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ANTIMICROBIAL PROPERTIES OF ENDOPHYTIC AND RHIZOSPHERIC FUNGAL SPECIES ASSOCIATED WITH SOME MEDICINAL PLANTS

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ABSTRACT: Nowadays, the development of multidrug-resistant human pathogenic microorganisms and the emergence of new diseases are the most challenging problems in public health care across the globe. Therefore, the objective of this study was to isolate and identify the antimicrobial properties of endophytic and rhizospheric fungi associated with some medicinal plants. A total of 150 plant parts and 50 soil samples were collected from five medicinal plants. In vitro antimicrobial activities were tested against common resistant pathogens (*Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Pseudomonas aeroginosa* and *Candida albicans*). A total of 582 fungal isolates were obtained. Accordingly, 78 (19.89%) isolates displayed antimicrobial activities by agar plug diffusion method. Ethyl acetate extracts of isolate 30CRS showed higher significant ($p \leq 0.001$) inhibition zones against *E. coli* (30.33 ± 0.57 mm), *E. faecalis* (25.33 ± 0.28 mm) and *S. aureus* (18.33 ± 0.57 mm) than the positive control, whereas fungal isolate 37BRaL showed significantly higher ($p \leq 0.001$) inhibition zone against *S. aureus* (19.16 ± 0.28 mm) and *C. albicans* (26.83 ± 0.76 mm). The mean minimum inhibitory concentration (MIC) result was 3.125 – 50 mg/ml for gram-positive bacteria, 6.25 – 50 mg/ml for gram-negative bacteria and 12.5 – 50 mg/ml for *C. albicans*. The minimum bactericidal concentration ranged from 6.25 – 50 mg/ml, while the minimum fungicidal concentration ranged from 12.5 – 50 mg/ml. The potent isolates (30CRS) and (37BRaL) were identified as *Penicillium simplicissimum* and *Talaramycetes flavus* var. *flavus* using Biolog microbial identification system. This research has confirmed the potential of endophytic and rhizospheric fungal species against resistant clinical isolates.

Keywords: Endophytic fungi, Medicinal plant, Multidrug-resistant, Rhizospheric fungi.

INTRODUCTION

Plants have served as a source of medicinal bioactive compounds against numerous forms of diseases for centuries. In recent years, rather than plants themselves, microorganisms associated with plants have proved to offer materials and products with high therapeutic potential (Subbulakshmi et al., 2012). Endophytes are

an endosymbiotic group of microorganisms often bacteria or fungi that colonize the intracellular locations of plants and have exhibited importance in antagonizing human pathogens (Singh and Dubey, 2015).

Endophytes produce bioactive compounds of biotechnological interest for pharmaceutical industries (Joseph and Priya, 2011). For instance, many endophytic fungi produce secondary metabolites which are very attractive in terms of their activity and chemical structure against human pathogens. Secondary metabolites such as alkaloids, phenols, terpenoids, and steroids play an important role as potential candidates for therapeutic compounds (Pandey and Malviya, 2014).

Diverse microbial populations also inhabit the rhizosphere region of many plants and they principally comprise fungal and bacterial species. In plants, organic materials from the root provide the driving force for the development of active microbial biomass in the rhizosphere region compared to the bulk soil (Qureshi et al., 2011). Different compounds secreted by plant roots into the rhizosphere serve as a source of energy and precursors of many metabolites produced by associated microorganisms (Solaiman and Anawar, 2015).

Antimicrobial agents are synthesized from microorganisms, plants, and animal products and are used to treat microbial diseases (Alkhyat and Al-Maqtari, 2014). However, currently, the development of multidrug-resistant human pathogenic microorganisms and the emergence of new diseases are the most challenging problems in the public healthcare system and a major challenge in Ethiopia (Reta et al., 2019). The development of resistant pathogenic bacteria against commonly used antibiotics due to their misuse and overuse in developing countries like Ethiopia is becoming a serious health problem in a hospital setting (Moges et al., 2014). Furthermore, the lack and high cost of new-generation drugs have escalated infection-related morbidity, mortality, losses in productivity, and affected the economy (Mulu et al., 2006). Infections caused by resistant bacteria also adversely affect treatment outcomes, treatment costs, disease spread, and prolonged illness (Moges et al., 2014). Therefore, these problems have prompted the need to search for new drugs with better efficacy from endophytes and rhizospheric microbes against drug-resistant pathogenic

microorganisms as well as for the better treatment of newly emerging diseases (Liang et al., 2012). There are a number of studies on medicinal plants and isolated endophytic fungi with antimicrobial activities which include, *Solanum incanum* (David et al., 2021), *Aloe vera* (Fuad et al., 2021), *Rumex abyssinicus* (Shifa, 2020), *Rumex nervosus* (Asma et al., 2022) and *Myrsine africana* (Hina et al., 2021). However, in Ethiopia, such studies are lacking. Thus, this research was aimed at isolating and identifying medicinal plant-associated fungi from five selected medicinal plants of Ethiopia (*Solanum incanum, Aloe vera, Rumex abyssinicus, Rumex nervosus* and *Myrsine africana* to evaluate their antimicrobial activities.

MATERIALS AND METHODS

Description of the study areas

The samples were collected from Bale, West Arsi, and Chancho Special Zones, Oromia Regional State (Figure 1). The samples were collected from Riverian Fasil Angeso natural forest and the surrounding grazing land in Bale Zone. The GPS coordinates of the sampling site were latitude 6°57'43" to 6°61'14"N and longitude 39°56'60" to 39°57'40"E and with an elevation of 1032 to 1080 masl.

West Arsi Zone is found in the central part of the Oromia National Regional State. GPS coordinates of the sampling site were Latitude 7°05'33" to 7°11'60.00"N and Longitude 38°22'41" to 38°38'03"E and an elevation 1877 to 1937 masl. The sampling sites covered grazing and farming land borderline.

Chancho, Oromia Special Zone, is another sample collection site of the current study. It is located 45 km north of Addis Ababa on the road to Gojam with an elevation of 2555 to 2600 masl. GPS coordinates of the sampling site were 9°15'59" to 9°18'59"N and 38°45'15" to 38°47'15"E.

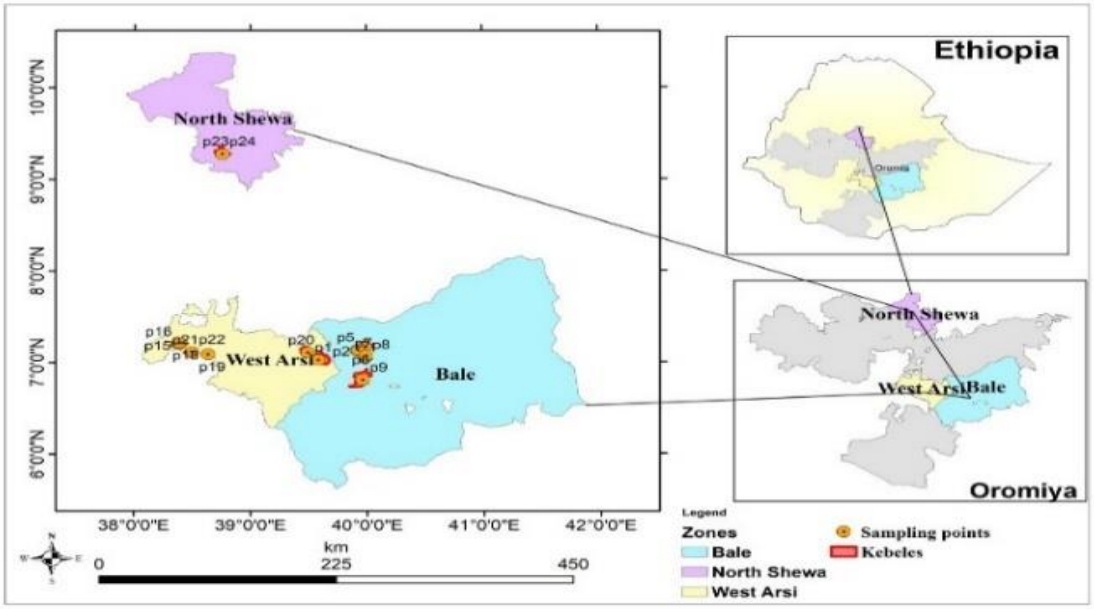


Figure 1. Map of sample collection areas.

Sample collection

Five healthy and young individual plants were selected randomly for each species per location and a total of 150 samples (stem, leaves, root/rhizome) and 50 rhizospheric soils from a depth of 5 cm were collected into sterile plastic bags separately (Table 1). All samples were kept in the refrigerator at 4°C and transported to Microbiology Laboratory of Ethiopian Biodiversity Institute using an icebox.

Isolation of endophytic fungi

About 3 g of plant samples (stem, leaves, root and rhizome) were transferred into a sterile petri dish and washed using running tap water three times to remove dust and soil. The samples were surface sterilized with 70% ethanol for 1 min and rinsed three times with sterile distilled water. Plant samples again were washed with 2% sodium hypochlorite solution for 30 seconds followed by rinsing three times with sterile distilled water (Basha et al., 2012). The samples were allowed to surface dry on sterile filter paper and transferred to PDA (HiMedia) for culturing fungi. Also, suspensions from the third wash were plated onto PDA supplemented with 100 mg/L chloramphenicol to check the efficiency of surface sterilization.

Each leaf, stem, and root/ rhizome sample was cut into one-centimeter size using a sterile blade. A total of 900 surfaces sterilized pieces from different tissues of each plant (300 leaves, 240 stems, 300 roots, and 60 segments of rhizome) were taken for the isolation of endophytic fungi. Six pieces from each sample were transferred to PDA containing chloramphenicol (100 mg/L). Inoculated plates were incubated at 27°C for seven days until growth is visible (Deepthi et al., 2018).

Table 1. Selected medicinal plants for isolation of endophytic and rhizospheric fungi

Family	Scientific Name	Local Name (Afaan Oromo/Amharic)	Sample Type	Collection Site
Polygonaceae	*Rumex abyssinicus*	Mekmeko[A]	Leaf, stem, rhizome/root and rhizosphere soil	Bale Zone and West Arsi
Polygonaceae	*Rumex nervosus*	Embuwacho[A]	Leaf, stem, root and rhizosphere soil	West Arsi and Chancho
Myrsinaceae	*Mysrine africana*	Kechemo[A/AO]	Leaf, stem, root and rhizosphere soil	Bale Zone
Alliaceae	*Aloe vera*	Ret[A]	Leaf, root and rhizosphere soil	West Arsi and Chancho
Solanaceae	*Solanum incanum*	Embuayi[A]	Leaf, stem, root, and rhizosphere soil	Bale and West Arsi

[A]= Amharic, [AO]= Afaan Oromo

Isolation of rhizosphere fungi

One gram of rhizospheric soil samples were taken and added into a test tube containing 9 ml of sterile distilled water. A tenfold serial dilution (10^{-1} to 10^{-6}) was prepared by pipetting 1 ml from stock suspension into nine ml of sterile distilled water, and thoroughly shaking manually to mix the suspension (Nisha et al., 2017). From an appropriate dilution factor (10^{-4} and 10^{-6}), 0.1 ml of the suspension was spread plated onto PDA supplemented with chloramphenicol (100 mg/L) and incubated at 27˚C for 7 days.

Purification and maintenance of the fungal isolates

The purified fungal isolates were transferred separately to brain heart infusion preservative medium supplemented with 10% glycerol and were maintained at two different temperatures (4ºC and -20ºC) for screening and identification purposes.

Source of test organisms

Clinical isolates (CI) and reference strains (RS) of human pathogenic microorganisms, *Staphylococcus aureus* (ATCC25923), *Enterococcus faecalis* (ATCC 29212), *Pseudomonas aeruginosa (*ATCC 27853), *Escherichia coli* (ATCC 25922) and *Candida albicans* (ATCC 9690) bacterial and fungal cultures were obtained from Tikur Anbessa Specialized Hospital and Ethiopian Public Health Institute (EPHI), Microbiology Department, Addis Ababa, Ethiopia. The cultures were maintained on nutrient agar and Sabouraud dextrose agar (SDA) medium. The test bacterial and fungal strains were incubated at 37^{o}C for 24 hrs. and at 35^{o}C for 48 hrs., respectively.

Standard antibiotics

Standard antibiotics, 30 µg chloramphenicol, were used as a positive control for the antibacterial susceptibility test by disc diffusion, 25 µg fluconazole was employed for the antifungal test and 2% dimethyl sulfoxide (DMSO) was used as a negative control (Matilde, 2011).

Preliminary screening

The preliminary screening of antimicrobial activity was carried out using the agar plug diffusion method (Devaraju and Satish, 2011). 100 µl of tested pathogenic bacteria and yeast, at concentration of 0.5 McFarland standards, were inoculated into nutrient agar (HiMedia) and Sabouraud dextrose agar (HiMedia) and spread uniformly using a sterile swab. The mycelial discs (6 mm) of each fungal isolate (14 days old) grown at 27°C on PDA were obtained using a sterile cork borer and placed on the surface of the media that were seeded with the test organisms. Chloramphenicol (30 µg) and fluconazole (25 µg) were used as a positive control for bacteria and yeast, respectively. Moreover, nutrient agar inoculated with test bacteria was also used as a control. The plates were incubated at 37°C for 24 hrs. and 35^{o}C for 48 hrs. for bacteria and fungus, respectively.

Fermentation and extraction of fungal crude metabolites

The potent fungal isolates in primary screening were cultivated on the surface of sterilized PDA plates and incubated at 27°C for 7 days. At a log phase growth stage of the culture, five plugs (6 mm diameter) were transferred into sterilized 250 ml of potato dextrose broth medium (HiMedia) and incubated at 27°C for 14 days. Then, the cultures were filtered and the filterate was centrifuged at 10,000 rpm for 15 minutes. All the supernatants were filtered again using Whatman No. 1 filter paper with pore size 11 µm to remove the remaining culture. Thereafter, the filtrate was extracted with an equal volume of ethyl acetate. The solution was mixed well by vortexing for 10 min and kept for 5 minutes until two clear immiscible layers (medium layer and ethyl acetate layer) were formed. The extract was concentrated by removing the solvents under reduced pressure at 40°C using rotary evaporator. Finally, the extracts were dissolved in 2% DMSO at an equal concentration and stored at 4°C (Sutjaritvorakul, 2011).

Secondary screening by agar well diffusion method

The secondary screening of antimicrobial activities of the fungal extracts was carried out using the agar well diffusion method (Moussa et al., 2011). Concentrations of 50 mg/ml were prepared for all the fungal extracts by dissolving the extracts in 2% DMSO. Molten Mueller Hinton Agar (20 ml) and Sabouraud dextrose agar (20 ml) were prepared for bacterial and yeast, respectively. All the clinical suspensions of the test organisms were standardized based on 0.5 McFarland Standard and spread uniformly. Then, holes, 6 mm in diameter and 4 mm depth were made in the inoculated agar plates using a sterile cork borer. The diluted extracts (100 µl) were added into each hole by using a micropipette and kept at room temperature for one hour to allow the crude metabolites to diffuse into the agar medium. Chloramphenicol (30 µg) and fluconazole (25 µg) were used as a positive control, while 2% DMSO was used as a negative control. Inoculated plates were incubated at 37^{o}C for 24 hrs. but the SDA plates were incubated at 35^{o}C for 2 days.

Determination of the minimum inhibitory concentrations

The minimum inhibitory concentration (MIC) of the solvent extracts was determined by agar dilution methods as described by (ESCMID, 2000). Molten Muller Hinton and Sabouraud agar media were used for this test. Accordingly, 1 ml of crude fungal solvent extracts were prepared at different concentrations (50, 25, 12.5, 6.25, 3.125 mg/ml) and mixed thoroughly with the respective molten agar medium and poured into sterile petri dishes. One milliliter of bacteria and yeast suspensions was adjusted to 0.5 McFarland standards and swabbed on Muller Hinton and Sabouraud agar. The seeded plates were incubated at 37°C for 24 hrs. for bacteria and at 35°C for 48 hrs. for yeast culture, respectively. The MIC was determined by observing the growth of the test pathogens.

Minimum Bactericidal Concentrations (MBCs) and Minimum Fungicidal Concentrations (MFCs)

A loopful of the test cultures from the last MIC were sub cultured by streaking onto a fresh Muller Hinton and Sabouraud dextrose Agar media and incubated at 37°C for 24 hrs. and at 35°C for 48 hrs. for bacteria and yeast respectively. The lowest concentration of the extracts that showed no growth on the media was recorded as MBC/MFC (Takudzwa, 2013).

Qualitative screening of fungal metabolites

Wagner's test was performed to evaluate the presence of alkaloids. One milliliter of fungal crude extract was dissolved in 2 N HCl solutions. The mixture was treated with three drops of Wagner's reagent (3 ml of potassium iodide solution mixed with 2 ml of iodine solution). The red-brown precipitate indicates the presence of alkaloids (Handunnetti, 2009).

The flavonoids test was performed as described by Cai et al. (2004). Three drops of 20% NaOH solution were added to the test tube containing 1 ml of the fungal extract resulting in the formation of yellow color. Then two drops of concentrated H_2SO_4 solution were added to the mixture. Finally, the change of color from yellow to colorless solution depicts the presence of flavonoids.

To test the presence of phenol compounds in the fungi extracts, 1 ml of the fungal extract was dissolved in 5 ml of distilled water. To this mixture, 5 µl of neutral 5% ferric chloride solution was added. Dark green color indicates the presence of phenolic compounds (Cai et al., 2004).

The presence of tannin compound was tested using a ferric chloride test as described in Yadav and Agarwala (2011). Fungal extract (1 ml) was treated with 0.5 ml of 5% ferric chloride reagent. The occurrence of the blackish-blue color showed the presence of gallic tannins and a green-blackish color indicated the presence of catechol tannins.

Keller-kiliani test was performed to assess the presence of cardiac glycosides. A 1 ml fungal extract was treated with 1 ml of $FeCl_3$ reagent (a mixture of 1 ml of 5% $FeCl_3$ solution and 99 ml of glacial acetic acid). To this solution, 1 ml of concentrated H_2SO_4 was added. The appearance of greenish-blue color within a few minutes indicates the presence of cardiac glycosides (Yadav and Agarwala, 2011).

Libermann-Burchard reaction method was used to assess the presence of steroids. Fungal extract (1 ml) was added to 1 ml of chloroform solution. The mixtures were treated with 2 ml of acetic anhydride. Thereafter, 2 drops of concentrated H_2SO_4 was added. The appearance of a blue-green ring indicates the presence of steroids (Nameirakpam et al., 2012). The presence of saponins was determined by the Frothing test (Sujana and Sridhar, 2013). A 1 ml fungal extract was vigorously shaken with 3 ml of distilled water and allowed to stand for 10 min. Formation of more than 1.5 cm stable froth (foam) indicates the presence of saponins.

For testing terpenoids, 1 ml of the fungal extract was mixed in 2 ml of chloroform and then 3 ml of concentrated H_2SO_4 was added. The formation of a reddish-brown colored precipitate at the interface indicates the presence of terpenoids (Yadav and Agarwala, 2011).

Morphological characterization

Microscopic characterization was done based on the slide culture method. Pure PDA medium was cut (5 mm square) and picked up carefully to transfer to the center of a sterile slide in a sterile petri plate. The four sides of the agar square were inoculated with 7 days old culture grown at 28°C of the fungus to be examined.

A cover glass was placed on the inoculated slide and incubated at 27°C for 48 hrs. and, the cover glass was taken carefully and flooded with Lactophenol Cotton Blue (LCB). The glass slide was observed under 40x light microscope (Kumar et al., 2015). The microscopic study included conidia and conidiophores and their arrangements (Barnet and Hunter, 2000).

Identification of fungi isolates by using Biolog™ System

Fungal isolates were identified using the Biolog Microstation™ ID System at the National Animal Health and Diagnosis Center, Sebeta, Ethiopia, following procedures described in the manufacturer's user guide (Biolog™, Hayward, CA). The testing was performed in a pre-filled microplate format to measure metabolic reactions. The characteristic metabolic pattern generated by an unknown organism was recorded and compared to hundreds of identification profiles in a corresponding Biolog Database. Fungal isolates were cultured on PDA and grown at 27ºC for one week and transferred to malt extract agar medium (Biolog™) by incubating at 27ºC for 3 days. Pure colonies were transferred into a test tube containing filamentous fungus inoculation fluid (FF-IF, Biolog™) to prepare a fungal suspension. The optical density of the suspension was adjusted to 47% transmittance using the Biolog™ turbidimeter. A 100 µl of the fungal suspension was transferred into each well of FF microplates (Biolog™) using a multichannel pipettor and incubated at 27°C. The microplates were read using BioLog Microstation™ microbial identification system at every 24 hrs. incubation period for seven days.

Data analysis

All the experiments were carried out in triplicates and the results were expressed as mean ± SD using R stat version 3.6.3 statistical software. One-way analysis of variance was conducted to test the significance levels. Significant differences among treatment means were separated using the least significant difference (LSD) at 5% Fisher’s probability level.

RESULTS

Isolation of endophytic and rhizospheric fungi

A total of 582 (316 endophytic and 266 rhizospheric) fungal isolates were obtained from the entire samples of the current study (Table 2). From the total fungal isolates under the studied plant taxa, 151 (25.9%) were from *Solanum incanum*, 147 (25.3%) were from *Rumex abyssinicus*, 127 (21.8%) were from *Mysrine africana*, 117 (20.1%) were from *Rumex nervosus* and 40 (6.9%) were from *Aloe vera*. From a total of 266 rhizospheric fungal isolates, 69 (25.9%) fungal isolates were recovered from *Solanum incanum*, 62 (23.3%) were from *Mysrine africana*, 53 (19.9%) were from *Rumex nervosus*, 52 (19.5%) were from *Rumex abyssinicus* and 30 (11.3%) were from *Aloe vera*.

Table 2. Fugal isolates obtained from different sample types.

Plant Species	**Number of fungal isolates obtained from different sample types**				
	Rhizosphere	Root/Rhizome	Stem	Leaf	**Total**
Solanum incanum	69	0	28	54	151
Rumex abyssinicus	52	18	23	54	147
Rumex nervosus	53	0	38	26	117
Mysrine africana	62	0	31	34	127
Aloe vera	30	5		5	40
Total	**266**	**23**	**120**	**173**	**582**

Preliminary and secondary screening for antimicrobial activity

Out of 582 fungal isolates, a total of 18 fungal isolates revealed better antimicrobial properties based on measured inhibition zone diameters at least against one clinical and standard pathogenic test organism (Table 3; Figure 2).

Table 3. Antimicrobial activities of fungal isolates by plug agar method.

Isolate code	Antimicrobial potential fungal isolates against test organisms									
	E. coli		*E. faecalis*		*S. aureus*		*P. aeroginosa*		*C. albicans*	
	CI	**RS**	**CI**	**RS**	**CI**	**RS**	**CI**	**RS**	**CI**	**RS**
34WRaL	+++	+++	++	++	-	-	-	-	-	-
22BRaS	++	+++	++	++	++	++	++	++	-	-
54WSL	-	-	-	-	-	-	++	++	-	-
34BSSoil	-	++	-	-	-	-	+++	++	-	-
30CRS	+	+++	++	+++	++	++	++	++	++	+++
37BRaL	++	+++	+	++	+	++	-	-	+	+
1BRaS	++	+++	-	+	-		-	-	-	-
10WRaS	+	+	+	++	++	-	-	-	-	-
15BRaS	+	++	+	+++	-	-	-	-	-	-
41WRaS	++	++	+	+	-	-	-	-	-	-
68BMS	++	++	+++	+++	-	-	+	+	+++	+++
67BMSoil	-	+	+	+	-	-	+	+	+	+
74WSS	-	-	++	+++	-	-	+++	+++	-	-
75WRaR	-	-	-	+	+	++	-	-	++	+++
78BRaR	-	+	++	++	-	-	-	-	++	+
72BML	-	+	-	+	-	-	-	-	++	++
63BML	++	+++	-	++	-	-	-	-	-	-
28BML	+	++	-	+	-	-	-	-	-	-
Chloraphenicol(C_{30})	++	+++	++	+++	+	++	+	+	-	-
Fluconazole (FLC_{10})	-	-	-	-	-	-	-	-	+	++
2%DMSO	-	-	-	-	-	-	-	-	-	-

CI=Clinical Isolates, RS=Reference strains; + = 10mm-15 mm;++ = 15 mm -25 mm; +++ = >25mm

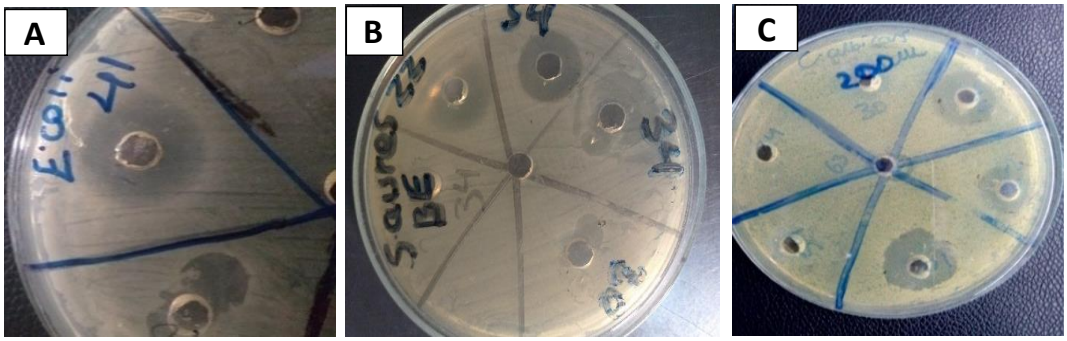


Figure 2. The inhibition zone of selected isolates against tested pathogens (A) *Escherichia coli,* (B) *Staphylococcus aureus*, (C) *Candida albicans* on Mueller Hinton agar medium by agar well diffusion method.

Table 4. Antimicrobial activity of crude ethyl acetate extracts of fungi using agar well diffusion method.

Isolates code	Inhibition zone of different human pathogenic microorganisms (Mean ± SD)				
	E. coli	***E. faecalis***	***S. aureus***	***P. aeruginosa***	***C. albicans***
1BRaS	20.33 ± 0.57^{e}	3.00 ± 0.50^{i}	4.16 ± 0.28^{efgh}	3.16 ± 0.28^{fg}	2.50 ± 0.00^{hi}
10WraS	15.16 ± 0.28^{g}	20.16 ± 0.28^{c}	23.33 ± 0.57^{b}	5.00 ± 0.00^{e}	2.00 ± 0.00^{i}
15BRaS	18.16 ± 0.28^{f}	18.33 ± 0.57^{d}	4.33 ± 0.28^{efg}	3.00 ± 0.00^{fg}	4.66 ± 0.57^{g}
22BRaS	23.70 ± 0.60^{c}	17.16 ± 0.28^{d}	19.25 ± 0.43^{c}	3.16 ± 0.28^{fg}	4.50 ± 0.86^{g}
28BML	22.33 ± 0.57^{d}	0.00 ± 0.00^{j}	0.00 ± 0.00^{k}	0.00 ± 0.00^{i}	0.00 ± 0.00^{j}
30CRS	30.33 ± 0.57^{a}	25.33 ± 0.28^{b}	18.33 ± 0.57^{c}	18.16 ± 0.28^{d}	20.33 ± 0.57^{d}
34WRaL	30.66 ± 0.57^{a}	25.33 ± 0.57^{b}	3.50 ± 0.50^{fghi}	3.33 ± 0.57^{f}	3.33 ± 0.57^{ghi}
34BSSoil	3.33 ± 0.28^{h}	5.00 ± 0.00^{g}	2.16 ± 0.28^{j}	32.33 ± 0.57^{a}	2.66 ± 0.28^{gh}
37BRaL	23.33 ± 0.57^{cd}	17.16 ± 0.28^{d}	19.16 ± 0.28^{c}	2.33 ± 0.28^{g}	26.83 ± 0.76^{b}
41WraS	20.33 ± 0.57^{e}	15.33 ± 0.57^{e}	5.000 ± 0.00^{e}	1.00 ± 0.00^{h}	2.00 ± 0.000^{i}
54WSL	1.83 ± 0.28^{i}	4.16 ± 0.28^{ghi}	2.833 ± 0.76^{ij}	29.36 ± 0.32^{b}	3.83 ± 0.76^{gh}
63BML	24.33 ± 0.57^{bc}	0.00 ± 0.00^{j}	0.00 ± 0.00^{k}	0.00 ± 0.00^{i}	0.00 ± 0.000^{j}
67BMSoil	0.00 ± 0.00^{j}	14.50 ± 0.70^{e}	5.00 ± 0.00^{d}	18.50 ± 0.70^{d}	12.50 ± 0.70^{f}
68BMS	14.33 ± 0.57^{g}	12.33 ± 0.57^{f}	3.16 ± 0.28^{ghij}	19.33 ± 0.57^{c}	35.33 ± 0.57^{a}
72BML	0.00 ± 0.00^{j}	0.00 ± 0.00^{j}	0.00 ± 0.00^{k}	0.00 ± 0.00^{i}	20.33 ± 0.57^{d}
74WSS	0.00 ± 0.00^{j}	20.33 ± 0.57^{c}	40.33 ± 0.57^{a}	0.00 ± 0.00^{i}	0.00 ± 0.000^{j}
75WRaR	0.00 ± 0.00^{j}	30.33 ± 0.57^{a}	3.00 ± 0.00^{hij}	0.00 ± 0.00^{i}	29.33 ± 0.57^{b}
78BRaR	0.00 ± 0.000^{j}	0.00 ± 0.00^{c}	0.00 ± 0.00^{k}	0.00 ± 0.00^{i}	15.33 ± 0.57^{e}
Chloramphenicol	25.33 ± 0.57^{b}	16.33 ± 0.28^{e}	15.33 ± 0.57^{d}	8.33 ± 0.57^{e}	0.00 ± 0.00^{j}
Fluconazole	0.00 ± 0.00^{j}	0.00 ± 0.00^{j}	0.00 ± 0.00^{k}	0.00 ± 0.00^{i}	24.00 ± 0.00^{c}

Note: Results displayed are representative of the mean of triplicate determinations ± sum of standard deviation (SD). Means followed by different letters (a, b, c, d, e, f, g, h, i, k) within the row are significantly different at $p < 0.05$.
Chloramphenicol: R = ≤ 12mm, I=13-17mm, S = ≥ 18mm

Selection of the best-performed isolates for identification

From 18 potential fungal isolates that showed higher inhibition zones against at least one clinical and standard pathogenic test organism, five were selected since they inhibited all the pathogenic test organisms with higher inhibition zone above 15.33 ± 0.57 mm (Table 5).

Table 5. Mean inhibition zones (mm) of the fungal isolates against test organisms using agar well diffusion method.

Test organisms	Mean inhibition zone in mm						MS	P-value	LSD
	30 CRS	37 BRaL	68BMS	22BRaS	34WRaL	Control			
E. coli	30.33^{a}	23.33^{c}	14.33^{d}	23.70^{c}	30.67^{a}	25.33^{b}	0.339	<0.05	1.60
E. faecalis	25.33^{a}	17.16^{b}	12.33^{c}	17.17^{b}	25.33^{a}	16.33^{b}	0.542	<0.001	1.30
S. aureus	18.33^{b}	19.17^{a}	3.166^{d}	19.25^{a}	3.500^{d}	15.33^{c}	0.212	<0.001	0.81
P. aeruginosa	18.16^{ab}	2.33^{c}	19.33^{a}	3.16^{c}	3.33^{c}	8.33^{bc}	34.875	>0.2	10.50
C. albicans	20.33^{d}	26.33^{b}	35.33^{a}	4.50^{e}	3.33^{f}	24.00^{c}	0.35	<0.001	1.05

Means followed by different letters (a, b, c, d, e and f) within the row are significantly different at $p < 0.05$. Resistant *E. coli, E. faecalis, S. aureus, P. aeruginosa* and *C. albicans*. LSD: Least Significant Difference at alpha=0.05.

Minimum Inhibitory Concentrations (MICs), Minimum bactericidal concentration and Minimum fungicidal concentration (MB/FCs)

For the selected fungal isolates (n=5), the MIC values ranged from 3.125 - 50 mg/ml for gram-positive bacteria, 6.25 - 50 mg/ml for gram-negative bacteria and 12.5 – 50 mg/ml for yeast test organisms. The fungal extracts showed different values of MBC against the tested microbes (Table 6).

Table 6. Minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration of EtOAc extract.

Isolates code	MIC and MB/FC against the test organisms (mg/ml)									
	E. coli		***E. faecalis***		***S. aureus***		***P. aeruginosa***		***C. alcicans***	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC
30CRS	6.25	6.25	3.125	6.25	50	50	50	50	50	50
34WRaL	25	25	50	50	-		-	-	50	50
37BRaL	12.5	12.5	12.5	12.5	50	50	-	-	25	50
68BMS	6.25	6.25	25	25	-	-	50	50	12.5	12.5
22BRaS	50	50	12.5	25	25	25	-	-	-	-

MIC=Minimum Inhibitory Concentration (MIC); MBC= Minimum Bactericidal Concentration; MFC= Minimum Fungicidal Concentration

Qualitative screening of fungal metabolites

Out of the 18 tested fungal isolates' ethyl acetate extracts, 12 of them were found to be positive for at least one secondary metabolite group. Fungal isolate 37BRaL extracts contained flavonoid, phenol, cardiac glycosides and saponin where as 30CRS fungal isolate extracts had only alkaloid and saponin (Table 7).

Table 7. Phytochemical analysis of endophytic and rhizospheric soil fungi.

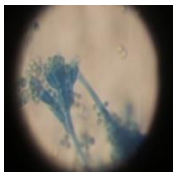
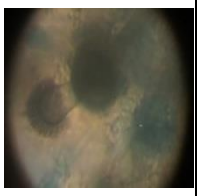
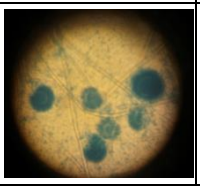
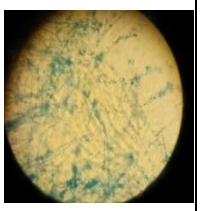
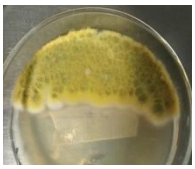
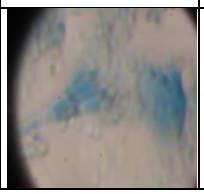
Fungal Extract	Alkaloid	Flavinoid	Phenol	Tanin	Cardiac glycosides	Steroid	Saponin	Terpenoid
34WRaL	-	-	-	-	-	-	+++	++
22BRaS	-	+	-	-	-	+++	-	+++
54WSL	+	-	-	-	-	+++	-	++
34BSSoil	-	-	-	-	-	++	-	+++
30CRS	++	-	-	-	-	-	+++	-
37BRaL	-	+++	+++	-	+++		++	-
1BraS	-	-	-	+	-	+++	+	+++
10WRaS	-	-	-	-	-	+++	-	++
15BRaS	-	++	-	+	-	-	-	++
41WRaS	-	-	-	-	-	+++	-	-
68BMS	-	-	-	-	-	+++	++	-
67BMSoil	-	-	-	-	-	-	-	-
74WSS	-	-	-	-	-	-	-	-
75WRaR	-	-	-	-	-	-	-	-
78BRaR	-	-	-	-	-	-	-	-
72BML	-	-	-	-	-	+	-	-
63BML	-	-	-	-	-	-	-	-
28BML	-	-	-	-	-	-	-	-

+++ = Potent activity; ++ = Moderate activity; + = Less activity; -, No activity

Identification of the fungal isolates

Morphological and biochemical tests of the five top fungal isolates with antimicrobial activities assigned the isolates to *Penicillium sp., Talaramycetes sp., Aspergillus sp*. and *Trichoderma sp*. (Table 8). Fungal isolate 30CRS was identified as *Penicillium simplicissimum*, while fungal isolate 37BRaL was identified as *Talaromyces flavus* var. *flavus* with similarity of 99.8% and 56.46%, respectively.

Table 8. Characterization and identification of efficient fungal isolates.

Isolate code	Morphological characteristic				Species identification		
	Fungi in a culture plate	**Macroscopic characteristics**	**Fungi under microscopic**	**Microscopic characteristic**	**Morphological identification**	**Identity using Biolog**	**Similarity**
30CRS		Green colour, colonies later turned into cream colour, filaments medium colony size, circular form/shape and flat elevation.		Conidiospore with metulae and phialides and the phialides branched. Conidia were budded from the phialides and oval shape medium size, blue color arranged in chains. The conidia ellipsoidal in shape and the walls smooth	*Penicillium*	*Penicillium simplicissimum* (oudemans) Thom BGA	99.8%
22BRaS		Gray color colonies, medium size, raised elevation and filamentous form		Smooth and colorless conidiophores and spores. Biseriate phialides	*Aspergillus*	Unidentified	
68BMS		Black colony color, large colony size, filamentous form, circular shape.		Conidia circular, branched phialides, smooth conidiophores and spores. Bi-seriate phialides	*Aspergillus*	Unidentified	
34WRaL		Gray color colonies, medium size, raised elevation and filamentous form.		Conidia were globose and Phialides were flask shaped	*Trhichoderma*	Unidentified	
37BRaL		Gray color colonies, medium size, raised elevation and filamentous form.		Conidiospore branching Monovalent, phialides branched, condia	*Talaramyces*	*Talaromyces flavus* var. *flavus*	56.46%

Penicillium simplicissimum and *Talaromyces flavus* var. *flavus*, those shows that fungal species has ability to utilize and oxidize different carbon sources (Table 9).

Table 9. Metabolic profile of *P. simplicissimum* and *T. flavus* var. *flavus* using Biolog FF Microplate.

Different carbon source utilized by endophytic fungi	Identified fungal species		Different carbon source utilized by endophytic fungi	Identified fungal species	
	P. simplicissimum	*T. flavus* var. *flavus*		*P. simplicissimum*	*T. flavus* var. *flavus*
Water	-	-	D-Ribose	+	+
Tween 80	-	-	Salicin	+	+
N-Acetyl-DGalactosamine	-	-	Sedoheptulosan	-	+
N-Acetyl-DGlucosamine	+	+	D-Sorbitol	+	+
N-Acetyl-DMannosamine	-	-	L-Sorbose	+	+
Adonitol	-	-	Stachyose	+	+
Amygdalin	+	-	Sucrose	+	+
D-Arabinose	+	-	D-Tagatose	-	-
L-Arabinose	-	+	D-Trehalose	+	+
D-Arabitol	-	+	Turanose	+	+
Arbutin	+	+	Xylitol	+	+
D-Cellobiose	-	+	D-Xylose	+	+
α-Cyclodextrin	-	-	γ-Amino-butyric Acid	-	+
β-Cyclodextrin	-	-	Bromosuccinic Acid	+	+
Dextrin	+	+	Fumaric Acid	+	+
i-Erythritol	-	+	β-Hydroxy-butyric Acid	+	+
D-Fructose	-	+	γ-Hydroxy-butyric Acid	-	-
L-Fucose	-	-	p-Hydroxyphenylacetic Acid	+	+
D-Galactose	-	+	α-Keto-glutaric Acid	+	+
D-Galacturonic Acid	-	-	D-Lactic Acid Methyl Ester	-	-
Gentiobiose	-	+	L-Lactic Acid	+	
D-Gluconic Acid	+	+	D-Malic Acid	+	+
α-D-Glucose	-	+	Quinic Acid	+	+
Glucose-1- Phosphate	+	+	D-Saccharic Acid	+	+
Glucuronamide	-	-	Sebacic Acid	+	+

Note: + = Utilization of carbon source; - = No utilization of carbon source

DISCUSSION

The current study demonstrated that a single plant part is colonized by more than one cultivable endophytic fungi. Similarly, previous studies have demonstrated that one species of plant can be inhabited by various groups of fungi (Ilyas, 2009). Corresponding to the current study, 30CRS isolate, later identified as *Penicillium simplicissimum* fungal species, was reported from some other medicinal plants such as the root

of *Alnus glutinosa* (Fisher et al., 1991), twig of *Eucalyptus nitens* (Fisher et al., 1993), stem of *Melia azadarach* (Geris dos, 2003), and roots of *Panax ginseng* (Hao, 2013). Similarly, isolate 37BRaL identified as *Talaromyces flvus* var. *flavus* that was previously retrieved from leaves of *Sonneratia apetala* (Li et al., 2011).

According to Nisha (2017), several antibiotic-producing fungi (*Aspergillus* sp. and *Penicillium* sp.) were isolated from the rhizosphere soil. Presence of these fungi depends on the nature of the environment and the texture of the soil. Generally, rhizosphere-associated microbes play a very important role in improving the medicinal values of plants (Guo et al., 2006; Raha and Shagufta, 2019). Soil microbial communities play several important ecological and physiological functions (Narula et al., 2009).

Five fungal extracts having antimicrobial properties were selected during secondary screening against more than two clinical test organisms. Accordingly, *P. simplicissimum* crude extract of the current study had broad-spectrum antimicrobial activities by inhibit the growth of gram-positive, gram-negative, and yeast at 50 mg/ml. Previous research conducted in Malysia by Yenn et al. (2014) revealed that endophytic fungi *Penicillium minioluteum* showed 17.3 ± 1.2 mm zone of inhibition against *S. aureus,* 5.7 ± 1.2 mm zone of inhibition against *E. coli,* and 17.3 ± 1.5 mm zone of inhibition against *P. aeruginosa* but did not inhibit *C. albicans* at 50 mg/ml concentration.

Bibin et al. (2016) have reported that the extract from *T. flavus* SP5 was found to be more active against various human pathogens at 10 g/100 ml of the biomass of ethanol extract, *E. coli* ATCC 52922 (18.3 ± 0.3 mm), *E. feacalis* ATCC 29212 (14.2 ± 0.7 mm), *P. aeruginosa* ATCC 27853 (17.8 ± 0.1 mm) and *C. albicans* ATCC 90028 (15.7 ± 0.7 mm). These results revealed that the extract from *T. flavus* SP5 showed strong antibacterial and antifungal activities. The current result showed a higher inhibition zone than that of the previous findings on *E. coli, E. faecalis* and *C. albicans.* This difference may be due to the application of different concentrations during antimicrobial assay and the use of different extraction methods. Besides, test organisms that were used in antimicrobial assay were from different sources.

The mean MIC of the current study exhibited by *P. simplicissimum* was higher than the finding by Amina et al. (2018) for crude ethyl acetate extract of *P. griseofulvum*, which reported MIC of 50 µg/ml for *E. coli* ATCC 25922 and 100 µg/ml for *S. aureus* ATCC 25923. Akanksha (2015) has reported that *Penicillium frequentans* inhibited *C. albicans* with less MIC (10 mg/ml).

The mean MIC of *T. flavus* var. *flavus* at 25 mg/ml against *C. albicans*, 50 mg/ml against *S. aureus* and 12.5 mg/ml against *E.coli* were higher when compared with the previous research finding by Fang et al. (2012) using *Talaromyces verruculosus* with MIC at 15.6 µg/ ml against *C. albicans*, 2.5 µg/ml against *S. aureus* and 5.0 µg/ml against *E. coli*. The variations might be due to differences in secondary metabolite production among different fungal species and the different susceptibility levels of test organisms for a fungal extract.

Penicillium simplicissimum was positive for alkaloid and saponin secondary metabolite. These results were similar to the findings by Akanksha et al. (2015) who reported that a study on *Penicillium frequentans* showed alkaloid, saponin, flavonoid, phenol, tannins, terpenoid and steroids as major secondary constituents of the crude extracts. Also Tan and Zou, (2001) reported that alkaloid was produced from grass endophytic *Penicillium* species. The result of *T. flavus* var. *flavus* was supported by a previous research conducted by Ming and Zhai (2016). *Talaromyces flavus* var. *flavus* has a remarkable potential for its secondary metabolites with unique biological activities (Bohumil, 2010). According to Lai (2010), endophytes and rhizospheric fungi have shown the presence of different secondary metabolite profiles to possess strong antimicrobial activities. They often have unusual structures and their formation is regulated by nutrients, growth rate, enzyme inactivation, and enzyme induction (Suni, 2009).

Morphological characterization was supported by the Biolog identification systems that gave similar results at the genus level. Suhaila et al. (2018) also found similar identification results of microscopic and the Biolog ID system with the molecular identification using ITS for *P. oxalicum*. *T. flavus* var. *flavus* also utilize carbohydrates. The substrate assimilation fingerprint obtained from the Biolog FF, Microplate analysis is useful in selecting components for media optimization of maximum biomass production in vitro

condition. The remaining unidentified isolates might be species not available in the Biolog database which did not utilize different carbon sources that were tagged into Biolog microplates.

CONCLUSION AND RECOMMENDATION

This research has confirmed the essential role of endophytic and rhizospheric fungal species that could exhibit antimicrobial activities against resistant clinical and reference human pathogenic microbes. Fungi associated with some medicinal plants as endophytes and/or rhizospheric organisms could be an excellent source of diverse biologically active compounds with pharmaceutical importance. The ethyl acetate extracts of *P. simplicissimum* isolated from the stem of *R. nervosus* and *T. flavus* var. *flavus* isolated from the leaf of *R. abyssinicus* exhibited the stronger antibacterial potential against test organisms. Further detailed research works on the characterization of the endophytic fungal species which are associated with different *R. nervosus* and *R. abyssinicus* genotypes/populations on potential antimicrobial secondary metabolite using NMRI and chromatography techniques is recommended. Moreover, further studies of the mode of action for a fungal metabolite of current isolates that have antimicrobial activities are important.

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LANDRACES DIVERSITY, POTENTIAL CHALLENGES AND A TRADITION OF CONSERVATION OF *ENSETE VENTRICOSUM* IN ENEMORINA EANER WOREDA, SOUTHERN ETHIOPIA

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ABSTRACT: Enset is a multipurpose crop that supports the livelihood of over 20 million people in Ethiopia. This study was aimed to assess the diversity and challenges of enset landraces in Enemorina Eaner Woreda. Six rural *kebeles* were purposively selected for the study based on their enset cultivation potential. A stratified and systematic sampling methods were used to select a total of 364 farmers for the household survey. Data were obtained through households and key informants' interviews, focus group discussions, and field observation. Shannon diversity indices and both descriptive and inferential statistical means were used to analyze the data. A total of 50 enset landraces were identified, of which 11 had medicinal importance. Enset occupied about 17% of the cropland area share in the Woreda, whereas the mean Shannon diversity and evenness indices were 2.61 and 0.78, respectively. The diversity of landraces among households was found to be significantly different ($P<0.000$) among agro-climatic zones as well as wealth groups. Wild animal pests, enset bacterial wilt, introduction of commercial crops, and labour constraints were identified as major challenges to the sustainability of enset production and landrace diversity. Furthermore, eucalyptus plantations occupied about 24% of the major cropland area share, posing a threat to enset sustainability. The establishment of an appropriate land use policy at national level, and effective collaboration of the local community, government, and academia in searching for technologies to are recommended to alleviate the observed threats and establishment of in-situ conservation sites at different agroclimatic areas of the country.

Keywords: Distribution, Diversity, Enemorina Eaner, Enset, Enset landraces.

INTRODUCTION

Enset (*Ensete ventricosum* (Welw.), Cheesman) is a multipurpose, perennial, herbaceous and monocarpic crop belonging to the family Musaceae (Cheesman, 1947). According to Brandt et al. (1997), enset supports the livelihood of more than 20% of Ethiopia's population though some succeeding researchers (Fetene and Yemata, 2018; Mengesha et al., 2022) have discussed that enset supports more than 20 million people with

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its significant role in food security in the south and southwestern parts of Ethiopia. The ethnobotanical information on intraspecific enset diversity and community knowledge on farmers' use and management is crucial for enset conservation and sustainable use (Mengesha et al., 2022). It was reported that the agricultural systems in southern Ethiopia maintain a greater level of enset intra-specific diversity than any other crop species (Olango et al., 2014). Enset farmers' varieties or landraces have a great range of genetic and phenotypic variations (Yemataw et al., 2012). Farmers' rich knowledge of enset, accumulated over years, plays a significant role in the characterization and maintenance of the existing genetic diversity. Enset growers can distinguish one enset variety from the other phenotypically by examining the leaf orientation, color of petiole and midrib, size, circumference, and length of pseudostem (Shumbulo et al., 2012; Yemataw et al., 2014; Maryo et al., 2018).

Ensete ventricosum is distributed as a wild form in the central and eastern Africa including Congo, Mozambique, Uganda, Tanzania and Zambia (Brandt, 1996). In Ethiopia, wild *E. ventricosum* is mainly found in Kaffa Zone, some area along the Omo river, and in Gamo Gofa Zone (Birmeta et al., 2004) as well as in Sheka forest (Garedew et al., 2017). Enset is distributed at altitudes between 1200 -1600 m.a.s.l., and propagated naturally by seed (Brandt and Vorobyev, 1997). *Ensete ventricosum* is cultivated only in Ethiopia (Tsegaye, 2002), occurring in the south and southwest Ethiopia (Tsegaye and Struik, 2002; Maryo et al., 2018). The domesticated enset is distributed at altitudes between 1500-3100 m.a.s.l. (Tekalign and Suneetha, 2012) and performs best at elevations of 2000-2750 m.a.s.l. (Brandt and Vorobyev, 1997), and predominantly reproduce vegetatively (Negash et al., 2002).

In Ethiopia, research on enset started in the 1970s and the major activities were clone collection, evaluation of the food and fiber values of the crop and agronomic traits, and maintenance of germplasm (Fetene and Yemata, 2018). A total of 163 enset farmers' varieties were collected initially, of which 103 and 60 were established at Holeta and Bishoftu, respectively. In 1986 a field gene bank was established at Areka Agricultural Research Center, Wolaita Zone, to conserve the diversity of enset landrace. Currently, a total

of 623 distinct enset farmers' varieties sampled from 12 major enset growing areas of Ethiopia are maintained in the center (Yemataw et al., 2017). More than 170 enset landraces have been conserved currently under the ex-situ system at Angacha center by the Ethiopian Biodiversity Institution (Personal observation and communication by the corresponding author).

Different researchers have reported different numbers of enset landraces based on morphological characterization from various enset growing areas in Ethiopia, which indeed was aided by local farmers, which include, 76 enset landraces by Shigeta (1990) from South Omo, 146 by Negash et al. (2002) from Hadiya, Keffa-Sheka, Sidama, and Wolaita; 312 by Yemataw et al. (2016) from eight enset growing Zones, 111 by Maryo et al. (2018) from Kembatta-Tembaro, and 34 by Mengesha et al. (2022) from Guji Zone .

All parts of *E. ventricosum* are utilized for various purposes. It is used for human food, animal forage, and traditional medicine. The major foods from enset are *Kocho* and *Bulla*, acquired from pseudostem and leaf petioles (Tsehaye and Kebebew, 2006) while *Amicho* is obtained from the underground corm (Yemataw et al., 2014; Maryo et al., 2018). Enset is the major food and revenue source in the highly populated southern and south-western parts of Ethiopia. The high yield per unit area linked with its ability to endure drought makes it an ideal and strategic crop for the inhabitants (Shumbulo et al., 2012). However, the sustainability of enset cultivation, enset diversity, and productivity are threatened by factors such as enset bacterial wilt (Shumbulo et al., 2012; Ayele and Sahu, 2014; Maryo et al., 2018), wild animal pests, such as porcupine and mole rat (Maryo et al., 2018), degradation of the soil (Bayu, 2016), cash-oriented crop production trends (Negash, 2001; Maryo et al., 2018), as well as poor post-harvest technology (Tekalign and Suneetha, 2012). The Gurage zone is one of the major enset production areas in the Southern Nations and Nationalities People Region (SNNPR) (Shank, 1996). Studies conducted from Kebena, Cheha and Ezha woredas (Mojo, 2017) and Gedebano Gutazer Welene woreda of Gurage Zone (Nudego, 2016) revealed that enset landrace diversity in these localities is under serious problems. In Enemorina Eaner woreda, enset is extensively cultivated for environmental, social, economic and medicinal benefits. From enset producing areas, data on

area share of enset plantation and its current status is required in order to make decisions related to conservation. In the current study area, enset is the major food crop. Like any other densely populated enset farming areas, the crop suffers from pests, diseases, lack of labor power, fragmentation of land and other similar factors (Maryo et al., 2018). Similarly, the diversity, distribution, uses, challenges, and management practices of the local communities on enset production have not been studied exhaustively and were poorly documented. Therefore, the objective of this study was to examine the enset landrace diversity, challenges, and the cultural management practices of enset in the study woreda.

MATERIALS AND METHODS

Description of the study area

The study area, Enemorina Eaner, is one of the woredas in the SNNPR, located between 7° 58′ N to 8° 6′ N latitude and 37° 45′ E to 37° 56′ E longitude (Figure 1). Its altitude ranges from 800 to 3400 m.a.s.l with bi-modal rainfall distribution (short rain from January to April and the main rain from June to September) and an average annual rainfall of 1100.5 mm. The average minimum and maximum temperature are 13°C and 25°C, respectively (EEWFEDO, 2019). The woreda has a population of 168, 183 (49% men and 51% women), and over 85% of the people live in the rural area. The average population density was 200 per km^2. The total land area of the woreda is 107,584 hectares, of which *Woyna-Dega* (mid-highland) is 57.3%, *Kola* (low land) 26.88%, and *Dega* (highland) 15.82 %. The major soil types are clay (26%), sandy (16%), and silts (58%). Agriculture is the dominant economic activity in the woreda; crop production is the leading means of livelihood supplemented by livestock production. The major crops of the study area are enset (49.89%), coffee (23.01%), ch'at (6.2%), and fruit crops (6.05%). The dominant livestock types are cattle, sheep, poultry, and goat (EEWFEDO, 2018).

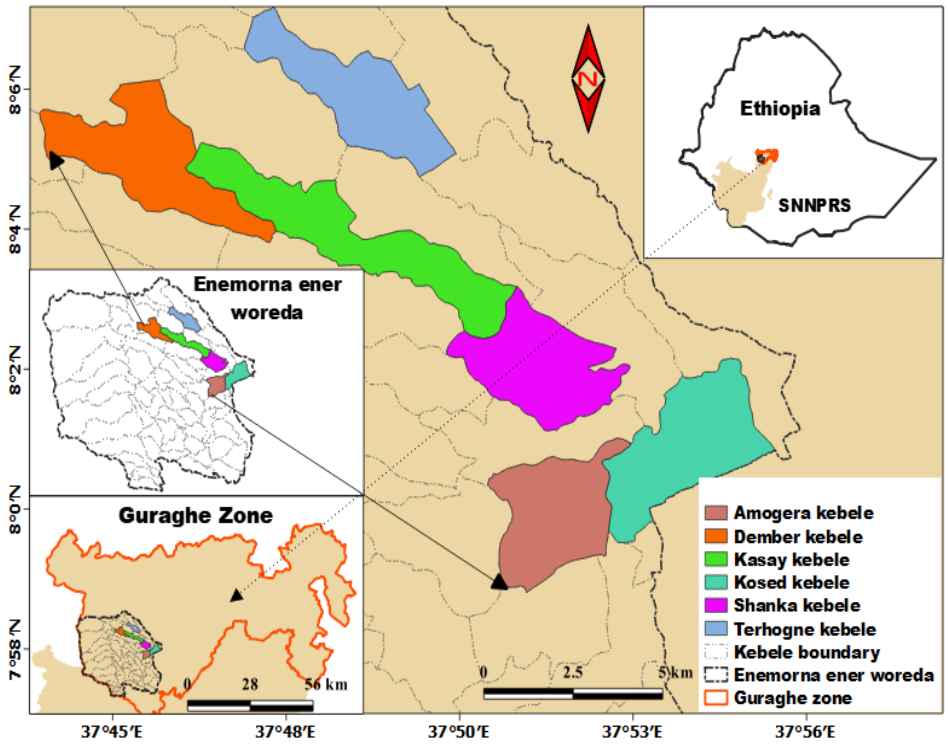

Figure 1. Location map of the study area.

Study sites and sample size

A reconnaissance survey was conducted from October 2019 to November 2019 in Enemorina Eaner woreda (district) of the Gurage zone. The woreda consists of 42 *Kebele*s (the lowest administration level in Ethiopia) that grow enset though they vary in the quantity of land size and landrace diversity. Six *Kebele*s were selected purposively based on enset cultivation potential and diverse agro-climatic zones (*Dega, Woyna-Dega*, and *Kola*). Two k*ebele*s were considered from each zone for the study.

The total sample size was determined through a stratified sampling technique, based on different agroclimatic zones and the socioeconomic background of the households. Accordingly, a total of 364 (287 males and 77 females) households (HH) were selected at 95% confidence level and acceptable sampling error (e=5%) using a simplified formula provided by Taro and Israel (1992). Three hundred thirty-four household respondents were selected using simple random sampling through the lottery method. However,

30 key informants' selection was done based on the snowball method with the help of knowledgeable farmers, *Kebele* leaders, and developmental agents.

Data collection

Data were collected through household interviews, key informants' interviews, focus group discussions, and field observation. From December 2019 to May 2020 364 household surveys were conducted. The use, challenges (such as pests and socioeconomic), and cultural management practices were recorded with the help of farmers. Since there was no clear and common identification technique for the identification of enset landraces (Elias, 2003; Maryo et al., 2018), this study utilized traditional method used by farmers. Farmers identify enset landraces just by looking at the colors of the midrib, petiole color, leaf color, leaf orientation and circumference, and length of pseudostem (Olango et al., 2014; Yemataw et al., 2014). The data collection was conducted primarily through individual interviews direct on-farm participatory observation, and key informant and focus group discussions. The participants were at the age range of 20-90 years old. Data about the wealth status of the farmers was collected with the help of key informants, *Kebele* leaders, and development agents based on the context of the local farmland size, the number of livestock owned, diversity of enset landraces, amount of crop production (enset, coffee, and ch'at area) and eucalyptus tree coverage.

Field observation was carried out to identify enset farmers' varieties, traditional management practices and challenges. Finally, five key informant groups with six members in each group and six focus groups with eight members in each group were consulted and the information was used to triangulate data collected from the households' interviews.

Data Analysis

Data were analyzed and summarized using descriptive statistics such as percentages, pie charts, and bar graphs using Microsoft excel 2010 and SPSS version 20.0 for ANOVA test. To analyze the enset diversity,

the Shannon-Weaver diversity (H') and Evenness measure (E) indices were employed following Magurran (2004). Accordingly, Shannon diversity index was calculated using the formula,

$$H'=-\sum_{i=1}^{S} Pi \ln Pi$$

where, H′ *is* the Shannon diversity index, pi is the proportion enset landraces composed of species and ln is a natural logarithm. Shannon evenness is calculated as the ratio of H' to maximum diversity (Hmax),

$$E = \frac{H\prime}{H'\max}, = \frac{H\prime}{lnS}$$

where, S= number of species and ln is a natural logarithm.

ANOVA test was conducted to examine any significant differences in enset farmers' varieties among farmers groups of different wealth status and the different agro-climatic zones.

RESULTS

Characteristics of the study group

Table 1 describes the characteristics of the study population and depicts that about half of the respondents lack the skill to read and write. About 79% of the respondents were male and the majority of the respondents (42%) belong to the 36-50 age groups, where > 96% were married. About 60% of the households (HHs) own a land ranging in size from 0.25 to1 hectare (ha). However, the enset planted area of most (>84%) of the respondents ranged between 0.25 and 1 ha. On the other hand, about 41% of the respondents confirmed that there were 5-10 enset landraces/HH.

Table 1. Characteristics of the study population.

Characteristics	Number of households	Percentage (%)
1. Age		
20-35	60	16.5
36–50	153	42.03
51–75	142	39.01
>75	9	2.5
2. Sex		
Male	287	78.9
Female	77	21.2
3. Educational status		
Cannot read and write	**173**	**47.5**
Read and write	83	22.8
Grade 1-4	26	7.1
Grade 5-8	68	18.7
Grade >9	*14*	*3.9*
4. Total land size in		
0.25-1	213	58.51
1_2	102	28.02
>2	49	13.45
5. Enset land size (ha)		
0.25-1	307	84.34
>1	57	15.66
6. No. of enset landraces		
< 5	98	26.92
5–10	148	40.65
11–15	81	22.25
>15	37	10.16

According to respondents, out of the land share of major crops, that of enset accounts for about 17%, followed by ch'at and coffee at 15 and 12%, respectively (Table 2). However, there is a tendency of the eucalyptus tree to occupy the greatest share of cropland, where about 24% of the study area of the cropland was occupied by eucalyptus tree, which is also considered as potential income generating plant in the area.

Table 2. Area share of enset Vs other agricultural crops and tree plantation.

Crop/tree type	Area in hectare	Percentage (%)
Enset	74.52	16.55
Wheat	19.75	4.39
Food barley	14.25	3.16
Teff	35.87	7.97
Maize	33.62	7.47
Potato	25.87	5.74
Avocado	14.54	3.23
Banana	0.403	0.09
Orange	0.146	0.03
Chat	67.79	15.05
Coffee	56.42	12.53
Eucalypts tree	107.15	23.79
Total	450.329	100

Diversity of enset farmers' varieties

In the current study, a total of 50 enset landraces were recorded based on farmers' local system of identification and classification (Annex I). The mean Shannon diversity (H') and evenness (E) indices were 2.61 and 0.78, respectively (Table 3). Table 3 also indicated that as enset land size increases, the enset landrace diversity and evenness also increases with altitude. Accordingly, high landrace richness and evenness were exhibited for Kosed *Kebele* (high altitude area) whereas landrace richness and evenness values were determined to be low at Dember *Kebele* (lower altitude area).

Table 3. The areas share of enset, mean number of enset landraces, Shannon (H') and the Evenness (E) values across the study *Kebeles.*

***Kebele*s**	**Altitude**	**Area share**	**Richness**	**Mean**	**Std.Dv**	**H'**	**Std.Dv**	**Evenness**
Kosed	2435	**0.35**	41(24.26)	**12.2**	4.51	**3.41**	0.074	**0.92**
Amogera	2290	0.20	32(18.93)	9.61	4.41	2.98	0.07	0.85
Shanka	2101	0.24	32(18.93)	5.56	2.84	2.79	0.081	0.8
Kasay	2018	0.23	37(21.89)	8.72	3.75	2.87	0.081	0.77
Dember	1762	0.1	15(8.88)	**3.98**	1.87	**1.77**	0.111	**0.64**
Terhogne	**1864**	0.08	12(7.1)	4.08	0.98	1.84	0.121	0.72
Average	2078	0.2	28.16	7.6	4.545	**2.61**	**0.089**	**0.78**

Note: Dega-agro climate =Kosed & Amogera, Woyna-Dega = Shanka & Kasay and Kola = Dember & Terhoghe

In *Dega* agro-climatic zones, higher numbers of enset landraces (mean=10.69 ± 0.365) were cultivated, while fewer enset landraces (mean=6.91) and (mean=4.04) were cultivated in *Woyna-Dega* and *Kola* agro-

climatic zones respectively. Based on the wealth-based grouping, a large number of enset landraces (11.72 ± 1.181) were obtained from the rich households, whereas it was the lowest (5.07 ± 0.295) among poor households (Table 4). However, the mean number of enset landraces in the study area was 7.6 ± 0.63. The number of enset landraces in each household exhibited significant difference (P<0.000) at 0.01 significant levels among agro-climatic zones as well as wealth groups (Table 5).

Table 4. Wealth status ranking of the study HHs across the three agroclimatic zones (N = 364).

Wealth Status	**Farmland Area (ha)**	**Enset Area (ha)**	**Coffee area (ha)**	**Ch'at Area (ha)**	**Eucalypts Tree area (ha)**	**No. of live stock**	**No. of enset farmers' varieties**			**mean**	**StD**
							Dega	***Woyna-Dega***	***Kola***		
Poor	0.711	0.079	0.083	0.069	0.171	1.717	9.4	3.99	3.91	5.07	0.295
Medium	1.276	0.241	0.167	0.207	0.267	3.577	10.59	7.18	4.09	8.18	0.4
Rich	2.939	0.351	0.318	0.442	1.04	10.957	12.43	8.67	6.00	11.72	1.18
Mean	1.237	0.204	0.154	0.186	0.294	3.57	**10.69**	**6.91**	**4.04**	**7.6**	**0.63**

The tropical livestock unit (TLU) is commonly taken to be an animal of 250 kg live weight. TLU conversion factors constitute a compromise between different common practices. 1 TLU = 250kg. Accordingly, Bull = 1.1, calves = 0.2, Chickens = 0.01, Cows (cross) = 1.2, Cows (local) = 0.8, Donkeys = 0.5, Goats/ sheep = 0.1, Heifers = 0.5, Horses/mule = 0.8, and Immature males 0.6.

Table 5. The diversity of enset landraces among agro-climatic zones and wealth groups.

Source of variation of enset farmers' varieties	Sum of Squares	df	Mean Square	F-value	P-value
Between agro-climatic areas	2741.393	2	1370.697	112.218	0.000
Within agro-climatic areas	4409.453	361	12.215		
Total agro-climatic areas	7150.846	363			
Between wealth groups	1138.005	2	569.003	34.162	0.000
Within wealth groups	6012.841	361	16.656		
Total wealth groups	7150.846	363			

Frequency of the distribution of dominant enset landraces across the agro-climatic zones

The study showed that there were many differences between enset landrace distributions across agro-climatic zones. In *Dega* agro-climatic zones the widely distributed enset farmers' varieties were *Agade*, *Nechiwe*, *Amerad*, *Lemare*, *Quashqashiye*, *Guarye* and *Sapara*, while in *Woyna-Dega* agro-climatic zones the widely distributed enset landraces were *Amerad*, *Sapara*, and *Eshirafriye*. In *Kola* agro-climatic zone

Eshirafriye and *Badaded* were widely distributed. The most cultivated enset farmers' varieties in the study areas are *Agade, Nechiwe, Eshirafriye,* and *Amerada* (Table 6). According to the respondents the wider availability of the aforementioned enset landraces in the study areas is due to their resistance to disease and drought.

Table 6. Most widely cultivated enset landraces /farmers' varieties in Enemorina Eaner woreda.

No.	Landrace Name	No. of respondents (N=364)	Percentage (%)	Distribution of enset landraces		
				Dega	*Woyna-*	*Kola*
1	*Agade*	245	67.3	√√√	√√	√
2	*Nechiwe*	224	61.5	√√√	√√	√√
3	*Eshirafriye*	200	54.9	√	√√√	√√√
4	*Amerad*	194	53.3	√√√	√√√	√
5	*Sapara*	186	51.1	√√√	√√√	√
6	*Lemare*	172	47.25	√√√	√√	√
7	*Bazeriye*	156	42.9	√√	√√	√
8	*Guarye*	142	39.01	√√√	√√	√
9	*Quashiquashiye*	142	39.01	√√√	√	-
10	*Badaded*	129	35.43	√√	√√	√√√

√√√= high frequency; √√ = average frequency, and √= low frequency of landrace distribution across the agroclimatic area.

Uses of enset by local communities

The present survey's result showed that enset crop is used for food, feed, fiber production, and medicinal uses. The major food products of enset are *kocho* and *bulla,* and the quality of these products varied from landrace to landrace. For instance, enset landraces selected for *Kocho* in the order of quality were *Amerad, Eshirafriye, Bazeriye, Nechiwe, Agade, Gezwed, Fereziye, Shertiye, Buaeche, Keswe, Anikofiye, Mishrad, Eweredіye, Bosere, Zobir,* and *Yiregye*. About fifty percent of the respondents reported that *Amerad* is the best quality enset landrace for *Kocho* production, of which, 40% of the respondents reported *Agade* as the best enset landrace for high *Kocho* yield in the study area. Enset landraces selected for *Bulla* production in their order of preference include *Lemare, Gimbuwe, Gumbura, Badaded, Yiregye, Nechiwe,* and *Bazeriye*. Among these, 29% of the respondents indicated *Lemare* as the best enset farmers' variety for *Bulla* production across the three agro-climatic zones.

Enset landraces preferred for *Amicho* production in their order of importance were *Guarye, Kibnar, Egendiye, Astara, Gimbuwee, Bazeriye, Agade, Gezwed, Yiregye, Quashqashiye, Dereeye, Agorgurkanchuwe, Tereriye, Edemerti, Enba, Tedrader, Abakita, Tegaded,* and *Wonadiye*. According to the survey result, about 38% of respondents indicated *Guarye* as the best quality enset landrace for *Amicho* production. Most respondents (60%) showed the enset landraces *Agade, Eaneragade, Eshirafrye, Eweredіye, Fereziye, Guarye, Keswe, Kibnar, Kinbat, Leamare, Ousmair, Quashqashiye, Tedrader, Tereriye, Wonade,* and *Yiregye* were preferred by growers for fiber production.

Traditional medicinal use of enset

The present study revealed that different enset parts such as a corm, pseudostem, and leaf are used by growers for traditional medicinal purposes to treat human and livestock ailments. Accordingly, 11 enset landraces were reported for their traditional medicinal values (Table 7).

Table 7. Enset farmers' varieties used for traditional medicinal value.

Ailment type	Name of enset farmers' varieties	No. of respondents	Part used	Used for	
				Human	Livestock
Healing Bone fracture	*Astara,*	101	Corm	The corm is cooked and feed with milk	Feed raw
	Agade	87			
	Kibnar	102			
	Gaurye	142			
	Sapara	67			
	Tereriye	30			
Initiate milk production	*Astara*	69			
Wound healing	*Dereye*	44			
Hepatitis	*Edemert*	17			
For healing the abscess	*Tegadedi*	12			
Remove specific implanted foreign body	*Quashiquashiye*	141			
Antiparasitic effect	*Badaded*	84	All part	Not common	Feed raw

Five landraces (the corm) were used to treat bone fracture both in humans and his livestock. The corm of different landraces was mentioned to treat wound, hepatitis, and abscess (one for each case) both in human

and livestock. Two landraces (all plant parts) were mentioned to treat pathogens both in human and livestock whereas one enset landrace (the corm) was reported to initiate milk production in livestock.

Challenges associated with enset cultivation and maintaining landrace diversity

From the interactions made with farmers to obtain information on the cultivation status of enset and the number of landraces maintained at farm and landscape level, it was found that both the production of enset and the diversity of enset varieties showed a declining trend. Respondents described that wild pest, diseases, focus on short period growing cash crops, labor shortage, lack of modern technology for processing enset, climate change, and land scarcity as major challenges that influence the production of enset and growing diverse varietal forms in the study area. In terms of magnitude, the impact of wild animals is mentioned by the highest proportion of respondents (40%, n= 364) and that of various diseases, cited by the second highest proportion (30%, n= 364), indicating that these two factors are the leading challenges of enset diversity and its production in the study area (Figure 2).

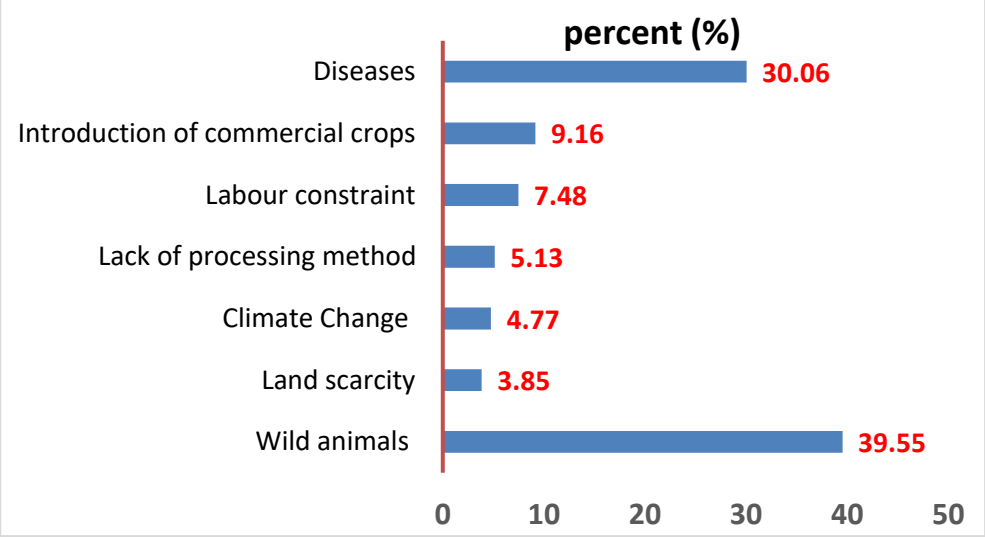

Figure 2. Major challenges of enset production in Enemorina Eaner woreda.

Among the enset pests and diseases, porcupine (30.85%), warthog (25.16%), enset bacterial wilt (18.67%), corm rot, locally called *Bure* (8.07%), wilting due to fungal and insects (9.65%) and zi-ire (sheath rot by fungi) (7.59%) were reported as major problems in the study areas. The majority of respondents (31%)

confirmed that porcupine causes a great loss to enset landraces (Figure 3), that are highly preferred for edible corm (e.g., *Sapara* and *Astara*) and landraces that have traditional medicinal values (e.g., Astara). As a result, farmers are forced to abandon the production of enset landraces that are socioeconomically important and highly susceptible to diverse diseases and pests. Warthog, and porcupine, from animal pest category, and enset bacterial wilt, corm rot (the mealybug), and *Ziire* (sheet rot), form the list of diseases which were described as problematic which pose a serious threat to the sustainability of enset landrace diversity. Enset bacterial wilt was reported as a major constraint in *Dega* agro-climatic zones as it was cited by a fair proportion of respondents (19%, n=364) while in *Woyna-Dega* and *Kolla* agro-climatic zones porcupine was mentioned to be a major challenge for enset production by about one-third of the respondents (31%, n= 364). Some enset landraces such as *Eshirafriye*, *Nechiwe*, *Lemare*, *Gumbura*, *Bazerye*, *Badaded*, and *Gezwod* were found to show either recovery after infection or were less affected by the disease.

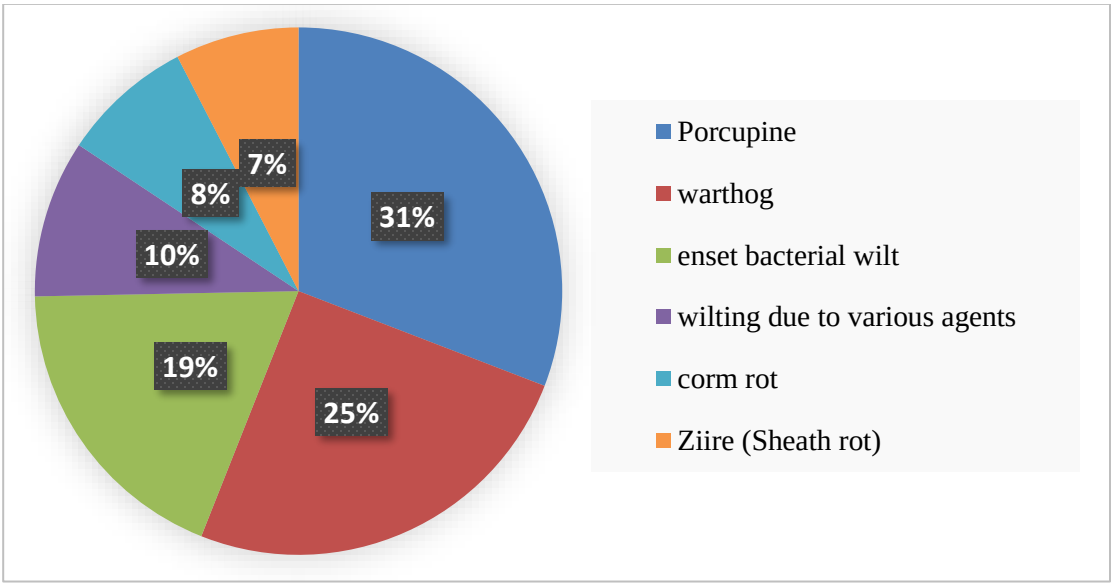

Figure 3. Major enset diseases and pest in the study area.

Local knowledge on the cultivation of enset and management of its diversity

The respondents reported that they differentiate enset landraces based on the use value (corm edibility, fiber, *Kocho* and bulla quality, etc.), color (leaf/petiole, midrib, and pseudostem), disease resistance, and fermentability. Nonetheless, the color of pseudostem was the dominant descriptor for identification. Almost

all informants agreed that planting material is obtained mainly from the three years old mother by distracting of the apical meristem of the corm. Enset is grown dominantly in the home garden. However, the local people intercrop enset crop with maize, coffee, cabbage, and other crops. Females' attachment to enset production is greater than the male's since they know the production nature of each enset landraces, the test of *Kocho* and *bulla*, fermentability, and the like. Accordingly, females are more knowledgeable and able to describe landraces better than males. About 73% of the respondents reported that they plow land three times before planting. The land preparation usually starts around October to December. Enset is propagated vegetatively using a corm of an immature plant. From December to March, farmers uproot 3 to 4 years old enset plant and cut off the pseudostem, remove the central growing bud, and rebury it covering with organic matter-rich porous soil (pit) so as to encourage the emergence of more than one sucker from the mother plant (after two to three months). Farmers in the study area transplant enset suckers (*Boshe*) from two to three times until their permanent fields are occupied. It is allowed to grow for one year, and then transplanted to the next stage called *Hiniba.* At this stage, the sucker is separated and planted in the individual hole called *Bekir* and allowed to grow for one to two years. Then, the most vigorous suckers will be transplanted into a permanent field as *Esed.*

Farmers practiced weeding by hand or with a sickle more frequently in earlier growth stages or during the rainy seasons (May-October). They used manure to fertilize the enset fields. Farmers use different local protection practices against pests and diseases to maintain the diversity, production, and health of enset landraces. These include fencing around the crop field, chasing the pest in a group of farmers organized at village level, capturing and killing the animal pest using traps, filling porcupine holes by soil, and sprinkling manure (usually the semi-solid form) around the enset stand.

DISCUSSION

The diversity and distribution of enset landraces

Farmers in the study area maintain a diverse range of enset local varieties on their home gardens for numerous reasons, mainly based on yield and quality of the products *Kocho, Bulla,* and *Amicho* and also for their traditional medicinal value, fiber quality, disease resistance, and low preference by wild animals. Since this finding is in agreement with previous reports (Olango et al.,2014; Yemataw et al., 2014; Maryo et al., 2018) it could be argued that the rational for maintenance of enset diversity by farmers across the country is related to its various use values.

Regarding intraspecific diversity, 50 different enset landraces were identified of which, the dominant landraces such as *Agade, Nechiwe, Eshirafriye, Ameradi, Sapara, Quashiquashiye,* and *Badaded* were widely distributed across-the agro-climatic regions. The dominance of these landraces across the agroclimatic areas of Gurage Zone was also reported by Mojo (2017), and such dominance could be linked with their resistance to disease and adaptation to local environmental situations. A similar study in Kembatta Tembaro zone (Maryo et al., 2018) also revealed the dominance and abundance of some enset landraces across agroclimatic areas; and this was interpreted in terms of the local varieties' resistance to various environmental conditions, including pests and diseases.

Comparison of the level of landrace richness of the study area (50) with other enset growing areas in SNNP showed that the diversity status is comparable with that reported for Aleta Chuko woreda in Sidama Zone (Seifu and Fitamo, 2016) and Offa woreda in Wolaita Zone (Shumbulo et al., 2012) since an equivalent number of landraces (55) was reported from each site. On the other hand, a lower number of landrace richness was reported from other studies; i.e. 42 landraces from Keffa Zone (Tsehaye and Kebebew, 2006), 42 landraces from Sidama zone (Abebe et al., 2010), and 33 landraces from three woredas of Gurage Zone (Mojo, 2017). This could be associated with the tradition of enset use for various purposes as well as its management practices and the variation of sample size among those studies. Furthermore, there were former

reports with high landraces diversity than the present study, and these include studies that yielded 67 landraces from Wolaita zone (Olango et al., 2014); 312 from Dawro, Gedeo, Gurage, Hadiya, Kembatta Tembaro, Sidama, Silte and Wolaita zones (Yemataw, et al., 2016); 111 from Kembatta Tembaro Zone (Maryo et al., 2018), and 93 from Yem special woreda (Zerfu et al., 2018). The variation in landrace diversity of enset among different localities might be associated with the variations in geographical locations, area coverage, and the sociocultural aspects of the local people.

In the present study, the number of enset landraces recorded ranged from 2 to 33/HH with the mean value determined being 7.6 ± 0.24. Accordingly, the maximum number of enset landraces per farm recorded from Kosed *Kebele* was 12, whereas the maximum altitude recorded was 2435 m.a.s.l. Furthermore, this *Kebele* exhibited the highest Shannon diversity (H'=3.4) and evenness (E=0.92) values showing the presence of a large diversity of enset landraces in this *kebele*. On the other hand, minimum number of landraces per farm (mean=3.98) was recorded from Dember *Kebele* with altitude of 1762 m.a.s.l, which exhibited the lowest landrace diversity (H'=1.77) and evenness (E=0.64) values, suggesting few numbers of enset farmers' varieties in this *Kebele*. The cultivation of fewer enset landraces in this *kebele* might be associated with the adaptation of the limited landraces to the environmental setting as well as low sociocultural dependence of the community on enset as a staple food in the lowland setting which favors cereal production (Maryo et al., 2018). In general, the mean values of enset Diversity (H') and Evenness (E) were found to be 2.61 and 0.78, respectively, and this is higher than previously reported (H'= 1.84 & E= 0.64) by Maryo et al (2018) from a study on Kembatta Tembaro Zone. The finding in this study therefore suggested a greater tendency by farmers to maintain diverse enset varieties with a fair abundance of each type.

As indicated in the result section, a significant difference in the diversity of enset landraces exists among agroclimatic areas ($P<0.001$). Furthermore, the result revealed a decreasing trend in the diversity of landraces as one goes from higher elevation to lower elevation areas. Therefore, the trend in the number of enset landraces across agro-climatic zones follows a similar pattern with what Maryo et al. (2018) reported

from an earlier study. The possible reason for this variation could be due to the differences in agro-climatic conditions in terms of temperature, moisture, soil fertility, cultural background, population pressure, and household assets (Tsegaye, 2002; Zeberga et al., 2014).

A highly significant difference ($P<0.001$), in the diversity of enset landraces among the wealth classes where the wealthy farmers grow twofold diverse enset varieties than that of the poor farmers was also observed. This finding is in agreement with a previous report by Tsegaye (2002) that stated the cultivation of enset on a large area is a sign of wealth among the community. The present study revealed that poor households possess a small plot of land and few livestock resources as a source of manures to fertilize their enset farms resulting in low landrace diversity in such farms. Similar findings have been reported by Shumbulo et al. (2012). Besides their low landholding size and lack of farm animals, poor farmers of the area are partly hired to serve as daily laborers on the farms of wealthy ones to fulfill their daily needs, and this might have favored the growing of diverse landraces by wealthy farmers as reported by Negash (2001) and (Jacobsen et al., 2018).

Uses of enset landrace

Enset is a multipurpose crop used as food for humans, fodder for livestock, traditional medicinal values, and fiber for the house construction material among others. The findings of this study agree with previous reports (Shigeta, 1990; Tsegaye and Struik, 2002; Olango et al., 2014; Maryo et al., 2018). The present study identified 11 enset landraces reported to have medicinal uses which is the largest reported so far from Gurage zone. In earlier studies from the zone, six landraces from Gedebano, Gutazer, Welene woredas (Nudego, 2016), and eight landraces from Kebena, Cheha, and Ezha woredas (Mojo, 2017) were reported to have medicinal uses. However, the reported enset landraces with medicinal values from Gurage zone is noticeably lower than the number of medicinal enset landraces (21) reported from Kembatta Tembaro zone (Maryo et al., 2018).

Challenges associated with enset production

The present study showed that major challenges for the diversity and production of enset in the study are wild animals and diseases. Porcupine is the leading enset pest followed by warthog. Porcupine causes the greatest damage on those enset farmers' varieties such as, *Astara*, *Kibnar*, and *Guarye*, that are highly preferable for their traditional medicinal values and edible corm. Similar results were reported by Negash (2001), Zeberga et al. (2014), Bayu (2016), Nudego (2016) and Maryo et al. (2018). Due to the animal pest attack related impact, and also climate change-related factors like drought, the number of enset landraces which are used for traditional medicine and edible corm were reported to have been degraded or lost. However, the various traditional methods which farmers use to prevent crop damage (fencing, filling holes, application of manure, collective hunting) have helped to reduce the impact. Similar methods are practiced in other parts of the country such as the Gamo highlands (Bayu, 2016), and Kembatta Tembaro Zone Maryo et al. (2018).

As the study put forward, enset disease, mainly enset bacterial wilt, is also the major challenge in the study area. The problem is more common in *Dega* (highland) areas (Amogera and Kosed *Kebele*s) while in low land areas (Terhogne and Dember *Kebele*s) the impact of enset bacterial wilt was reported to be minimum. This finding is in agreement with a previous report (Maryo et al, 2018) which stated that enset bacterial wilt infestation is high in relatively humid environment (high moisture) and low-temperature conditions. Additionally, respondent farmers confirmed that the impact of enset bacterial wilt disease is high during the rainy season than the dry season.

An additional threat to enset cultivation verified through the study is the competition for space by Eucalyptus, which is considered as a fast-growing commercial crop by many farming families. It has got attention and a relatively larger agricultural land allocation. This is a big threat to the sustainability of enset production in the study area. This study showed that enset makes only 17% of the crop land share in the area. A previous report however showed that the share of enset cover out of the total agricultural cropland

was about 50% in the study *Woreda* (EEWFEDO, 2019). The shrinkage in enset land, is assumed to have occurred due to the replacement of agricultural land by other fast-growing and cash-generating crops, including Eucalyptus and *Ch'at.*

Indigenous management of enset Diversity

Farmers identify enset farmers' varieties by employing indigenous phenotypic characterization. In this study, it was also noted that landraces are named by local people based on the origin, a location from which the farmers' variety was obtained. This traditional knowledge of identification is similar to the method of identification reported by Yemataw et al. (2014) in Wolaita, Kembatta, Hadiya, Sidama, Gamo Gofa, Gurage, and Dawro Zones; Olango et al. (2014) in Wolaita Zone, and Seifu and Fitamo (2016) in Sidama Zone. Farmers in the study area cultivate enset at their home gardens, which is located around the home where livestock and household members live together. Enset is used as fodder for cattle, and the livestock provides manure that is used to fertilize the enset plantation. A similar result was reported from Wolaita (Olango et al., 2014). Enset is grown as a sole crop or intercropped with other root crops, maize, and coffee (Garedew et al., 2017). The crop is reproduced vegetatively using the corm of a juvenile plant. Farmers in the study area transplant enset suckers from two to three times until they get permanent field. The finding is in agreement with previous reports of Mojo (2017) from Gurage Zone and Negash (2001) from Keffa Sheka Zone. Enset needs a series of follow up after planting. Maintaining sanitation, weeding, and application of manure are common practices done by the farmers as it was described in earlier works by Negash (2001) and Bayu (2016).

CONCLUSION AND RECOMMENDATION

The present study helped to assess the status of enset cultivation, its diversity, and the challenges encountered by local farmers. The study enabled the recording of 50 enset landraces that are differentiated and recognized by farmers via phenotypic characterization. The enset landrace richness, diversity, and evenness varied significantly across agro-climatic zones and wealth groups. Accordingly, it was verified

that *Dega* agroclimatic areas and wealthy classes in the community possessed higher number enset varieties. The distributional difference of enset landraces increases among agroclimatic zones is attributed to environmental variables as well as sociocultural aspects. The cultivation of enset in the study area and its intraspecific diversity is challenged by several factors including the attack by animal pests, diseases, the introduction and expansion crops with immediate economic benefit, labor shortage, lack of modern processing technology, climate change, and scarcity of land for the ever-increasing human population. Despite local efforts made to control or reduce losses caused by animal pests, the overall outcome is far from satisfactory. The rapid expansion of eucalyptus as a commercial crop will likely lead to its dominance on the enset agricultural landscape and this, in turn, will end up in a significant reduction of the area cover share of enset, threatening the sustainability of its production and diversity.

In the study area, local community members grow diverse landraces to meet objectives such as diversifying produce and thereby ensuring food and nutritional security, maintaining varieties used for medicine, and minimizing the risk of total yield loss. Although enset has been taking the greatest share of land in the local production system, a multitude of crops (e.g., cereals, legumes, tuber/root crops, vegetables, spices, and coffee) are grown alongside it. This concomitant cultivation of crops where enset forms the backbone of the production system has allowed locals to lead a sustainable living within a more or less stable environment. The present study, however, revealed that changes in the local production system, in general, and that of enset cultivation and diversity, in particular, have been occurring with a potential impact on the livelihood of local people and their environment. It is, therefore, recommended that all concerned bodies should consider and take appropriate measures that range from undertaking further research to implementing conservation interventions on the ground. Furthermore, it is advisable to develop an appropriate land use policy that aids in not converting enset land into other forms. Similarly, farmers need the means to build their capacity in areas of pest control and enset disease prevention to encourage them to grow diverse enset landraces.

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Annex I: **Diversity of enset landrace along the three agro-cimatic areas in Enemorina Eaner Woreda.**

No	Local name of landrace	Dega	Woinadega	Kolla	Total
		Frequency (Percent)			
1	*Abakita*	3(0.1)	13(0.4)	0	16
2	*Agade*	121(3.9)	87(2.8)	37(1.2)	245
3	*AgorgurKanchuwe*	1(0.001)	36(1.1)	0	37
4	*Amerad*	83(2.6)	87(2.8)	23(0.7)	193
5	*Anikofiye*	28(0.9)	0	0	28
6	*Astara*	61(1.9)	40(1.3)	0	101
7	*Badaded*	37(1.2)	29(0.9)	63(2)	129
8	*Bazeriye*	84(2.7)	71(2.3)	1(0.0001)	156
9	*Bosere*	43(1.4)	8(0.3)	0	51
10	*Buaeche*	19(0.6)	1(0.0001)	0	20
11	*Chohedye*	0	1(0.001)	0	1
12	*Dereeye*	37(1.2)	7(0.2)	0	44
13	*Eaner Agade*	0	1(0.001)	0	1
14	*Edemerti*	4(0.1)	9(0.3)	4(0.1)	17
15	*Egendiye*	43(1.4)	44(1.4)	20(0.6)	87
16	*Emiriye*	0	1(0.0001)	7(0.2)	8
17	*Enba*	2(0.1)	5(0.2)	8(0.3)	15
18	*Eshirafrye*	58(1.8)	76(2.4)	66(2.1)	200
19	*Eweredіye*	14(0.4)	20(0.6)	10(0.3)	44
20	*EzerBadadedit*	3(0.1)	3(0.1)	0	6
21	*Fereziye*	42(1.3)	0	0	42
22	*GebenaBadadede*	4(0.1)	2(0.1)	0	6
23	*Gebenaesed*	0	2(0.1)	0	2
24	*Gezwed*	54(1.7)	28(0.9)	0	82
25	*Gimbuwee*	60(1.9)	34(1.1)	0	94
26	*Guarye*	84(2.7)	49(1.6)	9(0.3)	142
27	*GudKanchuwe*	2(0.1)	1(0.0001)	0	3
28	*Gumbura*	84(2.7)	30(1)	9(0.3)	123
29	*Kembeto*	2(0.1)	0	0	2
30	*Keswe*	8(0.3)	20(0.6)	0	28
31	*Kibnar*	65(2.1)	31(1)	6(0.6)	102
32	*Kinbat*	1(0.0001)	0	0	1
33	*Lemare*	97(3.1)	73(2.3)	2(0.1)	172
34	*Mishrad*	19(0.6)	5(0.2)	1(0.0001)	25
35	*MoherKanchuwe*	1(0.0001)	4(0.1)	0	5
36	*Muyed*	2(0.1)	5(0.2)	0	7
37	*Natasibr*	0	1(0.0001)	0	1
38	*Nechiwe*	85(2.7)	84(2.7)	55(1.8)	224
39	*Ousmair*	0	2(0.1)	45(1.4)	47
40	*Quashqashiye*	96(3.1)	45(1.4)	0	141
41	*Sapara*	97(3.1)	84(2.7)	5(0.2)	186
42	*Shertiye*	26(0.8)	6(0.2)	0	32

No	Local name of landrace	Dega	Woinadega	Kolla	Total
		Frequency (Percent)			
43	*Tedrader*	14(0.4)	0	0	14
44	*Tegaded*	16(0.5)	0	0	16
45	*Tereriye*	37(1.2)	0	0	37
46	*Wonadiye*	24(0.8)	0	0	24
47	*Yirengye*	24(0.8)	0	47(1.5)	71
48	*Zewiyred*	4(0.1)	1(0.0001)	0	5
49	*Zobir*	36(1.1)	20(0.6)	0	56
50	*Zogired*	18(0.6)	6(0.2)	0	24

INDIGENOUS FORAGE SPECIES COMPOSITION, BIOMASS YIELD AND THEIR NUTRITIONAL QUALITY ACROSS GRAZING TYPES, IN NORTH SHEWA ZONE, ETHIOPIA

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ABSTRACT: This study was undertaken in Wachale district of North Shewa Zone with the aim to assess species composition, aboveground biomass production and nutritive values of indigenous forage species across three grazing types. Six free grazing, four controlled grazing and four enclosures areas were selected purposively from three kebeles. One transect with a length of 100 meters was laid on each management type from which forage samples were collected at every 25 m interval. Five plots of size 0.5m x 0.5m each were assigned along each transect. A total of 70 plots (30 plots for free grazing, 20 plots for controlled grazing and 20 plots for enclosure areas) were used throughout the study. A total of 16 indigenous herbaceous forage species were identified of which 11, 13 and 16 were found from free grazing, controlled grazing and enclosure areas, respectively. The highest relative frequency was obtained for *Andropogon abyssinicus*, 21.23% from free grazing, 20.82% from controlled grazing and 19% from enclosure. Dry matter yield was the highest ($P<0.001$) in enclosure followed by controlled grazing. The metabolizable energy (6.64 MJ/DM) and in vitro digestibility (44.29%) of *Sporobolus africanus* were lower ($P<0.05$) than *Andropogon abyssinicus, Pennisetum thunbergii, Eleusine floccifolia* and *Cyperus rotundus* values. The indigenous forage species in the study area were found to be poor in terms of diversity, composition yield and quality. It can be concluded that enclosures could be considered as better grazing management options in terms of maintaining species diversity, and dry matter yield.

Keywords: Dry matter yield, Grazing management types, Indigenous forage, Species composition

INTRODUCTION

Grasslands are found in all climatic zones except high mountains, extremely arid zones and the polar regions of the earth (Hedberg et al., 1995; Faber-Langendoen and Josse, 2010). The majority of grasslands are located in tropical developing countries where they are particularly important to the livelihoods of billions of poor people. Grasslands have many biodiversity values as they possess high species richness and provide

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numerous ecosystem functions and services (Faber-Langendoen and Josse, 2010). Grasslands provide feed for wild and domestic animals and they also play vital role in nonagricultural services, such as water supply, carbon storage, climate mitigation and offer natural habitats for both common and threatened species (Boval and Dixon, 2012; Bengtsson et al., 2019).

The grassland region of Ethiopia is found extensively in the central plateau, western, southern and southeastern semi-arid lowlands of the country (Hedberg et al., 1995; Mengistu, 2006). There are different types of grasslands that are used for livestock grazing in the highlands of Ethiopia. These include privately and communally owned, enclosures, riverside, lakeshore, and roadside grazing areas (Zewdu, 2005). The grassland harbors major feed resources in most of the highlands of Ethiopia (Keba et al., 2013; Yadessa et al., 2016; Abebaye et al., 2019), they are rich in indigenous forage species and mainly constitute grasses and various forb and shrub species (Kahurananga, 1986). According to CSA (2021) report, natural grazing land and hay accounts for 54.54% and 7.35% of the total feed utilized in Ethiopia, respectively.

However, the natural grazing lands in the highlands of Ethiopia are seriously overloaded with stocks beyond their optimum carrying capacity causing overgrazing, erosion and overall land degradation (Tolera and Abebe, 2007; Feyissa et al., 2015). Moreover, the current management and utilization of grazing lands have caused a reduction in biodiversity and the gradual replacement of better-quality indigenous forage species with unpalatable species; and caused rapid rates of genetic erosion on rare and endemic forage species and hampered germplasm exploration, collection, and conservation activities (Mengistu, 2004).

North Shoa zone of Oromia Regional State is one of the highlands of Ethiopia that occupies the central part of the country. The dominant feed resource for cattle in this area is natural pasture from communal grazing lands and enclosures which accounts for 49.8% of the basal feed (Brandsma et al., 2013). According to Feyissa et al. (2014), out of the total land owned and contracted in the area, 25% was used for hay production from indigenous and endemic forage species. From this area, substantial amount of hay was made available

for sale each year and transported to other places. It was also reported that hay prices have increased significantly from year to year.

To conserve and sustain the present forage diversity in the natural grasslands, substantial identification of the driving factors and evaluation of their effects must be conducted. Therefore, assessment of the status of grassland resources is the key to putting in place a strategic plan for appropriate utilization of grassland ecosystem of the area, as there were gaps on the knowledge of indigenous forage diversity and the effects of grazing management of the district. The objective of the study was to assess endemic and indigenous forage species for their species composition, above ground biomass yield across grazing management types and their nutritional quality in the natural grassland of Wachale woreda of North Shewa zone.

MATERIALS AND METHODS

Study area

The study was conducted in Wachale woreda of North Shewa, Oromia Regional State, Ethiopia, which is located at 84 km North West of Addis Ababa (Figure 1). The district (woreda) is located between 9°25'2.13" to 9°48'44" North and 38°38'49.02" to 39°08'41" East. The altitude of the district ranges between 1200 and 2880 m.a.s.l. The mean annual rainfall of the area is about 1000 mm that ranges from 1000 to 1800 mm. The maximum and minimum annual temperature is 30^0C and 25^0C, respectively. In this district, livestock production is the most important agricultural activity next to crop production.

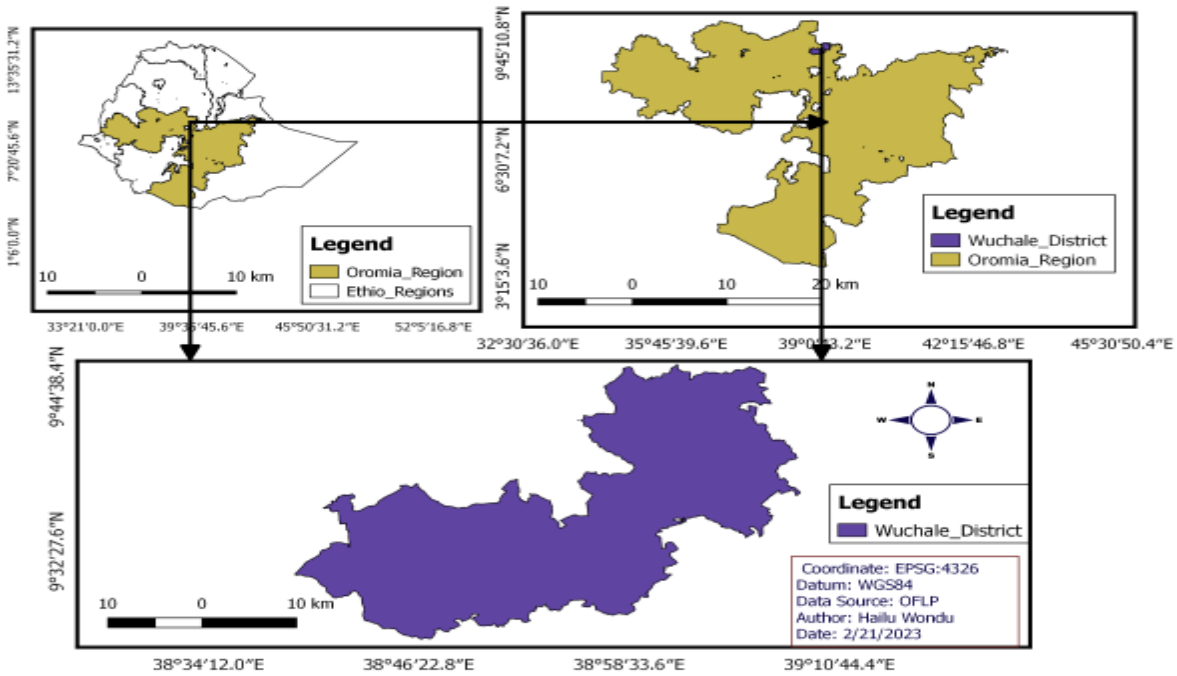

Figure 1. Maps showing the study area.

Sampling procedures

Sampling was conducted once both in wet and dry seasons at end of October and February, respectively. The study focused on communal, private free grazing, controlled grazing and enclosure grazing lands and comparing to each other with respect to herbaceous biomass production and nutritional qualities of the forage species. Kebeles were selected on their potential grazing types (free grazing, controlled grazing and enclosures). Thus, six free grazing, four controlled grazing and four enclosure areas were selected purposively from three kebeles: namely, Bosoke jate, Gora keteba and Wachala worto following discussions with district experts and knowledgeable community representatives.

One transect with a length of 100 meters was constructed on each grazing management and the forage samples were collected at every 25 m interval using 0.5m x 0.5m plots. Systematic sampling was used for the study (Kenkel et al., 1989). A total of 70 plots (30 plots for free grazing, 20 plots for controlled grazing and 20 plots for enclosure) were used throughout the study. The forage samples found inside the quadrats were clipped using a sickle at above 5 cm height. In each study quadrant, knowledgeable people were

consulted to identify the local name of each herbaceous forage species. They were identified in the field and specimens were collected, pressed and dried properly using plant presses and transported to the herbarium of Ethiopian Biodiversity Institute for further identification and nomenclature. Nomenclature of the forage species followed the Flora of Ethiopia and Eritrea (Hedberg et al., 1995).

Thereafter, samples were hand-separated into different species, labeled, shade dried and then fresh weight of forage samples were measured in the field and kept in paper bags. Both fresh and dried weights were measured in grams using an electronic kitchen scale of 5000 g weighing capacity. Samples were subjected to air dry until transportation for laboratory analysis.

Dry matter (DM) weights obtained from sample sites, the percent composition of each species of grasses, legumes, Cyperus and Sedge, and forbs of herbaceous species for each quadrant were calculated and the total biomass production capacities of the area were obtained following Tothil et al. (1978), as cited in Ayele et al. (2022).

$$\text{TDW of individual species} = \text{TFW of a species} \times \text{SDW of species x SFW of a species}$$

$$\%\ \text{Composition of each species at a site} = \frac{\text{TDW of species x 100}}{\text{GTDW of all the species}}$$

Where TDW is total dry weight, TFW is the total fresh weight of individual species, SDW is sub-sample dry weight, SFW is sub-sample fresh weight, and GTDW is the total dry weight of all species.

Dry matter yield (DMY) and crude protein yield (CPY) were calculated according to Mengistu and Mekasha, (2007).

$$\text{Dry matter yield (t/ha} = \frac{\text{Green forage yield (t/ha) X dry matter content (\%)}}{100}$$

$$\text{Crude protein yield} = \frac{\text{Dry matter yield } \left(\frac{\text{t}}{\text{ha}}\right) \text{X crude protein (\%)}}{100}$$

Laboratory analysis

The samples were analyzed using Near Infrared Reflectance Spectroscopy (NIRS) at the Animal Nutrition laboratory of the International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia. The NIRS instrument, Foss Forage Analyzer 5000 with the software package WinISI II in the 1108-2492 nm spectra range was used to scan dry forage samples and a good-of-fitness NIRS equation was used for the prediction of dry matter (DM), total ash (Ash), nitrogen, neutral detergent fiber (NDF), acid detergent fiber, metabolizable energy (ME) and in vitro digestibility (IVOMD).

Statistical analysis

The proportion of the different forage species were calculated using percentage. The data obtained from the dry matter production were subjected to ANOVA using the General Linear Model procedure of Statistical Analytical System (SAS) computer software (Wicklin, 2010). Grazing management and species were considered independent variables. Grass species' nutritive values, crude protein yields, dry matter yield, fresh weight and their dry weight were considered response variables. General Linear Model procedure of statistical analysis system (SAS) version 9.1 (Wicklin, 2010) was used to conduct statistical analysis. Duncan was used to determine mean differences at $P \leq 0.05$.

The model used was; $Yijk = \mu + Ai + Bj + (AB)ij + eij$

Where Y is the response variable,

μ is the overall mean,

Ai is the forage species effect,

Bj is the grazing management effect,

(AB)ij is the interaction between species and grazing management and

eijk is the random residual error assumed to be normally and independently distributed.

RESULTS

Species composition and biomass production

A total of sixteen indigenous herbaceous forage species were obtained from the study area, of them 10 were grasses (62.50%), 4 forbs (25%) and different Cyperus and sedges species (12.5%). The total percent frequencies of occurrence were as follows: 15.66 % *Pennisetum thunbergii*, 6.63% *Trifolium cryptopodium*, 1.81% *Pennisetum riparium*, 3.01% *Cynodon dactylon*, 0.06% *Chloris gayana*, 20.48% *Andropogon abyssinicus*, 9.04% *Alchemilla abyssinica*, 3.61% *Trifolium* sp., 0.06% *Medicago ploymorpha*, 0.6 % *Pennisetum longistylum*, 5.42% *Cyperus rotundus*, 16.27% Cyperus and Sedge sps., 1.81% *Eragrostis botryodes*, 7.83% *Sporobolus africanus*, 5.42% *Eleusine floccifolia*, and 1.2% *Hyparrhenia* sp.

From sixteen identified forage species, 11 (68.75%) were recorded from free grazing, 13 (81.25%) from controlled grazing and 16 (100%) from enclosure (Table 1). The most frequent herbaceous forage species in the study areas, across the three grazing management types, were *Andropogon abyssinicus*, *Pennisetum thunbergii* and *Cyperus* and sedge sps. In the present study the forage species *Chloris gayana*, *Medicago polymorpha* and *Pennisetum longistylum* were not encountered in both free and controlled grazing management types, while *Pennisetum riparium* and *Hyparrhenia* sp. were not observed in free grazing management type. In free gazing management type, the highest relative frequency was recorded for *Andropogon abyssinicus* (21.23%), followed by *Pennisetum thunbergii* (15.14%) and Cyperus and sedge sps. (12.12%), while the rest had values below 10%.

Table 1. Compositions (%) based on frequencies of occurrence forage species found in three grazing managements types.

Species	Relative frequency of each species		
	Free grazing (communal)	Controlled grazing (private)	enclosure
Alchimella abyssinica	9.10%	8.33%	8.41%
Andropogon abyssinicus	21.23%	20.82%	19.00%
Cyperus and *sedge sps*.	12.12%	13.59%	16.83%
Chloris gayana	0.00%	0.00%	1.17%
Cynodon dactylon	3.02%	2.07%	2.54%
Cyperus rotundus	7.10%	5.26%	5.54%
Eleusine floccifolia	9.10%	6.26%	5.54%
Eragrostis botryodes	5.02%	5.07%	5.17%
Hyperrhia sp.	0.00%	2.07%	1.17%
Medicago polymorpha	0.00%	0.00%	1.17%
Pennisetum longistylum	0.00%	0.00%	1.17%
Pennisetum riparium	0.00%	2.07%	2.34%
Pennisetum thunbergii	15.14%	16.60%	15.29%
Sporobolus africanus	6.04%	8.33%	5.20%
Trifolium sp.	3.02%	4.15%	3.54%
Trfolium cryptopodium	9.10%	6.26%	5.88%

The dry matter biomass yield of seven forage species, namely, *Andropogon abyssinicus* (31.81%), *Pennisetum thunbergii* (25%), *Alchimella abyssinica* (5.18%), *Sporobolus africanus* (5.16%), *Eleusine floccifolia* (5.21%), *Trifolium cryptopodium* (5.11%) and *Cyperus rotundus* (5.1%) had 82.57% contribution of the total identified forage species (Figure 2). Therefore, the highest dry matter biomass production was recorded by *Andropogon abyssinicus*, followed by *Pennisetum thunbergii* (25%). However, the most preferred forage species by the communities with their quality feed, *Pennisetum riparium, Trifolium sp.* and *Pennisetum longistylum*, had low % biomass dry matter production in the study area.

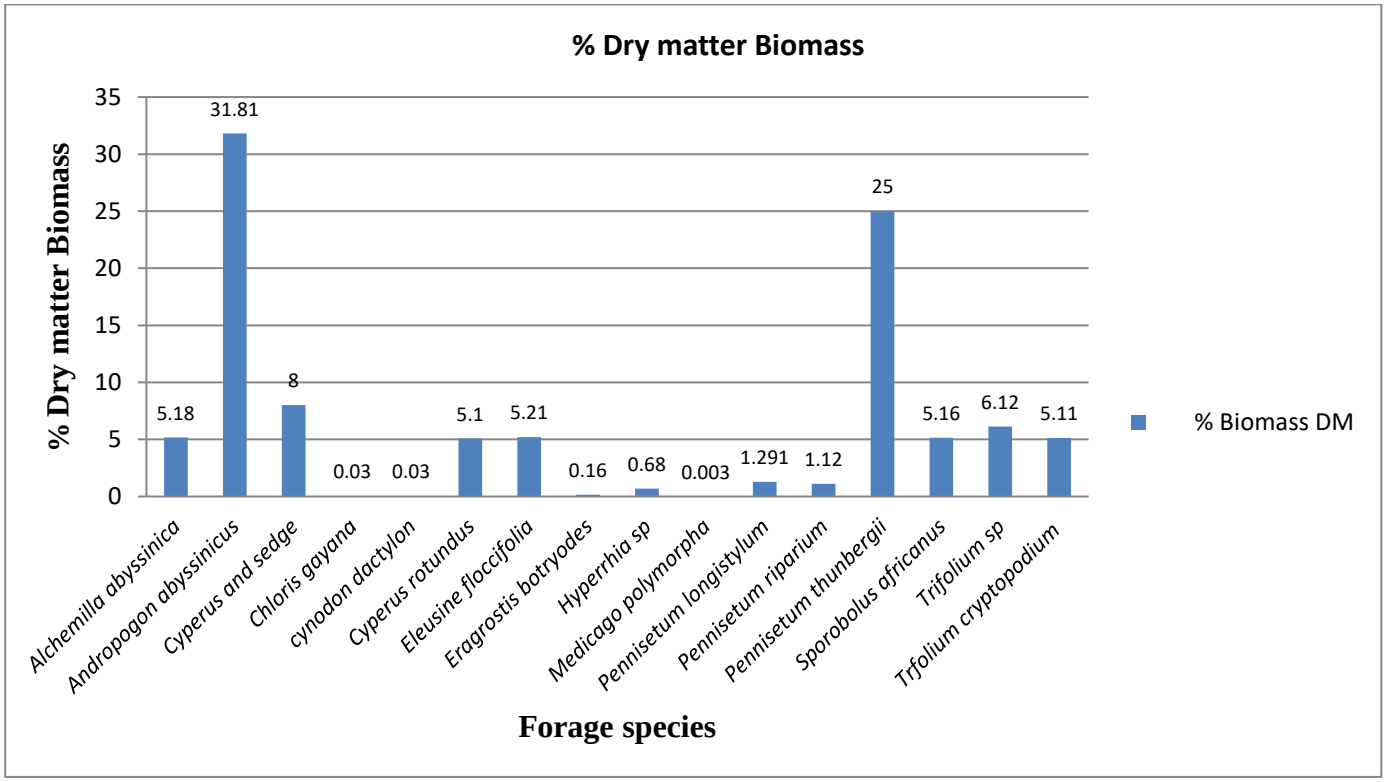

Figure 2. Percent dry matter biomass yield of indigenous forage species obtained in the study area.

Forage production

Analysis of variance (Table 2) showed that species and grazing management had highly significant ($P<0.001$) effects on dry matter yield (DMY), crude protein yield (CPY), fresh weight and dry weight of the forage species studied. Similarly, the interaction between species and grazing management had also significant effects ($P\leq0.05$) on DMY, CPY, fresh weight and dry weight of selected forage species.

The value of dry matter yield was the highest ($P<0.001$) in enclosure followed by controlled grazing, but free grazing management system had the lowest dry matter yield (0.40 t/ha). When the crude protein yields of the three grazing managements were compared, there were no significant difference ($P<0.05$) between controlled grazing and enclosure.

Considering fresh and dry weight of selected forage species, enclosure had very high significant ($P<0.001$) difference from free and controlled grazing managements (Table 3).

Table 2. Combined analysis of variance for dry matter yield, crude protein yield, fresh weight and dry weight of selected indigenous forage species.

S.O.V	DF	Variables (kg/ha)			
		DM	CPY	Fresh wt.	Dry wt.
		F-value			
Species	6	27.84***	4.70***	17.73***	17.73***
Grazing management	2	31.39***	8.91***	20.63***	20.63***
Species x grazing management	12	2.95**	2.20*	2.54**	2.5**

S.O.V= Source of variation; DMY=dry matter yield; CPY=crude protein yield; wt= weight; ***= (P<0.001); **= (P<0.01); *= (P<0.05)

Table 3. Mean comparisons of fresh weight and dry weight of selected forage species across grazing management.

Grazing management type	**Fresh weight (kg/ha)**	**Dry weight (kg/ha)**
Free grazing	1650[b]	460[b]
Controlled grazing	1880[b]	530[b]
Enclosure	4870[a]	1360[a]
Significant level	***	***

Kg/ha= kilogram per hectare, means with different letters within rows are significantly different at P<0.001

Dry matter and crude protein yields

The dry matter yields of *Andropogon abyssinicus, Pennisetum thunbergii* and *Eleusine floccifolia* were significantly higher (P<0.001) than *Alchemilla abyssinica*, *Cyperus rotundus*, *Sporobolus africanus* and *Trifolium crypsopodium*, in the three grazing management systems. The highest dry matter yield was obtained from *Andropogon abyssinicus* in free (830 kg/ha) and controlled (1220 kg/ha) grazing systems, while the highest dry matter yield was obtained from *Pennisetum thunbergii* (2130 kg/ha) harvested from enclosure. On the other hand, the dry matter yield of *Trifolium crypsopodium, Cyperus rotundus, Alchemilla abyssinica* and *Sporobolus africanus* was not significantly (P<0.05) different among each other in the free grazing, controlled grazing and enclosed grassland. Crude protein yields (CPY) of all selected forage species collected from free grazing and enclosed grasslands had statistically similar values (P≤0.05), while in

controlled grazing land the values of CPY in *Sporobolus africanus, Alchemilla abyssinica, Cyperus rotundus, Trifolium crypsopodium* and *Eleusine floccifolia* had similar results (Table 4). In the present study, the highest mean dry matter and crude protein yields were obtained from enclosure followed by controlled grazing. The crude protein yield of selected forage species harvested from free grazing and enclosure were not significantly different between each other ($p \leq 0.05$). The highest % biomass was obtained from *Andropogon abyssinicus* in free grazing and enclosure grasslands followed by *Pennisetum thunbergii* even though no significant difference was observed between them ($P \leq 0.05$).

Table 4. Mean comparisons of dry matter production (kg/ha), crude protein yields (kg/ha) %, dry matter biomass of selected forage species in the three grazing management systems.

Study parameters	Forage species						
	Sporobols africanus	*Alchemilla abyssinica*	*Cyperus rotundus*	*Trifolium crypsopodium*	*Eluesine floccifolia*	*Pennisetum thunbergii*	*Andropogon abyssinica*
Free grazing							
DMY (kg/ha)	30^{b}	110^{b}	110^{b}	80^{b}	380^{a}	690^{a}	830^{a}
CPY (kg/ha)	196^{a}	9.8^{a}	58.9^{a}	9.1^{a}	20.20^{a}	148.5^{a}	37.1^{a}
% Biomass	1.38c	1.79c	5.57b	0.32c	1.06c	36.51^{a}	53.41^{a}
Controlled grazing							
DMY (kg/ha)	170^{b}	200^{b}	350^{b}	210^{b}	970^{a}	997^{a}	1220^{a}
CPY (kg/ha)	21^{b}	22^{b}	223^{b}	129^{b}	47^{b}	600^{a}	540^{a}
% Biomass	4^{bc}	5^{bc}	3^{c}	5^{bc}	10^{b}	41^{a}	32^{a}
Enclosure grassland							
DMY (kg/ha)	750^{b}	180^{b}	320^{b}	520^{b}	1640^{a}	2130^{a}	1790^{a}
CPY (kg/ha)	111^{a}	34^{a}	81^{a}	326^{a}	79^{a}	640^{a}	563^{a}
% Biomass	3.17^{c}	2.72^{c}	2.5^{c}	5.20^{b}	6.13^{b}	35.25^{a}	45.04^{a}

Means with different letters within rows are significantly different at $P \leq 0.05$, DMY; dry matter yield, CPY; crude protein yield and % dry matter biomass yield.

Nutritive quality of forage species

Grasses and Cyprus

The DM contents of *Eleusine floccifolia* (90.80%), *Sporobolus africanus* (90.42%), and *Cyperus rotundus* (90.53%) were significantly higher than *Pennisetum thunbergii* (89.99%) ($P<0.05$) (Table 5). The crude protein (CP) contents of the four species of grass and *Cyperus rotundus* had statistically similar values

(P<0.05). Metabolizable energy (ME) (6.64MJ/DM) and Invitro organic Matter Digestibility (IVOMD) (44.29%) of *Sporobolus africanus* were lower (P<0.05) than *Andropogon abyssinicus, Pennisetum thunbergii, Eleusine floccifolia* and *Cyperus rotundus* values. Neutral Detergent Fiber (NDF) value of *Sporobolus africanus* (77.54%) also had higher (P<0.05) than *Andropogon abyssinicus* (72.59%), *Pennisetum thunbergii* (72.35%) and *Cyperus rotundu*s (70.63%) except *Eleusine floccifolia* (74.52%).

Table 5. Overall mean comparisons of chemical composition and nutritional values of collected forage species

Forage types	DM (%)	Ash % DM	CP % DM	NDF % DM	ADF % DM	ME MJ/kg DM	IVOMD (%)
Gasses and Cyperus							
Andropogon abyssinicus	90.25^{ab}	11.01^{ab}	5.21^{a}	72.59^{bc}	40.24^{bc}	7.07^{a}	47.86^{a}
Pennisetum thunbergii	89.99^{b}	11.78^{a}	5.48^{a}	72.35^{bc}	39.70^{b}	7.20^{a}	48.94^{a}
Eleusine floccifolia	90.80^{a}	10.44^{b}	4.80^{a}	74.52^{ab}	44.04^{a}	7.15^{a}	47.98^{a}
Cyperus rotundus	90.53^{a}	10.27^{ab}	5.62^{a}	70.63^{bc}	38.32^{c}	7.38^{a}	50.20^{a}
Sporobolus africanus	90.42^{a}	9.66^{c}	5.36^{a}	77.54^{a}	44.32^{a}	6.64^{b}	44.29^{b}
Forb and legume							
Trifolium cryptopodium	91.48^{a}	7.99^{b}	9.84^{a}	47.54^{b}	34.70^{a}	8.77^{a}	60.83^{a}
Alchimella abyssinica	88.32^{b}	12.88^{a}	9.77^{a}	62.05^{a}	28.38^{b}	8.59^{a}	59.89^{a}
Combined forage							
Mixed green forage	90.09^{a}	12.23^{a}	6.63^{a}	68.56^{b}	37.57^{b}	7.29^{a}	49.90^{a}
Mixed dry forage	89.96^{a}	12.07^{a}	4.94^{b}	76.20^{a}	48.91^{a}	6.16^{b}	41.54^{b}

Means within column followed by the same letter (s) are not significantly different at P<0.05 significant level of Duncan multiple tests. CP; crude protein, NDF; Neutral detergent fiber, ADF; acid detergent fiber, ADL; Acid detergent lignin, ME; metabolizable energy (MJ/kg DM), TIVOMD; true in vitro organic matter digestibility (gm/kg DM).

Forb and legume

Trifolium cryptopodium had (P<0.05) higher Dry Matter (DM) and Acid Detergent Fiber (ADF) contents than *Alchimella abyssinica*, on the other hand, *Alchimella abyssinica* had higher (P<0.05) Ash and NDF values than *Trifolium cryptopodium.* Crude protein, ME and IVOMD values of *Trifolium cryptopodium* and *Alchimella abyssinica* were not significantly different (P<0.05).

Combined forage species

Dry matter and Ash contents of mixed green forage and mixed dry forage were not significantly differed with each other (P<0.05). CP, ME and IVOMD values of mixed green forages were significantly higher than mixed dry forages (P<0.05). However, mixed dry forages had higher NDF and ADF values than mixed green forages (P<0.05).

Nutritional values across functional groups

The nutritional values of the families of the identified forage species (Table 6), has shown that the highest (12.88%) Ash value was obtained from Rosaceae, which was significantly higher (P<0.05) than Cyperaceae (10.72%) and Fabaceae (7.99%); but was not significantly different from Poaceae (11.13%) (P<0.05). Poaceae, Cyperaceae and Fabaceae had statistically similar values (P<0.05) in terms of CP; while Rosaceae had shown higher CP value than Cyperaceae and Poaceae. NDF and ADF contents of Poaceae obtained from the present study were the highest (P<0.05) of the rest three families. The NDF value of Fabaceae and Rosaceae were the lowest, while the ADF values of Cyperaceae and Fabaceae had similar values (P<0.05). The ME and IVOMD values were high (P<0.05) in Fabaceae and Rosaceae the lowest value being from Poaceae.

Table 6. Analysis of chemical composition and nutritional values based on families

	% of DM					
Family	Ash	CP	NDF	ADF	ME MJ/kg DM	IVOMD % of DM
Poaceae	11.13^{ab}	7.55^{b}	73.24^{a}	40.81^{a}	7.09^{c}	48.02^{c}
Cyperaceae	10.72^{b}	7.59^{b}	67.64^{b}	33.73^{b}	7.67^{b}	52.18^{b}
Fabaceae	7.99^{c}	9.84^{ab}	47.54^{c}	34.70^{b}	8.77^{a}	60.74^{a}
Rosaceae	12.88^{a}	10.86^{a}	62.38^{c}	27.56^{c}	8.67^{a}	60.10^{a}

Means within column followed by the same letter (s) are not significantly different at P <0.05 significant level of Duncan multiple tests CP; Crude protein, NDF; Neutral detergent fiber, ADF; acid detergent fiber, ADL; Acid detergent lignin, ME; metabolizable energy (MJ/kg DM), TIVOMD; true in vitro organic matter digestibility (gm/kg DM).

DISCUSSION

Species composition and Biomass contribution of forage species

The highest proportion of forage species in the present study was observed by grass species, which may be related to the management and utilization aspects of grasslands. It also could be a result of their ecological competitiveness and resilience to various adverse conditions (Linder et al., 2018). The present result is similar to the finding reported by Getachew (2005) and Bekele et al. (2010). In the present study among the forage species, *Andropogon abyssinicus, Pennisetum thunbergii* and Cyperus and sedge sps. were recorded as dominant with frequency percentage value of >15% and *Alchemilla abyssinica, Sporobolus africanus*, *Cyperus rotundus* and *Eleusine floccifolia* were commonly occurring species as their frequency of percentage was between 5% and 15%; while all the rest were recorded as rare species (Beyene and Mlambo, 2012). On the other hand, better quality forage species like mixture of *Trifolium* sp, *Trifolium cryptopodium*, *Alchemilla abyssinica, Pennisetum riparium, Pennisetum longistylum* and *Medicago ploymorpha* with lower percent frequency and biomass yield might indicate the poor forage quality and availability of the grassland in the district (Bekele et al., 2010). Indigenous forage species composition occupied by a few species made up the bulk of biomass yield and high values of relative frequency. This is because of the fact that in grassland community one or a few species are able to tolerate the multidimensional environmental factors of the area that they are spatially stable (Abule et al., 2007). Increased abundance of these species in this study area may be due to response to disturbance such as moderate to heavy grazing, competition, and water logging tendency of the area which is especially favored by Cyperus and sedge sps. (Edwards et al., 1997). Moreover, Asrat et al. (2015) reported that the composition and abundance of herbaceous species were influenced by increased grazing pressure. In the present study, the identified herbaceous forage species were lower than previously reported in various parts of Ethiopia (Zewdu, 2005), which may be due to water logging status of the area and acidity of the soil and small sampling area. It may also be attributed to high

altitude of the study area, since higher altitudes have lower vegetation diversity according to Aynekulu et al. (2012) and Gebrewahid and Abrehe (2019).

Out of sixteen indigenous forage species identified from the study area, 11 and 13 were available in free grazing and controlled grazing management types, respectively; while all the sixteen species were present in enclosure. This is an indication that grazing regime can influence botanical composition of herb species. High herbaceous biomass in enclosures could be linked with low grazing disturbance by livestock (Mengistu et al., 2005). Previous studies also indicated that grazing intensity is one of the most primary factors that result in reducing forage composition and diversity over time (Mengistu, 2006). The intensity of grazing can cause difference in botanical composition and relative abundance of important forage species. Moreover, Sternberg et al. (2000) and Keba et al. (2013) also stated that overgrazing affects the botanical composition and species diversity and causes strong influence on the structure and organization of forage species in different ways. This result coincides with the report of (Angassa, 2014) and Ayele et al. (2022), as they indicated that frequent and heavy grazing pressure may cause a reduction in herbaceous forage species composition, diversity and basal cover. Skornik et al. (2010) also suggested that heavy and long-term grazing caused both decline in floristic richness and above-ground biomass yield, ultimately altering species composition.

Determination of forage yield

The variation in mean DMY, CPY, Fresh weight and Dry weight were highly significant ($P<0.001$) due to species composition, biomass yield of the different forage species collected and grazing management types. The variation in DMY, CPY, fresh weight and dry weight were significant ($P\leq0.05$) due to the interaction effect between species and grazing management types. This indicated that forage production can be influenced by forage species and grazing management practices (Bekele et al., 2010). The effect of grazing type on DMY, CPY, Fresh weight and Dry weight depends on the available forage species of the grassland.

This indicated that forage yield can be influenced by different grazing management practices and species of forage available in the specific grassland.

Forage dry weight recorded from enclosure in the present study was similar with the results of Ayele et al. (2022) from private grazing, communal grazing, and fallow land and roadside. Moreover, fresh weight (4.87 t/ha) of forage species collected from enclosure grassland had similar value with the report of communal grazing land. Dry matter yield of grazing land increased as grazing intensity decreased. Moreover, growing under a condition without cattle grazing and human disturbances, the plants were able to complete a normal life cycle of growth, flowering, setting seeds and the likes (Wang et al., 2018; Liu et al., 2020).

Andropogon abyssinicus, Pennisetum thunbergii and *Eleusine floccifolia* resulted in higher dry matter yield than *Alchemilla abyssinica, Cyperus rotundus, Sporobolus africanus* and *Trifolium cryptopodium*, in the three grazing management systems. This could be due to high frequency, availability and distribution of these species in the study area. Even though, the sampling time was at full flowering stage the mean dry matter yields in the three grazing types were below the values of previously reported results (Mengistu, 1987; Agza et al., 2013; Zewdie and Yoseph, 2014), which may be due to species composition, interactions within and among species and increased degree of degradation, but comparable with Abule et al. (2007).

Nutritional values

Forage quality can be affected by a variety of biological and environmental factors. In general, the nutritional value of forages is the highest when the plant is young, have actively growing leaves but declines as the plant nears maturity. Good quality forage is associated with high CP and low fiber, the CP values of grass species in the present study ranged from 4.80-5.62% which is below some findings elsewhere (Teklu et al. 2010; Gebremariam and Belay, 2021), but comparable to the values of natural mix hay and maize straw reported by Gebremariam and Belay (2021). The crude protein contents of grasses, Cyperus, mixed green and mixed dry forages were below the minimum (7%) requirements for optimum microbial growth and maintenance (Van Soest et al., 1991). Moreover, the chemical composition of selected forage species

was below the results of earlier studies (Keba et al., 2013; Asrat et al., 2015). The CP decreased because of the accumulation of structural carbohydrates. According to Feyissa et al. (2014), CP content, IVOMD and ME content significantly declined with delaying harvesting from mid-October to late October.

The NDF values of grasses, Cyperus, mixed green forage and mixed dry forage were higher and categorized in the range of low-quality forage (>65%), while forbs (*Trifolium cryptopodium* and *Alchimella abyssinica*) had medium forage quality (45-65%). The ADF values of the studied forage types were within the medium quality (31-45%) with the exception of mixed dry forage which had a value within the range of low quality (Singh and Oosting, 1992). Similarly, Leng (1990) indicated that forage species of CP and digestibility lower than 8% and 55% are categorized under low-quality forages. The ME and IVOMD values of all the analyzed forage species were below the records of native hay (Geleti et al., 2013) with the exception of IVOMD of *Trifolium cryptopodium* (60.83%). The mean ME for the selected grass, Cyperus, mixed green and mixed dry forages were below the critical threshold levels (7.5 MJ/kg DM). Moreover, the IVOMD values of all the studied grasses and mixed dry forages were below the critical threshold level (50%) required for feed digestibility (Owen and Jayasuriyat, 1989). The present study generally demonstrated that grasses, Cyperus, mixed green and mixed dry forages that are widely used as roughage feeds for dairy animals in the study area are of inferior quality containing high fiber fractions, low CP, ME and IVOMD.

Nutritional values across functional groups

The CP, ME and IVOMD values of Fabaceae and Rosaceae were comparable. But the chemical and nutritional composition of Cyperaceae was higher than the values of Poaceae, that may be due to seasonal water logging tendency of the study area. The mean values of CP in Poaceae, Cyperaceae and Fabaceae in the present study were lower than the mean values reported by Keba et al. (2013) and Mosisa et al. (2021), but higher than the values of NDF and ADF obtained by the same authors. Moreover, ME and IVOMD values of the studies function group were lower than the values of native hay and herbaceous legumes (Geleti et al., 2013). Generally, the feed quality of Fabaceae and Rosaceae were comparable. Likewise, the

chemical composition and nutritional values of Cyperaceae and Fabaceae were also comparable in the major nutritional parameters. Generally, as the forage sampling was conducted during hay harvesting time the relative quality of hay produced in the study area has been assessed to be of poor quality.

CONCLUSIONS

The study gave information on botanical composition, above ground biomass and nutritional profiles of main forage species available in the Wahcale district of North Shewa zone, Oromia regional state. The result showed that the botanical composition of indigenous forage species was dominated by a few species, but with high biomass yield and high values of relative frequency. Comparison with previous studies revealed low diversity and compositions in the present study area. There were variations among the forage species, grazing types in terms of species composition, biomass yield and nutritional qualities. Some forage species with varied occurrence belonging to different families were identified from the three grazing management types. Wide variations were not recognized between the nutritive values of most indigenous forage species, especially on grasses, Cyperus, and mixed green and mixed dry forages. The CP contents of grasses, Cyprus, mixed green and mixed dry forages were found to be below the critical level required for maintenance, optimum rumen function and feed intake, resulting in low livestock productivity. Even though, the mean DM yield of indigenous forage species in study area was high and comparable with most of earlier reports, the feed quality tested according to the current result was very poor. Moreover, the nutritive values of most of the forage species during wet and dry season in the present study were below the requirement of ruminant livestock. Few forage grass species were dominant; therefore, to improve diversity, composition and availability of other crucial indigenous forage species, proper grazing land management has to be conducted. In addition, collection, conservation and multiplication of rarely available forage species should be widely undertaken. Overall, the indigenous forage species in the study area were found to be poor in terms of diversity, composition, yield and quality, while enclosures could be considered as better grazing management option in terms of maintaining species diversity and DM yield.

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THE MULTI-LOCATION STUDY OF SIX-ROWED BARLEY LINES FOR MALT QUALITY

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ABSTRACT: In Ethiopia, both two- and six-rowed barleys can grow in the highlands. However, the country is not self-sufficient in malt barley production to satisfy the growing demands of malt factories, and it depends on imports from other countries. Little effort has been made to study the potential of Ethiopian six-rowed barley genotypes for malt quality traits. Hence, a two-year (2017/18-2018/19) experiment with 12 six-rowed type barley genotypes including one two-rowed malt barley standard check (IBON174/03) was conducted in Bekoji, Holata and Kofale research plots to assess the potential of six-rowed barley genotypes for malt quality. Randomized complete block design with three replications was used. Malt quality data were collected and the data was analyzed using R statistical software, R version 4.1.3 (2022-03-10), agricolae package. Tukey HSD means comparison showed that the 2.8-mm slot sieve test (42.57%) and the dry matter-based crude protein of barley line 17148-16 (11.8%) are significantly greater than the check but the fine grind hot water extract of the line (79.8%) is slightly greater than for the check (78.25%). Therefore, the findings from the two-year experiment in the three locations indicated that the six-rowed barley line 17148-16 fulfilled the malt quality requirements and can be used in the future malt quality improvement of six-rowed barleys in Ethiopia.

Keywords: Line 17148-16, malt quality, six-rowed barleys, Tukey HSD.

INTRODUCTION

Barley is a major crop in the highlands of Ethiopia, where it is grown by more than four million smallholder farmers in an area of about one million hectares of land (CSA, 2022). Ethiopia has a growing malt beverage sector and beer production has grown nearly 20% annually, from 1 million hectoliters in 2003 to roughly 4 million hectoliters in 2012 (Abu and Teddy, 2014). Nevertheless, the favorable environment and market opportunity and the share of malt barley production is relatively low (about 15%) compared to food barley (Lakew and Fekadu, 2015). Generally, barley productivity in Ethiopia remained significantly lower than global and regional averages (FAO, 2013). Stress factors like poor distribution of rainfall, low soil fertility (Mulatu and Stenfania, 2011), low productivity of landraces and unavailability of improved barley

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technologies to farmers (Lakew and Fekadu, 2015), diseases (scald, net blotch, spot blotch and rusts) and insect pests (aphids and barley shoot fly) (Yirga et al., 1998) have been reported as major causes of significant yield reduction.

In Ethiopia, barley is cultivated in either *meher* (the main rainy season) or *belg* (the short rainy season) mainly for food, though the country has a huge potential for malt barley production. Both two- and six-rowed types can be used for malt intended for beer production. It has been reported that national production meets only 35% of the domestic demand (Molla et al., 2018). Breweries like Heineken Ethiopia import 67% of raw barley and malt (Gerrit van Loo, personal communication). A four-year (2017-2020) data obtained from the Ministry of Trade and Industry showed that Ethiopia imported 57,588,420.83 kg of barley malt from abroad (Woolfrey et al., 2021) which is worth 30,287,369.60 USD. The domestic demand for malt barley is likely to increase.

Malt barley research in Ethiopia has been engaged in evaluating the local barely collections (farmers' varieties) and screening introduced malt barely from Europe, the USA, and ICARDA for their suitability for malting purposes (Lakew and Fekadu, 2015). The introduction and/or development of new high-yielding malt barley varieties would greatly contribute to satisfying the growing domestic demand, boost export earnings, and substantially generate income for farmers. Recognizing the little effort made in two-rowed malt barley improvement, absence of any work done in six-rowed barley for malting, and the low barley malt supply to domestic breweries in Ethiopia, research in six-rowed barley for malting contributes to better supply of barley malt to breweries in Ethiopia. The need to do research on six-rowed barley malt quality improvement arose from low malt supply and poor quality in two-rowed barley (Aychew Bekele, personal communication). Research in the area of quality improvement is very young as compared to malt barley producing countries of the world. The lack of six-rowed barley varieties with good malting quality is considered as a gap in malt barley research in Ethiopia. Malting barley must meet specific quality requirements which are affected by several biotic and abiotic factors. Therefore, this study was conducted

to investigate the malting quality of six-rowed barley genotypes in three locations and to identify six-rowed barley lines fulfilling malting quality.

MATERIALS AND METHODS

Site description

The experiment was carried out in three locations (Holata, Bekoji and Kofale) for two consecutive years (2017/18-2018/19). The Holata Agricultural Research Center (HARC) is located 09°03'N and 38°30'E at an altitude of 2400 m.a.s.l. Its mean annual rainfall is 1044 mm, mean maximum and minimum temperature of the area is 22^{O}C, and 6.1^{O}C respectively and has a mean relative humidity of 60.6% (HARC, 2005). Bekoji is located at latitude of 7° 34'N and longitude of 39° 09' E with an elevation of 2810 m.a.s.l. Kofale is located at 7°04' N latitude, 38°78' E longitude and 2515 m.a.s.l. These locations are potential areas for barley production.

Plant material

Forty-eight six-rowed barley genotypes, the mother accessions of which were obtained from the Ethiopian Biodiversity Institute, were developed from the 2014/15 and 2015/16 cropping seasons. The selection was based on scald ratings and yield performances. These were multiplied ear-to-row in HARC's experimental plots during the main cropping season of 2016/17. Twelve lines were selected from the 48 genotypes. The grains of barley lines were sorted as mealy or glassy as described in the Reynolds (1909) Method 935.28. Before planting the lines, grains were cut cross-sectionally using a cutter. The cut surface of each grain from the lines was grouped as mealy or glassy depending on the amount of flour present. The 12 six-rowed barley lines and one two-rowed standard check (IBON174/03) were sown in a randomized complete block design (RCBD) with three replications. Each plot area was divided into four rows with a spacing of 20 cm between rows and a row length of 2.5 m.

Data collection

Grain quality analysis

Sieve test

Hundred grams of dockage-free barley grains from each line was measured and taken to a sieve. For five minutes, the grains were shaken on 2.8-mm, 2.5-mm, and 2.2-mm diameter slots. The grains retained on 2.8-mm, 2.5-mm, and 2.2-mm and those passing through the 2.2-mm slots were separately measured and recorded as percentages (Mastanjevic et al., 2017).

Moisture content and test weight

The moisture content and the test weight of dockage-free six-rowed barley grains from each line were measured using a grain analysis computer (GAC 2100)-DIKEY-john corporation, USA.

Crude protein content and fine grind hot water extract

Crude protein content and fine grind hot water extract data of the barley grains of each line were generated using Opus 7.5 software. The crude protein and fine grind hot water extract analyses instrument was tango Bruker optics (calibrated FT-IR near-infrared spectroscopy). The working principle is the interaction of electromagnetic radiation with protein and carbohydrates. The interaction produced spectra of a specific organic compound from which the models for crude protein and fine grind hot water extract were developed by Opus 7.5 software.

Data analysis

R version 4.1.3 (2022-03-10), agricolae package, (R Core Team, 2022) was used to examine the grain quality data obtained from the barley lines. For the quality-related measures, Tukey HSD was used to compare the means of the barley lines.

RESULTS

The analysis of variance (ANOVA) for sieve test (2.8-mm, 2.5-mm and 2.2-mm slot sieves) (Table 1 and 2), dry matter-based test weight, moisture content (Table 3), dry matter-based crude protein, and dry matter-based fine grind hot water extract (Table 4) showed very highly significant difference (100% confidence) among the barley lines, line-location, line-year, and line-location-year interactions. Therefore, the mean comparison for the lines was possible.

Table 1. Analysis of variance for sieve test (> 2.8-mm and > 2.5-mm slot sieves).

> 2.8 mm						> 2.5 mm					
	Df	Sum Sq	Mean Sq	F value	Pr. (>F)		Df	Sum Sq	Mean Sq	F value	Pr. (>F)
Trt	12	24486.3	2040.5	91.6731	< 2.2e-16***	Trt	12	4483.1	373.6	10.2843	<2.114e-14***
Loc	2	12513.6	6256.8	281.0941	< 2.2e-16***	Loc	2	22991.9	11495.9	316.4630	< 2.2e-16***
Year	1	12.3	12.3	0.5547	0.4576058	Year	1	3755.1	3755.1	103.3715	< 2.2e-16***
Trt:loc.	24	7972.2	332.2	14.9235	< 2.2e-16***	Trt:loc.	24	16145.3	672.7	18.5188	< 2.2e-16***
Trt:year	12	764.3	63.7	2.8614	0.0014736**	Trt:year	12	2077.1	173.1	4.7650	1.563e-06***
Loc:year	2	401.2	200.6	9.0126	0.0002059***	Loc:year	2	1420.3	710.1	19.5487	3.125e-08***
Trt:loc:year	24	2391.6	99.6	4.4768	6.735e-09***	Trt:loc:year	24	4365.2	181.9	5.0069	3.773e-10***
Loc:year:rep	12	811.2	67.6	3.0369	0.0007891***	Loc:year:rep	12	4035.9	336.3	9.2584	4.709e-13***
Residuals	143	3183.0	22.3			Residuals	143	5194.7	36.3		

Trt – barley line; Loc – location; rep-replication

Sign. codes: 0 '*** '0.001 '**'0.01 '*'0.05

MS error=22.2587; DF=143; Mean=11.39996; CV=41.38537

Sign. codes: 0 '*** '0.001 '**'0.01 '*'0.05

MS error=36.32633; DF=143; Mean=40.94755; CV=14.71915

Table 2. Analysis of variance for sieve test (> 2.2-mm and < 2.2-mm slot sieves).

> 2.2 mm						< 2.2 mm					
	Df	Sum Sq	Mean Sq	F value	Pr (>F)		Df	Sum Sq	Mean Sq	F value	Pr (>F)
Trt	12	12136.6	1011.4	49.2310	< 2.2e-16***	Trt	12	9033.2	752.8	20.9274	< 2.e-16***
Loc	2	12387.5	6193.7	301.4912	< 2.2e-16***	Loc	2	28236.4	14118.2	392.4938	< 2.e-16***
Year	1	36.7	36.7	1.7847	0.1837	Year	1	4833.0	4833.0	134.3593	< 2.e-16***
Trt:loc.	24	3437.3	143.2	6.9715	1.691e-14***	Trt:loc.	24	7365.8	306.9	8.5322	< 2.e-16***
Trt:year	12	1920.1	160.0	7.7888	4.954e-11***	Trt:year	12	1635.2	136.3	3.7883	< 5.269e-05***
Loc:year	2	416.1	208.0	10.1263	7.709e-05***	Loc:year	2	3030.6	1515.3	42.1262	4.132e-15***
Trt:loc:year	24	1390.8	57.9	2.8208	7.535e-05***	Trt:loc:year	24	4374.6	182.3	5.0674	2.729e-10***
Loc:year:rep	12	1018.0	84.8	4.1296	1.535e-05***	Loc:year:rep	12	4541.9	378.5	10.5224	1.046e-14***
Residuals	143	2937.7	20.5			Residuals	143	5143.8	36.0		

Trt – barley line; Loc – location; rep-replication

Sign. codes: 0 '*** '0.001 '**'0.01 '*'0.05

MS error=20.54369; DF=143; Mean= 30.060736; CV=15.07786

Sign. codes: 0 '*** '0.001 '**'0.01 '*'0.05

MS error= 35.97046; DF=143; Mean=17.52124; CV=34.23009

Table 3. Analysis of variance for dry matter-based grain test weight and moisture content.

Test weight (kg/hl)					
	Df	Sum	Mean Sq	F value	Pr (>F)
Trt	12	6574	547.8	15.0444	< 2.e-16***
Loc	2	55018	27508.9	755.4418	< 2.e-16***
Year	1	7239	7239.4	198.8063	< 2.e-16***
Trt:loc.	24	7241	301.7	8.2853	< 2.e-16***
Trt:year	12	2866	238.9	6.5598	< 2.957e-09***
Loc:year	2	9725	4862.7	1333.5386	< 2.e-16***
Trt:loc:year	24	4040	168.3	4.6227	< 3.026e-09***
Loc:year:rep	12	808	67.3	1.8492	0.04568*
Residuals	143	5207	36.4		

Trt – barley line; Loc – location; rep-replication
Sign. codes: 0 '*** '0.001 '**'0.01 '*'0.05
MS error=36.41429; DF =143; Mean=43.21897; CV=13.96245

Moisture content (%)					
	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Trt	12	10.8474	0.90395	9.9868	5.137e-14***
Loc	2	3.5738	1.78689	19.7416	2.686e-08***
Year	1	0.2912	0.29121	3.2173	0.0749753
Trt:loc.	24	6.1318	0.25549	2.8227	7.455e-05***
Trt:year	12	3.9624	0.33020	3.6481	8.748e-05***
Loc:year	2	1.1753	0.58763	6.4921	0.0020010**
Trt:loc:year	24	5.9492	0.24788	2.7386	0.0001197***
Loc:year:rep	12	0.8542	0.07119	0.7865	0.6636944
Residuals	143	12.9435	0.09051		

Sign. codes: 0 '*** '0.001 '**'0.01 '*'0.05
MS error =0.0905; DF=143; Mean=11.66687; CV=2.578717

Table 4. Analysis of variance for dry matter-based crude protein content and fine grind hot water extract.

Crude protein (%)					
	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Trt	12	2181.70	181.808	79.4845	< 2.2e-16***
Loc	2	109.10	54.552	23.8494	1.153e-09
Year	1	13.41	13.409	5.8624	0.01672*
Trt:loc.	24	210.78	8.783	3.8397	2.342e-07***
Trt:year	12	270.38	22.531	9.8505	7.742e-14***
Loc:year	2	93.27	46.637	20.3891	1.620e-08***
Trt:loc:year	24	295.72	12.322	5.3869	5.004e-11***
Loc:year:rep	12	53.73	4.477	1.9574	0.03236*
Residuals	143	327.09	2.287		

Trt – barley line; Loc – location; rep-replication
Sign. codes: 0 '*** '0.001 '**'0.01 '*'0.05
MS error=2.28734; DF=143Mean= 17.38021; CV= 8.701823

Fine grind hot water extract (%)					
	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Trt	12	6074.2	506.19	34.2396	< 2.2e-16***
Loc	2	774.2	387.09	26.1836	2.045e-10***
Year	1	171.6	171.59	11.6068	0.0008535***
Trt:loc.	24	1282.4	53.43	3.6144	8.356e-07***
Trt:year	12	778.2	64.85	4.3868	6.073e-06***
Loc:year	2	955.0	477.49	32.2987	2.662e-12***
Trt:loc:year	24	1406.6	58.61	3.9645	1.162e-07***
Loc:year:rep	12	247.9	20.66	1.3972	01736210
Residuals	143	2114.1	14.78		

Sign. codes: 0 '*** '0.001 '**'0.01 '*'0.05
MS error=14.78366; DF=143; Mean= 71.07996; CV=5.409334

Mean Comparisons

Sieve test

On 2.8-mm diameter slots, the barley line 17148-16 was significantly different from all lines, including the check. The percentage of barley grain retained on the 2.8-mm sieve was 42.57, but the check gave 23.98, followed by line 3257-16, resulting in 12.62 (Table 5). Line 17204-5 grain plumpness on 2.8-mm slot sieve is the least (3.865) of all lines. The malt barley standard specifies that 35-40% of the grain be retained on 2.8-mm slot sieve, 40-50% on 2.5-mm, 5-10% on 2.2-mm and the amount passing through 2.2-mm slot sieve be 1%.

Table 5. Mean comparison for sieve test.

Sieve Test (%)											
> 2.8 mm			> 2.5 mm			> 2.2 mm			< 2.2 mm		
17148-16	**42.568333**	**a**	CHECK	51.76882	a	CHECK	51.76882	a	17204-5	27.233333	a
CHECK	23.979412	b	16820-16	43.90056	b	16820-16	43.90056	b	16863-2	24.002222	ab
3257-16	12.621111	c	16822-12	42.98444	b	16822-12	42.98444	b	3462-12	22.581667	abc
3436-9	10.772222	cd	3257-16	42.23056	bc	3257-16	42.23056	bc	3436-9	21.711667	abc
16910-19	9.963333	cde	16734-6	42.10833	bc	16734-6	42.10833	bc	3257-16	19.793333	bcd
16863-2	7.715556	cdef	**17148-16**	**41.72000**	**bc**	**17148-16**	**41.72000**	**bc**	16812-4	18.496111	bcd
16734-6	7.575000	cdef	16814-7	41.67222	bc	16814-7	41.67222	bc	16734-6	17.826111	bcd
16814-7	6.808333	def	3436-9	41.64556	bc	3436-9	41.64556	bc	16910-19	17.053889	cd
16822-12	6.787222	def	16910-19	40.36389	bcd	16910-19	40.36389	bcd	16814-7	16.857222	cd
16812-4	5.846667	def	16812-4	40.36389	bcd	16812-4	40.36389	bcd	16822-12	16.508333	cd
16820-16	5.631111	def	3462-12	36.08167	cde	3462-12	36.08167	cde	16820-16	14.928333	d
3462-12	4.765000	ef	16863-2	34.81444	de	16863-2	34.81444	de	CHECK	6.710588	e
17204-5	3.865000	f	17204-5	33.26500	e	17204-5	33.26500	e	**17148-16**	**3.472778**	e

Means with different letters are significantly different according to Tukey HSD test.

Test weight

Line 17148-16, which is the best in plumpness on 2.8-mm slot sieve, showed no difference in test weight compared to the check (Table 6). Line 17148-16 exhibited a test weight of 50.5 kg/hl and the check gave a test weight of 54.2 kg/hl.

Moisture content

For moisture content, there was no significant difference among the lines and the check except lines 17204-5, 3257-16, 16863-2, 16820-16, 16814-7 showing difference when compared to line 16910-19 (Table 6).

Table 6. Mean comparison for dry matter-based test weight, moisture content, dry matter-based crude protein and dry matter-based fine grind hot water extract.

Parameters											
Test weight (kg/hl)			Moisture content (%)			Crude protein (%)			Fine grind hot water extract (%)		
CHECK	54.19765	a	16910-19	11.98333	a	16910-10	20.44444	a	**17148-16**	**79.81111**	a
17148-16	**50.48778**	**ab**	16822-12	11.86667	ab	16812-4	20.31667	a	CHECK	78.25294	ab
16820-16	45.94889	bc	3436-9	11.81611	ab	16822-12	20.23889	a	3462-12	75.57111	abc
16910-19	44.48611	bcd	16734-6	11.79444	abc	3436-9	20.11667	a	3257-16	75.41611	bc
3436-9	44.19167	bcd	CHECK	11.79412	abc	16734-6	19.85000	a	17204-5	75.33333	bc
3462-12	44.19056	bcd	3462-12	11.77278	abc	16863-2	18.97222	a	16910-19	73.07222	cd
16812-4	44.01944	bcd	16812-4	11.72778	abc	16814-7	18.85000	a	16820-16	69.35000	de
16814-7	43.33944	cd	**17148-16**	**11.70000**	**abc**	16820-16	18.82778	a	16734-6	67.47778	ef
16734-6	43.31833	cd	16814-7	11.63333	bc	3462-12	14.79611	b	16822-12	67.28333	ef
16822-12	41.53000	cd	16820-16	11.62222	bcd	CHECK	13.95294	b	16863-2	67.22222	ef
3257-16	38.03500	de	16863-2	11.46111	cde	3257-16	13.82000	b	16812-4	66.38333	ef
16863-2	34.57944	e	3257-16	11.29333	de	17204-5	13.76667	b	16814-7	65.38333	ef
17204	34.13222	e	17204-5	11.21111	e	**17148-16**	**11.80000**	**c**	3436-9	63.88111	f

Means with different letters are significantly different according to Tukey HSD test.

Crude protein

The mean comparison among the lines for crude protein exhibited a difference when assessed using Tukey HSD. Barley line 17148-16 excelled all other lines, including the check. This line was found to have 11.8% crude protein, whereas the check variety's protein content was 13.95% which is beyond the malt barley protein limit of 13% for six-rowed barley and 12.5% for two-rowed barley (Table 6).

Fine grind hot water extract

The mean comparison among the lines, including the check, showed that line 17148-16 fulfilled crude protein requirement and gave a fine-grind hot water extract value slightly greater than the check even if the difference was statistically insignificant (Table 6). However, the result showed a statistically significant

difference between line 17148-16 and lines 3257-16, 17204-5, 16910-19, 16820-16, 16734-6, 16822-12, 16863-2, 16812-4, 16814-7 and 3436-9.

DISCUSSION

Grain plumpness indicates the amount of starch contained and more plump malt barley grain is preferred to thinner ones. The sum of the percentages of grains from the best performing line 17148-16 on the exact sieve slot sizes was found to be 96.43%. Plump grains have more starch directly related to the amount of fine grind hot water extract. Line 17148-16 is beyond the EBC (European Brewery Convention) standard for the 2.8-mm slot sieve test. Two-rowed malt barley varieties released in Ethiopia, Singitan (IBON-MRA P# 26) (Tamene et al., 2016) and HB 1454 (Lakew and Fekadu, 2015) gave 98.3% and 93%, respectively when the percentage of grains retained on 2.8-mm, 2.5-mm and 2.2-mm slot sieves are summed.

Malt barley grain has to be uniform in plumpness, crude protein content, grain size, moisture content, test weight, thousand kernel weight and color for the malting process. Malt barley grains of different plumpness must not be malted together because grains of different plumpness imbibe water differently (Henry and Kettlewell, 1996). This leads to uneven germination, which results in different rates of enzymatic action and, therefore, different grain modification in the malting process and the occurrence of problems in the brewing process afterward. Therefore, sieve test results for 2.8-mm, 2.5-mm and 2.2-mm slot sieves are separately provided to decide whether a malt barley variety fulfills the plumpness specifications in the malt barley standard or not.

Test weight and thousand grain weight have a highly significant positive correlation with fine grind hot water extract (Sarkar et al., 2008). The test weight result obtained from the six-rowed barley lines evaluated in the present study (50.5 kg/hl) is comparable to a study conducted in India. A test weight in the range of 50.5-71 kg/hl in a study on 131 genotypes of two-rowed and six-rowed barley of Indian and exotic origin was reported by Sarkar et al. (2008). The EBC specifies malt barley test weight in 48-75 kg/hl. Therefore,

line 17148-16 fulfilled the standard for the parameter test weight despite the absence of significant difference when compared to the check.

Moisture content is critical from harvest and storage to the final sale of malt barley. If the moisture content is beyond the set standard (11-13%), there is a risk of quality reduction or even grain loss in-store by molding and heating. Moisture level needs to be low to prevent heat damage and the growth of disease-causing microorganisms. The moisture content of malt barley should be less than 13% when stored (Henery, 2004). Cardoso et al. (2010) have found that malt barley samples with moisture content 11-11.5% after harvest and kept in plastic bags for five months resulted in the lowest germination percentage from higher moisture content. The bag with a higher moisture content range had a maximum CO_2 value of 13%, indicating higher respiration which is not suitable for safe storage. The moisture content obtained from the barley line 17148-16 (11.7%) and the check (11.8%) are not significantly different. These moisture content values are within the range required for this parameter.

There is a strong relation between grain crude protein content and the resulting malt and beer quality (Robinson et al., 2007). In this study, lines 3462-12, 3257-16, 17204-5 and the check did not show a significant difference in crude protein. All have about 14% crude protein content. The mean crude protein content of the rest of the lines ranged from 18.8% (line 16820-16) to 20.4% (line 16910-19). Excess crude protein in malt barley grain means the proportion of barley starch is less than the minimum requirement. Less starch content leads to less fine-grind hot water extract from the corresponding malt. Reduced amount of extract results in less volume of beer at the end of the brewing process. Crude protein content above the brewery standard causes haze which reduces beer quality (Robinson et al., 2007). The development of beer haze is greater when higher protein malt is used (Paynter, 2015).

The present study is in agreement with the previous results reported by Galano et al. (2008) which reported crude protein content that ranged from 8.4% to 9.5% for two-rowed barley varieties Beka, HB 120, HB 52 and Holker. The result also conformed to the crude protein standard set by the EBC (10.5-12.5% for two-

rowed malt barley and 10.5%-13% for six-rowed barley). The crude protein exhibited by the promising line 17148-16 was also comparable to the experimental result obtained from different malt barley varieties (Liben et al., 2011; Tazebchew et al., 2018; Deme et al., 2019; Bekele et al., 2020).

Crude protein content is affected by several factors. Studies by different researchers show that grain crude protein content is affected by the rate of nitrogen fertilizer applied, the variety used, seed rate and the location where the crop is grown (Liben et al., 2011; Cai et al., 2013; Kassie and Tesfaye, 2019; Bekele et al., 2020; Ojha et al., 2020). Cai et al. (2013) revealed that grain protein content is significantly and positively correlated with soluble protein in malt and diastatic power and negatively correlated with fine malt grind hot water extract. According to Paynter (1996), high protein barley grains contain higher levels of gel proteins that can limit the separation of the fine grind hot water extract from the husk by blocking filter pores. This increases fine grind hot water extract filtration time and reduces the throughput of beer through the brewery. The gel proteins also limit the amount of starch that can be broken down into malt fine grind hot water extract during modification. Another consequence of excess protein barley intended to be malted for brewing is the change of flavor profiles of packaged beer with time. Bitterness decreases with time, while sweetness increases with time after packaging (Paynter, 1996).

Malt barley protein content must not be shallow to ensure that fermentation is not limited. Yeast requires soluble proteins which are obtained from the degradation of protein in the grain during malting. For stable foam, there should be sufficient protein in beer. Beer made from low protein malts may have foam stability problems that the foam disappears rapidly (Paynter, 1996). Lacing (adhesion of foam to the side of the glass) is also limited by a lack of protein in the grain. Since the crude protein content of the promising barley line in this study (17148-16) is neither too much nor too low, the beer from the corresponding malt is expected to be free from the protein-related problems mentioned.

The barley line 17148-16 have shown comparable fine grind hot water extract value with research findings in Ethiopia and other countries. In Ethiopia, Lakew and Fekadu, (2015) registered two-rowed malt barley

variety HB 14541 developed at Holata to have an acceptable grind hot water extract value of 76%. Tamene et al., (2016) reported that the registered two-rowed malt barley Singtan (IBON-MAR p# 26) produced a fine grind hot water extract value of 78% in an experiment conducted for two consecutive years at Sinana, Gobba, Dinsho and Dodola areas. Galano et al. (2011) has also shown a fine grind hot water extract of 76.8-79.2% from two-rowed malt barley varieties Beka, HB 120, HB 52 and Holker grown at Holata. In Croatia, Mastanjevic et al. (2017) have obtained fine grind hot water extract of > 80% from malt barley varieties Tifanny and Vanessa. In a study done in India, Sarkar et al. (2008) reported a mean fine grind hot water extract of 77.93% and found a significant positive correlation with test weight, thousand-grain weight, bold grains (%), malt friability and homogeneity. In an assessment on spring malt barley in Poland, Gorzelany et al. (2019) found a mean fine grind hot water extract of 81.15%. Fine grind hot water extract requirements of the Asella Malt Factory in Ethiopia and the EBC is 77-79%. Therefore, the 79.8% fine grind hot water extract value exhibited by line 17148-16 fulfilled brewery requirement, making this line a potential material for six-rowed malt barley quality improvement endeavors in Ethiopia.

CONCLUSION AND RECOMMENDATION

Based on this study, six-rowed barley line 17148-16 fulfilled sieve test, test weight, moisture content, dry matter-based crude protein and dry matter-based fine grind hot water extract requirements. This line can be a potential source of genes responsible for grain plumpness, test weight, dry matter-based crude protein and dry matter-based fine grind hot water extract. Therefore, malt barley breeders, malt barley agronomists, malt factories and breweries in Ethiopia are advised to exploit this line. Similar work must also be carried out in the future to exploit the barley genetic resources in Ethiopia.

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COMPARATIVE ASSESSMENT OF CARBON STOCK UNDER *EUCALYPTUS GRANDIS* AND *EUCALYPTUS CAMALDULENSIS* STANDS AT KIBRIT PLANTATION FOREST, NORTHWESTERN ETHIOPIA

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ABSTRACT: *Eucalyptus* species are the dominant plantation species with greater economic and environmental values in Ethiopia. Nevertheless, little is known about the carbon stock of *Eucalyptus* species and hence, this study was aimed at estimating the carbon stock of *Eucalyptus grandis* and *Eucalyptus camaldulensis* stands under Kibrit plantation forest. Systematic random sampling was used and a total of 60 plots (10 m×20 m size) were systematically established. Trees ≥ 5 cm Diameter at Breast Height (DBH) were measured. Within each sample plot, (1 m×1 m) subplots were designed for litter and soil sample collection. Species specific allometric equations were used to estimate the tree biomass. Soil organic carbon determination was done using the Walkley Black method. The mean total carbon stock (biomass plus soil organic carbon) was significantly higher ($P< 0.05$) in *E. grandis* (351.72 ± 72.72 t/ha) compared to the adjacent *E. camaldulensis* stand (192.16 ± 24.9 t/ha). The mean total biomass carbon stock was also significantly higher in *E. grandis* (267.78 ± 73.1t/ha) than in *E. camaldulensis* stand (105.52 ± 22.8 t/ha). The mean total soil organic carbon stock was 83.94 ± 1.5 t/ha and 86.64 ± 6.2 t/ha for *E. grandis* and *E. camaldulensis* stands respectively. This study indicated the presence of significant difference in carbon storage potential between the two stands and, therefore, planting *E. grandis* is rewarding in terms of climate change mitigation.

Keywords: Biomass carbon, *Eucalyptus* plantation, Kibrit plantation, Soil organic carbon

INTRODUCTION

Forests store more than 650 billion tons of carbon, 44% in the aboveground biomass, 11% in dead wood and litter, and 45% in the soil globally (Feng et al., 2016). The carbon stock of plantation forest varies with stand age and species. A study by Du et al. (2015) reported a tree biomass carbon stock of 70.1 t/ha in six to eight-year-old eucalyptus stands. Scalenghe et al. (2015) estimated that 550 t/ha, stored in the 50-year-

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old *Eucalyptus camaldulensis* stand in Italy. Plantation forests in Ethiopia store a total of 114.48 t/ha carbon (Metz et al., 2007). Eucalyptus plantations are very efficient at carbon sequestration with average annual fixation rates of 10 ton of carbon per hectare (Marcolin et al., 2002). Plantation forests can make a very significant contribution to a low-cost global climate change mitigation and provide synergy for adaptation and sustainable development, including extending the carbon retention in harvested wood products. Plantation forest has been promoted as a strategy for carbon sequestration under afforestation and reforestation programs as well as Clean Development Mechanisms of the Kyoto Protocol (Smith, 2007). United Nations Framework Convention on Climate Change (UNFCCC) has recognized the importance of plantation forests as a greenhouse gas mitigation options, as well as the need to monitor, preserve and enhance terrestrial carbon stocks (van Kooten, 2000). Carbon sequestration projects in developing nations could receive investments from companies and governments wishing to offset their emissions of greenhouse gases through the Kyoto Protocol's Clean Development Mechanism (Fearnside,1999).

Plantation forestry is an age-old practice widespread in different forms across the diverse agro-ecology of Ethiopia. Plantation forest includes industrial/commercial, wood-lots and peri-urban plantation (Tadesse et al., 2019). Eucalyptus is the dominant genus among plantations in Ethiopia and it is a source of fuelwood, construction material, and income generation for smallholder farmers. According to (FAO, 2011), there are about 55 species of Eucalyptus in the country of which *E. globulus, E. camaldulensis, E. citriodora, E. grandis* and *E. saligna* are widely distributed across the country. Eucalyptus covers 58% (500,000 ha) of the total plantation followed by *Cupressus* (29%), *Juniperus procera* (4%) and *Pinus patula* (2%) (Gil et al., 2010). *Eucalyptus* species are superior in their growth performance compared to other exotic and native species which encourages farmers to plant large numbers on small areas of land and manage to yield a variety of products (Dessie et al., 2019; Tesfaw et al., 2021).

Although extensive studies have been done on the importance and management of eucalyptus in Ethiopia (Mekonnen et al., 2007; Gil et al., 2010; Dessie et al., 2019), information on its carbon stock potential is

lacking. Moreover, prior studies have indicated that carbon stock potential of eucalyptus varies among species (Madeira et al., 2002; Keith et al., 2012). To understand the significance of eucalyptus species for climate change mitigation, carbon stock quantification needs to be considered. Therefore, this study was initiated with the objective to estimate and compare the carbon stock in *E. camaldulensis* and *E. grandis* stands of Kibrit Plantation Forest.

MATERIALS AND METHODS

Study area

This study was conducted in Kibrit Plantation Forest Awi Zone, Amhara Region, Ethiopia (Figure 1). It is situated between 10°56'40"N and 10°57'10"N latitude and 36°31'50"E and 36°32'20"E longitude.

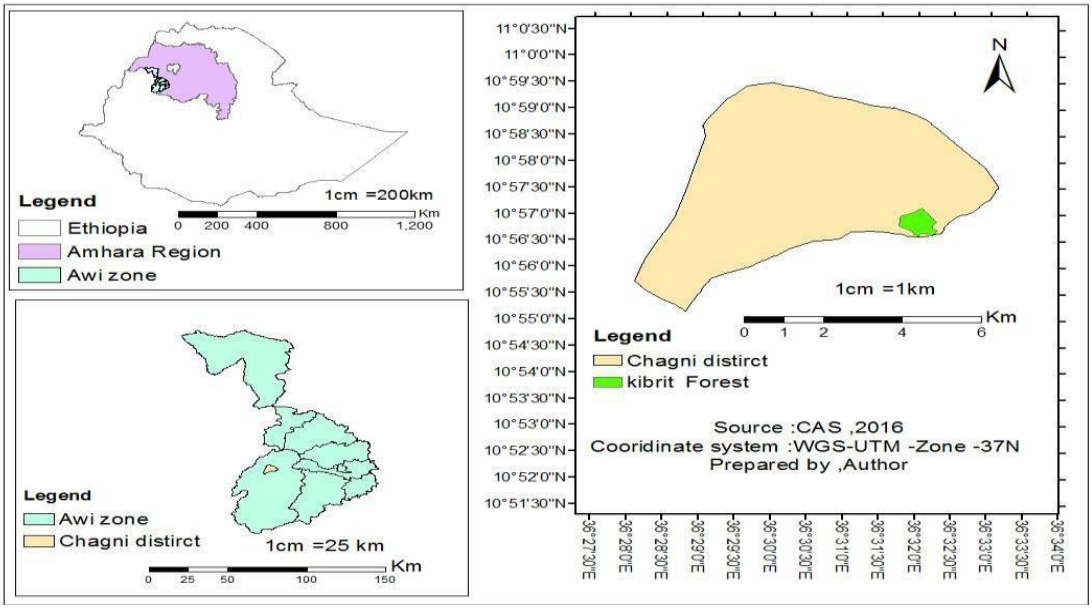

Figure 1. Map of Ethiopia showing the study area.

The study area is characterized by a unimodal rainfall distribution with the rainy season occurring from June to September and often continuing usually continued with a less pronounced wet period up to October. According to the weather data obtained from Chagni Meteorological Station, the mean annual rainfall and monthly temperature of the study area range from 1300 mm to 1800 mm and from 18.6°C to 28°C, respectively. Elevation ranges from 1627 m to 1793 m.a s.l The soil type of the study area is grouped under

Nitosols. Kibrit Plantation Forest covers an area of 57 ha and consists of *Eucalyptus grandis* (10 ha), *E. camaldulensis* (8 ha), *Grevillea robusta* (12 ha), and *Pinus patula* (11 ha) stands. Both *E. grandis* and *E. camaldulensis* are 28-year-old stands and were selected for this study due to similar climatic, topographic, edaphic, age and similar silvicultural management intervention systems.

Eucalyptus grandis is an evergreen tree 40-55 m tall, growing to a diameter of 2 m; with an excellent straight trunk and wide-spreading thin crown, and self-pruning of branches in plantations. It grows successfully in Moist and Wet *Weyna Dega* Agro climatic zones of Ethiopia and performs well on light and medium neutral to acid soils that are free draining and moist up to 1700-2500 m above sea level (Tesema, 2007).

Eucalyptus camaldulensis is a tall evergreen tree to 30 m, deeply branched but with a long straight pole. It is widely distributed in its native Australia and is one of the first Eucalyptus species used elsewhere. It grows well in semi-arid regions and tolerates a long dry season as well as some salinity. It does well in deep silt or clay soil in Dry and Moist *Kolla* Agro climatic zones up to 1,200 - 2,800 m a.s.l. (Tesema, 2007).

Sampling techniques

Systematic random sampling technique was employed and a total of 60 sample plots (30 for each stand) with 45 m distance between plots were selected using pragmatic approach. Considering the shape of the two stands, 16 transect lines (10 for *E. camaldulensis* and 6 for *E. grandis* stand) were laid with the space of 45 m between stands. Rectangular sample plots with an area of 200 m^2 (10 m×20 m) were used for the measurement of DBH and total height. Moreover, 1 m×1 m sub-plots were used for litter and soil sample collection. Soil samples were collected from the center and corners of each sub-plot (Figure 2).

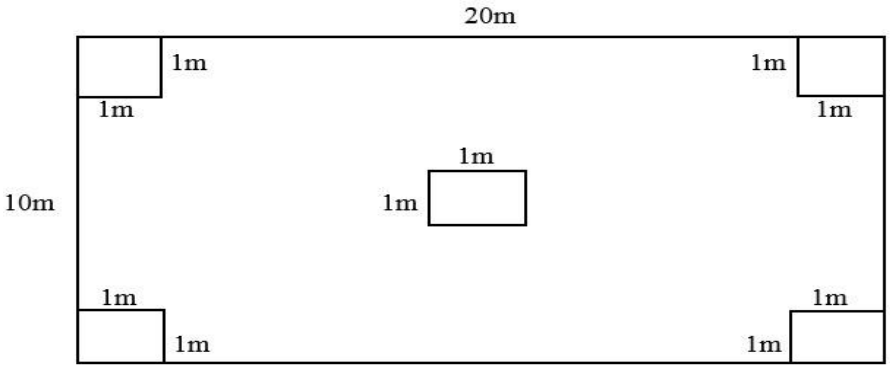

Figure 2. Sample plot design.

All trees of *E. grandis* and *E. camaldulensis* with DBH ≥5 cm in main plots were measured using 50 cm graduated caliper and Laser Ace 1000 rangefinder for DBH and height, respectively. DBH was measured from two perpendicular directions and an average value was recorded (Snowdon et al., 2002).

Litter sampling

The dead leaves, branches, twigs, flowers, and dead wood with a diameter of less than 10 cm was considered as litter and a total of 180 litter samples (90 for each stand) were collected from 3 sub-plots chosen randomly using the lottery method out of 4 sub-plots. Samples were weighed, coded and then evenly mixed to prepare composite samples. From each sample 100 g of composite samples were taken to the laboratory for the determination of oven dry mass (Pearson et al., 2007). Composite litter sub-samples, were air-dried for one day and then, oven-dried at 70°C for 24 hours to determine constant oven dry mass (Ullah and Al-Amin, 2012; Negash and Starr, 2015). The samples were weighed, grinded using mortar and pestle, then sieved by 2 mm mesh. The Loss on Ignition (LOI) method was used to estimate the percentage of carbon in the litter (Pearson et al., 2005). From the oven dried grinded sample, 3.00 g of each litter subsamples were taken in pre-weighed crucibles, and then put in the furnace for two hours to ignite. Then, the crucibles were cooled slowly for two hours inside the furnace. After cooling, the crucibles along with ash were weighed and litter organic matter fraction was calculated (Allen et al., 1986).

Soil sampling

Soil samples from three sub-plots were chosen randomly using the lottery method from four sub-plots at the corners of sample plots and the center. A total of 540 soil samples were collected across the study plots from three soil depths (0–20 cm, 20–40 cm, and 40–60 cm) using soil Auger. All wet soil samples were coded and evenly mixed per sample plot to prepare 180 composite samples. From each sample 500 g was taken to Debre-Markos Soil Laboratory Center for the analysis of carbon content. Bulk density samples were collected using core sampler with a volume of 392.5 cm^3 (20 cm length and 5 cm diameter) and samples were taken to the laboratory for the determination of soil bulk density. Soil samples were oven-dried at 105°C for 48 hours and weighed (Pearson et al., 2007) and bulk density was determined following the core method (Blake and Hartge,1986). Soil organic carbon content analysis was done following the Walkley and Black method (Schnitzer, 1982).

Carbon stock estimation

Aboveground biomass carbon stock estimation

Locally developed allometric equations give reliable biomass estimate than generic equation and hence the aboveground biomass of *E. grandis* stand was calculated using species specific allometric equation developed by Fantu et al. (2007) (Equation 1) and that of *E. camaldulensis* stand was calculated using an allometric equation developed by Hailu (2002) (Equation 2).

$$logY = -1.381 + 2.893(logDBH) \dots\dots\dots\dots\dots\dots \quad Equation\ 1$$

$$AGB = 0.0155 * (DBH^{2.5823}) \dots\dots\dots\dots\dots\dots\dots \quad Equation\ 2$$

Where, logY = aboveground biomass (kg/tree), AGB = aboveground biomass (kg/tree), DBH = diameter at breast height (1.3 m).

Belowground biomass was estimated using IPCC root –to- shoot ratio value of 0.26 for tropical dry forests (IPCC, 2006) as follows.

$$BGB = AGB * 0.26 \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots Equation\ 3$$

Where, BGB = belowground biomass (kg/tree), AGB = aboveground biomass (kg/tree) and 0.26 is conversion factor.

The biomass was converted to units of carbon stock by multiplying by a carbon fraction of 0.5 (Pearson et al., 2007).

Litter biomass and carbon stock estimation

According to Pearson et al., (2005), estimation of the amount of biomass in the litter is calculated as:

$$LB = \frac{Wfield}{A} * \left(\frac{Wsubsampledry}{Wsubsamplefresh}\right) * \frac{1}{10000} \quad ---- \quad Equation\ 4$$

Where, LB = Litter biomass (t/ha); Wfield = mass of wet field sample of litter sampled within an area of size $1m^2$ (g);

A = size of the area in which litter samples were collected (ha);

Wsubsample (dry) = mass of the oven-dry subsample of litter taken to the laboratory to determine moisture content (g)

Wsubsample (fresh) = mass of the fresh sub-sample of litter taken to the laboratory to determine moisture content (g).

Carbon stock of litter was then calculated by multiplying the biomass of litter per unit area with the percentage of carbon determined for each sample.

$$LBC = LB * \%C \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Equation\ 5$$

Where, LBC= total carbon stocks in the litter (t/ha) and %C = carbon fraction which was determined in the laboratory.

Soil organic carbon stock estimation

According to Pearson et al. (2007), the soil organic carbon was calculated as follows:

$$SOC = BD * SD * \%C * 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Equation\ 6$$

Where: SOC = soil organic carbon (t/ha), BD = bulk density (gcm^{-3}), SD = soil depth (cm) and %C = carbon fraction and expressed as a decimal fraction.

The total carbon stock density was calculated by adding the carbon stock densities of the individual carbon pools using Pearson et al. (2005) formula as follows;

$CT = AGC + BGC + CL + SOC$ ……. $Equation\ 7$

Where,

CT= Carbon stock density for all pools (ton/ ha),

AGC= Carbon in above -ground tree biomass (ton C/ ha),

BGC = Carbon in below-ground biomass (ton C/ ha)

CL= Carbon in dead litter (ton C/ ha) and

SOC = Soil organic carbon (ton C/ha).

Data analysis

Tree DBH, total tree height, wet and oven dry mass of soil and litter samples were analyzed using Statistical Package for Social science (SPSS version 23). Prior to the statistical test, all data were subjected to the Kolmogorov–Smirnov test to check normality. Independent sample t- test was used to compare the mean carbon stock of the two plantation stands. A 95% confidence level was used to evaluate the statistical tests.

RESULTS

Stand characteristics

The mean DBH was insignificant between stands whereas height, basal area and stem per hectare were significant ($P< 0.05$). The mean basal area of *E. grandis* was higher than *E. camadulensis* by a factor of 1.1 while its number of stems per hectare was relatively lower (Table 1).

Table 1. Stand characteristics of *E. grandis* and *E. camadulensis* (Mean ± standard deviation)

Stand characteristics	*E. grandis* (n=30)	*E. camaldulensis* (n=30)	*P-value*
DBH (cm)	29.6 ± 2.3	28.3 ± 2.3	0.052
H (m)	34.4 ± 1.0	24.7 ± 1.7	0.037
BA (m2ha-1)	35 ± 0.3	32 ± 0.1	0.023
Stem/ha	1507 ± 18	1555 ± 16	0.000

Carbon stock

There were significant differences in total carbon, AGC, BGC and SOC between the stands with significantly higher values of total carbon, AGC and BGC in *E. grandis* and a significantly higher value of SOC in *E. camadulensis*. On the other hand, there is no significant difference between the stands for litter carbon stock (Table 2).

Table 2. Mean ± standard deviation carbon stock of the different carbon pools of *E grandis* and *E camaldulensis* stands (30 plots each)

	Mean C (t/ha) of the different Carbon pools				
Plantation stands	AGC	BGC	LC	SOC	Total
E. grandis	212.50 ± 58	55.26 ± 15.1	0.02 ± 0.00	83.94 ± 1.52	351.72 ± 72.72
E. camaldulensis	83.73 ± 18.1	21.77 ± 47	0.02 ± 0.00	86.64 ± 6.23	192.16 ± 24.9
P value	0.000	0.000	0.079	0.000	0.000

DISCUSSION

Although the two stands belong to the same age, similar silvicultural management system and agro-ecology, their height, basal area and stem number were different. This difference might be, due to the difference in species characteristics. Silvicultural management system and agroecology could result in differences in the same species. Alem et al. (2015) recorded a height of 16.9 ± 5.3, basal area of 5.3 ± 0.03 and stem/ha of 822 ± 244 for a 27-year-old *E. camadulensis* stand in southwest Ethiopia which is quite smaller compared to our results.

Eucalyptus grandis stand stored a substantial amount of carbon than *E. camaldulensis* stand. The variation may be due to difference in aboveground tree biomass allometric equation used. There was no significant difference in litter carbon stocks between the two stands. The mean biomass carbon stock of *E. grandis* (267.78 t/ha) and *E. camaldulensis* (105.52 t/ha) was higher than the mean biomass carbon stock of Eucalyptus plantations (92.26 t/ha) in Ethiopia (Metz et al., 2007). Moreover, the mean aboveground carbon stock of *E. grandis* stand was higher than the mean aboveground carbon stock of plantation forests reported in Woody Biomass Inventory Strategic Planning Project (WBISPP) in Ethiopia (WBISPP, 2004). However, the mean aboveground carbon stock of *E. camaldulensis* stand was less than that reported in WBISPP (2004). This variation may be attributed to the difference in the allometric equations applied, silvicultural management system, climate and soil type of the plantation stands. Carbon sock estimation using species specific- allometric equation provides better and relatively reliable results than generic equation.

CONCLUSION

This study indicated that *E. grandis* and *E. camaldulensis* species stored substantial amount of carbon in their biomass (aboveground, belowground, litter and soil). *E. grandis* had stored enormous amount of total carbon than *E. camaldulensis*. Thus, planting *E. grandis* would be encouraging compared to *E. camaldulensis*. Overall, the species can be considered in plantation developments for climate change mitigation.

ACKNOWLEDGMENTS

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REVIEW OF THE STEPS TO ESTABLISH A SHEEP AND GOAT COMMUNITY-BASED CONSERVATION AND BREEDING PROGRAM (CBCBP) IN ETHIOPIA

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ABSTRACT: This review paper presents the prominent steps to develop a set of principles for breeding criteria as a step wise application of Community Based Conservation and Breeding Program (CBCBP) in Ethiopia. Sheep and goats are an important commodity for smallholder farmers in Ethiopia and are considered a crucial source of cash income. They are sources of meat, milk, wool, hides, and manure. The 2021 national average sheep and goat populations in Ethiopia were 96.4 (range 95.3–97.7) million. Nine sheep and eight goat breeds are identified in the country. However, scientific breeding, conservation, and production activities are lagging. To maximize conservation and productivity of small ruminants in Ethiopia, stakeholders need to take action on limitations and leverage on available opportunities urgently. Thus, this paper provides a set of principles to guide the establishment of CBCBP to contribute to the sustainable production of sheep and goats in the country. Improving management includes increasing the efficiency of production, controlling the use of resources, and maintaining animal health and welfare. Selecting breeding stock requires assessing genetic parameters and economic value of traits to be included in the breeding program which uses a recorded data in the selection process. Moreover, facilitating marketing through the value chain can add value to the sustainability of the sector. Steps required for the successful implementation of sheep and goats conservation and improvement through participation of the community were also addressed.

Keywords: Breeding, Community based, Conservation, Goat, Production, Selection, Sheep

INTRODUCTION

In Ethiopia, indigenous sheep and goats play an important role in the lives of resource-poor farmers, providing a variety of tangible (income, meat, milk, skins, and manure) and intangible benefits (savings, insurance against emergencies, and cultural, and ceremonial values). These benefits vary depending on the culture, socio-economy, and agroecology, of the farmer. Thus, sheep and goats are an important asset for smallholder farmers in Ethiopia, providing much-needed resources for their households (Sherif and Alemayehu, 2018).

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Ethiopia has one of the largest populations of small ruminants in the world, with an estimated 42.9 million sheep and 52.5 million goats (CSA, 2021). This accounts for around 10% of Africa's and 4% of the world's small ruminant population (FAO, 2019). The small ruminant's population is large and rapidly growing contributing about 2% of the national annual GDP (Assefa et al., 2021). Nine sheep and eight goats were characterized and identified as breeds (Gizaw et al., 2007; Mekuriaw, 2016). Despite the presence of large number of indigenous sheep and goats in the country however, their economic value is far lower than it should be (Legese and Fadiga, 2014). This is due to the high kid mortality rates (up to 40%), a low offtake rate, and a limited range of production outputs with no signs of intensification (Jemberu et al., 2022).

To effectively conserve animal genetic resources, it is necessary to identify and document the breeds adequately (Assefa et al., 2021). Therefore, the objective of this review was to compile information on the sheep and goat production systems for establishing community base breeding and conservation programs (CBCBP). It also identifies the opportunities and limitations of research-based production and develop the basic steps of breeding and conservation criteria that would fulfill the gaps in the design of CBCBP. It is hoped that this review and its recommendations will help in improving the existing sheep and goat production systems and establishing more efficient CBCBP in different parts of the country. Such improved systems would help to increase the sustainability of livelihoods of the communities and reduce the environmental impacts of livestock production.

The research conducted in Ethiopia on small ruminant production and reproduction has been well-documented in various forms. To give a better understanding of the counry's small ruminant production and reproduction, a variety of different sources were used in this review which include policy documents, published guidelines, and working papers from agricultural research centers and institutes. Furthermore, to provide further insight into the work, supportive references were included from the research results of characterizations conducted in the country. Papers from organizations like CSA (Central Statistical Agency), ICARDA (International Center for Agricultural Research in the Dry Areas), ILRI (International

Livestock Research Institute), EIAR (Ethiopian Institute of Agricultural Research), EBI (Ethiopian Biodiversity Institute), and FAO (Food and Agriculture Organization of the United Nations) have been extensively used to gain a greater understanding of the data.

Sheep and goat production systems in Ethiopia

In Ethiopia, sheep and goat production systems are an important component of the country's agricultural sector, with livestock contributing more than 25 percent of the country's agricultural GDP, and being the major source of food and income for the majority of rural households (Alemu and Bantider, 2020). They are primarily used for meat, milk, and wool production, and also provide important services such as soil fertility improvement, and manure production for crop production (Admassie, 2019). The two major production systems are, the mixed crop-livestock production system and the pastoral and agro-pastoral production systems.

Mixed Crop-Livestock Production Systems (MCLPS)

Mixed Crop-Livestock Production System (MCLPS) is a type of agricultural system that integrates crop and livestock production activities. It involves the integration of multiple crops and livestock species, grown in a diversity of production systems, including intensive, semi-intensive, and extensive systems (Edwards et al., 2020). This type of integrated system attempts to optimize the use of available natural resources and maximize the economic and environmental benefits of agricultural production. MCLPSs can play a key role in promoting agricultural sustainability, by providing a productive and efficient use of resources, while also reducing the negative impacts that agricultural activities can have on the environment (MacMillan et al., 2018). Moreover, the integration of crops and livestock can also increase the efficiency of nutrient cycling, by improving the availability of nutrients for crop production, while at the same time reducing the need for external inputs, such as chemical fertilizers (Kumar et al., 2019). A crop-based mixed farming system is often found in highland agro-ecological zones where the altitude ranges between 1500 and 3000 meters above sea level (masl). This production system is favored by the favorable climate for farming crops and

raising livestock. Crop production is the main focus of this type of farming system, with livestock production playing a secondary role. Furthermore, the presence of livestock in a farming system can also help to break pest and disease cycles, thus helping to increase crop yields (Solomon et al., 2014).

Pastoral and Agro-Pastoral Production Systems

Pastoral and agro-pastoral production systems are common in Ethiopia. These production systems are based on the use of animals (livestock) as a source of food and income. The pastoral system involves keeping of cattle, sheep, goats, and camels, while the agro-pastoral system is a combination of animal husbandry and crop cultivation.

Pastoralists in Ethiopia usually move their herds from one area to another in search of water and grazing land. This is in contrast to agro-pastoralists, who tend to stay in one area and use animals to supplement their crop production. Livestock provides food, such as milk and meat, and is also an important source of income for many households. In addition, animal products, such as skins and hides, are used for trade.

The majority of small ruminants, including 40% of sheep and 40% of goats, are concentrated in pastoral and agro-pastoral areas (Asfaw and Jabbar, 2008). These production systems are often kept under extensive systems, which make them major sources of livestock products for the Ethiopian export market (Legese and Fadiga, 2014). The system can either be transhumant (the whole system moves periodically), or sedentary (the system has limited movement) (Solomon et al., 2008). This production system has a relatively low human pressure on natural resources and a higher land holding per household than in the mixed farming system.

Significance of Sheep and Goat Production in Ethiopia

The importance of sheep and goat production in Ethiopia lies in its environmental, social and economic contributions. Sheep and goat production can have a positive impact on the environment; by reducing reduce land degradation by providing a source of food and by providing fertilizer for the land (Lemma et al., 2018). Socially, sheep and goat production play a major role in the lives of many Ethiopians. It is an important part

of the traditional culture, an important source of nutrition and income and providing employment opportunities in rural areas (Mesfin et al., 2017). Economically sheep and goats contribute to income generation as well as to the country's GDP. It contributes 154,000 tons of meat, about 40% of fresh skins, and 92% of the value of semi-processed skin and hide export trade (Mourad et al., 2015). Ethiopia has the potential to export 700,000 sheep and 2 million goats annually as well as supply 1,078,000 sheep and 1,128,000 goats for the domestic market (Sheriff and Alemayehu, 2018).

The future growth in Ethiopian small ruminant meat production is likely to come from an increase in the number of slaughtered animals or slaughter volume. This is because the current dressing percentage of Ethiopian sheep and goats is very low, which has been reported to be 42.5% for sheep and between 42 and 45% for goats. Furthermore, this low dressing percentage of indigenous small ruminants has been found to occur mainly due to poor nutrition and husbandry practices, which have been further exacerbated by the effects of drought (Legese and Fadiga, 2014). The demand for animal products is expected to increase as a consequence of urbanization, population growth, and increased income (Westhoek et al., 2011). To fulfill the demand, designing and implementing appropriate genetic conservation and improvement programs that can boost productivity (Solomon, 2014). Community based conservation and breeding program of small ruminants is advantageous since the breeding flocks are located within the production environment and have potential genotype-environment interactions.

Limitations and Opportunities for Production

Despite the sector's importance, small ruminant production is faced with several challenges that limit its potential for development. Especially, animal genetic resource conservation and sustainable utilization programs have faced many limitations in the country. The major limitations include lack of adequate financial resources to support existing programs, lack of technical capacity and infrastructure to effectively implement such programs, lack of coordination between different stakeholders to implement successful conservation program, lack of legal and policy frameworks to protect animal genetic resources (Assefa et

al., 2021). According to Aklilu (2008) and Hassen and Tesfaye (2014), disease, lack of access to veterinary services, access to good breeding stock, lack of animal records, and an established marketing chain also limit the utilization of these resources. Inappropriate livestock development policies may also be another factor that contributes to the low productivity of small ruminants (Getachew et al., 2010; Gizaw et al., 2013; Aleme and Lemma, 2015; Jemberu et al., 2022).

To improve small ruminant's productivity, it is necessary to address all of these factors in a coordinated and effective manner. This can be achieved through the implementation of appropriate policies and interventions on nutrition, veterinary services, infrastructure, technology, financial services, genetics, selection, and livestock development.

Improvement of sheep and goats - selection versus cross-breeding

The selection of indigenous versus cross-bred animals for breeding purposes has been an ongoing debate. Indigenous breeds of sheep and goats have been selected over the years based on their hardiness and ability to survive in harsh conditions. These animals are considered to be well adapted to the climate and environment of Ethiopia and their meat is of high quality. On the other hand, cross-bred animals are known to have higher yields and have become increasingly popular in the country (Kebede and Bekele 2014). The Government of Ethiopia has provided subsidies to farmers for the purchase of high-yielding and disease-resistant animals and has also established a national animal identification and traceability system. Additionally, research into the genetic improvement of sheep and goats has been carried out to improve the quality of the animals (Alemu and Stevenson, 2017).

Village-based cooperative breeding programs have been established for Menz, Horro, and Bonga sheep breeds to improve their reproductive and growth performance (Gizawu et al., 2013). This program had a positive effect on the litter size, litter weight per ewe, and pre-weaning lamb mortality of the Doyogena sheep. The litter size at birth (LSB), litter size at weaning (LSW), total live weight at birth (TLWB), and total live weight at weaning (TLWW) of the Doyogena sheep managed under this program were 1.57±0.02

lambs, 1.50±0.02 lambs, 5.24 ±0.09 kg, and 24.14± 0.69 kg, respectively (Kebede et al., 2022). This indicated that the community-based breeding program had a positive influence on the reproductive and growth performance of the Doyogena sheep. The authors also noted that the ongoing selection program has increased the survival rate of lambs and proposed that the improvement of the environment in the flock and special care for multiple-born lambs and small lambs would lead to further lamb survival (Kebede et al., 2022). Village-based cooperative breeding programs are could thus be an effective way to improve the reproductive and growth performance of the Doyogena sheep, as well as improve the survival rate of lambs. For a long time, Ethiopia has attempted to improve goat production by crossing exotic breeds with local breeds, but the results were not satisfactory. A review on Boer goat's impacts in Ethiopia showed that that the growth rate of Boer goats and their crosses was lower than that of native breeds (Mustefa (2022). Furthermore, local goats had a higher conception, kidding, and abortion rate than Boer and its crosses. At the smallholder level, a sustainable genetic improvement without sacrificing diversity, such as within breed selection, is recommended since exotics had a high mortality rate and low survival rate compared to pure local breeds (Mustefa, 2022). Thus, community-based breeding programs that focus on breed selection may be used to improve goat production. To this end, the Ethiopian Biodiversity Institute (EBI) is collaborating with other research and higher learning institutes are implementing community-based conservation and breeding programs.

The selection requirement for the breeding program

Participatory identification of breeding objectives, animal identification, performance recording, and selection of the best animals based on recorded performance and farmer criteria, pooling small flocks, and arranging sire use and sharing systems are integral components of the breeding programs (Haile et al., 2018). These components are essential to ensure the success of any breeding program, as they enable farmers to select the animals with the highest genetic merit based on performance and their criteria. However, for resource-poor farmers, recording individual animal performance and their pedigree is often too complex to

apply, and as such, they rely on their criteria when selecting animals to be parents of the next generation. Pooling of small flocks and arranging sire use and sharing systems can also help resource-poor farmers to increase their access to superior genetics, as they can share the cost of their own sires and/or use sires from other flocks (Sherif and Alemayehu, 2018).

Selection criteria for smallholder farmers reflect their breeding activities and farming philosophies, and the criteria vary among different production systems and species (Roessler et al., 2008). The criteria are also different between sexes. For instance, for males, appearance (Getachew, 2008), body size (Zewudu et al., 2012), tail type, color, and height (Gizaw, 2008) are given due emphasis during selection. On the contrary, rams and bucks with black color, poor body condition, and small size are not preferred for breeding purposes, and male animals of such character are usually culled at a young age or sold or slaughtered at home (Gizaw, 2008; Zewudu et al., 2012). Similarly, in selecting ewes and does, appearance, coat color, and lamb survival (Getachew, 2008; Niggussie et al., 2013) and litter size and lamb growth (Gemeda et al., 2011) were reported as the most important selection criteria, yet those that are black-colored, old-aged, poor-conditioned, and have a long lambing interval are culled (Zewudu et al., 2012; Yenesew et al., 2013). This is because such features are deemed to be associated with low reproductive performance and poor productivity (Gemeda et al., 2011; Niggussie et al., 2013).

Despite variations in production systems and sex, selection criteria for small ruminants in Ethiopia are generally geared towards a single market-driven trait. For example, in crop-livestock mixed production systems, the primary focus is usually on a fast growth rate to produce sheep and goats that can fetch a higher market price (Zergaw et al., 2016; Haile et al., 2018).

Community-based conservation and breeding programs

The Community-based Breeding Program (CBCBP) is a village-based breeding activity planned, designed, and implemented by smallholder farmers, either individually or in cooperatives, to effect genetic improvement in their flocks and conserve indigenous genetic resources. Unlike conventional crossbreeding

and nucleus-based selection, CBCBP involves the local community at every stage (from planning to operation) and takes their indigenous knowledge of breeding practices and objectives into account (Gizaw et al., 2013; Mustefa, 2022). The CBCBP was designed as an alternative to the conventional crossbreeding and nucleus-based selection programs, which had failed to achieve the desired outcomes in terms of genetic improvement and conservation of indigenous genetic resources in Ethiopia (Gizaw et al., 2013).

To design an appropriate and feasible CBCBP, the basic steps involve the selection of the communities and breeds, analysis of the production system (including livelihood strategies), characterization (phenotypic and molecular) of the breeds, definition of the breeding objectives, and evaluation of the breeding programs (Haile et al., 2011; Wurzinger et al., 2011). Despite the attempts made as early as 2003, with Washera and later with Gumz sheep (Amhara region agricultural research institute (ARARI) research directory), the development of these CBBP has been unsuccessful due to a lack of proper knowledge among researchers on the new approach (Gizaw et al., 2013). Therefore, it is essential to understand the production system, the socio-economic factors, and the genetic resources of small ruminants prior to implementing CBCBP.

In this regard, EBI is working on the development of breeding programs that are based on the conservation of animal genetic resources. The main focus of the breeding program is to develop animal breeds that are adapted to local conditions and have genetic diversity that can withstand environmental changes (Mustefa, 2022). The breeding program is also designed to create breeds that can produce higher yields of milk, meat, and eggs, and to improve animal health and welfare (Getachew et al., 2016). To achieve this goal, EBI has created a network of community-based animal genetic resources conservation areas (CAGRCAs) in different regions of Ethiopia (Assefa et al., 2021). These CAGRCAs are managed by local communities and are used for breeding, raising, and protecting animal genetic resources. In addition, EBI has launched various capacity-building activities to promote the conservation of animal genetic resources. These activities include training local communities on animal breeding and management techniques, organizing seminars, workshops, and educational trips to increase awareness, and providing technical and financial support to

local communities that are involved in the conservation of animal genetic resources (Getachew et al., 2016). The Institute also guides communities on how to use animal genetic resources sustainably (Assefa et al., 2021).

Several community-based sheep breeding programs (CBCBP) have been implemented in Ethiopia by the national agricultural research centers (Bako, Bonga, Debre Berhan, and Worer) and some higher institutions in collaboration with ICARDA-ILRI (Gutu et al., 2015). The programs have been implemented in four sites (Horro, Bonga, Menz, and Afar), and detailed information has been provided by Gemeda (2011), Haile et al. (2011), and Mirkena (2011). The CBCBP programs have achieved some major successes, such as increased body weights at birth, increased number of births, a better market outlet, lambs with a bigger size, an attractive color that fetch a better market price, reduced mortality rates, better awareness about inbreeding and the need for breeding rams, and the formation of well-functioning cooperatives (Haile et al., 2011; Gizaw et al., 2013; Gutu et al., 2015).

Despite these efforts, the development of breeding programs in line with the conservation of animal genetic resources is still limited in the country. To address this issue, EBI is working on strengthening the legal and institutional framework for the conservation of animal genetic resources (Mustefa, 2022). The Institute has also developed programs to increase awareness and promote research activities in the area of animal genetic resources conservation and aims to further build the capacity of local communities to better protect and manage these resources (Getachew et al., 2016). The Institute is also working on establishing an animal genetic resource database that will enable researchers, breeders, and policymakers to gain access to accurate and up-to-date information on the characteristics and diversity of animal genetic resources (Assefa et al., 2021).

This would enable the institutes and research centers to maximize the genetic gains of the breeding programs through their research and community service endeavors (Getachew et al., 2016; Haile et al., 2018).

Moreover, policy documents and literature need to be consulted to provide further insight into the challenges and solutions related to the implementation of the CBCBP programs.

Criteria for establishing CBCBP in Ethiopia

Communities have a central role to play in biodiversity conservation, and a community-based breeding and conservation program is one way to ensure the protection of valuable species in a given area. This type of program involves the collaboration of local stakeholders in identifying, managing, and conserving threatened species, as well as developing a breeding program to increase the populations of those species. CBCBP is designed to be a low-input system in which smallholder farmers take a leading role and fully participate in developing and implementing the program (Gizaw et al., 2013; Haile et al., 2018; Assefa et al., 2021). The community must be given ownership of the program and benefits must be shared among members of other communities, as well as their traditional knowledge being utilized and upgraded. Additionally, clear and measurable output must be set (Meuller et al., 2015; Assefa et al., 2021).

The success of a community-based conservation and breeding program also depends working with local, regional, and national governments to provide resources and support for the program. It also requires the establishment of effective communication and coordination among stakeholders, including the community, local and regional governments, NGOs, universities, and research institutes, to ensure that the program is successful (Kamal et al., 2018).

This guideline was prepared based on the experience of community-based breeding programs that have been implemented in Ethiopia (Menz, Bonga, and Horro sheep breeding), Mexico (goat breeding), and Peru (llama breeding), the experience of Debre Berhan Agricultural Research Center in improving the Menz and Wollo sheep breeds, (Gizaw et al., 2013) and an in situ conservation of animal genetic resources by EBI. The major steps are: breed, site, and community selection, awareness creation for participants in the community, identification of breeding objectives, animal identification, data collection, and recording,

recruitment of enumerators from farmers' communities, animal selection and mating, sire use arrangement and organizing cooperatives and encouraging community ownership.

Breed, site, and community selection

When selecting a site and a community for a community-based breeding program, it is important to consider the environment, local resources, and the needs of the target population. A breeding program should be established in an area that has suitable environmental conditions to support the species of interest and is close to existing resources such as water and food sources, as well as veterinary and animal husbandry services. The site should be in a location that is easily accessible to the target community (population), so that the program can be properly monitored and managed. The target community should also have the capacity to be able to provide the necessary resources and support to sustain the breeding program.

A targeted breed for CBCBP needs to be selected based on the risk status and economic contribution of the breed to society, the contribution of the breed to the genetic diversity of the species, and previous characterization work on breeds to identify the targeted breed (Assefa et al., 2021). Once the breed has been defined, an appropriate site should be identified, considering the breeding tract of the breed. Local development agents and other stakeholders working on the breed should participate in site selection (Gizaw et al., 2013). The relative accessibility of the site to roads and markets, the presence of institutions supporting the community, the potential of the area for the conservation and improvement of the selected breed(s) need to be considered (Mueller et al., 2015). The breeders should be trained in the best practices of conservation and sustainable use of the breed and the techniques of artificial insemination and other reproductive technologies (Tiwari et al., 2018). Local institutions should be involved in the project, and the local government need to be consulted. It is important to ensure that the breeders are not only aware of the importance of genetic diversity but also that they understand the risks of inbreeding and the need to select animals with good reproductive performance (Kumar et al., 2019). It is also essential to prioritize the conservation and improvement of the breed to ensure its long-term sustainability (Rani et al., 2020).

Awareness creation for participants in the community

Awareness creation is a key element of any successful community-based breeding program. An effective program must ensure that the participants in the program, including farmers, extension workers, researchers, and other stakeholders, are aware of the objectives, activities, and outputs of the program. Awareness creation is an ongoing process that should be addressed at all stages of the program, from planning to implementation (Kumar and Rajaram, 2015).

At the initial planning stage, it is important to create awareness among the participants about the objectives, scope, and potential benefits of the program. This will help to ensure that all stakeholders are involved in the planning process and are aware of the activities and outputs of the program (Mbogga and Wambugu, 2019). Implementing community-based breeding programs, mainly, animal identification and performance recording, is a new concept for most Ethiopian livestock keepers. Initially, some farmers may refuse to collaborate in data collection and recording. Hence it is important to raise awareness about the importance of such activities to ensure the success of the breeding program.

Identification of breeding objectives

The first step in identifying breeding objectives is to understand the local production system. In Ethiopia, the majority of sheep and goats are kept under extensive management systems with limited inputs hence the production system relies heavily on natural resources, such as pasture and browse, and the animals have limited access to veterinary care and supplementary feed (Bantider et al., 2017). As a result, the productivity of sheep and goats is largely determined by their ability to survive and reproduce in the local environment. Thus, a major breeding objective for sheep and goats should be to select traits that enhance their ability to survive and thrive in the local environment. This could include selecting traits such as disease resistance, heat tolerance, and good foraging ability (Kassa et al., 2014).

Another important breeding objective for sheep and goats in Ethiopia is to select traits that increase the productivity of the animals. This could include selecting traits such as increased milk yield, increased

growth rate, and improved reproductive performance and as well as to maximize their profits (Tizazu et al., 2017; Eshete et al., 2018).

Finally, it is important to consider the socio-economic implications of breeding objectives. In Ethiopia, smallholder farmers are the main producers of sheep and goats, and their livelihoods are dependent on the success of their herds. Thus, it is important to select traits that enable the animals to perform effectively in the local environment, while also being economically viable for the farmers (Gebre et al., 2016). To determine the selection criteria of the farmers, a mix of approaches may be used, such as individual interviews, group discussions, and workshops. Additionally, the rankings of the live animals can be assessed, including the ranking of known and unknown animals to the farmers (Haile et al., 2011; Gizaw, 2013).

Animal identification, data collection, and recording

Animal identification, data collection, and recording are important aspects of animal husbandry. Animal identification includes the marking of individual animals, species-based identification, and breed-based identification. Data collection is the process of collecting, analyzing, and recording information about animals, such as age, sex, breed, health status, production, and performance. Recording is the process of registering and tracking information about animals and their environment. It includes tracking animal movements and interactions, recording animal health histories, and documenting the environmental conditions in which the animal is kept (Gebreyohanes et al., 2022).

Animal identification is used to track individual animals and can involve the use of tags, ear tags, tattoos, microchips, and even DNA. This helps to ensure that animals are properly accounted for and can be identified in the event of theft, disease, or injury. Species and breed identification can also be used to help in the selection of breeding stock and to ensure that animals are properly matched for health and performance. Data collection is used to track animal behavior, health, production, and performance. This information can then be used to make decisions about management, breeding, and health protocols (Fogarty,

2009). The recording is used to track changes in the environment, such as changes in temperature, humidity, and air quality. This information is then used to adjust management practices, such as feed, water, and shelter requirements.

Acquiring good records on the pedigree and performance of animals is a primary component of the breeding program. The accuracy of selection highly depends on performance and pedigree records. Linking genetic relationships with performances enables the estimation of the genetic worth or breeding value of animals for selected traits and allows ranking and selecting the best animals among the candidates to be used for the next generation. Ear tags are the most commonly used and preferred animal identification method. Ear tags are relatively cheap and easy to apply and should be identified by their dam and sire immediately at birth. However, some farmers might resist this; in this case, it is possible to postpone tagging of kids for a few days with proper identification and recording of the dam tag number, sire tag number, kid coat color, and unique identifiers (Haile et al., 2011).

To properly utilize these records, a good numbering system should be devised for decision-making and analysis purposes. As a general rule, a unique number should be given for each animal, and the number of offspring should be greater than the number of sires and dams. This is a prerequisite for software to estimate breeding value. To ensure accuracy and traceability, a reliable record system should be established and updated regularly. Proper record-keeping is essential for the progress and success of the breeding program. Collecting data is essential for successful genetic selection, as it forms the basis of the decision-making process. According to Getachew et al. (2010) and Haile et al. (2011), the data to be collected should be carefully chosen based on the identified selection criteria. Generally, the data should be kept as simple as possible to make it applicable to a farmer's situation. This could include birth weight, litter size at birth, weaning weight, six-month weight, doe post-partum weight, and linear body measurements to increase the growth of the breed. Mothering ability, reproductive performance, and traits related to adaptation should also be considered. Milk yield and lactation length should be assessed to increase milk yield. To make

genetic selection decisions, records for relatives of the current generation should be collected, including progeny of selected sires and dams, and the ancestors of the current generation. It is important to collect data for both sexes to estimate heritability and genetic correlations. To ensure the data is not biased, it should be collected by a trained and knowledgeable staff, and stored securely in an easily accessible manner. A good numbering system should also be devised for decision-making and analysis purposes. Following these guidelines will ensure that data collection is successful and can be used to make informed selection decisions.

Recruitment of enumerators from farmers' communities

Enumerator recruitment from farmers' communities is an important step in data collection. It allows researchers to access valuable information that can be used to inform policy decisions. Enumerators can provide insight into the current challenges and successes of the sector by conducting interviews with farmers, gathering data and monitoring changes over time (Tran et al., 2015). Enumerator recruitment can be a complex process, requiring a thorough understanding of the communities and their needs. Researchers should be aware of the cultural and language differences between the communities and make sure to recruit enumerators who can communicate fluently with the target population. Furthermore, researchers should make sure to provide adequate training to ensure that the enumerators have the necessary skills and knowledge to collect data reliably (Moghimi et al., 2019).

According to the EBI (2016a), enumerators are important for taking responsibility for animal identification, data collection, and technical follow-up of the breeding program. As a bridge connecting researchers and the community, enumerators need to have good conduct approved by the community, be honest, and be committed to serving the community. Routine (daily) monitoring and follow-up are required so that the enumerator can live and interact with the community respectfully. Furthermore, additional two to three hours of labor are also required to spend in data collection. This is important to ensure that the research is performed ethically and efficiently (EBI, 2016a).

Animal selection and mating

Performance records and pedigree information are key tools used to select the best animals to be parents of the next generation, which is essential for maintaining the best animals within the community (Gizaw et al., 2011; Mourad et al., 2015). This process of selection is necessary to ensure that the genetic merit of these animals is disseminated to the next generation. Furthermore, it is important to select and maintain the best animals at an early age to avoid prior sales (Mourad et al., 2015). The appropriate time and age of selection for the animals depend on the breed and market situation, and should be carefully taken into consideration (Gizaw et al., 2011). In addition, traditional flocks have uncontrolled and non-seasonal mating, which leads to kidding distributed throughout the year, yet there is still variation among seasons in the number of kids produced (Gizaw et al., 2011).

Selection of buck lambs should be conducted in batches while considering the market age of the breed, the marketing season associated with annual feasts, and the frequency of available candidates. Gizaw et al. (2007) and Mekuriaw (2016) recommend that sires should be ranked based on their estimated breeding value, and then farmers should make the final selection based on their phenotypic assessment. To achieve this, it is recommended to select buck kids immediately before major feasts (eg. New Year, Christmas, Ester, Ed-Al-Adha). This two-step selection process, selecting a larger number of candidates at an early age (immediately before market age) and then approving the selection among candidates at breeding age, allows for adjusting for known environmental variations (like age, management, dam parity, postpartum weight, etc.) and using farmers' criteria to select the best buck kids. Furthermore, sires that fail the breeding soundness test need to be rejected automatically.

Sire use arrangement

Participatory scheming of the system to maintain and use breeding sires is a crucial step for the community (Getachew et al., 2010). A successful breeding program requires a well-thought-out plan that considers both short-term and long-term goals. The first step is to identify the breeding goals of the community and select

sires that have the best genetic merit to meet these goals. This involves availing and ensuring the functionality of selected sires to serve all flocks of the community fairly. Buy-in from the community members is a key factor in the sustainability of the breeding program. To ensure success, the selected sires should produce offspring that fetch above-average income for their owners. To make this possible, a revolving fund from the project or the contribution of community members is required in the beginning to acquire selected sires and other inputs for the community (Getachew et al., 2010). Once the program is successfully launched, it should be monitored and evaluated regularly to ensure that it meets the desired goals. Small-holder flocks are usually too small to make a selection of sires within the flock, so the selection of sires is implemented at the community level to maximize genetic gain.

The farmers in the community should be allocated to different breeding groups based on their neighborhood and communal grazing land and these groups should be decided with the full participation of the community. As few sires as possible should be used without affecting the accessible breeding dams. If sires are needed for more than one generation, it is important to rotate the sires among the groups or family flocks within the community to reduce inbreeding (Getachew et al., 2010; Haile et al., 2011). Unselected sires should not be used when mating the breeding dams, instead, technologies like conditioning or fattening unselected sires should be imposed to add value to them. Additionally, agreements regarding the communal use of selected sires, rotation of sires among flocks, and generations, and the use of a revolving fund for best sire selection should be set in place with the full participation of the community (EBI, 2016b). Once the sires have been selected, they should be castrated and fattened for breeding before being sold at a good price. The money obtained from the auction of the culled breeding sires should be used to buy replacement buck lambs.

Organizing cooperatives and encouraging community ownership

Organizing cooperatives and encouraging community ownership can provide a unique and powerful model for communities to not only gain economic autonomy but also to promote democracy and social justice. Cooperatives are members-owned and democratically-run businesses, which pool resources and share

profits among members. Such organizations can provide a way for communities to gain autonomy and ownership over their economic destiny. For example, by pooling resources, communities can better access capital, build resilience, and gain control of their resources and assets. Additionally, cooperatives create jobs and can provide a way for communities to become more self-sufficient. According to the United Nations International Year of Cooperatives, "Cooperatives are a reminder to the international community that it is possible to pursue both economic viability and social responsibility." (United Nations, 2012). Furthermore, when communities can own and control the resources, it allows them to have a greater say in the decision-making process, further promoting democracy and social justice. (Weeks et al., 2017).

Organizing farmers into a well-functioning cooperative is important to facilitate in situ community-based conservation and breed improvement activities. Breeder cooperatives shall be organized based on the guidelines of cooperative formation (Solomon, 2014; Getachew et al., 2016; EBI et al., 2016). Assefa et al., 2021). A cooperative unit under the wereda (district livestock agency) or agricultural office, research centers, community providers, universities, cooperatives, NGOs and Ethiopian Biodiversity Institute or the district livestock agency, is responsible for organizing farmers. The cooperative is expected to be run by a committee selected with the full participation of the community, which will perform duties like facilitating data collection and recording, discussing with the community and those responsible for the formation of breeding groups, arranging and monitoring buck utilization and rotation among groups, facilitating input supply (like feed and drugs) for goat production, being a main actor during buck selection and the animal show, discussing with the community and assisting in the implementation of the culling of unselected bucks, facilitating the fattening and marketing of culled breeding bucks, searching the market for breeding bucks, and approving the sale of bucks for breeding purposes in collaboration with the enumerator, district experts, and researchers. The committee will also be responsible for controlling the finances and properties of the community (Getachew et al., 2016; Assefa et al., 2021). It is important to note that the success of the cooperative will depend on the level of participation and commitment of the members. Therefore, the

committee needs to ensure that the members are well informed about the benefits of the cooperative and that they are given the necessary training and support to build capacity and ensure that the cooperative functions effectively.

Monitoring and evaluation

The ultimate goal of the CBCBP is to achieve genetic improvement for selected traits while maintaining the genetic diversity of the breed. Monitoring and evaluation is hence a vital component of CBCBP. Through regular monitoring, corrective measures can be taken on time. Defining key indictors to measure the progress, in achieving the goals set by the program and assess the outputs' contribution to the outcome is critical (Haile et al., 2011).

CONCLUSION

Sheep and goat production in Ethiopia has a long history and practice, with the population of these animals increasing over time, albeit not at the same rate as the human population. While researchers have identified limitations to breeding and conservation activities in the country, the practical application of community-based conservation and breeding programs (CBCBP) is still very limited. Thus, concerned institutes and organizations, as well as researchers, must strive to fill gaps and carry out CBCBP initiatives to the best of their ability, taking into consideration the available potential and resources in the country. Participatory identification of the breeding objectives, animal identification, performance recording, and selection of the best animals based on recorded performance and farmer criteria, pooling small flocks, and arranging sire use and sharing systems are all essential components of these programs. To successfully establish CBCBP, the community must be fully involved in a bottom-up, socio-cultural approach, with clear benefits for the members of the community and the use and upgrading of indigenous knowledge. Additionally, the activities of CBCBP should include the selection of breed, site, and awareness creation, recruitment of enumerators from farmers, animal selection and mating, organization of cooperatives to encourage community ownership, and monitoring and evaluation.

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Ethiopian Journal of Biodiversity
Guide for Contributors

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- **Review papers -** Critical and comprehensive reviews that provide new insights into or interpretations of the subject through a thorough and systematic evaluation of available evidence that should not exceed 10,000 words including Figures, Tables and References
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- **Book Reviews -** Book review which is a critical evaluation of published books in any discipline of biological sciences/biodiversity will be published under this column

2 Manuscript preparation

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Manuscripts should be written in American English, typed double-spaced, on A4 size, with margins of 1.5 cm on top and bottom sides of the paper, 2 cm on left and 1.5 cm on the right. A font size of 12 points (Times New Roman) should be used throughout the manuscript. The major sections of the manuscript include title, abstract, keywords, introduction, materials and methods, results, discussion, conclusion and recommendation, acknowledgements and references. Those sections having headings and sub-headings should not have more than three levels. All pages and lines should be numbered with the title page being page1

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- **Abstract:** The abstract of the manuscript should not exceed 250 words. It should give the reader the objectives of the study, how the study is conducted, the main findings and major conclusions. There should be no reference citations and abbreviations
- **Keywords:** Four to six words and/or phrases should be listed in alphabetical orders at the bottom of the abstract
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EthJBD follows referencing style described below. Unpublished results and personal communications are not recommended on the reference list, but maybe mentioned in the text and indicated in footnotes. Citation of a reference as 'in press' implies that the item has been accepted for publication. Moreover, citation in the text should follow the same referencing style. The citation styles described below is also applicable for Ethiopian Authors' work.

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For Chapters in Edited Books:

Harlan, J. R. 1971. On the origin of barley: a second look. In: R. A. Nilan, ed., *Barley Genetics vol.* II *Proc. 2nd Barley Genetics Symposium*. Washington State Univ. Press, Pullman, pp. 45 - 50.

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Brown, D. 2003. Deception point. New York: Atria Books.

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Engels, J. M. J. 1994. Genetic diversity in Ethiopia in relation to altitude. *Genetic Resources and Crop Evolution*, **41: 61-73.**

Lemessa, D., Hylander, K. and Hambäck, P. 2013. Composition of crops and land-use types in relation to crop raiding pattern at different distances from forests. *Agriculture Ecosystems and Environment*, **167:71-78.**

Mewded, B., Negash, M. and Awas, T. 2020. Woody species composition, structure and environmental determinants in a moist evergreen Afromontane forest, southern Ethiopia. *Journal of Forestry Research*, **31(4): 1173-1186.**

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One author: The last name of the author followed by year of publication will be cited in the text.

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Tables should be as editable text and be placed on a separate pages at the end of the manuscript. Number tables consecutively (i.e. Table 1, Table 2, etc.) in accordance with their appearance in the text and avoid vertical lines and shading in the table cells. Table captions should be descriptive and appear above the table. Footnotes and sources to tables should be placed under the table. Larger datasets can be uploaded separately as Supplementary Files

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