3.60^{b}	7.75^{e}	26.75^{e}	1.75^{n}	4.03b	6.70^{e}	25.05^{e}	2.50^{n}	4.75^{c}
2	2	8.13^{i}	28.75^{f}	1.53^{h}	3.02^{c}	7.13^{g}	26.50^{e}	3.05^{l}	3.28^{e}	6.45^{f}	24.00^{g}	2.75^{m}	3.53^{f}
3	2	8.38^{gh}	28.25^{h}	1.75^{g}	2.25^{e}	7.63^{ef}	26.05^{f}	5.50^{h}	2.50^{h}	6.75^{e}	23.53^{h}	4.53^{i}	2.50^{h}
4	2	8.62^{efg}	28.50^{g}	2.50^{e}	2.00^{f}	7.65^{ef}	26.08^{f}	6.25^{g}	2.05^{i}	7.10^{d}	23.13^{i}	5.03^{h}	2.25^{i}
5	2	8.82^{e}	29.13^{e}	3.75^{b}	1.50^{h}	8.13^{d}	25.50^{g}	7.50^{e}	1.75^{j}	7.65^{bc}	22.50^{j}	7.00^{e}	1.73^{j}
6	2	9.28^{cd}	29.50^{d}	3.50^{c}	0.75^{k}	8.63^{b}	27.75^{c}	9.02^{d}	1.12^{l}	7.75^{b}	25.53^{d}	6.07^{fg}	0.80^{l}
SE±		0.14	0.13	0.11	0. 09	0.14	0.15	0.11	0.10	0.11	0.13	0.12	0.09
CV		1.54	0.44	4.55	3.96	1.74	0.56	1.68	3.69	1.55	0.52	2.10	2.88

N.B, DS= No. of days for single shoot initiation, DM= No. of days for multiple shoot induction, NS= shoot number per explant, LS = Shoot length (cm), SE=standard error, and CV= coefficient of variation.

6.3.5 Rooting

Well-developed shoots from multiplication media were transferred to a half-strength MS medium containing *bulla* or agar as a gelling agent and supplemented with IBA or NAA at a concentration of 1.0, 2.0, and 3.0 mg/l for root induction. Among all studied genotypes, the highest root number was observed in *bulla*-gelled medium supplemented with 2.0 mg/l IBA (5.5) for *Sisqella* followed by *Gimbo* (4.5) after 11.0 and 12.5 days of culture, respectively (Table 6.4). The same genotypes produced the second-highest number of roots on a medium containing 1.0 mg/l NAA gelled with *bulla.* The rooting medium supplemented with 3.0 mg/l IBA as well as 2.0 and 3.0 mg/l NAA produced the lowest root number (1.45) per shoot. These revealed that the rate of root formation was gradually reduced with increasing concentrations of IBA and NAA. Similarly, no root induction was observed on growth regulator-free medium gelled with agar in all examined enset genotypes until a month. However, rooted plantlets were observed on both growth regulator-free media gelled with *bulla*. Moreover, spontaneous root induction was also observed on multiplication media gelled with *bulla* in all genotypes.

Table 6. 4 *In vitro* root induction and growth of three enset genotypes on *bulla*- gelled MS media containing different concentrations of IBA (Indole-3-butric acid) and NAA (α-Naphthalene acetic acid)

mg/l		Enset genotypes								
		Astara			***Gimbo***			***Sisqella***		
IBA	**NAA**	**DS**	**NR**	**RL**	**DS**	**NR**	**RL**	**DS**	**NR**	**RL**
0	0	24.53^{a}	1.07^{g}	1.69^{g}	22.53^{a}	1.20^{f}	1.95^{g}	21.13^{a}	1.73^{f}	2.25^{f}
1	0	14.50^{e}	1.90^{e}	5.50^{a}	13.60^{e}	2.18^{d}	6.25^{a}	13.00^{e}	2.33^{e}	7.02^{a}
2	0	13.25^{g}	3.03^{a}	4.85^{c}	12.50^{g}	4.50^{a}	5.77^{c}	11.05^{g}	5.55a	6.05^{c}
3	0	15.53^{d}	2.52^{c}	4.50^{d}	14.50^{d}	2.50^{c}	5.02^{d}	13.57^{d}	2.75^{c}	5.88^{c}
0	1	14.00^{f}	2.88^{b}	5.25^{b}	13.05^{f}	3.60^{b}	6.02^{b}	12.53^{f}	5.03^{b}	6.50^{b}
0	2	17.50^{c}	2.20d	3.95^{e}	16.53^{c}	2.25^{d}	4.13^{e}	15.50^{c}	2.50^{d}	4.77^{d}

		Table	6.4	Cont						
0	3	19.05[b]	1.45[f]	3.00[f]	17.05[b]	1.60[e]	3.47[f]	16.08[b]	1.85[f]	3.85[e]
SE±		0.14	0.08	0.07	0.12	0.10	0.08	0.13	0.08	0.08
CV		0.84	3.76	1.73	0.77	3.73	1.70	0.90	2.58	1.62

N.B: DS=days for single root initiation, NR =mean number of root /explant, RL= mean root length (cm), SE= standard error, and CV= coeffient of variation.

The highest root length was recorded from shoots planted on *bulla*-gelled media containing 1.0 mg/l IBA for *Sisqella* (7.02 cm), followed by *Gimbo* (6.25 cm) and *Astara* (5.50 cm) (Table 6.4 and Figures 6. 3H to J). The MS media supplemented with 1.0 mg/l NAA also resulted in slightly comparable root length, of 6.5, 6.0, and 5.25 cm for *Sisqella*, *Gimbo,* and *Astara*, respectively. However, the lowest root length per shoot was observed for all the genotypes on agar-gelled media (Table 6.5) as compared to *bulla*-gelled media. According to this study, 2.0 and 3.0 mg/l NAA and 3.0 mg/l IBA showed the lowest root length followed by both auxins-free media (Table 6.4). In general, the examined genotypes showed a different response.

Table 6. 5 *In vitro* root induction and growth of three enset genotypes on agar- gelled MS media containing different concentrations of IBA (Indole-3-butric acid) and NAA (α-Naphthalene acetic acid)

		Enset genotypes								
(mg/l)		***Astara***			***Gimbo***			***Sisqella***		
IBA	**NAA**	**DS**	**NR**	**RL**	**DS**	**NR**	**RL**	**DS**	**NR**	**RL**
0	0	33.50[a]	0.87[e]	1.22[d]	32.30[a]	1.05[g]	1.42[f]	31.25[a]	1.10[f]	1.57[f]
1	0	17.05[f]	1.52[c]	3.15[a]	16.10[f]	1.65[e]	3.93[a]	15.13[f]	2.15[d]	4.82[a]
2	0	16.10[g]	2.78[a]	2.90[b]	15.08[g]	3.20[a]	3.89[ab]	14.13[g]	4.08[a]	4.08[c]
3	0	18.05[e]	1.73[b]	2.82[b]	17.50[d]	2.62[c]	3.53[c]	16.25[c]	3.12[c]	3.90[d]
0	1	18.50[d]	2.75[a]	3.08[a]	16.50[e]	3.00[b]	3.82[b]	15.72[e]	3.72[b]	4.30[b]
0	2	19.13[c]	1.60[bc]	2.80[b]	18.10[c]	2.07[d]	3.15[d]	16.05[d]	2.10[d]	3.83[d]
0	3	21.05[b]	1.15[d]	2.52[c]	19.00[b]	1.24[f]	2.80[e]	18.05[b]	1.60[e]	3.25[e]
SE±		0.10	0.08	0.08	0.16	0.09	0.05	0.10	0.08	0.09
CV		0.52	4.69	2.98	0.85	4.45	1.60	0.56	3.05	2.43

N.B: DS=days for single root initiation, NR=mean number of root /explant, RL= mean root length (cm), SE= standard error, and CV= coeffient of variation.

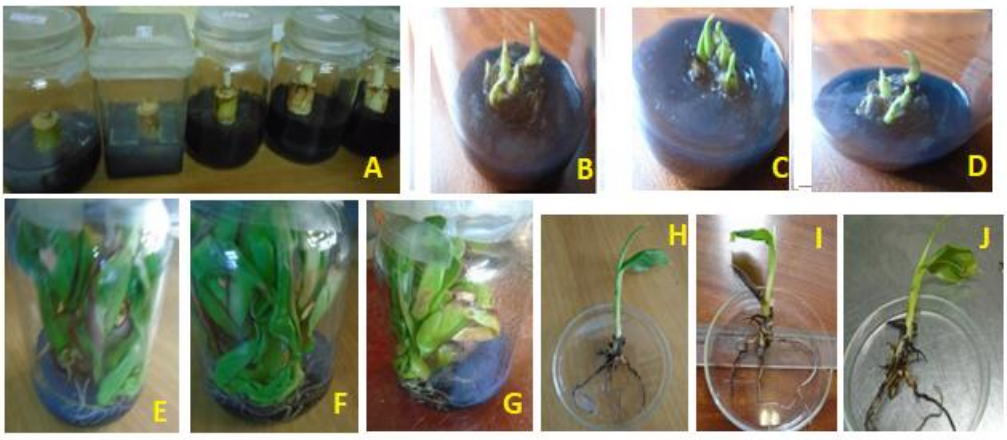

Figure 6. 3 Shoot initiation, multiple bud proliferation and rooting of three enset genotypes: (A) Shoot tip initiated after five days of culture on the agar (two jars) and bulla (three jars) gelled MS media containing 2.0 mg/l BAP; (B- D) Multiple proliferated shoots from the enset genotype *Astara*, *Gimbo* and *Sisqella*, respectively, on the 8% *bulla* gelled MS medium containing 5.0 mg/l BAP with 1.0 mg/l IAA after two to three weeks; (E-G) In vitro elongated multiple micro-shoots of the genotype *Astara*, *Gimbo* and *Sisqella*, respectively, on the 8% of *bulla* gelled MS medium after eight weeks; (H-J) Roots of single plantlets developed from the genotype *Astara*, *Gimbo* and *Sisqella*, respectively, on the 8% of *bulla* gelled 1/2 MS medium containing 1.0 mg/l IBA.

6.3.6 Acclimatization

After a month of acclimatization (Figure 6.4), 92.50%, 83.35%, and 71.15% of *Sisqella*, *Gimbo*, and *Astara* plantlets that were taken from *bulla*-gelled media survived, respectively. While, the survival percentage of the same genotypes obtained from agar-gelled media was found to be 85.50% for *Sisqella*, 74.75% for *Gimbo*, and 65.50% for *Astara*.

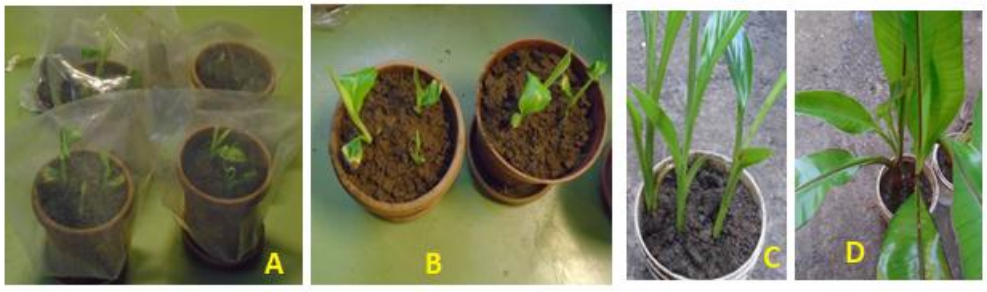

Figure 6. 4 Acclimatization of some *in vitro* grown plantlets of enset genotypes: (A) Plantlets in the pot of sterilized soil composted of sand: loam: red (1 : 2 : 1) covered with plastic bags; (B) Established plantlets after 8 days of plastic cover; (C) Acclimatized *Sisqella* plantlets in the greenhouse after 30 days; (D) *Astara* plantlets that were acclimatized in the greenhouse after 60 days.

6.3.7 Cost analysis

Based on this simple comparative cost analysis, prices for agar standard (plant propagation agar) and enset flour (*bulla*) were compared (Table 6.6). Considerable cost reduction was obtained by using *bulla* as a gelling agent instead of agar. Accordingly, using enset *bulla* for micro-propagation of enset shoot tips as an alternative source was efficient and resulted in a cost reduction of 73.3% (Table 6.6).

Table 6. 6 Simple comparative cost analysis for agar standard and enset flour (*bulla*)

Gelling agents	Amount used per litre	Cost per Kg ETB	Cost per g ETB	Cost per L ETB	Cost saved per L in %
Agar	6 g/l	4000	4	24	
Bulla	80 g/l	80	0.08	6.4	73.3

Note: ETB = Ethiopian birr

6.4 Discussion

Enset farmers in many enset growing regions have been cultivating the crop in the traditional method for many generations. However, nowadays its production is limited by various man-made and natural factors. Thus, in addition to on-farm maintenance appropriate tissue culture approaches were found indispensable for the rapid propagation, distribution, and conservation of high-quality planting material as well as for improving the production and productivity of the crop.

6.4.1 *Bulla* as a gelling agent

The result of this study revealed that *bulla* flour at a concentration of 80 g/l was the optimal amount to solidify and stabilize the MS medium in the *in vitro* culture of enset shoot tips. Similar results have been reported by Biruk Ayenew *et al*. (2012), Ayelign Mengesha *et al.* (2012), and Manaye Ayalew et al. (2017). They observed that a medium supplemented with 80 g/l *bulla* powder was optimum and can stabilize the MS media in all phases of *in vitro* culture of pineapple, vanilla, and cassava, respectively. Hirose *et al*. (2017) confirmed the high gelatinization property of enset starch for food and industrial uses. Similarly, ESTC (2003) reported that starch produced from enset can be used for the paper, textile, and adhesive industries. Furthermore, Tisge Gebre-Mariam and Nikolayev (1993) and Tisge Gebre-Mariam and Schmidt (1996) observed that enset starch has a better potential to be used in binding and disintegrating compressed tablets. In most enset-growing regions of Ethiopia, farmers also consider *bulla* to be the best quality of enset food (Pijls *et al*., 1995; Tadessa Daba and Shigeta 2016) and consume it mainly as porridge, gruel, and in crumbled form (Brandt *et al*., 1997; Temesgen Olango *et al*., 2014). However, its proximate and mineral content were lower than those of corm and *qocho* (unpublished data).

6.4.2 Surface sterilization

Initially, during this experiment, the presence of microbial contaminations was a major challenge, especially in the culture initiation steps. This could be attributed to the fact that explants may hold a wide range of microbial contaminants. Thus, to eliminate this source of contamination, in the present study, the explant tissues were sterilized carefully on their surfaces before culturing on the MS media. The results of the current study showed that surface disinfection experiments were effective when shoot tips of enset were sterilized with 70%

ethanol for 5 minutes, followed by 20% NaOCl for 20 and 10 minutes. This is in line with the findings of Genene Gezahegn and Firew Mekbib (2016), who reported 95% clean explants resulted when shoot tip explants were treated with 70% ethanol for 5 minutes, followed by double sterilization with 20% chlorox for 10 minutes, and then 20 minutes for all the clones until 10 days of culture in MS medum. However, Dejene Zinabu *et al*. (2018) reported that enset shoot tip explants were disinfected with 20% NaOCl for 20 and 10 minutes after being treated with 70% ethanol for 10 minutes. But in the present study, during the process of surface sterilization, the treatment of enset shoot tips with 70% ethanol for 10 minutes resulted in the loss of explants from all three genotypes. Likewise, after three weeks of culture, endophyte contamination caused more than 50% of the explants in the culture to be destroyed for all genotypes examined in this study. Previous researchers also mentioned that besides surface contaminants of explants, endogenous contaminants were the key problem during *in vitro* propagation activity of enset (Almaz Negash, 2001; Genet Birmeta and Welander, 2004; Genene Gezahegn and Firew Mekbib, 2016; Dejene Zinabu *et al*., 2018). In this study, to avoid contamination caused by endogenous microbes, we applied 500 mg/l cefotaxime to the culture media. Similarly, Dejene Zinabu *et al*. (2018) reported that 99% clean explants were obtained when 500 mg/l cefotaxime was included in a medium on the *Bededet* cultivar of enset. According to Khan *et al*. (2018), endophytic microbes residing within the explants are recognized as a main constraint to the establishment, growth, and multiplication of tissue-cultured plants, as they are more challenging to eradicate by using standard surface sterilization procedures. However, the endophytic contamination could be removed by supplying different antibiotics to the culture media (Fang and Hsu, 2012; El-Banna *et al*., 2021). In a similar study, numerous antimicrobial compounds have been thoroughly used to prevent the growth of endophytes in media during in vitro plant

cultures of different plant species, such as *Bambusa nutans* (Ray *et al*., 2017), banana (El-Banna *et al*., 2021), *Solanum tuberosum* (Mora *et al*., 2022), and *Ipomoea batatas* (Pérez-Pazos *et al*., 2023). Generally, an effective sterilization procedure as well as using anti-microbial agents in some cases in media are important steps in the reduction of exophytic and endophytic contamination in *in- vitro* culture. According to Yildiz (2012) and Khan *et al*. (2018), success in tissue culture depends on the effectiveness of the sterilization methods used on the explants before culture initiation and the awareness of the endophytic nature of the contaminants.

6.4.3 Shoot initiation

We have been able to compare for the first time the use of locally available and low-cost gelling agent *bulla* flour with plant propagation agar for micropropagation of the three enset genotypes on MS media supplemented with different concentrations of BAP and IAA. The results of this study indicated significant variations were found in days to shoot initiation among the three enset genotypes. Genene Gezahegn and Firew Mekbib (2016) reported an almost similar period (5.25 to 9.0 days) for the single shoot initiation on MS media gelled with 6 g/l agar, but the medium was supplemented with 4.5-6.0 mg/l BAP and 1.0-1.5 mg/l NAA separately and in combinations. However, Mulugeta Diro *et al*. (2004) reported single shoot initiation after two weeks when enset shoot tip explants were cultured on MS medium gelled with 11 g/l of agar and supplemented with 2.5 mg/L BAP alone. The most likely reasons for this early induction in the present study were the types of *bulla* and the amount of solidifying agent (6 g/l of agar) used. A more concentrated gelling agent can reduce nutrient uptake by explants since the medium becomes very compact. Moreover, Matheka *et al*. (2019) reported higher levels of BAP to induce buds that grew slowly and were highly blackened. This is in line with the present study, relatively low BAP concentrations (2.0 and 3.0 mg/l) with or without the combination of 1.0

mg/l IAA supplemented and the fewest number of days for shoot induction. The results of this study also revealed that significant variations existed among the tested genotypes in terms of the length in both gelling agents with different concentrations of BAP alone and combined with two levels of IAA. Similarly, Kahia *et al.* (2015) indicated that *in vitro* bud differentiation and development in bananas of the same family as enset were genotype-dependent.

6.4.4 Shoot multiplication

In this study, to obtain optimum conditions for multiple shoot formation from all explant types examined, after one month of shoot induction, the central part of the shoot tip at the base of corms was carefully removed to avoid apical dominance of enset shoot explant. Then, the excised explants were subcultured on MS media supplemented with BAP and IAA separately or in combination. This experiment was done after several preliminary trials in which different levels of BAP (from 1.0 mg/l to 6.0 mg/l) and IAA (1.0 mg/l and 2.0 mg/l) were compared with or without removing the central core of shoot tips. Thereafter, all non-excised explants of shoot tips gave rise to one complete normal shoot rather than multiple shoots until 45 days of sub-culturing on MS multiplication media (data not shown). But the explants that lack apical dominance produced multiple shoot buds at different levels based upon concentrations of growth regulators, genotypes, and gelling agents. A similar observation was also reported by Genet Birmeta and Welander (2004) and Mulugeta Diro *et al*. (2004).

The results of this experiment showed the effects of the two gelling agents (*bulla* and agar) for multiple shoot induction on MS medium supplemented with BAP and IAA at different concentrations for the three enset genotypes. All the examined genotypes have shown differences in the period of multiple shoot induction, the number of shoots, and the length of shoots per

shoot tip the explants. For all tested enset genotypes, a better growth response was observed with *bulla* flour gelled media than agar. Almost similar results have been reported in pineapple, vallina, and cassava by Biruk Ayenew *et al*. (2012), Ayelign Mengesha *et al*. (2012), and Manaye Ayalew *et al*. (2017), respectively, using *bulla* flour as an alternative gelling agent on MS media. However, their reports did not indicate the kinds of plant growth regulators or their concentration levels in the MS medium. In terms of the number of days for multiple shoot induction, the lowest number of days (13-22) were observed in the MS media supplemented with 5.0 mg/l BAP and 1.0 mg/l IAA on *bulla*-gelled media. However, on agar-gelled media with the same level of growth regulators and genotypes, it took from 22-26 days in the present study (Table 6.3). However, Genene Gezahegn and Firew Mekbib (2016) reported slightly shorter durations (11.67 to 25.33 days) for Mazia, Arkiya, and Digomerza enset cultivars cultured on agar-gelled media having 4.5- 6.0 mg/l BAP and 2.0 mg/l NAA for multiple shoot induction. This variation could be a result of genotype differences, the types and amounts of macro- and mirco nutrients, and growth regulators that were used.

Successful multiplication is one of the most essential steps in micro-propagation. The current findings revealed that the highest and lowest number of shoots were 18 and 6 for *Gimbo* and *Astara* genotypes on *bulla*-gelled media containing 5.0 mg/l BAP and 1.0 mg/l IAA in one sub-culture after 30 days, but on agar-gelled media, the two genotypes produced 14 and 4 shoots per explant, respectively. Similarly, Genene Gezahegn and Firew Mekbib (2016) reported 23 shoots per explant. However, Almaz Negash *et al.* (2000) reported one to two shoots per shoot tip from the *in vitro* regeneration of the three enset clones on MS medium containing 2.25 mg/l BAP in combination with 0.2 mg/l IAA. In addition, Mulugeta Diro *et al*. (2004) obtained 3.7 shoots per shoot after splitting the explants into two and culturing them separately. Furthermore,

Genet Birmeta and Welander (2004) reported about 75 shoot buds that can potentially grow to shoots per explant in one subculture through meristem wounding of initiated explants. This variation may occur due to differences in genotypes, size of explants, media types and their gelling agents, the concentration of growth regulators, and light intensity in the growth room. Generally, our results show that *bulla*-gelled media produce better responses in all aspects for all the tested genotypes of enset.

6.4.5 Rooting

The formation of the root is also a crucial step in micro-propagation because its success could depend on the number and length of roots per plantlet. In the present study, well-developed shoots from multiplication media were transferred to half-strength MS medium gelled with *bulla* or agar supplemented with IBA or NAA at concentrations of 1.0, 2.0, and 3.0 mg/l for root initiation. The result of this study shows that the fewest number of days (11.0–13.25) was observed on MS media gelled with *bulla* and supplemented with 2.0 mg/l IBA, but 14–16 days for root induction were recorded on the same media gelled with agar for all studied genotypes of enset. This result is slightly in agreement with Genene Gezahegn and Firew Mekbib (2016), who reported that 10.5 to 12.83 days are required for root induction on MS medium containing 1.0–2.0 mg/l IBA. In addition, Almaz Negash *et al.* (2000) reported that root formation occurred two weeks after transfer to root induction medium supplemented with 5 μM IBA, 1 μM IAA, and 1 μM BAP in combination for all three enset clones. However, Matheka *et al*. (2019) reported 3-5 days for root development after the culture of shoots on media gelled with 3 g/l gelrite. The most probable reasons for this early initiation are the type of gelling agent, and the concentration of chemicals and hormones in the media employed (Saraswathi *et al*., 2016; Bhat *et al*., 2022).

In terms of root number per shoot, our results are in agreement with the report of Genene Gezahegn and Firew Mekbib (2016). They reported the production of an average of 3.6 roots per explant after 12–14 days post-culture of explants on MS media with 1.0 mg/l IBA. On the other hand, Matheka *et al*. (2019) reported a maximum of 12 roots per shoot on a medium containing 1.0 mg/l IBA for *Bededet* enset genotypes. This is contrary to current findings of a maximum of 5.5 roots per plantlet. This might be due to genotype differences, the concentration of growth regulators and media, and the types of gelling agents. For example, genotype *Sisqella* responded better for root number and length, whereas poor response was observed for *Astara* culturing on both media types separately in the present study. Root induction and development are dependent on several factors, including media type and plant genotype (Kahia *et al*., 2015).

6.4.6 Acclimatization

The results of this study showed that the survival rate of the plantlets varied among the examined genotypes and gelling agents after 30 days in the greenhouse. Generally, all the studied genotypes of plantlets from a *bulla*-containing medium grew better than those from an agar-gelled medium. This is in agreement with the work of Biruk Ayenew *et al*. (2012) on pineapple, who reported 95% and 90% survival from *bulla* and agar medium, respectively. This was also supported by Ayelign Mengesha *et al*. (2012), who reported that 90% of survival in greenhouse conditions for vanilla plantlets was derived from a *bulla* gelling agent. This might be attributed to the presence of different nutritional supplements in *bulla* compared to agar. The present study also indicated that a different survival rate was observed among genotypes, which agrees with the findings of Genene Gezahegn and Firew Mekbib (2016). They described that *Degomerza* performed better than *Mazia* and *Arkyia* enset genotypes. Similarly, Almaz Negash *et al*. (2000) reported that the *Nobo* enset genotype revealed a better survival rate

than the *Choro* and *Ketano* genotypes. This might be due to genetic differences in the enset genotypes.

6.4.7 Simple cost analysis of *bulla* as a gelling agent

Few reports have mentioned the use of *bulla* as a potential gelling agent for micro-propagation of crops such as pineapple (Biruk Ayenew *et al*., 2012), vanilla (Ayelign Mengesha *et al*., 2012), and cassava (Manaye Ayalew *et al*., 2017). However, this is the first report in which the use of *bulla* instead of agar as an alternative gelling agent has been reported on the *in vitro* growth of enset. The unit production cost of micro-propagation in most cases is limited to the full application of these techniques. According to Saraswathi *et al.* (2015) and Ebile *et al*. (2022), agar, which is an expensive gelling agent, has been widely used for solid media in tissue culture. Similarly, Kacar *et al.* (2010) stated that because of the high price of tissue culture- grade agar, attempts have been made to identify suitable alternatives. The result of the present study indicated that using enset *bulla* as an alternative source saves 73.3% in cost. In a similar study, Biruk Ayenew *et al*. (2012), Ayelign Mengesha *et al*. (2012), and Manaye Ayalew *et al.* (2017) reported the use of *bulla* as an alternative source of agar for micro-propagation of pineapple, vanilla, and cassava, and they obtained 76%, 72%, and 65%-86% of gelling cost reduction, respectively. Overall, the findings are in line with the original hypothesis that the gelling agent, enset product *bulla*, is locally available in farmers' homegardens and a less expensive alternative to agar that can be used in all stages of micro-propagation without compromising culture quality. Furthermore, the results of this study indicated that the supplement of *bulla* reduced the direct dependency on plant tissue culture agar. A similar observation was made by Daud *et al.* (2011), who studied alternative sources of agar such as potato starch, rice flour, cassava flour, and corn

flour and obtained a 66%–90% gelling cost reduction. Likewise, Kodym and Arias (2001) reported a 90% cost reduction by replacing sucrose and GelriteTM with locally available commercial sugar and starch-Gelrite^{TM} mixtures, respectively. Ebile *et al.* (2022) demonstrated that there is a great opportunity to use some commonly available resources that are within the means of smallholder farmers in developing countries for media preparation in tissue culture technology to propagate indigenous as well as endangered crops.

6.5 Conclusion and recommendations

The present study showed a successful protocol for shoot tip *in vitro* culture studies of the multi-use plant *E. ventricosum* employing *bulla* as the gelling agent. Moreover, this research has for the first time compared and verified the effect of two gelling agents, *bulla* extracted from enset and agar, on *in vitro* shoot initiation, multiplication, and root development for three enset genotypes. The results showed that *bulla* can provide a significantly higher number and length of shoots and roots per shoot and also early initiation of shoots and roots for all the studied genotypes when compared with agar-gelled MS media. In addition, *bulla* is a less expensive and locally available resource, which could substitute conventional agar and result in an overall cost reduction for micro-propagation of enset. Thus, it provides additional supplements and the possibility of a backup for on-farm maintenance as well as mass *in vitro* propagation of enset genetic resources. It is accordingly imperative to make all feasible efforts to establish the *bulla* production firms and small-scale enset producers with cost-reducing harvesting and processing technologies that link sustainable enset farming systems with market opportunity. Furthermore, there is the need for domestication and expansion of enset plants to different regions and

countries, as well as promoting the potential of enset *bulla* for micro-propagation and other industrial purposes.

Chapter Seven

Analysis of proximate composition, mineral contents, and anti-nutritional factors of enset (*Ensete ventricosum*) landraces commonly used for amicho preparation in Hadiya Zone, central Ethiopia:

Abstract

Enset [Ensete ventricosum (Welw.) Cheesman] is a primarily starchy staple food crop for over 20 million people. Some landraces are widely favored for amicho (boiled corm) preparation and consumption. However, little information is available on its nutritional profile. Therefore, this study was aimed at identifying the proximate, mineral, and anti-nutritional contents of the seven commonly consumed corms of the cultivated enset landraces. The proximate was determined using the Association of Official Analytical Chemists (AOAC) standard methods. Minerals, phytate, and tannin contents were determined using the different models of spectrophotometer method, and oxalate was analyzed using the standard procedure. Also, the physiochemical parameters and the molar ratios were estimated to the relevant standards. The results revealed that the proximate composition (%) ranged in moisture content from 68.2–79.4, crude protein (2.43–11.90), crude fat (0.61–0.89), crude fiber (2.42–4.11), and total ash (2.01–4.60), while the total carbohydrates came to 80.89–89.92, and gross energy was 369.96–385.12 kcal/100 g. The mineral concentrations (mg/100 g) were also varied and ranged: calcium (22.46–49.74), potassium (28.51–86.56), magnesium (16.46–29.34), phosphorus (3.10–13.58), sodium (7.13–8.67), iron (0.9–3.85), and zinc (0.38–1.44) on a dry weight basis. The anti-nutritional contents (mg/100 g) for phytate, tannin, and oxalate ranged from 221.75–276.12, 27.97–113.74, and 5.69–9.10, respectively. Hayiwona and Gishira had the highest values in most proximate and minerals than other tested landraces, respectively. Compared to other landraces, Astara had higher total carbohydrate and phytate contents. Except for phytate×calcium to zinc, and oxalate to calcium, the molar ratios were above the critical values, which indicated that the studied enset corms had a considerable phytate value, which reduces mineral bioavailability. Overall, the present study revealed that the corm of the evaluated enset landraces contains appreciable amounts of nutritional value and can subsidize Ethiopia's sustained food security.

Key words/phrases: anti-nutritional factors, mineral bioavailability, corm, mineral, proximate composition

7. 1 Introduction

Enset [*Ensete ventricosum* (Welw.) Cheesman] is a large herbaceous monocarpic evergreen perennial root crop that has pseudostems above the ground and an underground corm that closely resembles the banana plant. Borrell *et al*. (2019) described *Ensete* as a genus of the banana family domesticated and cultivated only in Ethiopia. Enset is a multipurpose, drought-tolerant, adaptive plant with a diverse range of altitudes and uses for both nutritional and non-nutritious purposes (Brandt *et al*., 1997), and the crop is traditionally ranked first in its significance for Ethiopian enset farmers.

Food insecurity prevails in parts of Ethiopia (Girma Gebre, 2021), facing frequent drought and other calamities with about 28.8% of the undernourished population as indicated by data from 2014–16 (Borrell *et al*., 2020). Increased and improved production and use of enset is an important choice to pull the country out of such a food insecurity loop. Enset cultivation is a straightforward strategy to facilitate people's achievement of independent livelihood security (Abate Senbeta *et al.*, 2022), and it is an important staple crop for more than 25 million people living in the central, south, and southwest highlands of Ethiopia (Zerihun Yemataw *et al*., 2016; Borrell *et al*., 2020). The region where enset is consumed as the main food is distinguished by a large number of people, which cannot be maintained by any other form of land use in Ethiopia and is inhabited by more than 45 ethnic groups with a great variation in culture and agricultural practices (Zemede Asfaw, 2018). Pijls *et al*. (1995), and Admasu Tsegaye and Struik (2001) stated that in comparison to the yields of other food crops, enset has a reasonably high yield.

The reports of Zerihun Yemataw *et al.* (2016) and Bloome *et al*. (2018) indicated that a wide diversity of cultivated enset landraces exist in Ethiopia. Of the different farmer's landrace

selection criteria in enset, quantity and quality of its food product are two of the most important criteria (Tesfaye Dilebo *et al*., 2023b). The edible part of enset is the pseudostem and corm after processing and fermenting into *qocho and bulla,* and fresh or unfermented *amicho* (cooked corm) foods (Tadessa Daba and Shigeta, 2016). Some prior studies indicated that enset products are rich in carbohydrates and mineral sources. However, its yield and nutrient contents differ among enset varieties, the age of enset plants, management practices, and environmental factors (Abraham Bosha *et al*., 2016; Tadessa Daba and Shigeta, 2016), as do its different organs such as leaves, pseudostem, and corm.

The majority of enset landraces are produced mainly for processing starch through fermentation, *qocho,* or *bulla,* while some others are produced entirely for their *amicho* or cooked corm (Admasu Tsegaye and Struik, 2002; Tadessa Daba and Shigeta, 2016; Zerihun Yemataw *et al.,* 2016)**.** *Amicho* is an unfermented form of *enset* obtained from the immature enset plant corm, and it is boiled before consumption, similar to many other root and tuber crops (Admasu Tsegaye and Struik, 2002; Borrell *et al*., 2020). *Amicho* is a trustable food source since it may be uprooted and used at any time during the plant's growth (Mohammed *et al*., 2013). Moreover, corms of several different enset landraces are considered traditional medicinal values for various health problems in humans and cattle in the south and southwest parts of Ethiopia (Yemene Tsehay and Fassil Kebebew, 2006; Tesfaye Dilebo *et al.,* 2023a).

Although enset landraces are more diverse in terms of farmers' selection, cultivation, and consumption rate in Ethiopia, few scientific studies have been performed on the nutritional constituents of widely cultivated and commonly used enset landraces. The available information is more restricted to fermented products of enset, known as *qocho*. As far as we know, only a very few published articles (Yewelsew Abebe *et al.*, 2007; Ayalew Debebe *et al*.,

2012; Sirawdink Forsido *et al*., 2013; Mohammed *et al*., 2013; Admasu Tsegaye, 2015; Tadessa Daba and Shigeta, 2016; Gizachew Nuraga *et al*., 2019) are available on the nutritional and other compositions of the corm of enset as a general or unspecified small number of landraces also with limited parameters. Thus, it is necessary to have an understanding of the nutritional profile of such diverse and multi-use landraces for local and regional consumption. Moreover, that will contribute to food security and the sustainable use of the enset plant. Furthermore, the tedious harvesting and processing steps, as well as the sour flavor of its fermented *qocho*, may make it difficult for some new consumers. Hence, consuming *amicho* is appropriate for overcoming these challenges; furthermore, its preparation does not involve special skills and procedures to ensure the community's food security. Therefore, considering these facts, the present study was designed to identify the proximate composition, mineral content, anti-nutritional factors, mineral bioavailability, and physicochemical properties of the seven different widely consumed and locally favored corms of enset landraces representing the sweet, moderate, and bitter but traditionally medicinal corm types of the cultivated enset landraces.

7. 2 Materials and Methods

7. 2.1 Source of plant materials

Seven healthy enset landraces: *Astara*, *Gishira*, *Hayiwona*, *Leqeqa*, *Qiniwara*, *Separa*, and *Soqido* were selected based on their suitability as corms' consumption and other purposes for identified by local farmers (Table 7.1) (Tesfaye Dilebo *et al*., 2023b). All of them were gathered from one farmer’s enset field to minimize the variability that could happen due to management practice and soil fertility at the age of five years from the Lemmo district Shurmo

Dacho *kebele* in Hadiya Zone, Ethiopia, at the end of September 2020. The district lies at an altitude between 2105 and 2510 masl, and according to the meteorological data obtained from the Hossanna (administrative town of Hadiya Zone) meteorology station, the maximum and minimum mean annual temperatures (22.57 °C and 10.38 °C) and the mean annual rainfall range between 950 and 1540 mm, with a bi-modal pattern (March to May short and June to September main rain seasons). Most of the soil types are vertisol, with pH ranges of 5–6, according to reports from the Agricultural Office of the Lemmo district (unpublished data).

Table 7. 1 Enset landraces suitable for *amicho* consumption and used for nutritional and anti-nutritional analysis with their major characteristics and additional use values

Local names of enset landrace	**Characteristics of their corms (*amicho*)**	**Recommended use value**	**Additionally, recommended by farmers to treat to ailment**
Astara	Sweet	Food and medicinal for humans	Bone fracture and joint displacements
Gishira	Bitter	Medicinal for humans, food and medicinal for cattle	Bone fracture and joint displacements
Hayiwona	Moderate	Food and medicinal for humans and cattle	a painful infected swelling, to heal the damaged part of the body, and stimulate milk production in cattle
Leqeqa	Sweet	Food for humans	-
Qiniwara	Sweet	Food, medicinal for humans	Bone fracture and joint displacements
Separa	Moderate	Food for humans	-
Soqido	Sweet	Food for humans	-

Note: - no known additional recommendation by farmers of the study area

The rationale for selecting these landraces was based on their: local community favorites and frequency of consumption, wide distribution, and well-known for their consistent vernacular names in different districts of the Hadiya Zone and other adjacent zones of southern Ethiopia (Tesfaye Dilebo *et al*., 2023b). The parent landraces of these enset crops had traditionally been

vegetatively propagated over several generations, and the plants were not supplied by chemical fertilizers rather by animals' dung twice a year.

7.2.2 Sample preparation for laboratory analyses

For the laboratory analysis (Figures 7. 1A–D), the corms were trimmed carefully by using locally modified sharp knives to remove all roots, soil residues, and other adhering materials (Figures 7. 2A–C). Then the corms were pulverized by using a *jango* (a locally made instrument) until the parts became a small portion about two kg each (Figure 7.2D). Each of the remaining central non- pulverized corm samples was placed in a zipped polyethylene bag, preserved in an ice box to avoid moisture loss, and delivered to the Addis Ababa University Center for Food Science and Nutrition Laboratory. The cleaned and washed corms were sliced to a uniform size of about 2 mm in thickness using stainless steel knives. Before drying, the moisture content of each enset corm was measured. Corm samples were laid out on an aluminum foil tray and dried in an oven (DHG 9055A) at 60 ^{o}C for 24 hours prior to analysis for further laboratory tests with little modification (Gizachew Nuraga *et al*., 2019). Each dry sample was ground in an electric grinder to a fine powder and filtered using a 0.425 mm sieve. The powdered samples were then put in zipped polyethylene bags and kept at room temperature until they were needed for proximate, mineral, and anti-nutritional analysis.

Figure 7. 1 Some examples of enset landraces used for *amicho* consumption and laboratory analysis: A) before uprooting, B-D) after uprooting *Astara*, *Soqido*, and *Hayiwona*, respectively.

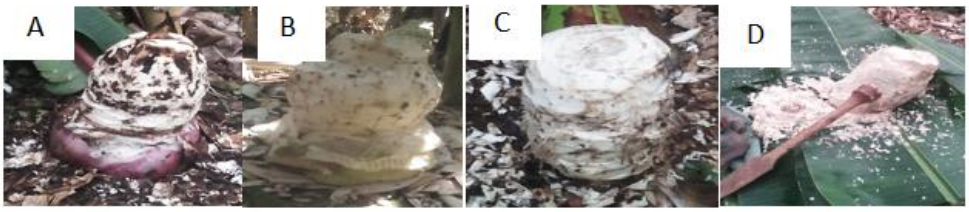

Figure 7. 2 Some examples of corms of the enset used for the laboratory analysis: A) *Astara*, B) *Gishira*, C) *Hayiwona*, D) *Leqeqa*

7.2.3 Proximate analysis

All analyses were carried out on a dry weight basis since the majority of the fresh leafy and root crops are made up of water (Punchay *et al*., 2020). Therefore, the samples' dry matter contents were determined after they had been dried and turned into flour. Each sample was examined in triplicate.

7.2.3.1 Determination of moisture content

The moisture content of the samples was determined by the drying air oven (DHG 9055A) method according to the official method 925.09 of the AOAC (2000). A crucible was dried in an oven at 105 °C for an hour and placed in desiccators to cool. The weight of the crucible (W_1) was determined. Samples (5 g) were weighed in the crucible (W_2) and dried at 105 °C for 3 hours. After cooling in a desiccator to room temperature, it was again weighed (W_3). The moisture content was determined as follows:

Moisture%= W_2-W_3/W_2-W_1 x100

7.2.3.2 Determination of crude protein

Crude protein was determined by the Kjeldahl method according to AOAC (2000) using the official method 979.09. A 0.5 g sample was digested by heating with 6 ml of concentrated sulphuric acid (H_2SO_4) and mixed with 3.5 ml of 30% hydrogen peroxide (H_2O_2) solution step by step. After the violent reaction stopped 3 g of catalyst mixture prepared from 10 g of Copper Sulfate ($CuSO_4$) and 150 g of Potassium Sulfate (K_2SO_4) was added to the digestion flask and digested at 370 ^{0}C for 4 hours in nitrogen determination apparatus until a clear solution was obtained. The digested samples were transferred into the fume hood for cooling, and the content in each flask was diluted with distilled water and then neutralized with 35% sodium hydroxide (NaOH) to make the solution slightly alkaline. Finally, samples were distilled, and ammonia was received in flasks containing excess 2% boric acid (H_3BO_3) solution for reaction with ammonia. The reacted solution of ammonia borate was then titrated with 0.1N hydrochloric acid (HCl), to determine the total nitrogen. The nitrogen content was calculated using the following equation:

% of N=S-B x 14 x NHCl/10 x W

Crude Protein (%) = % N x 6.25

Where S: is sample titration reading

B: is blank titration reading and 14 is the molecular weight of nitrogen

N: is the normality of HCl

W: Sample weight

7.2.3.3 Determination of crude fat

The crude fat test was carried out on the Soxhlet extraction (SZC-D fat determination meter, YLC 2000) method utilizing diethyl ether according to official method 920.39 of the AOAC (2000). 2.00 g of dried samples were added to the extraction thimbles and then covered with about a 2 cm layer of fat-free cotton. The thimbles with the sample content were placed into the Soxhlet extraction chamber. The cooling water was switched on, and 50 ml of diethyl ether was added to the extraction flask through the condenser. The extraction was conducted for about 3 hours. The extraction flasks with their contents were removed from the extraction chamber and placed in the drying oven at 90 °C for about 30 minutes, cooled to room temperature in the desiccator for about 30 minutes, and re-weighed with the extract.

The total crude fat was calculated as a percentage by weight:

% of crude fat= W_2-W_1/W x 100

Where W_1= weight of the extraction flask (g)

W_2= weight of the extraction flask plus the dried crude fat (g)

W= weight of the sample (g)

7.2.3.4 Determination of total ash

Total ash content was determined according to AOAC (2000), using the official method 923.03. The clean drying dish was dried at 105 °C in a hot air oven, cooled in a desiccator, and weighed using an analytical balance (W_1). Then, 2.5 grams of sample were put into the dish and weighed (W_2). The sample was charred on a hot plate until the contents turned black under a hood. The dish with its contents was transferred to a muffle furnace (S30 2RR England) and ignited at 550 °C until the sample changed to grayish white ash, which took about five hours, and then the samples were cooled inside desiccators. Finally, the residue was weighed (W_3), and the total ash was expressed as a percentage on a dry basis as follows:

Total ash (%) $=W_3-W_1/W_2-W_1 \times 100$

Where: W_1: weight of the dried dish in g

W_2: weight of the dish and the sample in g

W_3: weight of the dish and the sample after ashed

7.2.3.5. Determination of Crude Fiber

Crude fiber content was determined by using Fibertec (Fibertec 2010). The sample was analyzed using the steps of digestion, filtration, washing, drying, and combustion according to the official method 962.09 of the AOAC (2000). About 1 g (W_3) of the sample was transferred to a pre-dried crucible, which contains 1 g of celite sand to simplify filtration. Then the crucible with its contents was placed in the Fibertec and the sample was digested. After digestion, with 1.25% sulfuric acid for 37 minutes, washed with distilled water, and then digested with 1.25% NaOH for 37 minutes, the sample was filtered in a coarse porosity crucible in an apparatus at a vacuum of about 25 minutes. The residue was then dried in an oven at 130 °C for 2 hours, cooled in

desiccators, and weighed (W_1). Finally, the crucible was placed in a muffle furnace, and the sample was ashed at 525 °C for 3 hours, cooled in desiccators, and weighed again (W_2). The total crude fiber was expressed in percentage as:

Crude fiber (%) =W_1-W_2/W_3 x 100

W_1 is the weight of the crucible and dried sample, W_2 is the weight of the crucible and dried ash sample and W_3 is the sample weight

7.2.3.6 Determination of total carbohydrate content

The total carbohydrate content was determined by the difference from other nutrients using the formula as follows: Carbohydrate (%) = 100 – (% crude protein + % crude fiber + % total ash + % crude fat).

7. 2.4 Gross energy value

The gross energy content of enset corm samples was determined from the values of protein, fat, and carbohydrates using Atwater's conversion ratios: 16.7 kJ/g (4 kcal/g) for protein, 37.4 kJ/g (9 kcal/g) for fat, and 16.7 kJ/g (4 kcal/g) for carbohydrates (Nguyen *et al*., 2007). Gross energy can be expressed mathematically as follows:

Gross energy Kcal/100 g = (4 x g protein) + (9 x fat) + (4 x g carbohydrate)

7.2.5 Mineral analysis

The mineral content of the powdered corm samples of seven enset landraces was ignited to ash at 550 °C for 5 hours in a muffle furnace, dissolved in 20% HCl, boiled to bring the ash into solution form. The digested sample was filtered into an acid-washed volumetric flask of 100 ml after cooling, and the volume was then adjusted with distilled, deionized water. The

concentrations of minerals were determined with calibration curves prepared with their standard solutions. The calcium, magnesium, iron, and zinc content in the sample were determined using an atomic absorption spectrophotometer (Shimadzu, model AA-6800), while sodium and potassium contents were determined using flame photometry (Jenway model; pfp7, UK) as per Osborne and Voogt (1978). Phosphorus was analyzed by using a UV-VIS spectrophotometer (Thermo Scientific model; Evolution 220, USA).

7.2.6 Anti-nutrient analysis

The phytate content of the samples was determined using Latta and Eskin (1980) as modified by Vaintraub and Lapteva (1988). About 1.0 g of corm sample was extracted with 10 ml of 0.2 N HCl for 1 hour, followed by 30 minutes of centrifugation at 3000 rpm. To 3 ml of the supernatant solution, 2 ml of Wade reagent was added, and the mixture was centrifuged. The absorbance at 500 nm was measured using an ultra violet spectrophotometer (Lambda 950) (Thermo Scientific model Evolution 220, USA).

Tannin content was determined using the method of Burns (1971), as modified by Maxson and Rooney (1972). The extracted supernatant solutions from 1 g of corm were mixed with 5 ml of vanillin-HCl reagent after centrifuging at 1000 rpm for 5 minutes. After the reaction was accomplished, the absorbance was measured using a spectrophotometer at 500 nm. The oxalate content of the corm sample was determined using the procedures described by Ukpabi and Ejidoh (1989), which include three steps: digestion, oxalate precipitation, and permanganate titration.

7.2.7 Determination of molar ratios and bioavailability of minerals

The bioavailability of calcium, iron, and zinc was predicted by the molar ratios between anti-nutrients and minerals, that is, phytates: calcium, phytates: iron, phytates: zinc, phytates x calcium: zinc, and oxalates: calcium, using the method described by Norhaizan and Nor Faizadatul Ain (2009).

7.2.8 pH value and Titratable acidity analysis

After calibrating the pH meter at 7.00, 4.00, and 9.2 with a known buffer solution, the pH of the corm samples of the enset flour was determined by homogenizing a 1/10 dilution of sample (10 g of flour samples in 90 ml of distilled water) and by attaching a glass electrode to a digital pH meter at room temperature. The experiment was conducted in triplicate.

The total titratable acidity of corm flour of the enset was determined by titrating the sample with the addition of a standard base (0.1N NaOH) to 3–4 drops of 1% phenolphthalein indicator until the endpoint identified by the color of the sample changed to pink. The equation below uses the volume of NaOH to calculate the percent titratable acidity based on lactic acid, which is the main organic acid in enset samples (Tiruha Karssa *et al*., 2014).

$$\text{Titratable acidity}(\%) = \frac{Vol.NaOHused(ml) * 0.1NNaOH * 0.009 * 100}{W}$$

Where Vol = volume of the 0.1N NaOH used, N= normality of the NaOH used, 0.009= milli- equivalent factor for lactic acid, W = weight of the corm sample.

7.2.9 Statistical analysis

Data obtained through laboratory tests for proximate composition, mineral contents, and anti-nutritional factors of seven enset landraces suitable for corm consumption were computed using the analysis of variance (ANOVA) procedures using SPSS version 26.0 (IBM Corporation, Armok, USA), software for Windows. Mean differences were statistically significant at $p<0.05$ (5% probability levels), and the means of each parameter were compared using Duncan's multiple range test procedures to separate the means that existed among the enset landraces.

7. 3 Results and Discussion

7.3.1 Proximate composition

7.3.1.1 Moisture contents

The mean values for moisture content among the corms of seven enset landraces showed significant variations, ranging from 68.2–79.4 wet bases (Table 7.2). The highest value was obtained for raw corm samples prepared from the *Hayiwona* landrace, while the lowest moisture content was recorded for corm samples prepared from *Qiniwara* landraces (Table 7.2). Moisture measurement is one of the most representative tests in foods since the amount of water in foods has a significant effect on preservation and the occurrence of chemical, physical, and microbiological changes during storage (Punchay *et al*., 2020). The moisture content of the enset corm in the current study is consistent and slightly higher than the value (65–72%) reported by Gizachew Nuraga *et al.* (2019), but this is lower than the 85% reported by Mohammed *et al*. (2013). Such differences may be attributed to genetic variation, environmental factors, and agronomic practices. Our result indicated that the moisture content of enset corm is comparable with other root and tuber crops, such as anchote (*Coccinia abyssinica*) and sweet potatoes

(*Ipomoea batatas*) (Parmar *et al*., 2017). Crops, particularly roots and vegetables with high moisture content, provide a greater role for water-soluble enzymes and coenzymes required for metabolic processes (Punchay *et al*., 2020). Besides, a higher moisture level helps to eliminate hydrogen cyanide (HCN) content by stimulating glycosidase enzymatic activity (Safdar *et al.*, 2020).

7.3.1.2 Protein content

The mean crude protein content of corm samples obtained from seven different enset landraces is summarized in Table 7.2. The mean value of crude protein content of the corm samples analyzed ranged from 2.43 to 11.90% (Table 7.2). Our result showed the existence of large differences within landraces in the protein content of the studied enset corms. On a dry matter basis, the highest crude protein value was found from the corm sample of enset landrace *Hayiwona* (11.90%), followed by *Qiniwara* (9.63%) and *Separa* (9.1%), whereas the lowest value was documented from the corm sample taken from *Astara* (2.43%) landraces. This value was higher even compared to 8.23% in the *Nobo* landrace by Sirawdink Foesido *et al.* (2013) and 8.3% in *Neqaqa* corm by Tadessa Daba and Shigeta (2016). The higher protein contents of *Hayiwona* in this study indicate that its consumption may be useful for healing and replacing the tear and wear of body tissues. Farmers also claim that this landrace is bodybuilding in humans and stimulating milk production after delivery in cattle (Tesfaye Dilebo *et al*., 2023a). The mean value of crude protein content obtained from *Astara* (2.43%), *Gishira* (2.45%), and *Leqeqa* (4.36%) landraces in the present study is almost comparable with the findings of Mohammed *et al*. (2013) and Gizachew Nuraga *et al.* (2019), which range from 2.40 to 4.74%. Admasu Tsegaye (2015) indicated that the protein contents of corm ranged between 0.9 and 2.39%, which is lower than the findings of this study and what was noted in different literature. The crude protein content

obtained in this study from the *Hayiwona, Qiniwara, Separa, and Soqido* landraces is higher than the value reported for other root and tuber crops from Ethiopia. For instance, Tesfaye Abebe *et al*. (2012), Atnafua Bekele and Endashaw Bekele (2018), Demelash Mitiku and Tilahun Teka (2017), and Adugna Bayeta (2020) reported 4.8% of Irish potato (*Solanum tuberosum*), 3.1–5.4% for yam (*Dioscorea* spp), 2.1–2.8% for sweet potato (*Ipomoea batatas*), and 1.3–1.9% for cassava (*Manihot esculenta*) crude protein in percentage, respectively. This indicated that the corm of some enset landraces has appreciable value in protein content; hence, it is important for the growth and replacement of the lost body tissues and may have a good potential to serve as an affordable supply of protein in areas where protein-energy deficiency is an issue.

7.3.1.3 Total ash contents

The mean values of total ash contents from corm samples prepared varied between 2.01 and 4.6% (Table 7.2). Corm prepared from the *Gishira* landrace (4.6%) had the highest total ash content, followed by *Astara* and *Soqido* (4.2%) for each landrace. But the lowest total ash value was recorded at 2.01% from the corm sample prepared by *Qiniwara* (Table 7.2). According to Samydurai and Thangapandian (2012), ash content represents the total mineral content in foods, and it plays a key role in the physicochemical and nutritional content, which represents a small proportion of dry matter, often less than 7% of the total. In the current study, the total ash content of the corms of three enset landraces; *Astara* (4.2%), *Gishira* (4.6%), and *Soqido* (4.2%), increased almost twice in comparison with the *Hayiwona* and *Qiniwara* landraces (Table 7.2), and it exhibited higher values than the reports of Admasu Tsegaye (2015), Tadessa Daba and Shigeta (2016), and Gizachew Nuraga *et al*. (2019). However, Sirawdink Forsido *et al*. (2013) reported the ash content of the corm of enset as 5.12%. These differences may be attributed to

genotypic, environmental, agronomic practices, and the age of the enset crops. The corm of the enset showed comparable ash contents with most tubers and roots and an even better amount with most cereals. This implies that the higher ash content of corm means enset plants have the potential to raise mineral consumption in the diet, which directly contributes to the health of consumers. According to Jacob *et al*. (2015), high mineral elements in foods enhance growth and development and also catalyze metabolic processes in the human body.

7.3.1.4 Crude fat contents

The crude fat content in corm samples obtained from the seven enset landraces was not significantly different ($p<0.05$). All the corms had low crude fat contents below 1.0% and they oscillated between 0.61 and 0.89%, as presented in Table 7.2. A relatively higher crude fat content of 0.89% was recorded from the corm sample of the *Hayiwona* landrace, but all other landraces showed a nearly equal amount of crude fat content. This value is higher than the previous study by Admasu Tsegaye (2015) (0.18%) and Mohammed *et al.* (2013) (0.4%) but comparable with the reports of Tadessa Daba and Shigeta (2016) (0.6%). However, Sirawdink Forsido *et al*. (2013) found a relatively higher fat content of 1.24%. The observed differences may be due to genetic or environmental factors. In most root and tuber crops, the crude fat content is less than one. In like manner, enset corm contains a higher fat value in comparison to the anchote (*Coccinia abyssinica*) (0.26%), cassava (*Manihot esculenta*) (0.26%), and sweet potato (*Ipomoea batatas*) (0.05%) (Parmar *et al*., 2017).

7.3.1.5 Crude fiber content

The mean crude fiber content in the raw corm samples of the seven enset landraces was shown (Table 7.2). The crude fiber value of corm samples revealed a remarkable variation, ranging

from 2.42 to 4.11%. The corm sample made from *Hayiwona* had the highest crude fiber content, followed by *Gishira* (3.76%) and *Separa* (3.45%) landraces, while the lowest crude fiber contents were observed from the corm sample of *Leqeqa* (2.42%) landrace. Our results revealed that crude fiber was found to be the fourth largest proximate composition present in the corms of enset in this study after total carbohydrate, crude protein, and total ash contents on a dry weight basis. The percentage of crude fiber in the present study was in agreement with the findings of Gizachew Nuraga *et al*. (2019), who reported fiber content in the range of 2.38–4.47%. Likewise, Mohammed *et al*. (2013) reported slightly higher crude fiber (5.65%), but a very high value was observed by Tadessa Daba and Shigeta (2016) (17.4%) from the corm of *Neqaqa* enset landrace. On the other hand, a lower crude fiber value that ranged from 0.8 to 1.1% was reported by Admasu Tsegaye (2015). Such a high variation may occur due to the age of the plant or analytical procedures. Our findings and also the literature reveal that enset corm has an appreciable amount of crude fiber and also better contents than some other tuber and root crops like Irish potato, cassava, and anchote (Parmar *et al*., 2017). According to Mackowiak *et al.* (2016), the presence of crude fiber in the diet is essential for the digestion and removal of wastes. Similarly, Millar *et al*. (2019) reported that dietary fiber maintains normal bowel function and can prevent gastrointestinal disorders. Crude fiber is becoming more widely acknowledged as a helpful instrument for the regulation of oxidative processes in food substances and serves as a functional component of foods (Barber *et al*., 2020). Jacob *et al*. (2015) and Millar *et al*. (2019) also suggested that fiber lowers blood cholesterol levels and the risk of several diseases in humans.

7.3.1.6 Total carbohydrate content

The mean value of the total carbohydrate value on the dry basis of the corms of the seven enset landraces is shown in Table 7.2 and differs significantly ($p<0.05$). The mean total carbohydrate contents of raw corm samples ranged from 80.89% for the *Hayiwona* landrace to 89.92% for the *Astara* landrace. However, the total carbohydrate contents of *Astara, Leqeqa*, and *Gishira* landraces with the value of 89.92%, 89.81%, and 88.51% on a dry weight basis, respectively, did not show significant differences ($p > 0.05$). Likewise, no significant difference ($p > 0.05$) was also observed for the total carbohydrate (%) in the corms samples of *Qiniwara*, *Soqido*, and *Separa* enset landraces. Admasu Tsegaye and Struik (2001) and Mohammed *et al*. (2013) stated that enset corm has nutritional values similar to potatoes and is rich in carbohydrates. The laboratory analyses in the present study also revealed that the corm samples taken from each enset landrace showed high values of carbohydrate content; this supports the notion that root crops are typically high in carbohydrates. Similarly, Padhan *et al*. (2020) described that most root crops have a dry matter content that is 60% to 90% carbohydrate. Tadessa Daba and Shigeta (2016) reported the total carbohydrate contents (64%) of the corm sample of *Neqaqa* enset landrace, which is by far lower than the total carbohydrate contents of enset corm in this study. However, the present value is closely comparable with the findings of Mohammed *et al*. (2013) and Gizachew Nuraga *et al*. (2019). In the same manner, the carbohydrate content in corms of enset in the present study was comparable to those reported for other root and tuber crops from Ethiopia, such as sweet potato (*Ipomoea batatas*) (83–89%), anchote (*Coccinia abyssinica*) (74–84%), and taro (*Colocasia esculenta*) (73–76%) Endrias Dako *et al*. (2016), Yenenesh Ayalew (2016) and Melese Yirmaga (2017), respectively.

7.3.2 Gross energy value

The gross energy value (kcal/100 g) of samples prepared from the raw corms of seven enset landraces ranged from 369.96 to 385.12, which is differed significantly ($p<0.05$) (as presented in Table 7.2). The highest and lowest total energy values have existed in raw corm samples prepared from *Qiniwara* and *Gishira* enset landraces, respectively. According to Lowe (2021), the body requires food according to its energy value, which assesses the chemical energy contained in the bonds of food's organic compounds such as its protein, carbohydrate, and fat constituents. Similarly, roots and tubers are typically great providers of dietary energy from a nutritional point of view (Endrias Dako *et al*., 2016). The energy values of the corm samples of enset found in this study are higher than the values described by Tadessa Daba and Shigeta (2016) for the corm sample of *Neqaqa* (333 kcal/100 g) enset landrace. This variation might be attributed to genetic, environmental, and management differences. However, the energy values of corms of enset observed in the present study were nearly comparable with the values reported from other energy-rich roots and tubers: sweet potato (*Ipomoea batatas*) 361.86–376.90 kcal/100 g; taro (*Colocasia esculenta*) 376.30 kcal/100 g; and anchote (*Coccinia abyssinica*) (349–368) kcal/100 g (Endrias Dako *et al*., 2016; Yenenesh Ayalew, 2016; Melese Temesgen, 2017). Hence, the corm samples taken from each landrace revealed substantial levels of energy content. This shows that enset corms are an excellent source of energy for both humans and livestock. A similar finding was also reported by Melese Temesgen and Negussie Ratta (2015). In general, roots and tubers provide the least expensive source of energy for the underprivileged in developing countries.

Table 7. 2 Proximate composition (%) and gross energy (Kcal/100g) of corms of seven enset landraces suitable for *amicho* consumption

Name of Landrace	Moisture[1] content	Total ash	Crude protein	Crude fat	Crude fiber	Total[2] CHO	Gross energy
Astara	72.42±0.91^{b}	4.21±0.32^{a}	2.43±0.17^{d}	0.61±0.12^{b}	2.87±0.05^{c}	89.92±0.70^{a}	374.77±2.17^{b}
Gishira	71.35±1.10^{b}	4.60±0.28^{a}	2.45±0.23^{d}	0.68±0.12^{b}	3.76±0.02^{b}	88.51±0.85^{a}	369.96±3.05^{c}
Hayiwona	79.43±0.78^{a}	2.20±0.41^{b}	11.90±0.68^{a}	0.9±0.25^{a}	4.11±0.10^{a}	80.89±0.90^{c}	379.26±1.18^{b}
Leqeqa	78.65±0.85^{a}	2.80±0.50^{b}	4.36±0.32^{d}	0.61±0.15^{b}	2.42±0.06^{c}	89.81±0.80^{a}	382.17±1.68^{a}
Qiniwara	68.25±0.65^{c}	2.01±0.32^{b}	9.63±0.8^{b}	0.60±0.33^{b}	2.47±0.03^{c}	85.32±0.65^{b}	385.12±2.14^{a}
Separa	77.05±0.88^{a}	2.63±0.35^{b}	9.11±0.8^{b}	0.59±0.25^{b}	3.45±0.02^{b}	84.26±0.70^{b}	378.75±3.18^{b}
Soqido	76.25±0.90^{a}	4.21±0.25^{a}	6.67±0.48^{c}	0.62±0.15^{b}	2.75±0.03^{c}	85.76±0.88^{b}	375.33±3.20^{b}

Reported values in each cell are triplicate analysis. Means with different letters in the same column are significantly different ($P<0.05$). [1] Wet basis, [2] Total Carbohydrate.

7.3.3 Mineral content

The results of the mean mineral contents (calcium, potassium, magnesium, phosphorus, sodium, iron, and zinc) of raw corm samples from seven selected enset landraces are compiled in Table 7.3. The mean values for calcium content among analyzed corms of enset landraces showed statistically significant variations, ranging from 22.46 mg/100 g for *Hayiwona* to 49.74 mg/100 g for *Gishira* landraces on a dry weight basis. Yewelsew Abebe *et al*. (2007) reported the calcium contents of unspecified enset landrace corm at 25 mg/100 g, which is in the range of our findings in this study. However, Sirawdink Forsido *et al*. (2013) and Tadessa Daba and Shigeta (2016) reported higher calcium content of *Nobo* (100 mg/100 g) and *Neqaqa* (99.7 mg/100 g) respectively. On the other hand, Ajebu Nurfeta *et al*. (2008), Ayalew Debebe *et al*. (2012), and Gizachew Nuraga *et al.* (2019) reported far higher calcium contents of different enset landraces ranging from 3,610–11,400 mg/100 g. The variation could be attributable to environmental or genetic factors and analytical apparatus used for determination. The calcium contents in the current study were also slightly comparable to those previously published for other common root

and tuber crops by Parmar *et al*. (2017), who reported 30 mg/100 g in sweet potato (*Ipomoea batatas*) and 55 mg/100 g in taro (*Colocasia esculenta*) but it was better than cassava (*Manihot esculenta*). Calcium concentrations are necessary for bone constituents, tooth development, and blood coagulation in humans (Jacob *et al*., 2015; Tedeschi *et al*., 2023), and they also maintain the biological roles of nerve transmission, muscle contraction, and glandular secretion (Ooi *et al*., 2012).

The mean value for potassium content among corms of seven enset landraces (Table 7.3) indicated significant variations, ranging from 28.51–86.56 mg/100 g on a dry weight basis. *Hayiwona* landrace had the highest value of potassium content, while the lowest value was observed in *Qiniwara* landrace raw corm samples. The current finding appeared to be far lower than the potassium contents of raw corms of enset landraces reported by Ayalew Debebe *et al*. (2012), Sirawdink Forsido *et al.* (2013), and Mohammed *et al.* (2013), which ranged from 2000 to 3200 mg/100 g. Unlikely, Tadessa Daba and Shigeta (2016) reported a far lower potassium content (1.24 mg/100 g) in the unfermented enset corms of *Neqaqa* landraces. Such higher variation seemed to be due to analytical procedures rather than environmental or genetic factors. Potassium is essential in the regulation of heartbeat, neurotransmission, and water balance in the human body and in controlling hypertension (Alinnor and Oze, 2011; Jacob *et al.*, 2015).

The magnesium content in the corms of the enset ranged from 16.46 mg/100 g for *Leqeqa* to 29.34 mg/100 g for *Qiniwara*, as shown in Table 7.3. The observed values showed no significant difference between the landraces of *Leqeqa* and *Hayiwona*, *Separa*, *Soqido*, and *Astara* (Table 7. 3). The differences most probably appear due to be genetic factors rather than environmental ones since the crops had grown within the same environmental and management systems. The

magnesium content of corm samples in the present study was lower than that reported by Tadessa Daba and Shigeta (2016) (59.6 mg/100 g) and Sirawdink Forsido *et al.* (2013) (80 mg/100 g). This could be attributed to many factors, including the environment in which the enset grows, its genetic makeup, and the analytical methods used. However, the values found from this study are consistent with Parmar *et al*. (2017), who provided a comparable range of values for cassava (*Manihot esculenta*) and sweet potato (*Ipomoea batatas*). Magnesium is necessary for the healthy functioning of the nervous and muscular systems. It aids in maintaining a strong immune system, strengthens bones, and aids in blood sugar regulation, which supports appropriate blood pressure (Olatunde *et al*., 2016).

Mineral analysis of the examined raw corm samples of the enset landraces exhibited a remarkable difference ($p < 0.05$) in phosphorous content; the highest value of 13.58 mg/100 g was recorded from *Gishira* landrace, followed by *Astara* (12.16 mg/100 g) and *Soqido* (12.08 mg /100 g) landraces on a dry weight basis. These landraces provided three times higher phosphorous contents than those of *Qiniwara*, *Separa*, and *Hayiwona* (Table 7.3). The content of phosphorous in this work was lower than the value indicated by previous authors. Sirawdink Forsido *et al*. (2013) and Tadessa Daba and Shigeta (2016) reported phosphorus values of 90 mg/100 g and 80.4 mg/100g, respectively, when they analyzed the corm of enset landraces. The observed variation in the earlier study and the current findings might be attributed to the genetic differences of landraces and/or environmental factors such as soil types, agronomic practices, and the age of enset plants. A similar idea had been reported by Laya *et al*. (2018). Phosphorous plays a crucial role in the formation of nucleic acid, normal cell growth and repair, and the ossification of bones by being deposited as calcium phosphate (Vance *et al*., 2003).

There was no significant difference in sodium content ($P > 0.05$) among the landraces from a range of 7.13 mg/100 g in *Leqeqa* to 8.67 mg/100 g in *Gishira* (Table 7.3). The results of the present mineral analyses revealed slightly higher sodium contents than the finding by Tadessa Daba and Shigeta (2016) for the *Neqaqa* enset landrace (5.2 mg/100 g), but these values were significantly lower than the reported figures for the *Nobo* enset landrace by Sirawdink Forsido *et al.* (2013) (15 mg/100g) and by Mohammed *et al.* (2013) (30 mg/100 g) for the corm samples of unspecified enset landraces. The variation may result from a difference in genetics or the growing environment (Ajebu Nurfeta *et al*., 2008; Mugo *et al*., 2020). Sodium controls bodily fluid and maintains the tissue's electric potential (Alinnor and Oze, 2011).

In this study, the iron contents also differed significantly ($P<0.05$) among the landraces in raw corm samples of enset, ranging from 0.9 mg/100 g in the *Leqeqa* landrace to 3.85 mg/100 g in *Hayiwona*. The corms of *Hayiwona* and *Gishira* provided three times more value than other landraces based on their iron contents (Table 7.3). Compared to other minerals analyzed in this study, iron and zinc were found in low amounts in raw corms on a dry weight basis, as shown in Table 7.3. Admasu Tsgaye (2015) reported that the iron contents of the corms of different enset landrace ranged between 1.1 mg/100 g and 4.3 mg/100 g, which, except for landraces *Leqeqa*, is closely consistent with our results. Similarly, this study exhibited a higher iron value than the level (0.7 mg/100g) reported by Yewelsew Abebe *et al*. (2007). However, Tadessa Daba and Shigeta (2016) reported far higher iron contents (12.3 mg/100 g) in the raw corms of *Neqaqa* enset landrace. It appears that genetic and environmental factors were responsible for such higher variation (e.g., application of organic or inorganic fertilizers). The human body requires a smaller quantity of key trace elements, like iron and zinc, for vital biochemical processes. Iron is important for the production of hemoglobin, which is crucial for oxygen

transport throughout the body and for the oxidation of carbohydrates, proteins, and fats (Mlitan *et al*., 2014; Naz *et al*., 2018; Zahedi *et al*., 2020).

Table 7. 3 Mineral composition (mg/100g) of corms of seven enset landraces suitable for *amicho* consumption on a dry weight basis

Landrace	Calcium	Potassium	Magnesium	Phosphorous	Sodium	Iron	Zinc
Astara	38.48±1.15^{b}	64.44±2.03^{b}	23.84±0.70^{b}	12.16±1.20^{a}	8.19±1.10^{a}	1.11±0.25^{b}	0.38±0.05^{b}
Gishira	49.74±1.25^{a}	57.25±1.30^{c}	26.16±1.03^{ab}	13.58±1.08^{a}	8.67±1.00^{a}	3.82±0.60^{a}	1.09±0.25^{a}
Hayiwona	22.46±0.50^{d}	86.56±2.05^{a}	19.06±0.80^{c}	3.83±0.65^{b}	8.52±0.81^{a}	3.85±1.00^{a}	1.44±0.20^{a}
Leqeqa	22.51±0.75^{d}	31.63±1.05^{d}	16.46±0.50^{c}	4.17±1.15^{b}	7.13±0.55^{a}	0.90±0.15^{c}	0.58±0.03^{b}
Qiniwara	29.30±1.09^{c}	28.42±0.82^{d}	29.34±1.15^{a}	3.10±0.88^{b}	7.71±0.45^{a}	1.29±0.40^{b}	0.50±0.06^{b}
Separa	29.87±0.86^{c}	37.29±1.05^{d}	22.59±0.50^{b}	3.29±0.90^{b}	7.42±0.78^{a}	1.23±0.38^{b}	0.52±0.02^{b}
Soqido	36.24±1.20^{b}	59.37±1.25^{c}	23.46±1.00^{b}	12.08±1.50^{a}	7.87±0.88^{a}	1.06±0.15^{b}	0.74±0.06^{b}

Values are mean of triplicate analysis. Means not followed by the same superscript letters in the same column are significantly different ($P < 0.05$).

As shown in Table 7.3, zinc content differed significantly ($p < 0.05$) among the enset landraces and ranged from 0.38 to 1.44 mg/100 g. The *Hayiwona* landrace had the highest zinc content, and *Astara* had the lowest. However, the zinc contents of the analyzed corms of enset landraces showed no significant difference ($p > 0.05$) between the remaining five landraces. Yewelsew Abebe *et al*. (2007) also reported the zinc contents (1.33 mg/100 g), which was slightly comparable with the value of the *Hayiwona* landrace in this study. On the other hand, higher zinc content that ranged between 6.2 and 15.6 mg/100 g and 22 mg/100 g was also reported by Tsegaye (2015) and Tadessa Daba and Shigeta (2016), respectively, which may be related to various factors such as agronomic practice, genetic factors, environmental variations, and analytical procedures used for determination. Zinc is a vital trace element that is crucial for many cellular functions, such as healthy growth, brain development, behavioral response, bone production, and wound healing (Mlitan *et al*., 2014). Minerals are important constituents of the human diet as they serve many physiological and metabolic processes (Soetan *et al*., 2010;

Hossain *et al.*, 2021). According to reports by Ratsavong *et al*. (2020) and Ajebu Nurfeta *et al*. (2008), the mineral content of crops varies greatly depending on factors including disease, several transplantations, hygiene, plant variety, and soil contamination.

7.3.4 Anti-nutritional factors

Anti-nutritional factors (anti-nutrients) are chemical substances generated in natural feedstuffs by the normal metabolism of plants (Soetan and Oyewole, 2009; Habtamu Gemede and Negussie Ratta, 2014). Some of these plant chemicals can be beneficial or harmful to human and animal health, depending on the amount of intake (Sinha and Khare, 2017). Previous studies on anti-nutritional factors (e.g., phytate, tannin, and oxalate) on enset corm (*amicho*) have not been conducted so far, except Yewelsew Abebe *et al*. (2007) reported for unspecified enset phytate content from the Sidama region, Sirawdink Forsido *et al.* (2013) for *Nobo* landrace total phenolic contents, and Gizachew Nurage *et al.* (2019) for five enset landraces phytate and tannin from the Wolkite area of Ethiopia. The phytate, tannin, and oxalate contents of the corm samples of the seven enset landraces (*Astara*, *Gishira*, *Hayiwona*, *Leqeqa*, *Qiniwara*, *Separa*, and *Soqido*) are shown on a dry weight basis in Table 7.4. The mean phytate levels of the studied enset landraces were significantly different ($P< 0.05$) and ranged from 221.75 to 276.12 mg/100 g for *Astara* and *Soqido* landraces, respectively, as presented in Table 7.4. These values are far below the values reported by Gizachew Nuraga *et al.* (2019) for phytate (14,900–19,500 mg/100 g) for corms of five enset landraces on a dry weight basis and by a far higher value (0.9 mg/100 g) from the reports of Yewelsew Abebe *et al*. (2007). Such a high variation seems likely to be due to analytical procedures rather than environmental or genetic factors. Hiwot Bekele (2015) indicated that fresh *bulla* (extracted enset product) contents of four enset varieties ranged between 84.25 mg/100 g and 112.56 mg/100 g, which is lower than the phytate contents of enset

corm in this study. This implies that the various enset products may have different phytate contents. Similarly, raw enset corm exhibited a higher phytate value than the level (115.43 mg/100 g) reported by Adane Tilahun *et al*. (2013) on raw taro (*Colocasia esculenta*) flour. However, Habtamu Fekedu *et al.* (2013) reported a higher value of phytate in raw anchote (*Coccinia abyssinica*) tuber, which contained 389.30 mg /100 g on a fresh weight basis compared with the mean phytate contents of raw corm of enset landraces on dry weight basis. According to Chandrasekara and Kumar (2016), the level of phytic acid in a crop can differ based on the crop variety, location, climate, irrigation system, type of soil, and growing season of the plant. The principal mineral absorption inhibitor in plant-based diets is phytate, which also lowers the bioavailability of dietary minerals by producing insoluble mineral chelates in food and the body (Sefa-Dedeh and Agyir-Sackey, 2004; Zohora *et al*., 2018). Therefore, phytate levels must be minimized to concentrations of less than 200 mg/100g DM to mitigate the negative impact of phytates on mineral absorption (Hurrell, 2004). For vegetarian diets, the daily consumption of phytate was reported to be between 2000 and 2600 mg and 150–1400 mg for mixed diets (WHO, 2003).

Table 7. 4 Anti-nutritional factors (mg/100g) of corms of seven enset landraces suitable for *amicho* consumption (dry weight basis)

Landrace	Phytate	Tannin	Oxalate
Astara	276.12±1.35^{a}	77.64±1.21^{b}	5.97±0.25^{a}
Gishira	235.92±0.87^{c}	27.97±1.30^{e}	9.11±0.52^{a}
Hayiwona	232.51±1.05^{c}	113.74±2.38^{a}	6.26±0.33^{a}
Leqeqa	229.04±1.15^{cd}	67.27±1.35^{c}	5.79±0.13^{a}
Qiniwara	241.75±0.87^{b}	71.93±1.16^{b}	7.97±0.28^{a}
Separa	230.21±0.17^{c}	59.86±0.94^{c}	5.69±0.13^{a}
Soqido	221.75±0.75^{d}	37.78±1.27^{d}	6.54±0.30^{a}

Values are mean of triplicate analysis. Means not followed by the same superscript letters in the same column are significantly different ($P < 0.05$).

Results from statistical analysis showed that the mean values for tannin content among seven enset landraces varied significantly ($p<0.05$); they ranged from 27.97–113.74 mg /100 g in raw corm samples on a dry weight basis. The maximum tannin value (113.74 mg/100 g) was recorded in the *Hayiwona* landrace, and it was followed by the *Astara* (77.64 mg/100 g*)* and *Qiniwara* (71.93 mg /100 g) landraces. The lowest tannin level was observed in the *Gishira* (27.97 mg/100 g) landrace (Table 7.4). The tannin content for the raw corms of the enset landrace (5,048–10,356 mg/100 g) reported by Gizachew Nuraga *et al.* (2019) was too high as compared to the present study on a dry basis. These variations might be due to analytical approaches, environment, and age differences in the enset-crop. Tannin, which has a high molecular weight and is a water-soluble compound, greatly affects nutritional values. Because they precipitate proteins and prevent the activity of digestive enzymes and reabsorption, foods high in tannin are regarded as having low nutritional value (Jacob *et al*., 2015). For humans, a daily maximum of 560 mg of tannin is acceptable (WHO, 2003). Accordingly, based on the results of this study, all of the enset corm samples had low tannin contents and are safe for human consumption.

In the present study, the oxalate content obtained from corm samples of seven different enset landraces ranged from 5.69 to 9.11 mg/100g but did not differ significantly ($p > 0.05$) among enset landraces. A relatively high oxalate content (9.11 mg/100g) was obtained in the corm sample of the *Gishira* landrace, while a lower amount (5.69 mg/100g) was found in the corm sample of the *Separa* landrace on a dry weight basis. These contents are less than those for other root and tuber crops from Ethiopia that have been reported. High oxalate diets can be detrimental to human nutrition and health, particularly since they decrease calcium absorption and promote the development of kidney stones (Massey, 2007; Jacob *et al*., 2015; Millar *et al.,* 2019).

According to the reports of Massey *et al*. (2001), patients are recommended to consume fewer foods with a daily oxalate intake of no more than 50–60 mg. In humans, 3000–5000 mg of oxalate is fatal (WHO, 2003). As a result of the findings of this study, oxalate levels in enset corm are not high enough to pose a risk to human health.

7.3.5 Molar ratios and bioavailability of minerals

The molar ratios of phytates to calcium, iron, zinc, phytates x calcium to zinc, and oxalate to calcium for corm samples of the seven enset landraces were shown significant variation ($p < 0.05$) among landraces in the present study, and the results for each of the landraces are presented in Table 7.5. The highest phytate to calcium molar ratios (0.63) and (0.62) were recorded for the landraces *Hayiwona* and *Leqeqa*, respectively, and the lowest value belonged to the *Gishira* (0.28) landrace. Relatively, the highest phytate to iron (21.69) and (21.13) molar ratios were recorded for *Leqeqa* and *Astara*, whereas the lowest was recorded for the *Gishira* and *Hayiwona* landraces, respectively. Similarly, the highest and lowest phytate to zinc ratios were exhibited in the landraces *Astara* and *Hayiwona*, respectively. A significant difference ($P<0.05$) was observed among the analyzed corm samples of enset landraces in the phytate x calcium: zinc molar ratio; the highest and lowest ratios were recorded for *Astara* and *Hayiwona* varieties, respectively. The oxalate to calcium molar ratios of the corm samples of enset landraces ranged from 0.07 to 0.13. The highest value was observed in landrace *Hayiwona*, followed by *Leqeqa* and *Qiniwara* with the same molar ratio value (0.12), whereas *Astara* had the lowest value (Table 7.5).

The molar ratios of anti-nutrients to minerals are used to assess their inhibitory effect on the bioavailability of minerals in food and diet (Castro-Alba *et al*., 2019). Bioavailability is the

proportion of an ingested nutrient in food that is absorbed and utilized through normal metabolic pathways, particularly minerals (Gibson *et al*., 2010). It is influenced by the presence of phytates, oxalates, tannins, and dietary fiber (Frossard *et al*., 2000), and due to these, only a small portion of the minerals in the diet will be absorbed in the gastrointestinal tract (Norhaizan and Nor Faizadatul Ain, 2009). The phytate to calcium, iron, and zinc molar ratios above 0.24, 1, and 15, respectively, will impair these minerals and be indicative of poor bioavailability (Ma *et al*., 2007; Norhaizan and Nor Faizadatul Ain, 2009). According to WHO (1996), phytate×calcium to zinc is a more reliable assessment of zinc bioavailability than the phytate to zinc molar ratio alone.

In the present study, the molar ratio of phytate to calcium, iron, and zinc of all the analyzed corms of enset landraces was above the critical values **(**Table 7.5). This suggests that phytates may have a negative impact on the dietary materials of the corms of the analyzed enset landraces. However, the phytate×calcium to zinc molar ratio in corm samples of enset landraces was all below 200. This is good in terms of bioavailability (Norhaizan and Nor Faizadatul Ain, 2009). Likewise, the oxalate to calcium molar ratio in corm samples of enset landraces in this study was less than the critical value (1), and this implies that the bioavailability of calcium in corm samples is good, as indicated by Bhandari and Kawabata (2004).

Table 7. 5 Calculated molar ratios of phytate to Ca, Fe, and Zn and oxalate to Ca of corms from seven enset landraces suitable for *amicho* consumption

Landraces	**(Phy:Ca) [1]**	**(Phy:Fe)[2]**	**(Phy:Zn)[3]**	**(Phy·Ca:Zn)[4]**	**(Oxa:Ca)[5]**
Astara	0.43±0.01^{b}	21.13±0.27^{a}	72.14±0.53^{a}	69.41±0.62^{a}	0.07±0.01^{b}
Gishira	0.28±0.00^{d}	5.24±0.12^{d}	21.28±0.08^{c}	26.46±0.42^{c}	0.08±0.00^{b}
Hayiwona	0.63±0.02^{a}	5.12±0.09^{d}	15.87±0.21^{d}	8.91±0.08^{d}	0.13±0.02^{a}
Leqeqa	0.62±0.03^{a}	21.69±0.24^{a}	38.99±0.32^{b}	21.94±0.28^{c}	0.12±0.03^{a}
Qiniwara	0.50±0.00^{b}	15.93±0.32^{c}	41.16±0.73^{b}	34.85±0.54^{b}	0.12±0.00^{a}
Separa	0.47±0.01^{b}	15.88±0.16^{c}	43.58±0.41^{b}	32.54±0.32^{b}	0.09±0.01^{b}
Soqido	0.37±0.00^{c}	17.68±0.37^{b}	29.47±0.62^{c}	27.07±0.52^{c}	0.08±0.02^{b}

[1]mg of phytates/molecular weight of phytates: mg of calcium/molecular weight of calcium,
[2]mg of phytates/molecular weight of phytates: mg of iron/molecular weight of iron,
[3]mg of phytates/molecular weight of phytates: mg of zinc/molecular weight of zinc,
[4](mg of calcium/molecular weight of calcium)*(mg of phytates/molecular weight of phytates)/(mg of zinc/molecular weight of zinc),
[5]mg of oxalates/molecular weight of oxalate: mg of calcium/molecular weight of calcium.

7.3.6 pH and total titratable acidity

The pH and total titratable acidity of the seven enset landraces are presented in Table 7.6. Among enset landraces, the highest pH value (6.48) of corm flour samples was recorded from *Astara*, but the lowest pH value (5.34) was obtained from *Separa* landraces. This shows that the pH values of the corm samples of this study were slightly acidic, which met the standards for quality and was acceptable. Since flour with a pH of 4 or less would have a distinctively sour aroma and taste from fermentation, which is undesirable for use in culinary items (Eriksson, 2013), the pH is a suitable quality indicator for corm flour. Similarly, Hiwot Bekele (2015) reported that the pH value of fresh unfermented *bulla* flour from four varieties of enset ranged between 5.23 and 6.01. However, the fermented enset product *qocho* exhibited a 3.79 pH value after 30 days of fermentation (Melese Temesgen, 2013).

Table 7. 6 The pH value and titratable acidity (%) of corm obtained from seven different enset landraces

Landraces	pH value	Titratable acidity(%)
Astara	6.48±0.02[a]	0.28± 0.03[c]
Gishira	5.81±0.04[b]	0.39±0.04[a]
Hayiwona	6.13±0.07[a]	0.28± 0.03[c]
Leqeqa	5.85±0.04[b]	0.24±0.02[d]
Qiniwara	5.79±0.05[b]	0.33±0.04[b]
Separa	5.34±0.11[c]	0.39±0.03[a]
Soqido	6.25±0.03[a]	0.29± 0.03[c]

Values are mean of triplicate analysis. Means not followed by the same superscript letters in the same column are significantly different ($P < 0.05$).

Concerning the total titratable acidity, in this study, there was a significant difference in percent among the tested enset landraces (Table 7.6). The highest titratable acidity percent (0.39%) was recorded for corm prepared from *Gishira* and *Separa,* while the lowest titratable acidity percent (0.24%) was found from *Leqeqa* landraces. Hiwot Bekele (2015) reported the titratable acidity content of fresh *bulla* (0.22–0.30%), which was comparable with the corm of enset titratable acidity, in our study. However, our result is lower than the titratable acidity reported by Melese Temesgen (2013) (0.87%) for the 30-day fermented enset product *qocho*.

7.4 Conclusion

This study has shown notable variation in corm samples prepared from the seven cultivated enset landraces in the proximate, mineral, and anti-nutritional contents. Among the analyzed landraces, *Hayiwona* had the highest mean values of moisture, crude protein, crude fat, crude fiber, potassium, iron, zinc, and tannin contents compared to other tested landraces. On the other hand, the corm sample prepared from the *Gishira* landrace had high total ash, calcium, phosphorous, and sodium contents, but its gross energy content was significantly lower than the other tested landraces. Total carbohydrates and phytate contents obtained from the corm samples of *Astara* were relatively higher than those of other tested landraces in this study. Commonly, the corms of these landraces were highly considered by the farmers for their high *amicho* consumption as food as well as traditional medicine. *Qiniwara* was one of the most favored landraces by local users for its sweet *amicho* and was also traditionally suggested to treat bone-related problems in humans. However, its laboratory results indicated lower contents in total ash and most minerals except gross energy value and magnesium contents. The current study also

shows that all of the analyzed corm samples of enset landraces contained a low level of anti-nutritional contents, which is favorable from a nutritional point of view. On the other hand, the molar ratios of the enset corms in this study were above the standard values, except for phytate×calcium to zinc and oxalate to calcium, which show low mineral bioavailability. Enset corm is typically eaten after being cooked with various leafy vegetables and dairy products, which raises the mineral content and boosts the bioavailability of minerals in the enset diet. Therefore, our findings will provide a basis for continued identification and nutritional evaluation, which is necessary to distinguish the nutritious corm for *amicho* preparation from different enset landraces to sustain and improve enset cultivation for food security.

Chapter Eight
Summary, Conclusions and Recommendations

8.1 Summary

This dissertation offers insight into methods of on-farm (farmer-based) diversity, ethnobotanical documentation, and the genetic characterization of enset using molecular markers. The development of a micro-propagation protocol using a locally available enset product called *bulla* as a gelling agent, along with the proximate, minerals, anti-nutritional, and bioavailability values of the selected enset landraces. Hence, the farmers' indigenous knowledge of the production, conservation, and utilization systems of enset crops was accompanied by the study of genetic diversity using molecular markers and the determination of key nutritional and anti-nutritional compositions of multi-purpose enset landraces. The development of effective micro-propagation techniques was considered essential to the issues farmers encounter with biotic, abiotic, or human-made problems and could act as a backup approach to conventional propagation and on-farm preservation of the enset crop.

Farmers are knowledgeable and skilled enset-growers who understand a great deal about enset cultivation, including how to plant, manage in the field, harvest, use, and preserve its diversity within traditional systems of cultivation and production (Chapters 3 and 4). For regular needs such as dietary, medicinal, fodder, and other purposes, enset farmers depend on various forms of enset products. They also profit from the enset agrisystem's broad ecology of products and services. According to Edilegnaw Wale *et al*. (2011), farmers need to further be aware of the best practices for growing, storing, and caring for particular varieties in order to fully realize the qualities that they appreciate and for fulfilling their special needs. This is necessary for the on-farm maintenance of biodiversity. Abeli *et al.* (2020) also indicated that on-farm conservation of

plant diversity can minimize risk, stabilize yields, promote nutritional diversity, and optimize profits by utilizing limited resources and low levels of technology. Moreover, the diversity of plant species offers plant breeders the chance to develop new and enhanced varieties with desirable traits, such as those valued by farmers and breeders (Govindaraj *et al*., 2015).

In Ethiopia, farmers are the main generators and custodians for the diversity of enset landraces. In this study, we have recorded 282 landraces from the four administrative zones, which is a comparatively higher number (Chapter 3). Similarly, some previous studies have also showed that farmers conserved hundreds of diverse enset landraces in different farming locations or regions of Ethiopia (Chapters 3 and 4). Furthermore, the presence of high enset landrace diversity in the studied zones is shown by various diversity indices, including Shannon-Weaver, Simpson's, and evenness (Chapter 3). This work is consistent with prior reports (Awol Zeberga *et al*., 2014; Zerihun Yemataw *et al*., 2016).

Considering distribution, only a small percentage of enset landraces are more widely distributed and extremely abundant than most moderately frequent or uncommon landraces. This could be because of the ecological adaptability of the landraces and/or the diverse needs of farmers (Chapters 3 and 4). Several factors, including household resources, cultural background, pressure from population growth, and agro-ecology, influenced the abundance and distribution of enset landraces (Almaz Negash, 2001; AdmasuTsegaye, 2002).

Indigenous knowledge is the common resource of the indigenous communities, which is developed over years, transferred verbally as well as through practices (Villa *et al*., 2005). The present study also showed the incredible knowledge of the enset plant possessed by the local farmers, which has been amassed over many years and passed down orally from generation to

generation (Chapters 3 and 4). In study areas, local farmers have been involved in the identification practice, local naming, classification, utilization, and maintaining the genetic diversity of enset through traditional means (Chapter 4). For the identification reasons, farmers were applied four morphological features, agronomic characters, and the quality and quantity of the end-use values, this trend are also agrees with the indigenous knowledge of biosystematics for enset landraces reported from Wolaita and Sidama by Bizuayehu Tesfaye and Ludders (2003) and Temesgen Olango *et al*. (2014). All the identified enset landraces have their own local names, which have been given by enset-growers based on several attributes to distinguish each landrace from others (Chapters 3 and 4). Since enset cultivators always refer to the local names of enset landraces when propagating, planting, managing, harvesting, utilizing, and exchanging enset planting material in the study area. However, the meanings of the names for majority of the documented enset landraces were unexplained by the enset farmers in the studied areas (Chapter 4). This may partly be attributed to the loss of indigenous knowledge, the modification of the original name over a long period, or language dynamics. Similar findings have also been reported for other crops (Firew Mekbib, 2007; Hapsari *et al*., 2017; Nakabonge *et al*., 2020).

Different mixtures of enset landraces are consistently cultivated by farmers in the study regions to preserve the diversity of landraces on the farm and to balance the harvest of enset for various uses throughout the year (Chapters 3 and 4). They typically go through three successive phases of cultivation: vegetative propagation, transplanting, and harvesting (Chapter 4). Compared to specific-use landraces, multi-purpose enset landraces were typically more cultivated and highly chosen (Chapters 3 and 4). Nonetheless, in certain circumstances, there were slight variations among the regions or study zones regarding the perceptions of end-product users (Chapter 3). However, in all studied zones, commonly enset-growers provide more attention and protection

for the medicinal enset landraces than others, which also agrees with reports by Almaz Negash (2001), Yemane Tsehay and Fassil Kebebaw (2006), and Melese Maryo *et al*. (2014).

It is generally accepted that the application of molecular markers, such as SSR markers, may enhance enset crop conservation and improvement significantly. Due to their great abundance, co-dominant nature, high polymorphism, uniform distribution in the genomes of plant species, and good reproducibility in different research labs (Oliveira *et al*., 2006; Kalia *et al*., 2011). In the Chapter 5, the identification of 289 alleles using the 12 SSR markers, which suggests that the populations under consideration had a high level of genetic diversity. In addition, in terms of gene diversity, Shannon's information index, heterozygosity, and polymorphic information content (PIC), this study indicated a comparatively high extent of genetic diversity within populations. This could be explained by the highly diverse landrace resources that the local farmers have long managed and cultivated for both their own livelihood and various other sociocultural reasons (Chapters 3, 4, and 5). As a result, the areas may be seen as hotspots for genetic diversity and as possible locations for *in-situ* enset crop conservation. This finding corroborates the high levels of farmer or on-farm diversity found in the regions under study, which have been preserved sustainably for many generations (Awol Zeberga *et al.*, 2014; Zerihun Yemataw *et al*., 2014; Tesfaye Dilebo *et al.*, 2023a).

The AMOVA in Chapter 5 also showed a greater variance within the populations than among the populations, indicating a lesser influence of the origin of zones or regions. This shows how crucial within-population diversity is to starting conservation efforts and utilizing the existing enset landraces diversity. The low genetic differentiation among the examined enset populations is further evidenced by a low F_{ST} value (0.05) among the genotypes under analysis. This is most likely connected to the tight relationships between farming households and the long-standing

custom of sharing and exchanging enset germplasm by cultivating suckers of desired genotypes, which contributes to the high rate of gene flow within and among enset farming communities in different zones (Chapters 3 and 4). Other researchers have also documented a similar trend and observation (Admasu Tsegaye and Struik, 2002; Zerihun Yemataw *et al*., 2016). The low degree of population divergence in the current study is partly due to the comparatively high gene flow (mean Nm = 5.14) (Chapter 5). Farmers' extensive use of vegetative propagation methods in enset crops may further limit the level of gene flow and, as a result, reduce population differentiation (Chapters 3, 4 and 5). According to Nkhata *et al*. (2020), genetic divergence among populations can be low if the F_{ST} value is less than 0.05.

Based on pair-wise population genetic distances, the present study revealed that the populations of Silte and Gurage had the lowest genetic distance; similarly, Kembata and Hadiya populations had the second-lowest genetic distances (Chapter 5). This implies that the farmers of the neighboring administrative regions share a high degree of enset genotypes as a planting source (Chapters 3 and 5). In the same way, the Sorenson similarity coffecient (Chapter 3) demonstrated that there was greater plant material sharing and exchange among the zones of enset farming communities, confirming a high level of gene flow and, ultimately, less population divergence (Chapter 5). It is due to the deep-rooted practices and cultures of enset-growers, which have developed over many years and been passed down from generation to generation (Chapters 3 and 4).

The conservation of plant diversity is essential for future crop protection due to growing problems of biotic and abiotic stresses (Alexandratos and Bruinsma, 2012; Gupta *et al*., 2016). In this context, *in vitro* approaches allow for the enhancement of various features related to plant development and yield that may then be applied to *ex-situ* conservation (Lavanya *et al*., 2014). It

is a quick process because thousands of seedlings can be developed from tiny plant fragments in a short period in comparison to conventionally grown flora (Mattick, 2018). The genetic stability built with this method is one of its benefits (Chapter 5). In these cases, tissue culture can be used for conserving plants in their vegetative state, like enset crops (Chapter 6).

Enset cultivation mainly benefits the rural communities of the enset-growing regions of Ethiopia as food directly indirectly (feed) and non-food materials mostly from parts not eaten by people (Chapters 4 and 7). Hence, recognizing the related challenges involved in producing it and developing effective methods for addressing these issues are paramount and crucial. According to our survey (Chapters 3 and 4), there has been considerable interest among farmers in cultivating healthy genotypes of enset crops. This indicates that its production is at risk to some extent due to various pathogenic organisms. Thus, to sustain enset production, the development and preparation of disease-free enset genotypes for its plantations are very reasonable. Therefore, micro-propagation methods have been applied to grow healthy, disease-free enset plants year-round that also perform better in the field. There are only a few reports available on the micro-propagation of different genotypes of enset (Chapter 6).

The gelling agent is one of the most costly components of the tissue culture media (Ebile *et al*., 2022). Agar is widely used as a gelling agent in micro-propagation due to its suitable gelling properties (Jain-Raina and Babbar, 2011). Nevertheless, its high cost limits its application, especially in underdeveloped nations (Pati *et al*., 2011; Sanchez-Cardozo *et al*., 2019), since it accounts for almost three-fourths of the media's overall cost per unit (Teixeira Da Silva, 2019). Consequently, studies have been conducted to find less costly substitutes for agar (Babbar *et al*., 2005; Daud *et al*., 2011).

Bulla is one of the main starchy products of enset (Chapter 4) and has been employed as a gelling agent in the *in vitro* propagation of the enset genotypes (Chapter 6). The present study showed that *bulla* flour at a concentration of 80 g/l was the ideal extent to solidify and stabilize the MS medium in the *in vitro* culture of enset shoot tips and also applying it as an alternative source saves 73.3% in cost without compromising culture quality. A comparable observation was made by Ayelign Mengesha *et al*. (2012), Biruk Ayenew *et al*. (2012), and Manaye Ayalew *et al*. (2017), who studied alternative sources of agar in vanilla, pineapple, and cassava, respectively. However, this is the first report in which the use of *bulla* instead of agar as an alternative gelling agent has been reported on the *in vitro* growth of enset (Chapter 6).

The results of the present study exhibited that some enset landraces are cultivated primarily for their *amicho* (cooked corm) consumption for nutritional or traditional healing purposes (Chapters 3, 4, and 7). However, there is limited information on the nutritional and anti-nutritional compositions of enset corm in the study area or even in the country. In an attempt to fill up this information gap, the seven distinct extensively consumed and locally preferred corms of enset landraces were examined for their proximate contents, mineral values, anti-nutritional factors, mineral bioavailability, and physicochemical properties (Chapter 7).

The analysis has shown some remarkable differences in the proximate and mineral contents (Chapter 7). This study also implies that, from a nutritional perspective, it is desirable to note that all of the evaluated corm samples of enset landraces had low levels of anti-nutritional components. However, all of the enset corms' molar ratios were higher than the recommended values, with the exception of phytate × calcium to zinc and oxalate to calcium, which indicates limited mineral bioavailability (Chapter 7). But enset corm is usually consumed after being cooked along with a variety of leafy vegetables and dairy products (Chapters 3, 4, and 7), which

increases the mineral content and improves the bioavailability of minerals in the enset diet. According to Castro-Alba *et al.* (2019), the molar ratios of anti-nutrients to minerals can be used to assess the bioavailability of minerals in food and diet as well as their inhibitory effect.

8.2 General Conclusions

The enset system is a widely recognized, environmentally sound, and sustainable farming system that contributes to the food security of farmers who live in the central, southern, and southwestern regions of Ethiopia. It is one of the important crops that could be used to positively respond to the US Sustainable Development Goals (SDGs) that relate to food, agrobiodiversity, environment, and climate change in particular. Understanding the indigenous knowledge of local farmers regarding the cultural, social, and economic values of enset and its production system is crucial for considering how local community members traditionally maintain, utilize, and manage the existing enset landrace diversity in their homegardens, which can in turn enhance the crops diversity and usability.

Farmers' indigenous knowledge has continued to be the fundamental source of information concerning all issues and historical data for the existing enset crops. This implies that a great extent of enset landraces and most of their genetic diversity are also traditionally conserved on-farm by farmers'. Moreover, farmers understand the need to grow a mixture of enset landraces, as this has roles in the crop's adaptability as well as the socioeconomic and cultural life of communities. Our results have shown that a small amount of the cultivated enset landraces are broadly dispersed and abundant across the study zones. On the other hand, more landraces were extremely confined to one or two research zones. This shows that abundance and utilization of enset are positively correlated, which indicates that enset farmers have established their method

of choosing and characterizing landraces with some minor variations depending on their traditions and cultures in terms of use patterns and values. Some enset landraces were frequently referred to by the same local names by different farmers throughout all study zones. However, farmers within and across the study zones also gave different names to morphoanatomically /phenotypically the same landraces in some cases.

Our results indicated that local farmers have a great wealth of knowledge and a number of their own techniques for naming, classifying, utilizing, and managing enset landraces in their homegardens. For them, as enset is an indispensable and versatile crop, households use every part of it during the growing season for both food and non-food purposes. Therefore, for the enset farming community, enset could not be substituted by other crops. For many centuries, farmers have been cultivating and preserving various enset landraces in their homegardens through their everyday interactions and the knowledge they have acquired and developed over time. They have named their different landraces based on numerous attributable features; the main and the key features are the phenotypic features (e.g., color, size, and length of different parts of the plant and the angle of leaf orientation). Some other features were also used for naming, such as origin or sources, culinary features, maturity period, and functionality. However, farmers were unaware of the meaning behind the names mentioned in the interviews for most of their enset landraces.

Considering the significance of enset in the main enset-growing regions of Ethiopia's farming and food production system, sustaining current traditional knowledge and genetic variety is essential for achieving food security and sustainable development. Our findings have shown high genetic diversity among the enset samples with very small fixation indices among the populations. It has also been observed that there is a weak association between region of

cultivation and the patterns of genetic variability in enset landraces. Hence, the SSR marker system has been successfully used to determine genetic diversity between genotypes of enset, which supports the implementation of appropriate breeding, crop improvement, and conservation plans. Moreover, it inspires researchers to develop other genomic tools for this vital crop.

The production of enset crops is threatened by several problems nowadays, including diseases, pests, farmer selection pressure, and other factors. Therefore, farmers require a system that may act as a backup for the current conventional techniques for enset landrace propagation and on-farm maintenance. Thus, it's crucial to assess farmers' propagation issues and support them by giving them adequate, suitable enset suckers, which will ultimately allow them to produce more and expand their alternatives. Then the suckers can be conserved *ex-situ or in vitro,* or introduced or re-introduced into farmers' homegardens. This improves the connection between the formal and farmers' conservation programs and helps in maintaining and utilizing genetic diversity. Thus, this study has for the first time confirmed the effect of *bulla* extracted from enset on *in vitro* shoot initiation, multiplication, and root development for three multi-use enset genotypes. The results revealed that *bulla* was able to offer a noticeably higher value in all tested parameters. Moreover, *bulla* is a locally accessible and less expensive resource; this might substitute conventional agar and lower the cost of enset micro-propagation overall. It thus offers additional supplements and has the potential to serve as a backup for both mass *in vitro* propagation and on-farm maintenance of enset genetic resources.

Analysis of corm samples made from the seven cultivated enset landraces has revealed significant differences in the contents of nutritional and anti-nutritional components. The results demonstrate that the corms of enset landraces contained high concentrations of proximate, comparable amounts of minerals, and lower levels of anti-nutritional components than most root

and tuber crops. Hence, it is favorable from a nutritional standpoint. On the other hand, the bioavailability of calcium, iron, and zinc was found to be higher than the reference values. This suggests a restricted bioavailability of minerals. However, *amicho* (cooked corm) is typically eaten with various kinds of dairy-based products and vegetables, which enhances the bioavailability of minerals in the body. Thus, in order to maintain and enhance enset cultivation for food security, our findings will serve as a foundation for ongoing identification and nutritional evaluation. This is required to differentiate the nutritious corm for amicho production from various enset landraces.

In our review and analysis of the work done on enset so far over a period of more than three decades, we noted that many PhD degrees were earned and many scientific publications were made on the enset crop and its agricultural system. However, the impact of all these scientific works is hardly palpable at the farmer level. Either the research done was not focused on the needs and problems of the farmer, or there is a missing link between the agricultural extension and the overall research outreach system. This needs further discussion and debating with the goal of building and putting in place an effective redressing system or mechanism.

8.3 General Recommendations

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

- Establishing an enset *ex-situ* center with local, regional, and/or national institutions is crucial to supporting farming community efforts to conserve enset genetic resources in the Indigenous communities of the studied region.
- The cultural roots of the various administrative zones and localities in enset farming communities are different. This is useful for drawing parallels, classifying shared

ancestries, identifying commonalities, and examining the distinctive knowledge of various groups.

- The existing farmers' knowledge in enset crop diversity on naming, classification, and management practices should be further documented from uncovered areas and also complemented with research for building sustainable utilization and transmission of the knowledge. Moreover, special conservation attention needs to be given to the rare and unique types of enset landraces.
- To identify duplicates and clarify synonymies, as well as to support their on-farm conservation and sustainable use of enset farming communities and their improvement programs, a thorough analysis of the existing farm-based enset diversity (e.g., molecular characterization) is necessary.
- Providing enset farmers with sufficient planting materials for desired landraces that are either absent from their fields or rare in farms crucial. *In vitro* propagated materials might be essential backup collections or sources of support for enabling farmers to obtain plant genetic resources.
- The continued identification and more thorough analyses of nutritional and chemical components are improtant to differentiate the nutritious corm for *amicho* consumption from different enset landraces to sustain enset cultivation for food security.

References

Abate Senbeta, Getachew Sime and Struik, P. (2022). Enset farming system–a resilient, climate-robust production system in South and South-Western Ethiopia. *Cogent Food Agric*. **8**(1): 2074626. https://doi.org/10.1080/23311932.2022.2074626.

Abdelhamid, S., Lê CL, Conedera. M., and Kuepfer, P. (2014). The assessment of genetic diversity of Castanea species by RAPD, AFLP, ISSR, and SSR markers. *Turk. J.Bot*. **38**(5): 835-850. https://doi.org/10.3906/bot-1303-30.

Abebe Yimer, Sirawdink Forsido, Getachew Addis and Abebe Ayelign (2023). Nutritional composition of some wild edible plants consumed in Southwest Ethiopia. *Heliyon* **9(**6): https://doi.org/10.1016/j.heliyon.2023.e16541.

Abeli, T., Dalrymple, S., Godefroid, S., Mondoni, A., Müller, J.V., Rossi, G., and Orsenigo, S.(2020). *Ex-situ* collections and their potential for the restoration of extinct plants. *Conserv. Biol*. **34**(2):303-13.

Abraham Bosha (2018). Propagation practices and food values of enset [*Ensete ventricosum* (Welw.) Cheesman] Genotypes. Ph.D. Thesis, Haramaya University, Ethiopia.

Abraham Bosha, Abitew Dalbato, Tamado Tana, Wassu Mohammed, Buzayew Tesfaye and Karlsson, L.M. (2016). Nutritional and chemical properties of fermented food of wild and cultivated genotypes of enset (*Ensete ventricosum*). *Food Res. Intern.* **89:** 806 -811. https://doi.org/10.1016/j.foodres.2016.10.016.

Adane Tilahun, Shimelis Abebe, Negussie Reta, Tilahun Belayneh and Gulilat Haki (2013). Effect of processing method on the Proximate composition, mineral content and antinutritional factors of Taro (*Colocasia esculenta*, L.) growth in Ethiopia. *Afri. J. Food Agric. Nutr. Dev.* **13**(2). http://ajfand.net/AJFAND/copyrightstatement.html

Admasu Tsegaye (2002). On indigenous production, genetic diversity and crop ecology of enset (*Ensete ventricosum* (Welw.) Cheesman). Ph.D. Thesis, Wageningen University, The Netherlands, p.197

Admasu Tsegaye (2015). Identification and Analysis of Nutritional Components of Enset (*Ensete ventricosum*) Landraces Suitable for Corm Production in Southern Ethiopia. *Ethiop.J. Dev. Res*. **37**(2): 1-27.

Admasu Tsegaye and Struik, P.C. (2001). Enset (*Ensete ventricosum* (Wele.) Cheesman) *kocho* yield under different crop establishment methods as compared to yields of other carbohydrate rich foods. *Netherlands J.Agric. Sci.* **49**: 81-94. https://doi.org /10.1017 /S0014 47970 2003046.

Admasu Tsegaye and Struik, P.C.(2002). Analysis of enset (*Ensete ventricosum*) indigenous production methods and farm-based biodiversity in major enset growing regions of Southern Ethiopia. *Exp.Agric*.**38**:292–315. https://doi.org/10.1017/S001447970 2003046.

Adugna Bayata (2020). Determination of Proximate Composition and Anti-Nutritional Content of Cassava in Jimma Zone Ethiopia. *Academ. J. Chem*. **5**(8): 161-121. https://doi.org /10. 32861/ajc.58.116.121.

Agarwal, M., Shrivastava, N., and Padh, H. (2008). Advances in molecular marker techniques and their applications in plant sciences. *Plant Cell Rep.* **27**: 617–631.

Ajebu Nurfeta, Adugna Tolera, Eik, L. O., and Sundstøl, F. (2008). Yield and mineral content of ten enset (*Ensete ventricosum*) varieties. *Trop. animal health product*. **40**(4): 299-309. https://doi.org/10.1007/s11250-007-9095-0.

Albuquerque, U.P., da Cunha, L.V., De Lucena, R.F., and Alves, R.R. (2014). Methods and techniques in Ethnobiology and Ethnoecology. Springir. New York. USA.

Alexandratos, N., and Bruinsma, J. (2012). World agriculture towards 2030/2050: the 2012 revision (ESA working paper. hIn: Food Agric.Organ. UnitedNations). Available at: http://www.fao.org/fileadmin/templates/esa/Global_persepctives/world_ag_2030_50_2012_rev.pdf (Accessed 14 May 2018).

Alinnor, I. J., and Oze, R. (2011). Chemical evaluation of the nutritive value of *Pentaclethra macrophylla* tenth (African oil bean) seeds. *Pakis. J. Nutr.* **10**(4):355-359.

Almaz Negash (2001). Diversity and conservation of enset (*Ensete ventricosum* (Welw.) Cheesman) and its relation to household food and livelihood security in South-western Ethiopia. Ph.D. Thesis, Wageningen University and Research Centre, The Netherlands, p 247.

Almaz Negash, Admasu Tsegaye, Van Treuren R., and Visser, B. (2002). AFLP analysis of enset clonal diversity in south and Southwestern Ethiopia for conservation. *Crop Sci.* **42**: 1105–1111. https://doi.org/10.2135/cropsci2002.1105.

Almaz Negash, Puite, K., Schaart, J., Visser, B., and Krens, F. (2000). *In vitro* regeneration and micro-propagation of enset from Southwestern Ethiopia. *Plant Cell Tissue Organ Cult*. **62**(2):153–158. https://doi.org/10.1023/A:1026701419739.

Alvarez, A., Fuentes, J.L., Puldón, V., Gómez, P.J., Mora, L., Duque, M.C., Gallego, G. and Tohme, J.M. (2007). Genetic diversity analysis of Cuban traditional rice (*Oryza sativa L.)* varieties based on microsatellite markers. *Genet. Mol. Biol.* **30**(4):1109-1117.

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. *J. Plant Sci*. **4**(1): 1–7. https://doi.org /10. 11648/ j.jps.20160401.11.

Ambachew Zerfu, Sintayehu Gebre, Gezahegn Berecha and Kefelegn Getahun (2018). Assessment of spatial distribution of enset plant diversity and enset bacteria wilt using geostatistical techniques in Yem special district, Southern Ethiopia. *Environ. Syst. Res*. **7**(1): 23. https://doi. org/10.1186/s40068-018-0126-9.

Amiteye, S. (2021). Basic concepts and methodologies of DNA marker systems in plant molecular breeding. *Heliyon* **7**(10): https://doi.org/10.1016/j.heliyon.2021.e08093.

Amom, T., and Nongdam, P. (2017). The use of molecular marker methods in plants: a review. *Intern. J. Curr. Res. Revi.* **9**(17): 1-7.

Andrew, R. (2016). FigTree: Tree figure drawing tool, Version 1.4.3. Institute of Evolutionary Biology. United Kingdom: University of Edinburgh. http:// tree. bio.ed. ac.uk /software /figtree/.

AOAC (2000) Association of Official Analytic Chemists. Official method of analysis (vol. II 17th edition) of AOAC international Washington, DC, U.S.A.

Ashenafi Ayenew, Abiyselassie Mulatu, Bruk Lemma and Demissie Girma (2016). An Ethnobotanical Study of Enset (*Ensete ventricosum* (Welw) Cheesman) in Angacha Woreda, Kembata-Tembaro Zone, South Region, Ethiopia. *Amer. J. Life Sci*. **4**(6): 195-204. https://doi: 10.11648/j.ajls.20160406.18.

Ashenafi Kassaye, Shao, G., Wang, X., Eshetu Shifaw and Wu, S. (2021). Impact of climate change on the staple food crops yield in Ethiopia: implications for food security. *Theor. Appli. Climatol.* **145**(1-2): 327-343.

Ashenafi Tuffa (2019). Value chain and nutritional analyses of warqe food products in relation to post-harvest losses. PhD dissertation, Swedish University of Agricultural Sciences, Uppsala . p. 79.

Asnaketch Woldetensaye (1997). The ecology and production of *Enset ventricosum* in Ethiopia. PhD dissertation, Swedish University of Agricultural Sciences, Uppsala . p. 129.

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 Ethiopia. *J. Agric Dev.* **4**(1):63–85.

Ayalew Debebe, Chandravanshi, B.S., and Taddese Wondimu (2012). Metallic Nutrients in Enset (*Ensete ventricosum*) Corm Cultivated in Wolliso and Wolkite Towns in Ethiopia. *Ethiop. J. Sci.* **35**(2): 71–80.

Ayelign Mengesha, Biruk Ayenew, Elias Gebremariam and Tewodros Tadesse (2012) Micro Propagation of *Vanilla planifolia* using enset [*Ensete ventricosum* (Welw.) Cheesman] Starch as a Gelling Agent. *Curr. Res. J. Biol. Sci.* **4**(4):519–525.

Babbar, S.B., Jain, R., and Walia, N. (2005). Guar gum as a gelling agent for plant tissue culture media. *In vitro Cell Dev. Biol. Plant* **41**:258–261. https://doi.org/10.1079 /IVP 2005628.

Baker, R.E., and Simmonds, N.W. (1953). The genus *ensete* in Africa. *Kew Bullet*. **8**(3): 405-416.

Barber, T. M., Kabisch, S., Pfeiffer, A. F., and Weickert, M. O. (2020). The Health Benefits of Dietary Fibre. Review. *Nutrients* **12**(10): 3209. https://doi.org/10.3390/nu12103209.

Bayoudh, C., Labidi, R., Majdoub, A., and Mars, M. (2015). *In vitro* propagation of caprifig and female fig varieties (*Ficuscarica* l.) from shoot-tips. *J. Agric. Sci. Technol*. **17**:1597–1608.

Bernues, A., Rodríguez-Ortega, T., Ripoll-Bosch, R., and Alfnes, F. (2014). Socio-cultural and economic valuation of ecosystem services provided by Mediterranean mountain agroecosystems. *PloS one* **9**(7): e102479.

Berry, P.M., Fabók, V., Blicharska, M., Bredin, Y.K., Llorente, M.G., Kovács, E., Geamana, N., Stanciu, A., Termansen, M., Jääskeläinen, T., and Haslett, J.R. (2018). Why conserve

biodiversity? A multi-national exploration of stakeholders' views on the arguments for biodiversity conservation. *Biodiver. Conserv.* **27:** 1741-1762. https://doi.org/10.1007/s10531-016-1173-z.

Betelhem Abera, Menbere Berhane, Amsalu Nebiyu, Ruelle, M.L., McAlvay, A., Zemede Asfaw, Abush Tesfaye and Zerihun Woldu (2020). Diversity, use and production of farmers' varieties of common bean (*Phaseolus vulgaris* L., Fabaceae) in southwestern and northeastern Ethiopia. *Genet. Resour. Crop Evol*. **67**(2):339–356. https://doi.org /10.1007 /s10722-019-00877-4.

Bewuketu Haile, Bizuayehu Tesfaye and Temesgen Olango (2022). Indigenous biosystematics of Enset (*Ensete ventricosum* [Welw.] Cheesman) in its center of origin and diversity, Southwest Ethiopia: Folk nomenclature, classification, and descriptors. *Econ. Bot.* **76**(4): 382-413. https://doi.org/10.1007/s12231-022-09553-8.

Bhandari, M. R., and Kawabata, J. (2004). Assessment of antinutritional factors and bioavailability of calcium and zinc in wild yam (*Dioscorea spp*.) tubers of Nepal. *Food chem*. **85**(2): 281-287.

Bhat, M.H., Fayaz, M., Kumar, A., Dar, A.A., and Jain, A.K. (2022). Development of an efficient micropropagation system for *Dioscorea bulbifera* L. and phytochemical profile of regenerated plants. *J. Genet. Enginee. Biotech*. **20**(1): 1-12. https://doi.org/10.1186/s43141-022-00382-9.

Biruk Ayenew, Ayelign Mengesha, Tewodros Tadesse and Elias GebreMariam (2012). *Ensete ventricosum* (Welw.) Cheesman: a cheap and alternative gelling agent for pineapple (*Ananas comosus* var. Smooth cayenne) *in vitro* propagation Institute of Agricultural Research Jimma Plant Biotechnology Laboratory. *J. Microbiol. Biotech. Food. Sci.* **2**(2):640–652.

Biswas, M.K., Bagchi, M., Biswas, D., Harikrishna, J.A., Liu, Y., Li, C., Sheng, O., Mayer, C., Yi, G., and Deng, G. (2020). Genome-wide novel genic microsatellite marker resource development and validation for genetic diversity and population structure analysis of banana. *Genes* **11**(12):1479. https://doi.org/10.3390/genes11121479.

Biswas, M.K., Darbar, J.N., Borrell, J.S., Bagchi, M., Biswas, D., Gizachew Nuraga, Sebsebe Demissew, Wilkin, P., Schwarzacher, T., and Heslop-Harrison, J.S. (2020). The landscape of microsatellites in the enset (Ensete ventricosum) genome and web-based

marker resource development. *Sci. Rep.* **10**(1): 1-11. https://doi.org /10.1038/s41598-020-71984-x.

Bizuayehu Tesfaye (2008). On Sidama folk identification, naming, and classification of cultivated enset (*Ensete ventricosum*) varieties. *Genet. Resour. Crop Evol.* **55**:1359–1370.

Bizuayehu Tesfaye and Ludders, P. (2003). Diversity and distribution patterns of enset landraces in Sidama, Southern Ethiopia. *Genet. Resour. Crop Evol*. **50**:359–371.

Blomme, G., Jacobsen, K., Tawle, K., and Zerihun Yemataw (2018). Agronomic practices with a special focus on transplanting methods for optimum growth and yield of enset [*Ensete ventricosum* (Welw.) Cheesman] in Ethiopia. *Fruits* **73**(6): 349–355. https://doi.org /10.17 660/th2018 /73.6.5.

Blomme, G., Zerihun Yemataw, Tawle, K., Sinohin, V., Gueco, L., Kebede, R. and Lalusin, A. (2018). Assessing enset fibre yield and quality for a wide range of enset [*Ensete ventricosum* (Welw.) Cheesman] landraces in Ethiopia. *Fruits* **73**(6):328–341. https://doi.org/10.17660/th2018 /73.6.3.

Borrell, J.S., Biswas, M.K., Goodwin, M., Blomme, G., Schwarzacher, T., Heslop-Harrison, J.S., Wendawek Abebe, Admas Berhanu, Kallow, S., Janssens, S., and Ermias Lulekal (2019). Enset in Ethiopia: a poorly characterized but resilient starch staple. *Ann. Bot*. **123**(5):747–766. https://doi.org/10.1093/aob/mcy214.

Borrell, J.S., Goodwin, M., Blomme, G., Jacobsen, K., Wendawek Abebe, Dawid Gashu, Ermias Lulekal, Zemede Asfaw, Sebsebe Demissew, and Wilkin, P. (2020). Enset-based agricultural systems in Ethiopia: A systematic review of production trends, agronomy, processing and the wider food security applications of a neglected banana relative. *Plants people planet* **2**(3): 1–17. https://doi.org/10.1002/ppp3.10084.

Borsch, T., Hilu, K.W., Quandt, D., Wilde, V., Neinhuis, C., and Barthlott, W. (2003). Noncoding plastid trnT-trnF sequences reveal a well resolved phylogeny of basal angiosperms. *J. Evol. Biol.* **16**: 558–576.https://doi.org/10.1046/j.1420-Ne9101. 2003.005 77.x

Brandt, S.A. (1996). A model for the origin and evolution of enset food production. **In**: Enset-Based Sustainable Agriculture in Ethiopia, (Tsedeke Abate, Steven, C.H., Brandt, A. and

Seifu Gebremariam, eds.). Proceedings from the International Workshop on Enset held in Addis Ababa, Institute of Agricultural Research, Addis Ababa. pp 36–46.

Brandt, S.A., Spring, A., Hiebsch, C., McCabe, J.T., Endale Tabogie, Mulugeta Diro, Gizachew Wolde-Michael, Gebre Yntiso, Shigeta, M., and ShiferawTesfaye (1997). The "Tree Against Hunger": Enset- based agricultural systems in Ethiopia. American Association for the Advancement of Science, Washington D.C., p 56. http://users.clas.ufl. edu /aspring/ publications/enset.pdf

Brush, B., and Meng, E. (1998). Farmers' valuation and conservation of crop genetic resources. *Genet. Resour. Crop Evol*. **45**(2):139–150. https://doi.org/ 10.1023 /A:1008 65 0819946.

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

Castro-Alba, V., Lazarte, C.E., Bergenståhl, B., and Granfeldt, Y. (2019). Phytate, iron, zinc, and calcium content of common Bolivian foods and their estimated mineral bioavailability. *Food Sci. Nutr*. **7**(9):2854–2865. https://doi.org /10 .1002 /fsn3.1127.

Chandana, B.C., Nagaveni, H.C., Lakshmana, 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. *J. Pharmacogn Phytochem* **3**: 246-251.

Chandran, H., Meena, M., Barupal, T., and Sharma, K. (2020). Plant tissue culture as a perpetual source for production of industrially important bioactive compounds. *Biotechnol reports* **26**: e00450. https://doi.org/10.1016/j.btre.2020.e00450.

Chandrasekara, A., and Kumar, T. J. (2016). Roots and Tuber Crops as Functional Foods : A Review on Phytochemical Constituents and Their Potential Health Benefits. *Int. J. F. Sci*. 2016. https://doi.org/10.1155/2016/3631647

Chappell, M. J., and LaValle, L. A. (2011). Food security and biodiversity: can we have both? An agroecological analysis. *Agric.human values* **28:** 3-26.

Chase, R.R., Büchi, L., Rodenburg, J., Roux, N., Wendawek Abebe, and Borrell, J.S. (2022). Smallholder farmers expand production area of the perennial crop enset as a climate coping strategy in a drought-prone indigenous agrisystem. *Plants People Planet* **5**(2): 254-266. https://doi.org/10.1002/ppp3.10339.

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

Clegg, M.T. (1997). "Plant genetic diversity and the struggle to measure selection." *J. Hered.* **88**(1):1-7.

Collard, B.Y., Jahufer, M.Z., Brouwer, J.B., and Pang, E.C.K. (2005). An introduction to composition and anti-nutritional factors of fava-bean (*Vicia faba*), green-pea and yellow-compositions of melon (*Citrullus lanatus*) seeds. *Brit. J. Res.* **2**(5): 142-151.

Coste, A., Halmagyi, A., Butiuc-Keul, A.L., Deliu, C., Coldea, G., and Hurdu, B. (2012). *In vitro* propagation and cryopreservation of Romanian endemic and rare Hypericum species. *Plant Cell Tisse Organ Cult.***110** (2):213-226.https://doi.org/10.1007/s11240-012-0144-7.

Dagmawit Tobiaw and Endashaw 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. *Afr. J. Biotechnol.* **10** (70) :15697–15709. https://doi.org/10.5897/AJB11.885.

Daud, N., Than, R., Mohd, N., and Alimon, H. (2011). Potential of alternative Gelling Agents in Media for the *in vitro* Micro-Propagation of *Celosia* sp. In *Int. J. Bot.* **7**(2):183–188. https://doi.org/10.3923/ ijb.2011.183.188.

Deb, C.R., and Pongener, A. (2010). Search of alternative substratum for agar in plant tissue culture. *Curr Sci.* **98**: 99-102.

Dejene Zinabu, Endale Gebre and Jiregna Daksa (2018). Explants Sterilization Protocol for *In-vitro* Propagation of Elite Enset (*Ensete ventricosum* (Welw.) Chessman) Cultivars. *Asian J. Plant Sci. Res*. **8**(4):1-7 *Available online* at www.pelagiaresearchlibrary.com

Delfini, J., Moda-Cirino, V., dos Santos Neto, J., Ruas, P.M., Sant'Ana, G.C., Gepts, P., and Gonçalves, L.S.A. (2021). Population structure, genetic diversity and genomic selection signatures among a Brazilian common bean germplasm. *Sci. Rep.***11**(1): 2964.

Demelash Mitiku and Tilahun Teka (2017). Nutrient and anti-nutrient composition of improved sweet potato (*Ipomea batatas* (L) Lam) varieties grown in eastern Ethiopia. *Nutr. Food Sci.* **47***:* 369-380. https://doi.org/10.1108/NFS-07-2016-0098.

Dido, A.A., Krishna, M.S.R., Ermias Assefa, Dawit Degefu, Singh, B.J.K., and Kassahun Tesfaye (2022). Genetic diversity, population structure and relationship of Ethiopian

barley (Hordeum vulgare L.) landraces as revealed by SSR markers. *J. Genet*. **101**(1):9. https://doi.org/10. 1007/s12041-021-01346-7.

Earl, D.A, and VonHoldt, B.M. (2012). STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Cons. Genet. Resour*. **4**:359–361.

Ebile, P.A., Opata, J., and Hegele, S. (2022). Evaluating suitable low-cost agar substitutes, clarity, stability, and toxicity for resource-poor countries' tissue culture media. In *vitro Cell Dev Biol Plant* 1–13. https://doi.org/10.1007/s11627-022-10285-6.

Edilegnaw Wale, Drucker, A., and Zander, K. (2011). The Economics of Managing Crop Diversity On-farm: case studies from the genetic resources policy initiative; p. 157.

Edwards, S., and Lay, K.A. (1997). Musaceae. In: Edwards, S., Sebsebe Demissew, Hedberg, I. (Eds.), Flora of Ethiopia and Eritrea, Vol. 6. Addis Ababa University, Ethiopia and Uppsala University, Sweden, pp. 317-321.

El-Banna, A.N., El-Mahrouk, M.E., Dewir, Y.H., Farid, M.A., Abou Elyazid, D.M., and Schumacher, H.M. (2021). Endophytic bacteria in banana in vitro cultures: Molecular identification, antibiotic susceptibility, and plant survival. *Hortic*. **7**(12): 526. https://doi. org/10.3390/ horticulturae7120526.

Endale Tabogie (1997). Morphological characterization of enset (*Ensete ventricosum* (Welw.) Cheesman) clones and the association of yield with different traits. MSc., Thesis, Alemaya University, Alemaya, Ethiopia, p. 89.

Endashaw Bekele and Shigeta, M. (2011). Phylogenetic relationships between *Ensete* and *Musa* species as revealed by the trnT trnF region of cpDNA. *Genet. Resour. Crop Evol.* **58:** 259- 269. https://doi.org/10.1007/s10722-010-9568-2.

Endrias Dako, Negussie Retta and Gulelat Desse (2016). Comparison of Three Sweet Potato (*Ipomoea Batatas* (L.) Lam) Varieties on Nutritional and Anti-Nutritional Factors. *Global Journals Inc.* Global Journal of Science Frontier Research: D *Agric. Veterin*. **16**(4): 1-11.

Engels, J., and Hawkes, J. (1991). The Ethiopian gene center and its genetic diversity. **In**: Engels, J., Hawkes, J., Worede, M. (eds). *Plant Genetic Resources of Ethiopia.* Cambridge University Press, Cambridge.

Enjalbert, J., Dawson, J.C., Paillard, S., Rhoné, B., Rousselle, Y., Thomas, M., and Goldringer, I. (2011). Dynamic management of crop diversity: From an experimental approach to on-farm conservation. *Comptes rendus biologies*. **334**(5-6):458-68.

Eriksson, E. (2013). Flour from three local varieties of Cassava (*Manihot esculenta* Crantz): Physico-chemical properties, bread making quality and sensory evaluation. MSc. Thesis, Swedish University of Agricultural Sciences, Department of Food Science, SLU, p. 41.

Esekiel, A. (2010). Viable options and factors in consideration for low cost vegetable propagation of tropical trees. *Int. J. Bot.* **6**(2): 187-193.

Espinosa-Leal, C. A., Puente-Garza, C. A., and Garcıa-Lara, S. (2018). *In-vitro* plant tissue culture: means for production of biological active compounds. *Planta.* **248**: 1-18. https://doi: 10.1007/s00425-018-2910-1.

ESTC (Ethiopian Science and Technology Commission) (2003) Commission awards individuals for outstanding achievements. Available at: http://www.capitalethiopia.com/archive /2003/july/ week3/index.htm

Evanno, G., Regnaut, S., and Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol. Ecol*. **14**(8): 2611–2620.

Excoffier, L., and Lischer, H.E. (2010). Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol. Ecol. Resour*. **10**:564–567.

Fahad, S., and Wang, J. (2018). Farmers' risk perception, vulnerability, and adaptation to climate change in rural Pakistan. Land use policy. **79**:301–9.

Falush, D., Stephens, M., and Pritchard, J.K. (2003). Inference of population structure using multi-locus genotype data: linked loci and correlated allele frequencies. *Genet.* **164**(4): 1567-1587.

Fang, J.Y., and Hsu, Y.R. (2012). Molecular identification and antibiotic control of endophytic bacterial contaminants from micropropagated Aglaonema cultures. *Plant Cell Tiss. Org. Cult.* **110**: 53-62. https://doi.org/10.1007/s11240-012-0129-6.

Fekadu Gadissa, Kassahun Tesfaye, Kifle Dagne and Mulatu Geleta (2018). Genetic diversity and population structure analyses of *Plectranthus edulis* (Vatke) Agnew collections from diverse agro-ecologies in Ethiopia using newly developed EST-SSRs marker system. *BMC Genet.* **19**(1): 1-15. https://doi.org/10.1186/s12863-018-0682-z

Felsenstein, J. (1985). Phylogenies and the comparative methods. *Am. Nat*. **125**: 1–15.

Fetta Gerura, Beira Hailu, Martina K, Abush Tesfaye, Temesgen Olango and Nasser, Y. (2019). Genetic diversity and population structure of enset (*Ensete ventricosum* (Welw.) Cheesman) landraces of Gurage zone, Ethiopia. *Genet. Resour. Crop Evol*. **66**:1813–1824. https://doi. org/10.1007/s10722-019-00825-2.

Firew Mekbib (2007). Infra-specific folk taxonomy in sorghum (*Sorghum bicolor* (L.) Moench) in Ethiopia: folk nomenclature, classification, and criteria. *J. Ethnobiol. Ethnomed*. **3**:38. https://doi:10.1186/1746-4269-3-38.

Frossard, E., Bucher, M., Mächler, F., Mozafar, A., and Hurrell, R. (2000). Potential for increasing the content and bioavailability of Fe, Zn and Ca in plants for human nutrition. *J. Sci. Food Agric.* **80**(7): 861-879.

Futuyma, D.J. (2008). Sympatric speciation: norm or exception? **In**: Tilmon KJ. (ed): Specialization, speciation, and radiation: the evolutionary biology of herbivorous insects. Berkeley: Univ. of California Press. p. 136–48.

Garcia-Gonzales, R., Quiroz, K., Carrasco, B., and Caligari, P. (2010). Plant tissue culture: current status, opportunities and challenges. *Int. J. Agric. Nat. Res*. **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. *Afr. J. Biotechnol*. **15**(40):2192-2201. https://doi.org /10.5897 /AJB2016.15213.

Genet Birmeta (2004). Genetic variability and biotechnological studies for the conservation and improvement of *Ensete ventricosum*. Doctoral thesis, Swedish University of Agricultural Sciences, Alnarp, Sweden, p 91

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):315–325.

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

Genet Birmeta and Welander, M. (2004). Efficient micro-propagation of *Ensete ventricosum* applying meristem wounding: a three-step protocol. *Plant Cell Rep.* **23**:277-283. https://doi.org /10.1007/s00299-004-0832-9.

George, E.F., Hall, M.A., and Klerk, G.J. (2008). Plant tissue culture procedure: Plant propagation by tissue culture 3rd ed., Springer, Docrdrecht.

Getachew Selamawit, Firew Mekbib, Belayneh Admassu, Segenet Kelemu, Sisay Kidane, Kefyalew Negisho, Djikeng A, Nzuki I (2014). Look into genetic diversity of Enset (*Ensete ventricosum* (Welw.) Cheesman) using transferable microsatellite sequences of banana in Ethiopia. *J. Crop Improv*. **28**: 159–183. https://doi.org/10.1080 /15427528 .2013.861889.

Gibson, R. S., Bailey, K. B., Gibbs, M., and Ferguson, E. L. (2010). A review of phytate, iron, zinc, and calcium concentrations in plant-based complementary foods used in low-income countries and implications for bioavailability. *Food nutr. Bulle.* **31**(2- suppl2): S134-S146. https://doi.org/10.1177/15648265100312S206.

Girma Gebre (2021). Prevalence of household food insecurity in East Africa: Linking food access with climate vulnerability. *Clim. Risk Manag.* **33**: 100333.

Gizachew Nuraga, Tileye Feyissa, Kassahun Tesfaye, Biswas MK, Schwarzacher T, Borrell JS, Wilkin P, Sebsebe Demissew, Zerihun Tadele, Heslop-Harrison JS (2022). The genetic diversity of enset (*Ensete ventricosum*) landraces used in traditional medicine is similar to the diversity found in non-medicinal landraces. *Front. Plant Sci.* **12**: 756182. https://doi .org /10.3389 /fpls .2021.756182.

Gizachew Nuraga, Tileye Feyissa, Kassahun Tesfaye, Sebsebe Demissew and Zerihun Tadele (2019a). Phenotypic diversity of enset (*Ensete ventricosum* (Welw.) Cheesman) landraces used in traditional medicine. *Genet. Resour. Crop Evol*. **66**:1761-1772. https://doi.org /10.1007/s10722-019-00832-3.

Gizachew Nuraga, Tileye Feyissa, Kassahun Tesfaye, Sebsebe Demissew and Ashagire Zewdu (2019b). Comparison of proximate, mineral and phytochemical composition of enset (*Ensete ventricosum* (Welw.) Cheesman) landraces used for a diff erent purpose. *Afri. J. Agric. Res.* **14**(30): 1326–1334. https://doi.org/10.5897/AJAR 2019.13993.

Glato, K., Aidam, A., Kane, N.A., Bassirou, D., Couderc, M., Zekraoui, L., Scarcelli, N., Barnaud, A., and Vigouroux, Y. (2017). Structure of sweet potato (*Ipomoea batatas*)

diversity in West Africa covaries with a climatic gradient. PLoS ONE. **12**(5): e0177697. https://doi.org/10.1371/journal.pone.0177697.

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. *Genet. Res. Int. 2015*.

Grover, A., and Sharma, P. C. (2016). Development and use of molecular markers: past and present. *Crit. revi. biotechnol.* **36**(2): 290-302. https://doi.org /10.3109 /07388551 .2014.959891.

Gupta, P., Sharma, S., and Saxena, S. (2016). Effect of abiotic stress on growth parameters and steviol glycoside content in stevia rebaudiana (Bertoni) raised in vitro. *J. Appl. Res. Med. Aromat. Plants*. **3**: 160-167. https://doi:org/10.1016/j.jarmap.2016.03.004.

Habtamu Fekadu and Negussie Ratta (2014). Anti-nutritional factors in plant foods: Potential health benefits and adverse effects. *Int. J. Nutr. food Sci.* **3**(4):284-289. https://doi:10 .11648/j.ijnfs.20140304.18.

Habtamu Fekadu, Fekadu Beyene and Gullelat Desse (2013). Effect of traditional processing methods on nutritional composition and anti-nutritional factors of anchote (*Coccinia abyssinica* (Lam.) Cogn) tubers grown in Western Ethiopia. *J. Food Process Technol*. **4**(07*)*: http://doi.org/10.4172/2157-7110.1000249.

Habtamu Tegen and Wassu Mohammed, W. (2016). The role of plant tissue culture to supply disease free planting materials of major horticultural crops in Ethiopia. *J. Biol. Agric. Healthcare***. 6** (1): 122-129.

Hamza, H., Abederrahim, M.A., Elbekkay, M., and Ferchichi, A. (2013). Comparison of the effectiveness of ISSR and SSR markers in determination of date palm (*Phoenix dactylifera* (L.) agronomic traits. *Aust. J. Crop Sci*. **7**: 763–769.

Hapsari, L., Kennedy, J., Lestari, D.A., Masrum, A., and Lestarini, W. (2017). Ethnobotanical survey of bananas (Musaceae) in six districts of East Java, Indonesia. *Biodiv.***18**:160-174.

Harlan, J. R. (1969). Ethiopia: A center of diversity. *Econ. Bot.* **23**(4): 309–314. https://doi .org/10.1007/bf02860676.

Hasnain, A., Naqvi, S.A., Ayesha, S.I., Khalid, F., Ellahi, M., Iqbal, S., Hassan, M.Z., Abbas, A., Adamski, R., Markowska, D., and Baazeem, A. (2022). Plants *in vitro* propagation with its applications in food, pharmaceuticals and cosmetic industries; current scenario

and future approaches. *Front. Plant Sci*. **13**:1009395. https://doi.org/10.3389/fpls.2022.1009395.

Hedrick, P.W. (2007). Sex: differences in mutation, recombination, selection, gene flow, and genetic drift. *Evol.* **61**(12): 2750-2771.

Hinze, L.L., Hulse-Kemp, A.M., Wilson, I.W., Zhu, Q-H., Llewellyn, D.J., Taylor, J.M., Spriggs, A., Fang, D.D., Ulloa, M., and Burke, J.J. (2017). Diversity analysis of cotton (*Gossypium hirsutum* L.) germplasm using the CottonSNP63K Array. *BMC Plant Biol*. **17**(1):1-20. https://doi.org/10.1186/s12870-017-0981-y.

Hirose, R., Tezuka, Y., Kondo, T., Hirao, K., Hatta, T., Nemoto, S., Saio, K., Takahashi, S., and Kainuma, K. (2010). Characteristic Physico-chemical properties and potentaial uses of Enset (*Ensete ventricosum*) starch: Comparative Studies with Starches of Potato, Sago and Corn. *J. Appl. Glysosci*. **57**: 185-192. https://doi.org/10.5458/jag.57.185.

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

Hossain, M.M., Rahim, M.A., and Haque, M.R. (2021). Biochemical properties of some important underutilized minor fruits. *J. Agric. Food Res*. **5**: 100148. https://doi.org/10.1016/j.jafr.2021.100148.

Howells, E.J., and Willis, B.L., Bay, L.K., and Van Oppen, M.J.H. (2016). Microsatellite allele sizes alone are insufficient to delineate species boundaries in Symbiodinium, *Mol. Ecol*. **25** (12):2719–2723, https://doi.org/10.1111/mec.13631.

Hurrell, R. (2004). Phytic acid degradation as a means of improving iron absorption. *Int. J. Vit. Nutr. Res.* **74**(6): 445–452. https :/ /doi.org /10.1024/0300 9831.74.6.445.

Hussain, A., Qarshi, I. A., Nazir, H., and Ullah, I. (2012). Plant tissue culture: current status and opportunities. *Recent Advan. Plant Vitro Cult.* **6**(10): 1-28.

Jacob, A.G., Etong, D.I., and Tijjani, A. (2015). Proximate, mineral and anti-nutritional compositions of melon (*Citrullus lanatus*) seeds. *Brit. J. Res.* **2**(5): 142-151.

Jain-Raina, R., and Babbar, S. B. (2011). Evaluation of blends of alternative gelling agents with agar and development of xanthagar, a gelling mix, suitable for plant tissue culture media. *Asian J. Biotechnol.* **3**(2): 153-164. https://doi.org/10.3923/ajbkr.2011.153.164.

Jump, A.S., Marchant, R., and Peñuelas, J. (2009). Environmental change and the option value of genetic diversity. *Trends Plant Sci.* **14**(1): 51-58.

Justine, A.K., Kaur, N., and Pati, P.K. (2022). Biotechnological interventions in banana: current knowledge and future prospects. *Heliyon*. **8**:1-14. https:// doi.org /10.1016 /j.heliyon .2022 .e11636.

Kaçar, Y., Biçen, B., Varol, I., Mendi, Y., Serçe, S., and Çetiner, S. (2010). Gelling agents and culture vessels affect *in vitro* multiplication of banana plantlets. *Genet. Mol.Res.* **9**(1): 416-424.

Kaczmarczyk, A., Turner, S.R., Bunn, E., Mancera, R.L., and Dixon, K.W. (2011). Cryopreservation of threatened native Australian species—what have we learned and where to from here?. *Vitro Cell Dev. Biol. Plant.* **47**(1): 17-25. https://doi.org /10.1007 /s11627-010-9318-3.

Kahia, J., Ndaruhutse, F., Waweru, B., Bonaventure, N., Mutaganda, A., Sallah, P. Y., Kariuki, N. P., and Asiimwe, T. (2015). *In vitro* propagation of two elite cooking banana cultivars- FHIA 17 and INJAGI. *Int. J. Biotechnol. Mol. Biol. Res*. **6**(6): 40-47. https://doi.org/ 10.5897 /IJBMBR 2014 .0231.

Kalia, R.K., Rai, M.K., Kalia, S., Singh, R., and Dhawan, A.K. (2011). Microsatellite markers: an overview of the recent progress in plants. *Euphytica*. **177** (3): 309-334.

Kalinowski, S.T. (2005). HP-RARE 1.0: a computer program for performing rarefaction on measures of allelic richness. *Mol. Ecol. Notes*. **5**:187–9.

Kalinowski, S.T. (2002). How many alleles per locus should be used to estimate genetic distances?. *Heredity* **88(**1): 62-65. https://doi.org/10.1038/sj.hdy.6800009.

Karlsson, L.M., Abitew Dalbato, Tana Tamado and Yeshitila Mikias (2015). Effect of cultivar, traditional corm pre-treatment and watering on sprouting and early growth of *Ensete ventricosum* suckers. *Experi. Agric*. **51**(2): 232–243. https:// doi.org/10.1017 /S0014479 714000246.

Karp, A., Kresovich, S. Bhat, K.V. Ayad, W.G., and Hodgkin, T. (1997). Molecular tools in plant genetic resources conservation: a guide to the technologies. International Plant Genetic Resources Institute, Rome Italy.

Kefale Alemu and Sandford, S. (1991). Enset in North Omo region. FRP (Farmer's Research technical Pamphlet), No. 1. Farm Africa, Addid Abeba, Ethiopia. 49p.

Kefale Alemu and Sandford, S. (1996). Enset clone. In: Abate T, Hieb- sch C, Brandt S, Geberemariam S (eds) Enset-based sustainable agriculture in Ethiopia. Institute of Agricultural Research, Addis Ababa. pp 138–148.

Khan, T., Abbasi, B.H., Iqrar, I., Khan, M.A., and Shinwari, Z.K. (2018). Molecular identification and control of endophytic contamination during in vitro plantlet development of *Fagonia indica*. *Acta. Physiol. Planta*. **40**:1-9.https://doi.org /10.1007 /s11738-018-2727-3.

Khoury, C., Laliberté, B., and Guarino, L. (2010). Trends in ex-situ conservation of plant genetic resources: a review of global crop and regional conservation strategies. *Genet. Resour. Crop Evol.* **57**: 625-639. https://doi.org/10.1007/s10722-010-9534-z.

Kiran, U., Moahnty, S. K., Roy, P. S., Behera, L., and Chand, P. K. (2015). Genetic diversity among banana cultivars from Odisha using RAPD markers. *Sci. Res. Rep*. ***5***(2): 118-124.

Kodym, A., and Zapata-Arias, F.J. **(**2001). Low cost alternatives for the micropropagation of banana. *Plant Cell Tis. Org. Cult.* **66:** 67-71. https://doi.org/10.1023/A:1010661521438.

Kopelman, N.M., Mayzel, J., Jakobsson, M., Rosenberg, N.A., and Mayrose, I. (2015). Clumpak: a program for identifying clustering modes and packaging population structure inferences across K. *Mol. Ecol. Resour.*, **15**(5): 1179-1191. https://doi.org/10.1111/1755-0998.12387.

Krasteva, G., Georgiev, V., and Pavlov, A. (2020). Recent applications of plant cell culture technology in cosmetics and foods. *Eng. Life Sci*. **21**: 68-76. https://doi.org/10.1002 /elsc .202000078.

Krebs, C. (1999). Ecological methodology. Menlo Park, California: Addison-Wesley Educational Publishers; p. 620

Kujawska, M., Hilgert, N.I., Keller, H.A., and Gil, G. (2017). Medicinal Plant Diversity and Inter-Cultural Interactions between Indigenous Guarani, *Criollos* and Polish Migrants in the Subtropics of Argentina. PLOS ONE. **12**(1): e0169373. https ://doi.org /10.1371/ journal .pone.0169373.

Kumar, P., Gupta, V.K., Misra, A.K., Modi, D.R., and Pandey, B.K. (2009). Potential of molecular markers in plant biotechnology. *Plant omics* **2**(4): 141-162. https://doi.org/ 10.3316/090706285698938.

Kumar, S.J., Susmita, C., Sripathy, K.V., Agarwal, D.K., Pal, G., Singh, A.N., Kumar, S., Rai A.K., and Simal-Gandara, J. (2022). Molecular characterization and genetic diversity studies of Indian soybean (Glycine max (L.) Merr.) cultivars using SSR markers. *Mol. Biol. Rep.* **49**: 2129–2140. https://doi.org/10.1007/s11033-021-07030-4.

Kuria, P., Demo, P., Nyende, A. B., and Kahangi, E. M. (2008). Cassava starch as an alternative cheap gelling agent for the *in vitro* micro-propagation of potato (*Solanum tuberosum* L.). *Afr. J. Biotechnol.* **7**(3: 301-307.

Laishram, J., Saxena, K.G., and Rao, K.S. (2020). Rice cultivar diversity associated Indigenous knowledge and management practices in a lowland village landscape from north-eastern India. *Vegetos***. 33**:172-86.

Lambardi, M., Ozudogru, E. A., and Benelli, C. (2008). Cryopreservation of embryogenic cultures. In Plant cryopreservation: Pract. guide (Springer, New York, NY) pp, 177-210).

Latta, M., and Eskin, M. (1980). A simple and rapid colorimetric method for phytate evaluation. *J. Agric. Food Chem.* **28** (6): 1313-1315.

Lavanya, A. R., Muthukrishnan, S., MuthuKumar, M., Benjamin, J. F., Kumar, T. S., Kumaresan, V., and Rao, M. V. (2014). Indirect organogenesis from various explants of Hildegardia populifolia (Roxb.) Schott & Endl. A threatened tree species from Eastern Ghats of Tamil Nadu, India. *J.Genet. Eng. Biotechnol.* **12**(2): 95-101. https://doi.org/ 10.1016/j.jgeb.2014.09.002.

Laya, A., Koubala, B. B., Kouninki, H., and Nchiwan, N. E. (2018). Effect of harvest period on the proximate composition and functional and sensory properties of gari produced from local and improved cassava (*Manihot esculenta*) varieties. *Int. J. Food Sci.* 2018. https://doi.org/10.1155/2018/6241035.

Liu, K., and Muse, S.V. (2005). PowerMarker: an integrated analysis environment for genetic marker analysis. *Bioinformatics* **21**(9):2128-2129.https://doi.org/10.1093/bioinformatics /bti282.

Loko, L.E., Toffa, J., Adjatin, A., Akpo, A.J., Orobiyi, A., and Dansi, A. (2018). Folk taxonomy and traditional uses of common bean (*Phaseolus vulgaris* L.) landraces by the sociolinguistic groups in the central region of the Republic of Benin. *J. Ethnobiol Ethnomed.* **14**(1):1–5. https://doi.org/10.1186/s13002-018-0251-6.

Lowe, N.M. (2021). The global challenge of hidden hunger: Perspectives from the field, Proceed. *Nutr. Soci*. **80**(3): 283–289, https://doi.org/10.1017/S0029665121000902.

Ma, G., Li, Y., Jin, Y., Zhai, F., Kok, F.J., and Yang, X. (2007). Phytate intake and molar ratios of phytate to zinc, iron and calcium in the diets of people in China. *Europ. J. Clin. Nutr*. **61**(3): 368-374.

Maćkowiak, K., Torlińska-Walkowiak, N., and Torlińska, B. (2016). Dietary fibre as an important constituent of the diet. *Advances in Hygiene & Experimental Medicine/Postepy Higieny i Medycyny Doswiadczalnej*, *70*. http://www.phmd.pl/fulltxt.php?ICID=1195842.

Magurran, A.E. (1988). Ecological diversity and its measurement. London: Croom Helm. p.125.

Magurran, A. E. (2004). Measuring Biological Diversity. The standard reference for conceptual and quantitative aspects of diversity measurement. Oxford: Blackwell Publishing.

Manaye Ayalew, Vellaiyappan S, and Wedajo Gebre (2017). Micro-propagation of Cassava (*Manihot Esculenta* Crantz) using *Bulla* Flour [*Ensete Ventricosum*. Welw.) Cheesman] as an Alternative Source of Agar in Plant Tissue Culture Media. *Int. J. Res. Stud. Sci. Eng. Technol*. **4** (2): 23–34.

Massey, L. K., Palmer, R. G., and Horner, H. T. (2001). Oxalate content of soybean seeds (Glycine max: Leguminosae), soyfoods, and other edible legumes. *J. Agric. Food Chem.* **49**(9): 4262-4266. https://doi.org/10.1021/jf010484y.

Massey, L.K. (2007). Food oxalate: factors affecting measurement, biological variation, and bioavailability. *J. Am. Dietet. Assoc.* **107**(7):1191–1194. https://doi.org/10.1016/j.jada.2007.04.007.

Matheka, J., Tripathi, J., Ibsa Merga, Endale Gebre, and Tripathi, L. (2019). A simple and rapid protocol for the genetic transformation of *Ensete ventricosum*. *Plant Methods* **15**(1):1-17. https://doi.org /10.1186/s13007-019-0512-y.

Mattick, C. S. (2018). Cellular agriculture: the coming revolution in food production. *Sci. Bull. J.* **74**: 32–35. https://doi.org/ 10.1080/00963402.2017.1413059.

Matus, I.A., and Hayes, P. M. (2002). Genetic diversity in three groups of barley germplasm assessed by simple sequence repeats. *Genome* **45**: 1095–1106. https://doi.org/10.1139/g02-071.

Maxson, E.D., and Rooney, L.W. (1972). Evaluation of methods for tannin analysis in sorghum grain. *Cereal Chem*. **49:**719-729.

Maxted, N., Guarino, L. Myer, L., and Chiwona, E. A. (2002). Towards a methodology for on–farm conservation of plant genetic resources. *Genet. Resour. Crop Evol*. **49**:31–46.

McKey, D., Elias, M., Pujol, B., and Duputié, A. (2010). The evolutionary ecology of clonally propagated domesticated plants. *New Phytol.* **186**(2): 318-332. https://doi.org/10.1111/j.1469-8137.2010.03210.x.

Melese Temesgen and Negussie Retta (2015). Nutritional potential, health and food security benefits of taro *Colocasia esculenta* (L.): A review. *Food Sci. Qual. Manag.* **36(**0): 23-30.

Melese Temesgen (2013). Improving the Indigenous Processing of Kocho, an Ethiopian Traditional Fermented Food. *J. Nutr. Food Sci*. **3**(1):1-6. https://doi.org/10.4172/2155-9600.1000182.

Melese Temesgen (2017). Nutrient constituents of taro (*Colocasia esculenta*) corm and leaf for infant food development and chicken feed formulation. Ph.D. Dissertation in Food Science and Nutrition. Addis Ababa University.

Melesse Maryo, Sileshi Nemomissa and Tamirat Bekele (2014). Diversity and distribution of enset (*Enset ventricosum* (Welw.) Cheesman) Clones in Kambatta Tembaro Zone, Southern Ethiopia. In: Proceedingof the 4th National Conference on "Environment and Development", Dilla. pp 104-120.

Millar, K. A., Gallagher, E., Burke, R., McCarthy, S., and Barry-Ryan, C. (2019). Proximate Mineral Composition of *Peperomia pellucida* L. (Ketumpangan Air) Grown in Malaysia. *Molecules*, **17**(9): 11139-11145. https://doi.org/10.3390/molecules170911139.

Mlitan, A. M., Sasi, M. S., and Alkherraz, A. M. (2014). Proximate and minor mineral content in some selected basil leaves of *Ocimum gratissimum* L, in Libya. *Int. J. Chem. Eng. Appl.* **5**(6):502. https://doi.org/10.7763/IJCEA .2014 .V5.436.

MOA (Ministry of Agriculture) (2000). Agro eco‐logical zones of Ethiopia. Natural Resource Management and Regulatory Department. Addis Ababa, Ethiopia.

Mohammed, B., Martin, G., and Laila, M.K. (2013). Nutritive values of the drought tolerant food and fodder crop enset. *Afr. J. Agric. Res.* **8***:*2326-2333. https:// doi.org/10. 5897/AJA R 12.1296.

Mora, L.Y.C., Tarazona, D.Y.G., Bohórquez Quintero, M.D.L.A., Barrera, E.J.A., Ruíz, J.S.U., Moreno, D.M.A., and Pérez, Z.Z.O. (2022). Impact of initial explants on *in vitro* propagation of native potato (*Solanum tuberosum*, Andigena group). *Plant Cell Tiss. Org. Cult.* **150**(3):627-636. https://doi.org/10.1007/s11240-022-02317-1.

Mugo, J. N., Karanja, N. N., Gachene, C. K., Dittert, K., Nyawade, S. O., and Schulte-Geldermann, E. (2020). Assessment of soil fertility and potato crop nutrient status in central and eastern highlands of Kenya. *Sci. Rep*. **10**(1):7779, https://doi .org /10.1038 /s41598-020-64036-x.

Mukhopadhyay, M., and Pieri, C. (1999). Integration of women in sustainable land and crop management in Sub-Saharan Africa. Collaborative Effort between Gender in Rural Development Team and Sustainable Land and Crop Management Thematic Team, World Bank: Washington D.C

Mulugeta Diro, Van Staden J., and Bornman, C.H. (2004). Propagation of *Ensete in vitro*: a review. *S. Afr. J. Bot*. **70**(4):497-501. https://doi.org/10.1016/S0254-6299(15)30187-3.

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

Nadeem, M.A., Habyarimana, E., Çiftçi, V., Nawaz, M.A., Karaköy, T., Comertpay, G., Shahid, M.Q., Hatipoğlu, R., Yeken, M.Z., Ali, F., and Ercişli, S. (2018). Characterization of genetic diversity in Turkish common bean gene pool using phenotypic and whole-genome DArTseq-generated silicoDArT marker information. PloS one. **13** (10):e0205363. https://doi.org/10.1371/journal.pone.0205363.

Nakabonge, G., Samukoya, C., and Baguma, Y. (2018). Local varieties of cassava: conservation, cultivation and use in Uganda. *Environ. Dev. Sustain*. **20**:2427–45. https://doi.org /10.1007 /s10668-017-9997-6.

Naz, S., Anjum, M.A., Naqvi, S.A.H., Siddique, B., and Zulfiqar, M.A. (2018). Assessment of proximate, nutritional and mineral contents in some traditional vegetables consumed in Multan, Pakistan. *Pak. J. Agric. Res.* **31**(4):375–381. http://dx.doi.org /10.17582 /journal. Pjar /2018/31.4.375.381.

Nedelcheva, A., and Dogan, Y. (2009). Folk botanical nomenclature and classification in Bulgarian traditional knowledge, plants and culture: seeds of the cultural heritage of Europe. *Edipuglia Bari* 169–173.

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

Nene, Y.L., and Sheila, V.K. (1999). ICRISAT Newsletter, proceeding on international workshop on chickpea, improvement. pp. 172-180.

Nguyen, T. T. T., Loiseau, G., Icard-Vernière, C., Rochette, I., Trèche, S., and Guyot, J. P. (2007). Effect of fermentation by amylolytic lactic acid bacteria, in process combinations, on characteristics of rice/soybean slurries: A new method for preparing high energy density complementary foods for young children. *Food Chem.* **100**(2):623-631. https://doi.org/10 .1016/j.foodchem.2005.09.080.

Nkhata, W., Shimelis, H., Melis, R., Chirwa, R., Mzengeza, T., Mathew, I., and Shayanowako, A. (2020). Population structure and genetic diversity analyses of common bean germplasm collections of East and Southern Africa using morphological traits and high-density SNP markers. *PLoS One*. **15**(12): e0243238.

Norhaizan, M. E., and Nor Faizadatul Ain, A. W. (2009). Determination of phytate, iron, zinc, calcium contents and their molar ratios in commonly consumed raw and prepared food in Malaysia. *Malays. J. Nutr.* **15**(2): 213–222.

Nuru Mustafa, Fasiledes Asfaw, Emam Endris and Elias Bojago (2023). Evaluating the impact of productive safety net program on rural household food security achievement: Endogenous switching regression model. *J. Agric. Food Res*. **14:**100674. https://doi.org /10.1016 /j. jafr.2023.100674.

Olatunde, G. O., Ogunyinka, B. O., Ashimi, M., and Taiwo, A. G. (2016). Chemical composition and sensory quality of sweet potato crisps as affected by variety and frying conditions. *J.Nat. Sci. Eng.Technol*. **15**(1):108-121. https://doi. org /10.51406/jnset.v15i1.1770.

Oliveira, E.J., Padua, J.G., Zucchi, M.I., Vencovsky, R., and Vieira, M.L.C. (2006). Origin, evolution and genome distribution of microsatellites. *Genet. Mol. Biol.* **29**:294–307.

Ooi, D.J., Iqbal, S., and Ismail, M. (2012). Proximate Composition, Nutritional Attributes and Mineral Composition of *Peperomia pellucida* L. (Ketumpangan Air) Grown in Malaysia. *Molecules* **17**(9): 11139-11145. https://doi.org/10.3390/molecules170911139.

Ortiz-Barrientos, D., Engelstädter, J., and Rieseberg, L.H. (2016). Recombination rate evolution and the origin of species. *Trends Ecol. Evol.* **31**(3): 226-236. https ://doi.org /10. 1016 /j. tree .2015.12.016.

Osborne, D., and Voogt, P. (1978). The analysis of nutrients in foods. *A subsidiary of Harcourt Brack Jovanovich publisher*.

Özkan, G., Haliloğlu, K., Türkoğlu, A., Özturk, HI., Elkoca, E., and Poczai, P.(2022). Determining genetic diversity and population structure of common bean (*Phaseolus vulgaris* L.) landraces from Türkiye using SSR markers. *Genes*. **13**(8): 1410. https://doi.org/10.3390/genes13081410.

Padhan, B., Biswas, M., and Panda, D. (2020). Nutritional, anti-nutritional and physico-functional properties of wild edible yam (*Dioscorea* spp.) tubers from Koraput, India, Food *Biosci*. **34**: 100527, https://doi.org/10.1016/j.fbio.2020.100527.

Padmakar, B., Kanupriya, C., Latha, P. M., Prashant, K. S., Dinesh, M. R., Sailaja, D., and Aswath, C. (2015). Development of SRAP and SSR marker-based genetic linkage maps of guava (*Psidium guajava* L.). *Sci. Hortic.* **192:** 158-165.

Page, R.D. (1996). Tree View: An application to display phylogenetic trees on personal computers. *Bioinformatics* **12**(4): 357-358.

Pankhurst, A. (1996). Social consequences of enset production. In: Tsedeke, Abate, Hiebsch, C, Brandt, S.A., Seifu Gebremariam (Eds.), Enset-based sustainable agriculture in Ethiopia, Proceedings of the International Workshop on Enset. Addis Ababa, Ethiopia, 13-20 December 1993, pp. 69-82.

Parmar, A., Bilatu Gebre, Addisu Legesse, Yoseph Demelash, Fladung, K.,and Hensel, O. (2017). Nutritional comparison of white and red *Coccinia abyssinica* (Lam.) Cong. accessions: an under-utilised edible tuber of the Ethiopian highlands. *Foods*. **6**(8): 71. https://doi:10.3390/foods6080071.

Parra, F., Casas, A., Peñaloza-Ramírez, J. M., Cortés-Palomec, A. C., Rocha-Ramírez, V., and González-Rodríguez, A. (2010). Evolution under domestication: Ongoing artificial selection and divergence of wild and man‐aged *Stenocereus pruinosus* (Cactaceae) populations in the Tehuacán Valley, Mexico. *Ann. Bot*. **106**:483–496.

Pati, P. K., Kaur, J., and Singh, P (2011). A liquid culture system for shoot proliferation and analysis of pharmaceutically active constituents of *Catharanthus roseus* (L.) G. Don. *Plant Cell. Tiss.Org. Cult. (PCTOC)* **105**: 299-307.

Pawlak, K., and Kołodziejczak, M. (2020). The role of agriculture in ensuring food security in developing countries: Considerations in the context of the problem of sustainable food production. *Sustainability* **12**(13):5488.

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

Pegg, D.E. (2002). The history and principles of cryopreservation. *Semin. Reprod. Med*. **20**:5–13. https://doi.org/10.1055/s-2002-23515.

Pérez-Pazos, J., Rosero, A., Cardinale, M., and Gámez, R. (2023). Development of control strategies for bacteria and fungi associated with a micropropagated new cultivar of orange-fleshed sweet potato (*Ipomoea batatas* cv. Agrosavia–Aurora). *Hortic. Environ. Biotechnol*. **64**: 859–875 (2023). https://doi.org/10.1007/s13580-023-00521-2.

Phillips, G.C., and Garda, M. (2019). Plant tissue culture media and practices: an overview. *In-Vitro Cell. Dev. Biol.Plant* **55**: 242–257. https://doi.org/10.1007/s11627-019-09983-5.

Pielou, E.C. (1975). Ecological diversity. Wiley, New York; p. 165.

Pijls, L.T.J., Timmer, A.A.M., Zewdie Wolde-Gebriel, Z., and West, C.E. (1995). Cultivation, preparation and consumption of ensete (*Ensete ventricosum*) in Ethiopia. *J. Sci. Food Agric*. **67**: 1-11. https://doi.org/10.1002/jsfa.2740670102.

Prabakaran, A., Paramasivam, K., Rajesh, T., and Rajarajan, D. (2010). Molecular characterization of rice landraces using SSR markers. *J. Plant Breed*. **1**(4):512–516

Pritchard, J.K., Stephens, M., and Donnelly, P. (2000). Inference of population structure using multi-locus genotype data. *Genet*. **155:**945–59.

Punchay, K., Inta, A., Tiansawat, P., Balslev, H., and Wangpakapattanawong, P. (2020). Nutrient and mineral compositions of wild leafy vegetables of the Karen and Lawa communities in Thailand. *Foods* **9** (12):1748. https://doi.org/10.3390/foods9121748.

Purseglove, J. W. (1976). The origins and migrations of crops in tropical Africa. *Origins Afric. plant domest.* p 291-310.

Quinlan, M. (2005). Considerations for collecting free-lists in the field: examples from ethobotany. *Field methods* **17**(3): 219-234. https://doi.org/ 10.1177/1525822X05277460.

Raghu, A.V., Martin, G., Priya, V., Geetha, S.P., and Balachandran, I. (2007). Low-cost substitutes for the micropropagation of Centella asiatica. *J. Plant Sci*. **2**:592–599.

Rampersad, S.N., Perez-Brito, D., Torres-Calzada, C., Tapia-Tussell, R., and Carrington, V.F. (2013). Genetic structure and demographic history of Colletotrichum gloeosporioides sensu lato and C truncatum isolates from Trinidad and Mexico. *BMC Evol. Biol.* **13**:130. https://doi.org/10.1186/1471-2148-13-130.

Rao, K. N. (2004). Plant genetic resources: Advancing conservation and use through biotechnology. *Afr. J. Biotechnol.* **3:**136–145.

Rao, R., and Sthapit, B. (2012). Conservation of tropical plant genetic resources: **In** situ approach. In *Conservation of tropical plant species* (pp. 3-26). New York, NY: Springer New York. https://doi.org/10.1007/978-1-4614-3776-5_1.

Ratsavong, K., van Elsacker, T., Doungvichit, D., Siengsounthone, L., Kounnavong, S., and Essink, D. (2020). Are dietary intake and nutritional status influenced by gender? The pattern of dietary intake in Lao PDR: a developing country. *Nutr. J.* **19**: 1–16. https://doi.org/10.1186/s12937-020-00545-9.

Ray, S.S., Ali, M.N., Mukherjee, S., Chatterjee, G., and Banerjee, M. (2017). Elimination and molecular identification of endophytic bacterial contaminants during *in vitro* propagation of *Bambusa balcooa*. *World J. Microbiol. Biotechnol*. **33**: 1-9. https://doi.org/10.1007/s11274-016-2196-z.

Rieger, R., Michaelis, A., and Green, M. M. (2012). *Glossary of genetics and cytogenetics: classical and molecular*. Springer Science & Business Media.

Rosenzweig, M. (1995). Species Diversity in Space and Time. An overview and analysis of the geography of species richness, especially species area relations. New York: Cambridge University Press.

Safdar, B., Pang, Z., Liu, X., Rashid, M. T., and Jatoi, M. A. (2020). Structural and functional properties of raw and defatted flaxseed flour and degradation of cynogenic contents using different processing methods. *J. Food Proc. Eng.* **43**(6): e13406.

Salgotra, R. K., and Chauhan, B. S. (2023). Genetic diversity, conservation, and utilization of plant genetic resources, *Genes* **14**(1):174.

Samydurai, P., and Thangapandian, V. (2012). Nutritional assessment, polyphenols evaluation and antioxidant activity of food resource plant Decalepis hamiltonii Wight & Arn. *J. Appl. Pharmac. Sci.* **2** (5): 106-110. https://doi.org /10.7324/JAPS.2012.2518.

Sanchez-Cardozo, J., Quintanilla-Carvajal, M.X., Ruiz-Pardo, R., and Acosta-González, A. (2019). Evaluating gelling-agent mixtures as potential substitutes for bacteriological agar: an approach by mixture design. *Dyna*. **86** (208):171-176.

Sandhu, M., Wani, S. H., and Jiménez, V. M. (2018). *In vitro* propagation of bamboo species through axillary shoot proliferation: a review. *Plant Cell Tiss. Org. Cult.* (PCTOC). **132**(1): 27-53.

Saraswathi, M., Uma, S., Kannan, G., Selvasumathi, M., Mustaffa, M., and Backiyarani, S. (2016). Cost-effective tissue culture media for large-scale propagation of three commercial banana (*Musa* spp.) varieties. *J hortic. Sci. biotechnol*. **91**(1): 23–29. https://doi.org /10.1080 /14620316.2015.1117227.

Satori, D., Tovar, C., Faruk, A., Hammond Hunt, E., Muller, G., Cockel, C., Kühn, N., Leitch, I.J., Ermias Lulekal, Pereira, L. and Ryan, P. (2022). Prioritizing crop wild relatives to enhance agricultural resilience in sub- Saharan Africa under climate change. *Plants People Planet*. **4**(3):269-282. https://doi.org/10.1002/ppp3.10247.

Sefa-Dedeh, S., and Agyir-Sackey, E. K. (2004). Chemical composition and effect of processing on oxalate content of cocoyam *Xanthosoma sagittifolium* and *Colocasia esculenta* cormels. *Food Chem*. **85**(4): 479–487.

Shank, R. (1994). The Enset culture: A technical report on *Ensete ventricosum* or "False Banana". Addis Ababa, Ethiopia: United Nations-Emergencies Unit for Ethiopia.

Shank, R., and Chernet Ertiro (1996). A linear model for predicting Enset plant yield and Assessment of kocho production in Ethiopia. World Food Programme, Emergencies Unit for Ethiopia, Addis Ababa, Ethiopia.

Shannon, C., and Weaver, W. (1949). The mathematical theory of communication. Urbana: University of Illinois. p. 117.

Shara, S., and Mulugeta Diro (2012). Diversity and Indigenous management of enset (*Ensete venticosum* (Welw.) Cheesman) landraces in Gamo Gofa, Southern Ethiopia. In "Agrobiodiversity in Ethiopia: Risks and opportunities in a changing climate. Proceedings of a National workshop" (Seyoum Mengistu, and Fassil Assefa, Eds.), Biological Society of Ethiopia, Addis Ababa. pp 105

Shigeta, M. (1990). Folk *in- situ* conservation of enset (*Enset ventricosum* (Welw.) Cheesman): Towards the interpretation of indigenous agricultural science of the Ari, southwestern

Ethiopia. *Afr. Study monogra*. (Japan) **10(**3**):** 93-107

Shigeta, M. (1992). The Ethnobotanical study of enset (*Ensete ventricosum*) in the south-western Ethiopia. Dissertation, Kyoto University, Japan

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

Simmonds, N.W. (1960). Notes on banana taxonomy. *Kew Bullet*. **14**: 198-212.

Simmonds, N. W. (1962). The evolution of the bananas (2nd ed.).Longmans, London.

Simpson, E. (1949). Measurement of diversity.**163**: 688.

Sinha, K., and Khare, V. (2017). Review on: Anti-nutritional factors in vegetable crops. *The Pharma. Innov. J*. **6**(12): 353–358.

Sirawdink Forsido, Rupasinghe, H.P.V., and Astatkie, T. (2013). Antioxidant capacity, total phenolics and nutritional content in selected Ethiopian staple food ingredients. *Int. J. Food Sci. Nutr.* **64**(8*)*: 915-920. https://doi.org /10.3109 /09637486 .2013.806448.

Slatkin, M., and Barton, N.H. (1989). A comparison of three indirect methods for estimating average levels of gene flow. *Evol.* **43**(7): 1349-1368. https://doi.org/10.1111/j.1558-5646.1989.tb02587.x.

Sneath, P. H., and Sokal, R. R. (1973). Numerical Taxonomy. San Francisco: W. H. *Freeman.* p. 1–573.

Soetan, K. O., Olaiya, C. O., and Oyewole, O. E. (2010). The importance of mineral elements for humans , domestic animals and plants : A review. *Afr. J. Food Sci.* **4:** 200–222.

Soetan, K. O., and Oyewole, O. E. (2009). The need for adequate processing to reduce the anti-nutritional factors in plants used as human foods and animal feeds: A review. *Afr. J. food Sci.* **3**(9): 223-232.

Sorensen, T.A. (1948). A method of establishing groups of equal amplitude in plant sociology based on similarity of species content and its application to analyses of the vegetation on Danish commons. Kongelige Danske Videnskabernes Selskabs Biologiske Skrifter. **5**(4):1–34.

Spring, A., Hiebsch, C., Endale Tabogie, and Gizachew Weldmichael (1996). Enset needs assessment project phase I Report. Awassa, Ethiopia.

Stanley, S. (1996). Enset in Ethiopian economy. *Ethiop. Geograph. J*.**4**:30–37.

Sthapit, B.R., Joshi, K.D., and Witcombe, J.R. (1996). Farmer participatory crop improvement. III: Participatory plant breeding; a case study for rice in Nepal. *Exp. Agric*. **32**:479–496.

Taalat, K., Javed, M. A., Huyop, F. Z., and Kaya, Y. (2021). Plant tissue culture of nicotiana tabacum cv. TAPM 26 and its minimum inhibition against herbicide- dalapon. MANAS. *J. Eng.* **9**: 35-42. https://doi.org/10.51354/mjen.839516.

Tadessa Daba and Shigeta, M. (2016). Enset (*Ensete Ventricosum*) production in Ethiopia: its Nutritional and Socio-Cultural values. *Agric. Food Sci. Res.* **3**(2):66–74. http://www .asian onlinejournals .com/index. php/AESR.

Tadesse Kippie (2002). A Case Study on Gedeo Land Use (Southern Ethiopia). Ph.D Thesis, Wageningen University Promote, Heelsum, The Netherlands, p 296.

Tafesse Matewos (2019). Climate change-induced impacts on smallholder farmers in selected districts of Sidama, Southern Ethiopia. *Clim.* **7**(5): 70.

Takezaki, N., Nei, M., and Tamura, K. (2010). POPTREE2: software for constructing population trees from allele frequency data and computing some other population statistics with windows interface. *Mol. Biol. Evol*. **27**:747–52.

Taye Bezuneh and Asrat Feleke (1996). The production and utilization of genus *Ensete* in Ethiopia. *Econ. Bot*. **20**: 65-70.

Tedeschi, P., Brugnoli, F., Merighi, S., Grassilli, S., Nigro, M., Catani, M., Gessi, S., Bertagnolo, V., Travagli, A., Caboni, M.F., and Cavazzini, A. (2023). The Effect of Different Storage Conditions on Phytochemical Composition, Shelf-Life, and Bioactive Compounds of Voghiera Garlic PDO, *Antioxidants* **12**(2): 499, https://doi.org/10.3390/antiox 12020499.

Teixeira da Silva, J. A., Gulyás, A., Magyar-Tábori, K., Wang, M. R., Wang, Q. C., and Dobránszki, J. (2019). In vitro tissue culture of apple and other Malus species: recent advances and applications. *Planta*. **249**:975-1006.

Tella, R.D. (2007). Towards Promotion and Dissemination of Indigenous Knowledge. A Case of NIRD. *The Int. Inform. Libr. Revi*. **39**(3-4):185-193. https://doi .org/10.1016/j.iilr.2007. 06.002.

Temesgen Olango, Bizuayehu Tesfaye, Marcello, C., and Mario, E. (2014). Indigenous knowledge, use and on-farm management of enset [*Ensete ventricosum* (Welw.) Cheesman] diversity in Wolaita, Southern Ethiopia. *J. Ethnobiol. Ethnomed*. **10**:41. https://doi.org/10.1186/ s13002-016-0109-8.

Temesgen Olango, Bizuayehu Tesfaye, Pagnotta, M.A., Pè M.E., and Catellani, M. (2015). Development of SSR markers and genetic diversity analysis in enset (*Ensete ventricosum* (Welw.) Cheesman), an orphan food security crop from Southern Ethiopia. *BMC Genet*. **16**:98. https://doi.org/10.1186/s12863-015-0250-8.

Tesfaye Abebe (2005). Diversity in homegarden agro forestry systems of southern Ethiopia. Ph.D dissertation, Wageningen University, p 143.

Tesfaye Abebe, Wiersum, K.F., and Bongers, F. (2010). Spatial and temporal variation in crop diversity in agroforestry homegardens of southern Ethiopia. *Agrofore. syst*.**78**:309-22.

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. *Agricult. Nat. Resour.* **46**(6):837-850.

Tesfaye Dilebo, Tileye Feyissa, Zemede Asfaw and Ashagrie Zewdu (2023a). On-farm diversity, use pattern, and conservation of enset (*Ensete ventricosum*) genetic resources in southern Ethiopia. *J. Ethnobiol. Ethnomed.* **19**(1): 1-18. https://doi.org/10.1186/s13002-022-00569-x.

Tesfaye Dilebo, Tileye Feyissa and Zemede Asfaw (2023 b). Farmers' local knowledge on classification, utilization, and on‑farm management of enset (*Ensete ventricosum* [Welw.] Cheesman) landraces diversity in Hadiya, southern Ethiopia. *Genet. Resour. Crop Evol*. **70: 1-29**. https://doi.org/10.1007/s10722-023-01714-5.

Teshome Sirany, Esubalew Tadele, Tewabe Hibistu, Alemayehu Kefalew and Haimanot Reta (2022). Economic Viability and Use Dynamics of the Enset Food System in Ethiopia: Its Implications for Food Security. *Advan. Agric*. 2022. https://doi.org/10.1155/2022/5741528.

Teshome Yirgu (2016)**.** Land use dynamics and challenges of enset (*Ensete ventricosum*) agriculture in the upper reaches of Baso-Deme watershed, Gamo Highland, SW Ethiopia. *Global J. Interdiscip. Soci. Sci.* **5:**20–28.

Thorpe, T. (2007). History of plant tissue culture. *J. Mol. Microbial. Biotechnol.* **37**: 169-180. https://doi.org/10.1007/s12033-007-0031-3.

Tiago, A.V., Hoogerheide, E.S.S., Pedri, E.C.M., Rossi, F. S., Cardoso, E.S., Pinto, J.M.A., Pena, G.F., and Rossi, A.A.B. (2019). Genetic diversity and population structure of

cassava ethno-varieties grown in six municipalities in the state of Mato Grosso, Brazil. *Genet. Mol. Res.* **18** (4): gmr18357. http://dx.doi.org/10.4238/gmr18357.

Tibebu Habtewold, Sandford, S., and Kindness, H. (1996). The age and sex structure of enset plantations in north Omo. In: Tsedeke A, Hiebsch C, Brandt SA, Seifu Gebramaiam (Eds.) Enset-based sustainable agriculture in Ethiopia: Proceedings from the International Workshop on enset held in Addis Ababa, Ethiopia, 13-20 December 1993. pp 249-163.

Tileye Feyissa (2020). *Coccinia abyssinica* (Lam.) Cogn.(anchote) biology, productivity, and prospects of genetic improvement using biotechnological tools. *J. Hortic. Res.* **28**(2).

Tileye Feyissa, Welander, M., and Legesse Negash (2005). Micro-propagation of *Hagenia abyssinica*: a multipurpose tree. *Plant Cell Tiss. Org. Cult*. **80**:119-128. https://doi.org /10.1007/s11240-004-9157-1.

Timu, A., Mulwa, R., Okello, J., and Kamau, M. (2014). The role of varietal attributes on adoption of improved seed varieties: the case of sorghum in Kenya. Agriculture & Food Security. **3**: 9.

Tiruha Karssa, Kebede Ali and Edessa Gobena (2014). The microbiology of Kocho: An Ethiopian traditionally fermented food from Enset (*Ensete ventricosum*). *Int. J. Life Sci.* **8**(1): 7-13. https:// doi:dx.doi.org/10.3126/ijls.v8i1.8716.

Tomar, R.S., Parakhia, M.V., Patel, S.V., and Golakiya, B.A. (2010). Molecular Markers and Plant Biotechnology. *New India Publishing Agency, New Dehli.*

Tomilson, P. (1969). Anatomy of the monocotyledons III. Commelinales Zingiberales, Clarendon Press, Oxford, UK.

Tripathi, J., Matheka, J., Ibsa Merga, Endale Gebre, and Tripathi, L. (2017). Efficient regeneration system for rapid multiplication of clean planting material of [*Ensete ventricosum* (Welw.) Cheesman]. *Vitro Cell Dev. Biol. Plant*. **53**(6):624–30. https://doi .org /10.1007/s11627-017-9867-9.

Tsedale Waktola (2009). Globaliztion and Neo-liberalization: Challenges and Opportunities to Enset crop-based Agricultural communities and their farming system in Ethiopia. Ph.D. Thesis, Edmonton Alberta, Canada, p 217.

Tsige Gebre-Mariam and Nikolayev, A.S. (1993). Evaluation of starch obtained from *Ensete ventricosum* as a binder and disintegrant for compressed tablets. *J. Pharm. Pharmacol*. **45**(4):317–320. https://doi. org/10.1111/j.2042-7158.1993.tb05560.x.

Tsige Gebre-Mariam and Schmidt, P.C. (1996). Characterization of Enset Starch and its Use as a Binder and Disintegrant for Tablets. *J. Pharmazie*. **51**(5):303–311.

Tura Bareke, Zemede Asfaw, Zerihun Woldu, Medvecky, B., and Birhanu Amssalu (2018). Diversity of common bean (*Phaseolus vulgaris L*., Fabaceae) landraces in parts of southern and eastern Ethiopia. *Adv. Plants Agric .Res*. **8**(6):449–457. https://doi.org /10. 15406/apar.2018.08 .00365.

Ukpabi, u.j., and Ejidoh, j.i. (1989). Effect of deep oil frying on the oxalate content and the degree of itching of cocoyams (*Xanthosoma* and *Colocasia* spp). Technical paper presented at the 5th Annual Conference of the Agricultural Society of Nigeria, Federal University of Technology, Owerri, Nigeria, pp 3–6.

Ulian, T., Diazgranados, M., Pironon, S., Padulosi, S., Liu, U., Davies, L., Howes, M.J.R., Borrell, J.S., Ondo, I., Pérez- Escobar, O.A., and Sharrock, S. (2020). Unlocking plant resources to support food security and promote sustainable agriculture. *Plants People Planet.* **2**(5):421-45. https://doi.org/10.1002/ppp3.10145.

Vaintraub, I.A., and Lapteva, N. A. (1988). Colorimetric determination of phytate in unpurified extracts of seed and the products of their processing. *Analyt. Biochem*. **175**: 227-230. https://doi.org/10.1016/0003-2697(88)90382-X.

Vallejo- Marín, M., and Hiscock, S.J. (2016). Hybridization and hybrid speciation under global change. *New Phytol.* **211**(4): 1170-1187. https://doi.org/10.1111/nph.14004.

Vance, C. P., Uhde-Stone, C., and Allan, D. L. (2003). "Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource," *New Phytol*. **157**(3):432–449. https://doi.org/10.1046/j.1469-8137.2003.00695.x.

Vavilov, N.I. (1951). The origin, variation, immunity and breeding of cultivated plants. *Chron. Bot*. 13:1–366.

Vidyagina, E. O., Kharchenko, N. N., and Shestibratov, K. A. (2021). Efficient cryopreservation of populustremula by *In vitro*-grown axillary buds and genetic stability of recovered plants. *Plants*. **10** (1):77. https://doi.org/10.3390/ plants10010077.

Villa, T.C., Maxted, N., Scholten, M., and Ford-Lloyd, B. (2005). Defining and identifying crop landraces. *Plant. Genet. Resour*. **3**(3):373–384. https://doi.org/10.1079/PGR200591.

Wendawek Abebe, Sebsebe Demissew, Fay, M.F., Smith, R.J., Nordal, I., Wilkin, P. (2013). Genetic diversity and population structure of Guinea yams and their wild relatives in

South and South West Ethiopia as revealed by microsatellite markers. *Genet. Resour. Crop Evol*. **60**:529-41. https://doi.org/10.1007/s10722-012-9856-0.

Westphal, E. (1975). Agricultural systems in Ethiopia. Center for Agriculture Publishing and Documentation, Wageningen, The Netherlands, p 278.

Westwood, M., Cavender, N., Meyer, A., and Smith, P. (2021). Botanic garden solutions to the plant extinction crisis. *Plants People Planet* **3**(1): 22-32. https://doi.org/10.1002/ppp3.10134.

WHO (World Health Organization) (1996). Trace Elements in Human Nutrition and Health. Geneva. Retrieved from. https://apps. who. int/iris/ handle/10665/37931

WHO (World Health Organization), (2003). Feeding and nutrition of infants and young children: guidelines for the WHO European Region with Emphasis on the Former Soviet Countries, WHO Region Publication, European Series, No. 87.

Wright, S. (1951). The genetical structure of populations. *Ann Eugen* **15**:323–354.

Yan, L., Ogutu, C., Huang, L., Wang, X., Zhou, H., Lv, Y., Long, Y., Dong, Y., and Han, Y. (2019). Genetic diversity and population structure of coffee germplasm collections in China revealed by ISSR markers. *Plant Mol. Biol. Rep*. **37**:204-213.

Yemane Tsehay and Fassil Kebebew (2006). Diversity and cultural use of (*Ensete ventricosum* (Welw.) Cheesman) in Bonga *in- situ* Conservation Site, Ethiopia. *Ethnobot. Res. Appl.* **4**:147-157. https://doi.org/10.17348/era.4.0.147-158.

Yenenesh Ayalew (2016). Nutritional and Phytochemical Evaluation of Anchote *(Coccinia abyssinica)* (Lam.) (Cogn.) Accessions to Promote its Contribution for Food Security and Medicinal Use. A Ph.D Dissertation in Food Science and Nutrition.

Yewelsew Abebe, Alemtsehay Bogale, 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 Composit. Analy.* **20(**3-4): 161-168. http://doi:10.1016/j.jfca. 2006.09.003.

Yildiz, M. (2012). The prerequisite of the success in plant tissue culture: high frequency shoot regeneration. Recent advances in plant in vitro culture. *Intech Rijeka* 63-90. http://dx.doi.org/10.5772/51097.

Zahedi, S.M., Abdelrahman, M., Hosseini, M.S., Yousefi, R., and Tran, L.S.P. (2020). Physical and biochemical properties of 10 wild almond (*Amygdalus scoparia*) accessions naturally grown in Iran. *Food Biosci*. **37:**100721, https://doi.org/10.1016/j.fbio.2020.100721.

Zemede Asfaw (2018). Enset (*Ensete ventricosum*, Musaceae) ethnobotany: research status, gaps and key messages. *Ethiop. J. Biol. Sci*. **17**(Suppl.): 51–62.

Zerihun Teshome, Meseret Terfa, Bizuayehu Tesfaye, Eleni Shiferaw and Temesgen Olango (2020). Genetic diversity in anchote (*Coccinia abyssinica* (Lam.) Cogn) using microsatellite markers. *Curr. Plant Biol.* **24:** 100167. https://doi.org/10.1016/j.cpb.2020.100167.

Zerihun Yemataw, Hussein Mohamed, Mulugeta Diro, Temesgen Addis and Blomme G. (2014). Ethnic-based diversity and distribution of enset (*Ensete ventricosum*) cultivars in southern Ethiopia. *J. Ecol. Nat. Environ*. **6**(7):244–251. https://doi.org /10.5897 /JENE 2014.0450.

Zerihun Yemataw, Hussein Mohamed, Mulugeta Diro, Temesgen Addis and Blomme, G. (2014). Enset (*Ensete ventricosum*) clone selection by farmers and their cultural practices in southern Ethiopia. *Genet. Resour. Crop Evol.* **61**: (6):1091–1104. https://doi.org/10.1007 /s10722-014-0093-6.

Zerihun Yemataw, Kassahun Tesfaye, Awole 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. *J. Ethnobiol. Ethnomed*. **12**:34. https://doi.org/10.1186/s13002-016-0109-8.

Zerihun Yemataw, Kassahun Tesfaye, Grant, M., Studholme, D.J., and Alemayehu Chala (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. *Aust. J. Crop Sci*. **12**(12):1849–1858.

Zippel, K. (2005). Diversity over time and space in enset landraces (*Ensete ventricosum*) in Ethiopia. In African Biodiversity: Molecules, Organisms, Ecosystems. Edited by Huber BA, Sinclair BJ, Lampe K. Bonn, Germany: Springer. 423–438.

Zippel, K., and Kefale Alemu (1995). A field guide to enset landraces of North Omo, Ethiopia. FRP technical pamphlet No. 9.

Zippel, K, and Ludders, P. (2005). The Global food and product chain dynamics, innovations,

conflicts, strategies: *Ensete ventricosum* in Ethiopia: The need to grow more than one landraces. Deutscher Tropentag. pp 11-13.

Zohora, F., Bidad, K., Pourpak, Z., and Moin, M. (2018). Biological and immunological aspects of iron deficiency anemia in cancer development: a narrative review. *Nutr*. *Can*c. **70**(4): 546–556, https://doi.org/10.1080/01635581.2018.1460685.

Appendices

Appendix 1: Survey on Enset farming systems of respondents in Hadiya zone, south Ethiopia

Name of Interviewer	
Date of Interview	
Zone	
Woreda (District)	
Kebele (Sub-district)	
Name of Respondent	
Gender of Respondent	◯ Male ◯ Female
Age of respondent	
Educational level of respondent	◯ Illiterate ◯ Informal ◯Primary ◯ Junior ◯ Secondary ◯ College
Marital Status of the respondent	◯ Single ◯ Married ◯ Divorced ◯ Widowed

Status of enset and its production activities

1. Do you have enset in your homegarden?

A, yes B, no

2. If yes to question number 1, for what purpose do you plant the enset crop?

3. How would you compare the cultivation of enset with other crops grown in your homegarden?

Enset landrace diversity and distribution at local level

4. How many different types of enset landraces exist in your homegarden?

5. List all types of enset landraces cultivated on your homegarden

No	Local name	No	Local name	No	Local name	No	Local name
1		11		21		31	
2		12		22		32	
3		13		23		33	
4		14		24		34	
5		15		25		35	
6		16		26		36	
7		17		27		37	
8		18		28		38	
9		19		29		39	
10		20		30		40	

6. Which enset landraces are more frequently/common in your homegarden? Why?

7. Which enset landraces are moderately common in your homegarden?

8. Which enset landraces are rare or less frequent in your homegarden? Why?

9. Which enset landrace is unique (only one) in your homegarden? Why?

Farmers' identification, naming and categorization of enset landraces

10. Do you have any criteria by which you can identify or distinguish each enset landrace from your homegarden?

A, yes B, no

11. If yes to question number 10, list all the identification features for your landraces

12. Which identification features are more frequently used?

13. Which identification features are least frequently used?

14. Do the local names of the enset landraces have any meaning?

A, yes B, no

15. If yes to question number 14, please mention the meaning of the names

16. Do you categorize your enset landraces in different groups?

A, yes B, no

17. If yes to question number 19, what are the major criteria?

Indigenous use of enset

18. What is the best stage of harvesting most of the enset landraces for food?

A, before flowering B, after flowering C, at both stages D, at any stage

19. Which season is more preferred for harvesting enset?

A, dry season B, rainy season C, any time of the year

20. Which primary enset product(s) is/are used as human food?

A, *wassa* or *qocho* *B, buoo* or *bulla* C, *hamicho or amicho* D, all

21. Which primary enset product is more produced?

A, *wassa* or *qocho* *B, buoo* or *bulla* C, *hamicho or amicho*

22. Is there different types of *wassa* or *qocho*?

A, yes B. No

23. If yes to the number 22, would you tell me the types of *wassa* or *qocho*?

24. Which enset landrace (s) do you think is/are more preferred for the quality of *wassa* or *qocho*?

25. How long would it take for it to ferment?

26. What do you think determines the rate of fermentation?

27. Which types of enset landraces normally ferment faster?

28. Which enset landrace (s) is/are best for *buoo* (*bulla) production?*

29. Name the *buoo* cuisine, along with the occasions when it is typically prepared.

30. Which groups/categories of enset landraces normally ferment faster?

31. Which groups/ categories of enset landraces ferment slowly?

32. Which enset landrace (s) is/are normally used for *hamicho* or *amicho* (cooked corm) preparation in your homegarden?

33. Which of these landraces are regarded the best?

34. Which enset landraces are more preferred for the production of fiber in quality and quantity?

35. Do you have enset landraces particularly used for medicinal purpose?

A, yes B, no

36. If yes to question number 35, please mention landraces and the specific uses.

Name of enset landraces	Traditional treatment of ailments	Parts (products) of enset used

37. Do men and women practice different tasks in the production of enset?

A, yes B, no

38. If yes to question number 40, mention the types of occupation responsible for men, women and for both sexes.

Indigenous cultivation and management practice

39. When do you cut or propagate your enset landraces?

A, at rainy season B, at dry season C, at any season

40. How many enset landraces do you cut per year for *dubo* (sucker) production?

41. Which enset landrace (s) produce (s) the most *dubo* (suckers) from a mother corm?

42. Do you regularly transplant different ages of enset landraces?

A, yes B, no

43. If yes to question number 42, how often did you transplant suckers?

Specify their names and corresponding stages:

i. _____, ii, _____, iii, _____ iv. _____ _ _ _ v. _____

44. When do you transplant your enset landraces? Why?

45. Where do you obtain the suckers?

46. Do you need some inputs, such as fertilizers and pesticides, for enset production?

A, yes B, no

47. Do you equally care for enset types in your homegarden?

A, yes B, no

48. If yes to question number 50, please mention the reason

49. Which enset landrace(s) require more management practices? Why?

50. Which enset landrace(s) require least/less management practices? Why?

51. Does a farmer who has diverse enset landraces with mature and well-managed systems in your kebele or surroundings receive any societal recognition?

A, yes B, no

52. If yes to question number 51, please mention types of such social recognition.

Appendix 2: Local names of enset landraces or varieties with their code

Landrace Name	Code	Landrace Name	Code	Landrace Name	Code	Landrace Name	Code	Landrace Name	Code
Agade	G1	*Agade*	S1	*Aganche*	K1	*Alabite*	H1	*Endale*	R1
Ahiro	G2	*Ahiro*	S2	*Ashura*	K2	*Anchire*	H2	Gewada	R2
Astara	G3	*Ankfuye*	S3	*Banko*	K3	*Awunada*	H3	*Kelisa*	R3
Awunad	G4	*Ashaqite*	S4	*Badede*	K4	*Bamba*	H4	*Messena*	R4
Aywogna	G5	*Astara*	S5	*Cherquwa*	K5	*Beneje*	H5	*Yambule*	R5
Bededet red	G6	*Beneje*	S6	*Degoblack*	K6	*Bequcho*	H6	*Zereta*	R6
Bezeria	G7	*Darye*	S7	*Derqeta*	K7	*Boicho*	H7	Sucker 1	A1
Bitena	G8	*Dem-worad*	S8	*Dirbo*	K8	*BuchTor*	H8	Sucker 2	A2
Dem-wored	G9	*Fengo*	S9	*Etine*	K9	*Dirbo*	H9	Dawro 1	W1
Feraziya	G10	*Gariye*	S10	*Fecheche*	K10	*Fello*	H10	Dawro 2	W2
Gegered	G11	*Hanzana*	S11	*Gimbo*	K11	*Gimbo*	H11	Dawro 3	W3
Gimbuwa	G12	*Hiniba*	S12	*Gishira*	K12	*Ginjowona*	H12	Dawro 4	W4
Ginnad	G13	*Kembat*	S13	*Godorete*	K13	*Gogoricho*	H13	Kaffa-old-Red	W5
Gozoda	G14	*Megribe*	S14	*Gomorsa*	K14	*Gomorsa*	H14	Kaffa-old-white	W6
Gumbura	G15	*Merza Kembt*	S15	*Hella*	K15	*Gozoda*	H15	Kaffa-new-Red	W7
Hanzana	G16	*Orad*	S16	*Leqeqa*	K16	*Gudere*	H16		
Hiniwa	G17	*Qeshqeshe*	S17	*Merza-black*	K17	*Hella*	H17		
Kemele	G18	*Qiniware*	S18	*Mesmesa*	K18	*Kaseta mo*	H18		
Lemat	G19	*Separa*	S19	*Qeqille*	K19	*Kekera-red*	H19		
Nechewa	G20	*Sherafire*	S20	*Qorate*	K20	*Korina*	H20		
Oniya	G21	*Shigez*	S21	*Quina*	K21	*Laddare*	H21		
Qesewa	G22	*Shirtye*	S22	*Sebera*	K22	*Mariye*	H22		
Separa	G23	*Sino*	S23	*Shelleqe*	K23	*Meqelwesa*	H23		
Shewatia	G24	*Sisqella*	S24	*Sinera*	K24	*Moche*	H24		
Shewora	G25	*Tegeded*	S25	*Sorpie*	K25	*Oniya*	H25		
Shirafere	G26	*Wonade*	S26	*Torore*	K26	*Qeteqeta*	H26		
Wonadia	G27	*Woshemaja*	S27	*Unjame*	K27	*Qombotira*	H27		
Zobir-red	G28	*Zegizig*	S28	*Wachiso*	K28	*Quiena*	H28		
Zogirad	G29	*Zobir*	S29	*Wohe*	K29	*Separa*	H29		
				Xebere	K30	*Shate*	H30		
				Xessa	K31	*Sinera*	H31		
				Zobiro	K32	*Sisqella mo*	H32		
						Sisasura	H33		
						Soqido-Le	H34		
						Suwandiya	H35		

						Tegaded	H36		
						Xiggo	H37		
						Zobira	H38		
						Gariya	H39		
						Wonade	H40		
						Kaseta-Le	H41		
						Sisqella Du	H42		

G, Gurage, S, Silte, K, Kembata, and H, Hadiya administrative zones of farmers homegardens; R, released; A, sucker-wild and W, wild varieties, which are maintained in the Areka Agricultural Research Center (AARC) (*ex-situ* conservation site of enset crop) of Ethiopia.

Appendix 3. List of enset sample code, nucleic acid concentration and purity of DNA

Sample PCR ID	Nucleic Acid concentration	Unit	260/280	260/230	Sample Type	Factor
01	833.7	ng/µl	2.14	1.79	DNA	50
02	2709.3	ng/µl	2.17	1.4	DNA	50
03	1740.6	ng/µl	2.11	1.25	DNA	50
04	359.4	ng/µl	2.22	1.48	DNA	50
05	255.4	ng/µl	2.20	1.37	DNA	50
06	4693.9	ng/µl	2.17	1.27	DNA	50
07	1130	ng/µl	2.19	1.52	DNA	50
08	2295.5	ng/µl	2.14	1.26	DNA	50
09	980.4	ng/µl	2.23	1.43	DNA	50
10	1122.6	ng/µl	2.18	1.72	DNA	50
11	1315.7	ng/µl	2.15	1.51	DNA	50
12	717.1	ng/µl	2.19	1.45	DNA	50
13	1463.5	ng/µl	2.2	1.7	DNA	50
14	1220.5	ng/µl	2.21	1.47	DNA	50
15	2114.7	ng/µl	2.19	1.59	DNA	50
16	1348.6	ng/µl	2.21	1.23	DNA	50
17	1203	ng/µl	2.18	1.58	DNA	50
18	364	ng/µl	2.17	1.94	DNA	50
19	599.5	ng/µl	2.07	2.11	DNA	50
20	708.5	ng/µl	2.19	1.57	DNA	50
21	362.6	ng/µl	2.21	1.47	DNA	50
22	518.1	ng/µl	2.15	1.61	DNA	50
23	847.7	ng/µl	2.16	1.21	DNA	50
24	923.1	ng/µl	2.22	1.36	DNA	50
25	264.5	ng/µl	2.19	1.65	DNA	50
26	266.6	ng/µl	2.19	1.82	DNA	50
27	232.2	ng/µl	2.18	1.62	DNA	50
28	160.8	ng/µl	2.18	1.80	DNA	50
29	205.3	ng/µl	2.14	1.81	DNA	50
30	175.5	ng/µl	1.85	1.48	DNA	50
31	325	ng/µl	2.24	1.79	DNA	50
32	135.1	ng/µl	2.28	1.78	DNA	50
33	223.9	ng/µl	2.12	1.61	DNA	50
34	169.8	ng/µl	2.11	1.46	DNA	50
35	466.6	ng/µl	2.11	1.85	DNA	50
36	157.4	ng/µl	2.16	1.86	DNA	50
37	924.1	ng/µl	2.17	1.55	DNA	50
38	1308.3	ng/µl	2.16	1.73	DNA	50
39	346.5	ng/µl	2.17	1.64	DNA	50
40	311.9	ng/µl	2.18	1.77	DNA	50
41	332.4	ng/µl	2.27	1.34	DNA	50

Appendix 3. continued

42	603.6	ng/µl	2.19	1.48	DNA	50
43	418.1	ng/µl	2.17	1.28	DNA	50
44	314.5	ng/µl	2.09	1.98	DNA	50
45	313.9	ng/µl	2.15	1.71	DNA	50
46	208.8	ng/µl	2.23	1.61	DNA	50
47	380.6	ng/µl	2.22	1.48	DNA	50
48	351.6	ng/µl	2.14	1.55	DNA	50
49	164.1	ng/µl	2.21	1.64	DNA	50
50	296.5	ng/µl	2.14	1.36	DNA	50
51	705.6	ng/µl	2.14	1.58	DNA	50
52	686.2	ng/µl	2.16	1.37	DNA	50
53	534.2	ng/µl	2.03	1.92	DNA	50
54	371.8	ng/µl	2.11	1.73	DNA	50
55	1198.7	ng/µl	2.21	1.36	DNA	50
56	338.8	ng/µl	2.18	1.67	DNA	50
57	652.7	ng/µl	2.05	1.54	DNA	50
58	455.8	ng/µl	2.19	1.29	DNA	50
59	296.7	ng/µl	2.12	1.52	DNA	50
60	347.3	ng/µl	2.20	1.41	DNA	50
61	647.2	ng/µl	2.02	1.42	DNA	50
62	870.7	ng/µl	2.15	1.37	DNA	50
63	705.5	ng/µl	2.23	1.16	DNA	50
64	478.3	ng/µl	2.21	1.81	DNA	50
65	782.3	ng/µl	2.22	1.62	DNA	50
66	308.6	ng/µl	2.15	1.58	DNA	50
67	282.4	ng/µl	2.17	1.38	DNA	50
68	735.5	ng/µl	2.14	1.47	DNA	50
69	136.7	ng/µl	2.16	1.52	DNA	50
70	557.7	ng/µl	2.16	1.31	DNA	50
71	488.7	ng/µl	2.18	1.21	DNA	50
72	451	ng/µl	2.15	1.29	DNA	50
73	152.3	ng/µl	2.17	1.48	DNA	50
74	390.1	ng/µl	2.24	1.54	DNA	50
75	741.5	ng/µl	2.09	2.05	DNA	50
76	72.4	ng/µl	1.96	1.64	DNA	50
77	335.9	ng/µl	2.15	1.5	DNA	50
78	771.2	ng/µl	2.21	1.33	DNA	50
79	183	ng/µl	2.20	1.47	DNA	50
80	187.8	ng/µl	2.16	1.68	DNA	50
81	92.5	ng/µl	2.06	1.82	DNA	50
82	741.7	ng/µl	2.2	1.42	DNA	50
83	238.6	ng/µl	2.25	1.19	DNA	50
84	353.8	ng/µl	2.2	1.42	DNA	50

Appendix 3. continued

85	145.7	ng/µl	2.11	1.68	DNA	50
86	715.9	ng/µl	2.09	1.81	DNA	50
87	322.9	ng/µl	2.2	1.42	DNA	50
88	576.8	ng/µl	2.2	1.31	DNA	50
89	709.2	ng/µl	2.22	1.32	DNA	50
90	706.8	ng/µl	2.06	1.43	DNA	50
91	693.9	ng/µl	2.21	1.36	DNA	50
92	158.9	ng/µl	1.99	1.39	DNA	50
93	324.4	ng/µl	2.25	1.36	DNA	50
94	473.7	ng/µl	2.16	1.75	DNA	50
95	146	ng/µl	2.11	1.32	DNA	50
96	464.6	ng/µl	2.2	1.61	DNA	50
97	862.3	ng/µl	2.2	1.56	DNA	50
98	563.8	ng/µl	2.15	1.21	DNA	50
99	582.8	ng/µl	2.21	1.37	DNA	50
100	797.1	ng/µl	2.21	1.29	DNA	50
101	1248.1	ng/µl	2.18	1.41	DNA	50
102	587.3	ng/µl	2.16	1.25	DNA	50
103	542.4	ng/µl	2.15	1.39	DNA	50
104	1092	ng/µl	2.25	1.17	DNA	50
105	958.1	ng/µl	2.16	1.35	DNA	50
106	1587.2	ng/µl	2.23	1.26	DNA	50
107	833.8	ng/µl	2.29	1.02	DNA	50
108	565.4	ng/µl	2.16	1.28	DNA	50
109	512.4	ng/µl	2.21	1.62	DNA	50
110	1058.3	ng/µl	2.17	1.41	DNA	50
111	985.8	ng/µl	2.08	1.74	DNA	50
112	1323.7	ng/µl	2.15	1.32	DNA	50
113	1051.1	ng/µl	2.15	1.27	DNA	50
114	262.7	ng/µl	2.16	1.56	DNA	50
115	1150.6	ng/µl	2.18	1.41	DNA	50
116	1070.3	ng/µl	2.15	1.39	DNA	50
117	816	ng/µl	2.16	1.38	DNA	50
118	864.9	ng/µl	2.21	1.65	DNA	50
119	1563.4	ng/µl	2.11	1.49	DNA	50
120	949	ng/µl	2.18	1.49	DNA	50
121	852.5	ng/µl	2.14	1.31	DNA	50
122	822	ng/µl	2.19	1.79	DNA	50
123	677	ng/µl	2.21	1.47	DNA	50
124	897.9	ng/µl	2.18	1.23	DNA	50
125	1825.6	ng/µl	2.14	1.65	DNA	50
126	988.2	ng/µl	2.27	1.23	DNA	50
127	994	ng/µl	2.21	1.35	DNA	50

Appendix 3. continued

128	631.4	ng/µl	2.20	1.45	DNA	50
129	646.5	ng/µl	2.19	1.56	DNA	50
130	210.4	ng/µl	2.14	1.96	DNA	50
131	760.7	ng/µl	2.20	1.36	DNA	50
132	662.8	ng/µl	2.16	1.46	DNA	50
133	589.3	ng/µl	2.19	1.26	DNA	50
134	584.7	ng/µl	2.17	1.46	DNA	50
135	699.5	ng/µl	2.18	1.33	DNA	50
136	1072.5	ng/µl	2.22	1.33	DNA	50
137	499.5	ng/µl	2.23	1.29	DNA	50
138	1934.6	ng/µl	2.14	1.78	DNA	50
139	316.9	ng/µl	2.16	1.58	DNA	50
140	653.2	ng/µl	2.2	1.42	DNA	50
141	900.7	ng/µl	2.25	1.26	DNA	50
142	649.9	ng/µl	2.13	1.54	DNA	50
143	753.7	ng/µl	2.19	1.47	DNA	50
144	527.7	ng/µl	2.21	1.38	DNA	50
145	302.2	ng/µl	2.2	1.65	DNA	50
146	796.5	ng/µl	2.14	2.08	DNA	50
147	166.1	ng/µl	2.16	1.53	DNA	50

Appendix 4: The MS basal medium composition and concentration for full strength of MS media.

A. Macro-nutrient	mg/L	g/L	10X	20x per 1L	Stock volume ml per a litre of final solution
1. NH4NO3	1650	1.65	16.5g	33g	50 ml
2. KNO_3	1900	1.9	19g	38g	
3. $Cacl_2.2H_2O$	440	0.44	4.4g	8.8g	
4. $MgSO_4.7H_2O$	370	0.37	3.7g	7.4g	
5. KH_2PO_4	170	0.17	1.7g	3.4g	
B. Micro-nutrient	mg/L	g/L	1000x	2000x per 1L	Stock volume ml per a litre of final solution
1. H_3BO_3	6.2	0.006	6.2g	12.4g	5 ml
2. $MnSO_4.4H_2O$	22.3	0.022	22.3g	44.6g	
3. $ZnSO_4.7H_2O$	8.6	0.0086	8.6g	17.2g	
4. KI	0.83	0.00083	0.83g	1.66g	
5. $Na_2MoO_4.2H_2O$	0.25	0.00025	0.25g	0.5g	
6. $CoCl_2.6H_2O$	0.025	0.000025	0.025g	0.05g	
7. $CuSO_4.5H_2O$	0.025	0.000025	0.025g	0.05g	
C. Fe-Na-EDTA salt	40 mg/L	0.04 g/L	1000x=40	2000x=80 g	5 ml per a litre
D. Vitamins	mg/L	In 100 ml	In 100 ml		5 ml
1. Thiamine (HCl)	0.1	100 mg	0.1g		
2. Niacine	0.5	100 mg	0.1 g		
3. Pyrodoxine(HCl)	0.5	100 mg	0.1 g		
4. Glycine	2.0	100 mg	0.1 g		
5. Myo-inositol		100mg/L	0.1g/L		
E. Plant growth regulators	(mg/ml)				
1. BAP	25				
2. IAA	10				
3. IBA	10				
F. Sucrose	30g/L				
G. Agar	6g/L				
H. *Bulla*	80g/L				
I. Activated charcoal	2g/L				
J. pH	5.75				