



Addis Ababa University

College of Natural and Computational Sciences

Department of Plant Biology and Biodiversity Management

**Enset (*Ensete ventricosum* (Welw.) Cheesman, Musaceae): Ethnomedicinal applications,
nutritional quality and reproductive biology in selected districts of South and South
western Ethiopia**

**Dissertation submitted in fulfillment of the requirements for the degree of Doctor of Philosophy in
Plant Biology and Biodiversity Management**

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I, the undersigned, declare that this dissertation is my original work and it has not been submitted or presented to other universities, colleges or institutes for a degree or other purpose. All sources of the materials used have been duly acknowledged.

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Table of contents

Content	Page
List of tables.....	vii
List of figures.....	viii
List of acronyms and abbreviations.....	ii
Acknowledgements.....	iii
General Abstract.....	vi
CHAPTER ONE.....	1
1. General introduction.....	1
1.1. Background and justification.....	1
1.2. Brief literature review.....	4
1.2.1. Traditional medicinal applications of enset and some wild plants of the Ensete genus.....	4
1.2.2. Enset production in relation to food security: nutritional, biochemical and microbiological contents	6
1.2.3. Traditional versus modern enset propagation methods.....	10
1.3. Statement of the problem.....	13
1.4. Research questions.....	17
1.5. Objectives.....	18
1.5.1. General objective.....	18
1.5.2. Specific objectives.....	18
1.6. Significance of the study.....	19
1.7. Scope of the study.....	20
1.8. Limitations of the study.....	21
1.9. Structure of the dissertation.....	22
CHAPTER TWO.....	24
2. Ethnomedicinal applications of an indigenous food security plant Enset (Ensete ventricosum) in selected districts from Sidama, Wolaita and Gurage in Southern Ethiopia.....	24
Abstract.....	24
2.1. Introduction.....	25
2.2. Materials and Methods.....	27
2.2.1. Study design, description of the area and sampled population.....	27
2.2.2. Sampling of home gardens and informants.....	29
2.2.3. Collection of ethnobotanical data.....	30
2.2.4. Data analysis.....	32
2.3. Results.....	36
2.3.1. Characterization of farmers' landraces of enset used as traditional medicine.....	36
2.3.2. Plant parts and processed products of enset used as traditional medicine.....	37

2.3.3.	Modes of preparation and application of enset as traditional medicine	39
2.3.4.	Routes of administration of enset medicine	42
2.3.5.	Types of diseases and/or disorders treated with enset medicine	42
2.3.6.	Informant consensus factor and fidelity level indices	44
2.3.7.	Ethnoveterinary uses of enset for treatment of livestock ailments.....	45
2.3.8.	Ranking of multimedicinal and rare farmers' landraces of therapeutic values	48
2.3.9.	Preference or priority ranking to identify the rarest landraces used as medicine.....	48
2.3.10.	Factors affecting availability of medicinal enset and local conservation responses	49
2.3.11.	Effects of socioeconomic status on level of ILK of medicinal use of enset	51
2.4.	Discussion and conclusions	52
2.4.1.	Characterization of enset used in traditional medicine	52
2.4.2.	Applications of enset as medicine: plant parts and processed products used	54
2.4.3.	Mode of preparation and application of enset as traditional medicine.....	56
2.4.4.	Additives and a few of adverse effects associated to use of enset as medicine.....	57
2.4.5.	Ailments or disorders treated using enset as traditional medicine	59
2.4.6.	Informant agreements and relative healing potentials of enset medicine	62
2.4.7.	Ethnoveterinary applications of enset in treatment of livestock ailments.....	63
2.4.8.	Ranking of multi-medicinal and rare farmers' landraces of enset	66
2.4.9.	Factors determining local availability of medicinal enset.....	67
2.4.10.	Socioeconomic status as determinant factor for ILK of enset use as medicine	69
2.4.11.	Conservation approaches used to protect medicinal enset	72
CHAPTER THREE.....		75
3. Micronutrient composition and microbial community analysis across diverse landraces of the Ethiopian orphan crop enset.....		75
Abstract.....		76
3.1.	Introduction.....	77
3.2.	Material and methods.....	80
3.2.1.	Sample collection and preparation	80
3.2.2.	Micronutrient and mineral composition.....	82
3.2.3.	Crude protein and FAA composition	84
3.2.4.	Genomic characterization of enset fermentation.....	85
3.3.	Results and discussion	86
3.3.1.	Sample collection and processing	86
3.3.2.	Inorganic composition of enset and comparison with other regional crops.....	88
3.3.3.	Organic composition and the nutritional implications of enset fermentation	89
3.3.4.	Microbial community characterization and signatures of contamination	93
3.4.	Conclusion	97
CHAPTER FOUR.....		100
4. Reproductive biology of wild and domesticated Ensete ventricosum: Further evidence for maintenance of sexual reproductive capacity in a vegetatively propagated perennial crop.....		100
Abstract		101

4.1.	Introduction.....	102
4.2.	Materials and methods	107
4.2.1.	Sample collection.....	107
4.2.2.	Floral trait variance and allometry	109
4.2.3.	Seed morphology and viability	110
4.2.4.	Germination trials	111
4.3.	Results.....	113
4.3.1.	Observations, collection, processing and storage	113
4.3.2.	Floral trait allometry	114
4.3.3.	Seed morphology and viability	116
4.3.4.	Germination trials	116
4.4.	Discussion	120
CHAPTER FIVE		126
5.	General discussions, conclusions and recommendations.....	126
5.1.	General discussion	126
5.2.	Conclusions.....	131
5.3.	Recommendations.....	134
References cited.....		137
Appendices.....		158

List of tables

Table	Page
Table 1: Major plant parts and processed products of enset used in traditional medicine, %FC=Percent of citation frequency	39
Table 2: Cooking and fermentation state of enset parts or products used in traditional medicine, %FC=Percent of citation frequency	40
Table 3: Fementation of plant parts of enset used as remedies against human ailments	40
Table 4: Additives use with enset parts or their products in traditional medicine	41
Table 5: Route of administration of enset as traditional medicine in humans, %FC=Percent of citation frequency	42
Table 6: Parts and/or processed products of enset used in ethnoveterinary medicine	46
Table 7: Mode of preparation, route of administration and livestock treated in using enset in ethnoveterinary medicine	47
Table 8: Route of administration of enset as traditional medicine in treating livestock ailments	48
Table 9: Enset tissue and food product types evaluated in this study	82
Table 10: Wild & domesticated enset accessions used for seed morphology & germination analysis	108
Table 11: Wild and domesticated enset accessions sampled for floral morphology analysis.	109
Table 12: Comparison of variance and means for enset floral and seed morphology traits 1Holm correction applied to p-value significance	115
Table 13: Permutational multivariate ANOVA using distance matrices (PermANOVA) results for enset floral morphology with origin (domesticated versus wild) and landrace as explanatory factors	117

List of figures

Figure	Page
Figure 1: Map of the area showing Dara (Sidama), Kindo-Didaye (Wolaita) & Enemore & Enir (Gurage) districts all under the former SNNPRS	28
Figure 2: Species-accumulation curve adopted and used to estimate turnover of medicinal enset uses information per extra efforts made in interviewing	33
Figure 3: Cluster analysis for grouping farmers' landraces based on similarity ratios using traits from morphological, agronomic & end-user quality preferences	37
Figure 4: Therapeutic categories of human ailments & their citations frequencies (%FC)	44
Figure 5: Disease categories of ethnoveterinary importance treated using medicinal enset with their percent frequency of citations (%FC)	46
Figure 6: Concentration of inorganic nutrients (minerals) in enset corm tissues:	87
Figure 7: Comparison of enset tissue inorganic micronutrients (corm & pseudostem) with regionally occurring tubers and cereal crops.	90
Figure 8: Free AA composition and variation in enset.	92
Figure 9: Free AA composition in raw enset tissues (pseudostem and corm) and processed food products (kocho and bulla).	95
Figure 10: Microbial composition of enset kocho samples.	96
Figure 11: Enset cultivation in Ethiopia.	105
Figure 12: Redundancy analysis of male floral morphology in Enset. Solid points denote means aggregated by sample; lighter points show variation in floral traits.	117
Figure 13: Percentage germination across a range of environmental conditions for wild & domesticated enset (Experiment 2) after 120 days.	118
Figure 14: Germination behaviour and regional climate variables for wild and domesticated enset in Ethiopia.	119
Figure 15: Analysis of the influence of temperature shifts and dormancy on domesticated enset germination (Exp. 3 and 4).	120

List of appendices

Appendix	Page
Appendix 1: List of farmers' landraces reported & observed on crop fields (or home garden) with medicinally valuable landraces for treatment of human & livestock ailments	158
Appendix 2: Interactions with the local farmers during collection of ethnobotanical information through interviews, group discussions, guided garden walks and focus group discussions	161
Appendix 4: Interview items used for collection of indigenous knowledge on utilization, management and conservation of enset in the study area	162
Appendix 5: Items used for collection of ILK and perception of local people on enset use & management from the key informants	171
Appendix 6: Items used for collection of information on enset use through focus group discussion	172
Appendix 6: List of informants used as source of ethnobotanical information using semi structured interview interview	173
Appendix 7: Some of sample preparation and analysis events for nutritional composition studies	176

List of acronyms and abbreviations

AMT	Annual Mean Temperature
ANOVA	Analysis of Variance
CFSN	Center for Food Science and Nutrition
CIPD	Certain infectious and parasitic disease
DDS	Diseases of the digestive system
DGUS	Diseases of the genitourinary system
DMR	Direct Matrix Ranking
DMSCT	Diseases of musculoskeletal system & connective tissue
DBBFOIS	Diseases of blood and blood-forming organs & immune systems
DRS	Diseases of the respiratory system
DSST	Diseases of the skin and subcutaneous tissue
FAA	Free Amino Acid
FC (% FC)	Percentage frequency of citations
FL	Fidelity Level
GBIF	Global Biodiversity Information Facility
GCRF	Great Challenges Research Fund
ICF	Informant Consensus Factor
ILK	Indigenous Local Knowledge
IPCCEC	Injury, poisoning & certain consequences of external causes
MDR	Mean Diurnal Temperature
NCIB	National Center for Biotechnology Information
PCPD	Pregnancy, childbirth & the puerperium disorders
PermANOVA	Permutation Analysis of variance
RH	Research hypothesis
RQ	Research question
SNNPRS	Southern Nations, Nationalities and Peoples Regional State
SO	Specific objective
SWG	Sidama, Wolaita and Gurage study sites
tGBS	Tunable Genotyping-by-Sequencing

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General Abstract

Enset (*Ensete ventricosum* (Welw.) Cheesman, Musaceae): ethnomedicinal applications, nutritional quality, and reproductive biology in selected districts of South and South western Ethiopia

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Enset is a valuable food security crop consumed by over 20 million peoples among diverse cultures in Southern Ethiopia. Limited information exists on morphological characterization of landraces used in traditional medicine and the associated knowledge, nutritional contents, seed germination responses to various temperature regimes and floral biology of wild and domesticated populations. Morphological characterization of medicinally valued enset and documentation of Indigenous Local Knowledge (ILK) of their utilization was conducted using 100 informants and home gardens from nine study kebeles in the three selected districts of Sidama, Wolaita and Gurage (SWG) zones of the former SNNPRS. Nutritional analyses targeted on mineral and amino acid profiles was undertaken for various types of enset parts (corm, pseudostem, leaf petiole, leaf blade, fruit parts of exocarp, mesocarp and whole seed) and fermented products (Kocho, Bulla and whole Koho) (n=10) of 26 medicinally valued enset landraces. Fermentation was initiated using pre fermented enset tissue used as starter culture, and both external and pit fermentation were applied based on local recommendations. Germination responses of 20 accessions (seven wild and 13 domestic enset) originated from localities in the south and south western Ethiopia were tested against varied temperature regimes (10-°C, 15-°C, 20-°C, 25-°C, 30-°C, 40-°C, 20-°C/10-°C, 25-°C/10-°C, 25-°C/15-°C, 30-°C/-10°C, 30-°C/15-°C, 30-°C/20-°C, 35-°C/20-°C, 40-°C/20-°C & 40-°C/25-°C). The two enset populations were also evaluated for variations in traits of external and internal seed morphology (n=10 to 50) and seed viability (n=7 to 60 per accession). Diversity in floral

morphology of individuals from the two populations, was additionally, assessed using dimensions of 375 flowers, (i.e. 25 from each accession), sourced from 15 matured (nine domesticated and six wild) individuals. Medicinally valed enset landraces were clustered based on morphological similarities irrespective of sampling localities. Out of 184 farmers' landraces documented, 68 (37%) were used in traditional medicine. Boiled corms from 'Askala' (Sidama), 'Argama' (Wolaita), 'Astara' (Gurage), 'Kibnar' (Gurage), and 'Gowariye' (Gurage) are most frequently used as medicinal items in the treatment of human and livestock ailments. Delays in labor and/or placental discharge, bone fractures and wounds were the commonly treated disorders using enset medicine. Prenatal, natal and postnatal complications received the highest Informant Consensus Factor (ICF=0.86). Enset contains considerable calcium, iron, potassium and, zinc in comparison to commonly used tuber crops. Twenty of standard plant amino acids were detected with predominant arginine. Fermentation enhanced essential amino acids, though net loss overall were observed after the process. Enset showed significant variations in mineral concentrations across sampling sites and plant parts. Samples sourced from Gurage area and corms, hence, showed significantly higher amino acid. Alternating temperature, absolute changes from constant to alternating ($p=0.0159$), and prior cold stratification ($p \leq 0.001$) significantly improved germination. Wild and domesticated enset showed similarities in embryo viability, mean days to onset of germination, and morphological traits of seeds and flowers. Eventhough, different names are given in various cultures, medicinally valuable enset were morphologically similar across cultural variations in landrace naming and farm management, observed less frequent or rare; and used to treat diverse types of human and livestock ailments. Observations from ethnobotanical investigations imply the potential of enset for treatment of human and livestock ailments in the context of complimentary medicine to target the limitations of health care services through modern medicine. With the observed higher nutritional contents, enset products could be used to make up for deficiencies in essential minerals and functional amino acids. Conservation of sexual reproductive potential in enset was evidenced from similarities in germination and morphologies of reproductive organs of wild and domestic plants, with implications for future seed banking and varital development through conventional breeding.

Key Words/Phrases: Amino acid profiles, Crop domestication, Enset ethnobotany, Floral morphology, Indigenous Local Knowledge, Mineral composition, Seed germination

CHAPTER ONE

1. General introduction

1.1. Background and justification

Enset (*Ensete ventricosum* (Welw.) Cheesman) is a multipurpose, monocarpic, perennial herbaceous plant recommended for applications in traditional medicine against human and livestock ailments. Certain farmers' landraces were mentioned for local utilizations in various ways as traditional medicinal items. The diversity of ailments targeted using enset as traditional treatments include bone fractures, delays in childbirth and the placenta, stomach disorders, swellings, and unintended pregnancy (Kedir Abdella et al., 2017). Farmers' landraces with deeply colored pseudostems and leaves have been especially applied in the treatment of a diversity of ailments and health complications. 'Tayo' (Kefa), 'Choro' (Kefa), 'Lochingiya' (Wolaita), 'Askala' (Sidama), and 'Qeqille' (Kembata) have reddish, purplish or pinkish vegetative parts harvested for various medicinal applications (Yemane Tsehay and Fassil Kibebew, 2006; Temesgen Magule et al., 2014; Amare Seifu and Daniel Fitamo, 2016; Melesse Maryo et al., 2018). Not only the uniquely-coloured farmers' landraces, but also those lacking distinctive surface chroma provide medicinal values. 'Chacho' (Sidama), 'Tunako' (Sidama), 'Gena' (Wolaita), 'Hala' (Wolaita), 'Gefeteno' (Wolaita), 'Kibnar' (Gurage), and 'Gowariye' (Gurage) have medicinal properties though they lack distinctive surface pigments (Per. Comm and field observation, Solomon Tamrat). So far no clues were found for genetic differentiation between landraces used in traditional medicine and for other use values (Gizachew woldesenbet et al., 2022). The same report also mentioned that medicinal properties are restricted to a limited numbers of genotypes; and are sourced from interactions of these genotypes with the environment. Apart from these fragmented pieces of information, comprehensive investigation and documentation of the depth of ILK on medicinal applications and characterization of cultural diversity of the existing landraces used as traditional medicine were lacking.

The available reports on use of enset as traditional medicine indirectly highlight the presence of bioactive phytochemicals or micronutrients responsible for disease treatment and healing (Azene Tesfaye and Tesfaye Girma, 2017; Solomon Workineh and Neela, 2019; Ly et al., 2023). The biochemistry of enset ranges from primary cellular products like carbohydrates, proteins or amino acids to secondary cellular outputs including antioxidants (Holscher and Schneider, 1998; Sahaa et al., 2013; Vasundharan et al., 2013; Singh et al., 2016; Solomon Habtemariam and Varghese, 2017). These biochemical contents impart high nutritional and functional values indicated through utilizations in treatments of various ailments. A few studies exist on nutritional composition of fresh plant parts and processed food products of enset used as human diet or livestock feed (Mohammed et al., 2013; Abraham Bosha et al., 2016). High carbohydrate, and hence energy values, but lower proteins and fats were the common features of nutritional contents of enset (Ajebu Nurfeta et al., 2008a and b; Abraham Bosha et al., 2016; Borrell et al., 2020). Higher calcium, potassium, magnesium, zinc and physiological AAs were reported from enset products (Minaleshewa Atlabachew and Chandarvanshi, 2008; Mohammed et al., 2013; Admasu Tsegaye, 2015). Minaleshewa Atlabachew and Chandarvanshi (2008) evaluated minerals from ‘Kocho’ and ‘Bulla’, whereas Mohammed et al. (2013) analysed amino acid profiles for unspecified farmers’ landraces. In addition to these reports, Ajebu Nurfeta et al. (2008a) evaluated variations in mineral contents of enset used as fodder based on landrace variations, while Ayalew Debebe et al. (2012) quantified minerals from anonymous enset landraces. Previous studies on nutritional composition of enset were focused on common uses of the crop as food or fodder and no attempt was made in relation to its medicinal application. Hence, limited information existed towards characterization of enset used in traditional medicine at landrace level for mineral composition and AA profiles.

The germplasm for medicinally valued enset is maintained on cropping fields in a heterogeneous mix of landraces commonly used for food. Local people select and maintain farmers’ landraces with desired traits including high quality edible products, medicinal values, better agronomic traits, or preferred plant morphologies. The usual method of clonal propagation of enset locally by which partitioned corm for which the apical growing tip was removed is encouraged to produce profuse sprouts of seedlings (Karlsson et al., 2015) has several challenges. Different biotic and abiotic factors are reported so far to cause a rapid loss of the germplasm during propagation using saplings

generated from corms. Rapid onset of *Xanthomonas* wilt, root-mealy bugs and abrupt warming or cooling of local temperatures can lead to total loss of enset germplasm (Eckert, 2002; McKey et al., 2010; Barrett, 2015; Adane Abraham, 2018). The *in-situ* conservation practice of the existing enormous cultural diversity is, therefore, not sustainable. Hence, the situation demands or calls for alternative and efficient wayouts for germplasm multiplication and conservation of the crop plant. Propagation through seeds and banking these in cold rooms, on the other hand, are safer and untapped alternatives to address these challenges of *in-situ* conservation through clonal multiplication (Walter and Pence, 2021; Wamberg et al., 2023). Multiplication using seeds could maximise production to commercial scales, allows for genetic improvement through conventional breeding and favors for safer germplasm conservation.

Little attempts, however, had been made towards sexual propagation and conservation of the existing cultural diversity of enset via seed banking. Predation of mature fruits by frugivorous birds or primates and perceived lower rates of seed germination among the farmers (Pers. Comm, Solomon Tamrat) have impeded the seed-based production and banking of domesticated enset. Moreover, little information exists on studies related to comparative seed morphology and germination performance for cultivated and wild enset. Due to the above mentioned challenges, farmers entirely depend on clonal multiplication and hence regional seed banks lack seeds sourced from domestic populations (Guzzon and Muller, 2016). Contrary to this scenario, a few trials had been observed towards germination of enset using botanical seeds. Socking in cold or hot water and aqueous sulfuric acid (H_2SO_4), sodium hydroxide (NaOH), ammonium nitrate (NH_4NO_3), and sodium hypochlorite (NaOCl) showed no significant changes to germination (Karlsson et al., 2013). The same study showed that scarification, i.e, removal of the operculum or pretreatment with 70% ethanol showed significantly negative effects to seed germination in enset. Seeds of *Musa accuminata*, a related species of the same plant family produced viable embryo adapted to alternating day and night temperatures (Kallow et al., 2021). Following these observations, different sets of alternating and constant temperature regimes were targeted as key requirements for better germination in wild and domestic enset in the current study.

Prolonged clonal propagation is expected to lead to loss of sexual reproductive traits in enset as explained based on observation from other perennial crops (Denham et al., 2020; Kallow et al., 2021). Maintenance of sexuality is a key criterion for commercial seed production, selection and improvement of farmers' landrace for desired traits, and *ex-situ* conservation through seed banking. The conservation of sexual reproductive potential in domesticated enset against longer generations of clonal propagation could be investigated using trait variations in seeds and flowers. Diversity in seed weight and volume, total seed area, thickness of seed coat and the endosperm with seed viability and germination of wild and domestic enset can provide valuable evidences. Moreover, the hypothesis can be tested through analysis of variations in quantitative traits of floral morphology for wild and domestic enset (Elias et al., 2000, 2001; McKey et al., 2010). Evaluations of variations in length and width of floral parts including stamens, pistils, and the perianth of wild and domestic plants could also assist in testing conservation of sexual reproduction in cultivated populations.

1.2. Brief literature review

1.2.1. Traditional medicinal applications of enset and some wild plants of the *Ensete* genus

Pijls et al. (1995) reported that, enset has been used as traditional medicine among the Gurage people in southern Ethiopia, where root parts were used to treat nematodes. Farmers' landraces with reddish vegetative parts showed medicinal properties against cirrhosis and sexually transmitted diseases (Admasu Tsegaye, 2002). They were also used to induce birth or abortion and facilitate placental discharge after labour in humans and livestock (Temesgen Magule et al., 2014; Yemane Tsehaye and Fassil Kebebew, 2006; Amare Seifu and Daniel Fitamo, 2016). Boiled corms of a farmers' landrace of enset named 'Lochingiya' (Wolaita) and 'Qeqille' (Kembata) served as a birth control or abortifacient and cleansers of the uterus through facilitated placental discharge (Amare Seifu and Daniel Fitamo, 2016; Melesse Maryo et al., 2018). Moreover, some farmers' landraces were reported for being selectively used in the mending of bone fractures and the treatment of joint and muscle cramps and displacement of joints after trauma (Melesse Maryo et

al., 2018). ‘Tayo’ (Kefa), ‘Choro’ (Kefa), and ‘Officho’ (Kefa) are importantly utilized to treat trauma related complications (Yemane Tsehaye and Fassil Kebebew, 2006). Furthermore, ‘Bulla’ from ‘Agina’ (Wolaita) and ‘Gefeteno’ (Wolaita) have been used for strengthening of the maternal body after delivery and in mending bone fractures, respectively. ‘Laqaqqa’ (Kembata) has been used in the treatment of boils, while freshly harvested plant sap from ‘Hargama’ (Kembata) and ‘Moche’ (Kembata) has been used against skin fungal disorders (Melesse Maryo et al., 2018). Furthermore, ‘Chacho’ (Sidama) treats coughing, whereas ‘Tunayicho’ (Sidama) acts against malaria and complication with symptoms of discharging of bloody urine (Amare Seifu and Daniel Fitamo, 2016). A strongly fermented ‘Kocho’ from ‘Maziya’ (Wolaita) and ‘Hala’ (Wolaita) was also reported to relieve stomach cramps and related disorders (Temesgen Magule et al., 2014). This makes the available cultural diversity of enset valuable in the day to day life of local peoples in disease treatment for different human and livestock ailments.

Enset has been mentioned for its values in the treatment of some livestock ailments directly or indirectly through reports containing part of its biochemistry in a few of previous reports. Landraces commonly applied as livestock medicine are those having reddish or pikish vegetative parts such as ‘Askala’ (Sidama), ‘Mundiraro’ (Sidama), ‘Lochingiya’ (Wolaita), ‘Astar’ (Gurage), ‘Choro’ (Kefa), and ‘Qeqille’ (Kambata). The commonly reported ailments of ethnoveterinary medicine using enset as medicinal items include delayed delivery or ejection of the foetus and the placental tissue (Yemane Tsehaye and Fassil Kibebew, 2006). Minimal or poor milk secretion, if it was not production, muskuloskeletal disorders of bone fractures were also mentioned from previous works (Kedir Abdela et al., 2018). Some of its applications are related to the high nutritional contents of different fractions of enset parts used as fodder and they provide nutraceutical significances. Abraham Bosha et al (2016) reported higher energy, carbohydrate and crude protein values to wild and domestic enset which might imply applications as energy sources to facilitate delayed birth and placenta in cattle. Similarly, Ajebu Nurfet et al (2018) reported that enset contains higher amounts of calcium, phosphorus and other macro and micro minerals essential for animal health. The utilization of enset in the treatment of musculoskeletal disorders where bone fractures are the dominant might be associated to high minerals those are involved in the process of bone tissue formation.

These traditional medicinal properties and their cultural applications were reported not only for enset but also for plants of a sister species from the Musaceae family. Tangjitman et al. (2015) reported that *E. glaucum*, a close wild relative of enset, is used against food poisoning and associated diarrheal complications. That same species was reported for its therapeutic values to alleviate swelling and fatigue in Myanmar and Laos (Ochiai, 2012). Another wild relative of enset, *E. superbum*, was used against dog bites, calculi, leucorrhea, stomach aches, dehydration, appendicitis, chickenpox, measles, and urinary problems (Sethiya et al., 2019). *E. superbum* was also used to induce inflammatory responses and semen production. Applications as antipain, antidiabetic, antipneumonic, anticholera, antipsychosomatic, contraceptive, and wound healing were the other commonly mentioned uses of *E. superbum* (Solomon Habtemariam and Varghese, 2017). Plants of the same species are also used against disorders related to food poisoning, snake bites, diarrhoea, dysentery, jaundice, bone fractures, skin infections, fever, asthma, hiccups, leukoderma, and labour and delivery-related pains. Major bioactive phytochemicals with high pharmacological values were reported from *E. superbum* and *E. glaucum* (Vasundharan et al., 2013; Sethiya et al., 2019). These observations presuppose and provide evidence for the actual and potential utilisation of enset as an important ingredient in traditional medicine. These huge untapped potentials of traditional medicinal applications and pharmacological values should be studied to enhance maximised utilisation.

1.2.2. Enset production in relation to food security: nutritional, biochemical and microbiological contents

Enset is a valuable crop consumed as a staple food among different ethnic groups at various parts of southern Ethiopia (Zerihun Yemataw et al., 2016; Zerihun Yemataw et al., 2017; Borrell et al., 2020). These plants are dominantly cultivated among farming societies located in the former administrative ranges of SNNPRS and parts of Oromia and Gambella regions. Communities from several administrative units were involved for many years in the past in enset production that signify the value of the plant for subsistence use. About 20 administrative zones and two special districts

with diverse ethnic groups were engaged in enset-based farming for their livelihoods (CSA, 2015). The higher linguistic and cultural diversity and ranges of enset-based farming systems have led to the establishment of large arrays of diversity in farmers' landraces of the crop plant, its use cultures, and cultural conservation practices.

Due to its drought tolerance and high per-hectare production, enset has been identified as a food security item in Ethiopia (Admasu Tsegaye, 2002; Borrell et al., 2020; Sulaiman et al., 2022). Harvestability at any growth stage puts the crop suitable to serve as emergency food during losses in annual production. Moreover, traits of disease resistance (Befikadu Haile et al., 2022; Gizachew Wolde-Michael et al., 2008) and draught tolerance (Brandt et al., 1997) make the crop critically important in filling food gaps at times of massive wild fire or drought. Furthermore, the diverse gene pool of enset served to address food security problems during emergency times at the onset of war and human migrations in many years (Brandt et al., 1997; Oyen and Lemmens, 2002). Enset-based farming systems supported the highest human population densities on the highlands of south and south-west Ethiopia, rated up to 1300 people per square kilometre in the Gedeo zone (Abiyot Legesse, 2013). Such huge potential serves as a strong buffer for an ever-increasing human population in enset production areas. Furthermore, enset-based foods are known for their high stomach-filling capacity and higher starch contents as immediate sources of energy (Abraham Bosha et al., 2016). Hence, enset-based food products assist the local people in better handling their high-energy-demanding day-to-day activities, including farming and the preparation of enset foodstuffs.

The consumable products of enset used as human food originate from two basic food storage organs of the plants: the underground short stalks of the corm and the upright cylindrical pseudostem containing several leaf-sheathes clasped together forming a false stem. In the process of 'Kocho' preparation, corms are pulverised and made into smaller pieces using solid local items made of wooden or skeletal materials (Shigeta, 1990; Pijls et al., 1995; Admasu Tsegaye, 2002). Meanwhile, pseudostems are decorated with toothed bamboo or metallic items to collect the edible cortical tissues from the fibrous sheath. The scrapings will be pressed or squeezed fresh to extract

'Bulla'. 'Bulla' is an insoluble starchy powder (Tsigie Gebremariam et al., 1992), which settles from fluids collected from a fresh tissue of the scrapped pseudostem and gratted corms. Raw 'Kocho' is allowed for fermentation with a starter culture after it is mixed and buried in an earthen pit, while 'Bulla' is first rapped completely with fresh enset leaves and buried within the mass of fermenting 'Kocho'. The fermentation pit will be lined with fresh enset leaves and leaf sheaths prior to fermentation to enhance food quality avoiding of contamination with soil particles and enhancement of maturity as well as taste. Eventually, the fresh tissue mixture of 'Kocho' and separately stored 'Bulla' changes into a mesh of food products suitable for various preparations, including breads, pan cakes, porridges, and gruels (Temesgen Magule et al., 2014; Genet Birmeta et al., 2019). The process of enset fermentation varies among ethnic groups and cultures in south Ethiopia (Belachew Garedeew et al., 2017; Tariku Hunduma and Mogese Ashenafi, 2011). Some reports mentioned that starter cultures were prepared solely from plant parts sourced from a young enset plant (Tiruha Habte et al., 2014). Abraham Bosha et al. (2016) mentioned the application of *Rumex* spp., *Trigonella* spp., *Allium* spp., *Musa* spp., *Ocimum* spp., *Lippia* spp., and *Nigella* spp. to prepare the starter. Furthermore, a starter for fermentation was possibly induced using plant parts of enset left freely open in the environment for some days before pit fermentation (Genet Birmeta et al., 2019).

The available reports on use of enset as traditional medicine indirectly highlight the presence of bioactive phytochemicals and essential micronutrients those contribute for disease treatment and healing (Azene Tesfaye and Tesfaye Girma, 2017; Solomon Workineh and Neela, 2019; Ly et al., 2023). The nutritional composition of freshly collected plant parts or fermented products used in human diet or livestock feed have been reported (Mohammed et al., 2013; Abraham Bosha et al., 2016). High carbohydrate and hence energy, but lower values of protein and fat, were commonly reported nutritional properties of enset (Ajebu Nurfeta et al., 2008a, b; Abraham Bosha et al., 2016; Borrell et al., 2020). Higher essential minerals including calcium, potassium, magnesium, and zinc as well as functional AAs from plants are common attribute of enset (Minaleshewa Atlabachew and Chandarvanshi, 2008; Mohammed et al., 2013; Admasu Tsegaye, 2015). In addition to the above-mentioned contents, antioxidants, carotenoids, biogenic amines, phytosterols, alkaloids, saponins, phenols and tannins were detected in enset (Holscher and Schneider, 1998; Sahaa et al.,

2013; Vasundharan et al., 2013; Singh et al., 2016; Solomon Habtemariam and Varghese, 2017; Tewodros Birhanu et al., 2023). Holscher and Schneider (1998) have identified phenylphenalenone, a novel bioactive ingredient known for antibacterial, anticancer, and antinematocidal effects from enset. Abebe Dukessa et al (2021) reported drug induced hepatoprotective and anti-nephrotoxic activities in rodents from corm extracts of *E. ventricosum*. Sethiya et al (2017) reported antiurolithiatic activity induced by accumulation of calcium oxalate using pseudostem extracts of enset in rodents.

The diverse bioactive phytochemicals screened from enset are known for high antioxidant properties, biological activity that is necessary in the process of disease treatment. The traditional application of enset in the treatment of diverse types of health disorders is associated to these biological molecules abundant in fresh and fermented plant tissues. Minaleshewa Atlabachew and Chandarvanshi (2008) evaluated minerals from 'Kocho' and 'Bulla', whereas Mohammed et al. (2013) analysed amino acid profiles for anonymous enset landraces. Some reports also include landrace-based evaluations of mineral contents of enset used in fodder (Ajebu Nurfeta et al., 2008a), while others analysed enset minerals of unspecified farmers' landraces (Ayalew Debebe et al., 2012). Most of studies related to biochemical characterization of enset, however, focused on the general use of enset as food or fodder; and a few include landrace based characteriations (Ajebu Nurfeta et al., 2008; Hiwot Bekele et al., 2023). Hence, limited attempts were made towards characterization of minerals and AAs for farmers' landraces of enset selectively used in traditional medicine.

The chemical composition of enset-based products is altered via fermentation in various ways (Borrell et al., 2020). Fermentation increases the nutritional quality of enset by degrading anti-nutritional factors and complex polymers. Complex substances such as anti-nutritional factors, carbohydrates, and proteins are normally used as food sources for microbial growth in the course of the process (Tamang et al., 2016a). The pH and titrable acidity showed a decreasing and an increasing trend, respectively, with fermentation time (Addisu Fekadu et al., 2018). Antioxidant and phenolic contents of 'Kocho' were observed to be higher than those of non-fermented 'Kocho'

and fresh corms (Sirawdink Fikreyesus et al., 2013; Engeda Dessalegn, 2019). Amounts of dry matter, crude fiber, carbohydrate, sugars, crude fats, crude proteins, Ca, K, Mg and Mn were significantly reduced along fermentation time (Azera Tsegaye et al., 2020a, Azera Tsegaye et al., 2020b). On the contrary, total ash, Fe and Zn were depleted after fermentation. Chemical products of fermentation and the derived medium are important for the safety and quality of the resultant food products. For example, fermentation reduces or avoids the multiplication of spoilage microbes which are toxic to human health in the system in general (Tamang et al., 2016a).

The development of microbial communities in ‘Kocho’ leads to enhancement in the nutritional quality and bioavailability of food products during fermentation (Chelule et al., 2010). Considering the microbial dynamics which is poorly studied, however, different groups of microorganisms play roles during the start and end of the process (Genet Birmeta et al., 2019). *Streptococcus faecalis* and *Leuconostoc mesenteroides* start the dynamics of microbial communities in ‘Kocho’ fermentation (Birhanu Abegaz, 1987). However, during the process these microbes are suppressed by *Lactobacillus coryniformis* subsp. *coryniformis* and *L. plantarum*. The later bacterial populations lead to further reductions in pH, making the fermentation medium more acidic. From a recent investigation by Helen Weldemichael et al. (2019), it was known that the predominant microorganisms associated with enset fermentation are *Lactobacillus* and *Acetobacter* spp. In spite of this, microbial members of Enterobacteriaceae and *Leuconostoc mesenteroides* subsp. *cremoris* have a high relative abundance at the early stages of fermentation (Addisu Fekadu et al., 2018). Characterization of the microbiology of ‘Kocho’ is important for maintenance of safety and quality of food items derived through fermentation of enset.

1.2.3. Traditional versus modern enset propagation methods

Enset is commonly multiplied using conventional vegetative propagation through corm cutting in South Ethiopia. Plants of domesticated population are entirely propagated through corm cutting and sucker formation (Borrell et al., 2020). Burying of corms of which the actively growing meristematic tips are removed has invariably resulted in lateral buds producing multiple sprouts

(Karlsson et al., 2018). Sucker initiation is hence achieved through disturbing or killing of shoot tips to remove apical dominance, which otherwise leads to formation of a single shoot (Mulugeta Diro et al., 1996). Once the apical dominance of a young enset corm is removed, it could be buried in loose soil of about 20–30 cm deep. The corm can be buried in the soil either intact or subdivided into two, four, or eight parts, though the one divided to four parts produces more number of suckers (Karlsson et al., 2018; Bewuketu Haile et al., 2021). Some farmer's landraces have a high rate of sucker formation, providing clues for genetic differences in the success of sprout formation. Based on reports by the same authors, watering has a positive effect on the rapidity of sprout emergence lowering the average time required for suckering. Application of well-mixed dung also enhances nutrient release and facilitates shoot sprouting during clonal multiplication of enset (Taye Bezuneh and Asrat Feleke, 1966; Zerihun Yemataw et al., 2014). Based on reports by Abraham Bosha et al. (2019), types of farmers' landraces and the amount and depth of manure or fertiliser applied have a significant positive effect on the number of suckers produced. A 4-6 kg of dry weight, i.e., ca 7–11 dm³ of air-dried and pulverised manure thoroughly mixed with softened field soil and placed directly in the pit hole at Areka Enset Research Center, have been recommended for efficient suckering (Abraham Bosha et al., 2019). According to Zerihun Yemataw et al. (2018b) application of cattle manure and humus adjacent to a treated corm will help the immediate release of nutrients to the emerging sprouts. This also results in healthy and robust suckers with successful establishment after transfer to an independent plot.

Wild enset, on the other hand, spontaneously propagates through seeds in its wild habitats, while almost exclusively no attempts are known for seed-based multiplication of domestic enset. The starch-rich, massive endosperm of a well-matured seed contains a small embryo that would germinate in the seed coat. According to Karlsson et al. (2013), endosperm is utilised as an energy source during the start of germination, which continues to nourish the embryo after breaking of the seed coat. The same authors reported that the average germination success was not significantly different between seed lots from wild and domestic enset. The period to reach germination of 50% of the consumed seeds was 8.5 weeks, and germination ceased after 28 weeks of incubation (Karlsson et al., 2013). Neither morphological nor physiological seed dormancy was observed for enset seeds treated with different chemicals. The treatments include immersion in hot and cold

water, sulfuric acid (H₂SO₄), sodium hydroxide (NaOH), ammonium nitrate (NH₄NO₃), and sodium hypochlorite (NaClO) (Karlsson et al., 2013).

Studies related to the seed germination of enset are bottlenecked by the reduced availability of seeds (Guzzon and Müller, 2016). Access to seed materials of enset across its wild and cultivation ranges for genus *Ensete* in general and *E. vetricosum* in particular posed a major problem for conducting germination trials. Recently, an attempt was made to search for enset seeds in gene banks, seed banks, and databases of plant genetic resources. According to the same report, only an institute, i.e., the Millennium Seed Bank of the Royal Botanic Gardens (Kew), was found preserving some seeds of enset in cold rooms. Moreover, access to fresh and well-ripened fruits, matured enset seeds, and cultivation or harvesting of these was limited. This challenge has posed problems for studies targeted at sexual multiplication, seed production, varietal development, and seed banking of the valuable crop plant enset.

An alternative way of enset multiplication is through in-vitro embryo and tissue culture micropropagation techniques, which is a costly and high-tech demanding process. Genet Birmeta and Welander (2004) showed up to 75 healthy shoots per an ex-plant through meristem wounding and shoot initiation. Studies also reported optimal conditions for multiplication of enset using embryo and shoot tip culture. About 71% germination success for excised embryos and the formation of 100 to 254 shoots after 16 weeks of incubation were evident from another report (Almaz Negash et al., 2000). In a similar investigation by Tripathi et al. (2017), higher rates of shoot induction were observed in proliferation medium containing lower amounts of 6-Benzylaminopurine. Tissue culture was found more important for clonal propagation of enset than the conventional vegetative method of multiplication through corm cutting. In-vitro aseptic techniques, along with protocols of genetic engineering, assist in the preparation of disease-free lines of enset plants. It also helps in the control of the transfer of contagious diseases, including bacterial wilt (Genege Gezahegne and Firew Mekbib, 2016; Ibsa Merga et al., 2019; Matheka et al., 2019). Moreover, lab-based techniques generate enset with desired traits or farmers' landraces containing collections of desirable qualities (Mulugeta Diro and van Staden, 2003). Tissue culture

is also important in the process of conservation or preservation of the enset genome through the preparation of multiple seedlings at commercial scales (Saraswathi et al., 2016). Tissue culture supplements conservation of the germplasm through *in-situ* field gene banking or cryopreservation of excised embryos (Almaz Negash et al., 2000).

Multiplication of enset through in-vitro micropropagation techniques of tissue culture generates genetic variants after mutations via callus formation (Tripathi et al., 2017). The useful mutant traits generated could be conserved or applied for in-vitro somatic hybridization and recombinant DNA technology in the process of improving enset (Matheka et al., 2019). Application of these techniques would shorten the long generations of enset populations required for genetic modification through conventional breeding using botanical seeds (Almaz Negash, 2001; Ibsa Merga et al., 2019). Techniques of shoot tip, callus, and zygotic embryo culture also assist in clonal multiplication and genetic improvement of enset and other valuable crops (Mulugeta Diro and van Staden, 2003; 2004). A 100% successful acclimatisation of enset propagules was reported using young corms as ex-plants under the Murashige and Skoog (MS) medium (Tripathi et al., 2017). This observation has indicated that tissue culture is a better alternative for the development of enset germplasm and for *ex-situ* conservation through cryopreservation against clonal multiplication. However, biotic or abiotic challenges against corm based multiplication, technological demands and problems of contamination from *in-vitro* techniques calls for efficient protocols for propagation of enset. Seed based multiplication, hence, provides chances for genetic mixing, varietal selection and improvement as well as conservation in cost effective and efficient *ex-situ* conditions in cold rooms.

1.3. Statement of the problem

Enset plays great roles in the treatment of diverse types of human and livestock ailments in addition to its application as food security crop in southern Ethiopia. Beyond 300 farmer's landraces with food and medicinal values were maintained by local farmers (Zerihun Yemataw et al., 2016; Zerihun Yemataw et al., 2017). However, characterizations of farmers' landraces that are used for traditional medicine and the associated ILK for their application have not been studied. In addition

to lack of investigations targeted on medicinal use, management through utilization of existing diversity of the indigenous crop as remedies is under threat. The impacts of rapidly changing climates and the onset of disease and pests are the basic problems reported so far (Adane Abraham, 2018). Valuable farmers' landraces of enset are diminishing as a result of shifts in agricultural practices these days. Subsistence crops of higher food values are being replaced by cash crops including coffee and khat (Tesfaye Abebe, 2013; Teshome Yirgu, 2016; Beyene Teklu et al., 2018). Therefore, characterizations of medicinally important enset, and documentation of the associated ILK and their applications in disease treatment before complete loss of the germplasm and the associated ILK are parts of the present study.

Dominant applications of indigenous crop varieties and farmer's landraces are linked to the unique nutritional or phytochemical contents of plant tissues, organs, or their processed products (Ghosh-Jerath et al., 2023; Akinola et al., 2020; Mungofa et al., 2022; Sultanbawa et al., 2022). The traditional use of enset as medicinal items in southern Ethiopia presupposes a sort of promising biochemical content of selected farmers' landraces (Ajebu Nurfeta et al., 2008a; Mohammed et al., 2013; Abraham Bosha et al., 2016; Solomon Tamrat et al., 2020). These bioactive phytochemicals include dietary minerals, essential AAs, antioxidants, and other biosynthetic products. Hence, screening of selectively used medicinally valued farmer's landraces for nutritional contents is part of confirmatory test for the presence of useful biochemistry. So far, the nutritional composition of 'Kocho' and 'Bulla' has been reported for food products made from a mix of mature enset plants of different farmer's landraces. However, data on landraces having traditional medicinal use lacks evaluation of essential minerals, physiological AAs, crude protein and total ash contents. Descriptions on morphological characterization of medicinally valuable farmers' landraces of enset in addition to their biochemistry are also scanty, demanding further research attempts. Taking in to account of these conditions, evaluations of free or physiological AA profiles, mineral compositions, total ash, and crude protein contents, omitting carbohydrates, total energy and sugars for their higher values, and less fat contents in enset in general (Abraham Bosha et al., 2016; Mohammed et al., 2013), of farmers' landraces used as traditional medicine was proposed for the current study.

Domesticated enset is traditionally propagated asexually by cutting pseudostems at the junction of the corm and removing the centrally located, actively growing apical shoot tip to enhance sprouting (Karlsson et al., 2015; Abraham Bosha et al., 2019; Bewuketu Haile et al., 2021). The process stimulates lateral buds and multiple shoot formations from the underground stalk. However, genetic variation in enset that would result from mixing of genes during crossing via the process of fertilisation and the formation of seeds is a limitation during clonal propagation (McKey et al., 2010; White et al., 2023). Vegetative multiplication of enset also has further bottlenecks other than lack of genetic variability in the existing populations. These include the challenges of dispersal of insect pests and pathogens through vegetative parts used as saplings, including devastating bacterial, fungal and nematodal infections including the notorious *Xanthomonas* wilt and mealy bug infestations (Eckert, 2002; McKey et al., 2010; Barrett, 2015; Adane Abraham, 2018). In addition to pathogens and pests, the rapidly changing climates (Meron Aweke et al., 2019; Abera Jaleta et al., 2020; Abate Feyissa et al., 2022) are greatly challenging vegetative multiplication of enset using saplings of clonal sources. Collection and *in-situ* conservation of the diversity within this valuable crop is threatened from the rapidly changing local, regional or global climates (Mercer and Perales, 2010). Vegetative multiplication does not provide higher efficiency for safe germplasm protection than conservation via seed banking. Seeds have beneficial features including increased genetic variability, reduced moisture contents, and low space occupancy during storage. The high genetic diversity of seeds also favours varietal improvement through the processes of conventional breeding. Seeds also assure longer and safer storage of the germplasm through applications of *ex-situ* protocols in cold rooms (Dulloo et al., 2013). Evaluations of germination requirements of enset in the labs and establishment of conditions by which promising seed performance is an area with limited research.

Lack of scientific data on potentials of domesticated enset for seed germination has indirectly been challenging sexual multiplication of the crop via seeds. It has frequently been suggested that prolonged vegetative propagation can lead to loss of sexual reproductive capacity in clonal plants either through the mechanisms of deleterious mutations and genetic drift in traits of sexual reproduction as well as domestication bottlenecks (Eckert, 2002; Barrett, 2015; Denham et al., 2020; Kiflu Gebramichael et al., 2020). Prolonged and vegetative reproduction which brings to

suppression of sexual propagation leads to outcompetition of less adapted clones over expanding ramets of more adapted genotypes (Honnay and Bossuyt, 2005; McKey et al., 2010; Kifu Gebremichael et al., 2020; White et al., 2022). This an almost exclusive clonal propagation leads to sexual extinction or its rarity in perennial cops. Somatic mutation theory of clonality, which is stated as sexual reproductive success is inversely proportional to longevity, is the other mechanism for reduced sexual performance in clonal plants. It is more likely that mutations will be accumulated leading to decreased probability of successful sexual reproduction in the population when clones get older, larger and it took longer for periods of sexual reproduction between clones (Honnay and Bossuyt, 2005; Kifu Gebremichael et al., 2020; White et al., 2022). The existing high cultural diversity and various practice of farmers' selection of domesticated enset was hypothesised to cause poor seed development and germination response. Farmers in the local areas show the least tendency and interest in multiplication of the crop using botanical seeds. The scenarios put a perception for which cultivated plants have been thought of poor performances of seed formation associated to reduced potentials of sexual propagation. The evolutionary status of enset towards sexual reproductive potentials, however, can be tested by analysing diversity in seed and floral morphology and seed germination potentials. So far, little to no scientific data has been found evaluating the maintenance or loss of sexual reproductive capability in domesticated enset. Using samples collected from farmers' fields and wild habitats it has been reported that size of fruits and seeds as well as number of seeds per fruit have shown significant differences in wild and domestic accessions (Bewuketu Haile et al., 2023). The report has limitations in the control of growth conditions for wild and domesticated populations sampled, and those traits significant differing were subjective to variations in ecology or management which in turn alters soil environment that could have direct impact on growth, development and size of reproductive organs. Studies of this kind demand the establishment of laboratory protocols that provide improved seed germination. Successful germination, in turn, provides chances for commercial seed production, varietal development through conventional breeding, and future seed banking of the crop. Scientific evaluations of optimum conditions for seed-based propagation, including germination requirements for wild and domestic populations, have been limited. In connection with this, evaluation of variability in seed and floral morphology, embryo viability, and germination response against various temperature ranges of constant and alternating regimes for wild and domestic enset was targeted.

1.4. Research questions

The current study aspires to answer the following research questions, which are distributed and addressed under three study components:

- Which farmer's landraces of enset have traditional medicinal applications; what are the distinguishing features used to differentiate medicinally valued enset from those known for other uses; what are the ailments treated with; which enset parts or products are used for the treatment; what methods of preparation and application are followed; what are the factors threatening the abundance of medicinal enset and what local conservation measures are applied to protect these?

- What is the mineral and amino acid composition profile of farmers' landraces of enset having medicinal importance; is there any difference in micronutritional composition of these against differences in sample origin, landrace differences, raw tissues and fermented products; does medicinally valued enset contain higher values of minerals and amino acids to that of common root/tuber crops; what are the microbial communities which play roles in the process of 'Kocho' fermentation?

- Is there any evidence for changes in floral trait allometry, seed morphology or viability in domesticated as compared to wild enset; do domesticated enset seed and floral traits exhibit higher variance than under neutral expectations; what is the optimal temperature or its range for successful germination in enset seeds; does seed germination behaviour differ between wild and domesticated enset; and how do these germination requirements relate to the climate of wild and domesticated enset distributions?

1.5. Objectives

1.5.1. General objective

The overall study was to investigate ILK of medicinal applications along with morphological characterizations of farmers' landraces, evaluate their nutritional contents, variations in seed and flower morphology, seed viability and germination responses of wild and domestic enset.

1.5.2. Specific objectives

- To characterize farmer's landraces with traditional medicinal use, and document the associated ILK of enset ethnomedicine in southern Ethiopia; identify parts or products used, mode of preparation or application and ailments treated using medicinal enset; gather information on threats and conservation practices applied to protect rare enset.
- To evaluate mineral, AA, crude protein, and total ash contents of enset used as traditional medicine; whether medicinal enset shows variations in nutritional compositions across differences in sample origin, landraces types, and levels of processing of edible products; identify microbial communities that play roles in 'Koch' fermentation.
- To evaluate differences in floral traits, seed morphology and viability among wild and domesticated enset; whether seed and flower traits of domesticated enset exhibit higher variance than under neutral expectations; the range of temperature by which enset seeds perform the best germination success; whether wild and domesticated enset seeds show differences in germination behaviours; whether germination requirements of enset relate to the climates of distribution ranges of wild and domesticated populations.

1.6. Significance of the study

The planned investigations would generate important data on the medicinal applications of enset in a systematic way. The diversities of human and livestock ailments treated and farmer's landraces used against several ailments were ranked for their cultural values and documented. In addition to these locally rare farmers' landraces, factors that lead to their rarity and conservation approaches used were rated following key informant responses. This information would help to enhance the future utilisation of enset as alternative medicine in the treatment of human and livestock ailments in various ways. The data exhibits a depth of ILK of medicinal applications of enset among the surveyed enset-producing societies. The report in turn would call for related studies on this area among cultures not addressed by the current study where enset is applied as traditional medicine. The study also documented threats and local conservation methods recommended from farmers to protect the germplasm of medicinal enset from rarity or complete loss in the localities.

The higher nutritional contents of medicinally valuable enset landraces are part of the confirmation of their medicinal applications in the form of nutraceutical foods. The observed promising amounts of essential minerals, arginine, aspartic acid, glutamic acids, crude protein, and total ash put enset on the list of medicinally important items. The germplasm helps in the treatment of several ailments or disorders, including infectious and non-infectious diseases, as preventive or curative items. These findings also call for and pave a new research path towards further biochemical investigations in depth of the available cultural diversity of the crop plant. Some farmers' landraces with medicinal applications showed bitter corms and petioles, while others showed rose-coloured sap. Still other medicinal enset have pink-coloured vegetative parts of pseudostems, petioles, and leaf blades. These observations are clues to the presence of phytochemicals with therapeutic values. Special concerns for the future would be the screening and quantification of the available metabolites or bioactive ingredients in the different organs of enset plants which were not addressed in the current study for further requirements in time, budget, lab protocols and field conditions.

In relation to outputs of the investigation of its potential for sexual propagation, enset showed higher rates of embryo viability and seed germination. The lab conditions at which seeds germinate

at the highest rate were also identified. Wild and domestic enset showed similarities in the morphological traits of seeds and flowers, embryo viability, and germination responses. The findings indicate that prolonged vegetative propagation during the domestication process has not significantly altered the reproductive biology of the enset. This is evidenced through similarities in seed and floral morphologies and germination rates of wild and domestic seed accessions. This is a desired potential for future development of the crop in general. Those farmers' landraces of enset maintained in domestication are under threat from climatic changes, the onset of old and new diseases, and associated shifts in land use plans. Hence, the customary on-site conservation of the germplasm is a challenge and demands complementary actions from *ex-situ* protocols. The current findings also imply the possibility of seed banking of the diversity of domesticated enset germplasm in cold rooms in the future, based on these findings. The major bottleneck for seed-based conservation in enset was the lack of knowledge whether enset seeds could survive long durations of extreme dryness and germinate again. It is only recently that the orthodox behaviour of enset was reported showing neither physiological nor morphological seed dormancy (Karlsson et al., 2013; Borrell et al., 2021). The reported reduced potentials of seed viability or germination (not more than 55% in enset and a 49% viability in related plants of the *Musa* genus) was also a bottleneck for seed based propagation (Karlsson et al., 2013; Kallow et al., 2021). Though seed banking is a better alternative to fill the gaps from on-farm conservation of crops, quiescent seeds might lose the potential to recover or germinate when the required conditions would be adjusted back (Fleming et al., 2019). For this reason neither of the on-farm and the seed-banking techniques are risk free for conservation of enset germplasm and we need to follow the complimentary approach of in-situ and ex-situ to stand on the safest side.

1.7.Scope of the study

The current research traverses among three of the dominant enset-producing ethnic groups, the Sidama, Wolaita, and Gurage, to document ethnomedicinal applications of cultural diversity in the crop plant. A multistage sampling has covered three administrative kebeles (the lowest administrative units in Ethiopia) from each of the selected administrative districts under each of the three study zones. Plant organs or fermented food products of enset used for nutritional analysis

were collected from 26 farmers' landraces importantly used as traditional medicine, from which 19 landraces were sampled for total ash, macro and micro minerals and total crude protein analysis. Nine different enset parts of fermented products were sampled for nutritional profiling, where some represent edible parts or products whereas others belong to non-edible tissues. In total, 375 flowers were sourced from 15 individual inflorescences, of which nine domestic and six wild populations were collected for investigations of differences in floral trait diversity. From each of the fifteen inflorescences 25 male flowers from five consecutive matured and healthier bract axils were sampled yielding 225 (25 x 9) for domestic and 150 (25 x 6) for wild enset. A total of 45 female flowers were additionally sampled for domestic accessions and used in the analysis. On the other hand, 20 individual enset seed accessions were sampled for morphological study and germination tests, of which 13 belong to domestic and seven to wild populations. Commercial seeds of wild enset were purchased from an on-line distributor, Rare Palm Seeds (RPS) were also included in the study to screen for wider ranges of treatment conditions. In total more than 8000 seeds were harvested from wild and domestic sources from south and south west Ethiopia.

1.8. Limitations of the study

Indigenous Local Knowledge of the medicinal uses of farmers' landraces of enset by its nature is not restricted to the currently selected study localities or ethnic boundaries. Comprehensive documentation of ILK on traditional medicinal applications of enset among all peoples cultivating it would be of significance for enhanced use. In addition, only some segments of farmers' landraces with medicinal values have been documented. Provided that more time, finance, and lab facilities, including field and lab test kits, were available, the result would have been of greater value in exhibiting the potentials of enset in traditional medicine. Plant samples of medicinally used enset for laboratory analysis were obtained from a single individual for each of the farmers' landraces sampled. This has resulted from the reduced local availability of these farmers' landraces in the area; hence, the information generated may not be sufficiently representative. However, variation at the level of farmers' landrace is not confidently shown; the findings exhibited important clues for the higher nutritional contents of the selected farmers' landraces in general. We could seek out the missing information in our future research endeavour by sampling a larger number of

individuals from different localities as replicates to crystallise whether these findings are farmers' landrace-specific, site-specific, or both.

Sampling of reproductive structures was challenged by the reduced availability of farmers' landraces that had flowered and developed mature seeds during the data collection phase. Female flowers and fruits were reported as being hunted by large birds, primates, and rodents in the surveyed localities. We used data from the flowers of the male sexes for inferences, since enough intact samples of these were available for both wild and domestic populations. The collection of data for overall fruiting and seed formation potentials from total fruit counts and total seed counts was not a possibility because of the previously mentioned challenges of frugivory. The future direction would be the evaluation of samples from wild and domestic seeds and flowers, with continuous accumulation of the information through the years. With this practice, we could gather full or comprehensive data on the diversities of reproductive biology and evolutionary trends of enset among wild and domestic populations.

1.9. Structure of the dissertation

The research report is organised into five chapters. The first chapter contains a general introduction to the whole research project, background and justification, concise literature review, statement of the problem, research questions, objectives, significance, scope, and limitations of the study. The second, third and fourth chapters present the individual study components in the form of published articles and publishable manuscripts. Accordingly, the second chapter deals with ethnobotanical applications of enset in traditional medicine. The findings for this component are organised in the form of publishable manuscript and is under review by the editorial board. The third chapter contains a published article on the micronutritional composition and microbial community analysis of diverse farmers' landraces. The fourth chapter contains a second publication on the reproductive biology of wild and domesticated enset. The findings showed evidence for the maintenance of sexual reproductive capacity in a vegetatively propagated crop enset. The last chapter, chapter five, presents the general discussion, conclusions, and recommendations emanating from the overall study. This chapter is immediately followed by the overall references used throughout the

dissertation, including references cited and appendices. The preliminaries before the main body of the dissertation include the cover page, approval page, and statement of the author, table of contents, list of tables, figures, and appendices, as well as acknowledgements and the abstract.

CHAPTER TWO

2. Ethnomedicinal applications of an indigenous food security plant Enset (*Ensete ventricosum*) in selected districts from Sidama, Wolaita and Gurage in Southern Ethiopia

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Abstract

Enset is a valuable crop consumed by diverse nations and nationalities as a staple food in Ethiopia. Some farmers' landraces are locally used in the preparation of traditional medicine. However, scientific reports on medicinal use are limited to their absence. The aim of the study was to characterize medicinally valuable enset and associated ILK of their applications. Research sites of nine kebeles from the three selected districts of Dara (Sidama), Kindo Didaye (Wolaita) and Enemore and Enir (Gurage), and study informants were purposefully selected. Hundred home gardens from each district having enset cover of at least an eighth of a hectare based on recommendations from agriculture experts, were surveyed to document medicinal enset. ILK of medicinal use was collected from informants who attained not less than ten years of experience in enset gardening. Semi-structured interviews, focus group discussions, and guided garden tours were used to gather ethnobotanical information. Spot-captured photos, ocular observation, and key

informant interviews were used to characterise medicinally valued enset. Both qualitative and quantitative data including preferences or prioritization and direct matrix rankings (DMR), severity ranking for factors leading to reduced availability of medicinal enset, and ranks of sever ailments were recorded. To assess agreements for common ailments and relative healing potential of frequently used medicinal enset, we respectively evaluated using informant consensus factor (ICF) and fidelity level (FL). Among the 184 farmers' landraces recorded, 68 were medicinally valuable. Farmers' landraces differently named were clustered together based on morphological traits. Enset corms were commonly boiled and consumed orally as treatments for a variety of illnesses. Enset cures a long list of related and unrelated ailments both infectious and non infectious. Treatments against bone fractures, traumas, delayed labour or placenta were the most commonly reported. Complications related to delayed labour were associated with higher score of ICF. 'Argama' (Wolaita), 'Gowariye' (Gurage), 'Arkiya' (Wolaita), 'Astara' (Gurage) and 'Askala' (Sidama) obtained the highest FL. Frequent harvesting and lack of interest for multiplication through corms resulted in local rarity of medicinal enset. Medicinally valuable landraces of enset did not strictly cluster following their collection localities, were repeatedly used for treatment, and observed locally are. Boiled corm and whole plant parts are commonly used respectively for treatment of human and livestock ailments. The documented wider ILK and numbers of reported landraces exhibited remarkable medicinal properties of enset, which also demands biochemical confirmations for enhanced future use.

Key words/ phrases: Boiled corm, Bone fracture, Delayed labour, Injuries and wounds, Medicinal enset, Morphological traits, colour of vegetative parts

2.1.Introduction

Enset (*E. ventricosum*) is an herbaceous perennial crop indigenous to Ethiopia that serves as a staple food for a considerable number of populations. In addition to its food values, the crop plant has been variously mentioned for its traditional medicinal use among different ethnic groups in

southern Ethiopia. Farmers' landraces reported for such uses are maintained by local people in the home gardens of southern Ethiopia. People have used these for the treatment of fractured bones, back pain, and to ease labour and speed up the release of delayed placental tissue. Farmers' landraces with unique colours on their vegetative parts including reddish to pinkish pseudostems and leaves, were commonly used to treat human and livestock ailments. 'Tayo' (Kefa), 'Choro' (Kefa), 'Lochingiya' (Wolaita), 'Askala' (Sidama), and 'Qeqille' (Kembata) are among the colourful enset types that serve as medicinal items (Yemane Tsehaye and Fassil Kibebew, 2006; Temesgen Magule et al., 2014; Amare Seifu and Daniel Fitamo, 2016; Melesse Maryo et al., 2018). Apart from the farmers' landraces with colourful vegetative parts, several enset types lacking distinctive and unique chroma were reported for medicinal applications. 'Gefeteno' (Wolaita), 'Chacho' (Sidama), 'Hala' (Wolaita), 'Tunako' (Sidama), 'Kibnar' (Gurage), 'Gowariye' (Gurage), and 'Gena' (Wolaita) are among medicinal enset lacking unique colours on vegetative parts (Zerihun Yemataw et al., 2016; Tesfaye Dilebo et al., 2023a).

Kedir Abdella et al. (2017) reported a number of medicinally valuable farmers' landraces of enset from a district in the Gurage zone of southern Ethiopia. Farmers' landraces named 'Astara' and 'Qibnar' were most preferred for their medicinal use, where the former is used at early stages and the latter at late stages of fracture treatments. At an early stage of treatments for bone fractures, 'Astara' softens bones and associated muscle and nerve tissue, enhancing relaxation. Qibnar, on the contrary, fixes fractures once they are set in proper positions at later stages. Traditional applications of enset either to enhance or inhibit milk secretion are a common practice in southern Ethiopia (Newarinesh Feleke and Wondimagegnehu Tekalign, 2022). 'Astara' was used for facilitation of milk production and secretion, whereas 'Maymote' was used for inhibition of it when mothers planned to stop breast feeding (Kedir Abdella et al., 2017). Facilitation of milk production through feeding of enset parts was also reported for livestock. Enhancement of milk production in terms of amount and composition relates to positive effects on the growth of kids in goats up on feeding enset (Ajebu Nurfeta et al., 2012).

Enset is also used in the induction of abortions as a means of traditional birth control practices. ‘Qeqille’ (Kembata) is farmers’ landrace with such property that helps in birth control (Melesse Maryo et al., 2018). In addition to the above-mentioned ailments, joint displacements, muscle cramps, and boils containing pus are treated using ‘Tayo’ in Kefa, south Ethiopia (Yemae Tsehaye and Fassil Kibebew, 2008). In most cases, mentions of the medicinal application of enset include uses for the treatment of fractured bones, back pain, and trauma (Temesgen Magule et al., 2014; Kedir Abdella et al., 2017; Melesse Mriyo et al., 2018). Previous attempts to study traditional medicinal use of enset have some limitations of depth of treatment and coverage of the topic. Kedir Abdella et al (2017) is the only attempt, but having shallow survey report on a few of medicinal enset in a district from Gurage zone of south Ethiopia. Others tried either to only mention that enset has traditional medicinal applications or just listed some of medicinally valuable landraces mentioned in the surveyed areas in general (Zerihun Yemataw et al., 2016; Azene Tesfaye and Abebe Girma, 2017; Kedir Abdella et al., 2017; Melesse Mriyo et al., 2018). Therefore, a comprehensive investigation of the depth of ILK of medicinal use of enset, exhaustive documentation of valuable farmers’ landraces, ailments and symptoms of illness treated, parts used, mode of preparation, as well as threats and conservation of these landraces among different ethnic groups are worthy.

2.2. Materials and Methods

2.2.1. Study design, description of the area and sampled population

A multistage purposive sampling technique aimed at acquiring sufficient information on applications of enset in traditional medicine was used to select study zones, districts, kebeles, and home gardens after a reconnaissance survey. The volume of enset production at each of the administrative levels was used as a criterion for selection after recommendations from regional and zonal agriculture offices. Sidama, Wolaita, and Gurage administrative zones of the former SNNPRS were the broader sampling areas. A district from each of the selected administrative zones and three kebeles from each district composed the range of sampling localities (Figure 1). Dara, Kindo Didaye, and Enemore and Enir were the three districts representing the Sidama,

Wolaita, and Gurage sampling domains, respectively. The kebeles of Abera Bute, Aleme Korocho, and Safa were used to represent the highlands, midlands, and lowlands, or drier midlands, for Dara district, respectively. The equivalent kebeles from Kindo Didaye include Lasho, Koyisha Wamura, and Bosa Borto, whereas those from Enemore and Enir are Agata, Gaharad, and Horbet Zizo. Details of study localities with villages, kebele, geographic coordinates, and elevations were also collected and documented.

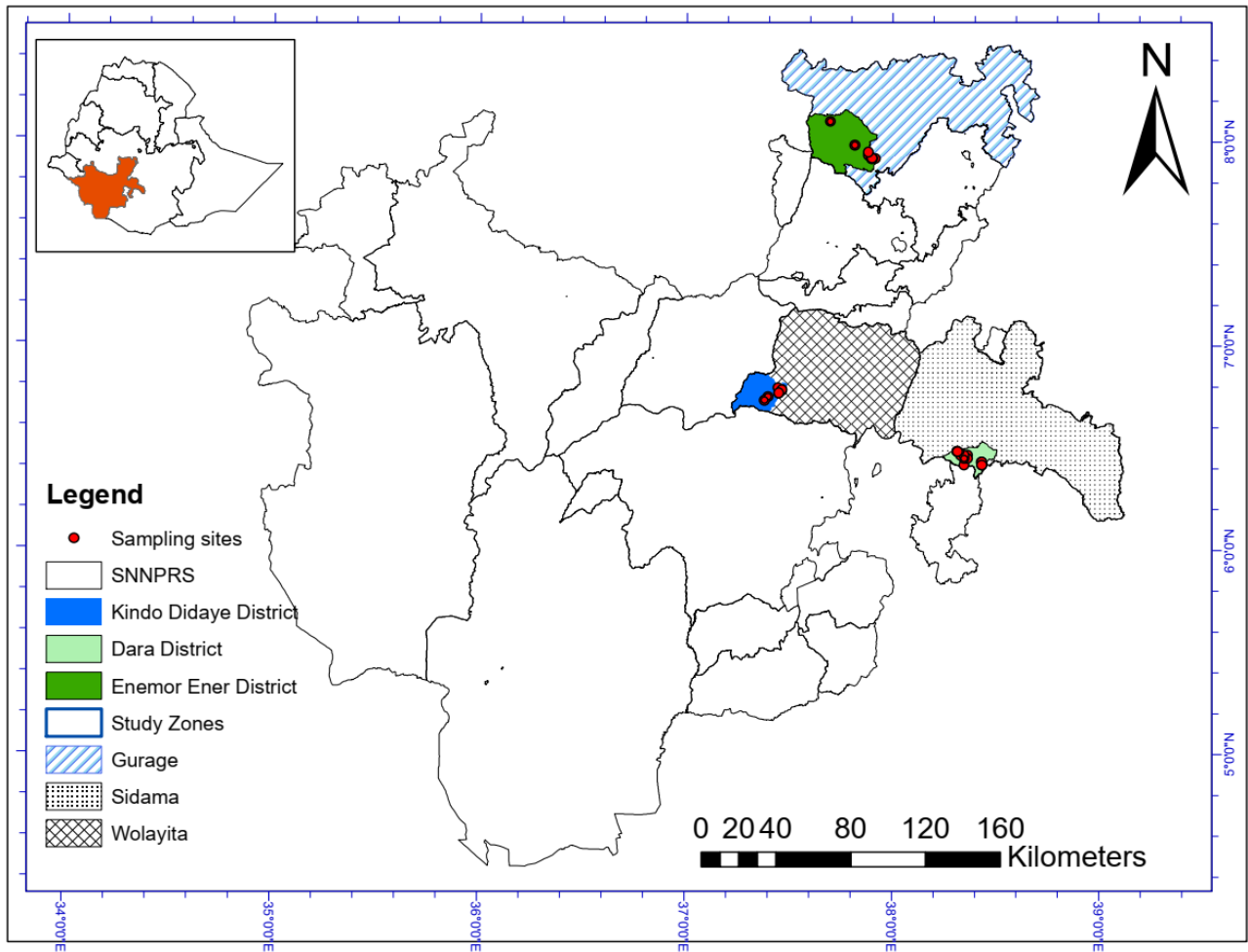


Figure 1: Map of the area showing Dara (Sidama), Kindo-Didaye (Wolaita) & Enemore & Enir (Gurage) districts all under the former SNNPRS

Kebado, a rural town used as the administrative centre for Dara, is situated respectively about 350 and 85 km south of Addis Ababa (ADD) and Hawassa (Dara District Financial Planning and Economic Development Office (DDFPEDO), 2018). The Sidama people, speaking ‘Sidamu afoo’, which belongs to the Cushitic language family, are the dominant inhabitants of the district. Kindo Didaye is centred at the rural town of Halaale, which is situated about 475 km south of ADD and 170 and 85 km west of Hawassa and Wolaita Sodo, respectively (Kindo Didaye District Financial Planning and Economic Development Office (KDDFPEDO), 2018). The Wolaita people, speaking ‘Wolaitato’, a member of the Omotic language family, dominated the area. Gunchire is a local town serving as the centre of the Enemore and Enir districts and is situated about 200 km south of ADD, 220 km west of Hawassa, and 60 km south of Wolkite (Enemore and Enir District Financial Planning and Economic Development Office (EEDFPEDO), 2018). The Gurage people, speaking ‘Guragigna’, a member of the Semitic language family, were the dominant occupants of the area. The major economic activities of the three selected districts are enset-based farming agriculture with integral livestock rearing activities (Southern Nations, Nationalities and Peoples Regional State Financial Planning and Economic Development Office (SNNPRFPEDO), 2018).

2.2.2. Sampling of home gardens and informants

One hundred home gardens were surveyed and selected via purposive sampling following recommendations from local agriculture experts that a farmer who managed at least an eighth of a hectare of it is considered as major producer of enset in the localities, and the associated garden visits during preliminary surveys. A similar approach to Zerihun Yemataw et al. (2016) has been followed for enset surveying during the current study, such that the sufficiency of collected information was checked using a multiple-use curve (Balick and O’Brien, 2004; Williams et al., 2005). Home gardens with enset plots of at least an eighth of a hectare (ca~1250 m²), and household heads with at least ten years of enset farming experience were used as informants after recommendations from local administrators for sampling of ILK. The global positions of the study sites, including latitudes and longitudes, with their elevations were recorded using a GPS receiver (Garmin-eTrex-12xGPS navigator, Germany). The names of

localities, i.e., villages and kebeles, were also recorded for each of the selected study sites. The heads of each of the selected households (HH) or his spouse, in the case the male HH head is not alive, were used for data collection as the general informants at each of the preferred home gardens.

A total of 49 male and 51 female household heads (Appendix 6) were sampled, alternating one after the other to ensure gender contributions and higher significance of both sexes towards enset management (Katie et al., 2013). The very first person nominated as an informant was determined by using the method of coin tossing, where consecutive sex alternations followed until the completion of all the numbers of respondents planned for sampling. The ages of the informants ranged from 25 to 80 years old. Ten key informants were purposively selected for reported experiences and knowledge of enset use and management out of the previously recruited informants in each district. Out of the total 30 key informants nineteen were males, while the remaining 11 were females. These informants were recommended for having better information on enset use and management from local experts, administrators and elders. Key informants participated throughout the study period as they were recommended for their accumulated knowledge.

2.2.3. Collection of ethnobotanical data

Standard ethnobotanical data collection methods were adopted (Martin, 1995; Höft et al., 1999, Cottton, 1996; Balik and Cox, 1995) to collect ILK on the traditional use of enset as medicinal elements in the area. A semi-structured interview was conducted with recruited informants to document indigenous utilisation and management of medicinally valued enset using interview items (Appendix 4 through 6). Open-ended and closed-ended questions were prepared in English and translated to the local languages of Sidama, Wolaita, and Gurage to facilitate easy delivery and information communication during interviews. Interviews were done after obtaining informed consent from each of the informants explaining purpose and significance of the study for utilization and conservation of diversity of this economically valuable crop plant.

Hence, before conducting the actual interview, an agreement was reached with each of the recruited informants to collect ethnobotanical data.

Three rounds of field visits to each of the selected districts were made to collect and synthesise ethnobotanical data. Demography or socioeconomic status of informants, landrace diversity and ailments treated using enset, threats to and conservation practices used are parts of the ILK collected. The first interview was made in the first fieldwork immediately after the selection of sampling sites. During the second fieldwork, medicinally important farmers' landraces were identified through guided garden walks and interviews. The last round trip was done to collect missing information, triangulate data, and identify vague or outlier responses. Morphological features or traits of medicinally important farmers' landraces of enset were documented (Appendices 2, 3, and 4), following spot ocular observation, informant suggestions, and following techniques used for morphological description (Bizuayehu Tesfaye, 2008; Temesgen Magule et al., 2014; Zerihun Yemataw et al., 2018a). A presence-absence data matrix of trait levels of the selected characteristic features was prepared. The matrix was used to record morphological traits for the purpose of classification (Bizuayehu Tesfaye, 2008; Zerihun Yemataw et al., 2018a). The Munsell colour charts (Munsell® Plant Tissue Colour Book-M50150, UK) for plant organs including pseudostems, petioles and mid ribs were used to assign the major colour types for vegetative parts and fluid sap from slashed petioles and corms.

Key informant interviews (Appendix 5) were employed by the researcher for collection of quantitative information on enset utilization, ranking of local preferences for medicinally valuable enset and verification of data. The tool was also used to rank frequencies of ailments or disorders, cultural values of multimedical enset, severity of treats frequently used medicinal enset, threats and locally applied conservation options. Focus group discussions (Appendix 6) were also employed using six groups, two for each district, which consisted of five key individuals in each locality. A key informant, a local women's representative, a local administrator, a knowledgeable elder, and a development agent were members of each focus group. These methods were used for data verification and for collection of criptic information

or issues taken as taboos and not exposed during individual interviews. Plant parts or fermented food products of enset used as traditional medicine, modes of preparation, routes of administration, and types of ailments or symptoms of disorders treated were also documented. It has been reported that age, educational status, levels of informant knowledge and plant biodiversity of an area determine significantly the distribution and ILK of use of medicinal plants (Ermias Lulekal et al., 2013; Zewdie Kassa et al., 2020). The socioeconomic status of informants, including gender, age, and educational status, was recorded additionally for each informant during interviews (Appendix 4) to test which social factors determine the distribution of ILK of enset use as traditional medicine in the study area. Causes of reduced local availability, following interview responses of medicinal enset and the associated local conservation practices were retrieved and ranked for their significance using the key informants.

2.2.4. Data analysis

Farmers' landraces of enset available in home gardens during the study period were documented for description of morphological features. Furthermore, these were described for detailed knowledge of medicinal applications using interview responses from key and general informants. Concepts of multiple use curves (Bizuayehu Tesfaye and Lüdders, 2003; Balick and O'Brien, 2004; Williams et al., 2005; Araújo et al., 2012) were adopted to determine the rate at which information is gathered and to check the sufficiency of the collected data or the available information in the area (Figure 2). The curve was plotted as the number of householders or home gardens accessed for data collection versus the number of additional medicinally valuable farmers' landraces recorded. Human and livestock ailments treated using enset were grouped into nine broad categories (Figure 5), following the international disease classification scheme, ICD-10 (WHO, 2019) and consultations to health experts at each of the three selected districts.

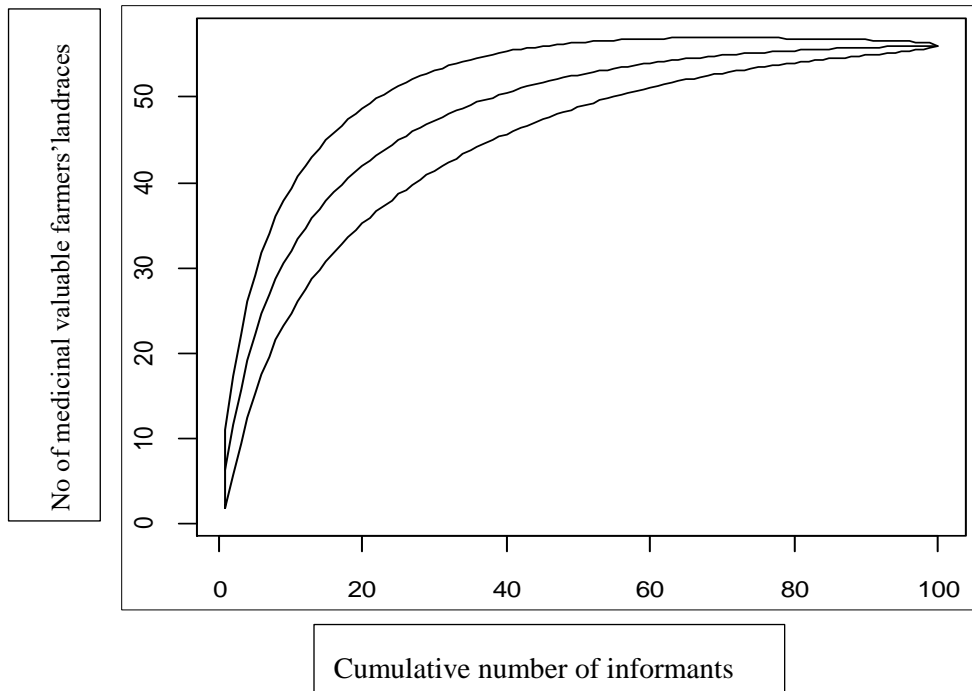


Figure 2: Species-accumulation curve adopted and used to estimate turnover of medicinal enset uses information per extra efforts made in interviewing

The collected data were organised and analysed using Microsoft Excel for percentages, frequencies, averages, and graphs presenting results. A one-way analysis of variance (ANOVA) test was computed using IBM-SPSS Statistics for Windows, Version 26 (IBM Corp., Armonk, N.Y., USA) to evaluate differences in knowledge level among the different informant classes towards medicinal use of enset. Differences in the numbers of reported medicinally valued farmers' landraces of enset among socioeconomic categories of level of knowledge among informants', gender (sex), age, educational status, sizes of total and enset land holding and studied ethnic classes were tested to check variables determining distribution of ILK of medicinal enset use in the community. A list of all the reported farmers' landraces and those mentioned medicinally valuable were tabulated (Appendix 1) after resolution of the synonyms or names referring to the same landraces following consultations from local elders and outputs offocus group discussions.

The major colours of vegetative parts of medicinally valued landraces were documented after repeated ocular observation of at least three individuals, key informant consultations and comparison with colour charts (Munsell® Plant Tissue Colour Book-M50150, UK). Patterns of colouration for non-uniformly distributed pigments were recorded. A colour pattern with wider longitudinal bands was referred to as a strand, while those with narrow bands were called strips. In addition to these patterns, enset having the narrowest vertical colour bands were referred to as streaks. The size of the unique colour pattern other than vertical patterns over a larger background was also grouped into different categories. We referred to large-sized colour islands over the major colour background as a “patch”, those with intermediate-sized colour islands as “spots”, and the finest dots of pigment as “dust”. A data matrix of the presence and/or absence of trait levels (McNeill, 1979; Inta et al., 2023) for morphological (abaxial color of pseudostem, petiole, mid-rib and leaf blade), agronomic (relative responses to disease and pest attacks), and consumer preference for taste of boiled corms was tabulated. Each sampled characteristic trait was resolved at all of its available levels for which data were collected. Trait levels were then coded for simplicity, to aid rapid recall, and for ease of recording and data analysis. Integer values of zero (0) and one (1) were assigned, respectively, for the absence and presence of a trait level for a given farmer's landrace. The data were fed into the statistical environment of R version 3.4.3 for cluster analysis. A hierarchical classification of medicinally valuable farmers' landraces was made using the dissimilarity ratio for separation of groups using library `vegan` by applying linkage rules through the Ward's method. Cluster analysis were used to evaluate whether landraces from similar sampling sites would really associate together or not. Farmers' landraces were taken as equivalent to species, while cultural features were considered equivalent to sites in ecological studies to facilitate analysis using R.

The informant's agreement towards ailment categories treated with enset was calculated using the index of the informant consensus factor (ICF) (Heinrich, 1998; Höft et al., 1999; Hoffman and Gallaher, 2007). The formula $ICF = [(Nur - Nt) / (Nur - 1)]$ is used for the calculations of ICF, where Nur is the number of use citations in each ailment category and Nt is the number of farmers' landraces used for each ailment category. The index of fidelity level (FL) was also calculated and used to rate levels of relative efficacy of medicinally valued enset following and

adopting methods described by Höft et al. (1999) and Hoffman and Gallaher (2007). The percentage of fidelity level index was calculated using the equation $FL (\%) = (I_p/I_u) * 100$, where I_p refers to the number of informants who independently cited the importance of a medicinally valued farmers' landrace for treating a particular disease, and I_u refers to the total number of informants who reported this medicinally valued farmers' landrace for treating any given disease.

Direct matrix ranking (Ashenafi Ayenew et al., 2016; Tesfaye Dilebo et al., 2023a) was used for rating the most important medicinal enset landraces used in treating several ailments. Five of the commonly reported medicinally important farmers' landraces treating at least three ailments were selected and presented to key informants in the respective study districts so that they assign scores to each farmers' landrace (Ashenafi Ayenew et al., 2016; Zerihun Yemataw et al., 2016; Tesfaye Dilebo et al., 2023). The method was used to identify culturally the most important medicinal enset in the respective study districts. The scores assigned range from one to five, where 5 represents the best preferred or an excellent farmers' landrace. In a similar way, 4 was assigned for those perceived as very good, 3 as good, 2 as fairly used, and 1 as rarely used or least preferred. The total sum of the average values assigned by all ten informants was used to rank the most important medicinally used enset treating several ailments.

Farmers' landraces of enset having medicinal importance have reduced frequencies, and were observed rare or least frequent and were also reported so by the informants during field surveys. The associated threats retrieved during semi-structured interviews were rated for the degree of severity (Abraham Shumbulo et al., 2012; Belachew Garedew and Aklilu Ayiza, 2018; Kutoya Kusse et al., 2021). The ten key informants were guided to assign numbers one to five to medicinally important farmers' landraces of infrequent occurrences. The frequently mentioned threats were also prioritized to rate their impacts on the production of enset in general and medicinally valued landraces in particular (Ashenafi Ayenew et al., 2016). Relatively, the least frequent or rare farmers' landrace received a score of 5, while the one with relatively abundant received a score of 1. Similarly, a threat reported as the most challenging was rated 5, while

those with comparatively less impact received 1. Intermediate numbers were assigned according to the degree of rareness or reduced frequency of farmers' landraces. The total sum of the average values assigned by the key informants was used to rank the the least frequent medicinally used enset to check the relative degree of rarity and a threat that is most challenging for conservation of medicinal enset.

2.3.Results

2.3.1. Characterization of farmers' landraces of enset used as traditional medicine

The study described and documented 68 medicinally valued farmers' landraces of enset out of a total of 184 recorded from home gardens and cropping fields (Appendix 1). Cluster analysis has resulted in six groups from the farmers' landraces for which enough information has been documented with no blanks for missing traits or trait levels on a data matrix (Figure 3;), since farmers were not willing to provide some valuable landraces for harvesting and dissection to record color of shalshed corms and petioles. The first cluster included farmers' landraces with light green or pale green pseudostems, light green petioles, and pale green mid-ribs. The second cluster represents those with brownish or dark brownish pseudostems, blackish, purplish, or pinkish petioles, and purplish or pale pinkish mid-ribs. Farmers' landraces with deep purplish, reddish, or light pinkish parts represented the third cluster. Medicinally valued enset which typically show reddish parts all over the pseudostems, petioles, and leaf blades (Figure 3), had been clustered together. Farmers' landraces known by the local names 'Mundiraro' (Sid.), 'Lochingiya' (Wol.), and 'Charkim' (Gur.) are good examples of such characteristic features. The fourth cluster belongs to those enset types with brownish, dark brownish, pinkish, dark pinkish, or blackish pseudostems and blackish or light to dark purplish mid-ribs. 'Astara' (Gur.), 'Yekembata-Astara' (Gur.), and 'Askala' (Gur.) composed this cluster. The fifth category accommodated farmers' landraces with purplish pink or rose sap exuding from slashed petioles or pseudostems and corms. These farmers' landraces included 'Suyitiya' (Wol.) and 'Edemert' (Gur.). The last group stands for enset with blackish or brownish pseudostems, otherwise having less likely related petioles and mid-ribs. Medicinally valuable farmers'

landraces were clustered irrespective of their collection sites and/or language groups, where those from different zonal administrations appeared in the same class.

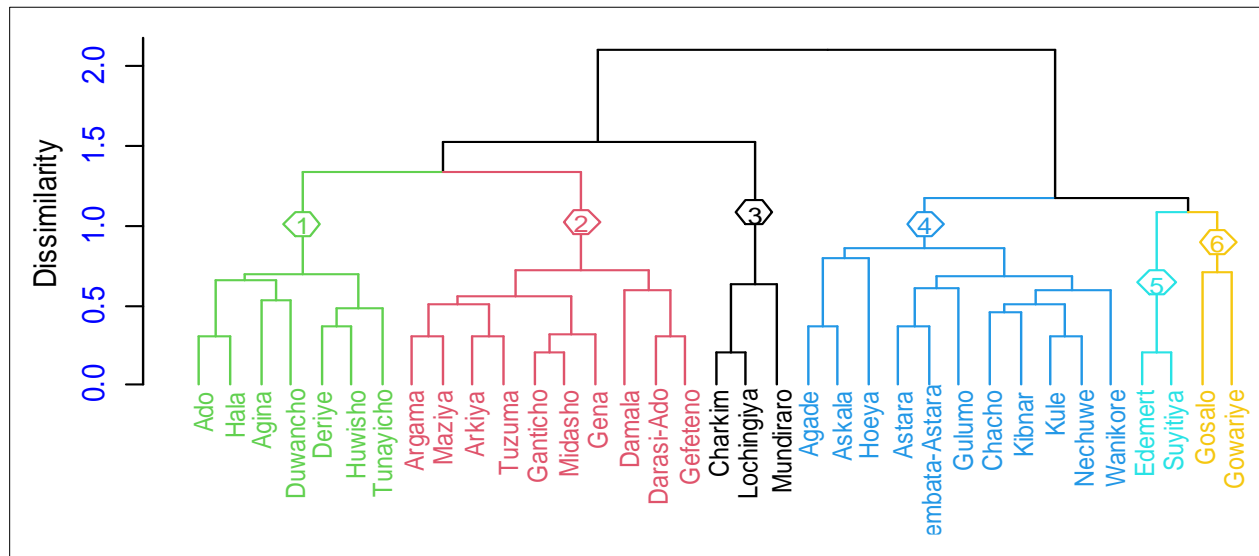


Figure 3: Cluster analysis for grouping farmers' landraces based on similarity ratios using traits from morphological, agronomic & end-user quality preferences

2.3.2. Plant parts and processed products of enset used as traditional medicine

Fresh or dried corms, pseudostems, roots, petioles, and their sap fluids from some farmers' landraces of enset were applied as traditional medicine. In addition to these raw plant parts, processed products of pseudostems and corms, including 'Kocho' and 'Bulla', also serve as traditional medicine (Appendices 9 and 12). The sediment and supernatant of 'Bulla' and 'Kocho' were also used to treat various ailments and disorders. Moreover, 'Kocho' from mature and flowered individuals, which is processed using scrappings from the central solid stalk of an inflorescence called 'Deleke' (Sidama), was reported for its medicinal importance. The general enset-based food stuff of 'Kocho' and 'Bulla', sourced from any of the farmers' landraces, which is also used as food items, was reported to treat some of the common ailments in the area. The fermentation starter called 'Gamancho' (Sidama) is used in the treatment of tonsillitis and oral infection in children having symptoms of discoloration of a tongue in to whitish dense mucoid

layer of infections. In addition to the starter culture, freshly scrapped and collected starch-like product from the tip of the apical shoot near the base of the pseudostem was applied as a medicinal item. It serves as a supplement for breast milk during a shortage or temporary lack of secretion immediately after the first birth. Moreover, this starch-like raw enst product is used by women as an immediate energy source or enery tonic and is consumed in the form of boiled or roasted tubers as snack food during 'Kocho' preparation.

The most commonly used product or plant part of enset used to treat human ailments or disorders are young corms (Table 1). 'Bulla', petiole, 'Kocho', and root-tip are the other parts or products of enset those are used in traditional medicine, the order of listing showing their respective rank of frequency of reports. Tissue sap from the petiole as well as the pseudostem and a fluidly exudate from the root tip are also used different medicinal purposes. The fluid byproduct of extremely fermented 'Kocho', known as 'Mocha' (Sidama) with reported medicinal properties, has the characteristic feature of fractionating into a settling solid and floating supernatant liquid. The sediment, which is known as 'Karta', (Sidama) and the supernatant, called 'Talala' (Sidama), also have traditional medicinal applications in the area. Fresh 'Mocha' is drunk to detoxify non-delebrate poisoning from toxic chemicals. These local toxic items include Dichloro-Diphenyl-Trichloro Ethane (DDT), Malathion (O-dimethyl-phosphorothioate) rat poison, and '*Wuha agar*' (a chemical used for water treatment locally). Poisoning was also reported from non-delebrate feeding of toxic mushrooms to children, and toxic grass or wild tuber plant by cattle in thestudy area.

Gruel from 'Karta' is used as a detoxifying agent in addition to its use in the treatment of weight loss and weak immunity in children. Moreover, the same product is used to target back pain and a cutaneous infection known as 'Chirt' (Amharic) translated to ringworm. Gruel is drunk hot to treat back pains; whereas the fluid is used to shower the affected skin part repeatedly till the ailment is cured. 'Kocho' fermented from scrapped pseudostems and gratted corms mixed with tissues squashed out of the central solid stalk of inflorescence is known as 'Deleke' (Sidama). 'Deleke' is used as a therapeutic food in the treatments of common health disorders in the study area. It is

chopped, squeezed to remove the unwanted products, prepared to make enset-based foodstuffs for treating disorders such as back pain, energy deficits, or debilitation. These disorders result from the challenges of ploughing virgin lands using hand tools, lifting extra heavy loads, and some illnesses that debilitate the physical and immunological states of a person. The Gurage peoples of the study area use corms as the most important medicinal components, while ‘Bulla’ and leaf petiole in Wolaita as well as root-tip in Sidama are also importantly applied as traditional medicine.

Table 1: Major plant parts and processed products of enset used in traditional medicine, %FC=Percent of citation frequency

Study site	Parts or product of enset reported & their %FC				
	Corm	Bulla	Kocho	Leaf petiole	Root tip
Sidama	61.07	12.98	3.44	7.26	10.31
Wolaita	42.00	39.00	7.00	12.00	0.00
Gurage	92.22	3.08	3.08	0.00	0.00
Average	65.00	18.00	4.50	6.40	3.40
Rank	1.00	2.00	4.00	3.00	5.00

2.3.3. Modes of preparation and application of enset as traditional medicine

2.3.3.1. *Practices of cooking and fermentation*

In the majority of cases, parts of enset plant or processed products applied as traditional medicine are prepared in similar ways to the commonly used enset-based food products. The basic modes of preparation of enset to use as medicinal treatments include the processes of cooking and fermentation. Out of the total informant citations from the selected localities of SWGs, cooking accounts for the highest average percent of citation frequency (% CF) (Table 2). ‘Kocho’ and ‘Bulla’ are fermented products from raw corms and pseudostems used in disease treatment. On average, the majority of enset parts or products used as medicine for SWGs were raw or fresh and

fermented in the ratio of three to one (Table 3). Some fermented enset products are used as traditional medicine, even though raw or unprocessed plant parts are commonly used as disease treatments. Corms are used in similar ways to the common modes of consumption for tropical root-tuber crops, through the process of boiling or roasting. Local peoples from Sidama and Wolaita areas also use enset in the form of raw or half-cooked tissues in the treatment of ailments. Fermented and non fermented tissues of enset are almost proportionally used in traditional medicine in Wolaita areas, while the majority of the later are used in Sidama and Gurage.

Table 2: Cooking and fermentation state of enset parts or products used in traditional medicine, %FC=Percent of citation frequency

Study site	Cooking state with %FC		
	Raw	Half-cooked	Cooked
Sidama	23.7	15.3	60.3
Wolaita	10.7	4.9	84.5
Gurage	1.0	0.0	99.0
Average	11.8	6.7	81.3
Rank	2.0	3.0	1.0

Table 3: Fementation of plant parts of enset used as remedies against human ailments

Fermentation status (% FC)	Study site			Average
	Sidama	Wolaita	Gurage	
Fermented	21.8	46.5	6.2	24.8
Non fermented	78.3	53.5	94.0	75.3

2.3.3.2. *Preparations of enset for remedies and additives used*

Diverse preparation and application methods of enset used as traditional medicine have been reported. Cooking or semi-cooking through boiling and making porridge were locally applied to make enset-based food for applications as traditional treatments for ailments. Corms are most commonly cooked in pots and used in the form of boiled tubers to target ailments and disorders in the area. Making in to porridge of ‘Bulla’ or ‘Kocho’ or in to various foodstuffs variously, and boiling or roasting to complete or partial cooking of corms are also commonly applied in the preparation of enset into medicinal preparations. Most frequently, raw plant parts of enset or their processed products are used as traditional medicine without additives (Table 4). In some instances, however, milk and milk products, including butter and beef, are used as additives in treatments. Minor additives used also include garlic and a few of spice plants. No strict dosage was determined for enset products as traditional medicine; however, the prescription of plant sap as a traditional medicine included dosages of a cup full for children and a glass full for adults until the issue was resolved. No side effects and antidots have been reported from use of enset as traditional medicine. However, there are abortive and antifertility activities in human beings and death from excessive feeding of certain landraces by livestock were reported as direct effects. Cooking through boiling and consumption of the fresh and hot corm immediately is the major mode of preparation and application of enset medicine among the Gurages. Cooking via boiling in to porridge and consumption of the fresh and hot corm immediately in Wolaita, and semi cooking via boiling and consumption of the fresh and hot corm immediately in Sidama are the most common ways of use of enset as medicine.

Table 4: Additives use with enset parts or their products in traditional medicine

% FC of additives used	Study site			Average	Rank
	Sidama	Wlaita	Gurage		
No additive use	98.00	69.00	77.00	81.00	1
Milk & its products	0.38	27.00	14.00	13.80	2
Minor additives	1.50	4.00	9.00	5.00	3

2.3.4. Routes of administration of enset medicine

Oral is the major route of administration used in taking enset as traditional medicine (Table 5), while ocular and dermal routes had a few reports of percent of citation frequency (%FC).

Table 5: Route of administration of enset as traditional medicine in humans, %FC=Percent of citation frequency

	Sidama	Wolaita	Gurage	Average	Rank
Oral	86	96	100	94	1
Ocular	5	0	0	2	3
Dermal	5	4	0	4	2

Enset-based food stuff from selected farmers' landraces serves as medicinal items whenever needed with a low incidence of side effects. Some informants, however, reported consumption of a few farmers' landraces have health concerns if their parts or processed products are used whether deliberately or non-deliberately. Focused group discussions revealed that farmers' landraces such as 'Askala' (Sidama), 'Mundiraro' (Wolaita), 'Lochingiya' (Wolaita), and 'Gowariye' (Gurage) are locally known for their abortifacient potential and are not served to pregnant women and livestock. Moreover, a farmers' landrace known as 'Deri'ea' (Gurage) was reported to cause sterility in women. This farmers' landrace was reported to cause a loss of fertility in adolescent girls who made the first attempt to get impregnated or for those women who had experienced birth previously. Preparations from this enset are only advised for consumption by sterile females or women who are in the menopause age.

2.3.5. Types of diseases and/or disorders treated with enset medicine

A number of ailments and health related complications that were treated using enset were reported from the study area. These ailments are used for either preventions or cures, and their impacts range from the provision of temporary relief to the complete cure of a disorder. Local people

diagnose ailments or disorders by physical examination or by asking for the symptoms and findings of the illness before the treatment is applied. Illnesses or disorders that are physically manifested in the victim and clinically exhibit deviations from the normal condition are directly examined. These include bone fractures, back pains, wounds and injuries, skin infections, bloody urine discharge, weight loss or debilitations, and the like. Complications of the internal organs are either explained by the patient's symptoms of pain or abnormalities in their health state. Gastro-intestinal issues, postnatal complications, muscle and joint cramps, and a lack of milk secretion were some of the reported internal ailments. These were diagnosed based on the symptoms and signs mentioned by the patient during treatments. Ailments treated with onset include complications that are infectious or non-infectious in origin, based on recommendations from local health experts. The common causative agents reported as the origins of infections include gut helminthes, bacteria, fungi, protozoans, and viruses, which were confirmed after expert consultations in the respective health centers of the study districts. Sources for noninfectious disorders reported include deficiency diseases, impacts of unsuitable climates, and debility disorders associated with malnourishment and heavy tasks such as ploughing and lifting heavy weights. Moreover, slipping from hillsides and rolling downhill, resulting in trauma and associated wounding were the most commonly reported disorders in the area.

Following the WHO's disease classification scheme (WHO, 2019) and expert-based identification, the retrieved human and livestock ailments and disorders were classified into nine categories (Figure 4, & 5). The major classes include musculoskeletal and connective tissue disorders (MSCTD), injury, poisoning, and certain consequences of external causes (IPCCEC), pregnancy, childbirth, and purpura complications (PCPC), diseases of the blood and blood-forming organs and certain immune diseases (DBBFOIS), certain infectious and parasitic diseases (CIPD), and digestive system disorders (DSD). Musculoskeletal system and connective tissue diseases were the most frequently reported ailment categories. The highest diversity of specific ailments or disorders, as a percent of overall specific ailments reported, was recorded under the categories of injury, poisoning, and certain consequences of external causes.

2.3.6. Informant consensus factor and fidelity level indices

Indices of ICF and FL were estimated to rate the relative level of agreement on the most commonly reported ailment categories and the relative healing potentials of frequently mentioned medicinal enset. The nine human ailment categories formulated were ranked for levels of informant agreements using index of ICF. Accordingly, disorders of MSCTD in Sidama and disorders of PCPC both in Wolaita and Gurage ranked the top in informant agreements. Moreover, the average ICF values for common ailments among localities of SWGs indicated PCPC as the most important ailment category treated with enset medicine. The highest average number of use citations was recorded for MSCTD, followed by that of PCPC on average for the three study localities.

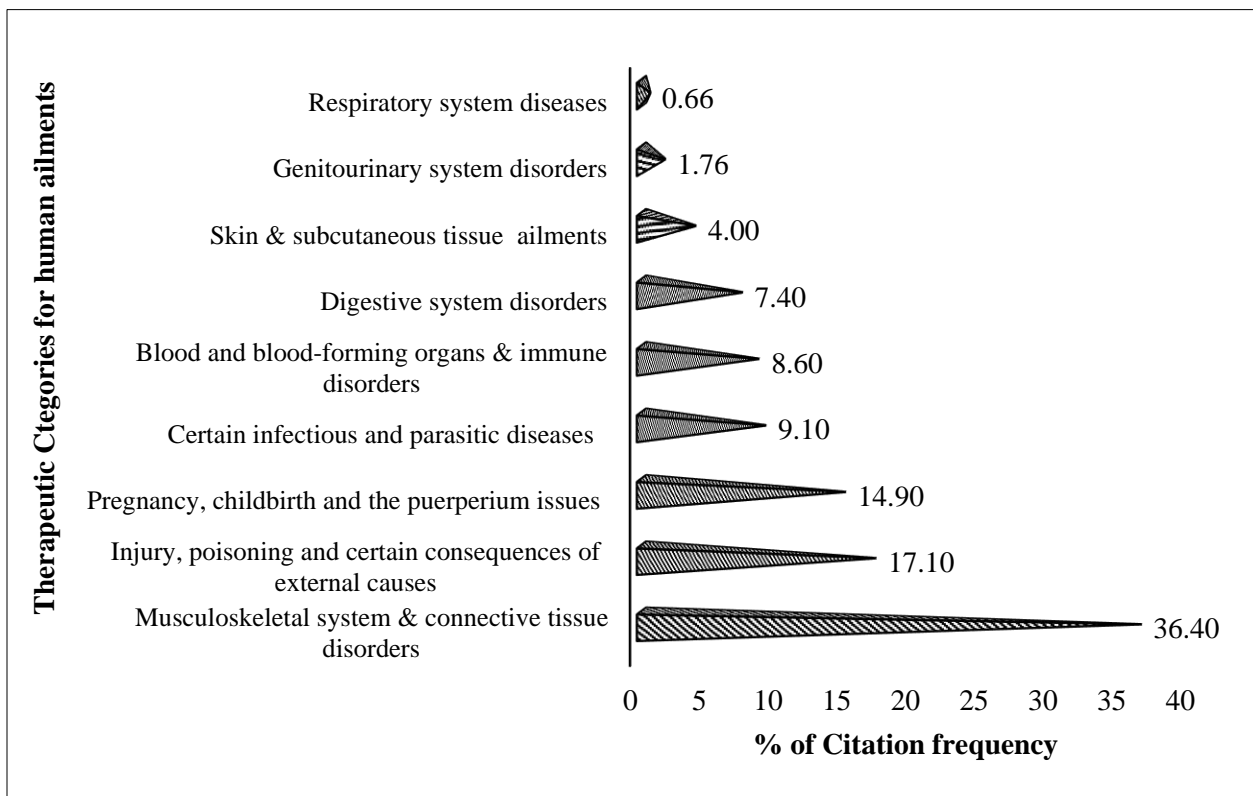


Figure 4: Therapeutic categories of human ailments & their citations frequencies (%FC)

The highest FL index value was obtained for farmers' landraces used for treating of ailments under the category of MSCTD. 'Argama' (Wolaita), 'Gowariye', 'Arkiya', 'Askala' (Sidama), and 'Astara' were farmers' landraces of medicinally valuable ensets receiving the top five fidelity levels in the study area under the ailments category of MSCTD (Figure 4). Health complications under PCPC and IPCCEC also received higher values of indices of FL for medicinal enset including 'Lochingiya' (Wolaita) and 'Astara' (Gurage).

2.3.7. Ethnoveterinary uses of enset for treatment of livestock ailments

The current study has identified ailments of veterinary importance, the majority of which were also common among humans and livestock (Figure 5). In a similar way to human ailments, veterinary diseases and disorders were classified into nine categories (Figure 5) following the WHO recommendations for disease classification (WHO, 2019). The majority of reported livestock ailments belong to PCPC in the area. Overall, 41 (60%) out of the 68 medicinally valued farmers' landraces documented as medicinally valuable were used to treat livestock disorders. 'Askala' (Sidama), 'Lochingiya' (Wolaita), and 'Astara' (Gurage) were the most frequently cited enset landraces among the three localities of SWGs, respectively, for their applications in ethnoveterinary medicine.

The intact plant or whole parts of enset are predominantly used for the purpose of treating livestock disorders under ethnoveterinary medicine (Table 6). Enset corms and to a lesser extent, some fermented byproducts from 'Kocho' called 'Mocha' (Sidama) and 'Bulla' are also served to livestock as ethnoveterinary medicine. Almost all of the respondents reported that unfermented and non boiled forms of enset products are used to treat livestock disorders (Table 7). Parts of enset plants are most frequently chopped and served (Table 7) to the animal through the oral route (Table 8) in the form of green forage for the treatment of disorders or complications in livestock.

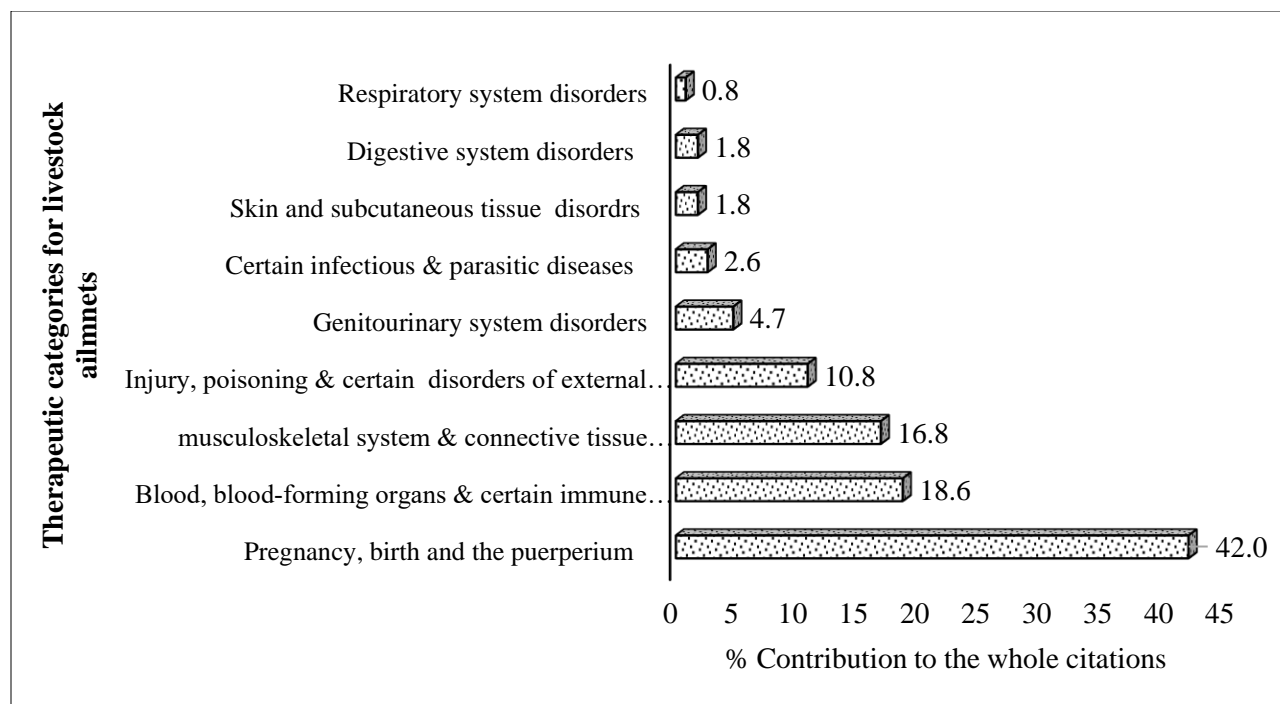


Figure 5: Disease categories of ethnoveterinary importance treated using medicinal enset with their percent frequency of citations (%FC)

Table 6: Parts and/or processed products of enset used in ethnoveterinary medicine

Study site	% CF Response of part or processed product used as medicinal items			
	Whole parts	Corm	Mocha	Bulla
Sidama	83.2	0.0	16.9	0.0
Wolaita	82.7	12.6	0.0	4.0
Gurage	75.7	24.3	0.0	0.0
Average	80.5	12.3	5.6	1.3
Rank	1	2	3	4

Framers' landraces of enset including 'Askala' (Sidama), 'Mundiraro' (Wolaita), 'Lochingiya' (Wolaita), 'Gowariye' (Gurage), 'Yekembata-Astara' (Gurage), and 'Yejjima-Astara' (Gurage), had abortive effects on pregnant livestock. And hence, raw plant parts or processed products from these farmers' landraces are not advised or served as traditional medicine during gestation. Moreover, 'Gowariye' (Gurage) was reported to cause animal death if it is solely served with a stomach full, and hence it is advised for mixed servings with other enset types. If this farmers' landrace is not deliberately served, it would cause stomach bloating. In such circumstances, the animal is treated with special products of enset in general used as antidotes. A fluid byproduct of thoroughly fermented 'Kocho', i.e., 'Mocha' (Sidama), will be mixed with mineral salt called 'Amole' (Sidama) and served for the cattle to drink to detoxify the poisonous effluents. In the wet seasons, enset is served to cattle as traditional treatments for ailments after some hours of exposure to solar radiation to facilitate the loss of excess moisture. This otherwise could lead to bloating or a swollen stomach after a hampered condition in the digestive tract.

Table 7: Mode of preparation, route of administration and livestock treated in using enset in ethnoveterinary medicine

Parameters	Conditions	Sidama	Wolaita	Gurage	Average	Rank
Fermentation condition	Fermented	14.0	4.0	0.0	6.0	2
	Not fermented	83.0	96.0	100.0	93.0	1
Mode of serving	Chopping into pieces	62.7	86.7	95.3	81.6	1
	Serving the fluid	16.9	4.0	0.0	7.0	3
	Foraged in any form	20.5	4.7	3.4	9.5	2

Table 8: Route of administration of enset as traditional medicine in treating livestock ailments

Route of administration	Study site			Average	Rank
	Sidama	Wolaita	Gurage		
Oral	100.0	100.0	95.0	98.0	1
Dermal	0.0	0.0	5.0	1.7	2

2.3.8. Ranking of multimedical and rare farmers' landraces of therapeutic values

In the study area, some farmers' landraces of enset are used to treat several ailments or disorders. Those farmers' landraces reported to treat at least three ailments in the study locality were ranked by the key informants. 'Askala' (Sidama), 'Chacho' (Sidama), and 'Mundiraro' (Sidama) were used to treat birth-related complications and musculoskeletal disorders in Sidama. In addition to farmers' landraces from Sidama, 'Argama' (Wolaita), 'Arkiya' (Wolaita), and 'Lochingiya' (Wolaita) were the three most frequently mentioned medicinal enset in Wolaita. These were used to treat bone fractures, broken cartilages, delivery-related issues, and early complications of trauma. Moreover, 'Astara' (Gurage), 'Kibnar' (Gurage), and 'Gowariye' (Gurage) are at the top ranks as farmers' landraces with multi-medicinal applications. These local enset types are known for the treatment of complications of the musculoskeletal system and those disorders related to early trauma in Gurage. The ranking of medicinal use diversity across multi-medicinal farmers' landraces showed the most prominent ailments in the area. Based on the ranking, exercise prenatal, natal and postnatal complications in Sidama and bone fractures and breakage of cartilage tissues, respectively, in Wolaita and Gurage are the most prominent.

2.3.9. Preference or priority ranking to identify the rarest landraces used as medicine

Relative rarity, i.e., relative reduced local frequency of access, was rated for selected medicinally valuable enset landraces in the area. These results showed that 'Askala', (Sidama) 'Chacho'

(Sidama), and ‘Mundiraro’ (Sidama) are the rarest medicinally valued enset types in Sidama. Similarly, ‘Arkiya’ (Wolaita), ‘Lochingiya’ (Wolaita), and ‘Sutiya’ (Wolaita) in Wolaita, and ‘Astara’ (Gurage), ‘Kibnar’ (Gurage), and ‘Gowariye’ (Gurage) in Gurage, are rated as the rarest landraces.

2.3.10. Factors affecting availability of medicinal enset and local conservation responses

Threat factors determining the local availability of enset or those that play greater roles in determining its multiplication were ranked after key informants’ prioritization. Poor interest of corm cutting and multiplication, or the reported habit of discriminating farmers’ landraces with poor agronomic traits and those with low consumers' tastes or preferences were the major determinant factors for the availability of farmers’ landraces. Accordingly, medicinal enset with a longer maturity period in Sidama and those prone to disease and drought in Wolaita and Gurage were less preferred for multiplication and hence were seen as locally rare. Reduced development of edible parts, i.e., slimness and/or wateriness, poor regeneration potential evidenced from the production of a few corm sprouts, and susceptibility to vertebrate pests were also reported as important limiting factors for enset production. These features or challenges led to reduced availability of some medicinally valuable enset types through farmers' discrimination for multiplication.

Features of individuals of some farmers’ landraces of enset also led to discrimination of farmers’ landraces for multiplication and contributed to reduced local abundances. These traits include reduced food reserves in edible parts, excessive hydration, bitterness, and poor ecological competitive abilities. Farmers’ landraces, including ‘Pelowa’ (Wolaita), ‘Woyisha’ (Wolaita), ‘Badadiya’ (Wolaita), ‘Lochingiya’ (Wolaita), and ‘Sorgiya’ (Wolaita) were reported to have less food reserve and hence were less preferred for multiplication. Enset types reported as excessively hydrated, including ‘Sorgiya’ (Wolaita) and ‘Godariya’ (Wolaita), and ‘Kesuwe’ (Gurage), also received fewer demands for multiplication. Others who had a bitter corm and hence ‘Kocho’ with poor taste, including ‘Tunayicho’ (Sidama), ‘Hanshicho’ (Sidama), and ‘Lemicho’ (Sidama), as well as ‘Lembo’ (Wolaita) and ‘Palakiya’ (Wolaita) are less preferred for multiplication. Similarly,

‘Shertiye’ (Gurage), ‘Woshemeja’ (Gurage), and ‘Shibe’ (Gurage) had bitter tissues, which resulted in reduced local frequency for less preference and poor multiplications in Gurage. Farmers also used some additional qualities to discriminate against enset for multiplication, including less competitiveness for nutrients and shade intolerance in enset-based mixed farming complexes.

Various local conservation measures were reported for the protection of farmers' landraces in the investigated localities. Farmers either borrow germplasm from neighbours or manage a few of those individuals with medicinal significance in their enset home gardens. However, they do not multiply them frequently through corm cutting, like those landraces commonly used for their food values. Moreover, local people bring some seedlings of selected farmers' landraces from the highlands and manage and adapt those receiving higher mentions for therapeutic values in their gardens for medicinal application.

The local people also collect farmers' landraces across ethnic groups or zones, adapt their use values, and multiply individuals to pass the germplasm to their neighbors. ‘Darasi-Ado’ (Sidama) was brought from the Gedeo zone to Sidama, while ‘Boroda-Wanadiya’ (Wolaita) and ‘Dawuro-Sirare’ (Wolaita) were exchanged between people from the Boroda and Dawuro areas. Similarly, a farmers' landrace morphologically similar to ‘Astarā’ (Gurage) was brought from Kembata and Jimma to the Gurage area and was named after its origin as ‘Kembata-Astarā’ (Gurage) and ‘Jima-Astarā’ (Gurage). There are also reports of the exchange of germplasm within each ethnic group. For example, ‘Alati-Ganticho’ (Sidama) was a ‘Ganticho’ (Sidama) type brought from Aleta-Wondo and distributed to the rest of the Sidama areas. Similarly, ‘Enir-Agade’ (Gurage) was a more similar enset type to a landrace called ‘Agade’ (Gurage) and was originally brought from the Enir sub-district to the Enemore area of the Gurage zone.

Disease protection practices were also applied by the local people to maintain the germplasm of an economically important but disease-prone enset. Farmers manage various plant species in enset gardens to prevent wilt disease. *Pycnostachys abyssinica*, *Euphorbia abyssinica*, *Canna indica*, *Croton macrostachyus*, and *Phoenix reclinata* are among those plant species. Locally, these were named respectively 'Tontona' (Sid.), 'Chare' (Sid.), 'Enset-abeba' (Amh.), 'Masincho' (Sid.), and 'Meti' (Sid.). The most probably mode of action of these plants against enset disease is through release of allelochemicals from the rhizosphere or above ground parts, which interfere in the multiplication of pathogens or pests. Local people clean their farms by slashing old and dried parts of enset leaf or leaf sheath and avoid sharing farm implements across gardens as a means of disease protection. They also cut and incinerate diseased plants on site and select disease-resistant enset types in the process of germplasm protection.

2.3.11. Effects of socioeconomic status on level of ILK of medicinal use of enset

A one-way ANOVA showed differences in the number of reported farmers' landraces of medicinally valued enset among study localities, informant's knowledge, age classes and educational status. The average number of reported medicinally important farmers' landraces showed significant differences ($p = 0.001$) between study localities. As a result, informants from Gurage reported a higher average number of medicinal enset types than those from Wolaita and Sidama. Similarly, respondents from Wolaita reported more medicinally valued farmers' landraces than those from Sidama. In addition to the local difference in the number of reported medicinal enset types in SWGs, key informants mentioned a higher average number of medicinal enset ($p = 0.000$) than the general or local informants. Age of informants was also observed as a determinat factor for the reported information on medicinal applications of enset. Elderly to old-aged informants have mentioned a higher average number of medicinal enset ($p = 0.032$) than the young and middle-aged respondents. The educational status of the informant was the other social factor that has significantly influenced the use of knowledge in the area. Informants with educational

backgrounds have reported a higher average number of medicinally valued farmers' landraces ($p = 0.004$) than those with no access to schooling. Total land holding and enset coverage of the total holding of households is directly related to the status of acquisition of ILK of enset use among informants.

Indigenous knowledge of the medicinal use of enset is transferred within the surveyed communities from one culture to another. This process was achieved by the local transfer of farmers' landraces from market places and farms. Reports from key informant interviews and focus group discussions indicated that midland and lowland farmers obtain enset germplasm and associated use knowledge from the highlands. Moreover, social interactions and communications during enset farm management by males provide advantages for the transfer of knowledge such as that of clone selection and planting for a specific purpose. Enset-based food preparation among female householders also provides a very good platform for knowledge exchange related to food taste, fiber quality, maturity time, e.t.c. These events are used to transfer knowledge vertically from parents to descendants with respect to gender lines and horizontally among the neighbouring families.

2.4. Discussion and conclusions

2.4.1. Characterization of enset used in traditional medicine

We have described and documented a considerable number of farmers' landraces of medicinally valuable enset from the study area. These accounted for about 37% of the total number of enset documented, indicating the high availability of ILK for medicinal applications of the plants. Higher medicinal plant species diversity was reported for conservation of huge ILK of plant use among local peoples (Ermias Lulekal et al., 2013; Zewdie Kassa et al., 2020). Medicinal enset collected from different study localities aggregated together and vice versa on clustering based on morphological traits, indicating reduced influences of farm management or geographical variations

on their morphology and hence genetic contents. This phenomenon is also related to the frequent transfer and flow of genetic materials of enset through introduction, conservation or exchange (Bizuayehu Tesfaye Tesfaye et al., 2002; Zerihun Yemataw et al., 2018). Higher within geographic region variations (84%) of the total genetic variance was reported through AMOVA explaining reduced impacts of geographic variations in genetic diversity (Temesgen Magule et al., 2015; Kiflu Gebramichael et al., 2020). Similar activities of material exchange were also reported in the study area with implications of transfer of ILK of landrace selection which has resulted in morphological similarities of some genotypes against regional variations (Almaz Negash et al., 2002, Temesgen Magule et al., 2015). Observations of the naming of identical farmers' landraces differently among different cultural groups were reported from a recent study (Tesfaye Dilebo et al., 2023a; Zerihun Yemataw et al., 2016). This indicates that linguistic variations in naming farmers' landraces or cultural differences in the management of farm components do not indicate strong differentiation among different morphological types of enset. The condition also explains that agricultural systems are neither isolated nor closed and germplasm and ILK of plant use are commonly transferred among distantly positioned localities and cultural groups (Bizuayehu Tesfaye et al., 2002).

Morphological traits were the basic features used by farmers for the identification of enset used in traditional medicine. The colours of pseudostems, petioles, midribs, and leaf blades were prominently used locally for identification of the diversity of farmers' landraces. Previous reports similarly indicated that morphological traits of colour in vegetative parts were important for the classification of enset cultivars (Yemane Tsehaye and Fasil Kibebew, 2006; Wendawek Abebe et al., 2022; Zerihun Yemataw et al., 2016). Gizachew woldesenbet et al (2020) reported 34% of total variations of the first principal component accounted for traits of color of vegetative parts. These observations explain the existing genetic variations among the different landraces used as traditional medicine (Zerihun Yemataw et al., 2018). However, it should be confirmed through exhaustive collections and in depth molecular characterizations. The chroma of the leaves and the presence of cuticular wax accumulation at the abaxial leaf surfaces has been used as a means of identification between wild and domestic enset (Borrell et al., 2019a). Domestic enset showed

foliar colours of green, yellowish green, reddish, and light pinkish, with prominent wax from the cuticle on abaxial surfaces. On the other hand, wild enset showed a green or light green lamina devoid of ashy wax-like whitish dust accumulation and smooth textures; indicating morphological, ecological or genetic variations among the wild and domestic populations.

So far, there is no clear understanding of the sources of medicinal properties within different plant parts and preparations of enset. The medicinal properties of plants of this species were thought to be only environmental impartations on their biochemistry (Gizachew Woldesenbet et al., 2022). The same report also hypothesised that the unique traditional medicinal applications of some farmers' landraces are just misidentifications of those used primarily as food items. Enset with dark pink parts were especially used as medicinally important items among the SWGs. Moreover, SWGs commonly use enset which produce plant sap of rose or light pink coloration in the treatment of ailments. Enset with distinctively reddish, brownish or pinkish colours all over their vegetative parts, including 'Askala' (Sidama), 'Lochingiya' (Wolaita), 'Astara' (Gurage), 'Yekembata-Astara' (Gurage), and 'Yejimma-Astara' (Gurage), are commonly used as medicinal items among the Sidama, Wolaita and Gurage peoples. The medicinal properties of enset are commonly practiced within each of the study localities across cultural variations. This indicates the rich accumulation of ILK of medicinal use of enset, and the ethnobotanical information is not yet fully understood and documented. This observation also indicates medicinal properties of enset has been less understood and researched. Hence, it demands further genetic, physiological, or agronomic studies to identify the source of these properties among the available farmers' landraces.

2.4.2. Applications of enset as medicine: plant parts and processed products used

Corms are most frequently utilised part of enset and are applied in the treatment of various ailments as traditional medicine. These were reported to detoxify drug-induced hepatotoxicity in mice. This condition shows the availability of some useful bioactive phytochemicals those neutralize or dilute

the chemical impacts of toxins within tissues of these plants (Abebe Dukessa et al., 2021). Some cultivated enset and wild genotypes from southern Ethiopia were reported for high antioxidant activities and total phenolic contents in from their pseudostems and corms (Aliye Tefera, 2023). Tubers of tropical root crops were reported as important sources of bioactive phytochemicals (Chandrasekara and Kumar, 2016; Kumar et al., 2017; Obidiegwu et al., 2020; Popola et al., 2023). The high protein content of a traditional root tuber called ‘Anchote’ (*Coccoloba abyssinica*) is evidence for the accumulation of high dietary contents in underground parts of tuberous crop plants (Yenenesh Ayalew et al., 2017). A variety of anti-ailment properties and associated bioactive phytochemicals were reported from taro, potato, sweet potato, yams, and coco yams that are traditionally used to treat diverse human ailments (Chandrasekara and Kumar 2016).

There were indirect reports on medicinal applications, therapeutic properties, and phytochemical contents of a wild relative to enset, i.e., *E. superbum*. Triterpenoid esters, proanthocyanidins, pelargonidins, glucosides, and anigorufones were detected in this plant species (Sethiya et al., 2019). Corms of *E. superbum* also contain bioactive compounds, including phenylphenalenone and β -carboline alkaloids (Hölscher and Schneider, 1998; Krishnamurthy et al., 2023). These reports, therefore, provide indirect clues to the availability of bioactive compounds in enset corms. Furthermore, phytochemicals including carotenoids, phenols, flavonoids, alkaloids, steroids, and tannins were evidenced in plants of the *Musa* genus (Vasundharan et al., 2013; Pereira and Maraschin, 2015; Zaini et al., 2022). These biochemicals are also known for their higher antioxidant properties. Previous biochemical composition tests from plants of the two related genera, *Ensete* and *Musa*, leave us with a task to search for bioactive phytochemicals in enset. Hence, we are keen to study the therapeutic significance of the consumption of corms and fermented products of enset. These observations would help to exhibit the actual and potential values of enset in disease prevention.

2.4.3. Mode of preparation and application of enset as traditional medicine

Foodstuffs made from enset corm, 'Kocho', and 'Bulla' were cooked before application as traditional treatments in the area. These processes enhance the bioavailability of bioactive ingredients they contain. It was reported that steam cooking of food items was applied to enhance palatability, taste, digestibility, shelf life, and micronutritional availability (Bradbury and Holloway, 1988). Moreover, the release and bioavailability of micronutrients and phytochemicals useful as dietary items and with medicinal properties were enhanced through cooking (Habtamu Fekadu et al., 2013; Esayas Ayele et al., 2015; Samatiya et al., 2020). Cooking orange-fleshed sweet potatoes enhances the retention of bioactive phytochemicals, including phenols, flavonoids, antioxidants, and carotenoids (Abong et al., 2020). Habtamu Fekadu (2013a) reported an increase and decreasing trend for flavonoids and phenols up to the cooking of tubers of *Coccinia abyssinica*. Breads, porridge, gruel, and corms from enset were baked, boiled, or roasted, most possibly to encourage the release of bioactive ingredients for use as medicinal items among SWGs.

The higher use rate of raw plant parts of enset in non-fermented forms relates indirectly to the dominant utilisation of boiled or roasted corms as traditional medicine. It has been reported that both the boiling and roasting of edible tubers enhance their nutritional contents. Boiled potato tubers retained more iron, while concentrated zinc, magnesium, and calcium were observed after roasting (Ikanone and Oyekan, 2014). Even though fermentation enhances the desired sensory properties of foodstuffs, local people in SWGs, in the majority of cases, preferred to use enset as boiled corms for traditional treatments. It was also reported that boiling food items brings about the breakdown of antinutritional factors and facilitates the release of nutrients, including minerals (Tamiru Kasaye et al., 2018). Despite its lesser frequency of use, fermentation of enset is also important in the application of the plant as traditional medicine. 'Kocho', 'Bulla', and the fluidy exuding byproducts of these products, including 'Mocha' and 'Karta', in Sidama areas are used to treat various ailments. The application of fermented byproducts of enset in the treatment of ailments is associated with the functional properties of microorganisms such as probiotics, antimicrobials and antioxidants (Tamang et al., 2016b; Patel et al., 2023).

Cooking in general and cooking through boiling in particular received the highest average percent frequency of citations as methods of preparation of enset into medicinal items. This indicates that utilisation in cooked tuber forms is the main mode of application of enset medicine in the area. The observation is linked to the accumulation of bioactive ingredients in underground plant parts. Then cooking assisted in the release of these therapeutic chemical ingredients (Bradbury and Holloway, 1988; Abong et al., 2020; Samatiya et al., 2020). Cooking ‘Bulla’ and ‘Kocho’ by boiling and making porridge stands out as the next important preparation method of enset medicine. This scenario is associated with the high starch or carbohydrate contents of enset-based foodstuffs (Tesfaye Dilebo et al., 2023b; Abraham Bosh et al., 2016). It also relates to the suitability of enset for the preparation of dishes with butter, milk, and other products (Pijls et al., 1995; Temesgen Magule et al., 2014). Hot-served food items like porridge as local treatments are important for instantly enhancing body heat in the form of thermotherapies. Therefore, these preparations relieve cramps around muscles in the thoracic cavity, joints, and back pains. Such types of foods that generate body heat are also used for the enhancement of muscle relaxation. In this regard, enset-based food preparations provide a relaxed uterine and hip muscle and create easy delivery and placental dropout during labour. The majority of enset products used in traditional medicine are in the form of edibles. This guided the major route of administration of enset as a medicine through the oral route. The scenario indicates that enset has functional or nutraceutical properties. Foods of this type are commonly consumed for their nutritional contents and treat various ailments at the same time (Azene Tesfaye and Abebe Girma, 2017; Solomon Workineh and Neela, 2019).

2.4.4. Additives and a few of adverse effects associated to use of enset as medicine

Most commonly, enset-based traditional medicines were used solely with little to no additives in the area. This is attributed to the high dietary, mineral, AA, and other phytochemical contents with promising amounts to target ailments. Different authors have reported higher nutritional and

phytochemical contents from enset so far (Mohammed et al., 2013; Vasundharan et al., 2013; Abraham Bosha et al., 2016; Sethiya et al., 2019; Solomon Tamrat et al., 2020). However limited in the frequency of use and amounts used, legumes, beef, milk, butter, and other milk products are used with enset in traditional medicine. Reports by Getahun Yemata (2020) and Pijls et al. (1995) mentioned the incorporation of animal-based additives such as beef, butter, and milk into enset-based products served as foodstuffs. Enset was known as a rich source of starch but claimed to have low protein content (Solomon Workineh and Neela, 2019; Borrell et al., 2020). However, recent findings indicated higher mineral and AA compositions (Mohammed et al., 2013; Abraham Bosha et al., 2016; Solomon Tamrat et al., 2020). This is the most probable scientific basis for why the majority of times enset medicine is used as a self-sufficient treatment for ailments with reduced use of additives.

Enset products, including boiled corms, ‘Kocho’, and ‘Bulla’, are consumed in their diverse forms with less likelihood of side effects than some of the known herbal traditional medicinal items. However, some farmers’ landraces were reported to have general abortive efficacy against pregnancy. The abortifacient activities of ‘Askala’ (Sid.), ‘Mundiraro’ (Sid.), ‘Lochingiya’ (Wol.), ‘Gowariye’ (Gur.), ‘Yekembata-Astara’ (Gur.), and ‘Yejima-Astara’ (Gur.) are common mentions in the area. These biological activities are linked to the presence of phytochemical constituents that interfere with the normal implantation process (Srikanth et al., 2013). Local people also indicated that products from these farmers’ landraces make the uterus slippery so that the foetus becomes loose and could spontaneously shed. Inhabitants in the study area are advised to avoid the use of these enset types for the purpose of treating ailments at any stage of pregnancy. A non-deliberate feeding of these leads to abortion; however, some people apply it deliberately to avoid unplanned pregnancy in the area. Previous reports also mentioned a considerable degree of abortifacient activity of extracts from a sister species of *Musa rosacea* (Srikanth et al., 2013). These mentions showed possible indications of similar phytochemical constituents in some enset types used as medicinally important items.

The fact that ‘Deri’ea’ (Gurage) was reported to cause sterility in females which is linked to the anti-fertility potential of its phytochemical constituents. Soni et al. (2013) reported the anti-ovulatory and antiestrogenic activities of a related species from a banana cultivar of *M. x paradisiaca*. Ethanollic extracts of banana stems exhibited anti-fertility properties via ant-ovulatory and anti-estrogenic activities in rats. Aqueous extracts of *M. balbisiana* seeds showed hypotesticular activity (Ghosh et al., 2017). This observation was evidenced by a significant reduction in sperm count, motility, and viability among experimental rats. The same phytochemical properties have been maintained among farmers’ landraces such as ‘Deri’ea’ (Gurage), which have anti-fertility properties under the same plant family of Musaceae. The medicinal preparation of enset-based food is consumed frequently at dining times until the disease or disorder is averted or cured, implying no serious dose rate is determined during oral prescription. However, for medicinal enset in which the sap is freshly drunk, a cup-full of fluid is recommended for children, whereas a glass-full of it is recommended for adults in treating stomach disorders.

2.4.5. Ailments or disorders treated using enset as traditional medicine

The diversity of ailments treated using enset exhibits the prevailing high cultural value of the germplasm and the residing ILK of ethnomedicinal applications. Enset was used to treat musculoskeletal disorders including bone fractures, back pains, muscle and joint cramps, trauma, and injuries or wounds. This observation is associated with higher concentration of micronutrients actively involved in the process of bone, skin, blood vessel, and muscle tissue reformation (Abraham Bosha et al., 2016; Ayalew Debebe et al., 2012; Minaleshewa Atlabachew and Chandravanshi, 2008; Solomon Tamrat et al., 2020). The high calcium and phosphorous contents of enset support the development of bone cells or tissues to fill the fractures and mend the gaps formed. Moreover, the high availability of zinc, magnesium, manganese, iron, and copper is responsible for the biosynthesis and strengthening of bone tissues. They act as co-factors or enhancers of bone density and strength after fractures (Karpouzou et al., 2017). In addition to higher mineral values reported, appreciably higher levels of essential and non-essential AAs were

reported from enset (Solomon Tamrat et al., 2020). Arginine, glutamine, and lysine are among these valuable amino acids involved in the healing of fractured bones (Polat et al., 2007; Wijnands et al., 2012; Sinha and Goel, 2009).

Birth-related complications, including delayed delivery and placenta dropping, as well as problems with either milk production or secretion, are the other prominent disorders threatened with enset. Diets with high energy and protein contents were recommended to overcome the negative energy balance during labour and heavy-duty tasks (Kurpad et al., 2005; Yeshambel Mekuriaw, 2023). Enset were reported to have higher carbohydrate and utilizable energy content and hence supply the required energy for cattle during labour for the ejection of the foetus and the placenta (Mohammed et al., 2013; Admasu Tsegaye and Struik, 2001; Pijls et al., 1995). In addition to higher starch or energy contents, enset contains other phytochemicals involved in delivery-related issues. The promising availability of functional AAs and minerals contributes to the facilitation of new birth and the dropping of placentas (Minaleshewa Atlabachew and Chandravanshi, 2008; Solomon Tamrat et al., 2020). Previous studies indicated that fruits from related plants, *M. acuminata* and *M. x paradisiaca*, were used to assist labour (Kamatenesi-Mugisha and Oryem-Origa, 2007). This observation is related to the reportedly high energy, calcium, iron, and zinc contents. Flower extracts from *M. x paradisiaca* and *M. balbisiana* showed improvements in the level of milk production in lactating rodents and women, respectively (Mahmood et al., 2011, 2012; Wahyuningsih et al., 2017). Stimulation of milk production is associated with higher abundance of lactose sugar enhancing production of lactation hormones which facilitates enhanced milk production (Cox et al., 1999). These reports are clues that phytochemical behaviour has been conserved among plants of *Ensete* and *Musa* (Lamxay et al., 2011; Mousa et al., 2019) within the same plant family; however, a confirmatory screening is important.

Injuries associated with wounding and related trauma under another ailment category received a high frequency of citations as disorders treated using enset as traditional medicine. The observation is associated with high phytochemical contents important in tissue repair and antimicrobial

activities. MacKay and Miller (2003) reported that food items rich in protein, arginine, glutamic acid, and zinc would enhance wound healing for patients with injuries and trauma. The higher contents of phytochemicals in enset are responsible for applications in the treatment of injuries, trauma, and wounding (Minaleshewa Atlabachew and Chandravanshi, 2008; Ayalew Debebe et al., 2012; Solomon Tamrat et al., 2020). Plants of the Musaceae family, including *M. x paradisiaca* and *M. sapientum*, were reported to show wound healing potential (Cheng et al., 2019; Agarwal et al., 2009). Glucosides, tannins, alkaloids, saponins, flavonoids, phenols, and terpenoids from these plants facilitate repairs in broken tissues (Shodehinde and Oboh, 2013; Pereira and Maraschin, 2015). Moreover, steroids, anthocyanins, fatty acids, carotenoids, and biogenic amines also provide the observed therapeutic properties (Mathew and Negi, 2017; Rex et al., 2018; Ghany et al., 2019). Mending of broken body parts and the healing activities were detected from various plant tissues or organs of *Musa* species. This discloses the conservation of biological activities in the plant family Musaceae, as evidenced through the treatment of fresh and mature wounds using enset.

The current study documented several ailments and disorders treated with enset. This has revealed the maintenance of a rich ILK of ethnomedicinal applications of the crop plant in traditional medicine. This ethnomedicinal knowledge in turn is linked to high metabolite contents, including dietary ash, minerals, crude protein, and essential AAs in enset. Significantly higher calcium, phosphorous, iron, potassium, magnesium, and zinc were detected from enset (Ayalew Debebe et al., 2012; Gizachew Woldesenbet et al., 2019). These biochemical contents have contributed to the setting of bone fractures, maintenance of the immune system, and other traditional medicinal values using enset. Apart from high dietary and mineral values, essential and functional AAs make enset-based foodtuffs or plant organs derivatives pivotal in maintaining immune strength and weight recovery (Solomon Tamrat et al., 2020). Farmers' landraces of enset including 'Askala' (Sidama), 'Gowariye' (Gurage), and 'Koshkowashiye' (Gurage) have high levels of arginine and calcium (the former landrace). These enset types are more dependable in their applications as treatments for the repair and reformation of broken musculoskeletal tissues.

2.4.6. Informant agreements and relative healing potentials of enset medicine

Higher indices of ICF were observed for the ailment classes of MSCTD and PCPC. The findings indicate a higher number of medicinally valued enset landraces commonly reported for the treatment of a diversity of ailments (Heinrich et al., 1998; Sharma et al., 2021; Zewdie Kassa et al., 2020). Moreover, these observations exhibit higher agreement levels among informants for farmers' landraces, which treat ailments under the two groups of ailments (Ermias Lulekal et al., 2013). The highest number of use citations was also reported for the ailment categories with the highest ICF. Ailments or diseases at the top positions of the ICF ranking are culturally most important for their prevalence and impact on the health status of people dwelling in the area. These ailments were observed in the top three to top five most frequently mentioned and most prevalent Outpatient Department (OPD) cases in the area. Such locally important health issues call for an integrated preventive approach in order to suppress their occurrences and consequences in the local economies.

So far, pieces of information have shown that enset is used in treating different ailments of the musculoskeletal tissues in southern Ethiopia (Melesse Maryo et al., 2018; Temesgen Magule, 2014; Pijls et al., 1995). Ailments of the musculoskeletal system were common, which is evidenced by their position among the top ten OPD cases. Since the majority of sampling sites were hilly and sloppy, local people and livestock are commonly exposed to falling and rolling downhill. The high frequency of citations for MSCTD is hence a result of frequent trauma, wounding, bone fractures, and back pain after sliding downhill. The higher frequencies of birth-related ailments are associated with traditional pregnancy handling practices. This is indirectly related to the remoteness of the majority of study sites from health centres. Reduced access to modern education, leading to poor awareness and attitudinal setups, is the backdrop of traditional pregnancy handling and associated complications. The dominant utilisation of enset in treating musculoskeletal ailments was surprisingly not supported by high ICF values. This was attributed to the utilisation of quite diverse types of medicinally valued enset of about 52.7% under the ailment categories of MSCTD.

The observations of higher FL values for ‘Askala’ (Sidama), ‘Argama’ (Wolaita), and ‘Gowariye’ (Gurage) indicate higher healing potentials of these enset types for specific ailments under MSCTD. This is an indirect evidence for the high biochemical composition of enset, including calcium, phosphorous, arginine, and glutamine, which facilitate bone and cartilage reformation (Abraham Bosha et al., 2016; Solomon Tamrat et al., 2020). Food items rich in some minerals, amino acids, and vitamins were reported to be important for the health of bones, cartilage, and ligaments (Karpouzios et al., 2027; Geiker et al., 2020; Devignes et al., 2022; Su et al., 2023). Moreover, observations of higher fidelity level values for some farmers’ landraces of enset call for phytochemical or micronutritional composition analysis.

2.4.7. Ethnoveterinary applications of enset in treatment of livestock ailments

In the study area, enset is not only used to treat human ailments but also for the targeting of livestock disorders. Utilisation of a considerable proportion (58%) of farmers’ landraces used in human medicine was observed as common remedies for livestock ailments. This scenario indicates higher integrity of livestock within enset farming systems and a strong association between medicinal uses of enset in the local cultures. The situation also exhibits the co-evolution of the ILK of disease treatment for humans and livestock side by side. The observation further explains that livestock rearing is an important complement for the conservation of medicinal enset in the area. Enset was reported for use in treating birth-related complications, bone breakage, and fractures both in humans and livestock (Pijls et al., 1995; Yemane G. Egziabiher et al., 2020; Temesgen Magule et al., 2014; Melesse Maryo et al., 2018). Though birth-related disorders were common, injuries and wounds were also treated using enset in both humans and livestock.

Preferential application of some farmers’ landraces over others in targeting livestock ailments is linked to differences in nutritional or phytochemical contents. Different enset varieties and plant

parts have shown significant differences for crude protein, dietary ash, and dry matter contents (Ajebu Nurfeta et al., 2008a). Abraham Bosha et al. (2016) reported higher starch from wild enset whereas cultivated landraces contained high levels of crude protein and minerals. Similar observations were reported for some species or cultivars of the banana (*Musa*) genus (Villaverde et al., 2013; Oyeyinka and Afolayan, 2020). The common farmers' landraces used in the treatment of livestock ailments have distinctive chromas of deep reddish, pinkish, or purplish colours on their vegetative parts (Yemane Tsehaye and Fassil Kibebew, 2006; Melesse Maryo et al., 2018). Unique colour pigments on surfaces of various plant organs are indications of special phytochemical constituents in plants. Orange-fleshed sweet potatoes contained high β -carotene, whereas purple-fleshed varieties showed high anthocyanins (Alam, 2021). However, these coloured enset types have not been screened for their phytochemistry, which seeks further biochemical confirmatory tests in the future.

The prevalence of PCPC, MSCTD, and IPCCEC as the most diverse livestock class of ailments is in line with their prevalence in the area, based on reports from veterinary departments in the study area. Shortage of milk secretion and weight loss through stunting are also important livestock disorders reported. Local experts reported that land fragmentation within enset-based agroforestry systems has resulted in a shortage of personal or communal grazing lands. Similar observations of land fragmentation and associated shortages were reported from previous studies (Corral et al., 2021; Leduc et al., 2021; Shimelis Mengistu et al., 2021). Trauma, wounds, and related musculoskeletal disorders, including bone fractures and breakage were among top priority for ethnoveterinary importance. This is linked to the dominant sloppy terrain and rugged topography of the enset-growing highlands. Livestock fall and roll down a hill and commonly get mechanical injuries. As a result of these conditions, trauma, wounds, and bone fractures were the common problems in the area.

Whole enset parts, chopped freshly, are primarily used in ethnoveterinary medicine, where enset is used as functional fodder for livestock. Plant parts from selected farmers' landraces of enset

served as medicinal treatments in the form of fodder and also targeted some disorders and health complications. The observation also dictates the distribution of bioactive phytochemicals and nutrients all over the plant. This is why the overall parts of enset plants are used as forage to enhance body weight, immune strength, and milk production (Ajebu Nurfeta et al., 2008b; Ajebu Nurfeta et al., 2012; Mohammed et al., 2013). Higher amounts of crude protein, AAs, dietary fibres, sugars, soluble carbohydrates, and starch were reported from different enset parts (Mohammed et al., 2013). This makes the plant important to enhance weight gain, develop immunity, and increase milk production in livestock. Solomon Tamrat et al. (2020) reported considerable amounts of calcium, iron, phosphorus, and zinc in addition to essential and functional AAs from enset. These findings in general support the utilisation of all parts and products of enset in the treatment of livestock ailments.

Enset is served to livestock in raw slices exposed to the sun without additives to target ethnoveterinary ailments. This mode of use has implications for the bioavailability of bioactive phytochemicals or food nutrients. The first is the presence of biochemicals in enset, which are readily available to the digestive system of the animal. The second indication is that enset contains a diversity of nutrients good enough for the treatment of health disorders in livestock with minimum additive use. Higher crude protein (in leaf blades), dry matter (in corms), and dry matter digestibility (in pseudostem and corm) were reported by Ajebu Nurfeta et al. (2008b). These attributes make all enset parts valuable as feed sources as well as traditional remedies against livestock ailments. This dietary value and bioactivity are maintained and reinforced using raw plant parts that have a higher nutritional content. A mineral salt called ‘Amole’ (Sid.) is used as an additive to enset to enhance taste and encourage effective foraging in the treatment of livestock ailments.

The reported abortive effects of feeding pregnant livestock with farmers’ landraces such as ‘Askala’ (Sidama), ‘Mundiraro’ (Sidama), ‘Lochingiya’ (Wolaita), ‘Yekembata-Astara’ (Gurage), and ‘Yejima-Astara’ (Gurage) are related to their phytochemistry and associated

bioactivities. The hydro-alcoholic extracts of *M. rosacea*, a related species of *Musa* genus, showed strong abortifacient properties (Srikanth et al., 2013). This report is evidence for similar phytochemical constituents in some farmers' landraces of enset under the same plant family of Musaceae. The reported death in cattle resulting from consumption of higher doses of plant parts from 'Gowariye' (Gurage) is linked to high ratios of indigestible to digestible organic matter in this specific farmers' landrace (Graber, 2012). The local people reported that this farmer's landrace is known as "dry enset" in the local contexts, which indicates the tissues are hardly digestible in the animal's stomach. In cases where this ratio gets higher, the rate of digestibility of stomach-full of the product served to an animal interferes with the normal digestive process (Graber, 2012). This in turn causes complications like stomach bloating, gas accumulation, hampered energy harvesting, and death. Enset-based feeds have shown differences in digestibility due to variations in farmers' landrace and plant parts. Feeds containing leaf lamina and corms, as well as 'Astara' and 'Gulumo' landraces, have shown the lowest and highest degradability, respectively (Ajebu Nurfeta et al., 2008b).

2.4.8. Ranking of multi-medicinal and rare farmers' landraces of enset

The high cultural value of medicinally important farmers' landraces of enset is manifested through their applications in treating several ailments. 'Askala' (Sidama), 'Argama' (Wolaita), and 'Astara' (Gurage) are the highest priority enset among the SWGs used as multimedicinal items. The possible scientific explanation is higher concentrations and diversity of bioactive phytochemicals or micronutrients. Frequent reports of targeting ailments are associated with higher antimicrobial, antioxidant, and other bioactivities within plants (Ermias Lulekal et al., 2013; Tauchen et al., 2015). In similar ways, multi-medicinally applied enset types have contributed to the treatment of diverse types of ailments and disorders. Based on a recent study, farmers' landraces used in targeting several ailment types contained higher levels of essential minerals, crude protein, and functional AAs (Solomon Tamrat et al., 2020). Supporting information about the availability of bioactive components from enset was also mentioned in

previous studies (Admasu Tsegaye, 2015; Azene Tesfaye and Abebe Girma, 2017; Sethiya et al., 2019; Gizachew Woldesenbet et al., 2019).

Prioritisation of farmers' landraces of enset as the most extracted and rare is allied to the trends of local trait factors reported as challenges of enset production. Those enset landraces with high demands for their medicinal values were reported as locally rare because of overharvesting and the associated lower frequency of replacement through corm cutting. Medicinal plants with high local consumption and multiple uses exhibited higher values of direct matrix ranking and were reported locally most frequently (Ermias Lulekal et al., 2008; Moa Megersa et al., 2013; Zewdie Kassa et al., 2020). 'Askala' (Sidama), 'Arkiya' (Wolaita), and 'Astara' (Gurage), the rarest farmers' landraces, were poorly multiplied, leading to a shortage of seedlings. Moreover, their tasty corm in high demand for consumption as delicious boiled tuber is harvested in addition to their medicinal consumption, making their access even rarer. A community norm was mentioned that a person in need of getting medicinal enset from one's garden is granted access free of charge. The perception involves thinking of medicinal enset as items of common use, and hence farmers are poorly motivated to multiply these for the benefit of a passersby, which was a common report among farmers. Therefore, local people are used to managing lower numbers of individuals of medicinal enset, leading to reduced local availability. A similar situation of production of income, land, and labour profit-oriented crop farming was observed in parts of the south where enset is being replaced for cash crops (Bizauyehu Tesfaye et al., 2008; Tesfaye Abebe et al., 2010; Mesfin Sahle et al., 2022). Negligence to multiply some valuable medicinal enset in the area is also a challenge that needs interventions before their total loss in the area.

2.4.9. Factors determining local availability of medicinal enset

Enset used in medicinal applications are locally threatened by lesser demands associated with various factors among the farmers for germplasm multiplication. Some medicinal enset types also

used as food items are hunted by females for tasty corms and hence get locally rare. This challenge of local availability is further aggravated by the few sprouts they produce upon corm cutting treatments. Moreover, medicinally valued plants that mature lately and others prone to challenges of disease and pests are poorly demanded for multiplication. Similarly, agronomic factors, including early maturity as well as disease and drought resistance, have affected farmers' crop varietal selection (Shikuku et al., 2019; Azu et al., 2021; Lidya Samuel et al., 2023; Miriti et al., 2023). The diminished interest in the multiplication of these enset landraces is further associated with the shortage of farming lands. Household farm size and other socioeconomic variables were reported to significantly determine crop diversity and varietal selection patterns (Belay Maru et al., 2022). Enset-producing farmscapes are known for the sharing of common paternal lands among descendants, leading to land fragmentation and higher population density, which is negatively affecting production and productivity of enset (Bizuyehu Tesfaye, 2008; Abraham Shumbulo et al., 2012; Abiyot Legesse, 2013; Belachew Garedew et al., 2017). Since enset takes longer to mature, farmers are less tolerant of waiting for crops, which take longer to mature. The local people adjust their varietal species preferences for multiplication to avoid crops that stay longer before maturity.

Challenges from wilt disease and pest attacks observed in the area also contributed to reduced availability or loss of medicinal enset. These valuable farmers' landraces were reported for their soft and tasty corms (Zerihun Yemataw et al., 2016, 2017; Melesse Maryo et al., 2018; Kutoya Kusse et al., 2021). This condition has led to challenges in hunting by both vertebrate and invertebrate pests, which had contributing to reduced local availability of certain landraces. Vertebrate pests, lack of quality "seedlings," bacterial wilt, corm rot, enset root mealy bugs, and recurrent drought are major factors. Farmers also use some criteria to discriminate against enset landraces primarily used for medicinal and food purposes that exhibit undesirable traits (Shikuku et al., 2019; Miriti et al., 2023). Sliminess, reduced accumulation of edible materials, poor regeneration potential exhibited through reduced sprouting, excessive hydration, and bitter corms were the major criteria used. Locally rare enset should be promoted for integrated *in-situ* and *ex-situ* conservation actions in the future. One of the possible management options is to find

alternative use values for the variously described farmers' landraces, including reclaiming drylands and wastelands and planting them as live fence or cattle fodder.

2.4.10. Socioeconomic status as determinant factor for ILK of enset use as medicine

Higher average numbers of medicinally valued enset were reported by informants from Gurage and Wolaita than from Sidama. This is associated with differences in the enset farming systems of the different localities sampled. Enset is intercropped with diverse agroforestry species in Sidama. The high species and crop functional group diversity managed within farms makes the continuum of Sidama-Gedeo-Amaro enset-coffee multi-species complex agricultural systems (Solomon Tamrat et al., 2011; Abiyot Legesse, 2013, Tadesse Kiphie, 2000; Fisseha Mesfin et al., 2014; Reta Regassa, 2016). A high diversity of 16 plant species and eight crop functional groups on average per farm was reported from enset-coffee-based home gardens in Sidama (Tesfaye Abebe et al., 2013). This indicates that the collection of medicinal values from plants other than enset has reduced the harvesting rate of enset for medicinal purposes. The multispecies home gardens of Sidama provide a wider range of economic benefits, from food to medicine, fodder, sources of cultural materials, and many others (Nigatu Tuasha et al., 2018; Banchiamlak Nigussie and Kim, 2019). This diversity of use categories with raised alternative plant species to treat ailments has led to the reduced application of enset as traditional medicine in Sidama. On the other hand, framers from the Gurage and Wolaita highlands maintain sole enset farms and manage other crops on separate plots of land (Talemos Seta et al., 2013; Mesfin Sahile et al., 2021). Sole cropping leads to reduced home garden species and crop functional diversity, as well as lower medicinal plant diversity. This would in turn call for a higher reliance on enset-based medicine in treating cultural ailments among the Gurage and Wolaita people.

The reports of higher average numbers of medicinal enset from elders are attributed to their age-long experiences in acquiring cultural knowledge of enset use and management. The observation also indicates that the youth tend to attend modern medicine rather than consult the valuable medicinally important enset in their gardens. The ILK of ethnomedicinal applications of enset is

not evenly distributed in the community, as depicted by the higher numbers of medicinally valuable farmers' landraces reported from key informants than general informants. This exhibits that, in addition to age factors, cognitive levels among informants contributed to variations in the acquisition of ILK for medicinal use of enset. Similar trends had been reported from previous studies, such as that key informants and elderly people mentioned significantly more medicinal plant species in the local contexts (Ermias Lulekal et al., 2013; Zewdie Kassa et al., 2020). The higher enset use tendency by older people is both an opportunity and a challenge for the conservation of medicinal enset. The opportunity comes when elders protect the information and the germplasm from loss, and the threat arises when the knowledge is threatened or lost before it is properly documented.

Literate informants had reported higher average numbers of medicinal enset types attributed to better awareness through schooling. This implies a better realisation of the medicinal values of enset in the studied community after schooling. Charts containing lists of the local names of farmers' landraces were posted on the walls of the staff rooms of primary schools. These were reported for their use as teaching aids. Hence, the inclusion of enset in early-age schools has contributed to better awareness of local diversity and utilisation, including traditional medicinal applications. Amanuel Berhanu et al. (2022) reported a positive and significant influence of education on enset production in the Jimma Zone of southern Ethiopia. Banchiamlak Nigussie and Kim (2019) also reported that formal education is positively correlated with the number of medicinal plant species reported in local areas. The higher number of reported medicinal enset from informants who attended schools, however, is contrary to global trends. In the case of our study, modern education has favoured the utilisation and conservation of medicinally valued enset. Farmers' field schooling and inclusion in formal education were recommended for high knowledge of cultural diversity, use, and conservation of enset (Temesgen Magule et al., 2014). Most commonly, in the cases of studies related to herbal medicine, a higher number of mentions for plant species are associated with the illiterates (Ermias Lulekal et al., 2013; Zewdie Kassa et al., 2020).

Male and female informants showed similarities in the average number of medicinal enset reported, attributed to uniformity in the sharing of ILK among genders. Indigenous knowledge on ethnomedicinal applications of enset is transferred from mothers to daughters and fathers to sons based on local reports. Previous reports explained that enset is a gender-sensitive crop, as evidenced through morphological classification into “male” and “female” (Temesgen Magule et al., 2014; Zerihun Yemataw et al., 2016). Females in the households were commonly involved in the selection and management of enset landraces known for high quality of tasty corms. These are called ‘female’ enset types in the localities and are more preferred among females, even though they develop pseudostem with shorter length and narrower girth. These landraces are reported for having softer pseudostem and are prone to disease, pests and drought. Female household members in the study localities were also reported for preparation of enset fermented products and making of various foodstuff. In the contrary, males are commonly involved in the farm management, overall selection and placement of farmers’ landraces in the farms and prefer the ‘male’ enset types over the ‘females’. Therefore the diversity of enset in the study area has been almost evenly distributed among the two sexes during the process of farm management, landrace selection, processing of enset based food and preparation of foodstuff from fermented and raw edible products. These and other unexplored factors have contributed to similarities in the use and knowledge of enset as traditional medicine among males and females in the area. The direct association of land holding and enset holding with the levels of ILK is attributed to a relaxed land accommodations to incorporate high diversity of enset in general and medicinal enset in particular. Those farmers having more hectareage of total holdings have more relaxed opportunities to allocate high coverage of the total land for enset production in general. Similarly, a household with wider enset farms get more chances for collection and maintain of more numbers of medicinally valued enset since they have less space constraints to plant these.

Transfer of ILK of enset use from highlands to lowlands was a common report. The observation is attributed to the suitability of the Ethiopian highlands for suckering and multiplication of enset.

The observation is supported by previous reports explaining the highlands of southern and southwestern Ethiopia as centres of origin and diversification of enset (Smeds, 1955; Simmonds, 1960; Bizuayehu Tesfaye et al., 2002). Households dwelling at different altitudes share ILK of enset use for general and/or special purposes. These include harvesting enset for medicinally valuable landcaes or the tasty corm. Doing so, they try to fill the knowledge and germplasm gaps across agro-climatic variations (Admasu Tsegaye and Struik, 2002; Bizuayehu Tesfaye and Ludders, 2003; Melesse Maryo et al., 2018). ILK for medicinal uses of enset is locally transferred within a household from fathers to elderly sons and mothers to elderly daughters in favor of maintainance of uniformity in enset use knowledge. The ultimate goal of this practice in the study area was conservation of enset against various biotic and abiotic factors those challenge its production and priductivity.

2.4.11. Conservation approaches used to protect medicinal enset

Multiplication of enset germplasm through corm cutting, and enriching farms with additional landraces indorporated through borrowing, or buying seedlings are among indigenous practices applied in the conservation of enset. Zerihun Yemataw et al. (2016) reported various conservation practices to protect the loss of valuable enset germplasm in southern Ethiopia. The practice of transferring and adapting farmers' landraces from one ethnic group to another was a commonly applied *ex-situ* strategy. Temesgen Magule et al. (2014) mentioned that regular propagation and harvesting cycles and maintenance of multiple type of enset on-farm were used as important conservation options. Among the Wolaita people, colourful and ornamental enset were placed in the front yards, and medicinal ones were hidden in the back yards as special germplasm protection practices (Temesgen Magule et al., 2014). Moreover, traditional practices of pest control, including disturbance of nesting grounds were followed for the conservation of enset (Melesse Maryo et al., 2018). Management of medicinal plants such as *Pycnostachyus abyssinica* and *Canna indica* was used to repel bacterial wilt and enset root mealybugs (Daniel Kasa et al., 2013; Getahun Yemata and Masresha Fetene, 2017). Rotation of annual crops intercropped within enset farms, use of

clean cutting tools, and management of disease-tolerant landraces were also applied as conservation measures among the Kembata people (Melesse Maryo et al., 2018). These traditional enset protection practices should be encouraged and supported to enhance the valuable cultural diversity of enset, especially the rare and locally diminishing medicinally useful farmers' landraces.

To summarize, the study has documented a high diversity of farmers' landraces of enset applied in traditional medicine. These valuable cultural diversities have been characterised by farmers' perceptions and morphological traits. The parts used as well as modes of preparation and applications of enset in remedy, along with the diversity of ailments treated, make up part of the knowledge compiled. These findings indicate the high potential of enset in disease management as preventive or curative items. The observations pave a new path for enset research directing into traditional medicine, phytochemical characterization, antimicrobial screening, and pharmacological evaluations.

A higher rate of utilisation of enset corms as traditional remedies indicates accumulations of higher concentrations of bioactive phytochemicals. The scenario also exhibits extractive use of the plant, i.e., uprooting and collecting the corm, an organ used as a means of multiplication. Excessive corm collection in association with reduced demands of farmers to multiply medicinally valuable enset puts these groups under threat of local loss. Since the plant species was also reported as the least concern by the IUCN red list, it is calling for further conservation efforts before it passes into the higher threat categories. Farmers' landrace-based multiplication of the valuable enset diversity and awareness creation towards reduced attitudes towards multiplication of medicinal enset should be future conservation approaches to follow.

The ailment classes of PCPC receiving reportedly the most efficacious enset and the highest informant citations went to DMSCT. The scenario indicates the prevalence and economic impacts of these disorders and complications in the area. Hence, it demands integrated

preventive and/or curative measures to minimise or avoid the economic impacts of these health issues. The observed difference in the levels of ILK on medicinal applications of enset among different social groups shows some level of localization. The situation dictates a tendency for future loss of ethnomedicinal use information and calls for immediate conservation efforts. Protection of ILK, along with *in-situ* or *ex-situ* conservation of the diversity of medicinal enset is an action in need. This could be achieved through collection of farmer's landraces on local or regional levels, both as gene banks and seed banks, in the future.

CHAPTER THREE

3. Micronutrient composition and microbial community analysis across diverse landraces of the Ethiopian orphan crop enset

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Abstract

Enset (*E. ventricosum*) is a major starch staple and food security crop for over 20 million people in southern Ethiopia. Despite substantial diversity in morphology, genetics, agronomy and utilization across its range, nutritional characteristics has only been reported in relatively few landraces. Here, a survey of nutritional composition in 26 landraces from three enset growing regions of Sidama, Wolaita and Gurage of the former SNNPRS were presented. Mineral and physiological AA characterization of raw or fresh (neither fermented nor cooked) and processed (fermented) tissues, and genomic analysis of the microbial community associated with fermentation were analyzed. Total ash and crude protein were evaluated using protocols from official methods of analysis (AOAC). Values of total ash was analyzed through complete combustion of samples at high temperature after charring. Total crude protein were evaluated applying the Kjeldahl digestion of samples at high temperature, followed by distillation and titration. Mineral analysis were conducted following the ICP-LC/MS, where internal and external multielemental calibration standrads, bespoke external multi-elemental calibration solutions, blank digests and certified reference materials were properly used. Physiological (free) AAs were deremined using a kit (EZ: faast™ kit) where derivatized AAs were quantified after comarision with calibration AA standards. Microbial communities of Kocho were determined via genotyping by sequencing (tGBS) using Cetyltrimetyl Ammonium Bromide (CTAB) buffer, and mapping retained sequences to reference bacterial and fungal genome from National Center for Biotechnology Information (NCBI). Enset showed significant varitions in mineral concentrarions across sampling sites and plant parts. Samples sourced from Gurage showed the highest AA, where as corms showed significantly higher AAs than pseudostems. In comparison to regionally important tubers and cereals, enset showed higher calcium, iron, potassium and zinc. We report changes in composition due to processing, with net loss of overall AAs, and enhanced essential AAs after fermetation. Arginine was observed at its highest amout in enset. Corms and leaf blades contained highes AA implying high nutritional and phytochemical values in disease treatment. We establish that bacteria genera *Acetobacter*, *Lactobacillus* and *Bifidobacterium*, predominate during fermentation. Nutritional and microbial variation presents opportunities to select for improved

composition, quality and safety with potentially significant impacts in food security and public health.

Keywords/ phrases

Ensete ventricosum, Ethiopia, Fermentation, FAAs, Food security, Kocho, Macronutrients

3.1.Introduction

Humans currently satisfy most dietary requirements with surprisingly few species (Borrell et al., 2019b), yet a much greater diversity of nutritionally suitable plants have been identified, often with narrow regions of utilization (Mayes et al., 2012). The food products of the large perennial herb *E. ventricosum* (Welw.) Cheesman (Musaceae) are an important dietary starch source in Ethiopia (Borrell et al., 2019a; Solomon Workneh and Neela, 2019; Almaz Negash and Niehof, 2004). Commonly known as enset (or alternatively as the false banana or Abyssinian banana), this major food crop is principally cultivated as a highly resilient staple that withstands a wide range of environmental conditions and can buffer seasonal variation in food availability. Enset contributes to the food security of over 20 million people, but is virtually unknown outside of its narrow zone of cultivation in South West Ethiopia, despite growing undomesticated and unutilized across much of East and Southern Africa (Borrell et al., 2019a). In addition to being processed for multiple food products, enset is also used for livestock fodder, packaging materials, fiber and traditional medicine (Borrell et al., 2020; Mohammed et al., 2013; Temesgen Magule et al., 2014).

Despite the importance of enset for food security and the existence of hundreds of diverse landraces (Borrell et al., 2019a), the nutritional composition of the raw plant tissues and processed food products (e.g. Kocho) has only been reported in a small number of landraces (Abraham Bosha et al., 2016; Tadessa Daba and Shigeta, 2016; Mohammed et al., 2013; Ajebu Nurfeta et al., 2008). These reported nutritional composition and the relative concentrations of certain micronutrients

show considerable variation in enset. Whilst this may be attributable in part to differing analysis methods, the extensive diversity of genetically differentiated enset landraces (Borrell et al., 2019a; Dagmawit Chombe and Endashaw Bekele, 2011), heterogeneity of farm management practices (Belachew Garedew et al., 2017; Temesgen Magule et al., 2014) and environmental conditions, particularly soil (Tilahun Amede and Mulugeta Diro, 2005; Borrell et al., 2019a), may also be a contributing factors. Therefore, we highlight the need to profile micronutrient composition across a representative subset of enset landrace diversity, whilst also characterizing the ubiquitous effects of fermentation on the composition, relevant to the quality and safety of enset foods, and the associated microorganism diversity responsible for mediating tissue processing.

The edible parts of enset comprise the starch rich pseudostems forming the pseudostem (overlapping leaf sheaths) which are decorticated, and the corm (the underground base of the stem that serves as a storage organ) which is pulverized and pressed (Borrell et al., 2019a). These two main tissues are collectively processed, using fermentation pits; into starch staples including Bulla and Kocho (see Genet Birmeta et al., 2018, for a detailed description). The precise fermentation practice is variable among regions and cultural groups (Belachew Garedew et al., 2017; Tariku Hunduma and Mogese Ashenafi, 2011). It may include a starter culture made from enset Karssa et al. (2014) or other co-occurring plant species (Abraham Bosha et al., 2016), or simply be initiated from mashed tissue left for several days at ambient temperature (Genet Birmeta et al., 2018). Kocho is the bulk of the fermented product and is baked into thin fibrous bread considered to have a good shelf life. Bulla is a small amount of water-insoluble starchy product separated from the Kocho during processing by squeezing and sometimes consumed separately.

The corm of enset is also occasionally consumed boiled, much like potato, and this is called *amicho*. The microorganisms responsible for fermentation alter the chemical composition of the raw substrate, which in some cases enriches the nutritional value of fermented products (Tamang et al., 2016) by removing antinutritionals and breaking down complex components. Furthermore, microbial communities introduced during processing are often critical to food safety and

preservation by preventing growth of spoilage and toxic organisms. These microorganisms, often occurring as communities in food products are poorly known in orphan and minor tropical crops cultivated by subsistence farmers (Tamang et al., 2016). However, improvement of these cultures represents a relatively accessible opportunity to enhance the nutritional consistency, bioavailability and quality of neglected food products (Chelule et al., 2010).

In a study on *Kocho* production from enset, Birhanu Abegaz (1987) reported that *Leuconostoc mesenteroides* and *Streptococcus faecalis* are responsible for initiating fermentation and reducing the pH. These are then superseded by the homofermentative bacteria *Lactobacillus coryniformis* subsp. *coryniformis* and *L. plantarum* which reduced the pH further. More recently, in an analysis of *Kocho* samples from the Wolkite area, Helen Weldemichael et al (2019) identified *Kocho*-associated bacteria as predominantly from the genera *Lactobacillus* and *Acetobacteri*, whilst a survey of *Kocho* samples from the Gamo highlands identified high relative abundance of *Enterobacteriaceae* and *L. mesenteroides* subsp. *cremoris* in the early stages of fermentation (Addisu Fekadu et al., 2018). More generally, the process of fermenting enset has been reported to reduce total protein and carbohydrates, whilst increasing FAAs (FAAs) 1.6-fold (Kelbessa Urga et al., 1997). The presence of FAAs in fermented enset products, particularly those essential to maintain health, are important to understand as they represent those that are actually consumed and could have advantages with respect to their bioavailability and flavour (Bhutta and Sadiq, 2012; Kato et al., 1989). However, the specific FAAs in fermented products have not been characterized. Therefore whether enset is a source of essential amino acids (those the body cannot synthesize in sufficient quantities) in this form is unknown.

Significant genomic, phenotypic, environmental and agronomic variation in enset was hypothesised to result in variation in nutritional composition across landraces. In this study we investigate selected nutritional values with a focus on inorganic heavy metals, trace elements and FAAs in domesticated enset in Ethiopia. Both raw tissues and processed (fermented) products in samples from 26 farmers' landraces across three major enset growing regions was profiled, and a

quantitative genomic survey of the microbial community associated with enset fermentation was presented. Landraces were selected for nutritional composition analysis based on a contemporary study on traditional medicinal applications of enset where those receiving high frequency of mentions and fidelity level values were targeted. The results were placed in the context of other regionally available staples and the opportunities that nutritional and microbial variation presents for applied biotechnology to improve the nutritional quality, consistency and safety of enset derived foods.

3.2. Material and methods

3.2.1. Sample collection and preparation

Fresh samples of six different types of enset parts or plant organs (corm, pseudostem, leaf, fruit flesh, fruit peel (exocarp) and seed were harvested (Table 9; Appendix 7) from a selection of mature enset plants at the ages not less than five years ($n = 32$, landraces = 26). These samples were collected from three administrative areas of Sidama, Wolaita and Gurage zones in the former Southern Nations Nationalities and Peoples Region (SNNPR), Ethiopia. In selecting samples we sought to capture the full range of phenotypic and cultural variation. Selected plants were individually prepared, processed and fermented following traditional local practices, also following Habte Tiruha and Papini (2018) and using associated qualitative indigenous knowledge related to processing was recorded. The method followed does not follow the indigenous applications of a mix of any of matured enset plants irrespective of their landraces in order to check varietal differences in nutritional contents. Corms were grated using scapula of a cattle or a wooden (hard wood or the base of an old bamboo plant) material that is toothed for crunching of tissues in to pieces, and pseudostems were decorticated using rod of a halved toothed bamboo or a similar shaped metallic instrument. ‘Bulla’ is extracted after squeezing tissues or simply allowing the fluid to drain using a slopy drainage from the decorticated pseudoatem in to a smaller pit prepared for this purpose. In some instances when a high quality of fermented food product is targeted the bulla fluid is left with decortiacet pseudostem to produce whole ‘Kocho’ in Sidama

areas. The decorticated corm and pseudostem are left in open air for about 11 to 15 days for removal of excess moisture and to allow surface fermentation with addition of a small amount of starter culture. Subsequently pit fermentation will be started by thoroughly mixing the tissue mass with itself after adding of enough material of the starter culture for about three months ('Kocho' and 'Bulla') and six months (for 'whole 'Kocho', a product fermented for extended times commonly aimed for preparation of high quality food in Sidama area). The time required for fermentation depends on the immediacy of the food required to feed householders or the quality of the product required. In times of food shortage or scarcity, fasted fermentation will be established, whereas when the target is to get a high quality of 'Kocho', then fermentation could be allowed to stay even more than six months. The fermentain was done in Dara, Kindo Didaye and Enemore and Enir districts during the dry season in 2018. Subsequently, samples of three enset products Kocho, Bulla and Whole 'Kocho' (whereby the bulla liquid is not isolated during preparation, a common practice in parts of Sidama) from selected plants were collected post-fermentation, air dried and powdered using an electric grinder. The total dietary ash and total crude proein were analysed in the food analysis laboratory of Center for Food Science and Nutrition (AAU, Ethiopia) in 2018. Major, minor and trace metal analysis were conducted in the university of the Notingham, in London (UK) in 2019. Amino acid analysis were conducted in the Jodrell laboratories of Royal Botanic Gradens (Kew) in London (UK), whereas the genomic analysis of the microbiology of 'KOCHO' fermentation was conducted in the University of the Leicester (UK) in 2019.

Table 9: Enset tissue and food product types evaluated in this study

Type	Tissue	Sample	Age of plants sampled	Time of sampling after fermentation	Use & food products
Tissue	Corm	20	5 years	-	A raw, underground storage organ used to make both kocho and bulla. Also boiled and eaten as 'Amicho'
	Pseudostem	9	5 years	-	A raw above ground tissue comprising overlapping leaf sheaths. The pseudostem is the main tissue type making up the bulk of both kocho and bulla. Petioles and leaf midribs are fed to cattle as fodder.
	Leaf	1	5 years	-	Used as a protective layer in baking bread and to line the kocho processing area and fermentation pits
	Fruit flesh	1	fruting	-	Not currently utilized
	Fruit peel	1	fruting	-	Not currently utilized
	Seed	2	fruting	-	Not currently utilized
Product	Kocho	11	5 years	3 months	The fermented material of grated corm and pulverized pseudostem, used to prepare a range of food products. The most common of which is a type of dense flatbread
	Whole Kocho	7	5 years	6 months	Similar to kocho, above, but made without extracting the bulla liquid. In this study this practice occurs in Sidama
	Bulla	8	5 years	3 months	The water-insoluble starchy liquid extracted from kocho by squeezing. Bulla can be dried and used as flour, or made into a gelatinous porridge. It is produced in small amounts and often considered the most valuable enset food product.

3.2.2. Micronutrient and mineral composition

Micronutrient content used only raw corm tissue, as relative concentration of micronutrients should not be affected by fermentation, and taking in o account the major enset parts used as traditional medicine. Total ash content was estimated using 5 g of desiccated corm tissue. Samples were charred in a hot plate (Wagtech Model ST15, Sweden) and ignited at 550 °C for 5 hours in a Muffle furnace (Carbolite Model S302RR, Sweden). Residual ash was calculated as a percentage of the original desiccated sample weight. Mineral content was assessed using 0.5 g corm samples

digested in a closed high-performance microwave digestion system (ETHOS) with the addition of trace metal grade 65% HNO₃ (Fisher Scientific, UK) and H₂O₂ according to the manufacturer's recommendations under high pressure and temperature. The digest was washed with distilled water (Milli-Q). Multi-elemental analysis was conducted using an inductively coupled plasma liquid chromatography mass spectrometry/ ICP-MS/ (Thermo-Fisher Scientific iCAP-Q; Thermo Fisher Scientific, Bremen, Germany). Samples were introduced from an autosampler (Cetac ASX-520) incorporating an ASXpress™ rapid uptake module through a perfluoroalkoxy (PFA) Microflow PFA-ST nebuliser (Thermo Fisher Scientific, Bremen, Germany). Internal standards were introduced to the sample stream on a separate line via the ASXpress unit and included Ge (10 µg L⁻¹), Rh (10 µg L⁻¹) and Ir (5 µg L⁻¹) in 2% trace analysis grade (Fisher Scientific, UK) HNO₃.

External multi-element calibration standards (Claritas-PPT grade CLMS-2 from SPEX Certiprep Inc., Metuchen, NJ, USA) including Al, As, Cd, Ca, Co, Cr, Cs, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Pb, S, Se, and Zn, in the range 0–100 µg L⁻¹ (0, 20, 40, 100 µg L⁻¹) were used. A bespoke external multi-element calibration solution (PlasmaCAL, SCP Science, France) was used to create Ca, Mg, Na and K standards in the range 0–30 mg L⁻¹. In addition, KH₂PO₄ and K₂SO₄ solutions were used to calibrate the machine during determination of P and S, respectively. Sample processing was undertaken using Qtegra™ software (Thermo-Fisher Scientific) utilizing external cross-calibration between pulse-counting and analogue detector modes when required. Blank digests containing all except enset samples were used for quality control. In addition, a certified reference material (SRM 1567b-wheat flour) from the National Institute of Standards and Technology (NIST, USA), was used for standardization. To visualize the variation in micronutrient and mineral composition, we performed a principal component analysis and plotted variable loadings of the first two axes. We tested for a significant difference in sample composition between regions using permutational analysis of variance (PerMANOVA) to partition euclidian distance, implemented with the Adonis function in the R package vegan (Oksanen et al., 2019). Comparative plant nutritional composition data from other relevant species was sourced from the USDA Food Composition Databases (USDA, 2012) and Feedipedia (Heuze et al., 2017), and plotted together with data from this study.

3.2.3. Crude protein and FAA composition

Crude protein was estimated using 0.5 g of desiccated corm tissue, also considering frequent applications of these enset parts in the treatment of various ailments in the study area. Briefly, 6 ml of conc H_2SO_4 and 3.5 ml of H_2O_2 were added followed by 3 g of a catalytic mixture of CuSO_4 and K_2SO_4 (1:15) for 15 min. The solution was heated at 370°C using a kjeldahl digester until a clear solution was observed and distilled using the Kjeldahl distillation apparatus (Auto K9840 Analyzer, Kjeltex, BCR-Technology Co, Ltd., China). The auto distiller was adjusted to add 30 ml distilled water (to avoid precipitation of sulfate in the solution), and 40 ml NaOH (35%) in order to neutralize excess acid, break down ammonium sulfate and release ammonia gas. The distillate was collected for 8 min in a 250 ml Erlenmeyer flask containing 2% boric acid and five drops of methyl red indicator. The distillate was titrated with standardized 0.1 M HCl and sample nitrogen (N) content determined by subtracting the N content of blank control samples.

Free amino acid composition was assessed in a subset of samples using an EZ: faast™ kit (Phenomenex, Macclesfield, UK). Powdered samples (100 mg) were extracted in 100% water and sonicated for 20 min prior to centrifugation. Supernatants were derivatized using the method described in the manufacturer's instructions, and as described previously (Dziągwa-becker et al., 2018). Analyses of derivatized amino acids were performed with 2 μl sample injections on a Thermo Scientific system, consisting of an 'Ultimate 3000' UHPLC unit and an 'LTQ Velos Pro' mass spectrometer (Thermo Scientific, Waltham, MA, USA). Chromatography (qualitative and quantitative analyses) was performed as described in the EZ: faast™ kit's instructions, with amino acids assigned by comparison with the calibration amino acid standards provided with the kit. For each enset sample, all analyses were performed in triplicate, and the base peak areas for detected amino acid derivatives analyzed using Thermo Xcalibur (Thermo Fisher Scientific, USA) and R software (R Core Team), and averaged per sample. Differences in composition between tissues and products were evaluated using principal component analysis and PerMANOVA as described previously for micronutrients.

3.2.4. Genomic characterization of enset fermentation

DNA was extracted from four Kocho samples collected from culturally the most important enset landraces in the respective study districts using a standard the CTAB protocol. High quality DNA was successfully isolated from the Kocho of three landraces ('Ganticho', 'Agade', and 'Ado'), one sample failed ('Hala') following approaches used by Abraham Bosha et al (2018) and Tiruha Karsa and Papini (2018), where kocho samples of individual landraces were separately fermented. Library preparation followed the double digest restriction site associated sequencing protocol of tGBS of Data2Bio (Iowa, USA), an approach suited to minimizing missing data in highly heterozygous samples. Sequencing was performed using the Ion Proton platform (Thermo Fisher Scientific, USA). Raw reads were subsequently trimmed and quality controlled following the Data2Bio pipeline.

To identify microorganisms present in Kocho samples, retained sequences were mapped to 24,610 reference bacteria and 285 reference fungi genomes, obtained from the NCBI (<https://www.ncbi.nlm.nih.gov/>, accessed 20th of March 2019). A local blast database was built and blast searches performed with e-value threshold of e^{-20} and scoring (match/mismatch) = 1-2. The percentage of retained reads that aligned to a bacterial or fungal genome was recorded. As a control, DNA was also extracted from the leaf tissue of multiple farmer-grown enset accessions and a similar pipeline used to characterize bacteria or fungi present on the surface or within plant tissue. This approach provides a comparative line of evidence that where specific microbes identified in Kocho samples are present at high concentrations, this is indicative that they are associated with fermentation (rather than simply present). It also provides an indication on whether the microbial community responsible for fermentation originates from the plant surface/tissue or elsewhere, such as the wider environment (e.g. soil) or a starter culture.

3.3. Results and discussion

3.3.1. Sample collection and processing

Fermentation was initiated from mashed pseudostem tissue left for several days at ambient temperature without contrived additives of cultures or previous products. Kocho and Bulla were then fermented for three months, and whole Kocho was fermented for six months (Table 9). We recorded the ubiquitous use of fresh enset leaves as a work surface for processing and the prevailing views that fermentation will not be successful if the fermentation pit is not positioned within the enset growing area. Positioning of the enset fermentation pit within the enset growing area may indicate that temperature (or shade) is important, or alternatively may be related to the presence of specific microorganisms in the soil or elsewhere in the environment associated with enset plants. The resulting fermentation products can be stored for long periods (> 6 months). As expected, farmers reported that different enset landraces produce Kocho (or other products) of different qualities or preferred uses (including as medicines), however multiple landraces are normally processed together. In times of extreme famine, wild enset may be blended with harvested domestic plants, despite generally being considered unpalatable, though the resulting product is regarded as having lower quality, perhaps due to taste (Shigeta, 1990).

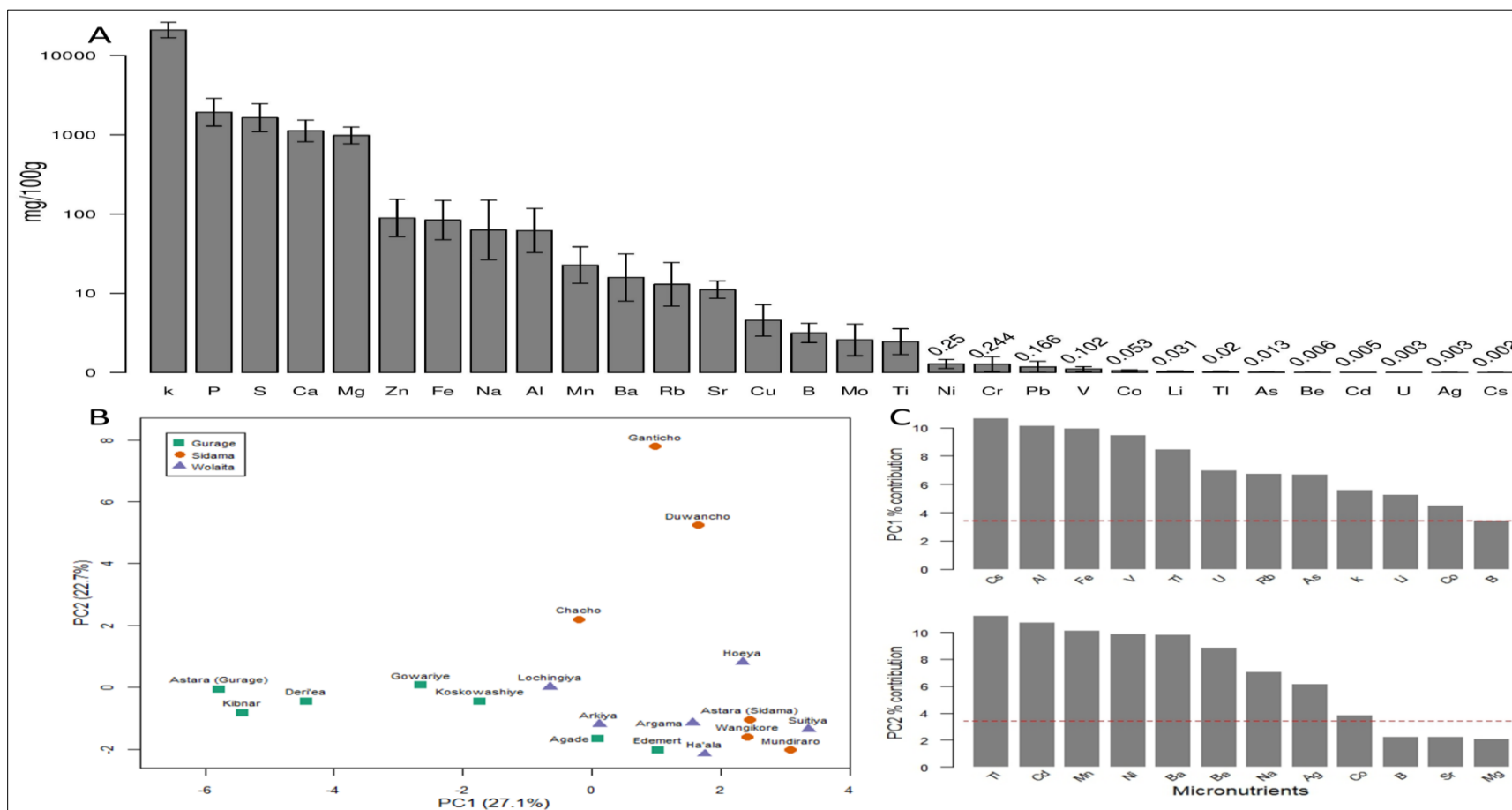


Figure 6: Concentration of inorganic nutrients (minerals) in enset corm tissues:

(A) Mineral values averaged across 19 enset corm samples. Bars denote standard deviation. (B) Principal component analysis of enset corm inorganic composition across three geographical regions. Group comparisons Gurage-Wolaita and Gurage-Sidama were significantly different. (C) Axis loading plots for the first and second principal components. The horizontal red line shows the level at which axis contributions would be equal. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.3.2. Inorganic composition of enset and comparison with other regional crops

Mean ash content across 19 corm samples was 5.0% (SD = 1.4%), which is similar to previously published ash content (4.5%) for enset corms (Nurfeta et al., 2008). Concentrations of inorganic micronutrients and trace elements in raw enset corm tissue were averaged across 19 corm samples, each with two replicates, and plotted in Fig. 6A. Inorganic micronutrient composition varied both by individual sample, and region (Fig. 6B). Samples originating from Gurage zone were most variable on the first axis (27.1% of the variation), whilst samples from Sidama vary on the second axis (22.7% of the variation). We found a significant difference in micronutrient composition of samples originating from different regions ($F(1, 17) = 2.57, p = 0.009$). Pairwise comparison of regions, after applying a Holm adjustment for multiple comparisons, identified significant differences between Gurage-Sidama ($F(1, 11) = 2.91, p = 0.033$), and Gurage-Wolaita ($F(1, 11) = 2.70, p = 0.033$), but not between Sidama and Wolaita ($F(1, 10) = 1.16, p = 0.28$).

These data illustrate important variation in micronutrient composition across enset landraces and growing regions, which mirrors the high vernacular and genetic diversity reported elsewhere for this crop (Borrell et al., 2019a; Zerihun Yemataw et al., 2014). This indicates the influence of genetic, environmental and/or management factors, and as a result, there are likely to be appropriate genetic targets for breeding to enhance nutritional composition. Similar performance gains have been achieved in a range of other species for which there is high variability in micronutrient composition (Welch and Graham, 2004). These data also suggest that climate or soil conditions, together with enset management practices may influence the uptake and accumulation of micronutrients in enset corm tissue, in particular because micronutrient composition appears to cluster weakly by region (Figure 6B)-though many landraces are region specific making further investigation of this pattern challenging. We note that consumption of some micronutrients in high amounts is not recommended (e.g. Nickel, Cadmium) and high levels of these metals in samples from Sidama (Figure 6B, 6C) may be associated with groundwater contamination.

In a comparison with regionally important tuber and cereal crops, enset reports particularly high values for calcium, iron, potassium and zinc and relatively low values for sodium (Figure 7). Both iron and zinc deficiencies are widespread in the region (Grebmer et al., 2018). Anemia (iron deficiency) is reported in 56% of children and 24% of adult women in Ethiopia (Gebru et al., 2018), with anemia also included in the World Health Organization's Global Nutrition Monitoring Framework for Ethiopia (WHO, 2019). This emphasizes the importance of enset as a potential dietary source of iron. Iron concentrations have previously been reported as higher in enset pseudostem compared to corm (Heuze et al., 2017) and we find further support for this pattern across a larger range of landraces (Figure 7), though we show almost 8-fold variation between samples. Zinc deficiency is also reported in diets in Ethiopia (Mestewat Gebru et al., 2018; Amha Kebede and Tibebu Moges, 2013). In this study we provide further evidence that enset is an important source of Zinc, and that enset corm contains higher levels of zinc than pseudostem tissue. Based on current data and previous reports (USDA, 2012; Heuzé et al., 2017) most micronutrients occur at higher concentrations in the pseudostem than the corm (Figure 7).

3.3.3. Organic composition and the nutritional implications of enset fermentation

Mean total protein content across 19 corm samples in this study was 4.6% (SD = 1.5%), broadly consistent with values published previously in Feedipedia as 3.5% with an SD of 1.1 (Heuzé et al (2017)). The mean concentration of FAAs across raw enset tissues and processed food products are reported in Figure 8A with overall concentrations for each FAA shown in Figure 8B. Of the edible components of enset, corm tissue contained the highest concentrations of FAAs, but was also most variable. FAA variation across samples is presented in Figure 8C with variable loadings for the first and second principal components reported in Figure 8D. Bulla and Kocho appeared most variable on the first axis, whilst corm concentrations were variable on both the first and second axes. PerMANOVA identified a significant difference in FAA composition of different tissue types ($F(3, 47) = 3.38, p = 0.001$). However, post-hoc pairwise comparison with a Holm adjustment only identified a significant difference between values for corm and pseudostem tissues ($F(1, 23)$

= 3.34p = 0.042). All other pairwise comparisons were non-significant. The relative concentration of individual FAAs before and after fermentation is reported in Figure 9.

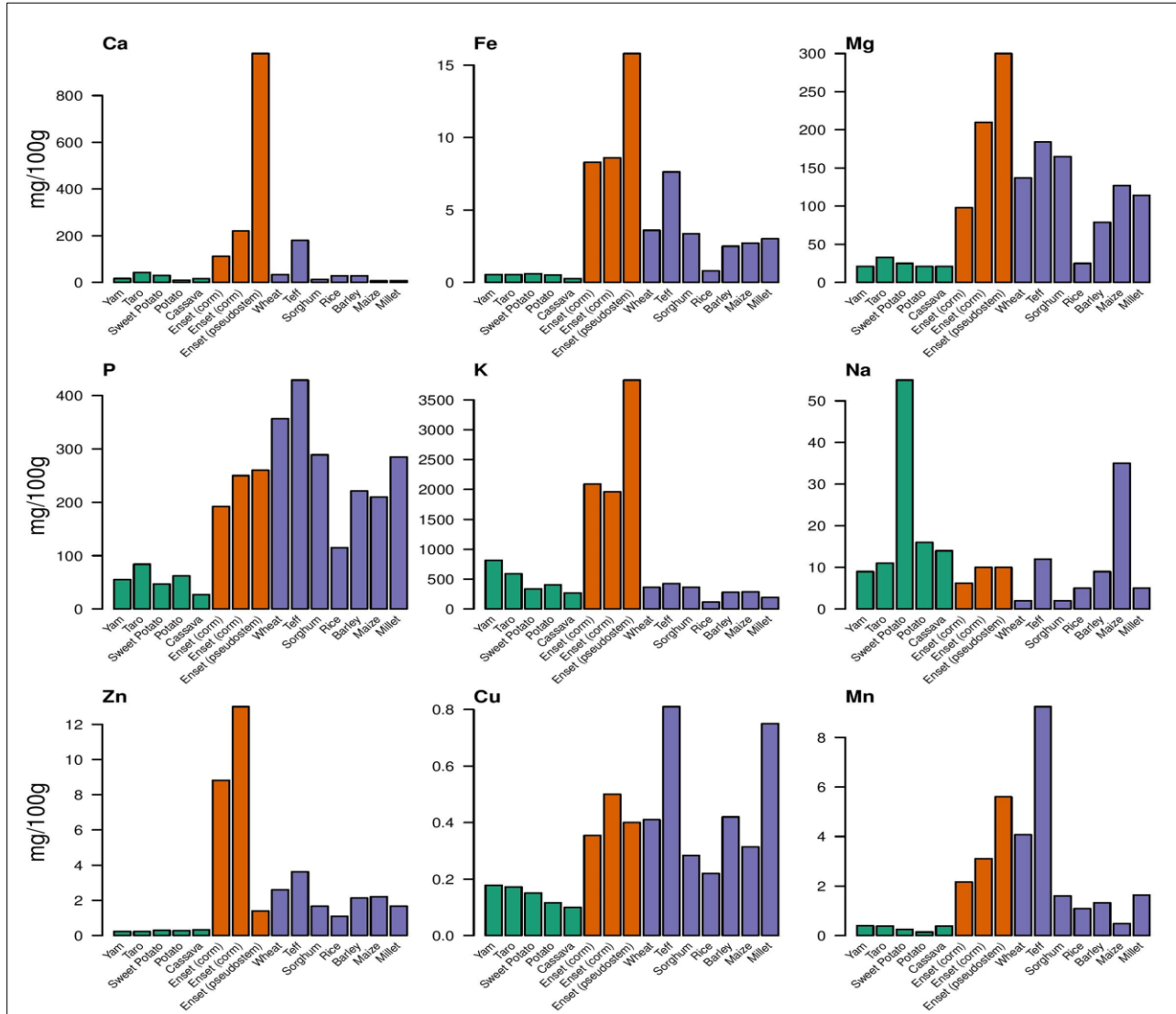


Figure 7: Comparison of enset tissue inorganic micronutrients (corm & pseudostem) with regionally occurring tubers and cereal crops.

For enset three values are provided (from left to right); (i) Enset (corm) results from this study, (ii) Enset (corm) from published sources and (iii) Enset (pseudostem) from published sources. Comparative values for tuber and cereal crops are sourced from Feedipedia and the USDA Food Composition Databases.

Processing of crops is normally performed to improve the digestibility of the product, removing anti-nutritionals and allowing storage without microbial or animal (including insect) contamination. However, there is no evidence that unprocessed enset is toxic, contrasting with cassava (cyanide) or many legumes (with both highly toxic lectin glycoproteins and frequent association with fungi producing mycotoxins). In this instance, enset processing appears to have a significant impact on the composition of derived food products, with FAAs present at higher concentrations in raw tissues (particularly leaf and corm tissue; Figure 8A), suggesting that microorganisms are net consumers of FAAs during enset fermentation. These data also show that certain FAAs may be localized to specific tissues, for example arginine and glutamine are both detected in corm, but at low concentrations or absent in other tissues both pre- and post-fermentation (Figure 9). Similarly, phenylalanine and glycine occur at higher concentrations in fermented products than raw tissues, whilst many other FAAs decline. Overall, several essential AAs (isoleucine, leucine, phenylalanine and valine) are increased in fermented products, particularly the main staple food, 'Kocho', suggesting that traditional fermentation practices may contribute to nutrition.

When protein or AAs are ingested, the vast majority of digestion products that reach the blood stream are single AAs, with the completeness of protein digestion dependent on metabolic factors (Bhutta and Sadiq, 2012). Thus in some cases ingested FAAs may be more bioavailable whilst in others a protein-rich diet may be poorly digested and of reduced nutritional value (Bhutta and Sadiq, 2012). Furthermore, certain AAs contribute to the flavour of foods (Kato et al., 1989), thus influencing the selection of processing or fermentation methods and dietary choices of consumers, with potential consequences for human nutrition. We note wild enset is not consumed because it is considered bitter and unpalatable. It is interesting; therefore, that fermentation appears to markedly reduce aspartic acid and glutamic acid, both of which produce a potent sour taste at relatively low concentrations, as well as reducing arginine and histidine concentrations, which are characteristic of bitter tastes. Whether development of processing techniques has been concomitant with domestication to produce food with improved palatability is an interesting area for further research.

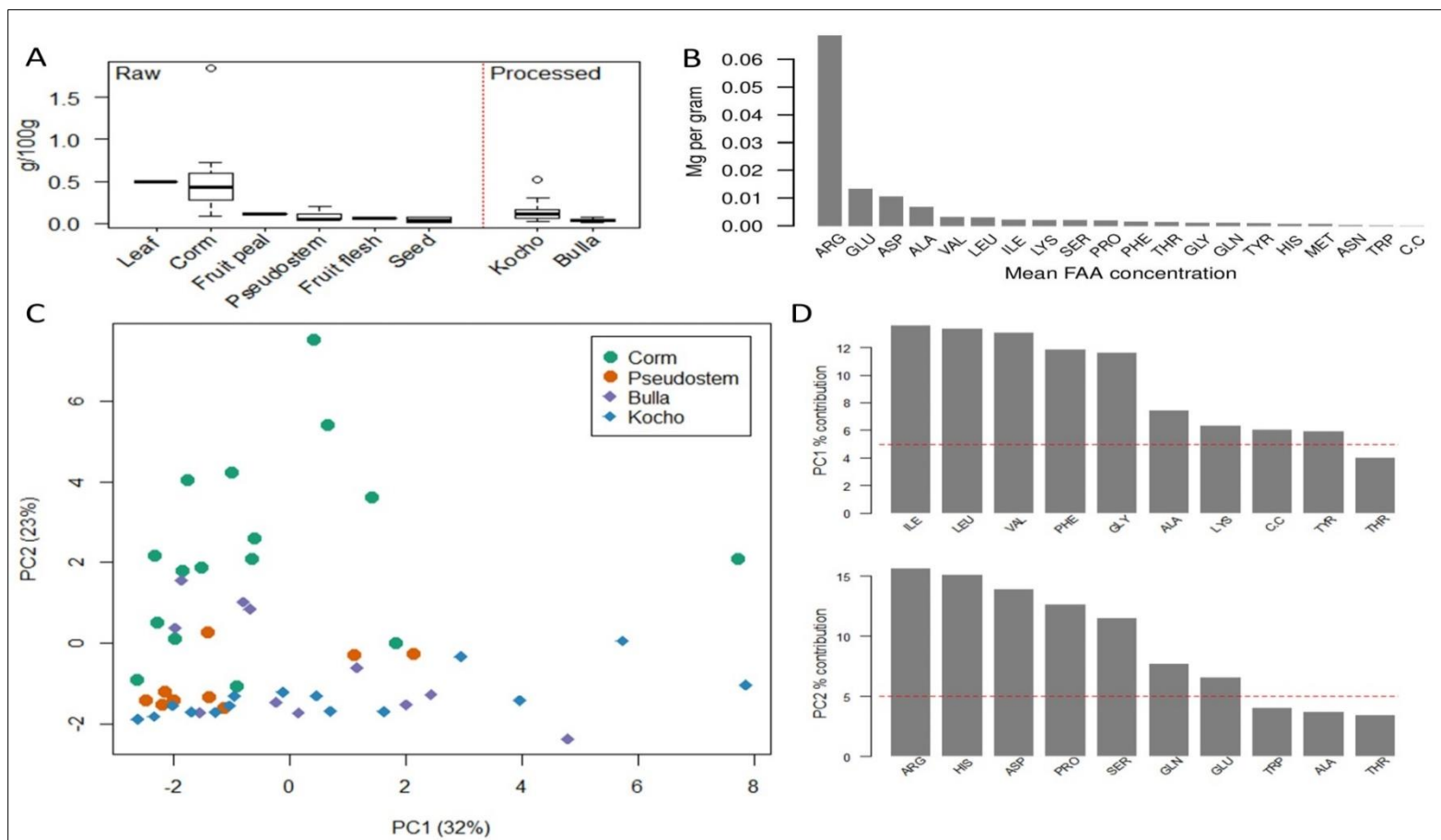


Figure 8: Free AA composition and variation in enset.

(A) Quantities of Free AAs (FAAs) present across raw enset tissues and fermented enset food products. (B) Mean concentrations of free AAs across all samples. (C) Principal component analysis of enset FAAs across four tissue types. We detected a significant difference in composition between corm and pseudostem samples. (D) Axis loading plots for the first and second principal components.

This study also reveals detection of varying concentrations of the essential AAs isoleucine, leucine, lysine, phenylalanine, threonine, tryptophan, and valine in different enset tissues (Figure 9) in the form of FAAs. Consequently, these data provide the first evidence that enset, as part of a broader diet, may contribute to intake of these essential AAs. The principal FAA detected across all enset samples analysed in this study was arginine (Figure 9), which occurred at the highest levels in corm tissue. Although arginine is not an essential AA, some evidence suggests that increased dietary arginine can improve outcomes in critically ill individuals (Emery, 2012) and it is considered essential for infant growth, with histidine also being important for the latter (Brayfield, 2019), and also detected in enset tissue, especially the corm (Figure 9).

A traditional use of enset in Ethiopia is its intake in the form of ‘Amicho’ (Sid.) (as boiled corms), which is reputed to heal bone fractures (Borrell et al., 2019a). Arginine is involved in collagen formation, tissue repair and wound healing via proline, which is hydroxylated to form hydroxyproline, and it may also stimulate collagen synthesis as a precursor of nitric oxide (Van de Poll et al., 2012). The analysis of free AAs in this study has revealed high levels of arginine, compared to other AAs, detected in different edible parts of enset. This finding provides a rational scientific basis for the first time that may explain the reputed traditional use of enset to aid bone healing after breakage. Intriguingly, three landraces often reported as having medicinal properties (Koshkowashiye (Gurage), Astara (Sidama) and Lochingiya (Wolaita)) report three of the four highest arginine values, whilst Lochingiya and Astara also report the highest calcium concentrations, a mineral critical for bone development if not healing.

3.3.4. Microbial community characterization and signatures of contamination

Sequencing of ‘Koch’o samples resulted in 9.6 M raw reads. After quality control 8.6 M reads were retained with a length of 50-209 bp. Of these, 1.9 M and 24,300 reads returned BLAST hits above a threshold of e^{-20} for bacteria and fungi genomes, respectively. The bacterial species *Acetobacter pasteurianus* was the most frequent in the ‘Kocho’ samples ‘Ganticho’ (Sidama) and

‘Ado’ (Sidama), whereas *Raoultella ornithinolytica* was found to be most frequent in ‘Agade’ (Gurage) (Figure 10-A). The fungal species *Penicillium chrysogenum*, *Pichia kudriavzevii* and *Aspergillus fischeri* were found most frequent in the ‘Ganticho’ (Sidama), ‘Ado’ (Sidama) and ‘Agade’ (Gurage) samples respectively (Figure 10-B). A total of 797 bacterial species were found in common across the three Kocho samples, though comparatively only eight fungi species were found across all samples (Figure 10-C). As a control, a BLAST result from leaf tissue is provided in Figure 10-D and largely identifies distinct species from the main ‘Kocho’ analysis.

Genomic analysis of the microbial community associated with enset fermentation showed that the most abundant genus of bacteria was *Acetobacter*, which is generally aerobic and known for producing acetic acid, reducing the pH of the fermenting enset pulp. It both gives a desirable flavor to the product and lowers the pH which inhibits growth of other organisms, thus allowing safe storage of the product. Whilst these species may occur as endophytes, the most common species identified in the ‘Kocho’ samples (*Acetobacter pasteurianus*) was not identified in raw leaf tissue (Figure 10-D). The second most abundant genus was *Lactobacillus*, a group of anaerobic bacteria often associated with controlled fermentation in foods. In a comparison with the bacteria identified by Birhanu Abegaz (1987) we also found *L. mensenteroides*, *L. coryniformis* and *L. plantarum* but not *S. faecalis*. In the genome sequencing of leaf-derived DNA, Harrison et al. (2014) reported extensive hits (> 8%) against *Pseudomonas fluorescens* and *Methylobacterium radiotolerans*, which they propose are endophytes associated with enset; we also found a small number of *P. fluorescens* hits in our ‘Kocho’ samples, but only one hit to *M. radiotolerans*. Overall, our analysis identified many more bacterial than fungal sequences. We also found a much higher proportion of bacteria species in common between samples (39.2%), compared to fungi (1.6%) including the yeasts. This suggests that bacteria are principally responsible for enset fermentation.

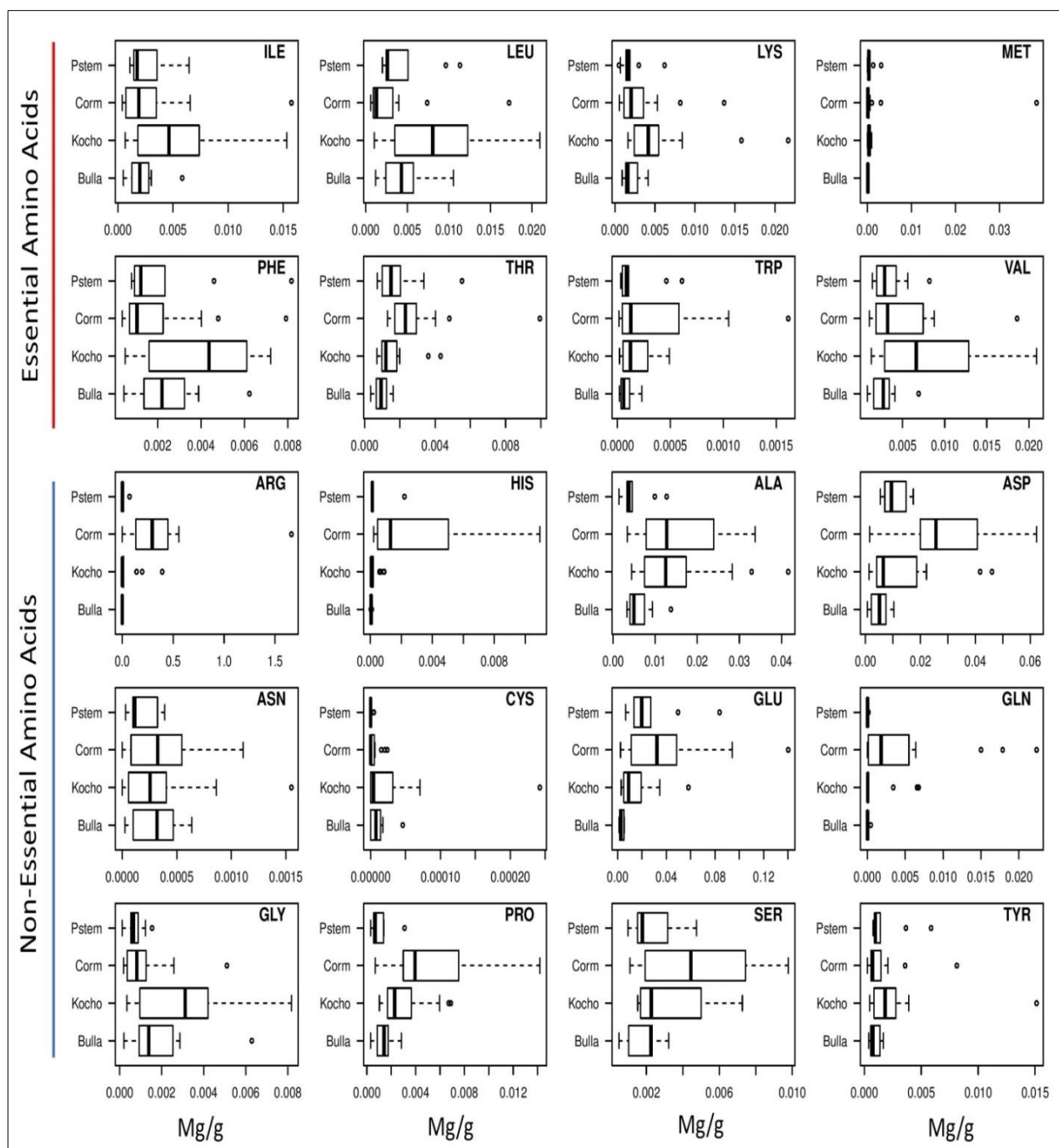


Figure 9: Free AA composition in raw enset tissues (pseudostem and corm) and processed food products (kocho and bulla).

Whilst essential and non-essential AAs are noted, conditionally essential AAs, required in certain circumstances, are presented and include arginine, histidine, glycine and glutamine.

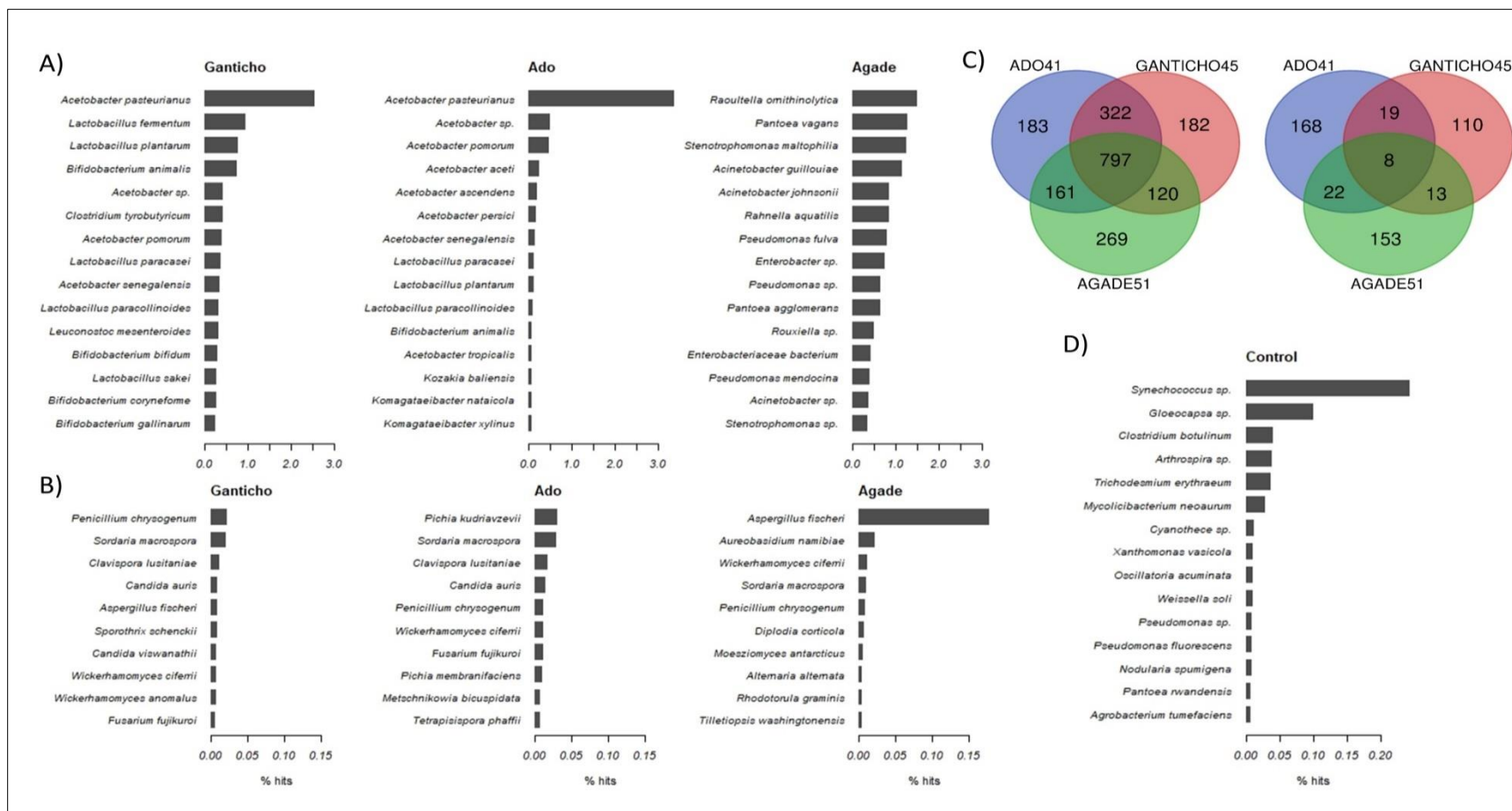


Figure 10: Microbial composition of enset kocho samples.

Bacteria (A) and Fungi (B) genomes with highest percentage hit rates across three kocho samples. (C) Venn diagrams illustrating the number of species in common between samples for Bacteria (left) and Fungi (right). (D) Top bacteria and fungi hits from enset leaf tissue.

These analyses provide an opportunity to identify potentially harmful microorganisms such as those associated with spoilage or food poisoning (Bhunja, 2018). The number of hits associated with *Escherichia*, *Campylobacter*, *Salmonella*, *Clostridium* (except in the Ganticho *Kocho* sample) and *Listeria* was generally very low in all samples. However, whilst the microbial community composition of ‘Ganticho’ (Sidama) and ‘Ado’ (Sidama) were very similar, ‘Agade’ (Gurage) was dominated by *Raoultella ornithinolytica*, a species associated with human infections. *Stenotrophomonas maltophilia*, *Acinetobacter johnsonii*, *Rahnella aquatilis* are other species potentially harmful to health were also among the most frequently identified bacteria in this sample. Similarly, the highest fungal hit for ‘Agade’ (Gurage) was *Aspergillus (Neosartorya) fischeri*, a close relative of the major pathogen *Aspergillus fumigatus* also associated with hypersensitivity pneumonitis (farmer’s lung, immunologically mediated inflammation). This suggests that in comparison to ‘Ganticho’ (Sidama) and ‘Ado’ (Sidama), ‘Agade’ (Gurage) could be characterized as a contaminated food product.

3.4. Conclusion

Ethiopia has historically been the world’s largest recipient of targeted food aid and is 93rd of 119 qualifying countries in the 2018 Global Hunger Index. Nationally, undernourishment affects 21.4% of the population and 38.4% of children under the age of five are affected by stunting. Therefore effective utilization of agricultural diversity is a priority to achieve food security and address public health needs, particularly in the context of climate change and population growth. However despite being a center of diversity for plant domestication, dietary diversity over much of Ethiopia is extremely low due to overdependence on starchy staples with major dietary deficiencies in iron and zinc. However, whilst child stunting prevalence in the enset growing region (reflecting ‘chronic’ undernutrition) is largely consistent with the national average, these areas have some of the lowest national levels of child wasting (an indicator of ‘acute’ undernutrition). This provides compelling indirect evidence of the potential of enset to mitigating acute food insecurity events. The results presented here show that there is significant potential for enhanced nutritional benefits

(e.g. iron, zinc, FAAs) from enset that could impact the chronic health and welfare challenges experienced by millions of Ethiopian farmers whom rely on enset as a resilient starch staple.

We show that there is significant variation in enset nutritional diversity, partitioned across multiple stages of enset cultivation and processing from the selection of landraces, environmental conditions and management practices, to the timing and selection of tissues for harvest and the microbial community associated with enset processing. Many of these sources of variation are not currently understood, controlled or investigated and represent significant opportunities for optimization or improvement. We also highlight that in addition to there being few enset germplasm collections, there are no collections of the microbial communities associated with Kocho processing. We therefore suggest that it is important to further document, collect and preserve their diversity as they would be lost should farm structures, agronomy or management change for social or economic reasons. In summary, more than 20 million Ethiopians rely on enset-derived products as a starch staple or co-staple, and this population is projected to grow significantly in the coming decades. Therefore, selection of enset landraces with improved raw nutritional content or enhanced processing techniques that improve the composition, quality or safety of enset based foods has the potential for significant public health impacts.

Ethics statement

All indigenous knowledge associated with enset was collected with prior informed consent and in accordance relevant Access and Benefit Sharing Agreements.

CRedit authorship contribution statement

Solomon Tamrat: Conceptualization, Investigation, Formal analysis, Data curation. **James S. Borrell:** Conceptualization, Data curation, Software, Formal analysis, Writing - original draft, Validation, Visualization, Supervision, Project administration. **Manosh K. Biswas:** Methodology,

Software, Formal analysis, Investigation. **Dawd Gashu:** Conceptualization, Data curation, Resources, Supervision, Funding acquisition. **Tigist Wondimu:** Supervision. **Carlos A. Vásquez-Londoño:** Investigation, Supervision. **Pat J.S. Heslop-Harrison:** Conceptualization, Resources, Supervision, Funding acquisition. **Sebsebe Demissew:** Supervision, Project administration, Funding acquisition. **Paul Wilkin:** Supervision, Project administration, Funding acquisition. **Melanie-Jayne R. Howes:** Conceptualization, Methodology, Resources, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodres.2020.109636>.

CHAPTER FOUR

4. Reproductive biology of wild and domesticated *Ensete ventricosum*: Further evidence for maintenance of sexual reproductive capacity in a vegetatively propagated perennial crop

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Abstract

Loss of sexual reproductive capacity has been proposed as a syndrome of domestication in vegetatively propagated crops, but there are relatively few examples from agricultural systems. In this study, we compare sexual reproductive capacity in wild (sexual) and domesticated (vegetative) populations of enset (*E. ventricosum* (Welw.) Cheesman), a tropical banana relative and an Ethiopian food security crop. Variations in floral and seed morphology, and germination ecology across 35 matured individuals of wild and domesticated enset were examined. Moreover, variations in floral traits of male flower (length of the styloid, pedicel, the five anthers and filaments, and the length and width of outer and inner tepals) and female flowers (length and width of pedicel, pistil outer and inner tepals, style and staminodes) were evaluated. Seed traits, measured including weight, size as diameter on an x, y and z axis, volume as product of the three linear dimensions, total seed area (cross-section), endosperm area and testa (outer integument) thickness. In addition to morphological traits, seed viability and germination response across a range of constant (10°C, 15°C, 20°C, 25°C, 30°C, 40°C) and alternating (20°C/ 10°C, 25°C/ 10°C, 25°C/ 15°C, 30°C/ 10°C, 30°C/ 15°C, 30°C/ 20°C, 35°C/ 20°C, 40°C/ 20°C, 40°C/ 25°C) temperature regimes were evaluated to characterize optimum requirements. A highly consistent floral allometry, seed viability, internal morphology and days to germination in wild and domesticated enset were reported. However, seeds from domesticated plants responded to cooler temperatures with greater diurnal range. Shifts in germination behaviour appear concordant with a climatic envelope shift in the domesticated distribution. These findings provide evidence that sexual reproductive capacity has been maintained despite long-term near-exclusive vegetative propagation in domesticated enset. Furthermore, certain traits such as germination behaviour and floral morphology may be under continued selection, presumably through rare sexually reproductive events. Compared to sexually propagated crops banked as seeds, vegetative crop diversity is typically conserved in living collections that are more costly and insecure. Improved understanding of sexual propagation in vegetative crops may have applications in germplasm conservation and plant breeding.

Keywords: Clonal reproduction; domestication; Ethiopia; germination biology; seed banking; vegetative propagation

4.1.Introduction

The erosion of plant genetic resources poses a substantial threat to food security and the diverse benefits we derive from useful plants (Powell et al., 2018; Borrell et al., 2019). To address this challenge, a significant effort has been made to conserve >2 million unique accessions, representing more than 16,500 plant species in 1750 gene banks worldwide (Commission for Genetic Resources on Food and Agriculture, 2010; Fu, 2017). However, an emphasis on conventional seed crops may overlook the majority of perennial fruit crops, for which vegetative propagation is the predominant means of replication and where seed generation may be rare or absent (Miller and Gross, 2011; Castaneda-Alvarez et al., 2016; Migicovsky and Myles, 2017). Vegetatively propagated crops are especially important in the tropics (Denham et al. 2020), where *ex situ* or *in vitro* germplasm collections are currently the only viable approach for conserving genetic resources (Thormann and Dulloo, 2006). Maintaining such collections is often logistically challenging and prohibitively expensive, particularly for developing countries (Dulloo et al., 2013). Improving our understanding of sexual reproduction in vegetatively propagated perennial crops, for example, through sexually reproducing wild progenitors (Miller and Gross, 2011), has potential to address these challenges by enabling a broader range of useful plants to be banked as seed and used in breeding programmes, enhancing conservation and use of genetic diversity (McKey et al., 2010; Borrell et al., 2019; Pironon et al., 2019; Denham et al., 2020).

Clonally propagated food crops encompass at least 34 families and a wide variety of morphological diversity (McKey et al., 2010). This diversity has hindered attempts to define a domestication syndrome (McKey et al., 2010; Miller and Gross, 2011; Denham et al., 2020), but one commonality is the hypothesis that prolonged vegetative reproduction can lead to the loss or attenuation of sexual reproductive capacity (Eckert, 2002; McKey et al., 2010; Barrett, 2015; Denham et al., 2020). For example, domestication of pineapple (*Ananas*), which involved both sexual and asexual selection, resulted in reduced seed production through lower fertility and self-incompatibility (Chen et al., 2019). A tendency to lose sexual reproductive capacity, if widespread across domesticated vegetative crops could significantly hinder future crop breeding programmes

and integration of useful alleles from crop wild relatives (Dempewolf et al., 2014; Migicovsky and Myles, 2017). Importantly, sexual reproductive capacity does not have to be completely lost – reduced fertility, viability, altered floral allometry or germination behaviour, as a result of deleterious mutations and genetic drift in reproductive traits, could still significantly hinder programmes that seek to recombine or conserve diversity as seeds (McClure et al., 2014; Iriondo et al., 2017; Migicovsky and Myles, 2017; Munguia-Rosas and Jacome-Flores, 2020). However, surprisingly few studies have attempted to survey the sexual reproductive capacity of vegetatively propagated crops in agricultural systems (Elias et al., 2001; Scarcelli et al., 2006).

Here, we investigate the impact of domestication on the reproductive biology of the perennial food security crop enset (*E. ventricosum* (Welw) Cheesman) (Figure 11). Enset is a giant monocarpic herb, in the sister genus to the more widely known banana (*Musa* L.), which provides a staple starch source for 20 million people of south and southwest Ethiopia (Borrell et al., 2019). Enset is a useful system in which to survey maintenance of sexual reproductive potential, because wild enset reproduction is exclusively sexual, whilst partly sympatric domesticated enset is exclusively propagated vegetatively (Borrell et al., 2020) (Figure 1B and 1C). The latter is achieved through removal of the apical meristem and meristematic tissue of a 2- to 3-year-old corm, resulting in the generation of adventitious buds (Karlsson et al., 2015). Domesticated enset is not currently banked as seed in international (or national) collections (Guzzon and Muller, 2016), and only a handful of local institutes maintain field germplasm collections (specifically, Areka, Yerefezy, Angacha and Hawassa, situated in southern Ethiopia).

With increasing pressures due to climate change (Conway and Schipper, 2011) and emerging pests and pathogens (Blomme et al., 2017), this represents a significant risk for the future sustainability of enset agriculture. Conserving domesticated enset diversity as seeds has been considered challenging for several reasons. First, enset is monocarpic and is harvested before flowering (unlike banana) to avoid reallocation of resources from edible storage organs to the inedible inflorescence (Borrell et al., 2020). This means that developed flowers and fruits are rarely

encountered in cultivation. Second, previous studies have found enset germination to be highly variable (0–90% success) (Tesfaye Messele, 1992; 1994; Mulugeta Diro et al., 2003).



Figure 11: Enset cultivation in Ethiopia.

A: Mature enset flowering in a neglected field near Checha. B: A farmer removing the meristematic tissue of a 2-year-old enset, as part of processing for vegetative propagation. C: Numerous adventitious buds sprouting from prepared corm, near Bonga.

The most detailed study to date by Karlsson et al (2013) found 5–55% germination, depending on the accession. Third, recent genomic analysis concluded that accumulation of mutations in genes associated with flower initiation and seed development may have contributed to enset's domestication (Kiflu Gebremichael et al., 2020). Finally, unlike other tuberous perennial genera like yams (*Dioscorea* L.) and cassava (*Manihot* Mill.), wild or sexually reproduced volunteer seedlings are not (knowingly) incorporated into cultivated populations (Rival and McKey, 2008; Wendawek Abebe et al., 2013; Borrell et al., 2020). Therefore, little to no indigenous knowledge pertains to enset sexual reproduction or seed germination.

In this study, we hypothesize that if sexual reproductive capacity has been conserved through domestication, we should observe consistent floral trait allometry and comparable seed morphology, viability and germination behaviour between wild and domesticated enset plants (see for example, maintenance of leaf silica for herbivore defence through domestication in grasses; Simpson et al., 2017), or potentially, moderately divergent selection for the domesticated environmental niche (Meyer et al., 2012). An alternative hypothesis would be that, after being released from selection pressure, enset reproductive traits would be susceptible to deleterious somatic mutations and genetic drift. Therefore, traits would be expected to display higher variance in domesticated individuals than wild plants. A third potential scenario entails bottlenecks, although these are likely weaker in perennial vegetative crops (Gaut et al., 2015) in which domesticated enset represents a subset of wild diversity and, therefore, a subset of the morphological or behavioral diversity in reproductive traits. To address these scenarios, we frame our analysis with three questions: (i) is there evidence for changes in floral trait allometry, seed morphology or viability in domesticated compared to wild enset; (ii) do domesticated enset seed and floral traits exhibit higher variance than under neutral expectations; (iii) does seed germination behaviour differ between wild and domesticated enset; and (iv) how do these germination requirements relate to the climate of wild and domesticated enset distributions? Finally, we discuss implications for maintenance of enset germplasm resources.

4.2. Materials and methods

4.2.1. Sample collection

Enset in Ethiopia is readily distinguished in the field as the only member of its genus and may be subdivided into (i) domesticated clonal landraces with farmers' vernacular names, and (ii) wild sexually reproducing populations. We made 20 seed collections from seven wild and 13 domesticated individuals (Table 10), in spring 2018. We also separately collected 15 complete inflorescences (Table 11), comprising six wild and nine domesticated individuals. Domesticated accessions were collected, with permission, from farmers' fields, and wild collections were made in river valleys, wet gorges and forest margins (Shigeta, 1990) at least 1 km from settlements cultivating enset, to mitigate the risk of feral or recently introgressed individuals. Broadly speaking wild enset is identified with its cylindrical base of pseudostems and the absence of ashy epicuticular wax on the abaxial surface of leaf blades in addition to its wild habitats. Maturity was determined based on visual inspection of inflorescence morphology, fruit and seed colour.

We sought to minimize collection distance between accessions to minimize differences arising from the maternal climatic environment, but due to only partly overlapping wild and domesticated distributions and the rarity of flowering individuals at the appropriate stage of maturity, we highlight that our disjunct sampling may introduce additional variance. To ascertain whether propensity to flower differs across landraces (enset domestication is hypothesized to select for delayed maturation; Borrell et al., 2020), we recorded (i) vernacular names of all observed and documented landraces during six field visits (2017–2020), and (ii) those observed flowering. We ranked both lists by the frequency at which landraces were observed and compared ranks using a Wilcoxon rank sum test to ascertain whether landraces observed flowering are a random sample of all observed landraces. All analyses were conducted in R software (R Core Team, 2017).

Table 10: Wild & domesticated enset accessions used for seed morphology & germination analysis

Origin	Landrace name	Study District	Elevation (m a.s.l.)	Latitude (°N)	Longitude (°E)	Tetrazolium viability (%)	Annual mean Temp. (°C)	Mean diurnal Range (°C)	Seed count
Domesticated	Ganticho	Dara Kebado	1840	6.47	38.35	30	18.10	14.28	100
Domesticated	Midasho	Dara Kebado	2787	6.47	38.54	14	12.89	13.67	7
Domesticated	Kiticho	Dara Kebado	2787	6.48	38.54	50	12.89	13.67	16
Domesticated	Gefeteno	Sodo Town	1930	6.83	37.75	75	20.24	14.72	300
Domesticated	Wanadiya	Sodo Zuriya	2125	6.86	37.79	48	18.78	14.44	530
Domesticated	Gefeteno	Sodo Zuriya	2125	6.86	37.79	46	18.78	14.44	540
Domesticated	Maze	Sodo Zuriya	2120	6.87	37.79	64	18.78	14.44	1267
Domesticated	Hala	Sodo Zuriya	2120	6.87	37.79	66	18.78	14.44	890
Domesticated	Suitiya	Sodo Zuriya	2120	6.87	37.79	0	18.78	14.44	75
Domesticated	Deri'ea	Enemore & Enir	2700	7.93	37.90	0	15.37	12.97	600
Domesticated	Lemat	Enemore & Enir	2053	8.45	38.03	67	17.97	13.49	100
Domesticated	Addis Ababa	Addis Ababa	2420	9.02	38.78	100	16.02	13.25	55
Domesticated	Addis Ababa	Addis Ababa	2430	9.03	38.76	84	16.02	13.25	1570
Wild	W1	Chena	1880	7.17	36.22	0	18.78	14.35	450
Wild	W2	Chena	1850	7.16	36.20	38	18.47	14.30	135
Wild	W3	Chena	1936	7.16	36.20	54	18.47	14.30	600
Wild	W4	Chena	1936	7.16	36.20	68	18.47	14.30	490
Wild	W5	Chena	1927	7.16	36.20	78	18.47	14.30	700
Wild	W6	Chena	1936	7.19	36.20	52	18.11	14.14	680
Wild	W7	Chena	1930	7.29	36.14	100	17.97	13.99	820
Wild (non-Ethiopian)	'Rare Palm Seeds'	-	-	-	-	94	-	-	1000

4.2.2. Floral trait variance and allometry

Five male flowers from each of five consecutive bracts, and where available, five female flowers, were sampled from each inflorescence and preserved in 70% ethanol. We recorded the position of flowers on the peduncle (i.e. the number of bracts and number of rows of flowers from the base of the inflorescence). Samples were carefully dissected, and morphological characters recorded using digital Vernier callipers, accurate to 0.01 mm. For the female flowers, we recorded the length and width of the pedicel, pistil outer and inner tepals, style and staminodes. For the male flowers, we recorded the length of the styloid, pedicel, the five anthers and filaments, and the length and width of the outer and inner tepals. Where specific tissues were absent and we were certain that this was not due to a damaged sample (e.g. four instead of five filaments), this trait was recorded as zero.

Table 11: Wild and domesticated enset accessions sampled for floral morphology analysis.

Plant origin	Landrace or enset type	Collection area (admin. Zone)	Collection site (garden)	Latitude (d.m.s)	Longitude (d.m.s)	Elivation (m.a.sl)
Domestic	Ado	Sidama	Choke	06 28 21.1	038 21 19.5	1826.00
Domestic	Ado	Sidama	Dambara	06 27 58.7	038 21 38.3	1846.00
Domestic	Ado	Sidama	Enatalem	06 28 38.1	038 20 35.7	1781.00
Domestic	Ganticho	Sidama	Baredo	06 27 53.5	038 21 38.4	1845.00
Domestic	Ganticho	Sidama	Bedilu	06 28 53.5	038 21 32.3	1856.00
Domestic	Ganticho	Sidama	Simbiro	06 28 05.1	038 21 29.9	1833.00
Domestic	Midasho	Sidama	Aynalem	06 28 45.2	038 20 34.2	1771.00
Domestic	Midasho	Sidama	Shu'na	06 28 07.7	038 21 17.7	1853.00
Domestic	Midasho	Sidama	Terefe	06 28 05.6	038 21 20.2	1847.00
Wild	Red_Type	Kefa	Wondimu	07 09 32.3	036 12 33.6	1950.00
Wild	Red_Type	Kefa	Gezmu	07 10 20.8	036 13 20.3	1855.00
Wild	Red_Type	Kefa	Gadisa	07 07 49.4	035 46 30.3	2200.00
Wild	White_Type	Kefa	Gichi River	07 09 55.0	036 12 55.0	1900.00
Wild	White_Type	Kefa	Gezmu	07 10 20.8	036 13 20.3	1855.00
Wild	White_Type	Kefa	Gadisa	07 07 49.4	035 46 30.3	2200.00

To investigate morphological variation, we first applied Spearman's rank non-parametric correlation to assess pairwise relationships for floral traits within domesticated and wild enset. We report pairwise tables of correlation coefficients. Second, we tested for a significant difference between domesticated and wild trait values. We aggregated measurements by individual plant to mitigate pseudo-replication, and then performed a Bartlett test for homogeneity of variances and an unpaired t-test for each trait, applying a Holm correction for multiple tests. All analysis was performed in R software version 3.4.1 (R Core Team, 2017). Finally to assess differentiation in overall floral morphology between wild and domesticated enset, we used redundancy analysis ('rda') implemented in the R package Vegan (Oksanen et al., 2019). We plotted data aggregated by each individual and applied permutational multivariate ANOVA using distance matrices (PerMANOVA) using the function 'adonis', to test the degree to which variance in floral traits can be explained by individual or group (i.e. domesticated or wild plants).

4.2.3. Seed morphology and viability

Seeds were extracted from ripe fruits by hand, and the pulp containing seeds was washed thoroughly until all the flesh was removed. After extraction and cleaning, seeds were air dried at room temperature (~20 °C) for 1 week, packaged and transferred to the Millennium Seed Bank, UK, where they were stored at a constant 60% relative humidity for 1-2 weeks (approximate humidity of collection region). In addition to field collected enset seed, we also used non-Ethiopian enset seeds legally sourced from the online horticultural distributor Rare Palm Seeds (<https://www.rarepalmseeds.com/>), which are available in large quantities (hereafter RPS), permitting us to screen a wider range of experimental conditions.

For each accession, 20-50 seeds were weighed individually, using a balance accurate to 0.001 g. Seed size (diameter on an x, y and z axis) was recorded for 10-20 seeds per accession using digital calipers accurate to 0.01 mm. Due to the highly non-uniform shape of enset seeds, with no

consistent long or short axis, seed volume (estimated as the cube of the three measured axes) was used for subsequent analyses. We used a Faxitron Ultrafocus X-ray (Faxitron Bioptics, LLC, Tucson, AZ, USA), to measure total seed area (cross-section), endosperm area and testa thickness (outer integument) in five seeds per accession. Seeds were positioned with the proximal end facing upwards, and results averaged by accession. We tested each dataset for homogeneity of variances and normality before applying an unpaired t-test to evaluate differences in population means between domesticated and wild seeds. Concurrently, tetrazolium tests were used to detect living tissue and viable seeds following the standard protocol of Leist et al (2003). Briefly, seven to 60 seeds per accession (mean = 55) were imbibed for 24 h, chipped to expose the endosperm and placed in 1% buffered 2, 3, 5-triphenyl tetrazolium chloride for 2 days in the dark at 30 °C. Subsequently, seeds were carefully dissected, and the embryo staining pattern recorded. Germination proportions were corrected for viability during subsequent analysis.

4.2.4. Germination trials

We performed four germination experiments, with temperature ranges selected based on WorldClim version 2 values for the region (Fick and Hijmans, 2017). We exclude treatments involving sulphuric acid, sodium hydroxide, ammonium nitrate, sodium hypochlorite or hot water as Karlsson et al (2013) reported that these had no significant positive effect on germination; and, indeed, scarification and 70% ethanol had significant negative effects. To mimic natural conditions, we draw on germination ecology in *Musa* (Laliberte, 2016), where alternating temperature regimes are known to be a key requirement in banana plants of *M. balbisiana* due to exposure of seeds on the soil surface to alternating day and night temperatures (Stotzky et al., 1962; Kallow et al., 2021) and similar behaviour reported by Tesfaye Messele (1992) in enset.

- i. Exp. 1: First, using RPS seed, we screened a range of 15 constant and alternating temperature regimes to guide our experimental design for subsequent germination tests. Constant conditions comprised six regimes: 10 °C, 15 °C, 20 °C, 25 °C, 30 °C and 40 °C. Alternating

conditions included 10-20 °C diurnal variation (12 h:12 h) to simulate larger temperature shifts, comprising an additional nine regimes: 20/10 °C, 25/10 °C, 25/15 °C, 30/10 °C, 30/ 15 °C, 30/20 °C, 35/20 °C, 40/20 °C, 40/25 °C.

- ii. Exp. 2: Based on results from the initial screening, seeds from seven wild accessions and seven domesticated accessions (those with sufficient seed available for full trials) were exposed to a refined range of 11 temperature regimes: 10 °C, 15 °C, 20 °C, 25 °C, 30 °C, 20/10 °C, 25/10 °C, 25/15 °C, 30/10 °C, 30/15 °C, 30/20 °C.
- iii. Exp. 3: To evaluate the relative importance of an absolute shift in ambient temperature (e.g. warming due to disturbance) and regular diurnal temperature shifts (i.e. our alternating temperature regimes) we exposed a subset of a subset of seven domesticated accessions plus RPS, to five constant temperatures for 3 months (10 °C, 15 °C, 20 °C, 25 °C, 30 °C), and then moved to the ‘optimum’ alternating temperature (25/10 °C) identified in earlier tests. Available wild seed was prioritized for Exp. 1 and 2.
- iv. Exp. 4: To evaluate the extent and influence of dormancy, a subset of seven domesticated accessions plus RPS, was stratified at 10 °C for 3 months, and then transferred to five other temperature regimes: 25 °C, 25/10 °C, 25/15 °C, 30/20 °C and 30/15 °C. We compare these to aggregated data from Exp. 2 that were not exposed to a period of stratification.

In all germination tests, seeds were placed on moist sand (300 g sand, 42 ml de-ionised water) and sealed in clear plastic boxes (120 to 180 mm). Boxes were then sealed in plastic bags to minimize moisture loss and contamination. Each box contained 60 seeds (except where specified), with a total of 235 unique condition x accession combinations evaluated in this study. Seed boxes were placed in the corresponding incubators with either constant temperature or 12-h alternating temperature cycles. All treatments included a 12-h photoperiod. Germination was defined by radicle emergence ≥ 2 mm, and tests were scored every 3-18 days, depending on activity, for 120 days. We calculated the number of days required for 50% of the final germination count in each experimental replicate. We aggregated these data by accession, excluding any replicate with zero germination.

To test for significant differences between wild and domesticated germination behaviour (Exp. 2), we fitted polynomial regression models for the logit transformed proportion germinated against (a) the daily temperature change and (b) the mean temperature the replicate was exposed to. For each variable we fitted two models, the first with all accessions and the second with an additional variable grouping the data by type (wild versus domesticated). We then used ANOVA to test whether grouping produced a significantly better model fit. To evaluate the role of stratification and dormancy (Exp. 3), we plotted the absolute temperature change between the first temperature and the mean of the second temperature of the treatments against germination proportion, and applied linear regression. The effect of a 10 °C stratification treatment was compared to non-stratification using an unpaired t-test (Exp. 4).

To understand whether differences in germination behaviour were concordant with differences in local climate, bioclimatic data for Ethiopia were sourced from WorldClim version 2 (Fick and Hijmans 2017) at 2.5 arc min resolution (~10 km). In the first instance, we extracted climate values for our study accessions, and tested for significant differences in Annual Mean Temperature and Mean Diurnal Range using unpaired t-tests. We then collated 472 enset localities from GBIF (GBIF.org, 2018) publications (Borrell et al., 2019; Pironon et al., 2019) and field observations, and subsampled these to a 10-km grid consistent with the environmental data layers; retaining 94 unique domesticated records and 19 unique wild records. We extracted climate data for these cells and aggregated them for domesticated and wild enset separately.

4.3. Results

4.3.1. Observations, collection, processing and storage

Summary information for the 15 floral and 20 seed accessions are reported in Tables 10 and 11. Overall, we surveyed 375 male flowers and 45 female flowers, and harvested >8000 seeds. During

field surveys, we documented 1864 observations of 453 named landraces from across the enset growing region. In addition, we recorded 39 flowering individuals of 26 landraces. After ranking by frequency of observation, we found no significant difference in rank order ($W = 6251.5$, $P = 0.60$).

4.3.2. Floral trait allometry

Out of the 120 pairwise comparisons for male flowers, 100 were significantly correlated in domesticated and 72 significant in wild enset. Traits such as anther length and filament length were highly correlated with each other in both wild and domesticated flowers, whereas sepal length and stylode length were highly correlated with anther length in wild flowers but only weakly correlated in domesticated flowers. Of 78 pairwise comparisons for female domesticated flowers, 34 were significant. Overall, we found a relatively weak correlation for pedicel length with other traits, whereas ovary length and outer whorl fused tepal width were highly correlated with other traits. Pairwise correlation coefficients for male and female flower morphological traits are also reported. Comparison of trait variance found few differences between wild and domesticated plants (Table 13). The only significant differentiation in trait means was for sepal width, which was significantly wider in domesticated flowers ($t = 7.7$, $DF = 12.4$, $P < 0.001$). With no wild female flowers, it was not possible to test for significant differences in female floral trait morphology, thus tissue means are reported in addition in the data set.

A redundancy analysis for male floral traits is plotted in Fig. 12. Domesticated enset tend to vary on the first axis, with sepal width the most important contributing variable. The majority of the wild variation is on the first axis, with sepal width the most important contributing variable. The majority of the wild variation is on the second axis, with filament length traits the most important contributing variable. PerMANOVA showed that the distinction between wild and domesticated origin explained a significant proportion of variation, whereas grouping samples by landrace did not (Table 14).

Table 12: Comparison of variance and means for onset floral and seed morphology traits 1Holm correction applied to p-value significance

Trait (mm)	Domesticated Trait mean (\pm sd)	Wild Trait mean (\pm sd)	Bartlett test				T-test			
			K-squared	df	p-value	p-value (corrected) ¹	T-value	df	p-value	p-value (corr.)
<i>Floral morphology</i>										
Pedicel (length)	8.18 (4.18)	12.62 (2.34)	1.69	1.00	0.19	0.97	2.63	12.77	0.02	0.17
Anther Length 1	30.18 (2.66)	29.56 (6.34)	4.34	1.00	0.04	0.59	-0.23	6.19	0.83	1.00
Anther Length 2	29.48 (2.66)	28.85 (6.18)	4.11	1.00	0.04	0.60	-0.23	6.25	0.82	1.00
Anther Length 3	28.69 (2.55)	28.25 (6.07)	4.34	1.00	0.04	0.59	-0.17	6.19	0.87	1.00
Anther Length 4	27.11 (2.52)	27.13 (5.72)	3.90	1.00	0.05	0.63	0.01	6.31	0.99	1.00
Anther Length 5	24.00 (3.32)	26.15 (5.6)	1.62	1.00	0.20	0.97	0.85	7.35	0.42	1.00
Filament Length 1	20.46 (6.24)	27.46 (2.96)	2.63	1.00	0.10	0.92	2.91	12.11	0.01	0.16
Filament Length 2	19.74 (6.11)	26.59 (3.01)	2.40	1.00	0.12	0.92	2.88	12.28	0.01	0.16
Filament Length 3	19.26 (6.1)	26.00 (2.88)	2.68	1.00	0.10	0.92	2.87	12.08	0.01	0.16
Filament Length 4	18.76 (6.05)	24.98 (2.62)	3.23	1.00	0.07	0.78	2.73	11.66	0.02	0.17
Filament Length 5	17.08 (6.07)	23.94 (2.52)	3.51	1.00	0.06	0.73	3.02	11.45	0.01	0.15
Sepal Length	55.36 (5.38)	54.85 (7.92)	0.88	1.00	0.35	1.00	-0.14	8.07	0.89	1.00
Sepal Width	13.39 (1.76)	8.06 (0.89)	2.26	1.00	0.13	0.92	-7.70	12.39	0.00	0.00
Petal Length	22.92 (3.95)	17.75 (2.75)	0.69	1.00	0.41	1.00	-2.99	12.93	0.01	0.15
Petal Width	16.97 (3.97)	12.27 (1.71)	3.25	1.00	0.07	0.78	-3.14	11.64	0.01	0.13
Stylode Length	24.69 (5.7)	18.16 (5.4)	0.02	1.00	0.90	1.00	-2.24	11.29	0.05	0.32
<i>Seed morphology</i>										
Volume (mm ³)	3156.00 (735)	3213.00 (569)	0.51	1.00	0.48	1.00	0.20	17.48	0.85	1.00
Weight (g)	1.29 (0.36)	1.74 (0.47)	0.56	1.00	0.45	1.00	2.25	12.63	0.04	0.21
Testa thickness	0.96 (0.21)	0.94 (0.13)	1.36	1.00	0.24	1.00	-0.29	11.79	0.78	1.00
Total area (mm ²)	173.20 (40.9)	200.10 (26.1)	1.29	1.00	0.26	1.00	1.57	11.87	0.14	0.43
Endosperm area (mm ²)	63.80 (12.7)	76.35 (14.5)	0.11	1.00	0.74	1.00	1.85	13.76	0.09	0.35

4.3.3. Seed morphology and viability

Seed volume ranged from 1.26-6.29 cm³ and seed weight from 0.20–2.85 g in domesticated accessions, and 1.15-5.05 cm³ and 0.78-2.66 g, respectively, in wild accessions. After correction for multiple tests, we found no significant difference in seed morphology traits between wild and domesticated seeds (Table 13). These patterns of none significance were consistent even if poorly germinating accessions were removed (i.e. Deri'ea, Sutiya landraces). Full morphological data are available in tables with the data provided with the final document. Tetrazolium tests showed high variation in viability across accessions, with both wild and domesticated accessions ranging from 0-100% viability. Mean viability was 55% and 49.5% for wild and domesticated, respectively, with no significant difference ($t = 0.12$, $DF = 12.9$, $P = 0.84$) (Table 10).

4.3.4. Germination trials

The mean time to 50% germination (T_{50}) was 36 days (SD, 15.7) for domesticated enset and 35 days (8.6) for wild enset, with no significant difference detected ($t = 0.51$, $df = 11.89$, $P = 0.62$). In Experiment 1, alternating temperature regimes outperformed constant temperatures, with the exception of constant 25 °C (Figure 13). Based on these data we reduced our suite of temperature regimes in subsequent experiments. ANOVA of polynomial regression models for germination behaviour in domesticated and wild enset were significantly different (i.e. grouping by accession type resulted in significantly better model fit) for both the alternating temperature range ($F_{136, 132} = 4.32$, $P = 0.003$) and the mean experimental temperature ($F_{136, 132} = 2.71$, $P = 0.033$) (Figure 14A).

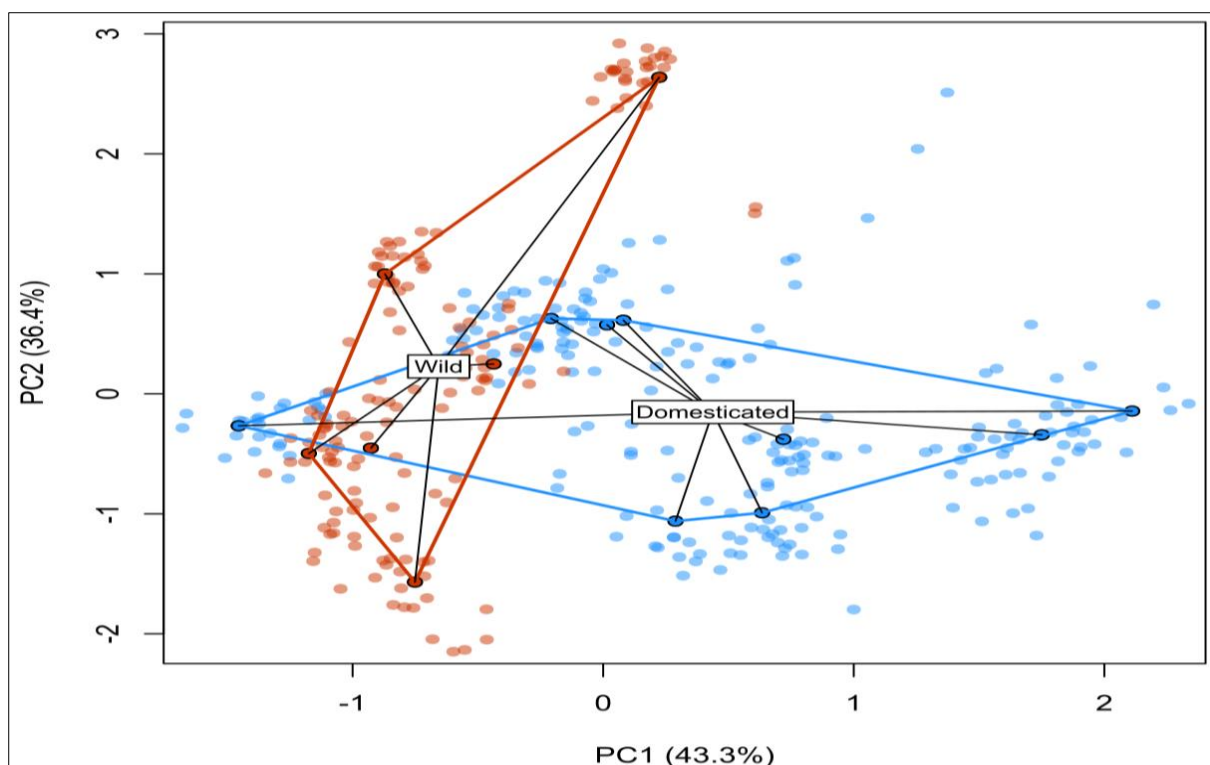


Figure 12: Redundancy analysis of male floral morphology in Enset. Solid points denote means aggregated by sample; lighter points show variation in floral traits.

Table 13: Permutational multivariate ANOVA using distance matrices (PerMANOVA) results for enset floral morphology with origin (domesticated versus wild) and landrace as explanatory factors

PerMANOVA	Df	Sums of squares	Mean squares	F model	R ²	Pr(>F)
Origin (wild vs domesticated)	1	49.22	49.22	3.43	0.23	0.01
Landrace	1	1.52	1.52	0.11	0.01	0.99
Both origin and landrace	1	1.36	1.36	0.09	0.01	0.99
Residuals	11	157.90	14.36		0.75	
Total	14	210.00			1.00	

Specifically, we found that domesticated accessions had an improved germination response in cooler mean temperatures with higher alternating temperature amplitude. Comparison of germination requirements to regional climatic conditions for wild and domesticated enset

showed that domesticated onset is found in local climates that have significantly cooler Annual Mean Temperatures (AMT) ($t = 5.52$, $df = 31$, $P = <0.001$), with significantly larger Mean Diurnal Range (MDR) ($t = 3.42$, $df = 19.2$, $P = 0.003$), than wild onset (Figure 14B). Importantly, there was no significant difference in AMT ($t = 1.77$, $DF = 12.56$, $P = 0.10$) or MDR ($t = 1.63$, $df = 13.96$, $P = 0.13$) of our collected accession sites, therefore this is unlikely to be solely a maternal effect.

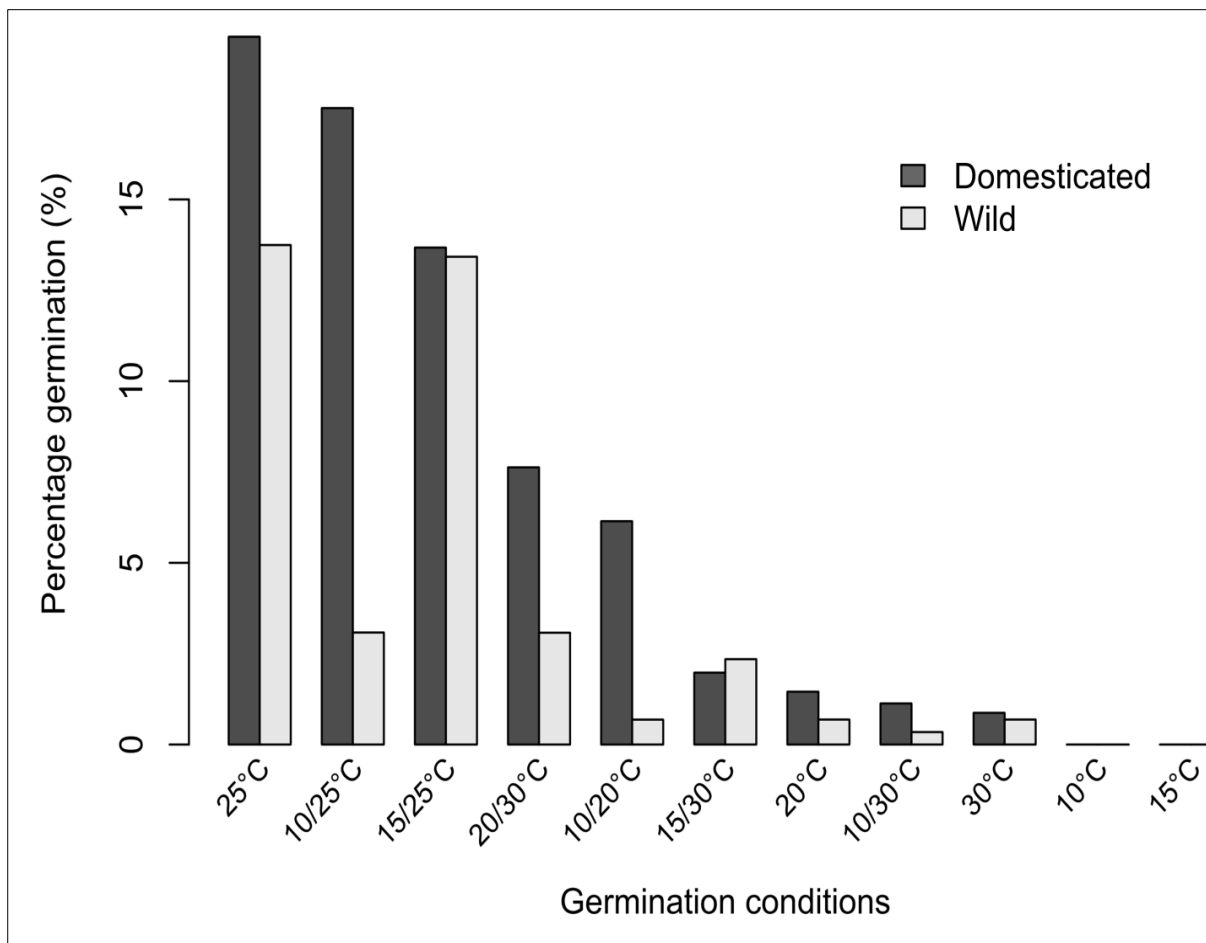


Figure 13: Percentage germination across a range of environmental conditions for wild & domesticated enset (Experiment 2) after 120 days.

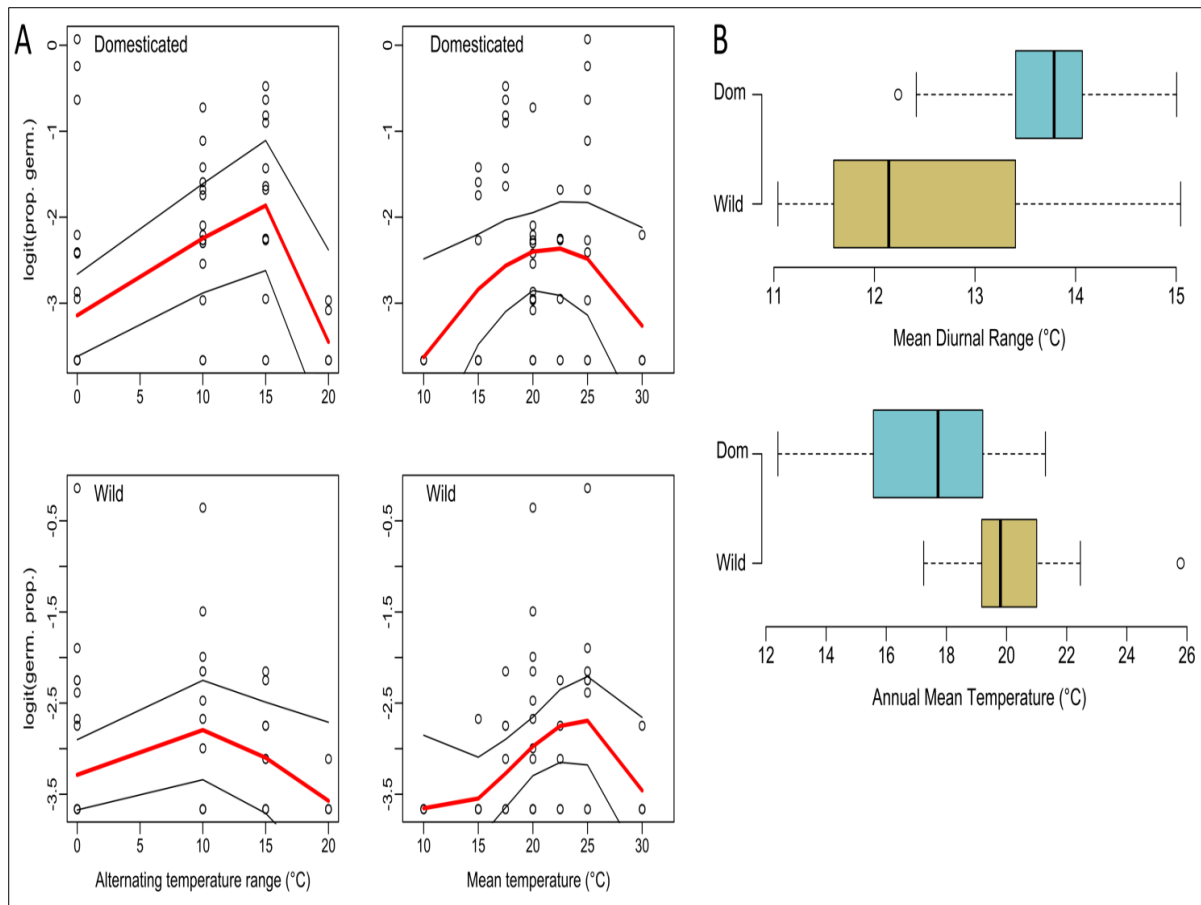


Figure 14: Germination behaviour and regional climate variables for wild and domesticated enset in Ethiopia.

A: Polynomial regression of logit-transformed germination proportion in wild and domestic accessions under varying mean temperature and alternating temperature regimes. Each point denotes a single treatment, corrected to account for variations in seed viability. B: Boxplots of regional climate for domestic and wild enset records in Ethiopia.

Analysis of Experiment 3 found a significant positive relationship between germination proportion and the absolute temperature change from a constant to an alternating temperature regime ($F_{1, 38} = 6.37, P = 0.0159$). Analysis of Exp. 4 found that a 3-month period of cold stratification at 10 °C prior to an experimental treatment also significantly improved germination compared to seeds immediately exposed to the experimental treatment ($t = 5.22,$

df = 26.4, P = <0.001) (Figure 15). Full germination data are available in tables on data provided with the final report.

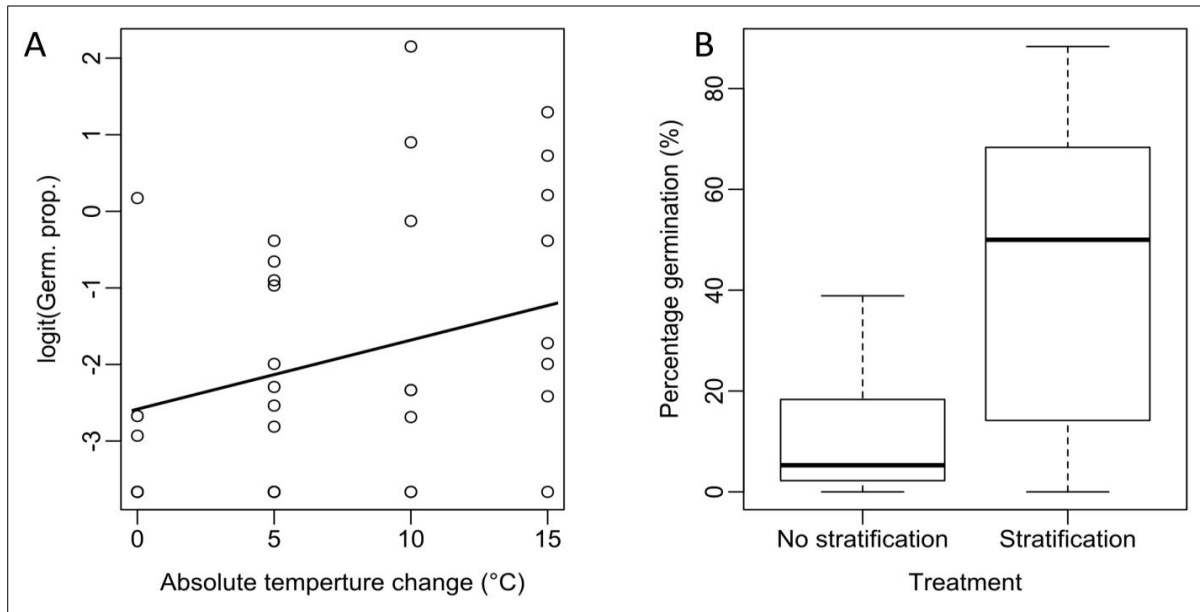


Figure 15: Analysis of the influence of temperature shifts and dormancy on domesticated enset germination (Exp. 3 and 4).

A: Influence of absolute temperature change from constant to alternating temperature, showing a positive relationship with increased germination response for larger temperature shifts. B: Comparison of germination response for stratified seeds (3 months at 10 °C) versus no stratification (immediate exposure to experimental conditions).

4.4. Discussion

Prolonged vegetative reproduction during domestication may be associated with the loss of sexual reproductive capacity (Eckert, 2002; Barrett, 2015; Denham et al., 2020; Kiflu Gebremichael et al., 2020). In this study, we show evidence that the indigenous Ethiopian vegetative crop enset has retained viable sexual reproductive potential through domestication. Currently, the duration over which enset has been domesticated, and the temporal advent of vegetative reproduction is unclear (Borrell et al., 2019). However, if we consider the extensive accumulation of indigenous knowledge associated with enset cultivation (Belachew Garedew

et al., 2017), and its origins in the Ethiopian centre of crop domestication (Harlan, 1971), it is reasonable to conclude that enset was not recently domesticated. Therefore, we suggest that the potential for sexual reproduction has been maintained despite a prolonged period of vegetative propagation. While these findings are concordant with Karlsson et al (2013), they differ substantially from those of Kiflu Gebremichael et al (2020), who suggested that selection has favoured vegetative growth over capacity for sexual reproduction. Whilst Kiflu Gebremichael et al (2020) applied a genomic approach to identify putative flowering-associated genes under selection, we noted that inference of the gene function is challenging and few resources are available in non-model species to facilitate this. For this reason, we suggest that the empirical analyses presented here may provide a better indication of enset reproductive capacity.

In our surveys, we found no evidence that certain landraces have lost the propensity to flower. Wild and domesticated floral morphology was significantly differentiated (Figure 12), but we did not find evidence for increased variance in domesticated enset or for loss of allometry, which could indicate inhibited function. The exception to this pattern was sepal width, which was significantly larger in domesticated flowers, even after correcting for multiple tests. It is difficult to ascertain whether this is functional or, more likely, developmentally linked to another gene under selection. We found no evidence of differing seed viability rate between wild and domesticated enset, although we did find high variability in seed viability across accessions, consistent with previous reports in both enset (Karlsson et al., 2013) and *Musa* (Kallow et al., 2021). Internal and external seed morphology was also consistent between wild and domesticated accessions.

Our germination trials indicate that, surprisingly, the optimum germination requirements significantly differ between domesticated and wild enset (Figure 15). Domesticated enset has an increased germination response at cooler mean temperatures (~22 °C) and with increased amplitude of alternating temperature (Figure 14A) compared to wild enset. Naturally, wild enset occupies consistently warm, moist tropical forest in western Ethiopia, whereas the predominant region of contemporary enset cultivation is a region with lower AMT and higher MDR (Figure 14B). This suggests that wild and domesticated enset are potentially locally

adapted to their respective environments. Importantly, there was no significant difference between the AMT and MDR of the wild and domesticated seed collection sites surveyed here, despite being approximately 170 km apart, suggesting that this observation is unlikely to be a strong maternal effect.

When we consider enset floral morphology and seed germination behaviour together, there are several evolutionary explanations for these observations. First, a scenario where selection pressure has been relaxed, as a result of domestication, would be expected to show increased variance in domesticated traits, perhaps as a result of deleterious somatic mutations and genetic drift, but we do not observe this pattern in these data. If pervasive genetic drift is indeed in progress, then potentially insufficient time or generations have passed for it to become apparent. Alternatively, in a bottleneck scenario where sexual reproductive potential was not selected for, seed and floral morphology might be expected to show reduced variance and display a subset of wild diversity. Our data do not provide strong evidence for this either.

Seeking a more parsimonious scenario, we suggest that despite an almost virtually exclusive clonal propagation in cultivation, it is possible that a small number of escaped or neglected domesticated plants are continuing to reproduce sexually (Baker and Simmonds, 1953; Mulugeta Diro et al., 2003; Karlsson et al., 2013). Whilst deliberate sexual propagation is not reported by enset farmers, agricultural practices that enable sexual progeny has been recorded through ennoblement in yam (*Dioscorea*) (Cornet et al. 2010; Wendawek Abebe et al., 2013) and tolerance of volunteer manioc seedlings in cassava (*Manihot esculenta*) (Rival and Mckey, 2008). Where this has occurred in a novel agricultural environment, it is possible that key functional traits have remained under balancing selection, while germination traits have been subjected to some degree of directional selection. This explanation is supported by both significantly different germination behaviours concordant with local environment, and lack of differences in trait means or variance. Previous work has shown that a comparatively low rate of sexual reproduction would be sufficient to maintain this balancing selection (Rice and Chippindale, 2001; Cutter, 2019).

More broadly, we note that enset germination requirements appear consistent with reports from *Musa* (Stotzky et al., 1962; Kallow et al., 2021). Specifically, in enset, alternating temperatures elicit a stronger germination response than constant temperatures, although in *Musa* this is virtually an absolute requirement. Using alternating temperatures as an environmental cue is hypothesized to be a strategy for detecting disturbance and canopy gaps, whereby solar radiation warms the seeds during the day followed by a cooler ambient temperature at night. Enset is also reported to colonize disturbed areas (Stotzky et al., 1962), and thus this trait appears to be conserved across the two major branches of the Musaceae. Surprisingly, the magnitude of the transition from constant to alternating temperature was also significantly associated with germination. A possible mechanism may involve alternating temperatures reducing the ratio of abscisic acid to gibberellic acid, reducing water potential and initiating elongation and cell growth. This suggests that future approaches involving application of GA to seeds or the growing medium may provide another mechanism for initiating germination. In addition, increased germination is also observed where seeds were stratified at a constant temperature prior to germination. Climate data indicate that the domesticated distribution of enset may reach a minimum of 8.2 °C in the coolest month, which coincides with higher rainfall. Whilst no seasonality has been reported in enset flowering, this may be an additional, putatively conserved, mechanism for optimizing germination timing. We anticipate that numerous factors, such as fruit maturity, length and type of storage, epigenetic and other factors may influence variability, although we do not have sufficient statistical power to resolve these in this study.

In conclusion, the continued function of sexual reproduction in enset, together with emerging examples in other species, enables us to reinterpret the reported tendency to lose sexual capacity in vegetatively propagated crops (Eckert, 2002; McKey et al., 2010; Barrett, 2015; Denham et al., 2020). As a result, the development of seed storage and germination protocols, underpinned by diverse seed collections, may be a feasible strategy to safeguard enset genetic diversity. This may also lead to a lower risk of provenance information loss than living plants in a germplasm collection (Thormann and Dulloo, 2006). We note, however, that conservation of enset diversity as seeds entails recombination, meaning that farmer's preferred vegetatively propagated genotypes may be shuffled. For this reason, seed banking for clonal crops is better considered as an insurance policy, over which maintenance in situ by farmers offers clear

advantages. Importantly, we also acknowledge that maintenance of genetic diversity may be insufficient to realize enset's food security benefits if strategies for the conservation of associated indigenous and traditional knowledge are not concurrently developed (Borrell et al., 2020).

Encouragingly, our analysis also provides initial evidence that very low levels of sexual reproduction may be facilitating the local adaptation of enset germination biology, influencing our interpretation of contemporary landrace diversification. An improved understanding of enset germination biology is a useful prerequisite for future crop development through sexual recombination of existing landraces, developing mapping populations and the breeding of novel genotypes, with significant reciprocal potential in bananas (*Musa*), and a closely related and globally important group of clonally produced crops. In summary, we advocate for a broader effort towards developing germination protocols for vegetatively propagated crops to provide an important alternative germplasm conservation strategy that is likely to disproportionately benefit tropical species and developing country agriculture in the global south.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. X-ray images of *Ensete ventricosum* seeds.

Table S1. Male floral morphology correlation matrix.

Table S2. Female floral morphology correlation matrix.

Table S3. Summary of female floral trait means.

Table S4. Seed morphology raw data – size.

Table S5. Seed morphology raw data – weight.

Table S6. Germination raw data experiments 1 and 2.

Table S7. Germination raw data experiments 3 and 4.

CHAPTER FIVE

5. General discussions, conclusions and recommendations

5.1. General discussion

The study documented a high cultural diversity of medicinally valuable enset landraces and a diversity of ailments treated using these plants. This indicates the prevalence of higher levels of ILK in traditional medicine and their actual and potential contribution to the health care system. Farmers' landraces of enset having medicinal importance were identified based on morphological features (Yemane Tsehaye and Fassil Kibebew, 2006; Zerihun Yemataw et al., 2016; Gizachew woldesenbet et al., 2020; Wendawek Abebe et al., 2022), especially color of their vegetative parts. Our findings also show that medicinal enset sampled from different ethnic groups or localities did not strictly cluster together; rather, they followed morphological similarities or differences. Therefore, it is less likely that the medicinal properties of enset result solely from environmental impacts (Tilahun Amede and Mulugeta Diro, 2005), cultural management types (Belachew Garedeew et al., 2017), or genetic differences (Bizuayehu Tesfaye et al., 2002; Genet Birmeta et al., 2004; Azera Tsegaye et al., 2020b; Lamesgen Yegrem et al., 2022) rather, might be most likely their interactions and demands further investigation. Medicinally valuable farmers' landraces of enset had been perceived and reported to be similar to those used as common food items (Gizachew woldesenbet et al., 2020). Their unique colors, which are used for identification of individual medicinal enset landraces and distinguishing them from those commonly used as food items have been considered to emanate either from differences in environmental conditions of soil nutrients, moisture or shade, genetics or management conditions. We found that medicinally valuable enset landraces were found morphologically distinct from one another and landraces of other uses, and it demands further investigations at the molecular level to answer sources for these variations.

Enset corms were the most frequently used item in the treatment of ailments and health disorders. The finding indicates the availability of higher concentrations of food nutrients or secondary metabolites of therapeutic value within the underground storage organs of plants Tewodros Birhanu et al., 2023, including enset corms. Novel phytochemicals have been

reported from enset corms, including phenylphenalenones (Holscher and Schneider, 1998) which exhibit antibacterial, anticancer, and antinematocidal effects. Enset corms have also been mentioned for hepatoprotective and anti-nephrotoxic activities in controlled experiments (Abebe Dukessa et al., 2021). These previous observations and the current findings of frequent application of enset corms in the treatment of several ailments together stand as promises for the availability of many phytochemicals of therapeutic value in these plants. The potential high diversity of bioactive phytochemicals in enset was also indirectly evidenced via abortive, female sterility or anti-fertility, and mortality effects after feeding tissues or products of some farmers' landraces of medicinal importance. Antioviulatory and antiestrogenic activities have previously been reported for plants of a sister species from the *Musa* genus, where tissues significantly reduced sperm counts, motility, and viability in vitro in rodents (Soni et al., 2013; Ghosh et al., 2017). These bioactivities from enset and sister plant species in the Musaceae family indicate the conservation of bioactive phytochemicals in enset which makes the plant a potential medicinal item for future use.

The high cultural values of enset towards their applications as traditional medicine are further evidenced by the higher diversity of ailments and disorders treated using products from selected farmers' landraces. Infectious or non-infectious ailments, external or systemic infections, and temporary to prolonged health disorders were treated using enset medicine (Kedir Abdela et al., 2017; Melesse Mariyo et al., 2018). Some medicinally valuable farmers' landraces have relatively higher cultural values, manifested through higher values of DMR and index of FL, and their applications in treating several ailments. These observations implied higher diversity and concentration of bioactive phytochemicals among these enset landraces. Medicinal plants of other species receiving higher FL values also showed higher efficacy and concentration of bioactive compounds (Asaye Asfaw et al., 2023). Ailments that received higher ICF, including birth-related complications, musculoskeletal disorders, and injuries (wounds), were serious problems reported in the area (Perso. Comm, Solomon Tamrat). The condition calls for integrated management to reduce their economic risks in the study localities. Applications of enset products as multimedical items used in the treatment of several ailments in the study area indicate high levels of selective harvesting pressure and, hence, local rarity of medicinal enset landraces. Ermias Luleka et al (2013) and Zewdie Kassa et al (2020) reported higher

pressures of harvesting on medicinal plants having higher DMR values with repeated extaction for several use values. Applications of enset as livestock medicine in addition to treatment of human ailments are further evidence for the integration of medicinal use of these plants in the cultures and traditions of local peoples (Cox et al., 1999; Mahmood et al., 2011, 2012; Wahyuningsih et al., 2017).

Lack of interest in multiplying landraces with poor agronomic traits of reduced sprouting, formation of reduced food products, both interms of quality and quantity, were the major factors hindering the propagation of medicinal enset (Bizauyehu Tesfaye et al., 2008; Tesfaye Abebe et al., 2010; Melesse Mariyo et al., 2018; Mesfin Sahle et al., 2022). Local people collect medicinal enset from other areas and manage a few of these individuals in their enset gardens as conservation measures (Bizauyehu Tesfaye et al., 2008; Temesgen Magule et al., 2014; Melesse Mariyo et al., 2018). The observed differences in the levels of ILK towards medicinal use of enset among the different social groups indicate a non-uniform distribution of this ethnomedicinal use knowledge in the area. Variations in medicinal pant use knowledge among informants for other medicinal plants were similarly reported for a non uniform distribution and vulunerability for loss (Ermias Lulekal et al., 2013). Hence, the knowledge and tradition of medicinal applications of enset and the germplasm of medicinal landraces should be documented before they are lost from the impacts of modern lifestyles of feeding cereals and inductrial food items, expansions of western medicine, and climatic changes affecting production rabges.

The current finding of high average ash content for medicinally valued enset landraces indicates the high prevalence of inorganic residues as sources of essential and non-essential minerals (Abebe Yimer et al., 2023). Higher levels of calcium (Ca), potassium (K), phosphorus (P), and magnesium (Mg) in enset products help our body maintain its health status, targeting ailments and health-related disorders (Minaleshewa Atlabachew and Chandarvanshi, 2008; Mohammed et al., 2013; Admasu Tsegaye, 2015). Treatment of bone fractures, cramps, acute blood pressure, mild diabetes, kidney infections, debilitations, delayed labor, and immune complications are attributed to these essential nutrients within enset plants. Enset also contains essential micronutrients (Ajebu Nurfeta et al., 2008a; Ayalew Debebe et al., 2012), including

iron (Fe), zinc (Zn), manganese (Mn), and copper (Cu). The applications of these enset landraces in the cultural treatment of blurry vision resulting from anemia, various diarrhea types, and surface wounds are associated with the abundance of essential micronutrients.

Medicinally valued enset landraces exhibited variations in mineral composition across their origins (or sampling sites) and individual samples sourced from different farmers' landraces (Ajebu Nurfeta et al., 2008a; Ayalew Debebe et al., 2012; Hiwot Bekeke et al., 2023). These differences in mineral content are attributed to variations in either or all of the confined factors or their combinations, including genetic, environmental, or management levels. Geo-spatial variations have been reported to significantly affect the nutritional contents of crop varieties (Dawd Gashu et al., 2021). Differences in soil chemistry and the culture of on-spot burning of slashed farm wastes or weeds would contribute to differences in the mineral composition of farms and, hence, mineral sequestration rate of enset plants. Different crop varieties have also been reported for differences in food chemistry, which are associated with biosynthetic potentials for sequestering or accumulating mineral nutrients (Ajebu Nurfeta et al., 2008a, 2012; Ayalew Debebe et al., 2012; Azera Tsegay et al., 2020a, b; Lamesgen Yegrem et al., 2019, 2022). The prevalent variation in mineral compositions among different enset types provides potential for selection and improvement of enset for superior qualities in mineral concentrations. The surveyed and evaluated enset landraces showed higher amounts of calcium (Ca), iron (Fe), potassium (K), and zinc (Zn) minerals than those of commonly used root tubers and cereals. This has useful potential for addressing iron-based anemia and zinc deficiencies previously reported in the enset-producing communities in the region.

The observed high total crude protein from our enset samples showed the potential of selected medicinal enset landraces as sources of dietary protein or functional foods (Abraham Bosha et al., 2016; Tesfaye Dilebo et al., 2023). The observation also presupposes the availability of a diversity of essential amino acids within enset-based food products sourced from selected farmers' landraces (Admassu Tsegaye, 2015; Solomon Tamrat et al., 2020). Various AAs were relatively localised among the different tissues, by which corms contained higher arginine and glutamine, while phenylalanine and glycine were common within fermented products. This indicates the higher potential of some enset parts for the accumulation of relatively higher

amino acid concentrations acting as sinks. The observation also indicates that fermentation alters the biochemistry of enset-based food items in favour of essential amino acids. We found a significant difference in amino acid concentrations between corms and pseudostems, where the former tissues contain higher values, reflected in their frequent applications as medicinal items. The observed higher values of total AA in corms and leaf tissues provide evidence for the applications of enset as a dietary and functional food for both humans and livestock. A diversity of food nutrients and bioactive phytochemicals have been reported from under ground storage organs of various crop plants (Mohammed et al., 2013; Chandrasekara and Kumar, 2016; Kumar et al., 2017; Obidiegwu et al., 2020; Popola et al., 2023). Arginine, at its highest level detected from medicinal enset, was a fundamental AA that contributed to the treatment of various health disorders after the utilisation of selected farmers' landraces. Observations of the microbial diversity of 'Kocho' revealed that bacterial communities are key players during fermentation dynamics in enset processing. Fermentation of 'Kocho' was primarily mediated by microbial populations from *Acetobacter* (*A. pasteurianus*) and *Lactobacillus* (*L. mensenteroides*, *L. coryniformis*, and *L. plantarum*) (Addisu Fekadu et al., 2018; Genet Birmeta et al., 2018). Microorganisms of the *Acetobacter* genus are aerobic and known for producing acetic acid, which maintains an acidic medium in the fermentation system. The created acidic medium is an important attribute for maintaining food safety through the production of desired food flavours, hindering microbial growth and spoilage. Applications of fermented food products including Kocho and Bulla and some byproducts in the disease treatments is associated with functional properties of microorganisms including probiotic, antimicrobial and antioxidant activities (Tamang et al., 2016b; Patel et al., 2023).

We found no evidence for increased variance in floral traits, any loss of allometry, or certain functions of sexual reproductive potential for domesticated enset. This was evidenced by the non-significant differences in seed viability, mean days to reach about 50% of germination, overall germination proportions, as well as internal and external seed morphological traits between the two populations (Solomon Tamrat et al., 2022). The observed similarity between seed and floral traits as well as seed viability and germination among wild and domestic enset hence exhibit maintenance of sexual reproductive potentials against prolonged almost exclusive vegetative propagation (Karlsson et al., 2013 & 2015). This finding exposes an

potential opportunity for seed-based multiplication or propagation, varietal development through conventional breeding, and ex-situ conservation of enset seeds under cold rooms of seed banks. Optimal enset seed germination was observed for alternating temperature regimes (Stotzky et al., 1962; Tesfaye Messele, 1992; Laliberte, 2016; Kallow et al., 2021), though 25 °C showed the best of all treatments. Improved seed germination was also observed for absolute changes from constant to alternating temperature and prior cold stratification treatments. These observations could be used as a baseline for future seed germination studies, from which various substrates, incubator conditions and their combinations could be prepared to enhance germination rate in enset. Wild and domesticated enset showed significant differences in optimum germination requirements. Domestic enset has an increased response at a cooler mean temperature and increased amplitudes of alternating temperature. The possible explanation for this observation is that wild and domesticated enset have been locally adapted to their respective environments. Wild enset inhabits the consistently warm and moist tropical forests of western Ethiopia. Contrary to this, domesticated enset are adapted to areas with lower annual mean temperatures and higher mean temperature durnal ranges.

5.2. Conclusions

The high proportion, enumerating to 68 (37% of the total surveyed enset landraces) medicinally valued farmer's landraces recorded, a diversity of human and livestock ailments treated using these, and depth of ILK maintained exhibit the significance of enset in the local health care systems where these germplasm would possibly fill the gaps of modern health care practices. Farmer's landraces of enset collected from different study localities did not strictly cluster together; rather they followed similarities in their morphologies, implying genetic variations rather than environmental or cultural managemnet types. These genetic diversities of especial values as traditional medicine should given due attention for conservation and effective utilization in the future development plans of agriculture, food security and health care systems. Lower demands by farmers for clonal multiplications of medicinal enset through corm cutting for their inferior agronomic and morphological qualities in association with applications of these in the treatment of several ailments implies local pressure of harvesting through repeated use with poor replacement rates exhibits a

need for conservation efforts. The condition has led to declined access and rarity of valuable farmers' landraces of medicinal importance in the surveyed localities.

Ethiopia has historically been the world's largest recipient of targeted food aid and is 93rd of 119 qualifying countries in the 2018 Global Hunger Index. Nationally, undernourishment affects 21.4% of the population and 38.4% of children under the age of five are affected by stunting. Therefore effective utilization of agricultural diversity is a priority to achieve food security and address public health needs, particularly in the context of climate change and population growth. However despite being a center of diversity for plant domestication, dietary diversity over much of Ethiopia is extremely low due to overdependence on starchy staples with major dietary deficiencies in iron and zinc. However, whilst child stunting prevalence in the enset growing region (reflecting 'chronic' undernutrition) is largely consistent with the national average, these areas have some of the lowest national levels of child wasting (an indicator of 'acute' undernutrition). This provides compelling indirect evidence of the potential of enset to mitigating acute food insecurity events. The results presented here show that there is significant potential for enhanced nutritional benefits (e.g. iron, zinc, FAAs) from enset that could impact the chronic health and welfare challenges experienced by millions of Ethiopian farmers whom rely on enset as a resilient starch staple.

We show that there is significant variation in enset nutritional diversity, partitioned across multiple stages of enset cultivation and processing from the selection of landraces, environmental conditions and management practices, to the timing and selection of tissues for harvest and the microbial community associated with enset processing. Many of these sources of variation are not currently understood, controlled or investigated and represent significant opportunities for optimization or improvement. We also highlight that in addition to there being few enset germplasm collections, there are no collections of the microbial communities associated with Kocho processing. We therefore suggest that it is important to further document, collect and preserve their diversity as they would be lost should farm structures, agronomy or management change for social or economic reasons. In summary, more than 20 million Ethiopians rely on enset-derived products as a starch staple or co-staple, and this population is projected to grow significantly in the coming decades. Therefore, selection of enset landraces

with improved raw nutritional content or enhanced processing techniques that improve the composition, quality or safety of enset based foods has the potential for significant public health impacts.

The continued function of sexual reproduction in enset, together with emerging examples in other species, enables us to reinterpret the reported tendency to lose sexual capacity in vegetatively propagated crops (Eckert, 2002; McKey et al., 2010; Barrett, 2015; Denham et al., 2020). As a result, the development of seed storage and germination protocols, underpinned by diverse seed collections, may be a feasible strategy to safeguard enset genetic diversity. This may also lead to a lower risk of provenance information loss than living plants in a germplasm collection (Thormann and Dulloo, 2006). We note, however, that conservation of enset diversity as seeds entails recombination, meaning that farmer's preferred vegetatively propagated genotypes may be shuffled. For this reason, seed banking for clonal crops is better considered as an insurance policy, over which maintenance in situ by farmers offers clear advantages. Importantly, we also acknowledge that maintenance of genetic diversity may be insufficient to realize enset's food security benefits if strategies for the conservation of associated indigenous and traditional knowledge are not concurrently developed (Borrell et al., 2020).

Encouragingly, our analysis also provides initial evidence that very low levels of sexual reproduction may be facilitating the local adaptation of enset germination biology, influencing our interpretation of contemporary landrace diversification. An improved understanding of enset germination biology is a useful prerequisite for future crop development through sexual recombination of existing landraces, developing mapping populations and the breeding of novel genotypes, with significant reciprocal potential in bananas (*Musa*), and a closely related and globally important group of clonally produced crops. In summary, we advocate for a broader effort towards developing germination protocols for vegetatively propagated crops to provide an important alternative germplasm conservation strategy that is likely to disproportionately benefit tropical species and developing country agriculture in the global south.

5.3. Recommendations

Based on the research findings, the following recommendations are proposed:

- Medicinally valued enset with observed high potential in the treatment of diverse types of human and livestock ailments should be incorporated and given due attention to the health and agricultural development policies, strategies, and actions of regional states those are directly involved in enset production.
- Enset landraces having medicinal values collected from different study localities did not strictly cluster together; rather, they followed similarities in their morphologies. Studies should be conducted to find the source of medicinal properties in order to check whether these colour variations and chemical compositions result purely from genetic or environmental variations or their interactions.
- Farmers' landraces of enset used in traditional medicine are under threat from repeated harvesting and poor demands for corm based multiplication by the farmers. The Ethiopian Biodiversity Institute (EBI), Areka Enset Research Centre, and universities residing within enset production ranges should create awareness about the significance of medicinal enset; and be involved in the process of collection, multiplication, and distribution of the germplasm to the local people.
- The ILK of utilisation of enset in traditional medicine and its remarkable cultural diversity are under threat of local rarity. Therefore, the plant enset should be involved in the curricula of schools and universities, at least in the production ranges, to promote this fundamental item for its economic, cultural, and environmental values.
- Those landraces of enset used as medicinal valuables exhibited higher concentrations and diversity of essential minerals and functional amino acids in comparison to regionally important root/tubers and cereals. This valuable germplasm should be promoted by regional governments, research centres, and the scientific community and identified as a potential

item to target regional and local malnutrients in essential minerals and functional amino acids.

- The sampled farmers' landraces of enset showed variations in nutritional contents across their origin, plant parts, or levels of processing (fermentation). Studies should be carried out to check whether variations in nutritional chemistry in medicinally valued farmers' landraces are variations sourced from environment, genetics, management, or the combinations of any of these.
- Bacterial strains of *Acetobacter* and *Lactobacillus* were the dominant communities and key players during the process of 'Kochof' fermentation. The diversity of these microbial populations should be collected, studied, and maintained *ex-situ* in the gene banks of the Ethiopian Biodiversity Institute, Areka Enset Research Centre, and the Ethiopian Institute of Agricultural Research.
- Enset has maintained sexual reproductive potential against prolonged clonal multiplications. Seeds of the existing cultural diversity of the plant should be collected by researchers from its cultivation ranges, studied for their physiological behaviours, and protected *ex situ* in the cold rooms of the seed banks of EBI.
- Better seed germination responses were observed for enset under temperature alternations, absolute changes from constant to alternating regimes, and prior cold stratification, however the overall germination was of low quantities. The observed promising seed viability and germination potential should be used as a basis for future studies searching for substrates, protocols, and growth conditions that result in maximum seed performance.
- Enset showed significant differences in seed germination behaviours between wild and domesticated accessions, because of adaptations to local climates. Seed germination trials should take these innate variations into consideration in order to achieve high germination

success for commercial seed production, varietal improvements through conventional breeding, and seed banking.

- Stablishment of a national institute as center of excellence to coordinate research and development for this key-stone plant which is pivotal for maintainance of food security should be promoted just like the Dilla University is satblishing the same for coffee, a basic source of foreign currency for Ethiopia at Yirgachefe in Gedeo Zone of southern Ethioia.

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Appendices

Appendix 1: List of farmers' landraces reported & observed on crop fields (or home garden) with medicinally valuable landraces for treatment of human & livestock ailments

Note that: Hum = Enset having medicinal value for treating huma ailments, Vet= those having applications in veterinary medicine

S/No	Wolaita landraces	HM/ LM	Gurage landraces	HM/ LM	Sidama landraces	HM/ LM
1	Adinona		Aawiyare		Ado	HM
2	Adowa	HM	Abakit		Adame_Ado	
3	Agina	HM, LM	Agade	HM, LM	Agana	HM
4	Anko Gena		Agelgil		Alati_Ganticho	
5	Ankowa		Amerand	HM	Amboma	
6	Argama	HM, LM	Ameriye		Arisho	
7	Arkiya	HM, LM	Angorgur Kanchuwe		Askala	HM, LM
8	Badadiya		Ankofiye		Awulcho	
9	Bala	HM, LM	Ashakit	HM	Bira	
10	Banga		Astara	HM, LM	Bora	
11	Bino		Ayiwogne		Borbodh'ancho	
12	Boroda Wanadiya	HM, LM	Badadet	HM, LM	Boso	
13	Bota Agina		Bangiye Agade	HM	Bowete_Ado	
14	Bota Arkiya	HM, LM	Bazeriye	LM	Bufare	
15	Bota Maziya	HM	Beneze		Busha	
16	Bota Tuzuma		Bosere		Chacho	HM, LM
17	Bulowa		Charkim	HM, LM	Damala	
18	Chama		Dere'ea	HM, LM	Dansite	
19	Chapariya		Edemert	HM, LM	Darasi Ado	HM

20	Chichiya		Egendiye		Digne	HM
21	Da'ana		Egetiye		Disho	
22	Dawuro Sirare		Eieimiriye		Dowirame	
23	Dokowa		Ekechiriye		Duwancho	HM
24	Earpa		Emiriye		Ganticho	MH, LM
25	Falakiya/Palakiya	HM, LM	Eniba / Eniwa		Garircho	
26	Filikiya/ Pilkiya		Enir Agade		Gedimo	
27	Gea'na Tuzuma		Erengiye		Gena	
28	Geena	HM	Eshirafre	HM, LM	Gesa	
29	Gefeteno	HM, LM	Esmaeael		Golloma	
30	Godariya		Eweret/Ewored	HM	Gosalo	HM
31	Gofiniya		Ezara Badadet	HM, LM	Goticho	
32	Ha'ala	HM, LM	Fereziye	LM	Gulumo	HM, LM
33	Hagila	HM, LM	Gezuwed		Hanshicho	HM
34	Hoea'ya	HM, LM	Gimbuwe		Heqeche'ne	
35	Kabariya	HM, LM	Gowariye /Gowadiye	HM, LM	Huwisho	HM
36	Kareta Argama		Gumbura		Kanda	
37	Kareta Tuzuma		Jimma Astara	LM	Kincho	
38	Karta	HM	Kanchuwe	HM, LM	Kiticho	
39	Kataniya		Kasayid/ Kisayit		Kora	
40	Kekerowa	HM	Kembata Astara	HM, LM	Kule	HM
41	Koltuwa		Kesuwe		Lemicho	
42	Kopa Zinkiya		Key Kanchuwe		Mentiwese	
43	Kua'ane		Kibnar/ Kimnar	HM	Midasho	
44	Lembo	HM	Koshkowashiye	HM, LM	Mundiraro	HM, LM
45	Lochingiya	HM, LM	Lemat/Lemare /Nemare/Neymare		Nifo	

46	Masa Maziya		Mahea Kanchuwe	HM	Shawite	
47	Matiya	HM	Moche		Shisho	
48	Maziya	HM	Mushirat/Mishrad		Siltite	
49	Mella Zinkiya		Muyed	LM	Tunayicho	HM
50	Mochiya		Nechu Kanchuwe		Wanikore	
51	Nakaka	HM, LM	Nechuwe			
52	Pellowa	HM	Nokowiye			
53	Sanka		Sapara	HM, LM		
54	Shalakume		Shertiye	HM, LM		
55	Shasha		Shewurat			
56	Shuchafiya		Shive /Shibe			
57	Sirariya		Tegaded			
58	Sorgiya		Tegowanere			
59	Suitiya	HM, LM	Tereriye	HM, LM		
60	Tarsakiya	HM	Woke	HM		
61	Tela		Wonadiye			
62	Titiya		Woshemeja			
63	Tufa		Zeguwired			
64	Tuzuma	HM, LM	Zobir			
65	Wanadiya					
66	Woyisha					
67	Yesha Maziya					
68	Zinkiya	HM, LM				
69	Zo'owa Agina					
70	Zo'owa Argama					

Appendix 2: Interactions with the local farmers during collection of ethnobotanical information through interviews, group discussions, guided garden walks and focus group discussions



Appendix 3: Interview items used for collection of indigenous knowledge on utilization, management and conservation of enset in the study area

I. General information on the study area:

Basic info on the study area	Response (Note: Sign in front of the name)
Name of interviewer	
Date & time of interview	Date: / / Time: _____AM/PM to _____AM/PM
Zone	1=Sidama 2=Wolaita 3=Gurage
District (Woreda)	1=Dara 2=Kindo_Didaye 3=Enemor_Enir
Kebele	1=Abera_Bute, 2=Aleme_Korocho, 3=Safa, 4=Lasho, 5=Koyisha_Wamura, 6=Bosa_Borto, 7=Agata, 8=Gharad, 9=Horbet_Zizo
Village	
Altitude	
Latitude	
Longitude	
Agro-ecology	1=Highland 2=Midland 3=Lowland

II. Information on the respondent's personal data

Name	
Gender (sex)	1=Male 2=Female
Age (in years)	
Marital status	1=Single 2=Married 3=Widowed 4=Divorced
Educational status	1=Illiterate-I (not able to read & write) 2=Primary-I (grade 1-4) 3=Primary-II (grade 5-8), 4=Secondary-I (grade 9-10) 5=Secondary-2 (grade 11-12) 6=other----- -----
Religion	1= Protestant 2=Orthodox 3=Muslim 4=other--- -----
Ethnicity	1=Sidama 2=Wolaita 3=Gurage 4=Others- -----
Family size (if married)	
No of years you lived in the area	
No of years you started planting enset	
Size of total land holding (<i>TIMAD</i>)_____;	
Size of enset holding from the total land_____	

III. Enset cultivation, use and management in the study area

1. What is the number of enset landraces you managed in your enset farm= -----?

2. What is your purpose of planting enset in your farm? (Circle one or more responses)

1=Food

2=Fodder

3=Traditional medicine

4=Income source

5=Garden beautification (aesthetic)

6=Making of culutral materials

7=Soil, water and climate devt/Environmental value

3. Which landraces are selectively and frequently used in your farm?

No	Landrace	Selectively used landraces

4. What is the reason of using these landraces in preference to others?

No	Reason for selective utilization

IV. Medicinal application (use) of enset

5. List down enset landraces used as traditional medicine from the above table (free listing) and mention their medicinal application

Note: (Part: C=corm, PS=Pseudostem, PT=petiole, LF=Leaf, RT=Root, SD=Seed, FR=Fruit; Condition; F=Fresh, D=dry; Prep & Application: CK=Cooked & eaten, RS=Roasted & eaten; SD= Sap drunken, SS=Sap shower, DAA=Dry as application; Route of admin: O=Oral, D=Dermal, N=Nasal, AU=Auricular; Disease of: H=Human, A=Animal, B=Both)

Landrace	Part used	Condition (Fresh/dry)	Preparation &/ or application	Dose rate	Route of admin	Additive (any)	Disease treated	Human/ livestock

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6. How many medicinally valued landraces have you managed in your farm? _____; Can you show me & mention their names & count from your farm?
7. Are medicinally valuable landraces rare in your locality? 1/ YES 2/ NO
8. If you respond yes for Q-7, What are the possible causes of rarity of medicinally valued enset landraces in your locality? (Circle one or more options)

Causes for rarity
1=Disease
2=Insect pests
3=Vertebrate pests
4=Lack of enough planting materials
5=Lack of interest to multiply these landraces
6=Selective extraction of these landraces
7=Lack of access of seedlings in markets
Others -----

9. Mention any of conservation measures you use traditionally apply to protect loss of medicinal enset in your locality

Conservation measures
1=Multiplication by corm cutting
2=Seed sowing after maturity
3=Sharing germplasm from neighbors
4=Disease management using traditional methods
5=Traditional protection from vertebrate pests
6=Avoid use as food/ fodder
7=Others-----

10. Do you add any other plant (herbs, shrubs or lianas) to a fermentation pit of enset?
1/ YES 2/ NO

11. If you say yes for the Q10, list local plants you add during enset fermentation with purpose of adding it

Local name of the plant	Part added	Mode of application and purpose of adding	Fresh or dry part	Plant added on	
				1=Kocho	2=Bulla

V. Agronomic and end user qualities taste of enset

12. Mention enset landraces that are fast maturing, disease resistant and draught tolerant

No	Landraces	Early maturing	Disease resistant	Draught tolerant	Rapidly ferment	Quality Kocho	Quality Bulla	Quality Corm	Quality fiber
1									
2									
3									

VI. Traditional classification of enset

13. Mention the major features of enset you use for identification of landraces in your locality

Feature used in landrace identification
1=Pseudostem color
2=Petiole color
3=Mid rib color (lower)
4=Flower (male bract) color
5=Leaf color (lower side)
6=Other (s)-----

14. Do you commonly practice classification of enset as male & female using locally
1/YES 2/NO

15. If you say yes for the above Q, list down the characteristics of male & female enset types

Criteria used to classify	Circle if selected			
	Male		Female	
1=Vigor of the plant:	1=strong	2=weak	1=strong	2=weak
2=Maturity rate:	1=fast maturity	2=late maturity	1=fast maturity	2=late maturity
3=Disease response:	1=resistant	2=vulnerable	1=resistant	2=vulnerable
4=Draught response:	1=tolerant	2=Susceptible	1=tolerant	2=Susceptible
5=Fiber content:	1=more fibrous	2=less fibrous	1=more fibrous	2=less fibrous
6=Kocho quality:	1=high quality	2=low quality	1=high quality	2=low quality
7=Corm quality:	1=tasty	2=not tasty	1=tasty	2=not tasty
8=Bulla quality:	1=high quality	2=low quality	1=high quality	2=low quality

16. Mention different diseases & pests of enset challenging enset production and productivity in your locality with local names, stage affected & traditional mode of protection

Disease/ pest name	Local name	Part/product affected	Stage (product) of most affected	Traditional prevention/
1=Enset leaf wilt disease				
2=Eset sheath rot				
3=Enset corm rot				
4=Enset root mealy bug				
5=Mole rats (AYTEMOGOT)				
6=Porcupines (JART)				
7=Monkey (ZINJERO)				
8=Velvet monkey (GUREZA)				
9 =Small monkey (TOTA)				

17. Do you commonly manage some plant species around or within enset farm to protect enset disease or pest attack? 1/YES 2/NO

18. If you say yes for the above Question; can you list down the plant species you manage in enset farm to protect enset disease or pests

Plant species to treat enset disease	Enset disease (pest) treated

19. What are the factors those determine the maturity rate and flowering of enset in your area?

Factors affecting maturity rate & flowering of enset
1=Altitude/agroecology (climate)
2=Landrace (genetic)
3=Soil type (mineral nutrient)
4=Manure application (nitrogen)
5=Transplanting
6=Intercropping
7=Smoking
8=Spacing
9=Others _____

20. What is the fastest, slowest and average number of years enset flowers in your area?

Flowering age of enset	Time in (years/months)
Fast flowering	
Average flowering	
Slow flowering	

21. What are the signs & indications by which you know that enset plant is matured and close enough to flowering before the inflorescence emerges?

Method of identification of enset at the verge of flowering
1=Corm enlargement & exposure above ground
2=delay of cigar (central) leaf
3=emergence of short and stout leaves
4=Others (mention!)

22. What are the benefits and harmful effects of flower formation on enset plant?

Benefits of flowering on enset plant	Harmful effects of flowering on enset plant

23. Mention animals (small animals, large animals, birds or insects) around or inside of enset inflorescence?

Animals in (around) enset inflorescence
1
2
3

24. What do you think is the ecological function of organisms dwelling in or around enset inflorescence?

Animals in (around) enset inflorescence	Organism observed
1=Feeding on the nectar or the pollen	
2=Preying other organisms for food	
3=Using inflorescence as a shelter & mating place	
4=As a playground to enjoy climbing on it	
5=Other_____	

25. What are the seasons and environmental conditions for enset flowering?

Seasons & conditions during enset flowering	
1=Season	1=Kiremt, 2=Bega, 3=Tseday, 4=Meher
2=Rain condition	1=High 2=Medium 3=low
3=Temperature (hot, mild, cold)	1=Hot 2=Mild 3=Cold

4=Major tree species flowering	
5=Major crop flowering	
5=Other- _____	

26. Have you ever seen ripened fruit of enset that turned in to orange colored small sized banana like fruits in your locality? 1/YES 2/NO

27. If you say yes, where (local ecology) and at what season of a year do you notice these fruits?

Local ecology & season to get ripened enset fruit	
Local ecology	Season of access
1=Cultivation farms (home gardens)	1=Kiremt, 2=Bega, 3=Tsedey, 4=Meher, 5=other
1=Old & stable enset farms	1=Kiremt, 2=Bega, 3=Tsedey, 4=Meher, 5=other
2=Sloppy & inaccessible river gorges	1=Kiremt, 2=Bega, 3=Tsedey, 4=Meher, 5=other
3=Deep Forest margins	1=Kiremt, 2=Bega, 3=Tsedey, 4=Meher, 5=other
5=Other _____	

28. Have you ever seen black colored asphalt-gravel-like seeds inside matured & orange-colored fleshy fruits of enset? 1/YES 2/NO

29. Have you ever seen enset produced matured fruits (with seeds) that had spontaneously fallen & died by ageing, sprout & seeds germinate in-situ? 1/YES 2/NO

30. If you said “Yes” for the above question (Q-No-18), relatively how many seedlings have you observed per fruit bunch of the fruiting stalk of a single enset plant? _____

31. Do you think all those seedlings emerging from a single plant (that germinates from a fallen down bunch of enset inflorescence) are similar to the mother landrace? 1/YES 2/NO

32. If you said “No”, mention any of those good and bad features you had observed (than the mother landrace) from the new progeny germinated spontaneously?

Good qualities than the mother landrace	Bad qualities then the mother landrace
1=	1=
2=	2=
3=	3=

33. Have you ever tried the sexual reproduction and multiplication of enset using seeds? 1/ YES 2/NO

34. If yes, what are the useful and harmful sides of reproduction of enset by seeds to you?

Advantages of reproduction by seeds	Disadvantages of reproduction by seeds
1=	1=
2=	2=
3=	3=

35. Have you ever noticed any of the wild animal (s) feeding on ripened fruits of enset? 1/YES 2/ NO

36. If you said “Yes”, mention any of those animals that use the ripened fruit of enset (on the erect plant or after it fall down) as food?

Animals feeding on ripened fruits of enset
1=
2=
3=

37. Do you feel any fruity & pleasant smell from blooming flower of enset inflorescence in your locality? 1/ YES 2/ NO

38. What time of a day is the habitual time of release of this aromatic smell?

Time of release of aroma from enset flower	
1=Early morning (12:00-2:00AM)	5=Mid-day -----
2=Late morning (2:00-3:00AM)	6=Mid-night -----
3=Early evening (12:00-2:00PM)	
4=Late evening (2:00-3:00PM)	

39. Have you ever seen enset in the wild habitats in your locality? 1/ YES 2/ NO

40. If yes, how do you think these seeds were transported to these wild areas?

Agents of transport for wild enset germplasm
1=Primates
2=Large Birds
3=Bats
4=Mole rats, wild rats
5=Water currents
6=Slope gradients
7=Others

41. At what especial times you or traditional people in your locality had used wild enset in history?

Time for feeding wild enset
1=Draught & hunger
2=War and mass-migration
3=Famine & epidemic diseases
4=Massive flooding & huge fires
5=Decline (fall) of agricultural productivity
6=Off season time for farm products
7=Others

42. How many landraces of wild types of enset have you ever encountered in your locality?__
43. Can you mention their distinguishing features and names you use to identify (if any name)?

Distinguishing features of wild races	Name used locally for identification
1=	
2=	
3=	

44. How long does it take for enset fruit to get ripened & develop black seeds once after emergence of the inflorescence? _____
45. Do you use color of bracts of the male bud of different landraces of enset in your locality? 1/YES 2/NO

Appendix 4: Items used for collection of ILK and perception of local people on enset use & management from the key informants

1. Do you know locally managed farmer's varieties of enset selectively used for especial purposes in your locality? What are their names?
2. What do you think are the most destructive harvesting modes (activities) to culturally preferred enset landraces of enset in your area?
3. Which of farmers' landraces of enset have been threatened (and locally rare) in your locality? What are the activities practiced so far for protection of landrace diversity of enset on-farm in your community?
4. Can you mention enset landraces used for application in traditional medicine?
5. What are the main human and livestock ailments and disorders at your locality?
6. How do you diagnose each of these ailments, and what are the major symptoms of the ailment?
7. How do you treat and prevent or control human and livestock ailments in your locality?
8. Which enset landraces(s) do you use for treating that particular ailment? (Please, try to provide the local name (s) and morphological characteristics of important parts of these races)
9. Provide tissue parts or processed products of medicinally valued enset used in treatment of ailments (disorders)
10. Does these parts or products of enset are used alone or mixed with other materials?
11. How enset parts or processed products are used, (can you provide a detailed preparation of enset for treatments)?
12. What amount of the remedy used (dose rate) is effectively used for treatments? Does it dose differ among genders, pregnant women and children?
13. Do you know or hear any noticeable adverse or side effects(s) of enset used in disease treatments?
14. Are there any threats to the medicinally important enset landraces in your area?
15. How is the knowledge on traditional medicinal applications of enset passed to a family member or younger generation?
16. How do modern lifestyles interfere with traditional medicinal knowledge of applications of enset in disease treatments?

Appendix 5: Items used for collection of information on enset use through focus group discussion

1. Can you please tell us your name, the ebele from where you come and your responsibility in the area?
2. How do you describe the traditional enset management practices of dwelling in your locality?
3. For what purpose do people use enset traditionally in your locality? Do they use for traditional treatment of disorders (ailments) of humans or livestock?
4. Do the local people uproot and sell some enset types to generate income? What are the major purpose people buy some enset landraces from your locality?
5. Which enset landraces are used importantly as sources of treatments for human or livestock ailments in y your locality?
6. What are the different products or raw tissues of enset used in treatment of cultural ailments?
7. What are the range of human and livestock ailments (disorders) that are treated using enset as source of traditional healings?
8. How do you perceive the current conservation states of the diversity of enset landraces in your locality?
9. What are the basic preparation methods used in applications of enset in traditional medicine in your area?
10. How do you rate the availability at present and past of enset landraces in your locality? Do you know enset landraces known by your predecessors, but locally not available or extremely rare to get today? Where do you think these rare enset landraces could be found at their highest diversity today (highlands, midlands or lowlands)?
11. What are the major factors (local conditions of abiotic or biotic entities) mentioned as responsible for the loss or diminishing of some valuable enset landraces in your area?
12. What are the local methods of conservation of enset landraces used in protection of the diversity maintained by the farmer as farmers' landraces?
13. What are the different roles of males & female in management of enset from farms to the plate?
14. How is the indigenous knowledge of management of enset is passed from your predecessors is passed to you and you also pass to your children (youth)? Is there any means through which the elders teach their children about traditional management of enset?
15. Do you think that traditional practices of enset management and utilization are declining gradually? Could you mention your opinion regarding this gradual loss in ILK? What are the factors contributing for the loss?

Appendix 6: List of informants used as source of ethnobotanical information using semi structured interview interview

Name	Age	Gender/ Sex	Educational status	Informant Class/type	Study zone
Dangamo Rusa	53	Male	Primary education	Key Informant	Sidama
Faneso Fako	58	Male	Primary education	Key Informant	Sidama
Jebena Yona	75	Male	Primary education	Key Informant	Sidama
Kalaltu Biftu	44	Female	Primary education	Key Informant	Sidama
Kontamo Kora	45	Male	No modern education	Key Informant	Sidama
Meselech Arero	55	Female	Primary education	Key Informant	Sidama
Tadelech Gergedo	60	Female	Primary education	Key Informant	Sidama
Nigist Barana	50	Female	Primary education	Key Informant	Sidama
Choke Balicha	48	Male	No modern education	Key Informant	Sidama
Baredo Wagiso	54	Male	No modern education	Key Informant	Sidama
Lagide Wojago	25	Male	Primary education	General Informant	Sidama
Ayu Shumbulo	45	Female	Primary education	General Informant	Sidama
Dilkamo Jifaro	29	Male	Primary education	General Informant	Sidama
Tdelech Kakabo	50	Female	Primary education	General Informant	Sidama
Adanch Bire	54	Female	No modern education	General Informant	Sidama
Alemnesh Wariyo	45	Female	No modern education	General Informant	Sidama
Bekele Tefera	68	Male	No modern education	General Informant	Sidama
Armitu Dogoma	36	Female	No modern education	General Informant	Sidama
Mamo Bire	55	Male	Primary education	General Informant	Sidama
Aster Darara	53	Female	Primary education	General Informant	Sidama
Amanuel Amaje	63	Male	No modern education	General Informant	Sidama
Ayantuu Shole	55	Female	Primary education	General Informant	Sidama
Adato Alalcha	44	Male	Primary education	General Informant	Sidama
Darado Yona	44	Female	No modern education	General Informant	Sidama
Elfinesh Mato	69	Female	No modern education	General Informant	Sidama
Terefe Badiso	60	Male	No modern education	General Informant	Sidama
Fantu Amido	67	Female	Primary education	General Informant	Sidama
Frenesh Regasa	57	Female	No education	General Informant	Sidama
Tafese Berasa	35	Male	No modern education	General Informant	Sidama
Harite Bunaka	51	Female	No modern education	General Informant	Sidama
Adafre Abayneh	50	Male	No modern education	Key Informant	Wolaita
Alaro Asale	78	Male	Primary education	Key Informant	Wolaita
Arzu Altaye	55	Male	No modern education	General Informant	Wolaita
Asa Fata	65	Male	No modern education	General Informant	Wolaita
Aster Falta	65	Female	Primary education	Key Informant	Wolaita
Aster Kusa	50	Female	Primary education	Key Informant	Wolaita
Batiso Balta	65	Male	No modern education	Key Informant	Wolaita
Berihannesh Babe	62	Female	No modern education	General Informant	Wolaita
Beyene Balta	49	Male	Primary education	General Informant	Wolaita

Bonja Borsamo	80	Male	No modern education	General Informant	Wolaita
Buche Gezuma	34	Male	No modern education	General Informant	Wolaita
Chinasho Salte	61	Male	No modern education	Key Informant	Wolaita
Chutulo Joba	51	Male	Primary education	General Informant	Wolaita
Daka Darebo	37	Male	No education	General Informant	Wolaita
Dashure Gonjofo	63	Female	Primary education	General Informant	Wolaita
Dorane Chade	55	Female	No modern education	General Informant	Wolaita
Dube Ololo	39	Female	No modern education	General Informant	Wolaita
Eana Koyo	75	Male	No modern education	Key Informant	Wolaita
Erbikan Basa	58	Female	Primary education	General Informant	Wolaita
Genet Dadiso	49	Female	No modern education	General Informant	Wolaita
Genet Doko	42	Female	No modern education	General Informant	Wolaita
Gige Gidebo	60	Male	Primary education	General Informant	Wolaita
Gonjofo Agago	60	Male	Primary education	Key Informant	Wolaita
Handeno Hadero	70	Male	No modern education	General Informant	Wolaita
Laa Kolbaye	65	Male	Primary education	General Informant	Wolaita
Matewos Taltamo	65	Male	No modern education	General Informant	Wolaita
Mekbibe Choka	70	Female	Primary education	General Informant	Wolaita
Moges Mota	67	Male	No modern education	General Informant	Wolaita
Nasa Fata	37	Male	No modern education	General Informant	Wolaita
Sata Sadebo	60	Male	Primary education	General Informant	Wolaita
Tayote Ololo	65	Female	Primary education	Key Informant	Wolaita
Timaro Damte	64	Male	No modern education	General Informant	Wolaita
Toma Dolato	75	Male	Primary education	General Informant	Wolaita
Ukumo Adare	35	Female	No modern education	General Informant	Wolaita
Wope Sorsa	40	Female	Primary education	Key Informant	Wolaita
Behailu Bizako	55	Male	Primary education	Key Informant	Gurage
Betrakech Murad	60	Female	Primary education	Key Informant	Gurage
Delil Amir	67	Male	Primary education	Key Informant	Gurage
Fikre Gebremariam	45	Male	No modern education	Key Informant	Gurage
Mengesha Haile	73	Male	Primary education	Key Informant	Gurage
Shikuriya Husen	56	Female	Primary education	Key Informant	Gurage
Temam Sabir	63	Male	No modern education	Key Informant	Gurage
Woletesenbet Akumel	70	Female	Primary education	Key Informant	Gurage
Muhajir Shamil	65	Male	Primary education	Key Informant	Gurage
Olewi Nekane	68	Male	Primary education	Key Informant	Gurage
Debebe Baksira	51	Male	Primary education	General Informant	Gurage
Tadese Badeta	66	Male	Primary education	General Informant	Gurage
Maymuna Sabir	48	Female	Primary education	General Informant	Gurage
Meshmun Sabit	44	Female	No education	General Informant	Gurage
Murga Echga	27	Female	Primary education	General Informant	Gurage
Muzidat Mehamed	44	Female	Primary education	General Informant	Gurage
Tadesech Dembi	53	Female	Primary education	General Informant	Gurage

Tenu Arega	39	Female	No modern education	General Informant	Gurage
Tsige Worku	37	Female	No modern education	General Informant	Gurage
Workinesh Bezaneh	48	Female	No modern education	General Informant	Gurage
Zeritu Abisir	29	Female	Primary education	General Informant	Gurage
Ajaeba Jemal	44	Female	Primary education	General Informant	Gurage
Bosena Nechmane	52	Female	No modern education	General Informant	Gurage
Kere Tesema	33	Female	Primary education	General Informant	Gurage
Kibu Wolde	42	Female	Primary education	General Informant	Gurage
Behiriya Ahimed	36	Female	No modern education	General Informant	Gurage
Abesha Ahmedin	42	Female	No modern education	General Informant	Gurage
Abdijuad Yasin	63	Male	Primary education	General Informant	Gurage
Mehbuba Shemsu	40	Female	No modern education	General Informant	Gurage
Bechene Endriyas	51	Male	No modern education	General Informant	Gurage
Zeytuna Yasin	58	Female	No modern education	General Informant	Gurage
Dembnineh Dibs	56	Male	No modern education	General Informant	Gurage
Zemzem Echaga	45	Female	Primary education	General Informant	Gurage
Dina Arga	65	Male	No modern education	General Informant	Gurage
Melesech Gebru	44	Female	Primary education	General Informant	Gurage

Appendix 7: Some of sample preparation and analysis events for nutritional composition studies

