



**ANALYSES OF PHENOTYPIC AND MOLECULAR DIVERSITY, GENOTYPE
BY ENVIRONMENT INTERACTION AND FOOD-FEED TRAITS IN TEF
[*ERAGROSTISTEF* (ZUCC.) TROTTER]**

**A THESIS SUBMITTED TO
THE DEPARTMENT OF MICROBIAL, CELLULAR AND MOLECULAR
BIOLOGY**

**BY
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**IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY IN BIOLOGY (APPLIED GENETICS)**

ADDIS ABABA UNIVERSITY

ADDIS ABABA, ETHIOPIA

DECEMBER 2018

ADDIS ABABA UNIVERSITY SCHOOL OF GRADUATE STUDIES

Analysis of phenotypic and molecular diversity, genotype by environment interaction and food- feed traits in Tef [*Eragrostics tef* (ZUCC.) TROTTER]

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*A Thesis Presented to the School of Graduate Studies of the Addis Ababa University in Partial
Fulfillment of the Requirements for the PhD Degree in Biology (Applied Genetics)*

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Dedidication

This thesis is dedicated to my father and grandmother who believed in the education of girls and boys during those early days and to my beloved mother, Gebi Shure, a strong woman who stood alone to raise and educate the whole family. In general, it is dedicated to those people with a genuine and kind heart to support and guide others.

Declaration

I declare that the thesis hereby submitted for the Degree of Doctor of Philosophy (PhD) in Biology (Applied Genetics) to the Department of Microbial, Cellular, and Molecular Biology, Addis Ababa University is my own work and has not previously been submitted at another University. The materials obtained from other sources have been duly acknowledged in the thesis.

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Acknowledgements

First of all, I would like to thank the **Almighty God**, for His absolute protection and the courage and patience He offered me to successfully accomplish my PhD study and research.

Next, I would like to express my sincere gratitude and appreciation to my supervisors: Dr. Kifle Dagne, Dr. Kassahun Tesfaye, Dr. Kebebew Assefa and Dr. Zerihun Tadele, for their guidance throughout my study. Dr. Kifle Dagne and Dr. Kassahun Tesfaye from Addis Ababa University guided me all the way from initiation of the research proposal to final write-up of this thesis. Besides, their visit to my field work at various locations in Ethiopia and their comments and suggestions are highly appreciated. My co-supervisor, Lead Scientist Dr. Kebebew Assefa, from the Ethiopian Institute of Agricultural Research (EIAR) has shared me his lifelong knowledge and experiences to conduct my thesis research without any difficulties. Hence, I deeply express my respect and appreciation to him. The role of my co-supervisor Dr. Zerihun Tadele, Tef Improvement Project Team Leader at University of Bern, Switzerland, is far beyond expectations. He facilitated a six-month fellowship opportunity to conduct my molecular study in his laboratory by covering from his project all costs related to my travel, laboratory and living expenses in Switzerland. Despite the various responsibilities he has at his home institute, he was assisting and guiding my laboratory research in Switzerland and field research in Ethiopia. Hence, he deserves special appreciation for his intelligent mind as well as a genuine and kind heart to support his students.

The inspiration of my academic achievement, a move from a little boy to a PhD holder, was first conceived when my father and grandmother placed me at Algae Elementary School in Illu Ababora zone, Ethiopia. My grandmother was an elite woman believing in the education of girls and boys. She brought my elder sisters and me to school when even most males in rural Ethiopian do not get such a chance. My parents nurtured and educated me with affection and love without being educated by their family. Sadly, they were not fortunate enough to live a little longer to see their ambitions being realized with the eventual success of their son. I would, therefore, wish to dedicate this thesis to their immortal love and memories which always reside deep in my heart.

My heartfelt thanks are to my dear wife, Gebaynesh Negash, for her love, encouragement, inspiration and perfectly shouldering all responsibilities during the entire period of my study. My beloved son Kennaol and my little daughter Eliyana have always been the pillars of my life and the spines of my courage. My deepest gratitude and appreciation should also go to my beloved mother, Gebi Shure, a strong woman who stood alone to raise me and my six brothers and sisters. She has been through many difficulties to grow and educate us without significant support from others. My brothers (Terefe and Tesfaye) and sisters (Tirunesh, Chaltu, Aregash and Shitaye) have all played their role to lift me up to this level.

Next to my supervisors and families, I would also like extend my sincere respect and gratitude to all individuals who contributed to my education that led me to a mature professional level. Among others, Workineh Nura (my elementary teacher), Ato Mekonnen Negeri, Ato Mengistu Worku and his wife w/ro Sinknesh Tefera, the late Ato Tesema

Yeneneh; my high school teachers (Yared Kebede, Dr. Assefa Abegaz, Markos Alem and Zemichael Abera); my former colleagues at Bako research center (Dr. Diriba Geleti, Dr. Firdissa Eticha, Dr. Girma Abera, Dr. Tolera Abera and Mr. Zewude Kelkay) had kindly devoted their precious time and resources to assist my study at various levels.

This PhD study was sponsored by Syngenta Foundation for Sustainable Agriculture and the University of Bern through its cooperation with Ethiopian Institute of Agricultural Research (EIAR). Besides, International Livestock Research Institute (ILRI) has covered the laboratory cost for analysis of feed quality traits. EIAR has granted me a study leave with full salary while Addis Ababa University offered me a studentship and various supports. Holetta and Debre Zeit Agricultural Research Centers provided me with immense material, financial and technical supports. The germplasm used for this PhD work were obtained from the Ethiopian Biodiversity Institute (EBI), University of Bern and National Tef Research Project at Debre Zeit Agricultural Research Center (DZARC). Hence, I would like to acknowledge them all for their kind cooperation and support.

All researchers and technicians on tef breeding, especially, Woynishet, Adane, Desta, Kitaw and Negus from Holetta; Negussu, Worku, Kidist, Mengistu and Girma from Debre Zeit; Belay from Alem Tena sub center; Atinkut, Gedefa and Sewagegne from Adet; Kidu, Alemat and Miruts from Axum, and Merera, Kassahun and Girma from Bako research centers are highly and duly acknowledged. My deepest gratitude also goes to Dr. Solomon Chanyalew (Director of DZARC) and his entire staff for allowing me to use their vehicles and field facilities during my field studies at Debre Zeit and Alem Tena. My heartfelt

thanks are also to staff members of the University of Bern; especially, Miss. Regula and Miss. Annette for kindly assisting and guiding my laboratory study, and Dr. Ginna, the Bioinformatician in tef project, for her kind guidance in selecting primers for the molecular study. I am also grateful to Dr. Michael, Head of the ILRI feeds and nutrition laboratory in Addis Ababa, for allowing me his laboratory to analyze my feed samples free of charge. It was due to Dr. Alemayehu Teressa that I can get Dr. Michael and hence, thank you very much Alex. I would also like to thank Mr. Yonas, Mr. Degusew and Mr. Alemeshet, who kindly assisted me even beyond their normal working times during sample preparation, NIRS scanning and wet chemistry analysis. Thus, it was due to their genuine assistance that I have been able to finish the entire laboratory activities within two months.

My special gratitude is to Dr. Zerihun's family (his wife Haymanot and children Biya and Beka) for hosting me and making my stay in Switzerland more enjoyable and memorable. Finally, I would also like to thank my friends, PhD students and colleagues who have assisted me in one way or the other to accomplish my study and research at various levels. Among others, my sister W/ro Abebaye Gezahegn; my colleagues: Dr. Temesgen Dessalegn, Dr. Berhane Lakew, Wondimu Fekadu, Fikadu Amsalu, Thomas Tsige, Atsede Solomon and Bezaye Gorfu from Holetta, Dagnachew Bekele and Wasihun Legesse from Debre Zeit, Tolera Kenno from Bako, Elizabeth Basilos, Abebe Kirub and Etagegn from EIAR head office, my PhD fellows (Dr. Fikadu Gadissa & Agegnehu Wasssie) from Addis Ababa University and Terefe Fita from International maize and wheat improvement center (CIMMYT) are highly acknowledged.

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Lists of Acronyms and Abbreviations

AARC	Adet Agricultural Research Center
AAU	Addis Ababa University
ADF	Acid detergent fiber
ADL	Acid detergent lignin
AEA	Average Environment Axis
AFLP	Amplified fragment length polymorphism
AMMI	Additive Main effect and Multiplicative Interaction
AMOVA	Analysis of molecular variance
ANOVA	Analysis of variance
BARC	Bako Agricultural Research Center
CA	Cluster analysis
CLUMPAK	Cluster Markov Package Across K
CSA	Central Statistics Agency
CTAB	Cetyl tri-ethyl ammonium bromide
CV	Coefficient of variation
EDTA	Ethylene diamine tetra acetic acid
DAP	Diammonium phosphate DAP
DF	Degrees of freedom
DZARC	Debre Zeit Agricultural Research Center
EIAR	Ethiopian Institute of Agricultural Research
EBI	Ethiopian Biodiversity Institute
GA	Genetic advance
GCV	Genotypic Coefficient of Variation
GEI	Genotype by environment interaction
GGE	Genotype and genotype by environment interaction
HARC	Holetta Agricultural Research Center
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
IPCA	Interaction Principal Component Axes
ISSR	Inter Simple Sequence Repeat
LSD	Least Significant Difference
MCMC	Markov Chain Monte Carlo
MSE	Mean square of error

NJ	Neighbor Joining
PAGE	Polyacrylamide gel electrophoresis
PCA	Principal Component Axis
PCV	Phenotypic Coefficient of variation
PIC	Polymorphic Information Content
RAPD	Random amplified polymorphic DNA
RCBD	randomized complete block design
RFLP	Restriction fragment length polymorphism
RILs	Recombinant inbred lines
SAS	Statistical Analysis System
SE	standard error of mean
SSR	Simple sequence repeat
TBE	Tris/Borate/EDTA
TE	Tris EDTA
TEMED	Tetramethylenediamine

Abstract

Analyses of Phenotypic and Molecular Diversity, Genotype by Environment Interaction and Food-feed Traits in Tef [*Eragrostis tef* (Zucc.) Trotter]

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Tef [*Eragrostis tef* (Zucc.) Trotter] is an indigenous major cereal crop widely grown and utilized in Ethiopia. It is an excellent source of healthy and nutritious human food and livestock feed. However, its productivity is significantly lower than that of major cereals grown in Ethiopia. Although diverse constraints contribute for its inferior grain yield, the huge diversity present in tef germplasm has not been exploited. This study was, therefore, conducted to assess the phenotypic and molecular diversity, genotype by environment interactions and variation of food-feed traits in tef genotypes from various sources. The field experiments were conducted at Holetta, Debre Zeit, Alem Tena, Adet, Axum and Shambu using randomized complete block design with three replications whereas, the laboratory analyses were conducted at the University of Bern in Switzerland, Sci-Co in South Africa, International Livestock Research Institute (ILRI) in Ethiopia and International Crops Research Institute for Semi-Arid Tropics (ICRISAT) in India. Phenotypic diversity was assessed based on 12 quantitative and five qualitative traits of 188 tef genotypes while, the genotyping study was conducted using 189 genotypes with 16 SSR markers. Results from phenotypic study revealed the existence of huge variability in plant height (60.7 to 107.1 cm), panicle length (19.5 to 39.5 cm), number of fertile tillers per plant (2.1 to 5.5), number of spikelets per panicle (156.7 to 441.7), thousand kernel weight (20.7 to 33.0 mg), grain yield (3.7 to 7.3 t/ha) and lodging index (44.7 to 79.3%). Molecular diversity study generated a total of 260 alleles for the total studied loci. Cluster and STRUCTURE analyses showed four gene pools, but with high level of admixtures due

to geographic proximity and/or trans human seed exchange. Analysis of molecular variance showed significant variation of 56.6% within individuals, 40.8% among individuals within population and 2.6% among populations. Based on SSR study, furthermore, a core collection composed of 64 genotypes (33.9%) is suffice to capture the entire alleles of studied genotypes. Significant variations were also detected for all traits due to genotypes, environments and their interaction. Based on AMMI, GGE and Eberhart Russell's model, G₆ (Melko) was identified as the highest yielding and moderately stable variety while, E₅ (Debre Zeit-1) was the highest yielding and ideal environment. GGE biplot grouped the test environments into four mega environments and the studied genotypes into seven groups. Analysis of food-feed traits revealed significant ($P < 0.01$) effect of genotypes, year, locations and their interactions. Based on this, the highest mean crude protein (CP), *in vitro* organic matter digestibility (IVOMD), metabolic energy (ME), total biomass (TBM) and straw yield (STY), and the lowest mean for neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were obtained from genotypes evaluated at Debre Zeit. Cluster analysis and PCA-biplot grouped the genotypes into four. These are, (i) those with improved feed quality traits such as Kena, Genet and Ambo Toke; (ii) those with high food-feed traits such as Dukem, Gimbichu and Dega Tef; (iii) those with dual purpose such as Melko, Koye and Key Tena; and (iv) those with low food-feed traits such as Simada, Etsub and Mechare. The overall results of the present study enabled to identify tef genotypes with sufficient morpho-agronomic and molecular variations, better yield and stability, and food-feed traits having implications for collection, conservation and proper utilization of germplasm which are the basis of tef improvement.

Key words: Allelic richness, *Eragrostis tef*, genetic diversity, genotypes, phenotypic diversity, population structure, simple sequence repeats, tef

Chapter 1

1. GENERAL INTRODUCTION

1.1 *Background and justification*

Tef [*Eragrostis tef* (Zucc.) Trotter] is an indigenous cereal crop widely grown and consumed in Ethiopia. Globally, it is also becoming a life style diet and potential grain of attraction due to its nutritional and health benefits. The tef crop has several merits in terms of husbandry and utilization (Ketema, 1993; Assefa *et al.*, 2011, 2013) to increase crop production and to feed the ever-growing population in an environmentally friendly and sustainable way.

The grain of tef is gluten free (Spaenij-Dekking *et al.*, 2005) and nutritious human food (Mengesha, 1966). It is the most important source of best quality and consumer-preferred “injera” which has long shelf-life, unique flavor, pliability, smooth and glossy texture (Bultossa and Umeta, 2013). It provides high returns in flour upon milling (99%) compared wheat (60-80%) while its flour has also good water holding capacity to give high returns in *injera* upon baking (Ebba, 1969). Furthermore, tef grain has high market price, long shelf life, low-post harvest pest problems (Ketema, 1993; Assefa *et al.*, 2011) and provides 600 and 200 kcal/ day per person in the Urban and rural areas of Ethiopia, respectively (FAO, 2015).

The straw of tef, on the other hand, is also the most preferred, nutritious and palatable livestock feed (Bediye and Fekadu, 2001; Yami, 2013) compared to other cereal crops

grown in Ethiopia (Bediye *et al.*, 1996; Yami, 2013). Besides, it is also useful as a binder of mud for plastering walls of local houses. Hence, both the grain and straw of tef generate high income for farmers in the major tef production areas of Ethiopia who do not have alternative cash crops like coffee, tea or cotton (FAO, 2015).

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In Ethiopia, cereals contribute for 81.3% of the total area and 87.4% of the total production of grain crops (CSA, 2014 ; CSA, 2017). Tef is one of the major cereals in terms of area and volume of production among grain crops. Among cereals, tef, maize, sorghum and wheat are annually cultivated on 29.5%, 20.9%, 18.4% and 16.6% of the total area under cereal crops (Fig. 1) indicating their large-scale coverage in the country. Regarding the total production, the contribution of the same cereals in the same order to the total cereal grain production is 19.8%, 31%, 18.7% and 17.9%, respectively. This shows that tef stands first in the total area and second to maize in the total grain production and the number of households involved in its production while, it ranked last in terms of productivity among major cereals grown in Ethiopia (CSA, 2014 ; CSA, 2017) (Figure 1). Thus, despite its large-scale cultivation and its preference by both growers and consumers, its national average productivity is the lowest and is only about 1.66 tons per hectare (CSA, 2017). Nevertheless, it is possible to get over 4.5 tons of grain per hectare by using improved varieties (Dukem) and management practices under non-lodging condition (Tefera and Ketema, 2001). Such low productivity of tef at national level is mainly resulting due to lodging, drought and different biotic stresses (Assefa *et al.*, 2011; Assefa *et al.*, 2015). Hence, a lot is expected from tef breeders and all stakeholders to improve the productivity and minimize the yield gap with other major cereals.

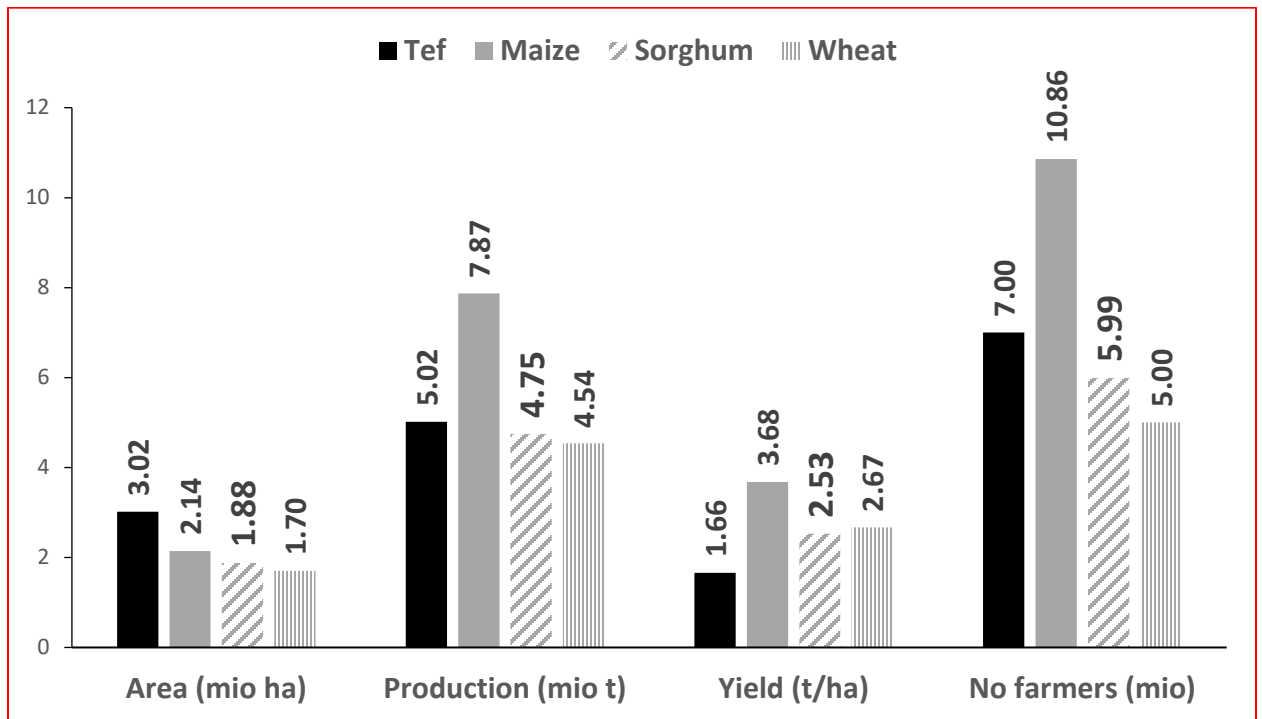


Figure 1.Area, production, productivity and number of farmers involved in the production of major cereal crops in Ethiopia (CSA, 2017).

Despite the various research efforts to increase production and productivity, there are still many gaps in tef breeding that require further research and investigation. Thus, the morphological, molecular and biochemical markers based genetic diversity studies conducted on tef germplasm was not as such exhaustive. The Ethiopian Biodiversity Institute (EBI) was reported to hold 5169 tef accessions in its gene bank (Tessema, 2013). Of the total accession in the EBI gene-bank, 3315 were collected directly by EBI, while the remaining 1854 accessions were acquired through donations and repatriations (Assefa et al., 2017). More than half of the total accessions at EBI have not yet been characterized (Tessema, 2013) and some of those characterized were found to be redundant (Chanyalew et al., 2013). Besides, the extent and pattern of genetic diversity in existing germplasm have not yet been exhaustively studied. Based on these constraints, the need for detailed

characterization of tef germplasm and the importance of establishing non-redundant working core germplasm were suggested (Chanyalew *et al.*, 2013). The EBI tef accessions collected from diverse growing regions in Ethiopia are expected to harbor genotypes with beneficial agronomic and nutritional traits for the improvement of tef crop. Hence, assessing the extent and pattern of genetic diversity among genotypes from various genetic and eco-geographic origins using a combination of markers is very essential.

Tef grows under various altitudes, soil and climate as well as crop management conditions whereby the same/or different genotypes may perform differently. Hence, the study of genotype by environment ($G \times E$) interaction is a vital research area in crop breeding and varietal development (Ketema, 1993). So far, limited studies have been made on $G \times E$ interaction in tef (Kefyalew, 1999; Ashamo and Belay, 2012). The study of $G \times E$ enables breeders to generate appropriate information on the magnitude and nature of $G \times E$ interactions and to design a workable future tef breeding strategy. Hence, it has long been suggested to conduct such study across the major tef growing areas of the country (Kefyalew, 2001).

The mid and highland areas characterized to have a mixed farming system are the major tef production environment in Ethiopia. In such system, the crop and livestock husbandry are practiced under the same management unit whereby the livestock provides draught power, cash and plant nutrition (manure) while crop residues are used as feed sources for livestock. As the contribution of natural pasture is declining due to continued increase of crop farming into grazing lands, feed shortage is becoming a serious constraint (Tolera

et al., 1999). Hence, proper use of crop residue is a good strategical option to be implemented. This is due to the fact that tef straw provides nearly half of the total annual cereal straw in the central highland (Yami, 2013) and a quarter of the straw in the whole country (Bediye *et al.*, 1996). However, the quantity and quality of the grain and straw of tef vary among varieties, and its growing climatic and soil conditions (Bediye *et al.*, 1996; Bediye and Dereje, 2001). So far, limited information is available on the straw quality of released tef varieties and the food-feed traits have not yet been investigated. The importance of tef as food and feed, the critical decline in grazing land, and the fact that farmers started to worry about crop residues and its grain require identification of tef genotypes with better grain and straw yield and quality. Hence, assessing the feed quality traits and identification of tef genotypes with high quality food-feed traits is essential in order to develop and release new varieties that fulfill these criteria.

1.2 Objectives

1.2.1 General Objective:

To investigate the genetic diversity, genotype by environment interaction and food-feed trait variation among tef genotypes from various genetic and eco-geographic origins.

1.2.2 Specific objectives:

- ❖ To characterize the phenotypic diversity of tef genotypes from various origins using morpho-agronomic traits;
- ❖ To assess the genetic diversity of tef genotypes and populations from various origins and to establish a non-redundant core tef germplasm using microsatellite (SSR) markers;
- ❖ To evaluate the extent of genotype by environment interactions, stability of improved tef varieties across locations and to characterize the tef growing environments.
- ❖ To assess and understand the association among food-feed traits of improved tef varieties evaluated across locations and years;

Outline of the thesis

This PhD thesis is based on four research areas on tef: morphological, molecular, genotype by environment interactions and food-feed traits analysis. There are seven chapters including the general introduction (Chapter I), the literature review (Chapter II), the four

experimental chapters (Chapters III to VI) and the conclusion and recommendations (Chapter VII). Thus,

- ✚ In chapter III and IV, diversity among tef genotypes from various origins in the country was assessed using morphological and molecular markers, respectively.
- ✚ Chapter V deals with the assessment of genotype, environment and genotype by environment interaction using 35 improved varieties evaluated at nine representative tef growing environments in the country.
- ✚ Chapter VI assesses the performance of 36 tef genotypes across two years and two locations for food and feed related traits.
- ✚ Chapter VII deals with the overall conclusion and recommendations drawn based on the results from various experiments conducted in this PhD study. It also summarizes major findings from experiments described in Chapters III to VI and provides recommendations as well as future directions in tef improvement.

Chapter 2

2. LITERATURE REVIEW

2.1 *Description of the tef crop*

Tef is an allotetraploid species with chromosome number ($2n = 4x = 40$) (Jones *et al.*, 1978; Ponti, 1978; Tavassoli, 1986) and is a cereal crop grown primarily in Ethiopia (Amanda and Doyle, 2003). The genome size of tef ranges from 714 to 733 Mbp (Ayele *et al.*, 1996) and 648 to 926 Mbp (Hundera *et al.*, 2000) based on different sets of cultivars suggesting that it varies with cultivars. Gina *et al.* (2014) also reported a draft genome containing 672 Mbp representing 87% of the genome size estimated from flow cytometry. Morphologically, tef has fibrous root system, fine stem and many tillers. It is a C_4 , self-pollinated chasmogamous annual cereal crop consisting of both the stamens and pistils in the same floret (Ketema, 1997). Tef has a very minute seed size ranging from 0.9-1.7 mm length and 0.7-1.0 mm diameter (Ebba, 1975) and the weight of its 100 to 150 grains is equal to a grain of wheat (Saturni *et al.*, 2010). According to Ketema (1997), tef spikelet has 2-12 florets consisting of a lemma, palea, three stamens, an ovary and mostly two feathery stigmas. The maturity periods of tef cultivars ranges from 60 to 180 days depending on its growing environments (Ketema, 1997; Assefa *et al.*, 2001; 2011; 2013).

Grain and lemma colour, panicle form and plant height as well as maturity period are the main distinguishing features among various tef cultivars (Berhe *et al.*, 1989; Tefera *et al.*, 1990, 1995; Ketema, 1997). Berhe *et al.* (1989) found that lemma and seed colour, and panicle forms are inherited independently whereby parents with colored lemma can

produce white or red lemma and colored lemma can be produced in the absence of coloration. Regarding dominance level, colored lemmas were reported to be dominant over non-colored ones (Berhe *et al.*, 1989). Furthermore, panicle form and maturity were reported to be linked among tef landraces (Tefera *et al.*, 1992) and thus, the late maturing genotypes have compact panicles and larger plants whereas the early maturing ones have very loose or open panicle forms. However, according to the same authors, seed and lemma colour are not linked to panicle form revealing that the seed and lemma color is not determined by the panicle form or vice versa.

2.2 *Origin and domestication of tef*

The center of origin and diversity of tef crop is believed to be in Ethiopia (Vavilov, 1951). The domestication of tef for human consumption was made by pre-Semitic inhabitants of Ethiopia between 4000 and 1000 B.C. (Ketema, 1997; Tessema, 2013). Tef is the only cultivated species in the genus *Eragrostis* which consists of 350 species (Tefera and Ketema, 2001; Tefera *et al.*, 2003). The fact that 14 of the 54 *Eragrostis* species found in Ethiopia are endemic (Tefera and Ketema, 2001; Tefera *et al.*, 2003) is strong evidence for Ethiopia to be considered as a center of origin and diversity of tef. About 43%, 18%, 12%, 10%, 9%, 6% and 2% of the 350 *Eragrostis* species were reported to be originated in Africa, South America, Asia, Australia, Central America, North America and Europe, respectively (Costanza 1974; Ketema, 1997). Amanda and Doyle (2003) suggested *E. pilossa* as the most likely wild progenitor of tef.

2.3 *The role of tef in Ethiopian farming system*

2.3.1 Agronomic role of tef

Agronomically, tef has several merits for the growers compared to other cereals. It possesses an excellent adaptation under various environmental conditions (altitudes, soils, rainfall and temperatures) and fits to different cropping systems, patterns and sequences (Assefa *et al.*, 2011, 2013; Ketema 1993). Tef crop can be grown from sea level to about 3000 meter above sea level (masl) and under various rainfall, temperature and soil regimes (Ketema, 1993). Therefore, it exhibits high level of phenotypic plasticity in phenology, morphology and agronomic performance depending on its growing environment (Assefa *et al.*, 2001c). However, the climate and soils under which it is grown affect both its quality and yield (Ketema, 1993) and hence, it gives a better yield and quality when it grows between 1,800 to 2,100 m a. s. l., receives 450 to 550 mm rainfall and gets 10 to 27°C temperature throughout its growing period (Ketema, 1993). Tef is a relatively resistant crop having no serious threats from disease and pest epidemics in the field and store (Assefa *et al.*, 2013) and hence has a long shelf life (Ketema, 1993; Assefa *et al.*, 2011). Furthermore, it can be used as a replacement crop at times of failures of long-season crops by drought, pest and/or other calamities. The fact that a smaller quantity of tef grains can sow larger area of land is also its additional advantage. Tef exhibits high level of phenotypic plasticity in phenology, morphology and agronomic performance depending on its growing environment (Assefa *et al.*, 2001). Hence, it is considered to be a reliable cereal for unreliable climate to enhance food security and crop diversification in the moisture stress and challenging agroecological areas of the country.

2.3.2 The utilization of tef

2.3.2.1 Nutritional content of tef

Tef is a very nutritious cereal grain having a relatively high content of minerals such as iron, phosphorous, and calcium (Melak-Hail, 1966; Asrat and Tekabe 2001; Baye, 2014). Besides, it contains more calcium, copper, zinc, aluminum, and barium than wheat, barley and sorghum. It has also a starch-rich endosperm, nutrient-rich germ and fiber-rich hull with much higher germ to endosperm ratio than any other grain due to its minute size (Patricia and Lisette, 2008). Tef grain provides human body with complete range of nutrients such as carbohydrates, fats, proteins, vitamins and minerals (Patricia and Lisette, 2008). It contributes 40-60% of the energy required by the body (Bultosa and Umeta, 2013) and approximately 60-80 percent starch (Bultossa *et al.*, 2008; Bultosa and Umeta, 2013; Baye, 2014). Surprisingly, a *dirqosh* or dried tef *injera* has a good digestibility and keeping quality compared to any other forms of *injera* (Bultossa *et al.*, 2008). Tef grain has significantly lower glycemic index (74) than white wheat (100) but comparable to sorghum (72) and oats (71) (Wolter *et al.*, 2013).

The iron and calcium content of tef ranges from 11 to 33 mg and 100 to 150 mg, respectively, revealing a higher content of both nutrients than those reported for wheat, barley, or sorghum (NAS, 1996). Besides, its zinc and copper content are also reported to be higher than sorghum and wheat (Baye, 2014). Furthermore, the grain and *injera* of tef provide high level of phosphorus, potassium and sodium than maize, sorghum, wheat and millet though fermented *injera* has more bio-available iron than the unfermented one (Bultosa and Umeta, 2013).

The protein content of tef grain ranges from 8 to 15 % with an average of 11% (National Academy of Sciences (NAS), 1996), which is a value higher than maize but comparable with wheat (Hundera, 1998; Bultosa, 2007). Tef grain is remarkably rich in albumins and globulins which is a digestible type of proteins (Bekele, 1995). Its composition of essential amino acids is comparable to an egg protein except for lysine content (Jansen *et al.*, 1962; Patricia and Lisette, 2008) which is still better than barley, millet and wheat (Bultosa and Umeta, 2013). Regarding its feed aspect, a mean crude protein (9 to 14%), acid detergent fiber (32 to 38%), neutral detergent fiber (53 to 65%) and Total Digestible Nitrogen (55 to 64%) was reported for tef forage (Miller, 2009).

2.3.2.2 The nutritional importance of tef

The fact that grain of tef is gluten free (Spaenij-Dekking *et al.*, 2005) has brought a global attention as a healthy diet for people suffering from gluten allergy or celiac disease. Besides, its slowly released carbohydrate is suitable for diabetic people and high iron content is very useful for pregnant woman and for people suffering from hookworm infestation related anemia (Spaenij-Dekking *et al.*, 2005). The minimum dietary energy (2100 kcal) requirements for food security for a person can be met by consuming about 546 g of dried *injera* per day (Bultosa and Umeta, 2013). In general, its better amino acid composition and gluten free nature potentially makes tef a very useful raw material for the development of new food products (Patricia and Lisette, 2008).

2.4 Constraints of tef production

Despite its major importance in Ethiopia as a source of food, feed, cash, and as a rescue crop, tef is reported to have several production constraints. Hence, its national average yield is only about 1.64 tons per hectare (CSA, 2017) which is far below its expected

genetic potential. The major reasons for such low yield of tef has been summarized by Assefa *et al.* (2011) as follows: low yield potential of the widely cultivated farmers' varieties; susceptibility to lodging especially under growth and yield promoting conditions; biotic stresses (diseases, insects, etc.); abiotic stress; the labor-intensive husbandry and the weak seed and extension system. Thus, the poor extension linkage has hampered the penetration of improved varieties and production package to reach the desired level. As a result, most farmers in the larger parts of the country are still using local landraces or traditional agronomic practices. Such wide gap between using improved production package and using local landraces and cultural practices, is therefore, seriously affecting the national productivity of tef. Besides, lodging is a major production bottlenecks causing a grain yield loss of 17 to 27% (Ketema, 1993) under natural conditions and 15 to 45% (Zhu *et al.*, 2012) depending on the weather condition and varietal inherent nature. The fact that tef production is a labor-intensive husbandry (requires up to five ploughing) and the difficulty of farmers to fulfill such requirement is another factor affecting the productivity of tef (Assefa *et al.*, 2011). Good land preparation usually allows tef to grow better and become more competitive to weed so as to increase yield (Hundera *et al.*, 2001; Fisehay and Tadele, 2001). Last but not least, inadequate research investment allocated to tef improvement at national level and lack of global attention due to its localized importance has been considered as another critical constraint of tef (Assefa *et al.*, 2011).

2.5 *Efforts and success in tef improvement*

2.5.1 Collection and conservation of tef germplasm resources

Existence of sufficient genetic variation forms the fundamental basis of any crop breeding. Indigenous germplasm resources are the primary source of variability for tef genetic

improvement. Hence, collection and conservation of these resources is essential to develop new varieties with desired traits. To this effect, the Ethiopian Biodiversity Institute (EBI) has collected about 5169 tef accessions. However, only 2533 of these accessions had the necessary passport data (Tessema, 2013) and most of them were not yet characterized. Based on their passport data, 42.6%, 39.6%, 11.8% and 5.9% of these accessions were collected from altitude range of 2001 to 2500m, 1501 to 2000m, 2501 to 3000m and 1001 to 1500 m above sea level (asl), respectively, while those from below 1000m and above 3000m asl is less than 1% (Tessema, 2013).

2.5.2 Genetic Characterization

Characterization of germplasm is the process of detecting genetic diversity existing within or among germplasm accessions using descriptor lists of morphological characters or at the level of gene expression or DNA sequences (de Vicente *et al.*, 2005). Plant breeders may put more focus on diversity within a crop species of interest (Rubenstein *et al.*, 2005) and hence, genetic characterization from breeding point of view involves the evaluation of agronomic performance of accessions for different morpho-agronomic and physiological characters under various environmental conditions (de Vicente *et al.*, 2005). Hence, genetic characterization and evaluation provides essential information for germplasm utilization, establishment of core collections and detection of duplications in collections (Carvalho, 2004). Genetic diversity studies are usually performed using morpho-agronomic characters, biochemical and molecular methods (Assefa *et al.*, 2001c; Carvalho, 2004; de Vicente *et al.*, 2005; Zeid *et al.*, 2012; Chanyalew *et al.*, 2013)) or combination of them.

2.5.2.1 Morphological and chemical characterization of tef

Various studies based on phenotypic traits have been conducted (Ebba, 1975; Tefera, 1988; Tefera *et al.*, 1990; Ketema, 1993; Bekele, 1996; Ketema, 1997; Teklu, 1998; Assefa *et al.*, 1999; Assefa *et al.*, 2000; Kefyalew *et al.*, 2000; Assefa *et al.*, 2001b; Assefa *et al.*, 2001a; Assefa *et al.*, 2001c; Adenew, 2002; Assefa *et al.*, 2002a; Assefa *et al.*, 2003b; Tefera *et al.*, 2003; Adinew *et al.*, 2005; Teklu and Tefera, 2005; Jifar, 2008; Assefa *et al.*, 2011; Jifar *et al.*, 2011; Plaza *et al.*, 2013). Thus, the existence of wide range of phenotypic variation in improved varieties (Hundera, 1998; Jifar, 2008; Jifar *et al.*, 2011), cultivars (Tefera, 1988; Tefera *et al.*, 1990) and germplasm accessions (Assefa *et al.*, 2000; Assefa *et al.*, 2001b; Assefa *et al.*, 2001a) for the various traits of tef have been revealed. Besides, wide range of phenotypic variability and heritability in grain yield and yield related characters were also reported (Tefera, 1988). Panicle form, seed and lemma color, embryo mark and basal stalk color are identified to be the major indicators of morphological variation in tef (Ketema, 1997; Tefera and Ketema, 2001). Variation in tef, furthermore, was also assessed using leaf and seed proteins (Bekele, 1995), grain chemical composition (Bekele and Lester, 1981; Hundera, 1998). Studies on grain chemical composition, for instance, revealed that a protein content and total nitrogen value ranging from 9.4% to 12.8% and 1.5% to 2.0% (Hundera, 1998).

Multivariate analysis such as cluster and principal component analysis have also revealed the existence of variation in tef (Assefa *et al.*, 2000; Assefa *et al.*, 2001b; Assefa *et al.*, 2001a; Adenew, 2002; Assefa *et al.*, 2003b; Adinew *et al.*, 2005; Jifar, 2008; Jifar *et al.*, 2011). In PCA, for instance, PCs with eigen values greater than unity accounted for 71 to 85% of the entire variation in tef (Assefa *et al.*, 2000; Adinew *et al.*, 2005; Jifar *et al.*,

2011). Reports based on cluster analysis have also documented the formation of three to seven clusters using various sets of tef (Assefa *et al.*, 2000; Assefa *et al.*, 2001b; Assefa *et al.*, 2001a; Adenew, 2002; Assefa *et al.*, 2003b; Adinew *et al.*, 2005; Jifar, 2008; Jifar *et al.*, 2011).

2.5.2.2 Molecular characterization of tef

Molecular markers are useful tools for assessing genetic variations, and provide an efficient means to link phenotypic and genotypic variations (Varshney *et al.*, 2005; Agarwal *et al.*, 2008). Understanding the molecular basis of essential biological phenomena is crucial for effective conservation, management, and efficient utilization of plant genetic resources (Mondini *et al.*, 2009). So far, the national tef improvement program in Ethiopia, Cornell University in USA and the University of Bern in Switzerland has been employing various modern genetic and genomic tools and techniques for tef crop improvement. Several molecular markers such as Restriction Fragment Length Polymorphism (RFLP) (Zhang *et al.*, 2001), Amplified Fragment Length Polymorphism (AFLP) (AYELE *et al.*, 1999; Bai *et al.*, 1999; Ayele and Nguyen, 2000), Random Amplified Polymorphism DNA (RAPD) (Bai *et al.*, 2000), Inter-simple sequence repeats (ISSR) (Assefa *et al.*, 2003a), simple sequence repeat (SSR) (Zeid *et al.*, 2012) and QTL mapping studies (Chanyalew *et al.*, 2005; Yu *et al.*, 2006; Yu *et al.*, 2007; Zeid *et al.*, 2011) and high throughput mutation discovery (Zhu *et al.*, 2012) have been employed to assess the genetic diversity in tef. Besides, the publication of tef genome sequences (Cannarozzi *et al.*, 2014) was another important breakthrough in tef improvement. Thus, it has confirmed that breeding targets identified for other cereal crops will also be a valuable breeding targets for tef. Besides, the draft genome and transcriptome enabled to identify those identified targets for genetic

improvement of tef. More recently, studies conducted by Girma *et al* (2018) using 40 wild *Eragrostis* species based on genotype by sequencing (GBS) confirmed 92% sequence representation of *E. pilosa*, *E. aethiopica*, *E. obtusa*, *E. ferruginea*, *E. lugens*, and *E. lehmanniana* in the tef reference genome. This is another confirmation to suggest *E. pilosa* as a potential diploid progenitor of tef.

Simple Sequence Repeats, commonly known as microsatellites, are the markers of choice in plant breeding (Gupta and Varshney, 2000). It has been widely in use to screen, characterize and evaluate genetic diversity in cereal crops (Tautz, 1989; Gous *et al.*, 2013) including tef (Zeid *et al.*, 2012; Abate, 2015; Abraha *et al.*, 2016). Co-dominant inheritance, high abundance, enormous allelic diversity and high reproducibility (Mondini *et al.*, 2009) are among the features increasing the potential use of microsatellites in large-scale genetic diversity studies.

2.5.2.3 Establishing core collection

Germplasm collections enable to maintain the genetic variation of crop plants and their wild relatives. However, efficient management of existing genetic variation is sometimes very difficult especially when we have large germplasm holdings. Nevertheless, one can reduce the problem by establishing a proper core collection. A core collection is a group of accessions from an existing germplasm collection being chosen to represent the genetic variations of the entire collection (Hao *et al.*, 2006). It should include the maximum genetic variation contained in the whole collection, with minimum redundancies (Brown, 1989; Anoumaa *et al.*, 2017). According to Paredes *et al.* (2010), knowing the level of genetic diversity and population structure are very essential in establishing a core set.

2.5.3 Development of tef varieties

The success of any crop breeding program depends on the level of genetic variability existing in its germplasm. The first step in the variety development is, therefore, knowing how variable the working germplasms are and designing strategies of creating variability if there is no sufficient variability. In tef, variety development relies on germplasm enhancement using indigenous germplasm, hybridization and induced mutation techniques. Thus, genetic characterization and evaluation, and targeted crossing among selected parental lines are the most common practices to develop and release varieties (Assefa *et al.*, 2011). So far, the existence of wide range of genetic variability in tef crop phenology, morphology and agronomic traits (Tefera *et al.*, 1992; Assefa *et al.*, 2001, 2011) that can be utilized for various improvements have been reported while, the variability observed for seed size, lodging, shattering, and leaf rust disease resistance was not sufficient (Assefa *et al.*, 2001, 2011). The use of induced mutation has also been employed to create variability for lodging resistance and seed size (Assefa *et al.*, 2011) though not succeeded to alleviate such critical problems.

Genotypes developed through various germplasm enhancement schemes are promoted for variety evaluation at various stage such as observation nursery, preliminary variety trial, national variety trial and variety verification trial. Commonly, there are two sets of variety evaluation (early and late sets) in which the late set is targeted for high potential environments, while the early set is targeted for terminal moisture stress areas and for double cropping (Assefa *et al.*, 2011; 2013). Variety verification trial is the last stage of variety evaluation whereby promising genotypes obtained from national variety trials are evaluated on larger plots both on station and on farm by the national variety release

committee. In the process of variety development, genotypes selected for a given trait are subsequently evaluated for direct yield tests or taken back as parental lines for hybridization or mutation schemes of germplasm enhancement (Assefa *et al.*, 2011, 2013, 2015).

2.5.4 Success in tef breeding

The discovery of tef floral biology and flowering time in 1974 by Tareke Berhe (Berhe, 1975) is a great breakthrough in tef breeding that enabled us know the time within 24hour cycle when tef flowers open (from about 6:00 to 6:45 am). This has allowed to start artificial crossing technique and incorporation of intra-specific hybridization into the pre-existing breeding methods. Personal communication with tef breeders at Debre Zeit agricultural research center confirmed the existence of over 650 crosses made by the national tef improvement program until 2018. Those crosses with high genetic variability were promoted for further selection and generation advancement (Tefera *et al.*, 2001). Thus, about 44 varieties with different genetic backgrounds have been released for use by small holder farmers (MoANR, 2017). Notably, varieties like *Dukem* have a yield potential of over 4.5 t/ha with improved management practices under non-lodging condition (Tefera and Ketema, 2001). The sequencing of dwarfing candidate genes homologous of *dw3* of sorghum and *rht1* of wheat has enabled to identify mutant tef lines to be combined and tested for improved lodging resistance (Zhu *et al.*, 2012). Thus, promising semi-dwarf and lodging tolerant tef lines have been developed using induced mutagenesis (Esfeld *et al.*, 2013). *Kinde* is one of those lines identified to have semi-dwarf stature, increased number of tillers, larger leaf size, stay green traits and strong tolerance to lodging (Jifar *et al.*, 2017). However, the yield of *Kinde* was very low compared to the locally adapted improved

varieties. But, introgression of the important agronomic merits of *Kinde* was possible by crossing it with the high-yielding and locally adapted tef cultivars in Ethiopia (Esfeld *et al.*, 2013). Such introgression has, therefore, enabled to release tef variety *Tesfain* 2017 (MoANR, 2017) and to develop several promising tef pipelines. Overall, however, the tef breeding has so far enabled to develop and release over 40 tef varieties to the farming communities in Ethiopia (MoANR, 2017).

2.6 *Genotype by Environment Interactions*

2.6.1 Assessing the suitability of environments

Presence of significant genotype by environment interactions (GEI) affects selection progress and makes variety development and recommendation more difficult (Allard and Bradshaw, 1964), and reduces the opportunity to get stable and high yielding genotypes across environments. Hence, understanding crop performance and its growing environment enables plant breeders and geneticists to increase agricultural production (Tolessa *et al.*, 2013; Yan and Kang, 2003). Besides, it enables to reduce the cost of genotype evaluation by eliminating unnecessary spatial and temporal replication of yield trials (Shafi *et al.*, 1992; Basford and Cooper, 1998). Furthermore, it is also very useful to establish additional testing environments when the existing ones are underrepresented (Piepho, 1996; Wakjira and Labuschagne, 2002). Multi environment trial (MET), generally, enables the identification of superior cultivar for a target region and to determine how the target region is subdivided into different mega-environments (Yan *et al.*, 2000). This gives breeders the opportunity to determine optimum breeding strategy to make informed choices of the locations and input systems to be used in the breeding efforts (Gruneberg *et al.*, 2005).

Hence, study of GEI and yield stability will continue to be important in checking varietal suitability for cultivation across seasons and ecological zones (Nassir and Ariyo, 2011).

2.6.2 Description of tools used to explain GEI

Various approaches have been used to quantify the genotype by environment interaction and to recommend the best genotypes for target environments. Joint regression (Eberhart and Russell, 1966), additive main effect a multiplicative interaction (AMMI) analysis (Gauch, and Zobel, 1988) and GGE biplot (Yan and Rajcan, 2002) are among these approaches. The AMMI biplot model is one of the widely used statistical methods for GEI and is relatively powerful for effective analysis and interpretation of multi environment data (Zobel et al., 1988; Yan et al., 2000; Samonte et al., 2005). Besides, its biplots are also useful to visualize relationships among genotypes and environments by showing both the main and interaction effects (Zobel *et al.*, 1988; Sadeghi et al., 2011).

GGE biplot methodology is another essential tool for plant breeders and researchers enabling the detection of genotype by environment interaction pattern, classification of mega environments, simultaneous selection of genotypes based on stability and mean yield. It is also useful to present the “which-won-where” pattern, mean performance and stability of genotypes, discriminating power and representativeness of environments, etc. (Gabriel, 1971; Yan *et al.*, 2000; Frutos *et al.*, 2013). Genotype by environment interaction analysis in R (GEAR) is free software developed by the CIMMYT (Alvarado *et al.*, 2016) and employed to analyze the AMMI and GGE biplot in the present study.

2.7 Food-feed traits

2.7.1 Food Value study in tef

Tef is one of the crops having high food and feed trait values. As a whole grain, its contribution to the human diet is of immense significance. Thus, tef a grain with higher fiber content than other grains due to its small size and whole grain nature (Bultossa and Umata, 2013) and also contain 20-40% resistant starches (Patricia and Lisette, 2008) which is of significant health benefits. Tef, therefore, is a source of nutritious and healthy diet for people having various health disorders such as celiac disease, diabetes, cardio vascular disease, obesity, etc. (Bultossa and Umata, 2013, Baye, 2014). Consumption of tef helps to manage celiac disease and possibly prevent and control iron deficiency and diabetes (Baye, 2013). Furthermore, tef is a good source of iron and vitamin B; especially, for women who are prone to anemic conditions (Bultossa and Umata, 2013). In general, according to NAS (1996), iron deficiency related anemia among Ethiopian people is very rare in the tef consumption area (especially, the red tef) have higher level of hemoglobin and a decreased risk of anemia related to parasitic infection (Bultosa and Umata, 2013).

2.7.2 Feed value study in tef

Natural pastures, crop residues, agro-industrial by-products, and to some extent improved forage and pasture species are the sources of animal feeds (Diriba *et al.*, 2013). Tef straw is reported to be a better feed resource than wheat and barley straws since it has lower lignin content and higher *in vitro* dry matter digestibility (IVOMD) and energy composition (Yami, 2013). As a result, farmers preferred to feed tef straw to lactating cows and working oxen (Ketema. 1997). Hence, its nutrient supply is close to that of a medium quality native grass hay (Ketema, 1993; Yami, 2013). Tef crop has been identified as a fast growing, low cost, high quality annual forage with multiple harvests during a growing

season compared to other common forage grasses (Roseberg *et al.*, 2008). According to Ketema (1997), it is possible to get up to three harvests of green products annually from tef. Forage quality is the appropriate consumption of the forage by the animal and the efficient conversion of its nutrients into animal products (Fulgueira *et al.*, 2007). The feed quality and food-feed yield traits are affected by the crop cultivar, climate, location, soil fertility, stage of maturity, techniques of harvest and storage conditions (Bediye *et al.*, 1996; Bediye and Dereje, 2001). The feeding value of crop residues also varies with the crop variety, content of anti-nutritional factors (tannins, lignin, silica etc.), stage of harvest, length of storage, leaf to stem ratio, soil fertility and fertilizer application as well as on the agronomic practices we follow (Yami, 2013). Thus, late harvesting reduces the protein content of a given feed compared to harvesting made at proper stage of growth and tef hay protein, therefore, ranged from 12 to 19.5% and 9.6 to 13.7% in South Dakota and Montana states respectively (Patricia and Lisette, 2008). This justifies that the protein content varies with location and crop growth stage of a given feed. Besides, studies conducted by Bediye *et al.* (1996) also reported that improved tef varieties had higher proportion of the more digestible leaf and panicle fractions compared to unimproved varieties indicating that palatability varies with genotypes. Studies conducted in USA reported a mean crude protein (9 to 14%), acid detergent fiber (ADF) (32 to 38%), neutral detergent fiber (NDF) (53 to 65%) and Total Digestible Nitrogen (TDN) (55 to 64%) for tef forage (Miller, 2009). Feed quality parameters can be analyzed by using either Near Infrared Reflectance Spectroscopy (NIRS) or the conventional wet chemistry method. The NIRS method is, however, a cheaper and faster method of analyzing larger samples whereas the latter is accurate and costly. It is one of the recent techniques applied for feed resource quality

determination and further characterization. NIRS is one of the robust applications to estimate chemical entity and parameters such as Dry matter (DM), organic matter (OM), Ash, Crude protein (CP), Acid detergent fiber (ADF), Neutral detergent fiber (ADF), Acid detergent lignin (ADL), Metabolic energy (ME) and *In vitro* organic matter digestibility (IVOMD).

Chapter 3

3. Agro-morphological traits diversity in Tef [*Eragrostis tef* (Zucc.) Trotter] genotypes from various genetic and eco-geographic origins

Abstract

Morphological characterization is the process of detecting genetic diversity existing within or among germplasm accessions using descriptor lists of morphological characters. Conducting further studies with this regard is essential as most of the tef accessions in the EBI gene-bank are not yet existively characterized. A total of 188 tef genotypes including 144 pure lines selected from germplasm collection, 35 released varieties, eight breeding lines and their parents were evaluated in three replications at two locations in Ethiopia. The objectives were to assess the magnitude and pattern of phenotypic diversity among tef genotypes, and to see association among major traits of tef from various sources. The results revealed existence of sufficient level of genetic variation for both qualitative and quantitative traits of tef. Combined analysis of variance revealed highly significant ($P \leq 0.01$) differences among genotypes, locations and genotype by environment interaction for all the studied traits. A wider range of variations were also observed for days to heading (40.3 to 60.8 days), days to maturity (101 to 122.5 days), plant height (60.7cm to 107.1 cm), panicle length (19.5 to 39.5 cm), second culm diameter (1.2 to 2.1 cm), number of fertile tillers per plant (2.1 to 5.5) and number of spikelet per panicle (156.7 to 441.7). Besides, 1000 kernel weights, grain yield and lodging index also ranged from 20.7 mg to 33.0 mg, 3.7 to 7.3 t/ha and 44.7 to 79.3 %, respectively. Genotypes collected from West Shewa and Central Tigray zones were found to have the highest plant height, second culm diameter, number of fertile tillers, number of spikelet per panicle, grain yield and shoot biomass. Cluster analysis also revealed the formation of six distinct groups for individual genotypes while the 14 populations were grouped into four distinct clusters.

Key words: *Eragrostis tef*, morphological markers, cluster analysis, genetic diversity, phenotypic parameters, tef, tef accessions

3.1 **Introduction**

Tef is the most important staple cereal having a dozen of agronomic, nutritional and health merits. Despite its various importance, the national productivity of tef (1.66 t/ha) is very low compared to the common cereals grown in Ethiopia (CSA, 2017). Poor dissemination of improved varieties and production packages, inability to tackle the problem of lodging and lack of capacity to characterize the available germplasm are among the major factors contributing to low productivity of tef.

Morphological markers are the earliest markers utilized in the assessment of genetic diversity within and among populations. Even though they have low polymorphism, heritability and expression, and are vulnerable to environmental influences (Smith and Smith, 1992; Mondini *et al.*, 2009), they are simple and direct measures of phenotypes and are cheap to characterize germplasm accessions. Hence, they are still very important methodological option to document the diversity at phenotypic traits levels when researchers/breeders have no access to the molecular or biochemical methods for detecting genetic variation.

Various studies conducted on morpho-agronomic characters revealed the existence of wide range of genetic variation in tef varieties (Hundera, 1998; Jifar, 2008; Jifar *et al.*, 2011), cultivars (Tefera, 1988; Tefera *et al.*, 1990) and germplasm accessions (Assefa *et al.*, 2000; Assefa *et al.*, 2001b; Assefa *et al.*, 2001a) for days to maturity, plant height, culm and panicle length, and number of tillers per plant. Thus, days to heading, days to maturity and grain filling period were reported to range from 25 to 81, 60 to 140 and 29 to 76, respectively (Assefa *et al.*, 2001c; Assefa *et al.*, 2003b). Besides, wide range of phenotypic

variability and heritability in grain yield and yield related characters were also reported (Tefera, 1988). The range of values reported for phenological traits is very useful for selection of genotypes for different maturity groups and adaptation (Assefa *et al.*, 2002a; Assefa *et al.*, 2002b). Panicle form, seed and lemma color, embryo mark and basal stalk color are the major indicators of morphological variation in tef (Ketema, 1997; Tefera and Ketema, 2001). Thus, genotypes having very loose panicle form were reported to have the highest yield and wider adaptation compared to those with other panicle forms (Tefera, 1988). Cluster and principal component analysis have also revealed the existence of variation in tef (Assefa *et al.*, 2000; Assefa *et al.*, 2001b; Assefa *et al.*, 2001a; Adenew, 2002; Assefa *et al.*, 2003b; Adinew *et al.*, 2005; Jifar, 2008; Jifar *et al.*, 2011). Despite previous diversity studies in tef germplasm using morphological traits, there is limited investigations using representative accessions from diverse tef growing regions. The objectives of this study were, therefore, to assess the magnitude and pattern of phenotypic diversity and to determine association among the major morphological traits in tef genotypes collected from representative tef growing areas in Ethiopia.

3.2 *Materials and methods*

3.2.1 Plant materials

A total of 188 tef genotypes including 144 pure lines selected from accessions collected from 12 administrative zones in the northern and central Ethiopia, 35 released varieties, eight breeding lines and their parents (mutant line *Kinde* and cultivar *Kaye Murri*) were studied. The collection year of the accessions ranged from 1979 to 2011 with the altitude range of 1260 m to 3090 m above sea level (a. s. l.). Description of the collection Woredas

for the 144 lines identified from germplasm is presented (Table 1; Figure 2). The germplasm was originally acquired from EBI in 2014 and sorted into pure lines in the same year after proper evaluation under field and greenhouse conditions. Thus, 12 pure lines were selected from each of the 12 administrative zones to make a total of 144 pure lines. The 35 tef varieties, on the other hand, were released by seven research centers in Ethiopia between 1970 and 2014 and obtained from the respective research centers. The source of the mutant line (*Kinde*) and the eight breeding lines from the crosses to *Kinde* were from the collaborative work between the University of Bern and the Ethiopian Institute of Agricultural Research (EIAR). *Kinde* is a mutant line identified to have shorter plant height and better lodging tolerance compared to improved tef varieties. *Kaye Murri* is a cultivar known to have several desirable agronomic properties whereas *Quncho* is a popular variety widely cultivated in the country. The remaining eight breeding lines were selected based on their performance from population involving two crosses (*Kinde* x *Kaye Murri* and *Quncho* x *Kinde*). The seeds of these breeding lines were obtained from Debre Zeit Agricultural Research Center (DZARC). The detailed description of all studied genotypes is given in Table 1. Regarding altitude of collections, 12, 49, 51 and 32 genotypes were collected from altitudes up to 1500, between 1501-2000m, 2001-and above 2500m, respectively (Appendix Table 1). As to the collection year, 42% and 58% of the studied genotypes were collections before 2000 and those from 2001 to 2011, respectively (Appendix Table 2).

Table 1. Description of the tef genotypes used in the present study

Origin/ Category	Collection Zones	No of genotypes (serial No.)	Name of genotypes (Accessions)	Altitude (masl)
Lines from landrace accessions	Central Tigray	12 (1-12)	Acc. nos. 19132-2, 19132-3, 19166-1, 19253-1, 19253-2, 234407-1, 234407-2, 237184-1, 237205-2, 243513-1, 243513-3 & 243520-2	1350-2640
	East Gojam	12 (13-24)	Acc. nos. 9545-1, 9556-1, 19516-1, 19516-3, 55221-1, 55221-2, 212698-2, 229768-1, 229768-3, 229768-4, 55046-2 and 55046-3	1470-2650
	East Shewa	12 (25-36)	Acc. nos. 15361-3, 17335-1, 18460-0, 18466-2, 18466-3, 236963-1, 236963-2, 236965-1, 236965-3, 236967-1, 236967-2 and 236972-0	1657-2303
	East Tigray	12 (37-48)	Acc. nos. 15297-1, 15297-2, 15299-1, 15299-3, 19201-1, 19201-3, 19202-2, 234460-1, 234460-2, 234460-3, 242540-1 and 242540-2	1979-2632
	North Gonder	12 (49-60)	Acc. 9448-1, 9448-2, 9451-2, 9469-2, 9472-2, 9472-4, 19343-2, 242186-3, 242186-4, 243540-1, 243540-3 and 243540-4	1840-2208
	North Shewa	12 (61-72)	Acc. nos. 9559-1, 9559-2, 15309-2, 15309-3, 15322-1, 15322-2, 18385-2, 212482-1, 236745-2, 236746-0, 236748-2 and 236957-1	1260-2670
	North Wello	12 (73-84)	Acc. nos. 55104-3, 215196-1, 215200-1, 215200-2, 215200-3, 234356-4, 234985-2, 234993-1, 234993-3, 237148-1, 237148-5 and 243501-2	1520-2950
	South Gonder	12 (85-96)	Acc. nos. 19341-2, 19341-3, 19367-2, 19374-1, 55293-2, 212717-0, 212720-1, 225919-2, 225919-3, 225919-4, 225919-7 and 242160-1	1804-2950
	South Wello	12 (97-108)	Acc. nos. 212607-2, 212612-3, 212614-1, 212614-2, 225898-1, 242214-1, 242214-2, 243491-2, 2433497-2, 243504-1, 243504-2 and 243504-3	1550-3090
	West Gojam	12 (109-120)	Acc. nos. 19394-1, 19443-3, 19452-4, 19506-2, 19506-4, 242140-3, 242144-1, 242144-3, 242155-1, 242155-3, 55029-2 and 55029-3	1890-2735
	West Shewa	12 (121-132)	Acc. nos. 17365-1, 17371-3, 18410-1, 18410-2, 18410-3, 18414-2, 18414-4, 18423-3, 236757-2, 236760-1, 236760-4 and 236760-6	1640-2674

Table 1. *Continued*

Origin/ Category	Zones	No of genotypes (serial No.)	Name of genotypes (Accessions)	Altitude (masl)
	West Tigray	12 (133-144)	Acc. nos. 9419-1, 9444-2, 9444-3, 19241-1, 19241-2, 234435-2, 237236-3, 237236-4, 237239-3, 243526-2, 243526-4 and 243526-5	1260-2054
Improved varieties by breeders	Improved varieties	35 (145-179)	Enatite (DZARC), Asgori (DZARC), Magna (DZARC), Wellenkomi (DZARC), Menagesha (DZARC), Melko (DZARC), Tsedey (DZARC), Gibe (DZARC), Ziquala (DZARC), Dukem (DZARC), Holetta Key (HARC), Ambo Toke (HARC), Gerado (DZARC), Koye (DZARC), Key Tena (DZARC), Gola (SARC), Ajora (ArARC), Genete (SARC), Zobel (SARC), Dima (AARC), Yilmana (AARC), Dega Tef (DZARC), Gimbichu (DZARC), Amarach (DZARC), Quncho (DZARC), Gudurru (BARC), Gemechis (MARC), Mechare (SARC), Kena (BARC), Etsub (AARC), Laketch (SARC), Simada (DZARC), Boset (DZARC), Kora (DZARC) and Were-Kiyu (SARC)	-
Lines from crosses of mutant by adapted cultivars	Breeding lines	10 (180-189)	Kaye Murri (cultivar, parent), <i>Kinde</i> (Mutant line, parent), Quncho X <i>Kinde</i> (RIL-85), Quncho X <i>Kinde</i> (RIL-91), Quncho X <i>Kinde</i> (RIL-96), <i>Kinde</i> X Kaye Murri (RIL-11), <i>Kinde</i> X Kaye Murri (RIL-302), <i>Kinde</i> X Kaye Murri (RIL-69) and <i>Kinde</i> X Kaye Murri (RIL-81)	-

- DZARC, HARC, SARC, ArARC, AARC, BARC and MARC refer to Debre Zeit, Holetta, Sirinka, Areka, Adet, Bako and Melkassa Agricultural Research Centers, respectively.

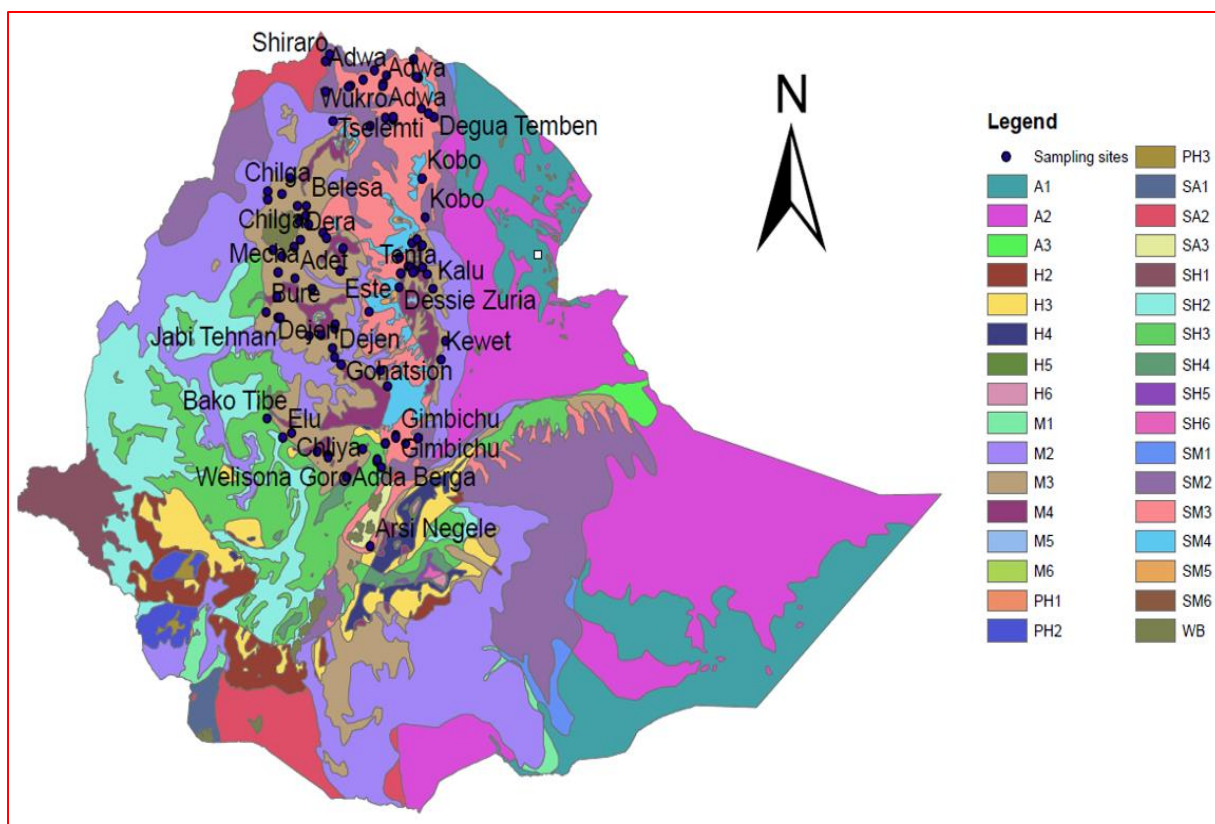


Figure 2. Map of Ethiopia and collection Woredas for the 144 tef germplasm lines based on passport data from EBI.

3.2.2 Description of study sites and design

The field experiment was conducted during the main cropping season of 2015/16 at Holetta and Debre Zeit Agricultural Research Centers which represent the major tef production areas in Ethiopia. Holetta Agricultural Research Center is located at 9°44' N, 38°30' E, at 2400 m above sea level (m a. s. l.) 39 km west of Addis Ababa and receives a mean annual rainfall of 1100 mm, with annual minimum and maximum temperatures of 6°C and 22°C, respectively (Jifar *et al.*, 2017). The majority of its rain is obtained between March and October with a peak in July and August. Debre Zeit Agricultural Research Center, on the other hand, is situated at 8°44'N and 38°58'E, 47 km East of Addis Ababa at an altitude of 1900 m. a. s. l. and receives a mean annual rainfall of 851 mm with a minimum and

maximum temperature of 8.9⁰C to 28.3⁰C, respectively (Jifar et al., 2017). The experimental soil at Holetta was acidic Nitosols, whereas the soil at Debre Zeit is a black clay soil (Pellic Vertisol) with high moisture holding capacity.

This experiment was laid in Randomized Complete Block Design with three replications. Each genotype was grown into two rows of 0.5 m length in each plot at a spacing of 0.2 m, 0.4m and 1.5m between genotypes, rows and replications, respectively. During the crop growing period, all agronomic and cultural practices recommended for tef production were applied at each location.

3.2.3 Data collection

3.2.3.1 Qualitative Data

Qualitative traits data were recorded for panicle form, number of internodes, and the colors of basal stalk culm, lemma and seeds (Table 2). The description of the various qualitative traits was partially made based on the classifications in the previous works (Tefera et al., 1992; Ketema 1997). The number and percentage frequency distribution of qualitative traits across genetic and ecogeographic origins were calculated using Microsoft Excel.

Table 2.Phenotypic classes of the qualitative characters used for diversity study

Character	No. Class	code	Classes
Lemma colour	5	1	Green
		2	purple
		3	Red
		4	Variegated
		5	Yellowish white
Panicle form	5	1	Compact
		2	Semi compact
		3	Fairly loose
		4	Loose
		5	Very loose
Basal stalk color	4	1	Dark blue
		2	Golden brown
		3	Green
		4	purple
Seed colour	3	1	Brown
		2	White
		3	Very white
Number of internodes	3	1	Three
		2	Four
		3	Five

3.2.3.2 Quantitative traits data

The following quantitative traits data including phenological and agro-morphological data were collected for the present study.

A. Phenological data

Days to heading/ panicle emergence (DH): Number of days it takes from seedling emergence to the appearance of the tips (about 5 cm) of the panicles in 50% of the plants

on a plot. Note that tef panicle appears without showing the booting stage, which is unlike the other small cereals like wheat and barley, but similar to that in rice.

Days to maturity (DM): Number of days it takes from seedling emergence to physiological maturity as judged by the change to straw color of the vegetative parts of 75% of the plants in a plot.

Grain filling period (days) (GFP): This is computed as the difference between days to panicle emergence and to maturity.

B. Agro-morphological data collected on five randomly selected plants

All traits under this section were recorded at crop maturity stage.

Plant height (PH): The length of the plant in centimeters from ground level to the tip of the panicle.

Panicle length (PL): Length in centimeters from the node where the first panicle branch starts to the tip of the panicle.

Peduncle length (Pdl): Length in centimeters of the top most spanning from the last culm node until the start of the first panicle branch. It stretches from the node where the flag leaf starts to where the first panicle branch starts.

Culm length (cm): Length in centimeters from ground level to the node where the first panicle branch starts.

Culm diameter (CD): The circumference of the second basal culm internode in millimeter measured using caliper.

Number of fertile tillers per plant (NFT): Counts of the panicle-bearing tillers of a single plant that have produced a productive panicle.

C. Agro-morphological data collected on plot bases

Shoot biomass yield (SBM): The dry weight in kilograms of the above ground biomass per plot before threshing

Grain yield/plot (GY): The dry weight in grams or kilograms of grains after threshing.

Straw yield/plot (STY): The dry weight in grams or kilograms of tef crop residues obtained after threshing and grain removal.

Harvest index (HI) (ratio): The ratio of grain yield to total biomass.

1000-kernel weight (TKW): The weight in milligrams of 1000 random kernels of tef.

Lodging Index (LI): The displacement of plants from the vertical up right position. The common method accepted by the tef program is the lodging index method of Caldicott and Nuttall (1979), which considers both the extent and severity of lodging.

- ✓ **Severity of lodging** can be measured from **0 to 5** based on the **angle of lodging** (0 = no lodging = 100% upright, and 5 = completely lodged = 100% flat). Intermediate readings of lodging are expressed as scores 1 to 4 according to the angle of lodging.
- ✓ The **extent of lodging** in a plot (percentage area of the plot affected for each severity score) is also recorded.

3.2.4 Statistical analysis of phenotypic traits

All quantitative traits data were subjected to analysis of variance (ANOVA) as per the procedure suggested by (Gomez and Gomez, 1984). (SAS, 2002), Minitab 17.1 (2013) statistical software packages were used for different analysis of quantitative traits data.

Variance homogeneity test and combined analysis of variance was performed using the general linear model (PROC GLM) procedure to determine the effect of environment (E),

genotype (G) and G x E interaction for the various traits of tef. Mean separation was performed using Duncan's multiple range test at 5% probability level. Genotypic and phenotypic association of major quantitative traits were analyzed using Multi Environment Trial Analysis with R for windows (META-R) version 6.0 developed by CIMMYT (Alvarado *et al.*, 2016). Principal component analysis and cluster analysis, on the other hand, were conducted using MINITAB software version 17 (MINITAB, 2013). Genetic distances between clusters as standardized Mahalanobis's D^2 statistics were calculated as:

$$D^2_{ij} = (\mathbf{x}_i - \mathbf{x}_j)' \mathbf{cov}^{-1}(\mathbf{x}_i - \mathbf{x}_j)$$

Where, D^2_{ij} = the distance between cases i and j; $\mathbf{x}_i$ and $\mathbf{x}_j$ = vectors of the values of the variables for cases i and j; and $\mathbf{cov}^{-1}$ = the pooled within groups variance-covariance matrix. The D^2 values obtained for pairs of clusters were considered as the calculated values of Chi-square (X^2) and were tested for significance at 1% and 5% probability levels against the tabulated value of X^2 for 'P' degrees of freedom, where P is the number of characters considered in each study (Singh and Chaudhary 1985).

3.2.5 Broad-sense heritability and genetic gains from selection

Partitioning of the total phenotypic variance into components due to genotype (σ^2_g), environment (σ^2_e) and genotype by location interaction (σ^2_{gl}) variances was performed from the analyses of variance by assuming various observed mean squares equal to their expected mean squares (Table 3) as suggested by Singh and Chaudhary (1985).

Table 3.Summary of the analysis of variance model

Source of variation	Degree of Freedom	Mean Square (MS)	Expected Mean Square (EMS)
Locations	L -1	MS ₁	$\sigma^2_e + G\sigma^2_r + GRL\sigma^2$
Rep within location	L (R -1)	MS ₂	$\sigma^2_e + G\sigma^2_r$
Genotypes	G -1	MS ₃	$\sigma^2_e + R\sigma^2_{gl} + RL\sigma^2_g$
Genotype x Location	(G -1) (L-1)	MS ₄	$\sigma^2_e + R\sigma^2_{gl}$
Error	L (G -1) (R -1)	MS ₅	σ^2_e

Thus,

$$\sigma^2_g = [(\sigma^2_e + R\sigma^2_{gl} + RL\sigma^2_g) - (\sigma^2_e + R\sigma^2_{gl})]/RL = (MS_3 - MS_4)/RL$$

$$\sigma^2_e = MS_5$$

$$\sigma^2_{gl} = [(\sigma^2_e + R\sigma^2_{gl}) - (\sigma^2_e)]/R = (MS_4 - MS_5)/R$$

Where: σ^2_g = genotype variance, σ^2_e = environmental variance and σ^2_{gl} = genotype by location interaction variance.

$$\text{Broad-sense heritability } (h^2_b) \text{ was calculated as: } h^2_b = \frac{\sigma^2_g}{[\sigma^2_g + \frac{\sigma^2_{gl}}{L} + \frac{\sigma^2_e}{RL}]} \times 100$$

The predicted response to selection or the expected genetic advance (GA) was calculated, assuming the selection intensity of 5%, as:

$$GA = K\sqrt{\delta^2 P} \times h^2$$

$$GAM = \left(\frac{GA}{X}\right) \times 100$$

Where, GAM= Genetic advance as percent of mean, X= grand mean, GA = expected genetic advance from selection and K = the selection differential (K = 2.06 at 5% selection intensity), σ^2_p = phenotypic variance and h^2 = broad-sense heritability (Singh and Chaudhary, 1985).

3.3 RESULTS

3.3.1 Qualitative traits diversity

The variations among qualitative traits of the studied genotypes were analyzed and are presented in Table 4 and Fig. 3. Based on this result, panicle form of the studied genotypes was grouped into five categories: loose, fairly loose, very loose, compact and semi compact types, and included 87, 72, 23, 6 and 1 genotypes, respectively. Thus, the majority of the genotypes had loose (46.0%) and fairly loose (38.1%) types while the semi compact type accounted for less than 1 % (Table 4). With regard to lemma colour, the studied genotypes showed green, purple, red, variegated and yellowish white lemma colour. The majority of these genotypes were represented by yellowish white (43.9%) and variegated (38.1%) lemma colour while the remaining classes accounted for about 18% of the variation. The basal stalk colour, on the other hand, were categorized into dark brown, golden brown, green and purple types whereby the green basal stalk colour covered 90.5% whereas the remaining three colours together constituted less than 10% of the variation.



Figure 3. One hundred and eighty-eight tef genotypes grown at Holetta Agric. Research Center (October 2015). Photo: Habte Jifar

In addition, the variation for number of internodes and seed colour among the tef genotypes are summarized in Table 4. Regarding number of internodes, the majority of the studied genotypes had four internodes (51.3%) followed by those having three (35.5%) and five (13.2 %) internodes. Furthermore, 55%, 42.9% and 2.1% of the studied tef genotypes were found to have white, brown and very white grain colour, respectively.

Table 4. Variation in qualitative traits of tef genotypes evaluated at two locations in 2015

Character	code	Classes	No of genotypes	%
Lemma colour	1	Green	16	8.5
	2	purple	12	6.4
	3	Red	6	3.2
	4	Variegated	72	38.1
	5	Yellowish white	83	43.9
Panicle form	1	Compact	6	3.2
	2	Semi compact	1	0.5
	3	Fairly loose	72	38.3
	4	Loose	87	46.3
	5	Very loose	23	12.2
Basal stalk color	1	Dark blue	3	1.6
	2	Golden brown	10	5.3
	3	Green	171	90.5
	4	purple	4	2.1
Seed colour	1	Brown	81	42.9
	2	White	104	55.0
	3	Very white	4	2.1
Number of internodes	1	Three	67	35.5
	2	Four	97	51.3
	3	Five	25	13.2

3.3.1.1 Seed colour distribution by origin

The seed colour distribution of the studied genotypes across genetic origin, region and zone of collection was assessed and presented in Table 5. Based on zones of collections, genotypes from the three zones of Tigray region were identified to have 66.7% white, 36.6% brown and 2.8% very white seed colour whereas, those from North and South Gonder of Amhara region were composed of 62.5% brown, 33.3% white and 4.2% very white seed colour. Besides, genotypes from North and South Wello zones of Amhara region showed 45.8% brown, 37.5% white and 16.7% very white seed colour while those from West and East Gojam zones of the same region occupy 41.7% brown, 45.8% white and 12.5% very white seed colour.

On the other hand, genotypes from North Shewa zone of Oromia region showed 58.3% brown, 25.0% white and 16.7% very white seed colour while those from West Shewa zone showed 41.7% brown, 50.0% white and 8.3% very white seed colour. Unlike other zones, the majority of genotypes from East Shewa zone had very white seed colour (50.0%) followed by 33.3% brown and 16.7% white seeded types. The improved varieties, on the other hand, were found to have 11.4% brown, 71.4% white and 17.1% very white seed colour while the breeding lines had 70% white and 30% very white seed colour (Table 5).

3.3.1.2 Panicle form distribution by origin

The distribution of panicle form of the studied genotypes across genetic origin, region and zone of collection was assessed and presented in Table 6. Based on zones of collections, genotypes from the three zones of Tigray region were identified to have 33.3% fairly loose, 47.2% loose, 11.1% very loose, 5.6% semi compact and 2.8% compact type panicle form.

Among the three zones, the loose type has the highest proportion in East and Central Tigray whereas the fairly loose accounted for the largest portion for West Tigray collections. In Amhara region, collections from North and South Gonder were composed of 37.5% fairly loose, 54.2% loose and 8.3% very loose panicle form whereas those from North and South Wello zones had 37.5% fairly loose, 50% loose, 8.3% very loose and 4.2% compact panicle form. Collections from West and East Gojam zones of the same region had, however, 29.2% fairly loose, 41.7% loose, 20.8% very loose and 8.3% compact panicle form (Table 6).

In Oromia region, collections from the three zones of Shewa had 27.8% fairly loose, 50% loose, 19.4% very loose and 2.8% compact panicle form. The improved varieties, on the other hand, were found to have 57.1% fairly loose, 33.3% loose and 8.3% very loose panicle form while the breeding lines had 30% fairly loose, 30% loose, 10% very loose and 20% compact panicle form (Table 6).

Table 5. Seed color distribution of 188 tef genotypes from various origins

Region	Zones	Class I (Brown)	Class II (White)	Class II (very white)	Total
Tigray	Central Tigray	3 (25%)	8 (66.7%)	1 (8.3%)	12
	East Tigray	4 (33.3%)	8 (66.7%)	0 (0.0%)	12
	West Tigray	4 (33.3%)	8 (66.7%)	0 (0.0%)	12
Amhara	North Gonder	7 (58.3%)	4 (33.3%)	1 (8.3%)	12
	South Gonder	8 (66.7%)	4 (33.3%)	0 (0.0%)	12
	North Wello	6 (50.0%)	5 (41.7%)	1 (8.3%)	12
	South Wello	5 (41.7%)	4 (33.3%)	3 (25.0%)	12
	East Gojam	7 (58.3%)	4 (33.3%)	1 (8.3%)	12
	West Gojam	3 (25.0%)	8 (66.7%)	1 (8.3%)	12
Oromia	North Shewa	7 (58.3%)	3 (25.0%)	2 (16.7%)	12
	East Shewa	4 (33.3%)	2 (16.7%)	6 (50.0%)	12
	West Shewa	5 (41.7%)	6 (50.0%)	1 (8.3%)	12
Improved varieties		4 (11.4%)	25 (71.4%)	6 (17.1%)	35
Breeding lines		0 (0.0%)	6 (66.7%)	3 (33.3%)	9
Total		67 (35.6%)	96 (51.06)	26 (13.8%)	188

NB. Numbers in bracket shows the percentage of grain colour in each class.

Table 6. Panicle form distribution of 188tef genotypes from various origins

Region	Zones	Panicle form					Total
		FL	L	VL	SC	Comp.	
gray	Central Tigray	4 (33.3)	6(50.0)	1(8.3)	1(8.3)	0(0.0)	12
	East Tigray	1(8.3)	7(58.3)	2(16.7)	0(0.0)	2(16.7)	12
	West Tigray	7(58.3)	4(33.3)	1(8.3)	0(0.0)	0(0.0)	12
Amhara	North Gonder	4(33.3)	6 (50.0)	2(16.7)	0(0.0)	0(0.0)	12
	South Gonder	5(41.7)	7(58.3)	0(0.0)	0(0.0)	0(0.0)	12
	North Wello	5(41.7)	5(41.7)	1(8.3)	0(0.0)	1(8.3)	12
	South Wello	4(33.3)	7(58.3)	1(8.3)	0(0.0)	0(0.0)	12
	East Gojam	1(8.3)	7(58.3)	2(16.7)	0(0.0)	2(16.7)	12
	West Gojam	6(50.0)	3(25.0)	3(25.0)	0(0.0)	0(0.0)	12
Oromia	North Shewa	3(25.0)	6(50.0)	3(25.0)	0(0.0)	0(0.0)	12
	East Shewa	3(25.0)	8(66.7)	1(8.3)	0(0.0)	0(0.0)	12
	West Shewa	4(33.3)	4(33.3)	3(25.0)	0(0.0)	1(8.3)	12
Improved varieties		20(57.1)	12(34.3)	3(8.6)	0(0.0)	0(0.0)	35
Breeding lines		3(33.3)	4(44.4)	1(11.1)	0(0.0)	1(11.1)	9
Total		70 (37.2)	86(45.7)	24 (12.8)	1(0.5)	7 (3.7)	188

NB. Numbers in bracket shows the percentage of grain colour in each class.FL= fairly loose, L= loose, VL= very loose, SC= semi compact, comp. = compact

3.3.1.3 Lemma colour distribution by origin

The lemmacolour distribution of the studied genotypes across origin, region and zone of collection was assessed and presented in Table 7. Based on zones of collections, the lemma colour for genotypes from the three zones of Tigray region were 41.7 % yellowish white, 47.2% variegated 5.6% red, 2.8% purple and 2.8% green. On the other hand, collections from the six zones of Amhara region were 36.1 % yellowish white, 38.9% variegated 1.2% red, 12.5% purple and 11.1% green in lemma colour. Furthermore, collections from three zones of Oromia region were 33.3% yellowish white, 44.4% variegated, 2.8% red, 5.6% purple and 13.9% green in lemma colour. In general, in all regions and zones, the majority of the lemma colour in the studied genotypes were of variegated and yellowish white type.

The improved varieties showed 71.4% yellowish white, 22.9% variegated, 2.9% purple and 2.9% green lemma colour while the breeding lines showed 50% yellowish white, 20% variegated, 10% purple and 10% red.

Table 7. Lemma colour distribution of 188 tef genotypes from various origins.

Region	Zones	Lemma Colour					Total
		Yellowish White	Variegated	Red	Purple	Green	
Tigray	Central Tigray	8(66.7)	4 (33.3)	0	0	0	12
	East Tigray	4(33.3)	8(66.7)	0	0	0	12
	West Tigray	3(25.0)	5(41.7)	2(16.7)	1(8.3)	1(8.3)	12
Amhara	North Gonder	4(33.3)	5(41.7)	0	1(8.3)	2(50.0)	12
	South Gonder	3(25.0)	6(50.0)	0	2(16.7)	1(8.3)	12
	North Wello	6(50.0)	4(33.3)	0	2(16.7)	0	12
	South Wello	3(25.0)	5(41.7)	1(8.3)	3(25.0)	0	12
	East Gojam	3(25.0)	5(41.7)	0	1(8.3)	3(25.0)	12
	West Gojam	7(58.3)	3(25.0)	0	0	2(50.0)	12
Oromia	North Shewa	6(50.0)	4(33.3)	0	2(16.7)	0	12
	East Shewa	2(16.7)	8(66.7)	0	0	2(16.7)	12
	West Shewa	4(33.3)	4(33.3)	1(8.3)	0	3(25.0)	12
Improved varieties		25 (71.4)	8(22.9)	0	1(2.9)	1(2.9)	35
Breeding lines		5(55.6)	1(11.1)	1(11.1)	1(11.1)	0	9
Total		83(44.1)	70(37.2)	5(2.7)	14(7.4)	15(8.4)	188

NB. Numbers in bracket shows the percentage of grain colour in each class.

3.3.1.4 Basal stalk colour distribution by origin

The basal stalk colour distribution of the studied genotypes across genetic origin, region and zone of collection are presented in Table 8. Based on zones of collections, the genotypes from central and East Tigray, East and West Gojam, East Shewa and North Wello showed entirely of green basal stalk colour. However, those from West Tigray, North Gonder, South Wello, North and East Shewa showed a combination of stalk colour with the majority still green colour. Similarly, 94.3% and 5.7% of the improved varieties were of

green and golden brown colour, respectively whereas the breeding lines had 70% green, 10% purple, 10% golden brown, and 10% dark brown stalk colour.

Table 8. Basal stalk colour distribution of 189 tef genotypes from various origins

Region	Zones	Basal stalk colour				Total
		DB	GB	Purple	Green	
Tigray	Central Tigray	0	0	0	12(100)	12
	East Tigray	0	0	0	12(100)	12
	West Tigray	0	3(33.3)	1(8.3)	8(66.7)	12
Amhara	North Gonder	1(8.3)	0	0	11(91.7)	12
	South Gonder	0	1(8.3)	1(8.3)	10(83.3)	12
	North Wello	0	0	0	12(100)	12
	South Wello	1(8.3)	2(16.7)	0	9(75.0)	12
	East Gojam	0	0	0	12(100)	12
	West Gojam	0	0	0	12(100)	12
Oromia	North Shewa	1(8.3)	0	1(8.3)	10(83.3)	12
	East Shewa	0	0	0	12(100)	12
	West Shewa	0	1(8.3)	0	11(91.7)	12
Improved varieties		0	2(5.7)	0	33(94.3)	35
Breeding lines		1(11.1)	1(11.1)	1(11.1)	6(66.7)	9
Total		4(2.1)	10 (5.3)	4(2.1)	170(90.4)	188

NB. Numbers in bracket shows the percentage of grain colour in each class.DB = Dark brawn, GB = Golden brown

The distribution of lemma colour across the various altitude is summarized in Table 9. Thus, the variegated and yellowish white lemma colour accounted for the largest proportion in all altitudinal classes. On the other hand, the red lemma colour occupied the least proportion and even collections from altitude class of 2001-2500 and above 2500m showed no such colour. The green and purple lemma colourwere equally represented by 6.25% in altitude above 2500m.

Table 9. Distribution of Lemma colour by altitude

Lemma colour	Altitude							
	<1500		1501-2000		2001-2500		>2500m	
	#	%	#	%	#	%	#	%
Variegated	6	50	20	40.8	19	37.3	17	53.1
Yellowish white	3	25	18	37.6	21	41.2	11	34.4
Purple	1	8.3	4	8.2	3	5.9	2	6.25
Red	2	16.6	2	4.1	0	0	0	0
Green	0	0	5	10.2	8	15.7	2	6.25
Total	12	100	49	100	51	100	32	100

3.3.2 Quantitative Traits Diversity

3.3.2.1 Analysis of Variance

The results of combined analysis of variance revealed highly significant ($P \leq 0.01$) differences among genotypes, locations and genotype by environment interaction for the studied traits (Table 10). Thus, the mean square due to genotypes was significant for all traits and that of location was also significant for all traits except culm length. Furthermore, GXE interaction was also highly significant ($P \leq 0.01$) for all studied traits except plant height, peduncle length, number of total and fertile tillers ($P > 0.05$) showing the differential performance of genotypes across different environments (Table 10).

Table 10. Combined ANOVA across two locations for 16 traits of 188 tef genotypes studied

Traits	Loc	Rep (loc)	Geno	GXE	Error
DH	298350.2***	86.8***	97.9***	31.9***	5.47
DM	510085.3***	41.3***	92.85***	63.9***	7.86
GFP	27415.5***	90.4***	98.7***	71.3***	9.34
PH	2313.89***	137.5***	392.4***	13.5ns	13.48
PL	1429.7***	54.2***	101.0***	2.0ns	5.64
CL	0.32ns	17.8ns	219.2***	15.5**	11.92
Pdl	362.0***	145.0***	36.6***	0.8ns	3.85
SCD	159.9***	0.37***	0.14***	0.10***	0.04
TT	140.2***	17.5***	2.41***	0.02ns	0.47
FT	73.1***	8.9***	2.14***	0.02ns	0.43
SPK	6432547.4***	3120.3**	21514.7***	13205.3***	896.7
SBM	36895.1***	23.4***	89.0***	56.4***	1.09
GY	666.1***	0.57***	2.5***	1.6***	0.05
HI	2574.4***	0.72ns	29.1***	27.8***	0.41
TKW	364.0***	32.2***	24.4***	22.2***	6.41
LG	4036.4***	669.0***	201.0***	242.8***	21.33

Abbreviations: DH: days to heading; DM: days to maturity; GFP: grain filling period; PH: plant height; PL: panicle length; CL: culm length; Pdl: peduncle length; SCD: second culm diameter; TT: number of total tillers; FT: number of fertile tillers; SPK: number of spikelet; TBM: total biomass; GY: grain yield; HI: harvest index; TKW: thousand kernel weight; LG: lodging index.

, * significantly different at 1% and 0.1%, respectively.

3.3.2.2 Patterns of quantitative traits variation in tef

In this study, a wider range of variations were observed for all quantitative traits of 188 tef genotypes evaluated at two locations (Table 11). Plant height and panicle length ranged from 60.7 cm to 107.1 cm and 19.5 to 39.5 cm, respectively. Thickness of the second culm diameter which had positive correlation with lodging resistance also ranged from 1.2 to 2.1 cm in this study. The number of fertile tillers per plant and spikelet per panicle ranged from 2.1 to 5.5 and 156.7 to 441.7 respectively and thus, the variation observed for latter trait was the widest of all studied traits. Thousand kernel weights and grain yield also ranged from 20.7 mg to 33.0 mg and 3.7 to 7.3 t/ha in that order (Table 11). The wide variation obtained for plant height, culm diameter and tillering capacity in the present study indicates the possibility to combat the problem of lodging. The variation observed for number of spikelet per panicle, harvest index, thousand kernel weight, total biomass and grain yield, on the other hand, imply the possibility to develop varieties with better grain yield and/or other biological yields. Finally, the lodging index value which ranged from 44.7 to 79.3 shows the possibility to identify lodging resistance among the studied genotypes (Table 11).

With respect to zones of germplasm collections, genotypes from North Gonder had the shortest mean plant height, length of panicle, culm and peduncle and smallest diameter of the second culm internodes (Table 12). Genotypes from South Gonder, on the other hand, had the longest days to heading and maturity whereas those from West Shewa zone had the highest value for plant height, culm diameter, harvest index, number of fertile tillers, panicle length and spikelet per panicle. Furthermore, the highest mean grain yield and total biomass was obtained for genotypes from central Tigray whereas East Gojam, North

Shewa and South Gonder had the least value of grain yield, shoot biomass and number of spikelet per panicle respectively. Genotypes from East Tigray had the highest mean value for 1000-kernel weight and lodging index while North Wello had the least mean value for both traits (Table 13). In general, among all zones, genotypes from Central Tigray and West Shewa were found to have several desirable traits to increase tef productivity.

Regarding altitudinal classes, collections from below 1500m a. s. l. had the highest mean value for days to heading and maturity, plant height, length of panicle and peduncle, culm diameter while those from 1500-2000m a. s. l. had moderate values for some studied traits. On the other hand, the highest mean value for spikelet per panicle, shoot biomass, grain yield and 1000-kernel weight were observed for genotypes collected from 2001-2500m a. s. l. whereas the highest value of lodging and number of fertile tillers was obtained for genotypes collected from altitude above 2500m a. s. l (Table 13). Thus, based on the altitude of collections, genotypes obtained from altitudes ranging from 2001-2500m had more desirable traits useful to increase tef productivity.

Table 11. Range, mean and Standard error (SE) of mean for 13 different traits of tef genotypes (based on average of 188 genotypes from 14 populations)

Variable	Range				Mean± SE
	Minimum		Maximum		
	Value	Genotype	Value	Genotype	
Days to heading (days)	40.3	Simada	60.8	Acc. 236760-6	51.1±0.30
Days to maturity (days)	101	Acc. 212614-2	122.5	Melko	114.0±0.29
Plant height (cm)	62.7	Acc. 19506-4	107.1	Acc. 212698-2	85.9±0.59
Panicle length (cm)	19.5	Acc. 55221-1	39.5	Acc. 18460-0	30.4±0.30
Peduncle length (cm)	12.5	Acc. 237205-2	26	Acc. 242160-1	18.9±0.18
Culm diameter (mm)	1.2	Acc. 19506-4	2.1	Acc. 243491-2	1.5±0.01
Fertile tillers (number)	2.1	Acc. 243540-3	5.5	Acc. 242186-4	3.2±0.04
Spikelet per panicle (number)	156.7	Acc. 236760-4	441.7	Acc. 15309-3	312.5±4.37
Shoot biomass (kg/ha)	15.8	RIL-11	36.7	Acc. 237184-1	26.3±0.28
Grain yield (kg/ha)	3.7	RIL-302	7.3	RIL-91	5.6±0.05
Harvest index (%)	14.7	Acc. 237205-2	24.3	Acc. 234407-2	21.73±0.10
Thousand kernel weights (mg)	20.7	Acc. 243513-1	33	Acc. 19241-1	26.7±0.15
Lodging index (%)	44.7	Key Murri	79.3	Acc. 242155-3	67.2±0.42

Table 12. Range, mean and standard error (SE) of mean for 13 different traits of tef genotypes (based on average of 188 genotypes from 12 zones of collections)

Variable	Range				Mean± SE
	Minimum		Maximum		
	Value	Origin	Value	Origin	
Days to heading (days)	49.0	East Shewa	52.9	South Gonder	51.±0.37
Days to maturity (days)	112.5	North Shewa	114.9	South Gonder	113.9±0.18
Plant height (cm)	80.4	North Gonder	87.7	West Shewa	85.7±0.62
Panicle length (cm)	28.0	North Gonder	31.4	East Shewa	30.0±0.30
Peduncle length (cm)	17.5	North Gonder	20.5	North Shewa	18.9±0.26
Culm diameter (mm)	1.4	North Gonder	1.6	West Shewa	1.5±0.02
Fertile tillers (number)	3.0	East Shewa	3.5	West Shewa	3.2±0.05
Spikelet per panicle (number)	289.6	South Gonder	344.1	West Shewa	305.9±4.4
Shoot biomass (kg/ha)	24.7	North Shewa	28.1	Central Tigray	26.7±0.30
Grain yield (kg/ha)	5.3	East Gojam	6.0	Central Tigray	5.6±0.06
Harvest index (%)	20.5	West Shewa	22.5	East Tigray	21.73±0.09
Thousand kernel weights (mg)	25.6	North Wello	28.2	East Tigray	26.7±0.23
Lodging index (%)	65.3	North Wello	69.4	East Tigray	67.6±0.38

Table 13. Range, mean and standard error (SE) of mean for 13 traits of tef genotypes (based on average of 188 genotypes from 4 altitudinal ranges of collections)

Variable	Range				Mean± SE
	Minimum		Maximum		
	Value	Origin	Value	Origin	
Days to heading (days)	51.1	15001-2000m	52.2	<1500m	51.6±0.25
Days to maturity (days)	113.7	15001-2000m	114.6	<1500m	114.1±0.19
Plant height (cm)	85.5	15001-2000m	87.7	<1500m	86.2±0.51
Panicle length (cm)	30.0	15001-2000m	30.5	<1500m	30.2±0.13
Peduncle length (cm)	18.5	15001-2000m	19.4	>2500m	18.9±0.19
Culm diameter (mm)	1.5	>2500m	1.6	<1500m	1.5±0.01
Fertile tillers (number)	3.0	>2500m	3.3	>2500m	3.2±0.05
Spikelet per panicle (number)	282.0	<1500m	310.2	15001-2000m	301.9±6.65
Shoot biomass (kg/ha)	25.5	>2500m	27.8	15001-2000m	26.3±0.55
Grain yield (kg/ha)	5.4	<1500m	5.7	15001-2000m	5.6±0.05
Harvest index (%)	21.1	2001-2500m	22.4	>2500m	21.73±0.09
Thousand kernel weights (mg)	26.2	>2500m	27.2	15001-2000m	26.7±0.21
Lodging index (%)	66.1	<1500m	68.6	>2500m	67.5±0.53

3.3.2.3 Principal component analysis

3.3.2.3.1 PCA based on individual genotypes

The principal component analysis (PCA) based on 12 quantitative traits of 188 tef genotypes from various genetic and eco-geographic origin was assessed and is presented (Table 14). Thus, the first three PCs with eigen value greater than unity contributed 60.1% of the total variation, with PC1, PC2 and PC3 accounted for 32.9%, 15.8% and 11.4% of the total variation, respectively. All traits other than peduncle length and 1000-kernel

weight contributed to most of the variation in PC1 (Table 14) whereas the variation in PC2 was mainly due to grain yield and total biomass, lodging index and peduncle length. Furthermore, peduncle length, 1000-kernel weight, days to heading and grain yield, respectively, were the major traits for the variation in PC3.

Table 14. Principal component analysis for 12 quantitative traits of 188 tef genotypes from various origins

Variable	PC1	PC2	PC3
Days to heading (days)	0.323	-0.050	-0.468
Days to maturity (days)	0.368	-0.196	-0.088
Plant height (cm)	0.443	0.051	0.113
Panicle length (cm)	0.359	0.213	-0.046
Peduncle length (cm)	0.076	-0.390	0.520
Culm diameter (mm)	0.387	-0.244	0.091
Fertile tillers (number)	-0.217	0.112	-0.262
Spikelet per panicle (number)	0.338	0.013	-0.100
Shoot biomass (kg/ha)	0.267	0.478	0.052
Grain yield (kg/ha)	0.142	0.513	0.399
Harvest index (%)	0.004	-0.135	0.473
Thousand kernel weights (mg)	-0.160	0.420	0.127
Eigenvalue	3.866	1.893	1.371
Proportion	0.322	0.158	0.114
Cumulative	32.2	48.0	59.4

3.3.2.3.2 PCA based on 14 genetic and eco-geographic origins

Principal component analysis based on 14 genetic and eco-geographic origins revealed that the first PCs with eigen value greater than one accounted for 75.8% of the total variations.

In this analysis, PC1, PC2 and PC3 respectively contributed for 39.5%, 21.6% and 14.7%

of the total variations (Table 15). Thus, the majority of variations in PC₁ were due to variations in lodging index, panicle length, shoot biomass, days to maturity, plant height and grain yield whereas those in PC₂ were due to peduncle length, culm diameter, shoot biomass and plant height. Likewise, the variation in PC₃ was contributed due to variations in number of spikelet per panicle, 1000-kernel weight and number of fertile tillers.

3.3.2.3.3 PCA based on 12 zones of germplasm collection

The first five PCs based on 12 zones of germplasm collection which had eigen value greater than one accounted for 89.2% of the total variation using 12 quantitative traits of tef. Thus, PC₁, PC₂, PC₃, PC₄ and PC₅ accounted for 30.7%, 23.7%, 15.3%, 10.7% and 8.7% of the total variation respectively (Table 15). Peduncle length, plant height, panicle length and second culm diameter and shoot biomass were the major contributors for variations in PC₁ whereas lodging index, 1000 kernel weight, days to maturity, grain yield and spikelet per panicle were the major contributors for PC₂. On the other hand, the major contributors for PC₃ were days to heading, lodging index, grain yield, shoot biomass and 1000 kernel weight whereas fertile tillers, lodging index and 1000 kernel weight were for PC₄. Furthermore, 1000 kernel weight, days to heading, second culm diameter and peduncle length were the major traits accounted for PC₅.

Table 15. Principal component analysis of 12 traits of tef genotypes based on 14 origins (left half) and 12 zones (right half) of germplasm collections

Variable	Based on Origins* (14)			Based on zones of collections** (12)				
	PC1	PC2	PC3	PC1	PC2	PC3	PC4	PC5
Days to heading	0.177	0.297	0.271	0.223	-0.187	-0.473	0.134	0.455
Days to maturity	0.366	0.142	0.196	0.198	-0.334	-0.187	-0.369	0.143
Plant height	0.36	-0.311	0.102	-0.411	-0.265	-0.129	-0.222	0.016
Panicle length	0.375	-0.281	0.091	-0.405	-0.291	0.129	0.054	0.179
Peduncle length	-0.001	-0.556	-0.098	-0.462	0.057	-0.077	-0.043	-0.335
Culm diameter	0.265	-0.4	0.05	-0.406	-0.181	-0.074	0.178	0.416
Fertile tillers	-0.235	0.073	0.451	0.22	-0.125	-0.198	0.637	-0.283
Spikelet per panicle	-0.042	-0.269	0.628	-0.125	-0.424	-0.281	0.294	-0.20
Shoot biomass	0.372	0.338	0.016	0.321	-0.317	0.274	-0.126	0.271
Grain yield	0.352	0.129	0.061	0.039	-0.396	0.397	-0.158	-0.213
1000- kernel weights	0.174	-0.084	-0.506	-0.16	0.319	0.328	0.336	0.461
Lodging index	0.38	0.177	-0.029	0.07	-0.329	0.488	0.337	-0.055
Eigenvalue	4.736	2.589	1.768	3.690	2.848	1.838	1.286	1.038
Proportion	0.395	0.216	0.147	0.307	0.237	0.153	0.107	0.087
Cumulative	0.395	0.61	0.758	0.307	0.545	0.698	0.805	0.892

* combined data of populations from 12 zones of collections plus one improved variety plus population of breeding lines. ** 12 populations from 12 zones of collections.

3.3.2.3.4 PCA based on four altitudes of germplasm collection

For altitude classes, the first two PCs with eigen values greater than one extracted 92.9% of the total variation. The first PC accounted for 63.9% of the total variation mainly due to almost all traits other than Peduncle length and 1000-kernel weight (Table. 16). PC₂, on the other hand, accounted for 29% of the total variation whereby peduncle length, 1000-kernel weight, shoot biomass and fertile tillers contributed for the largest proportion.

3.3.2.3.5 PCA based on woredas of germplasm collection

Based on collection Woredas, the first three PCs with eigen values greater than one extracted 62.5% of the total variation whereby PC1, PC2 and PC3 respectively accounted for 32.7%, 17.2% and 12.6% of the variations exhibited for collection Woredas. Thus, the variation in PC1 was mainly due to plant height, days to maturity, second culm diameter, panicle length and spikelet per panicle (Table. 16). PC₂, on the other hand, was based on the variation mainly resulting from shoot biomass, peduncle length, grain yield and lodging index whereas the variation in grain yield, days to heading, peduncle length and 1000-kernel weight were the major contributors to PC3.

Table 16. Principal component analysis for 12 traits of tef based on four altitudinal zones (left half) and 72 Woredas (right half) of germplasm collections

Variable	Based on altitude		Based on collection Woredas		
	PC1	PC2	PC1	PC2	PC3
Days to heading	0.298	-0.28	0.273	-0.147	-0.526
Days to maturity	0.324	-0.106	0.397	0.146	-0.065
Plant height	0.355	-0.083	0.45	0.053	0.071
Panicle length	0.328	0.148	0.345	-0.245	0.044
Peduncle length	0.001	-0.509	0.116	0.48	0.399
Culm diameter	0.346	0.15	0.381	0.285	-0.013
Fertile tillers	-0.286	-0.322	-0.248	-0.086	-0.2
Spikelet per panicle	-0.359	-0.023	0.343	-0.253	-0.003
Shoot biomass	-0.203	0.385	0.207	-0.485	0.144
Grain yield	-0.344	0.12	0.097	-0.36	0.542
Thousand kernel weights	-0.028	0.502	-0.001	0.179	0.392
Lodging index	-0.285	-0.284	-0.236	-0.339	0.21
Eigenvalue	7.6724	3.475	3.9205	2.0685	1.5094
Proportion	0.639	0.29	0.327	0.172	0.126
Cumulative	0.639	0.929	0.327	0.499	0.625

3.3.2.4 Cluster Analysis

3.3.2.4.1 Cluster analysis of individual tef genotypes

Cluster analysis based on 12 standardized traits of 188 individual tef genotypes from various sources resulted in the formation of six distinct clusters consisting of 19 to 50 genotypes. Similar number of clusters were reported previously by different authors using germplasm collections (Assefa *et al*, 2001, 2003), released tef varieties (Jifar *et al.*, 2015), brown seeded tef genotypes (Jifar *et al.*, 2017). Cluster I and V consisted of 26 genotypes whereas cluster II, III, IV and VI consisted of 32, 19, 35 and 50 genotypes, respectively (Table 17, 18; Fig. 4). The first cluster consisted of 26 genotypes from 11 pre-determined populations with the majorities from Central Tigray (5) North Gonder (3), North Wello (3), West Gojam (3) and improved varieties (3). Cluster II, on the other hand, consisted of 32 genotypes from 11 populations whereby the majority of the genotypes were from populations of breeding lines (7) improved varieties (5), West Gojam (4) and East Gojam (3). Cluster III consisted of 19 genotypes whereby, the majority were from populations of North Wello (4) followed by North Shewa (3), Central Tigray (2), South Gonder (2) and West Tigray (2) In cluster IV, genotypes from populations of improved varieties (7), South Wello (5) North Gonder (4), North Shewa (3) and South Gonder (3) covered the majority. Cluster -V consisted of 26 genotypes from 13 populations, with the majority from improved varieties (7) and East Shewa (5). Cluster-VI, on the other hand, consisted of the largest number of genotypes from all predetermined populations except the breeding lines. Thus, the highest number of genotypes were from improved varieties (12) followed by East Tigray (6), West Shewa (6) and East Gojam (5) populations.

Based on cluster mean, genotypes in cluster-I had the highest number of fertile tillers while it had the least peduncle length. Genotypes in cluster-II, however, were characterized to

have the shortest days to heading and maturity, plant height and panicle length, least value of second culm diameter, number of spikelet per panicle and shoot biomass and the highest value of lodging index. Cluster-III had the highest value of days to maturity, peduncle length and 1000-kernel weight and the lowest value of lodging index with moderate values for all remaining studied traits. Cluster-IV, furthermore, had the least value for grain yield and 1000-kernel weight while it had the longest days heading with moderate values for the remaining traits. Interestingly, cluster-V had the highest values for five of the twelve studied traits while it had the least value for number of fertile tillers only. Genotypes in cluster-VI were found to have the highest value of grain yield and average values of all other studied traits (Table 18). Thus, breeders dealing with high yield should use genotypes in cluster VI whereas those dealing with early maturity should focus on genotypes in cluster-II. Besides, in variability creation, excellent level of genetic variability and high heterosis can be obtained bycrossing genotypes in cluster 2 & 5 followed by cluster 1 & 3, Cluster 2 & 3 and cluster 1 & 5, respectively (Table 19).

The distribution of 12 investigated traits in tef genotypes across PC₁ and PC₂ was also assessed and revealed the formation of four distinct groups. Thus, the first group consisted of fertile tillers and lodging index; the second group consisted of grain yield and total biomass; the third group consisted of plant height, panicle length, culm diameter, days to maturity and days to heading whereas the fourth group consisted of 1000-kernel weight and peduncle length (Fig. 5). Similarly, the distribution of the studied genotypes across the first two PCs was also analyzed and it showed no distinct grouping pattern similar with that of the clustering of individual genotypes (Appendix Fig. 1) may be due to the fact that the two PCs together accounted for less than 60% of the total variations.

Table 17. Clustering of 188 tef genotypes into six clusters using mean of 12 morpho-agronomic characters

Cluster	No of genotypes	Name of genotypes
C1	26	Acc. 19166-1, 19253-1, 234407-2, 243513-3, 243520-2, 19516-1, 55221-1, 18466-3, 236972-0, 234460-3, 9448-1, 242186-4, 243540-1, 15322-1, 212482-1, 215200-1, 237148-1, 243501-2, 19506-4, 242140-3, 55029-2, 18410-2, 237236-4, Holeta Key, Key Tena, Yilmana
C2	32	Acc. 243513-1, 55221-2, 229768-3, 55046-3, 242540-2, 9472-4, 15309-2, 234993-1, 19341-2, 225919-3, 212614-2, 225898-1, 19394-1, 19452-4, 242155-1, 242155-3, 17365-1, 236760-1, 9444-2, 234435-2, Asgori, Tseday, Amarach, Simada, Boset, <i>Kaye Murri</i> , RIL-85, RIL-91, RIL-96, RIL-11, RIL-302 and RIL-69
C3	19	Acc. 19132-2, 19132-3, 236965-1, 19202-2, 9559-1, 9559-2, 236746-0, 55104-3, 215200-2, 234985-2, 234993-3, 19341-3, 19374-1, 242214-1, 242144-1, 237236-3, 237239-3, Magna and RIL-81.
C4	35	Acc.234407-1, 237205-2, 19516-3, 18466-2, 19201-1, 242540-1, 9448-2, 9469-2, 9472-2, 243540-3, 15309-3, 18385-2, 236745-2, 215196-1, 234356-4, 212717-0, 225919-2, 225919-7, 212607-2, 212612-3, 2433497-2, 243504-1, 243504-3 and 19443-3, 18423-3, 236757-2, 19241-1, 243526-2, Holenkomi, Melko, Gibe, Gerado, Dega Tef, Gemechis and Kena
C5	26	Acc.19253-2, 55046-2, 15361-3, 17335-1, 18460-0, 236963-1, 236965-3, 19201-3, 9451-2, 242186-3, 15322-2, 19367-2, 212720-1, 242214-2, 19506-2, 18414-4, 9444-3, 243526-5, Genet, Zobel, Quncho, Guduru, Etsub, Laketch, <i>Kinde</i> and Kora
C6	50	Acc.237184-1, 9545-1, 9556-1, 212698-2, 229768-1, 229768-4, 236963-2, 236967-1, 236967-2, 15297-1, 15297-2, 15299-1, 15299-3, 234460-1, 234460-2, 19343-2, 243540-4, 236748-2, 236957-1, 215200-3, 237148-5, 55293-2, 225919-4, 242160-1, 212614-1, 243491-2, 243504-2, 242144-3, 55029-3, 17371-3, 18410-1, 18410-3, 18414-2, 236760-4, 236760-6, 9419-1, 19241-2, 243526-4, Enatit, Menagesha, Ziquala, Dukem, Ambo-Toke, Koye, Gola, Ajora, Dima, Gimbichu, Mechare and Workiyu

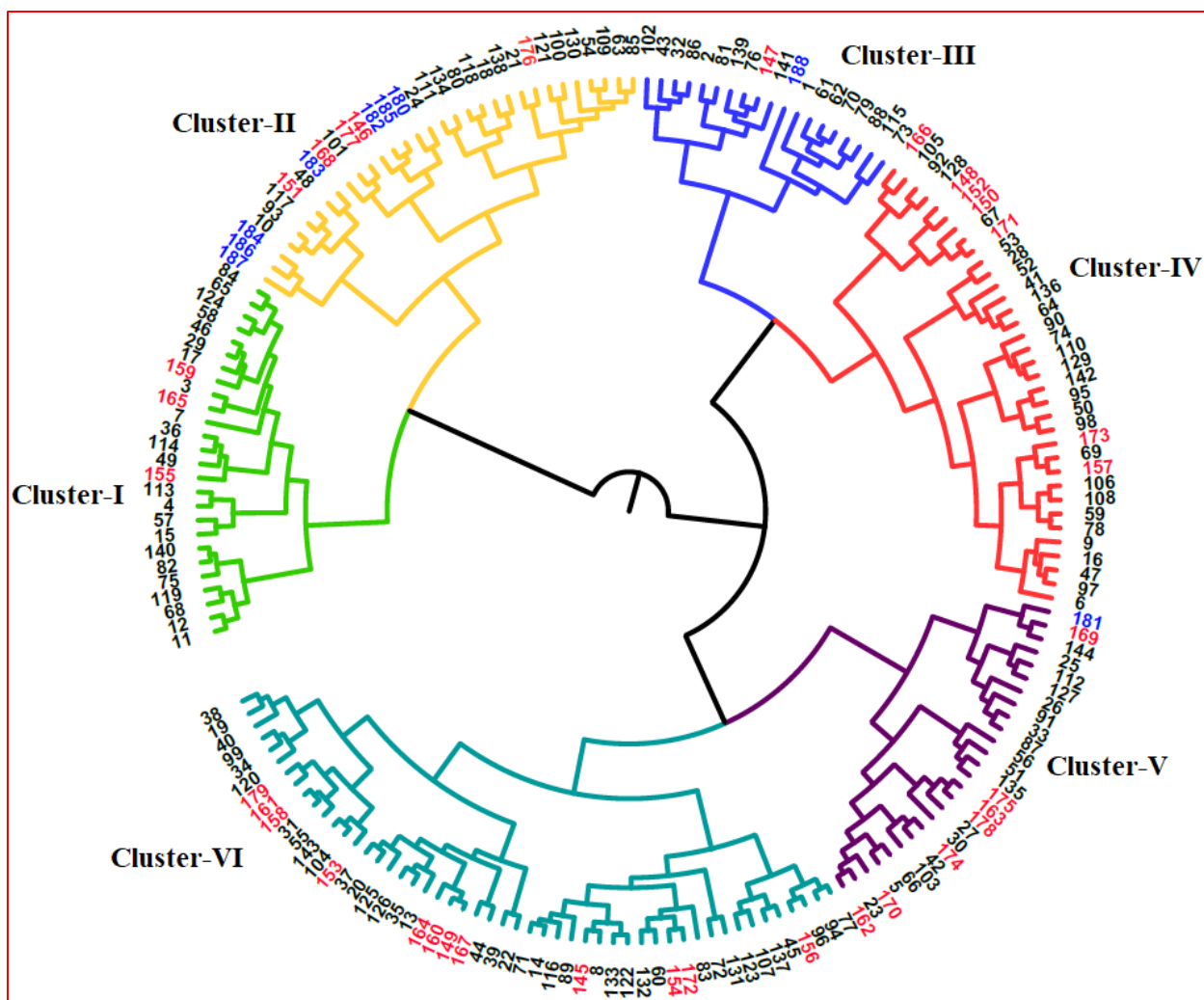


Figure 4. Dendrogram showing the genetic relationship among 188 tef genotypes from various sources in Ethiopia using 12 major traits. Genotypes and zones of collections are described as 1-12 (central Tigray), 13-24 (East Gojam), 25-36 (East Shewa), 37- 48 (East Tigray), 49-60 (North Gonder), 61-72 (North Shewa), 73-84 (North Wello), 85-96 (South Gonder), 97-108 (South Wello), 109-120 (West Gojam), 121-132 (West Shewa) and 133-144 (West Tigray). Improved varieties (145-179) and breeding lines (180-189) are written in red and blue color, respectively.

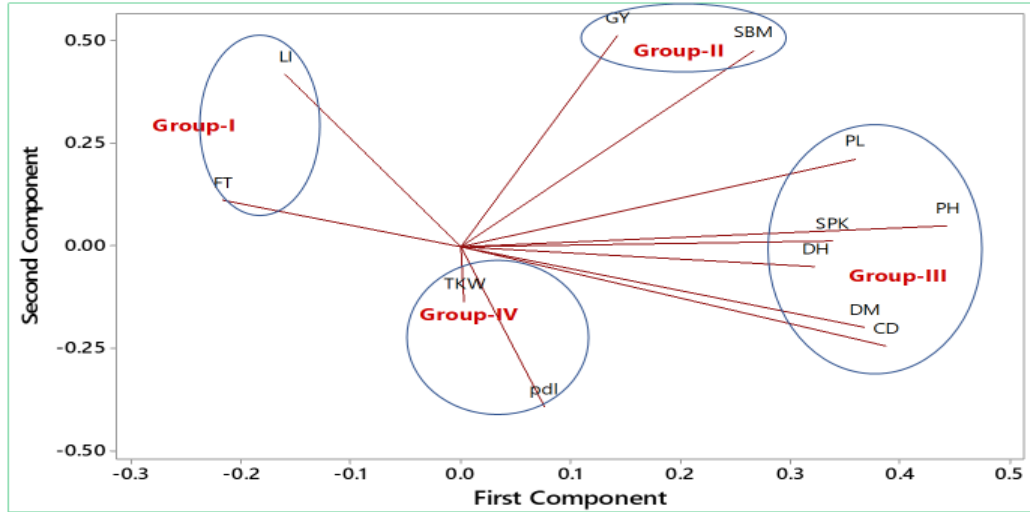


Figure 5. Distribution of 12 traits of 188 tef genotypes across PC₁ and PC₂

Table 18. Cluster means of 12 quantitative traits of 188 tef genotypes

Traits	Cluster Means					
	I	II	III	IV	V	VI
Days to heading	50.86	47.23	52.21	54.45	54.14	49.33
Days to maturity	111.97	108.92	116.32	116.17	115.51	115.22
Plant height (cm)	80.16	77.54	84.74	87.97	95.01	88.33
Panicle length(cm)	29.25	26.40	25.60	32.33	34.42	32.03
Peduncle length(cm)	16.16	18.88	22.06	18.19	18.85	19.58
Culm diameter(mm)	1.42	1.41	1.65	1.58	1.70	1.54
Number of fertile tillers	3.87	3.36	2.91	3.38	2.85	3.02
Number of spikelet per panicle	275.93	266.89	308.84	368.52	379.65	350.94
Shoot biomass (kg/ha)	27.57	23.53	24.17	24.99	28.99	27.87
Grain yield(kg/ha)	5.78	5.38	5.08	4.92	5.87	6.02
Thousand kernel weights	26.60	26.59	28.11	26.09	27.01	26.62
Lodging index	68.69	69.50	62.72	67.44	63.88	68.04

Table 19.Distance among six clusters of 188 tef genotypes

	Cluster1	Cluster2	Cluster3	Cluster 4	Cluster5	Cluster6
Cluster1	0					
Cluster2	9.73	0				
Cluster3	30.67**	26.65**	0			
Cluster4	12.51	20.96	17.14	0		
Cluster5	25.34**	38.98**	19.17	12.23	0	
Cluster6	12.97	19.55	16.07	9.63	6.81	0

*** Significant at 1% probability level

3.3.2.4.2 Clustering of tef populations

Clustering based on 14 populations of tef from various genetic and eco-geographic origin resulted in the formation of four distinct clusters (Fig. 6& 7). The number of genotypes in each cluster ranged from one to six genotype in cluster-I V & III, respectively. The first and second cluster, on the other hand, consisted of three and four genotypes, respectively. In this study, the breeding lines remained solitary whereas the improved varieties were grouped along with populations from the different zones of Shewa, and those from North Gonder and West Tigray zones. The grouping of populations from different parts of Shewa zones is due to geographic proximity along the three zones. Cluster-I consisted of three populations from Central Tigray, North Wello and West Gojam whereas, cluster-II consisted of four populations from East Gojam, East Tigray, South Gonder and South Wello. Here, the grouping of Central Tigray population with that of the West Gojam could be due to trans human seed exchanges among regions and zones. On the other hand, the

largest inter cluster distance was observed between Cluster 3 & 4 followed by Cluster 2 & 4, and 1 & 4 to provide best level of genetic recombination (Table 20).

Biplot analysis of the 12 traits of the 14 populations resulted in the formation of four major groups (Fig.8). Thus, group-I consisted of fertile tiller whereas group-II consisted of days to heading, days to maturity, lodging index, total biomass and grain yield. Group-III consisted of 1000-kernel weight, plant height, panicle length and culm diameter while Group-IV consisted of peduncle length spiklet per panicle.

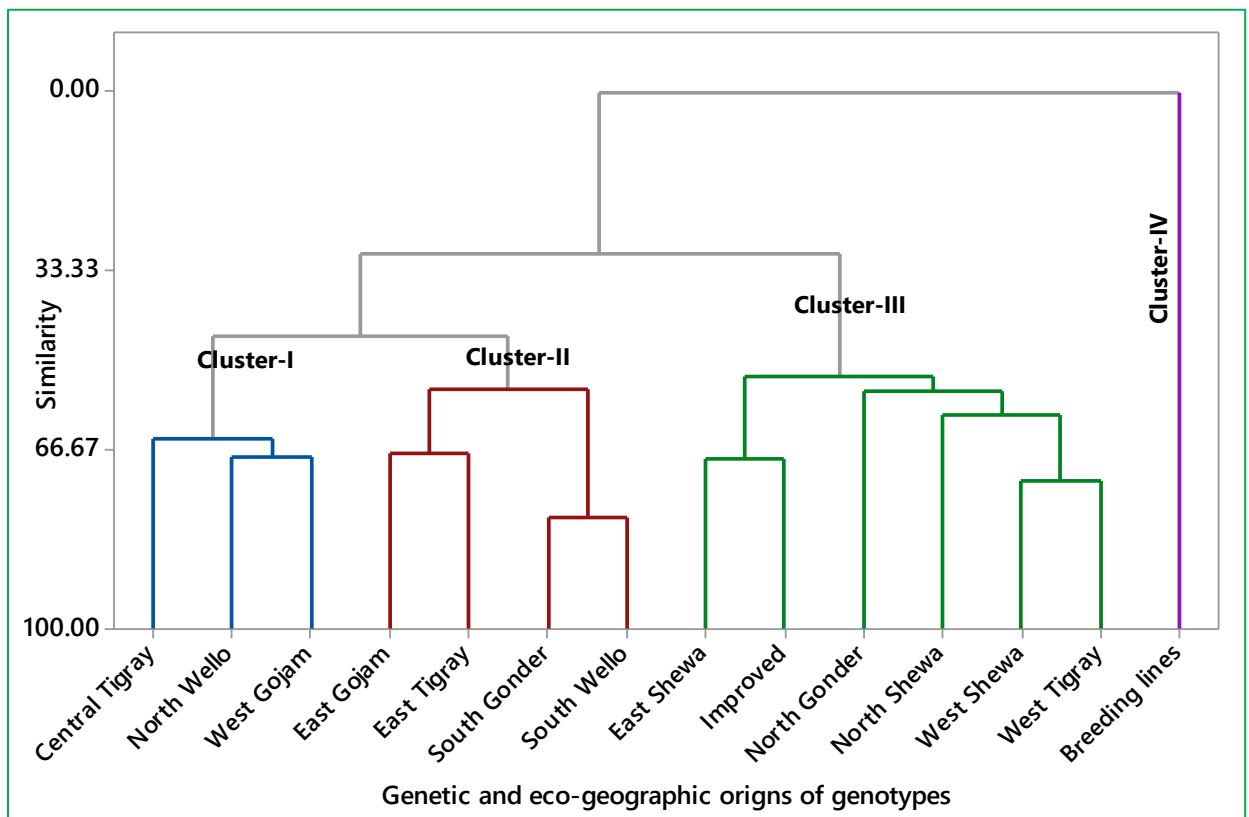


Figure 6. Clustering pattern of the 14 populations based on genetic and eco-geographic origins

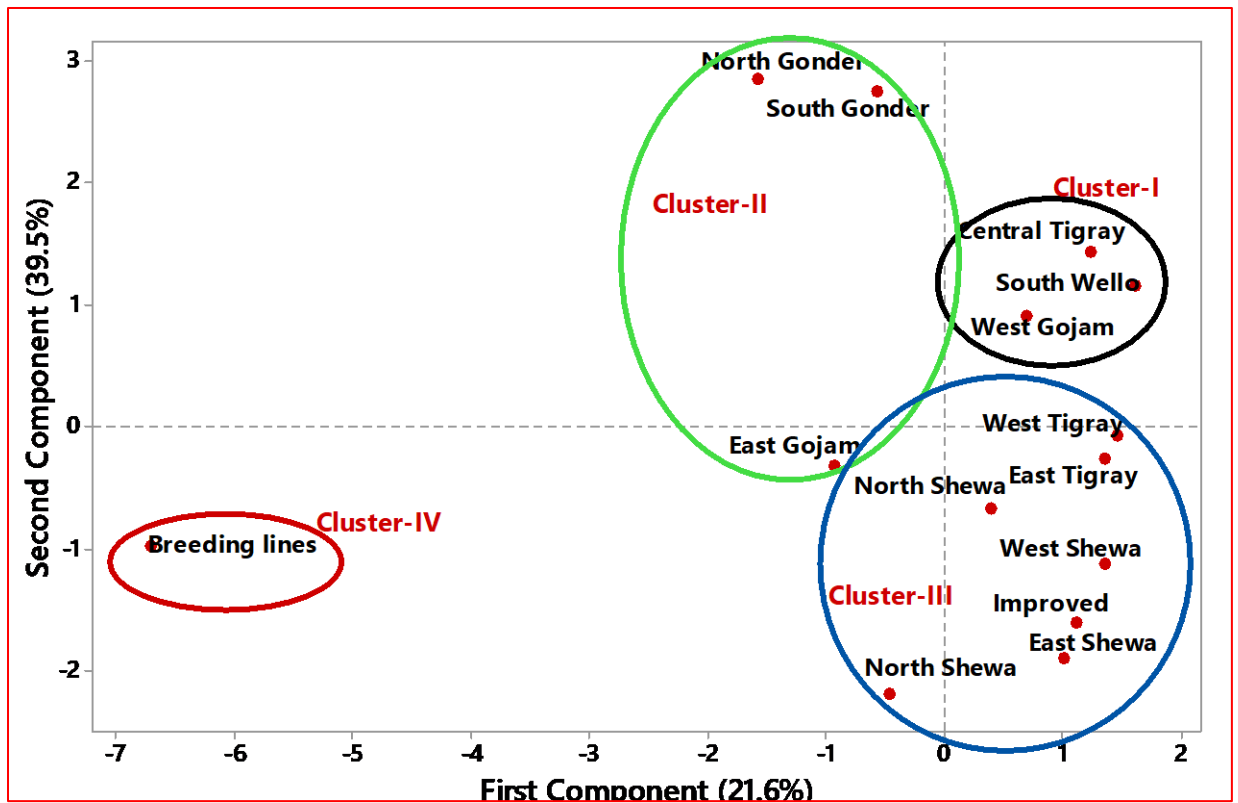


Figure 7. Biplot showing the distribution of 14 populations based on genetic and eco-geographic origins

Table 20. Distance among four clusters of 188 tef genotypes

	Cluster 1	Cluster 2	Cluster 3	Cluster 4
Cluster 1	0			
Cluster 2	3.21	0		
Cluster 3	3.84	2.97	0	
Cluster 4	6.08	7.01	7.78	0

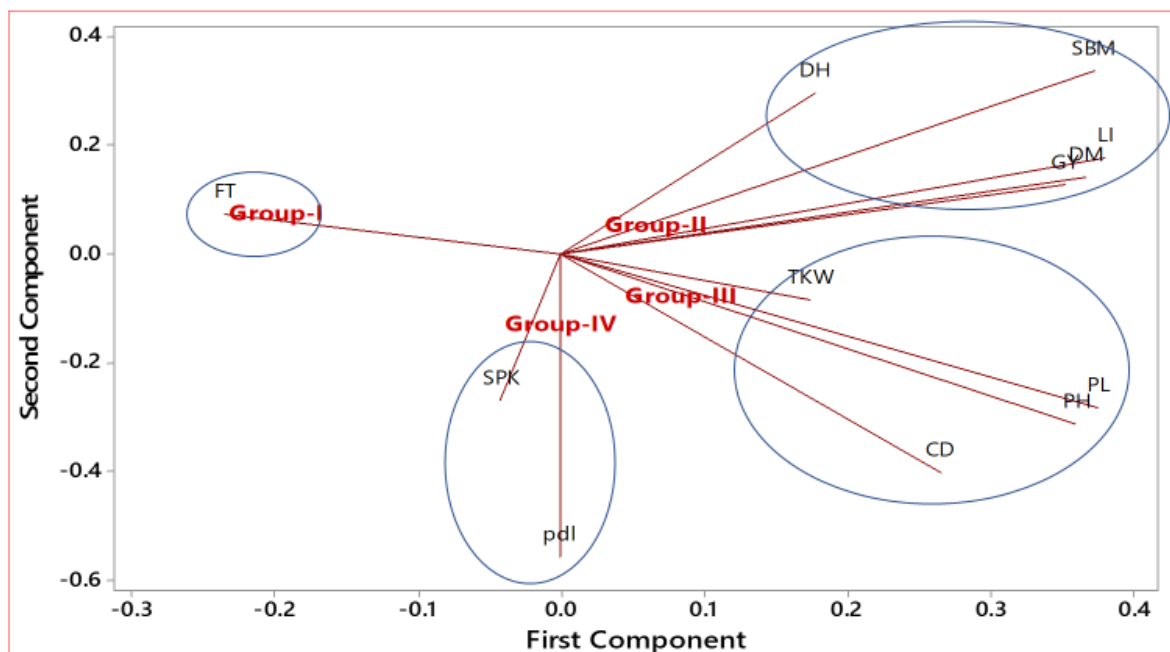


Figure 8. Biplot showing the distribution of 12 traits of the 14 populations across PC1 & PC2

3.3.2.4.3 Clustering of tef collection zones

Clustering based on 12 zones of tef germplasm collection grouped the 12 zones into three distinct clusters (Fig.9 and 10). Thus, cluster I, II and III consisted of three, four and five, respectively. The first cluster consisted of Central Tigray, North Wello and West Gojam zones whereas cluster-II includes East Tigray, East Gojam, South Gonder and South Wello zones. Cluster-III, on the other hand, consisted of the three Shewa zones as well as North Gonder and West Tigray zones. This grouping based of zones is in line with the origin-based clustering whereby the same zones were grouped under the three clusters (I, II & III).

Biplot analysis based on 12 traits of the 12 collection zones formed four major groups (Fig.11) whereby group 1 consisted of days to heading and days to maturity whereas group 2 consisted of culm diameter, spiklet per panicle, plant height, panicle length, total biomass, and grain

yield. Thousand kernel weight and lodging index constituted the third group while group four consists of fertile tillers only (Fig. 11).

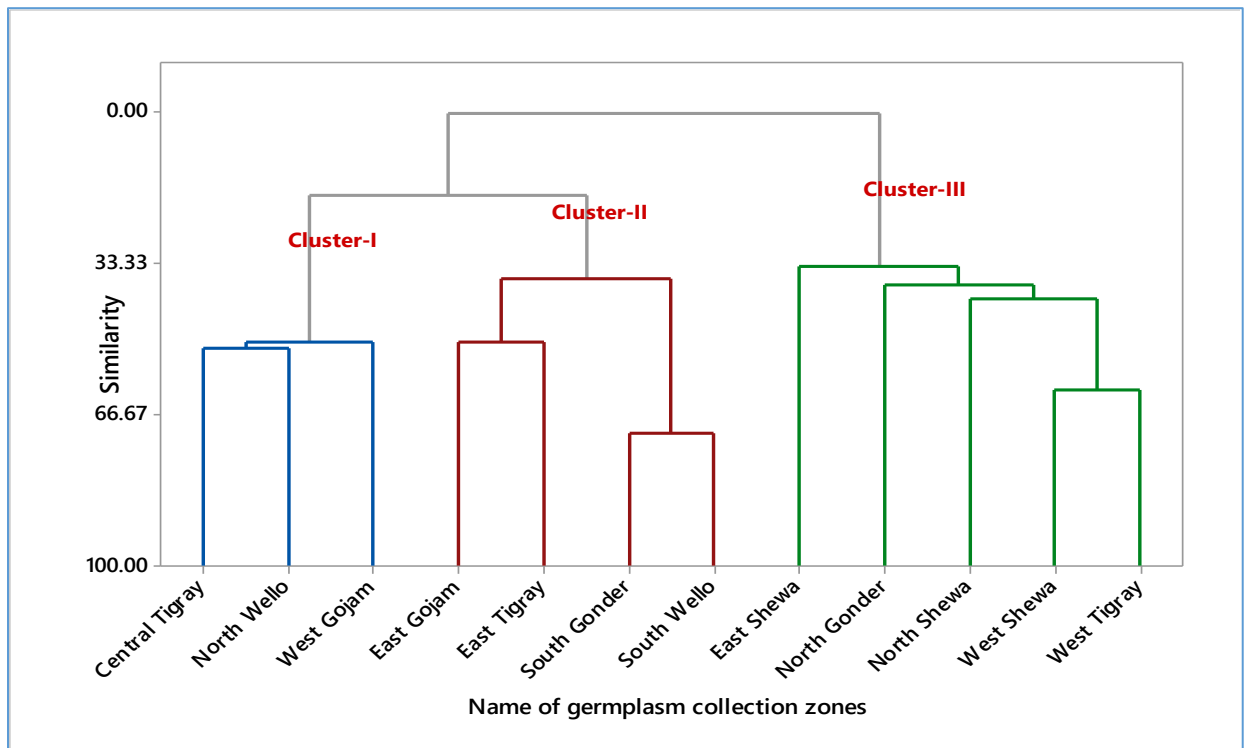


Figure 9. Clustering pattern of 12 zones of collection based on the variation in 12 major traits of tef.

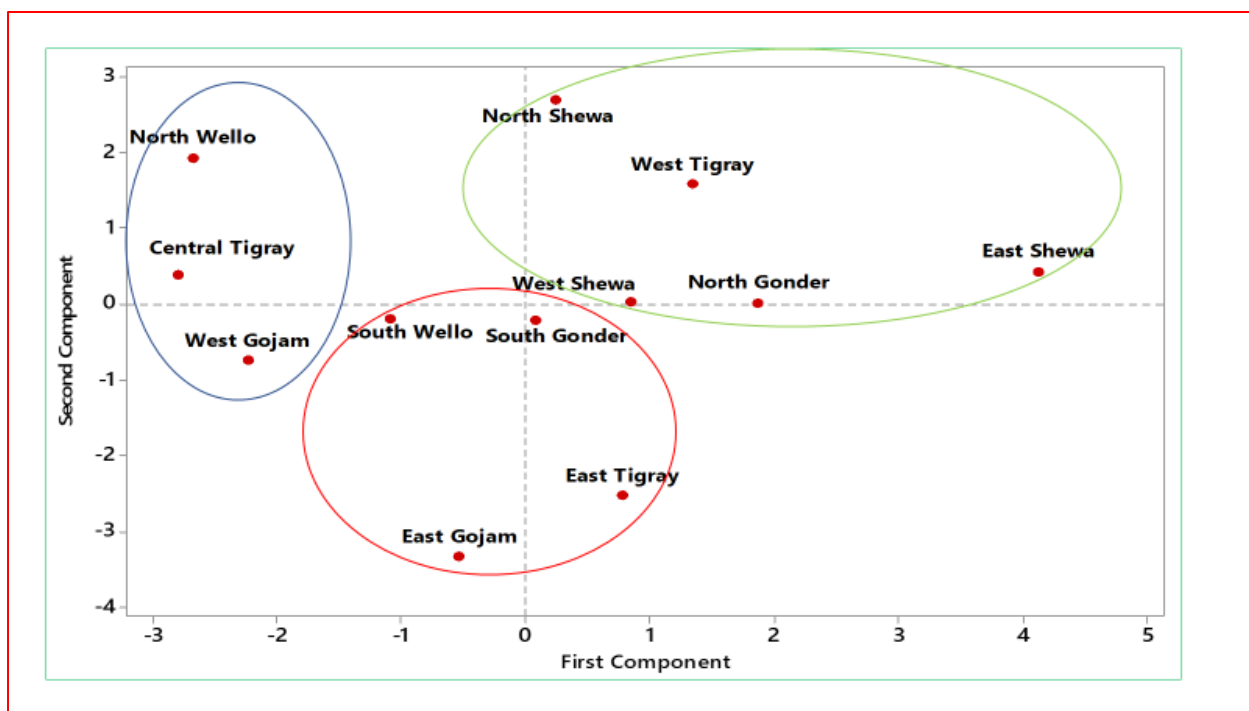


Figure 10. Biplot showing the distribution of 12 zones of tef germplasm collections

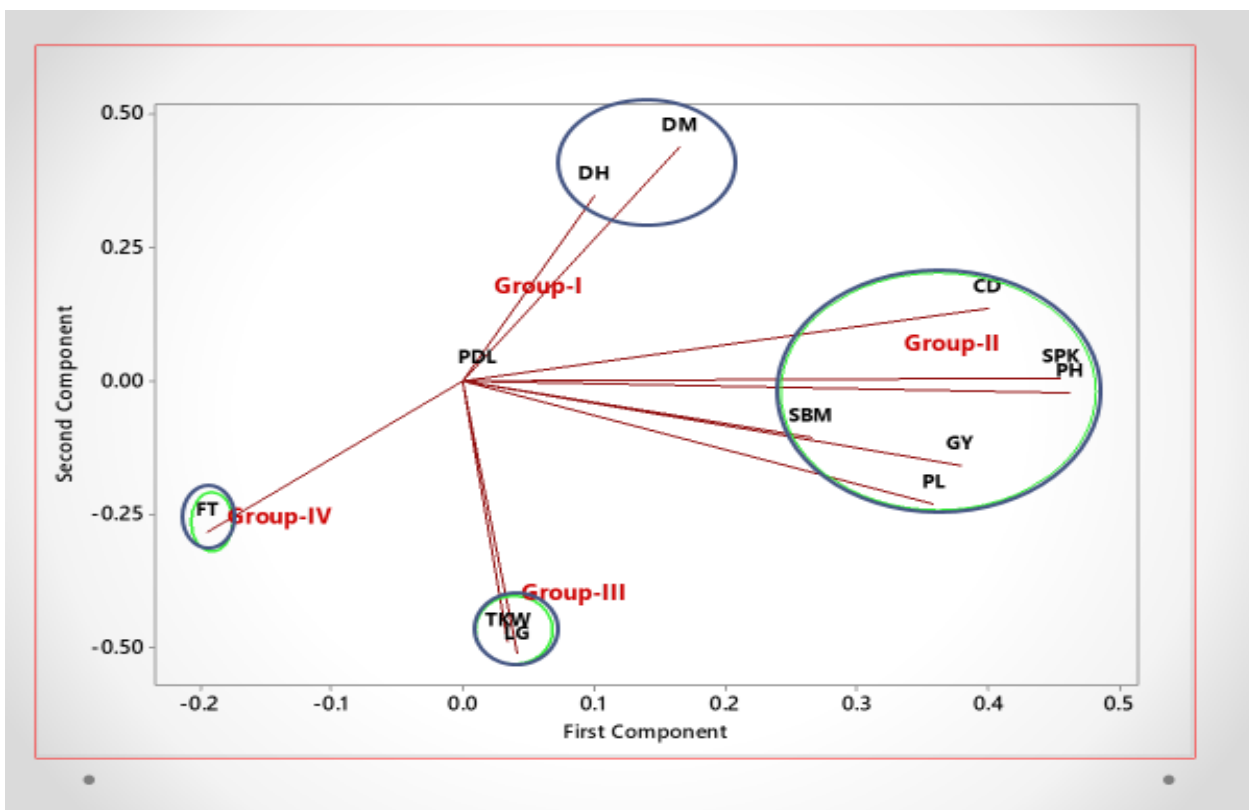


Figure 11. Biplot showing the distribution of 12 traits of the 12 collection zones on PC1 and PC2

3.3.2.4.4 Clustering of tef collection altitudes

Clustering based on the altitude of collections grouped the four populations into three distinct clusters (Fig.12 and 13). Thus, cluster I consisted of collections from below 1500m a. s. l while, cluster II consisted of collections from 1501-2000 and from 2001-2500m a. s. l. The third cluster, on the other hand, consisted of those collected from above 2500m. Thus, the upper (>2500m) and the lower (<1500m) altitudinal classes appeared solitary while the middle two altitudinal classes were grouped together.

Biplot analysis of the 12 traits of four altitudes of collections resulted in the formation of four major groups (Fig. 14). Thus, group-I consisted of four yield related traits such as number of spikelets per panicle, 1000-kernel weight, grain yield and total biomass while group-II consisted of panicle length, plant height, second culm diameter, days to heading, and days to maturity. Group-III, on the other hand, constituted of peduncle length only while, cluster IV consisted of lodging index and number of fertile tillers.

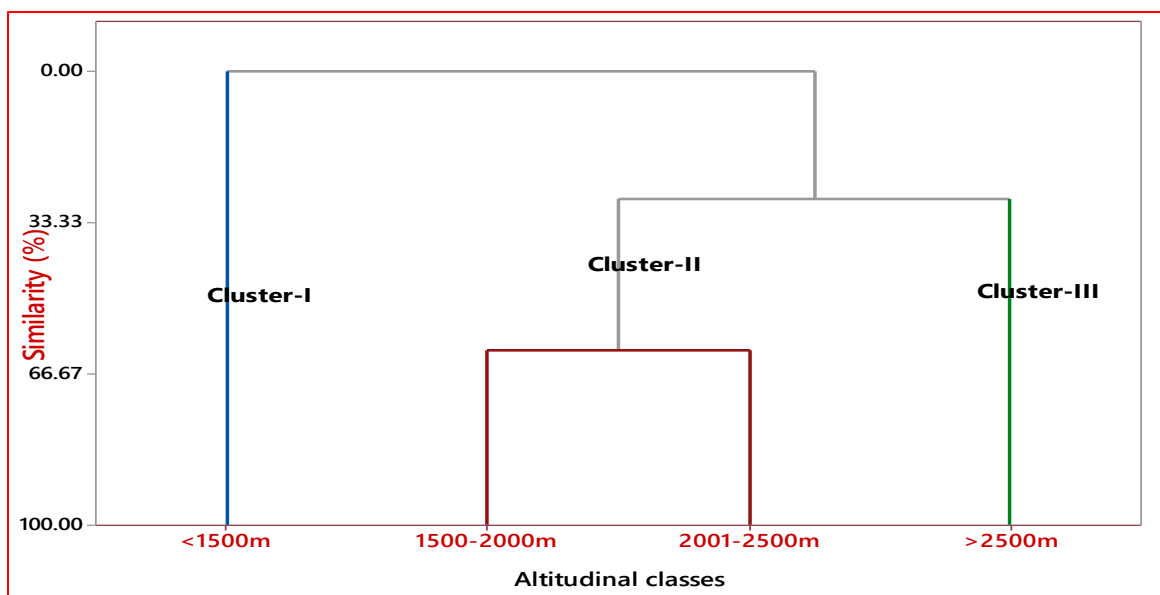


Figure 12. Dendrogram based on complete linkage Euclidean distance showing clustering pattern among four altitudes of collections using 12 major traits of tef.

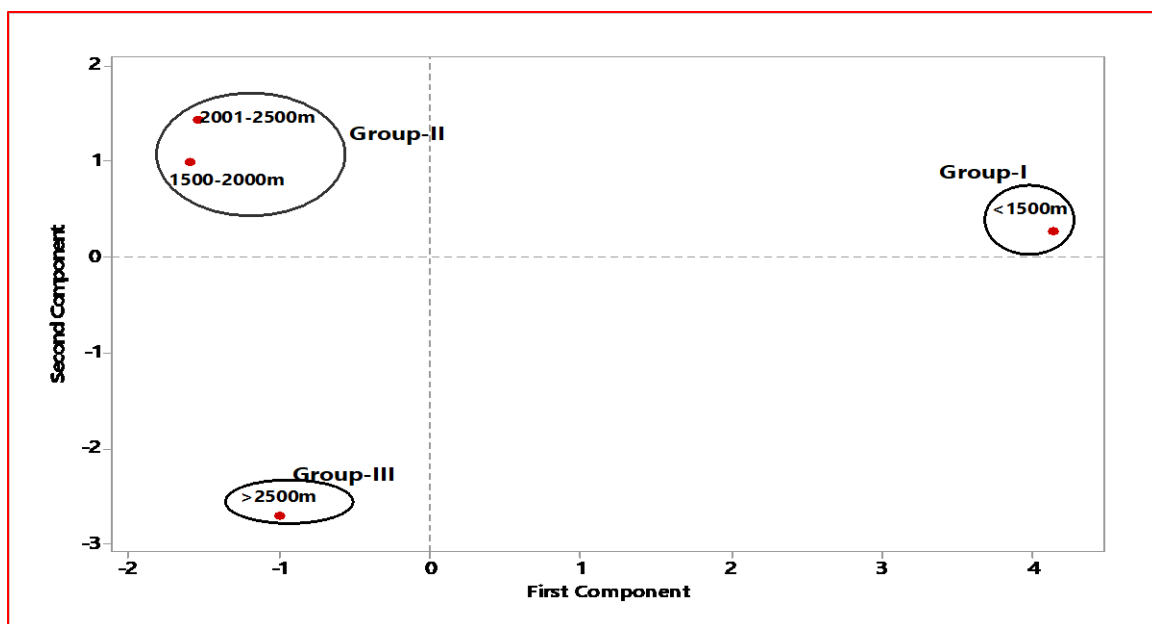


Figure 13. Biplot showing the distribution of four altitudes of tef germplasm collections

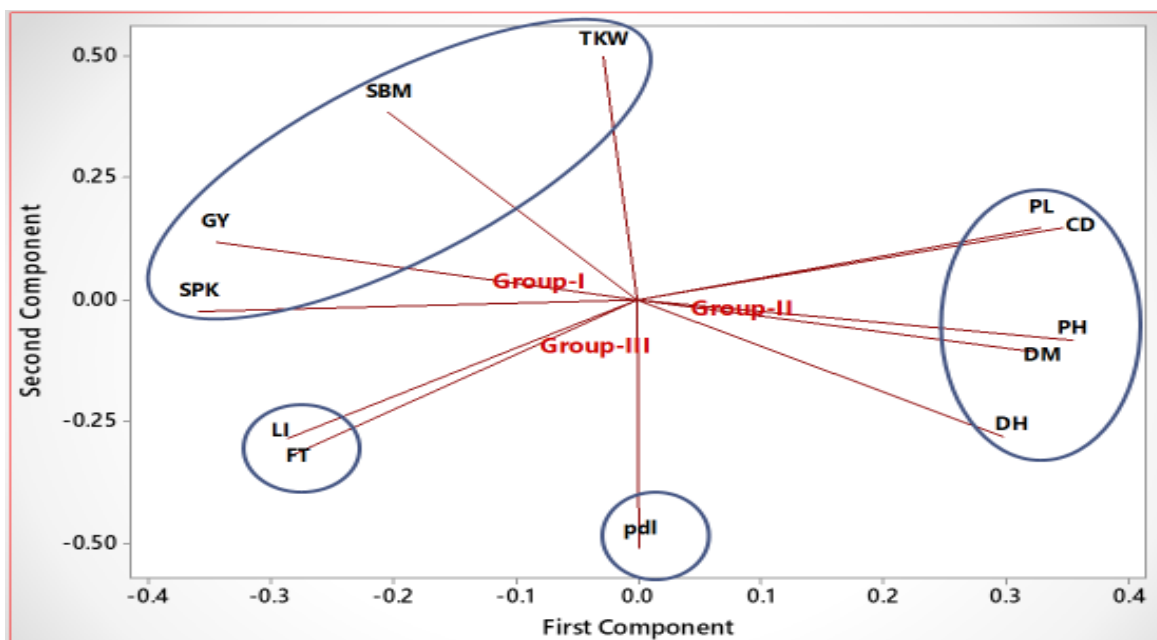


Figure 14. Biplot showing distribution of 12 traits of tef from four altitudes of collection across PC₁ & PC₂

3.3.2.4.5 Clustering based on germplasm collection Woredas

Cluster analysis based on collection Woredas formed five groups (Fig. 15 & Appendix Fig. 2). Thus, the first cluster consisted of 10 collection woredas which include Amhara (4),

Tigray (3) and Oromia (3) regions while the second cluster consisted of 23 woredas from Amhara (15), Tigray (4) and Oromia (4) regions. Cluster-III, on the other hand, consisted of 23 woredas from Amhara (11), Tigray (7) and Oromia (5) regions while, cluster-IV consisted of 14 woredas from Amhara (9), Tigray (2) and Oromia (3) regions. Cluster-V, however, consisted of two Woredas namely Lay Gayint from Amhara and Arsi Negele from Oromia region. In this grouping no two adjacent woredas were grouped together. In PCA-biplot analysis, the 12 investigated traits were grouped into five groups based on collection woredas (Fig. 16). The first group consisted of peduncle length and 1000-kernel weight while the second group consisted of culm diameter, days to maturity and plant height. The third group, however, consisted of days to heading, number of spikelet per panicle and panicle length while, the fourth group consisted grain and total biomass. The fifth group consisted of number of fertile tillers and lodging index,

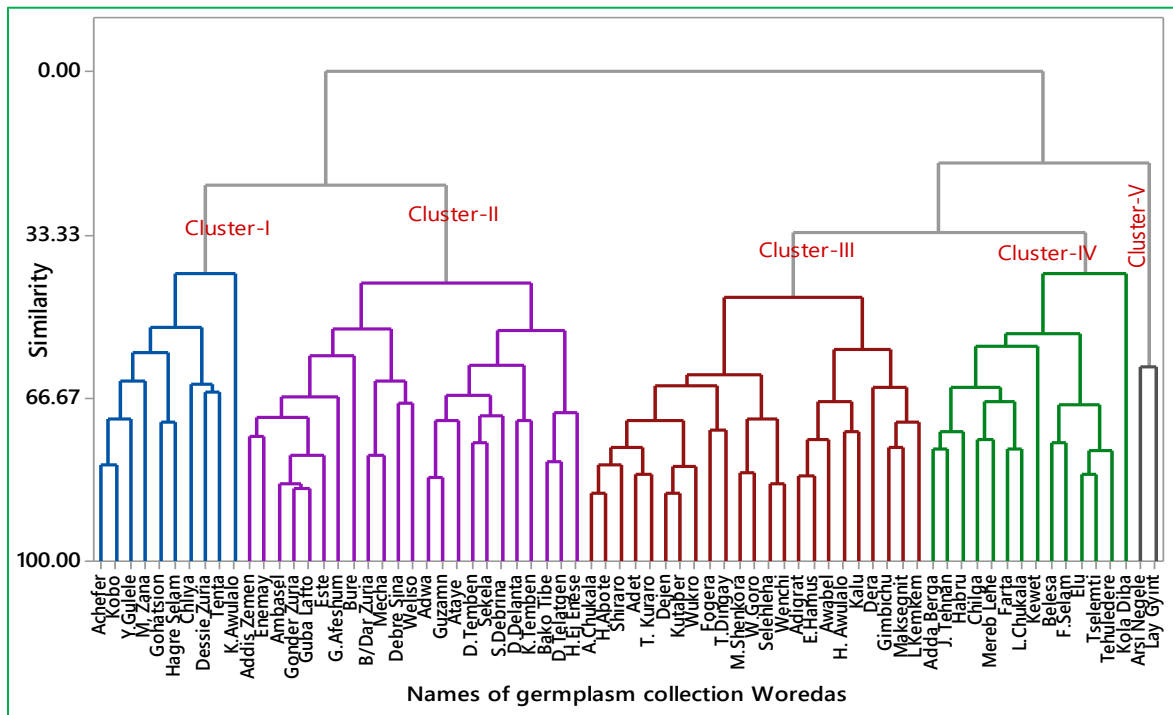


Figure 15. Dendrogram based on complete linkage Euclidean distance showing

clustering pattern among 72 wordas of germplasm collections.

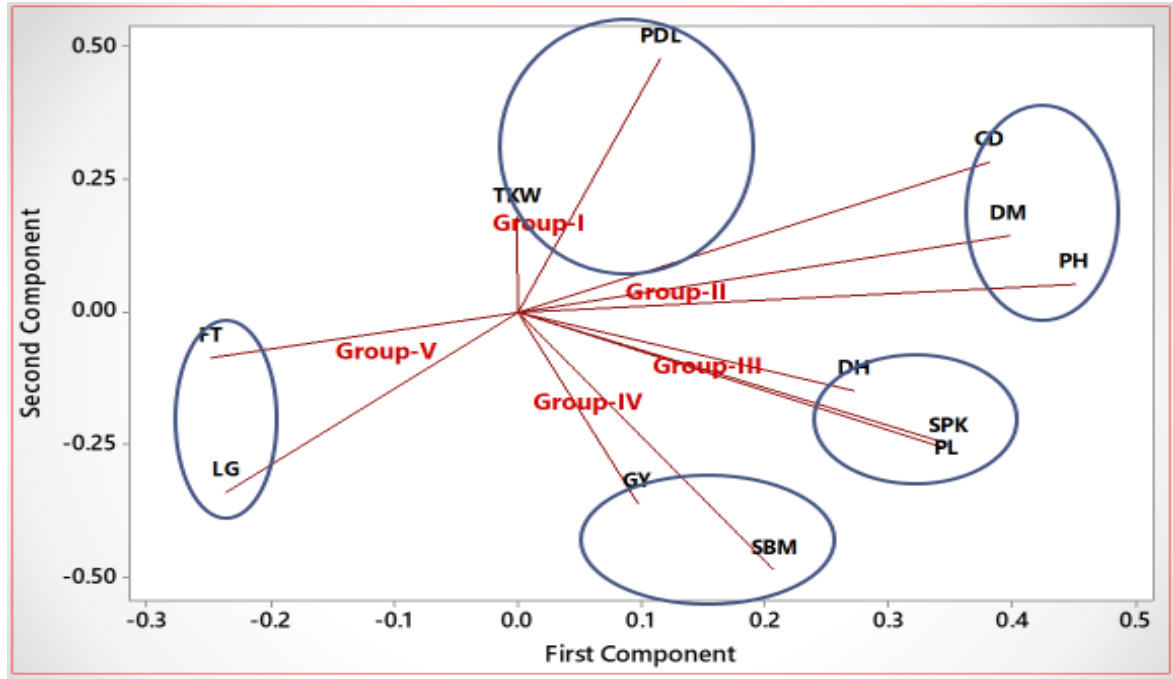


Figure 16. Clustering pattern of 12 major traits of tef based on collections of 72 wordas

3.3.2.5 Estimates of variance components, heritability and genetic advance

3.3.2.5.1 Phenotypic and Genotypic Coefficient of Variation

In this study, the PCV values ranged from 0.06% for lodging index to 19.2% for number of spikelet per panicle while GCV ranged from 0.0% for lodging index to 17.0% for number of fertile tillers (Table 21). Panicle length, peduncle length, number of fertile tillers and spikelet per panicle were found to have intermediate values of both GCV and PCV while second culm diameter, yield of shoot biomass and grain had such intermediate value at PCV level only. All remaining traits had low PCV and GCV while no traits showed high value in both cases.

3.3.2.5.2 Broad sense heritability (h^2) and Genetic advance

Heritability and genetic advance are important factors determining the success of selection in breeding programs. Estimates of heritability (h^2) in the present study ranged from near zero per cent for lodging index to 96.6% for plant height (Table 21). Thus, the highest heritability estimates were observed for plant height (96.6%), panicle length (95.1%), peduncle length (91.1%) and number of fertile tillers per plant (83.7%). Lodging index, 1000-kernel weight, days to maturity, grain yield and second culm diameter, on the other hand, exhibited relatively low heritability value of below 40%. Estimates of genetic advance as percent of mean ranged from 0% for lodging index to 32.0 % for number of fertile tillers (Table 21). In this study, highest heritability coupled with high genetic advance were estimated for plant height (96.6%, 18.8%), panicle length (95.1%, 26.4%), peduncle length (91.1%, 24.6%) and number of fertile tillers per plant (83.7%, 31.9%) showing that they are predominantly controlled by the additive gene action. This indicates that additive gene action plays key role in controlling the expression and existence of highly expected genetic gain through selection.

Table 21. Estimates of variance components, heritability and genetic advance for 188 tef genotypes evaluated at two locations

Traits	$g\sigma^2$	$gl\sigma^2$	$l\sigma^2$	$e\sigma^2$	$Ph\sigma^2$	σPh	σg	GCV (%)	PCV (%)	H ²	GA	GA (%)	Grand Mean
DH	11.00	8.81	528.42	5.47	16.32	4.04	3.32	6.49	7.91	67.42	5.61	10.98	51.09
DM	4.82	18.69	905.30	7.86	15.48	3.93	2.20	1.93	3.45	31.15	2.52	2.21	114.0
PH	63.14	0.02	3.86	13.48	65.40	8.09	7.95	9.25	9.42	96.55	16.1	18.73	85.86
PL	16.01	0.0	2.44	4.91	16.83	4.10	4.00	13.2	13.5	95.14	8.04	26.43	30.42
PdL	5.56	0.0	0.38	3.25	6.10	2.47	2.36	12.5	13.1	91.12	4.64	24.56	18.88
SCD	0.01	0.02	0.28	0.04	0.03	0.16	0.10	6.54	10.7	37.50	0.12	7.56	1.53
FT	0.30	0.0	0.11	0.35	0.36	0.60	0.55	17.0	18.5	83.72	1.03	31.94	3.23
SPK	1384.9	4102.9	11376.3	896.7	3585.8	59.9	37.2	11.9	19.2	38.62	47.6	15.25	312.5
SBM	5.44	18.42	65.23	1.09	14.83	3.85	2.33	8.85	14.6	36.68	2.91	11.04	26.34
GY	0.14	0.53	1.18	0.05	0.41	0.64	0.37	6.73	11.6	33.87	0.45	8.17	5.56
TKW	0.37	5.26	0.56	6.41	4.07	2.02	0.61	2.28	7.55	9.09	0.37	1.40	26.72
LI	0.0	66.85	5.62	21.33	36.98	6.08	0.00	0.00	0.06	0.00	0.00	0.00	67.15

Abbreviations: DH: days to heading; DM: days to maturity; GFP: grain filling period; PH: plant height; PL: panicle length; CL: culm length; Pdl: peduncle length; SCD: second culm diameter; TT: number of total tillers; FT: number of fertile tillers; SPK: number of spikelet; TBM: total biomass; GY: grain yield; HI: harvest index; TKW: thousand kernel weight; LG: lodging index.

3.3.2.6 Association among major traits of tef genotypes

Days to heading and maturity showed positive and significant ($P < 0.01$) phenotypic association with all traits other than number of fertile tillers per plant, number of spikelets per panicle, shoot biomass, grain yield and 1000-kernel weight which were found to be significant ($P < 0.01$) but negatively correlated (Table 22). Besides, plant height had positive and significant association with all traits except with number of fertile tillers which were significantly and negatively associated. On the other hand, the grain yield, 1000-kernel weight and lodging index were not significant ($P > 0.05$). Likewise, panicle length was positively and significantly correlated with all traits other than fertile tillers which was negatively correlated ($P < 0.05$). However, panicle length was negatively associated with shoot biomass, grain yield and 1000-kernel weight. Furthermore, peduncle length had positive and significant association with phenological and morphological traits whereas it had negative association with all agronomic and yield traits. However, its association with 1000-kernel weight and lodging index was not significant. Shoot biomass and grain yield had negative and significant phenotypic association with all traits other than number of fertile tillers, number of spikelets per panicle, 1000-kernel weight and with each other while their association with panicle length and lodging index was not significant ($P > 0.05$). The association of lodging was positive and significant with days to heading and maturity, and panicle length while all remaining traits other than number of spikelets per panicle were not significant ($P > 0.05$).

At genotypic level, the association of days to heading was negative and significant with all traits except with days to maturity, plant height, panicle length, second culm diameter, number of spikelets per panicle and shoot biomass which were positively correlated with

it (Table 22). Similarly, days to maturity had positive and significant association with all traits other than number of fertile tillers and lodging index whereas grain yield and 1000-kernel weight had no significant association with it. Plant height, on the other hand, had positive and significant association with all traits other than fertile tillers which had negative and significant association whereas its association with 1000-kernel weight and lodging index were not significant ($P>0.05$). The genotypic association of grain yield with all traits other than plant height, shoot biomass and panicle length was not significant ($P>0.05$). Furthermore, lodging index had no genotypic correlation with all traits ($P>0.05$) other than days to heading and maturity, peduncle length and second culm diameter which were negative and significant while its association with fertile tillers and grain yield was found to be positive and significant ($P<0.05$).

Table 22. Genotypic (upper) and Phenotypic (lower) correlation among 12 traits of 188 tef genotypes evaluated at Holetta and Debre Zeit in 2016

Trait	DH	DM	PH	PL	PdL	SCD	FT	SPK	SBM	GY	TKW	LI
DH	1	0.49***	0.48***	0.32***	-0.18*	0.45***	-0.15*	0.34***	0.34***	-0.13ns	-0.11ns	-0.25***
DM	0.95***	1	0.51***	0.38***	0.20***	0.51***	-0.26***	0.41***	0.26***	-0.01ns	0.03ns	-0.35***
PH	0.26***	0.24***	1	0.71***	0.24***	0.63***	-0.32***	0.51***	0.41***	0.28***	0.02ns	-0.10ns
PL	0.30***	0.29***	0.68***	1	-0.07ns	0.40***	-0.11ns	0.48***	0.37***	0.26***	-0.03ns	0.02ns
PdL	0.14***	0.20***	0.21***	0.01ns	1	0.28***	-0.17*	0.07ns	-0.24***	-0.04ns	0.17*	-0.16*
SCD	0.83***	0.85***	0.33***	0.33***	0.25***	1	-0.33***	0.42***	0.19*	0.04ns	0.13ns	-0.39***
FT	-0.30***	-0.32***	-0.19***	-0.06*	-0.16***	-0.30***	1	-0.20**	-0.14*	-0.14ns	-0.04ns	0.17*
SPK	-0.62***	-0.63***	0.14***	0.07*	-0.10***	-0.50***	0.14***	1	0.22**	0.14ns	-0.11ns	-0.14ns
SBM	-0.69***	-0.72***	0.07*	-0.01ns	-0.23***	-0.61***	0.17***	0.56***	1	0.66***	0.00ns	0.05ns
GY	-0.67***	-0.67***	0.04ns	-0.03ns	-0.14***	-0.57***	0.14***	0.51***	0.80***	1	0.00ns	0.19*
TKW	-0.17***	-0.15***	-0.02ns	-0.06ns	0.04ns	-0.10***	0.05ns	0.05ns	0.12***	0.11***	1	-0.02ns
LI	0.13***	0.11***	-0.02ns	0.06*	-0.06ns	-0.01ns	0.01ns	-0.17***	-0.04ns	0.00ns	-0.06ns	1

Abbreviations: DH: days to heading; DM: days to maturity; GFP: grain filling period; PH: plant height; PL: panicle length; CL: culm length; Pdl: peduncle length; SCD: second culm diameter; TT: number of total tillers; FT: number of fertile tillers; SPK: number of spikelet; TBM: total biomass; GY: grain yield; HI: harvest index; TKW: thousand kernel weight; LG: lodging index.

3.4 Discussion

3.4.1 Qualitative traits diversity

Panicle form, and the colour of grain, lemma and stalk are the most important qualitative traits to distinguish among various tef genotypes (Berhe *et al.*, 1989; Tefera *et al.*, 1990, 1995; Ketema, 1997). In the present study, the qualitative traits variation among tef genotypes were assessed and existence of wide range of variation was observed. Regarding seed colour, most improved varieties, breeding lines and genotypes from West Gojam, West Shewa and three zones of Tigray exhibited white seed colour while those from North and South Gonder, North and South Wello, East Gojam and North Shewa had brown seed colours. On the other hand, the majority of genotypes from East Shewa zone exhibited very white seed colour (Table 6). Such larger proportion of very white seed colour could be a justification for the premium market price for tef grain from in east Shewa zone (Belay *et al.*, 2008).

According to Tefera *et al* (1990), panicle forms are the determinant trait to know about the yielding potential of a given tef genotype whereby genotypes with loose panicle forms are high yielding and the most adapted type. In the present study, the loose and fairly loose panicle forms accounted for the largest proportion in almost all zones of tef germplasm collections & this is in line with the previous findings (Tefera *et al.*, 1990). This is, therefore, a good opportunity to make selection for yield related traits, earliness and better adaptation as loose panicle forms are early maturing, adapted and higher yielding compared to compact forms (Tefera *et al.*, 1992, 1995; Ketema, 1997). Thus, early maturing genotypes are useful to overcome the recurrent climate change problem as well as to use them for double cropping to increase tef productivity per unit area.

3.4.2 Variation in quantitative traits of tef

Quantitative traits are another important features to distinguish among tef genotypes. In this study, highly significant variation was revealed among genotypes, locations and their interactions for all studied traits of tef genotypes. Thus, the days to heading and maturity ranged from 40.3 to 60.8 and 101 to 122.5 days respectively. Assefa *et al.* (2001) also reported values ranging from 37- 46 and 83-101 for days to heading and maturity respectively based on study conducted using germplasm collections whereas, Tefera *et al.* (1990) reported a range of 82-113 for days to maturity. Such variations are very essential to augment the efforts to develop varieties fitting to various agro-ecologies and cropping systems to increase tef production and productivity in Ethiopia. Thus, focusing on traits for earliness enables breeders to develop variety that can escape late season drought happening due to climate change.

The values of plant height and panicle forms that ranged from 60.7cm to 107.1cm and 19.5cm and 39.5cm, respectively in the present study is in line with the previous findings of Tefera *et al.*, (1992) who reported 73.6 -123 cm for plant height and 41.2 to 56.3cm for panicle length. Tadesse (1993) also reported 71.3-93.7cm for plant height and 27.9-40.6cm for panicle form while, Assefa *et al* (2001) reported 28.1-38.8 cm for panicle length using germplasm collections from North and Central Ethiopia.

In the present study, a widerange of values were observed for number of spikelet per panicle (156.7 to 441.7) and harvest index (14.7 to 24.3%). These observed ranges are wider

than the previous report of 224.3 - 427.4 and 16.9-28.8% for number of spiklet per panicles and harvest index, respectively (Assefa *et al.*, 2001). Regarding altitudinal classes, collections from 2001 to 2500m asl exhibited most of the desirable traits in tef. Similar findings have also been reported previously (Ketema, 1993, 1997) whereby altitude from 1800-2400m asl was identified as ideal tef production environment.

3.4.3 Principal component analysis

In this study, the variation explained based on individual genotypes, 14 populations, 12 zones and four altitudes of collections were analysed. Thus, it showed that the first three PCs, three PCs, five PCs and two PCs with eigen value greater than unity accounted for 59.4%, 75.8%, 89.2% and 92.9% of their respective total variation based on twelve quantitative traits. Thus, the observed values for individual genotypes was very small while those for the remaining three factors was comparable to the previous reports. For instance, Assefa *et al.* (2003), reported 81% of the total variance explained for germplasm collected from south and western Ethiopia whereas Jifar *et al.* (2015) reported 78.3% based on 36 brown seeded tef genotypes. Besides, it is also far below the studies conducted using 28 semi dwarf tef genotypes (Jifar *et al.*, 2017) who reported 85% for the first five PCs. The variation in PC1 was due to all studied traits other than peduncle length and 1000-kernel weight whereas that of PC2 was mainly due to grain yield, shoot biomass, lodging index and peduncle length. However, grain yield, 1000-kernel weight and days to heading were the major traits contributing to the variation in PC3 (Table 15). Various authors also reported the contribution of different phenotypic traits to each PCs in tef (Assefa *et al.*, 2003; Lule *et al.*, 2011; Jifar *et al.*, 2013; Plaza *et al.*, 2013; Jifar *et al.*, 2015a & b; Jifar *et al.*, 2017).

3.4.4 Cluster Analysis

Cluster analysis based on 188 individual tef genotypes revealed the formation of six clusters consisting of 19 to 50 genotypes whereas the clustering based on 14 populations resulted in the formation of four clusters consisting of 1 to 5 populations. In this clustering, the grouping of individual genotypes or populations into a given cluster was neither purely based on geographic nor genetic origin. In population clustering, for instance, the improved varieties were grouped with East, West and North Shewa, North Gonder and West Tigray zones while the breeding lines remained solitary. The grouping of populations from three zones of Shewa in the same cluster could be due to geographic proximity and seed exchange along their borders. On the other hand, the grouping of Central Tigray with West Gojam zones or the grouping of East Tigray with East Gojam population could be due to seed movement by trans humans. Such seed movement may occur during resettlement program conducted about 40 years ago when people from Tigray were forced to move to the western parts of Ethiopia due to continuous failure of rainfall. In the present study, therefore, seeds from adjacent zones and/or distant origin was clustered together may be due to geographic proximity and/or trans human seed exchange.

In individual genotypes clustering, genotypes cluster-I exhibited the longest days to maturity, peduncle length, thicker culm diameter and the least value for lodging percentage. Cluster-II, however, consisted of 32 genotypes with the longest days to heading, plant height and panicle length, the highest number of spikelet per panicle, yield of grain and total biomass with the least number of fertile tillers. The third cluster had moderate performance for all studied traits while genotypes in cluster-IV exhibited the lowest value of peduncle length and 100-kernel weight with the highest grain yield while the genotypes

in cluster-V were identified to have the highest value for 1000-kernel weight and lodging percent. The sixth cluster, however, had the least value for all traits other than peduncle length, 1000-kernel weight and lodging percent with the highest number of fertile tillers (Table 18).

The PCA-biplot for the 188 individual genotypes, 14 populations, 12 zones of collections, four altitudes and 72 Woredas of germplasm collection also revealed similar patterns of clustering with that of their respective dendrogram (Fig. 3, 5, 6, 8, 10, 11 & 13). Furthermore, in this study, genotypes in cluster-II can be recommended for breeders dealing with high yield whereas those dealing with early maturity should focus on genotypes in cluster-VI. In variability creation, excellent level of genetic variability and high heterosis can be obtained between cluster 2 & 5 followed by cluster 1 & 3, and cluster 2 & 3, and cluster 1 & 4, respectively for individual clusters. In the case of population clustering, the best genetic recombination and high level of heterosis can be obtained by making crossing between cluster 3 & 4, Cluster 2 & 4, cluster 1 & 4, respectively.

3.4.5 Estimates of variance components, heritability (H²) and genetic advance

PCV and GCV values below 10%, 10%-20% and above 20% are considered to be low, intermediate and high, respectively (Khorgade *et al.*, 1985). In the present study, about 41.7% and 66.7% of the studied traits had low PCV and GCV values respectively whereas the remaining traits had intermediate PCV and GCV. Thus, panicle length, peduncle length, number of fertile tillers and spikelet per panicle had intermediate values of both GCV and PCV while second culm diameter, yield of shoot biomass and grain had such intermediate

value at PCV level only (Table 21). All remaining studied traits had low PCV and GCV while no trait was observed to have high PCV or GCV in this study. The range of values estimated in the present study is in line with the previous reports of 6.1 to 40.2% for PCV and 3.0 to 22.1% for GCV by Assefa *et al.* (1999), 4.3 to 21.7 for PCV and 4.0 to 20.3% for GCV (Jifar *et al.*, 2015a) while it is observed to be far below the previous report of 2 to 58% for PCV and less than 1 to 35% for GCV (Assefa *et al.*, 2000). Furthermore, the GCV (6.73%) and PCV (11.6%) estimated for grain yield in the present study is far below the previous report for the same traits (GCV=21.3 and PCV=21.7) (Jifar *et al.*, 2015b)

High heritability indicates high proportion of genetic variance that could be inherited and would be exploited by breeders to select superior genotypes based on phenotypic performance (Peter *et al.*, 2008; Tazeen *et al.*, 2009, Atnaf *et al.*, 2017). Singh (2000) suggested the importance of considering heritability along with genetic advance. In the present study, higher heritability coupled with high genetic advance was estimated for three morphological traits and number of fertile tillers. This shows the role of additive gene action in controlling their expression and the existence of high expected genetic gain through selection.

3.4.6 Association among major traits of tef

The existence of significant positive associations (Table 22) among grain yield and that of totalbiomass, thousand kernel weight and number of spikelets per panicle indicates that improving one of them would bring an improvement in the other trait. Thus, selection for such related traits can be made based on traits that can be assessed relatively easily. In line with these findings, significant positive association were reported between grain yield and

that of totalbiomass and harvest index in the previous studies by several researchers (Chanyalew *et al.*, 2006; Chanyalew *et al.*, 2009; Jifar and Assefa, 2013; Jifar *et al.*, 2015a; Jifar *et al.*, 2015b; Jifar *et al.*, 2017). Similarly, positive and significant phenotypic association observed between lodging and days to heading, days to maturity and panicle length is also in line with the previous findings of Jifar *et al.* (2015b) and Jifar *et al.* (2017). The positive phenotypic correlation between lodging index and that of phenological traits imply that long maturing genotypes expected to have higher yield is susceptible lodging and vice versa.

Chapter 4

4. Genetic diversity and population structure analyses in Tef [*Eragrostis tef* (Zucc.) Trotter] genotypes using microsatellite markers

Abstract

Tef [Eragrostis tef (Zucc.) Trotter] is a major cereal crop widely grown and utilized in Ethiopia. As more than half of our current germplasm holdings were not yet characterized conducting more studies are required with regard. One hundred eighty-nine tef genotypes from various sources in Ethiopia were studied using 16 simple sequence repeat markers to characterize and assess the genetic diversity and pattern of relationship among tef genotypes. A total of 260 alleles were identified with a mean of 16.25 alleles per locus. The number of alleles, polymorphic information content (PIC), gene diversity and allelic richness per locus ranged from 2 to 26, 0.30 to 0.92, 0.37 to 0.93 and 2 to 10.6, respectively. Improved varieties and collections from North Shewa and East Tigray respectively exhibited the largest average allelic richness (8.9), gene diversity (0.85) and private allele (0.63), while breeding lines had the least values for all genetic parameters. Furthermore, the rare and intermediate alleles constituted together 99.2% while abundant alleles accounted for 0.8%. Molecular analysis of variance revealed a variation of 56.6% within individuals, 40.8% among individuals within population and 2.6% among populations. Genetic differentiation (F_{ST}) showed the existence of high genetic variation among the various origins of genotypes. Clustering analysis of population and individual genotypes revealed formation of four clusters. The model-based approach of structure software also inferred four gene pools, but with high level of admixture among the various gene pools. A core collection consisting 64 (33.9%) of the genotypes and 100% of the alleles for the entire genotypes was identified. In general, existence of substantial polymorphism among genotypes from diverse sources shows the potential of using these variabilities in the future improvement of tef.

Key words: Allelic richness, cluster analysis, *Eragrostis tef*, genetic diversity, microsatellite markers, population structure, Tef,

4.1 *Introduction*

Tef [*Eragrostis tef* (Zucc.) Trotter] is a gluten free (Spaenij-Dekking *et al.*, 2005), nutritious human food (Ketema, 1993; Assefa *et al.*, 2011) and livestock feed (Bediye and Fekadu, 2001; Yami, 2013). It is an indigenous cereal domesticated by pre-Semitic inhabitants of Ethiopia between 4000 and 1000 B.C. (Ketema, 1997; Tessema, 2013). Tef is the only cereal cultivated for human food in the genus *Eragrostis* which consists of 350 species (Tefera and Ketema, 2001; Tefera *et al.*, 2003). The genus *Eragrostis* seem to have originated in Africa (43%), South America (18%), Asia (12%), Australia (10%), Central America (9%), North America (6%) and Europe (2%) (Costanza, 1974).

As indigenous germplasm rollectionsare the main source of variability for tef genetic improvement; collection and conservation of such resources is very essential to develop new varieties with traits of interest. To this effect, the Ethiopian Biodiversity Institute (EBI) collected about 6000 tef accessions of which only 2533 had the necessary passport data (Tessema, 2013). Thus, germplasm characterization and evaluation, and targeted crossing among selected parental lines have been the most common practices to develop and release improved tef varieties in Ethiopia (Assefa *et al.*, 2011). Currently over 35 varieties of tef with different genetic backgrounds have been released for use by small holder farmers (MoANR, 2016)

Molecular characterization of germplasm in gene bank enables the provision of relevant information to conserve and improve crop productivity. However, studies indicated that more than half of the tef germplasm holdings of EBI was not yet characterized, which

makes the effort towards improving this neglected crop less comprehensive (Chanyalew *et al.*, 2013; Tessema, 2013). Hence, it is essential to undertake intensive and comprehensive characterization of the available germplasm for further use. As part of this effort, this study was performed to characterize and assess the genetic diversity and pattern of relationship among tef genotypes from various genetic and eco-geographic origin using microsatellite markers.

4.2 *Materials and methods*

4.2.1 Plant materials and DNA extraction

A total of 189 tef genotypes including 188 genotypes from previous studies (Table 1) and a breeding line called RIL-44(a progeny of *Kinde* x *Kaye Murri*) were planted in a 40mm diameter pot using sterile soil in a long day growth room at the Institute of Plant Sciences, University of Bern, Switzerland. A total of 100mg fresh leaves were harvested from 3 to 4 weeks old seedlings and kept in 2 ml Eppendorf tube (at -80°C) until DNA isolation. Manual grinding of samples was done in Eppendorf tubes using blue pestle in the presence of liquid nitrogen. Genomic DNA was isolated using slightly modified Cetyltrimethyl-ammonium bromide (CTAB) protocol of Doyle and Doyle (1990). The quality and quantity of the isolated DNA was assessed on 2% agarose standard gel electrophoresis and using Nano drop-1000 spectrophotometer. A cyber-green dye was used instead of the carcinogenic ethidium bromide to facilitate the detection of the PCR products. A 1 kb standard DNA ladder was used to determine the size of DNA fragment. The gel products were visualized using gel documentation, 3UV-trans illuminator (Bio-Doc).

4.2.2 SSR marker selection

Twenty-five polymorphic simple sequence repeat (SSR) markers distributed throughout the *tef* genome were selected (Zeid *et al.*, 2011; Zeid *et al.*, 2012; Cannarozzi *et al.*, 2014) were screened for amplification of their target genomic region. Forward and reverse primers of these 25 markers were synthesized at Micro-synth (Switzerland). These markers were tested using 30 selected *tef* genotypes representing the diversity among eco-geographic and genetic origin. PCR optimization was performed using a thermal cycler (Bio-Rad) in a total volume of 20 µl containing 1 µl DNA, 0.5 µl 10µM dNTPs, 4 µl buffer 5x, 0.75 µl 10µM each forward and reverse primer and 0.1 µl Go-Taq DNA polymerase (Promega) and 12.9 µl dH₂O. The PCR machine was programmed for initial denaturation at 95°C for 2 minutes followed by 35 cycles at 95°C for 30 seconds, annealing temperature at 54°C or 50°C (depending on the melting temperature of the primers) for 30 sec, and an extension at 72°C for 50 sec. Then, a 5 min. final extension at 72°C was also employed. After screening for amplification of unambiguously visible and polymorphic SSR bands, 16 primer pairs showing polymorphism and good resolution were identified for further analysis (Table 23). SSR studies of all genotypes were carried out at the Institute of plant Sciences, University of Bern in Switzerland and at Sci-Corp laboratory in South Africa. Eight markers were analyzed at the University of Bern, whereas the remaining eight markers and two control markers from University of Bern were sent to Sci-Corp laboratory in South Africa.

4.2.3 PCR amplification using fluorescently labeled primers

For cost effectiveness and improved quality of the amplified products, a 19 bp M13 sequence (5'- CACGACGTTGTAAAACGAC -3') was added to the 5' side of each

forward primer of the 16 markers. Thus, separately fluorescent labeled (Dyomics681, Microsynth, Balgach, Switzerland) M13 sequence was included in the PCR together with the forward primer (described above) and a corresponding reverse primer (Schuelke, 2000). PCR amplification was performed using thermal cycler in a total volume of 10 μ l containing [1 μ l DNA, 0.2 μ l 10mM dNTPs, 2 μ l 10x buffer, 0.35 μ l 1 μ M forward primer, 0.35 μ l 1 μ M reverse primer, 0.35 μ l 1 μ M fluorescently labeled primer, 0.25 μ l Go-Taq DNA polymerase and 5.5 μ l dH₂O]. The labeled primer is sensitive to light and was always protected from direct light. The PCR was programmed to the following five steps: an initial denaturation of 95°C for 2 min; 40 cycles of denaturation at 95°C for 30 sec.; 30 seconds of annealing at 54 or 50°C (based on melting temperature for the pair of primers); initial extension at 72°C for 30 sec., and a final extension at 72°C for 5 min.

4.2.4 SSR study at Sci-Corp laboratory

Leaves of 2-week old seedlings from each genotype were pressed onto labeled FTA cards (Sigma, Germany) and were sent to Sci-Corp Laboratory in South Africa. Pestles were used to press the leaf sample until both sides of the FTA card were soaked with a leaf extract. The pressing board and pestle were cleaned with 70% ethanol between each sample to avoid cross-contamination. The DNA was extracted at the Sci-Corp laboratory in South Africa from the sample fixed on the FTA cards using Whatman FTA Card extraction protocol. A marker specific PCR was performed for the 10 polymorphic SSR markers (Zeid *et al.*, 2012) and the PCR amplicons were analysed via capillary electrophoresis using fluorescent dyes.

4.2.5 SSR data analysis

The generated fragment size data from all entries were summarized and converted into input files suitable for various analyses using convert software version 1.31 (Glaubitz, 2004). Analysis of molecular variance (AMOVA), determination of the number of alleles (Na) and effective alleles per locus (Ne), observed heterozygosity (Ho), gene diversity (UHe) and F_{ST} were carried out using GenAlEx software version 6.5 (Peakall and Smouse 2006, 2012). Besides, allelic richness (Ar) was estimated using the rarefaction method implemented in HP-Rare 1.03 (Kalinowski, 2005). Power Marker software v.3.25 (Liu and Muse, 2005) was also employed to compute polymorphic loci percentage, gene flow and Nei's unbiased genetic distance. Cluster analysis was carried out based on population and individual genotypes using Pop tree2 (Takezaki *et al.*, 2010) and DARwin software version 6.0.13 (Perrier and Jacquemoud-Collet, 2006), respectively. The dendrogram for individual genotypes was edited for visualization using Fig-Tree software version 1.4.3 (Rambaut, 2016).

Population structure was inferred by the Bayesian model-based clustering method using the Structure Software version 2.3.4 (Pritchard *et al.*, 2000; Falush *et al.*, 2007) assuming population admixture through inferred ancestry. Twenty independent simulations were performed by setting 50,000 for burn-in and 500,000 replications of Markov Chain Monte Carlo run to estimate the number of sub-populations for each K values from the K=1 to K=14 settings. The appropriate number of clusters (best K) was determined as suggested by Evanno *et al.* (2005) using the STRUCTURE HARVESTER online software developed by Earl (2012). Besides, CLUMPAK (Cluster Markov Packager Across K) software was also used to identify an optimal alignment of inferred clusters across different values of K.

Table 23. Characteristics of selected SSR markers developed for *Eragrostis tef*

No.	Primer	Forward primer 5'...3')	Reverse primer (5'...3')	Repeat type and size	Expected size (bp)	Source
1	CNLTs 27	TTGGAATGAGATGGCATTG	GAAGCGGGTAAGATTGAA	(TC)14	174	Zeid et al. (2012)
2	CNLTs 33	TTTGCACCTAGTCTCCCATG	ACGATCGGATGTTTTGCTTT	(GA)16	230	
3	CNLTs 42	ATGCATGGATGGATGGCTA	TTACCCAATTGCCCTAGCTG	(TC)27	179	
4	CNLTs 60	AGGGTGATAGCTGCCAGAC	CCCGAGTAATTGGTCGCTAA	(TC)20& (CA)7	297	
5	CNLTs 133	GGGGAGACTGCATTGGACTA	CAAGAGGGACTGCACAGTGA	(GA)9	249	
6	CNLTs 136	TGAGAAGGTAATAACTGGTGAAGC	CAAGGTTTACACACCGTGAAGC	(CT)18	246	
7	CNLTs 150	AACACGTCCTTGCCGTATTC	CGGGGTAGCCATAGCCTAAT	(AG)5& (AG)16	225	
8	CNLTs 157	GGATCCGACATGACGTGTAGT	CACAGAATGAGATTGGGGAGA	(CT)18	168	
9	CNLTs 216	GGAAATTCGCACGAGAGAGA	CGAGAGAGAAGCCTGTGAGG	(GA)15(AAG)6	191	
10	CNLTs 315	ATAGCTGCTCCGTTTTGCAT	GGTCCACTTGGCATTCTGTT	(AG)8 (TAG)6	233	
11	CNLTs 416	AACAGATACAGTTGGAGACAGAAATG	CTCTGAGTGCGTCGCAAG	(AG)19	151	
12	CNLTs 438	CTAACCGGCGGCGAGAGA	CTGCCACATGCGTCGTTAGA	(GA)14	153	
13	CNLTs 484	GAGATCCTACCACGGCGATA	CGCTTTCCCCTCCTTTTGTA	(GA)18	157	
14	CNLTs 538	CCATCTTAGCTTTGGCGAGA	ACAAGAGGCAACAAGCCAGA	(AG)18(AGA)20	176	
15	SSR3.3	GGGAAGAGGAGTGTACAGA	CCCTGGCAACTGCTTTAAGA	(AAG)6	226	Cannarozzi et al. (2014)
16	SSR9.4	CTCATCTCCCACCCTCACTC	GTAGCCCAGATCAAACGACC	(CTCCT)5	204	

N.B. All forward primers used for SSR study had an additional 19 bp (5'-CACGACGTTGTAAAACGAC-3') sequence at the 5'-end.

4.2.6 Establishing non-redundant core collection and construction of dendrogram

Development of core collection enables monitoring of germplasm conservation and maximizing the evaluation and utilization of these resources for breeding purposes. In other words, an ideal core collection is essential to maximize the genetic diversity in the large collection. A core collection was constructed by selecting accessions with higher diversity and capturing the total alleles present in the entire collection. Thus, the size of the core collection was determined by referring to the number of samples corresponding to the inflection point prior to the plateau of the redundancy curve. The individuals of the core collection were selected based on the number of alleles that they accounted for and based on the value of the standard deviation from the mean. As suggested by Anoumaa *et al.* (2017), when the removal of several individuals led to the same number of alleles in the collection, only the one with low standard deviation from the mean (which when removed from the core collection has little effect on the mean standard deviation) was selected. In constructing the genetic core collections, allelic data were used to calculate the dissimilarity matrix as implemented in Dissimilarity Analysis for Representation in Window (DARwin), v 6.0.13 software (Perrier and Jacquemoud Collect, 2006) following random sampling method. The efficiency of the strategy was assessed by comparing the total number of alleles captured for each run using the same software.

4.3 **RESULTS**

4.3.1 **SSR markers and its use for tef genetic diversity**

The 16 studied SSR markers generated a total of 260 alleles with a mean of 16.3 alleles per locus (Table 24). Besides, the number of effective alleles (N_e) was also in the range of 1.56 for SSR 3.3 to 13.60 for CNLT 42, while Shannon diversity index (I) ranged from 0.55 to 2.8 for the same markers in the same order. Thus, H_o per locus ranged from 0.01 (CNLT 157) to 0.97 (CNLT 484) with a mean of 0.51 whereas, H_e ranged from 0.36 (SSR 3.3) to 0.93 (CNLT 42) with a mean of 0.82. Furthermore, the polymorphic information content (PIC) of the studied loci also ranged from 0.30 (SSR 3.3) to 0.92 (CNLT 42) with a mean of 0.80 (Table 24).

In this study, rare alleles with frequency < 0.05 , intermediate alleles with 0.05 to 0.50 frequency and abundant alleles with frequency > 0.50 constituted for 61.5%, 37.7% and less than 1% of all detected alleles, respectively. The frequency of major alleles ranged from 0.15 (CNLT 42; CNLT 438 and CNLT 538) to 0.76 (SSR 3.3) with a mean of 0.28 per marker (Table 24). Analysis of allelic richness, on the other hand, showed the existence of high level of variation among loci that ranged from 2 (SSR 3.3) to 10.6 (CNLT 42), with a mean of 7.2 alleles (Table 24). Markers like CNLT 42, CNLT 216 and CNLT 484 had mean allelic richness of 10 or more. Besides, CNLT 484 had the highest number of private alleles (eight) followed by CNLT 416 which had seven private alleles while, CNLT 27, CNLT 150 and SSR 3.3 were found to have no private allele (Appendix Table 3). SSR 3.3 had the least value of PIC, uH_e , H_e , allelic richness and effective alleles suggesting that the sequence used for its development is highly conserved in the studied tef genotypes.

Table 24. Genetic diversity parameters of 189 tef genotypes assessed based on 16 SSR markers

Locus	Allele size range	Genetic parameters									
		MAF	Na	Ne	Ar	I	H _o	H _e	uH _e	F	PIC
CNLT 133	260-302	0.25	21	7.40	7.71	2.36	0.39	0.86	0.87	0.50	0.85
CNLT 216	186-235	0.19	24	11.12	10.21	2.69	0.60	0.91	0.91	0.32	0.90
CNLT 27	186-200	0.28	8	5.78	6.21	1.91	0.35	0.83	0.83	0.52	0.81
CNLT 538	162-198	0.15	26	11.84	9.57	2.73	0.33	0.92	0.92	0.61	0.91
CNLT 60	298-320	0.22	12	7.39	7.14	2.15	0.62	0.87	0.87	0.25	0.85
CNLT 33	228-268	0.23	19	7.96	7.64	2.38	0.41	0.88	0.87	0.51	0.86
SSR 9.4	200-225	0.33	6	3.73	4.14	1.42	0.61	0.73	0.73	0.10	0.68
CNLT 42	177-220	0.15	22	13.6	10.64	2.80	0.61	0.93	0.93	0.27	0.92
CNLT 150	232-269	0.24	14	6.50	7.29	2.13	0.82	0.83	0.85	-0.04	0.83
CNLT 157	181-199	0.35	10	4.80	5.21	1.80	0.01	0.79	0.79	0.99	0.77
SSR 3.3	228-243	0.76	2	1.56	2.00	0.55	0.47	0.36	0.36	-0.38	0.30
CNLT 136	252-281	0.27	17	7.17	5.79	2.27	0.05	0.86	0.86	0.90	0.85
CNLT 315	206-279	0.52	12	2.92	4.93	1.44	0.95	0.66	0.66	-0.48	0.62
CNLT 416	155-199	0.24	25	7.70	8.21	2.41	0.61	0.87	0.87	0.25	0.86
CNLT 438	158-214	0.15	18	11.21	7.64	2.59	0.27	0.91	0.91	0.67	0.90
CNLT 484	170-202	0.21	24	8.32	10.00	2.46	0.97	0.88	0.88	-0.17	0.87
Mean		0.28	16	7.44	7.15	2.13	0.50	0.82	0.82	0.33	0.80
SE		0.01	7.3	3.32	0.19	0.59	0.30	0.14	0.14	0.03	0.32

MAF = maximum allele frequency, Na = observed number of allele, Ne = number of effective allele, Ar = Allelic richness, I = Shannon's Information Index, H_o = observed heterozygosity, H_e = expected heterozygosity, uH_e = Un-biased heterozygosity, F = fixation index, PIC = polymorphic information content.

4.3.2 Tef populations diversity

Analysis of important genetic parameters among 14 populations revealed that improved varieties had the highest mean number of alleles (8.94), effective alleles (5.56), and Shannon's information index (1.80) (Table 25). North Shewa (0.83) and North Gonder

(0.81) had the highest gene diversity whereas the breeding lines had the least value for all genetic parameters (Table 25). Analysis of allelic richness revealed existence of high level of variation among populations which ranged from 4.9 for breeding lines to 8.9 for improved varieties (Table 25). The highest average number of private alleles in this study was detected for genotypes from East Tigray (10) followed by North Gonder (7) private alleles while the breeding lines had no private allele (Table 25).

Table 25. Summary of genetic diversity parameters obtained for 14 populations of tef genotypes based on 16 SSR markers

Population	Na	Ne	I	NPA	Ho	He	uHe	F	Ar	PPL
C. Tigray	7.38	5.09	1.71	1.00	0.49	0.75	0.79	0.33	7.38	100
E. Gojam	7.25	5.17	1.69	2.00	0.56	0.77	0.80	0.23	7.25	100
E. Shewa	6.94	4.76	1.64	2.00	0.51	0.75	0.78	0.31	6.94	100
E. Tigray	7.25	5.09	1.68	10.0	0.51	0.76	0.79	0.30	7.25	100
N. Gonder	7.88	5.51	1.78	7.00	0.52	0.77	0.81	0.29	7.88	100
N. Shewa	7.44	5.79	1.78	5.00	0.48	0.79	0.83	0.37	7.44	100
N. Wello	6.50	4.60	1.58	2.00	0.46	0.73	0.77	0.34	6.50	100
S. Gonder	7.50	5.47	1.72	1.00	0.41	0.76	0.80	0.43	7.50	100
S. Wello	7.06	5.01	1.67	4.00	0.53	0.75	0.79	0.28	7.06	100
W. Gojam	7.56	4.77	1.67	5.00	0.49	0.74	0.78	0.32	7.56	100
W. Shewa	7.19	5.23	1.70	3.00	0.53	0.76	0.80	0.28	7.19	100
W. Tigray	6.31	4.55	1.57	4.00	0.47	0.73	0.77	0.33	6.31	100
Improved	8.94	5.58	1.81	3.00	0.53	0.77	0.79	0.29	8.94	100
Breeding lines	4.88	3.57	1.32	0.00	0.59	0.67	0.71	0.13	4.88	100
Mean	7.15	5.01	1.66	0.22	0.50	0.75	0.79	0.30	7.15	100
SE	0.19	0.14	0.03	0.11	0.02	0.01	0.01	0.03	0.19	0.00

Na = No. of different Alleles, Ne = No. of Effective Alleles, I = Shannon's Information Index, NPA= No. Private Alleles, He = Expected Heterozygosity, uHe = Unbiased Expected Heterozygosity, Ar= Allelic richness, PPL= Percentage of polymorphic loci.

4.3.3 Population differentiation and gene flow

Molecular analysis of variance (AMOVA) showed highly significant ($P < 0.01$) variation among populations, among genotypes within populations and within genotypes from different genetic and eco-geographic origin (Table 26). Thus, 56.6%, 40.7% and 2.7% of

the total genetic variation was explained by variation within individuals, among individuals within populations and among populations, respectively.

Table 26. AMOVA showing variation among populations, among individuals within populations and within individual tef genotypes from various sources

Source	df	SS	MS	Variance		F- Statistic	P
				Estimated	%		
Among populations (AP)	13	180.74	13.90	0.175	2.7	$F_{ST}=0.027$	0.001
Among individuals within population (AI)	175	1618.49	9.25	2.729	40.7	$F_{IS}=0.418$	0.001
Within individuals WI)	189	716.50	3.79	3.791	56.6	$F_{IT}=0.434$	0.001
Total	377	2515.73	-	6.695	100		

F-statistic has also showed significant genetic differentiation among populations ($F_{ST} = 0.03$), among individuals within population ($F_{IS}=0.42$) and within individuals ($F_{IT} = 0.43$) (Table 26). On the other hand, the F_{ST} values for various markers in this study ranged from 0.03 to 0.14 with a mean of 0.08. For instance, CNLT 315 had the least F_{ST} (0.03) and highest gene flow (8.67) values whereas CNLT-136 had the highest F_{ST} (0.137) and lowest gene flow (1.573) (Table 27). Pair-wise population F_{ST} showed existence of significant differentiation between populations from various sources. Thus, the largest differentiation value was detected between the breeding lines and that of East Tigray (0.09), West Shewa (0.09) and West Tigray (0.085). On the other hand, very low pairwise differentiation was observed between North Wello and improved varieties; South Well and East Gojam; West Shewa and East Shewa, and improved varieties and North Gonderpopulations (Table 28). This shows the presence of high gene flow among populations with lower pair-wise F_{ST} values.

Table 27. Summary of F-Statistics and estimates of gene flow (Nm) over all populations for each locus

Locus	F _{IS}	F _{IT}	F _{ST}	Nm
CNLT_133	0.490	0.541	0.100	2.250
CNLT_216	0.321	0.372	0.075	3.101
CNLT_27	0.508	0.541	0.068	3.412
CNLT_538b	0.608	0.632	0.060	3.947
CNLT_60	0.258	0.306	0.065	3.594
CNLT_33	0.520	0.565	0.095	2.375
SSR_9.4	0.107	0.194	0.098	2.305
CNLT_42	0.271	0.327	0.076	3.032
CNLT_150	-0.037	0.040	0.074	3.117
CNLT_157	0.992	0.993	0.110	2.027
SSR_3.3	-0.438	-0.351	0.061	3.875
CNLT_136	0.906	0.919	0.137	1.573
CNLT_315	-0.486	-0.444	0.028	8.703
CNLT_416	0.241	0.299	0.076	3.035
CNLT_438	0.667	0.708	0.123	1.788
CNLT_484	-0.172	-0.104	0.058	4.087
Mean	0.297	0.346	0.081	3.264
SE	0.107	0.102	0.007	0.412

Furthermore, pairwise population Nei unbiased genetic distance (Nei, 1978) between population ranged from 0.045 (South Wello vs East Gojam) to 0.545 (breeding lines vs East Tigray) (Table 28). Thus, most large values were observed between the breeding lines and the other 13 populations whereas the least values observed between South Wello and East Gojam. This shows that the breeding lines derived from mutant line were substantially differentiated from population other than released varieties in the pair-wise comparisons. On the other hand, the relatively smallest distance observed between breeding lines and improved varieties could be due to the breeding lines were developed through hybridization of the mutant line with improved varieties.

Table 28. Pair-wise Nei's Unbiased Genetic Distance (above diagonal) and genetic differentiation (F_{ST}) (below diagonal)

Pop.	CT	EG	ES	ET	NG	NS	NW	SG	SW	WG	WS	WT	REL	BrL
CT	0.000	0.196	0.143	0.072	0.108	0.126	0.086	0.125	0.106	0.082	0.139	0.084	0.167	0.401
EG	0.050	0.000	0.208	0.168	0.154	0.110	0.195	0.099	0.045	0.115	0.145	0.212	0.177	0.350
ES	0.041	0.048	0.000	0.228	0.233	0.174	0.175	0.295	0.173	0.324	0.053	0.246	0.138	0.454
ET	0.033	0.043	0.049	0.000	0.172	0.139	0.188	0.180	0.126	0.120	0.128	0.129	0.241	0.545
NG	0.034	0.043	0.048	0.041	0.000	0.134	0.148	0.181	0.116	0.110	0.106	0.247	0.133	0.337
NS	0.039	0.035	0.042	0.038	0.038	0.000	0.109	0.155	0.167	0.067	0.082	0.201	0.179	0.362
NW	0.035	0.051	0.046	0.047	0.041	0.039	0.000	0.220	0.099	0.189	0.177	0.151	0.104	0.272
SG	0.038	0.036	0.054	0.043	0.041	0.039	0.050	0.000	0.169	0.113	0.244	0.152	0.223	0.423
SW	0.036	0.030	0.042	0.037	0.035	0.041	0.037	0.041	0.000	0.170	0.158	0.141	0.164	0.386
WG	0.031	0.040	0.058	0.036	0.034	0.031	0.046	0.036	0.042	0.000	0.170	0.153	0.230	0.433
WS	0.042	0.041	0.030	0.038	0.037	0.033	0.046	0.050	0.041	0.044	0.000	0.241	0.198	0.518
WT	0.034	0.051	0.053	0.040	0.050	0.047	0.045	0.041	0.041	0.041	0.052	0.000	0.253	0.474
REL	0.034	0.040	0.033	0.044	0.030	0.038	0.029	0.040	0.035	0.042	0.041	0.046	0.000	0.190
BrL	0.076	0.072	0.083	0.088	0.069	0.071	0.066	0.076	0.074	0.079	0.088	0.086	0.046	0.000

CT=Central Tigray, EG= East Gojam, ES= East Shewa, ET=East Tigray, NG=North Gonder, NS= North Shewa, NW=North Wello, SG= South Gonder, SW= South Wello, WG= West Gojam, WS=West Shewa, WT=West Tigray, Imp= Improved variety, BrL= Breedinglines

4.3.4 Patterns of genetic diversity and population structure

In this study, the 14 populations from various sources were grouped into four clusters consisting of one to nine populations (Fig. 17) whereby the different populations within a cluster are assumed to be more closely related with each other than those in different clusters. Thus, cluster one was solitary and consisted of the breeding lines alone whereas cluster two consisted three populations from West, Central and East Tigray zones. This clearly shows the existence of high gene flow among three zones of Tigray due to geographical proximity. The third cluster consisted of six populations from South and North Gonder, East and West Gojam, South Wello and North Shewa zones resulting may be due to exchange of germplasm resources along their border. The fourth cluster consisted of populations from East Shewa, West Shewa and North Wello zones along with improved varieties.

In this study, several germplasms from adjacent collection zones or various origin tend to cluster together. In this study, Delta K reached its highest pick at $K = 4$ and hence, it was decided to have four clusters (Fig. 18a). At $K=4$, the structure bar plot revealed the existence of four clusters with wide genetic admixtures (Fig. 19b). Thus, the major constituents of cluster I were populations from West Gojam (77.4%), East Tigray (61.9%), North Gonder (51.1%), South Gonder (44.2%) and North Shewa (43.3%) represented by light blue color. Cluster II, on the other hand, consisted of mostly population from North Wello (43.4%), West Tigray (29.5%), East Gojam (27.6%) and West Shewa (27.1%) and represented by a dark purple colour. The inferred cluster III had a significant admixture mainly from East Shewa (49.9%), South Wello (39.3%) and improved varieties (37.6%)

and represented by a orangecolor. Cluster IV constituted mostly the breeding lines (92.8%) with some admixtures from improved varieties (43%) and represented by a green color.

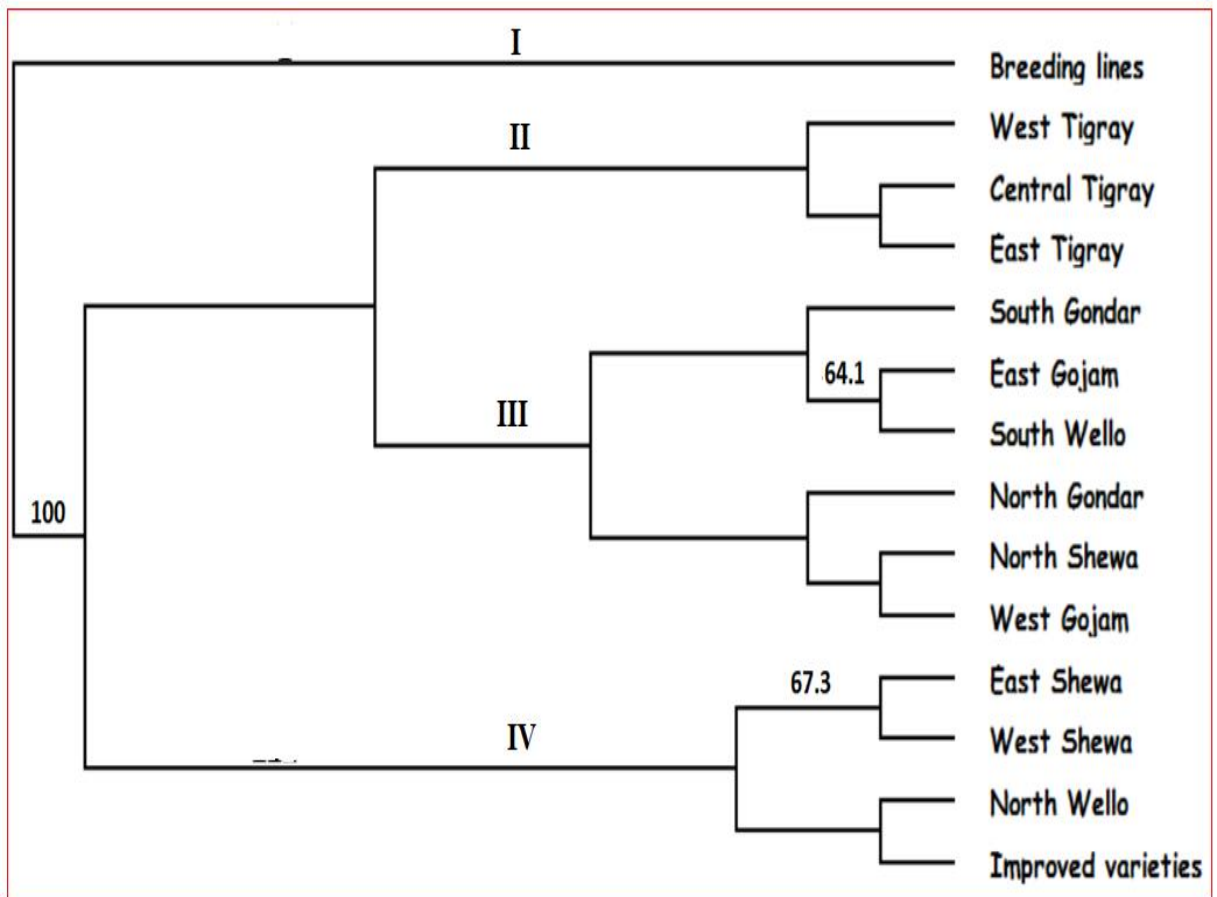
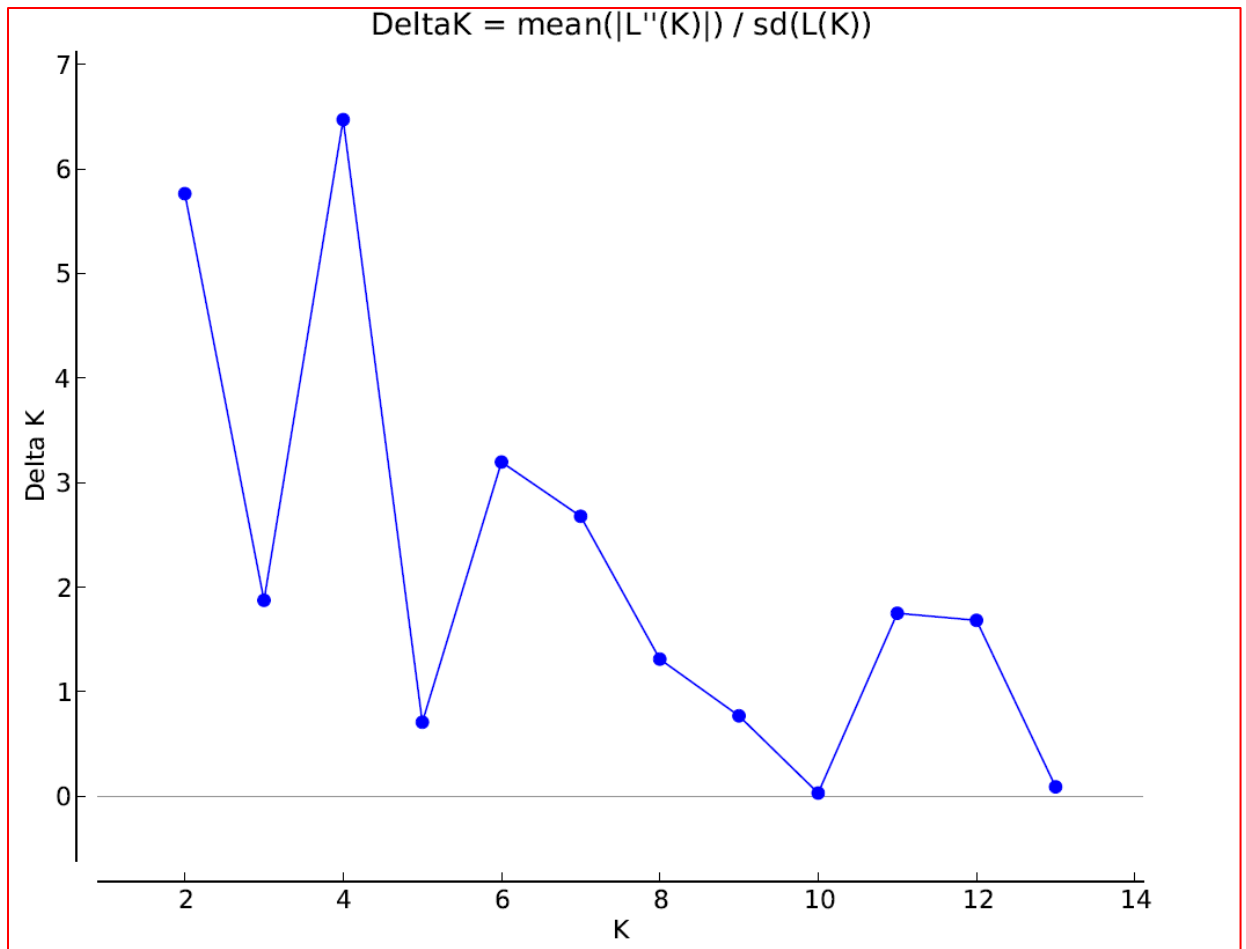
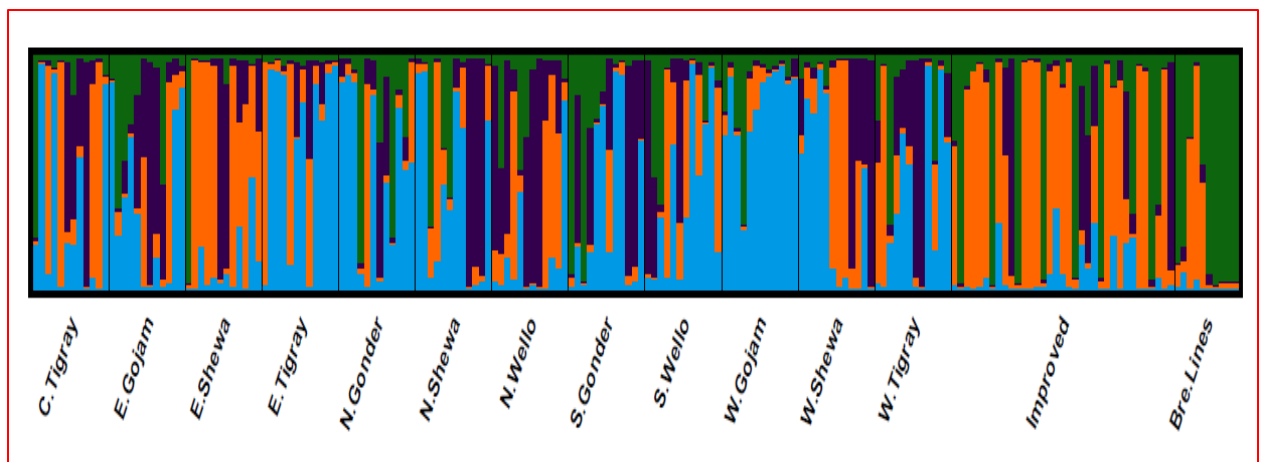


Figure 17. Dendrogram showing the relationship among 14 populations of tef from different genetic and eco-geographic origins based on 16 SSR markers. (A bootstrap value less than 50 was not written for all nodes in this figure).



(a)



(b)

Figure 18. Delta K showing the best K (a) and structure bar-plot (b) of tef genotypes from 14 populations showing the pattern of assignment into four clusters

4.3.5 Patterns of genetic diversity and clustering among individual genotypes

Cluster analysis of the 189 individual tef genotypes from various genetic and eco-geographic origins grouped into four distinct clusters with further major and minor sub clusters. Each cluster consisted of 40 to 60 genotypes (Fig. 19). Cluster I, for instance, consisted of 40 genotypes including 38 pure lines from all geographic zones and two improved varieties. In this cluster, large number of genotypes were represented from North Wello (6), North Shewa (5) and West Tigray (5) while all remaining population other than the breeding lines were represented by one to three genotypes. Cluster II, on the other hand, consisted of 60 genotypes from all populations other than West Gojam. Thus, the highest number of genotypes was represented from improved varieties (21), East Shewa (8), Central Tigray (4), North Gonder (4), North Wello (4), South Wello (4) and West Shewa (4) while the remaining populations were represented with 1 to 3 genotypes.

The third cluster consisted of 41 genotypes from all populations except East Shewa and the breeding lines. Thus, large number of genotypes was represented from West Gojam (8), East Tigray (6), East Gojam (4), North Gonder (4) and South Wello (4) while the remaining populations were represented with 1 to 3 genotypes. Cluster IV, consisted of 48 genotypes including 31 pure lines from germplasm collections, 10 improved varieties and seven breeding lines. The majority of the genotypes were from improved varieties (10), breeding lines (7), South Gonder (5), East Gojam (4) and North Shewa (4) whereas the remaining populations were represented by 1 to 3 genotypes.

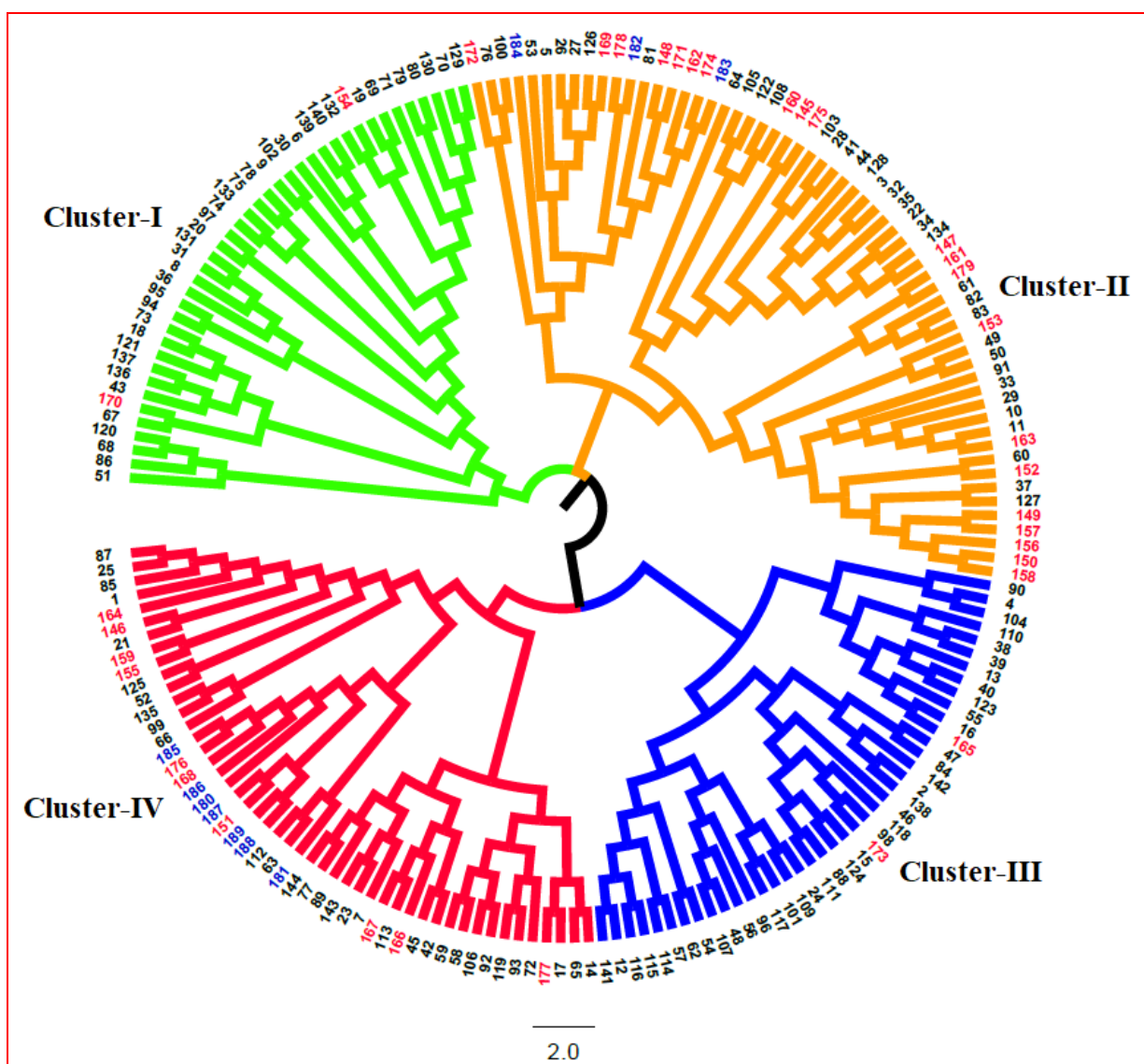


Figure 19. Dendrogram based on Unweighted Pair Group Method with Arithmetic Mean (UPGMA) showing the genetic relationship among 189 tef genotypes using 16 SSR markers. Genotypes and region of collections are described as 1-12 (central Tigray), 13-24 (East Gojam), 25-36 (East Shewa), 37-48 (East Tigray), 49-60 (North Gonder), 61-72 (North Shewa), 73-84 (North Wello), 85-96 (South Gonder), 97-108 (South Wello), 109-120 (West Gojam), 121-132 (West Shewa) and 133-144 (West Tigray). Improved varieties (145-179) and breeding lines (180-189) are written in red and blue color respectively.

4.3.6 Establishing core collections

In this study, a core collection consisting of 64 (33.9%) genotypes and capturing 100% of the alleles from the entire studied genotypes. The genotypes identified for core collections were from all the 14 populations with various proportions. Thus, the number of genotypes

captured for core collection from each population ranged from 1 to 9 for the breeding lines and East Tigray population, respectively. The second largest number of genotypes (8) was from improved varieties followed by those from North Gonder (6) and North Shewa (6) populations (Table 29, Fig. 20). Such large number of genotypes identified for core collection from East Tigray, North Gonder and North Shewa is in line with the number of private alleles detected in the entire studied genotypes for the same population.

A dendrogram showing the relationship among core collections was also constructed and compared to the dendrogram for the entire genotypes (Fig. 21). Thus, four clusters were displayed for both core set and entire studied genotypes. As it is clearly seen in Fig. 21, cluster I, II, III and IV consisted of 13, 8, 12 and 31 individuals, respectively. Thus, genotypes in cluster-I were composed of genotypes from seven populations with the majority from South Gonder and improved varieties. Cluster-II, on the other hand consisted of genotypes from eight populations with equal proportion. The third cluster was composed of genotypes from eight populations whereby the improved varieties and North Wello populations accounted for the vast majority. The fourth cluster, however, consisted of the largest number of genotypes from all the 14 populations with most of them from East Tigray (7) followed by North Gonder (4) and South Wello (3). The other seven populations were represented by two genotypes whereas the other three populations were each represented by one genotype. Based on altitude of collection, about 5.5%, 32.7%, 40 % and 21.8% of the genotypes of the core collections were from below 1500m, 1501-2000m, 2001-2500m and >2500m, respectively (Table 29).

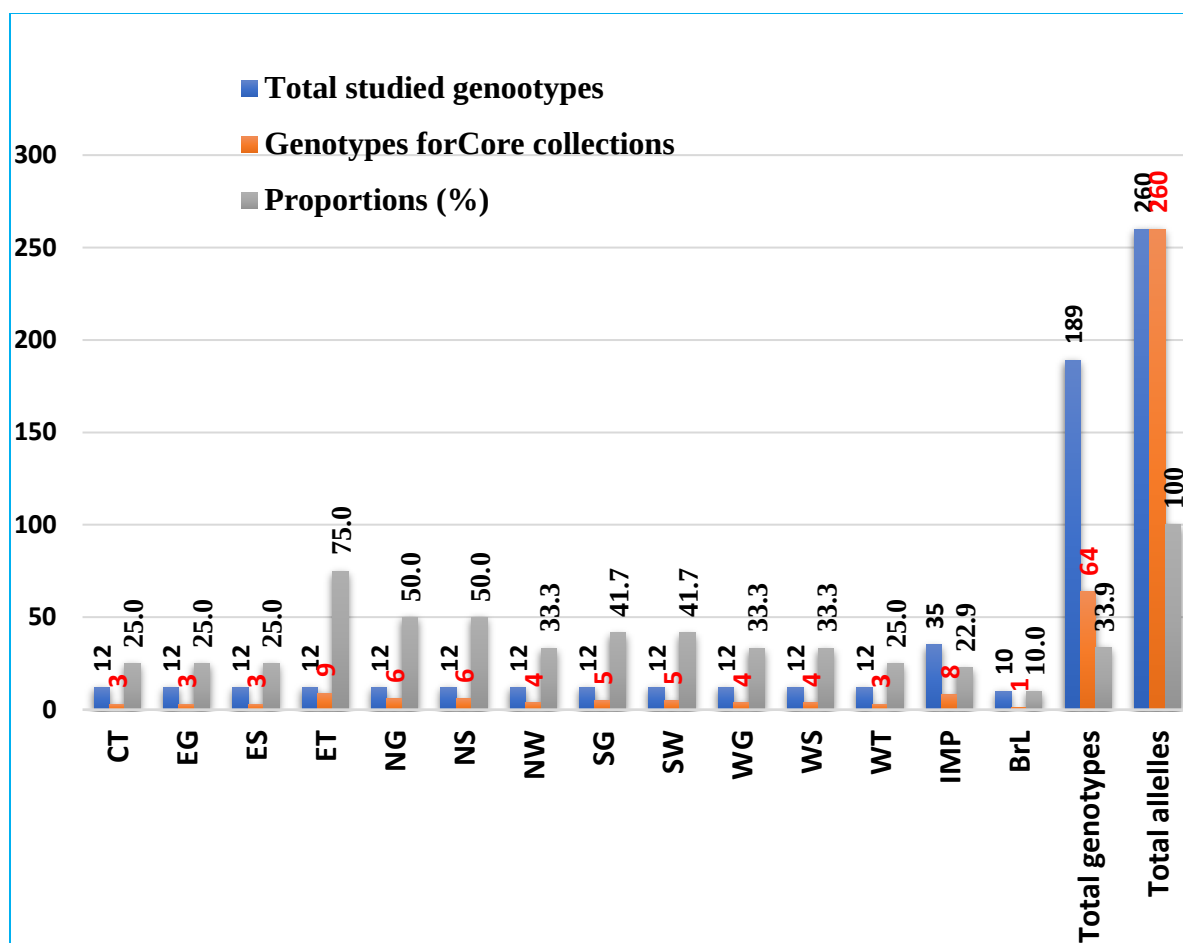


Figure 20. Proportion of core collection to entire studied genotypes and contributions of predefined populations from various sources to the core collection.

Table 29. Description of the tef genotypes proposed to construct a none redundant core collection

No.	Acc./ name	Zone	Altitude	No.	Acc./ name	Zone	Altitude
1	19132-3	Central Tigray	2644	33	234985-2	North Wello	1520
2	234407-1	Central Tigray	1830	34	234993-3	North Wello	2580
3	237184-1	Central Tigray	2300	35	55293-2	South Gonder	1900
4	9545-1	East Gojam	2481	36	212717-0	South Gonder	2650
5	9556-1	East Gojam	1470	37	225919-2	South Gonder	2360
6	229768-3	East Gojam	2570	38	225919-3	South Gonder	2360
7	18460-0	East Shewa	1961	39	225919-7	South Gonder	2360
8	236963-1	East Shewa	2300	40	212612-3	South Wello	1550
9	236963-2	East Shewa	2300	41	212614-1	South Wello	1560
10	15297-1	East Tigray	2632	42	225898-1	South Wello	2575
11	15297-2	East Tigray	2632	43	243491-2	South Wello	3090
12	15299-1	East Tigray	2517	44	243504-1	South Wello	2265
13	15299-3	East Tigray	2517	45	19506-2	West Gojam	2043
14	19201-1	East Tigray	2063	46	242140-3	West Gojam	2300
15	19202-2	East Tigray	1997	47	242155-1	West Gojam	1890
16	234460-1	East Tigray	2210	48	242155-3	West Gojam	1890
17	234460-2	East Tigray	2210	49	18410-2	West Shewa	1880
18	242540-1	East Tigray	2052	50	18410-3	West Shewa	1880
19	9448-1	North Gonder	2059	51	236760-1	West Shewa	2380
20	9448-2	North Gonder	2059	52	236760-4	West Shewa	2380
21	9451-2	North Gonder	1840	53	9444-3	West Tigray	1492
22	9472-4	North Gonder	2208	54	19241-1	West Tigray	1963
23	19343-2	North Gonder	1916	55	237236-3	West Tigray	2050
24	243540-3	North Gonder	2050	56	Melko	Improved Varieties	
25	9559-1	North Shewa	2575	57	Gibe	Improved Varieties	
26	9559-2	North Shewa	2575	58	Ziquala	Improved Varieties	
27	15322-2	North Shewa	1693	59	Holeta Key	Improved Varieties	
28	18385-2	North Shewa	1260	60	Yilmana	Improved Varieties	
29	236746-0	North Shewa	2650	61	Amarach	Improved Varieties	
30	236957-1	North Shewa	1700	62	Mechare	Improved Varieties	
31	215200-1	North Wello	2000	63	Worekiyu	Improved Varieties	
32	234356-4	North Wello	1580	64	RIL-44	Breeding Line (KindeX Kaye Murri)	

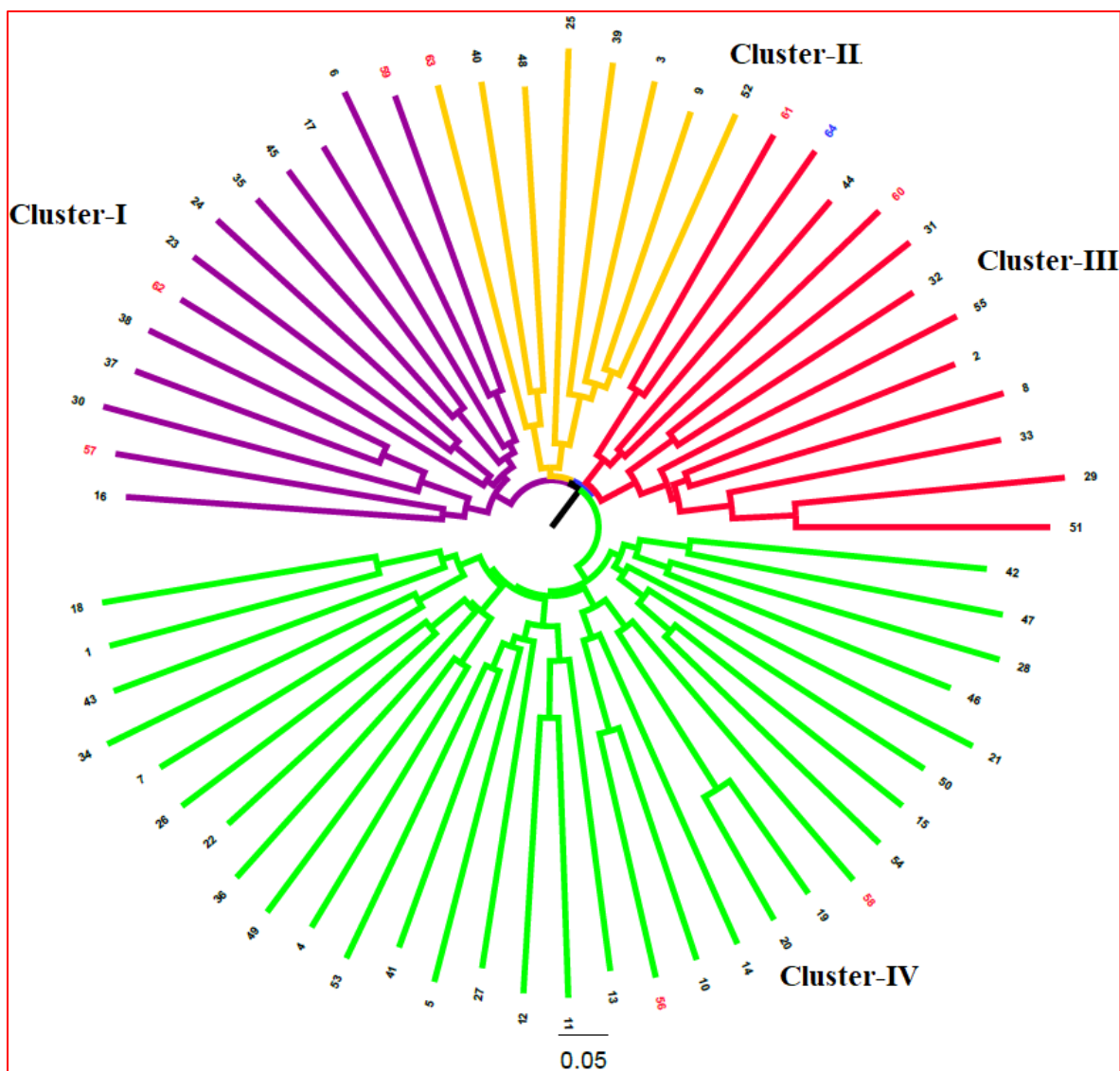


Figure 21. Dendrogram showing the clustering pattern of genotypes in the core collections. Genotypes and Zones of collections are described as 1-3 (Central Tigray), 4-6 (East Gojam), 7-9 (East Shewa), 10-18 (East Tigray), 19-24 (North Gonder), 25-30 (North Shewa), 31-34 (North Wello), 35-39 (South Gonder), 40-44 (South Wello), 45-48 (West Gojam), 49-52 (West Shewa) and 53-55 (West Tigray). The names of improved varieties (56-63) and breeding lines (64) are written in red and blue color respectively.

4.4 *Discussions*

4.4.1 **SSR markers and its use in tef genetic diversity**

The present study revealed that all the studied SSR markers were highly polymorphic (mean PIC = 0.80). The 16 markers generated a large total number of alleles (260) and average number of alleles per locus (16.3) (Table 24). Thus, the number of alleles generated per locus ranged from 2 (SSR 3.3) to 26 alleles (CNLT 538) which is by far more than the previously reported 148 total number of alleles (Abate, 2015) and 164 alleles (Abraha *et al.*, 2016) obtained using 16 and 10 markers, respectively. This could be due to use of different types and numbers of genotypes and markers. The number of alleles (N_a) generated per locus in the present study is, however, closer to Abate (2015) who reported 2 to 27 alleles per locus while it is slightly larger than the 8 to 23 alleles per locus reported by other authors (Zeid *et al.*, 2012; Abraha *et al.*, 2016).

Observed heterozygosity (H_o) is the frequency of heterozygous individuals in a population whereas expected heterozygosity (H_e) or gene diversity is the probability that two gametes, randomly chosen from the gene pool, are of different alleles (Greenbaum *et al.*, 2014). Thus, the value obtained for gene diversity in the current study (0.36 for SSR 3.3 to 0.93 for CNLT- 42) is a bit different from the previous reports of 0.75 to 0.91 based on most of the present markers (Zeid *et al.*, 2012); 0.00 to 0.85 (Abate, 2015) and 0.67 to 0.94 (Abraha *et al.*, 2016). Furthermore, the PIC value obtained in the present study (0.30 to 0.92 with a mean of 0.80), is different from those previous reports of PIC ranging from 0.05 to 0.86 (Zeid *et al.*, 2012), 0.02 to 0.95 (Abate, 2015) and 0.64 to 0.94 (Abraha *et al.*, 2016) using 39, 16 and 10 microsatellite markers, respectively. In general, more than 85% of the studied markers were selected from Zeid *et al.* (2012) and thus, generated similar PIC with the

previous report (Zeid *et al.*, 2012). In general, about 75% of the 16 studied SSR markers were found to be polymorphic with a mean PIC greater than 80% whereas Abate (2015) reported 81% polymorphic loci with PIC of over 50% for the number of SSR markers. The fact that about 75% of the loci in the present study had PIC values greater than 0.80 reveals the excellent discriminatory power of the studied loci. Thus, a positive and significant correlation was detected between the allele number and gene diversity ($r = 0.751$, $P = 0.01$) and PIC ($r = 0.764$, $P = 0.01$) for more than 80% of the studied loci in the present study showing that loci having a greater number of alleles (≥ 10) had also high PIC values. In general, the highest value of various genetic parameters observed for CNLT 42 in the present study is in line with most of the previous reports (Zeid *et al.*, 2012; Abate, 2015, Abraha *et al.*, 2016). Therefore, such SSR markers identified for high gene diversity are useful for the genetic diversity assessment of tef germplasm.

4.4.2 Tef populations diversity

The level of diversity among the various populations was assessed and it was possible to identify populations having better genetic diversity. All studied populations in the present study were polymorphic across all loci unlike the previous report of Abate (2015) which revealed a polymorphic value ranging from 69 to 100%. Populations from North Shewa and North Gonder were identified to have the highest gene diversity whereas the breeding lines had the least value for all studied genetic parameters (Table 27). Similar to this finding, Abate (2015) reported high level of genetic diversity among germplasm accessions compared to the breeding lines. This could be due to mutagenesis, hybridization and artificial selection that would reduce the level of some genetic parameters. The highest

gene diversity for most of the genotypes from germplasm collection, however, could be due to more chances of coevolving in nature than those under artificial selection or due to variation in sample size. In general, the present result suggests that collections from North Shewa and North Gonder Zones which had high gene diversity are important for future germplasm collections and conservation.

Allelic richness or allelic diversity is the total number of alleles in a population. It is a measure of genetic diversity indicative of population's long-term potential for adaptability and persistence (Greenbaum *et al.*, 2014). In the present study, allelic richness was the highest for improved varieties followed by North Gonder, West Gojam and South Gonder population whereas the breeding lines had the least value of allelic richness. This is in line with Abate (2015) who reported the least allelic richness for the breeding lines. As allelic richness is a raw material for evolution by natural selection, its decrease leads to a reduction in the population's potential to adapt to future environmental changes (Greenbaum *et al.*, 2014). In the present study, the estimated allelic richness for improved varieties was found to be the highest mostly due to the fact that the largest number of samples (35 genotypes for improved varieties) compared to the 12 genotypes for lines from germplasm accessions.

Private alleles are alleles that are found only in a single population among a broader collection of populations (Szpiech and Rosenberg, 2011). In the present study, the number of private alleles was the highest for germplasm collections while the breeding lines had no private allele. Such findings are inline with the previous findings of Abate (2015) who reported the least value of private alleles for the breeding lines and improved varieties.

Among the germplasm collections, the highest number of private alleles was observed for East Tigray population (10) followed by North Gonder population (7), North Shewa (5) and West Gojam (5). Therefore, it is important to give due attention for collection from these zones while establishing core collections.

4.4.3 Population differentiation and gene flow

In the present study, the variations explained by within individuals, among individuals within populations and among populations variation is in line with the results of Abraha *et al.* (2016) who reported a variation of 2%, 35% and 65% among population, among genotypes within population and within genotypes, respectively. Abate (2015), however, reported the highest value for variation among genotypes within population (60%) followed by within genotypes (31%) and among populations (9%). This could be due to the use of different number and type of genotypes and markers.

The F_{ST} values for various markers in this study ranged from 0.03 to 0.14, with a mean of 0.08 (Table 29) and it is according to Wright (1978) considered to be low to moderate. The detection of such low to moderate value could be due to high variability among individuals and within individuals as a result of gene flow. Because, Kent and Bruce (2009), reported the occurrence of little differentiation in large populations having extensive migration. In the current study, markers having small F_{ST} were observed to have the highest gene flow values (Table 29) since F_{ST} is inversely proportional to the gene flow. Lower values of F_{ST} according to Abraha *et al.* (2016) could appear due to exchange of genes through hybridization during varietal development.

Pair-wise population F_{ST} showed that the largest differentiation value was detected between breeding lines and East Tigray population whereas the least was observed between the improved varieties and North Wello populations. In general, very low differentiation was observed among population from adjacent region showing the presence of high gene flow among the border zone. On the other hand, most large values of Nei unbiased genetic distance were observed between the breeding lines and the populations from germplasm collections whereas the value observed between the remaining populations was quite lower. This shows that the breeding lines derived from the crossing of mutant lines with adapted cultivars created substantial variability in the pair-wise comparisons. As suggested by Abraha *et al* (2016), the relatively smaller distance observed between breeding lines and improved varieties could be due to hybridization during the development of the breeding lines.

4.4.4 Patterns of genetic diversity and population structure

Clustering of the 189 individual genotypes and the 14 populations revealed the formation of four distinct clusters whereby genotypes from populations of adjacent geographic zones and genetic origins were grouped together. This may be due to seed exchange resulting from geographical proximity and dissemination of improved tef cultivars via extension. The observed number of clusters in this study is in line with the previous reports of Abate (2015) and Abrha *et al.* (2016) who grouped 41 and 60 tef genotypes into three clusters based on 16 and 10 selected microsatellite markers, respectively.

In this study, in general, genotypes from various origins were grouped into different clusters with various proportions. For instance, the improved varieties were distributed in

all the four clusters whereas the breeding lines were grouped into two clusters. In the grouping of breeding lines, surprisingly, the mutant parental line (*Kinde*) and cultivar *Kaye Murri* were grouped in the first cluster together with all progenies of *Kinde* X *Kaye Murri* whereas the progenies of *Quncho* X *Kinde* were grouped in cluster two. The lines derived from germplasm collections, on the other hand, were grouped into all clusters and sub clusters with various proportion. Such groupings may be resulted from seed exchange due to geographical proximity; dissemination of improved tef cultivars via extension. In general, the clustering pattern observed based on molecular data is to some extent in line with the clustering pattern based on morphological traits.

4.4.5 Construction of core collections

Various researchers suggested different proportion of sample size to establish a core collection. For instance, Brown (1989) suggested a sampling percentage of 5% to 10% whereas a 33.9% of the entire population was identified to establish a core set in the present study. In previous studies, Atnaf (2017) reported 16% whereas; Liu and Hou (2010) reported a core collection size 27%. The present result demonstrated the great potential of using molecular data to construct a core collection for efficient conservation and utilization of the tef germplasm collections. However, the number of markers employed for genetic analysis in the present study is relatively low to give a concluding remark about the conservation of all available genetic resources. Therefore, it is very important to increase the number and kind of markers to be used.

Chapter 5

5. Genotype by environment interaction in major quantitative traits and yield stability of tef

Abstract

Tef adapts to a wide range of environmental conditions and hence, identification of adaptable, stable and high yielding genotypes under varying environmental conditions is the primary concern of crop breeders. In this study, 35 improved tef varieties were evaluated at Debre Zeit, Holetta and Alem Tena for two years and at Axum, Adet and Shamu for one year to assess the effect of genotype, environment, G x E interaction; existence of mega environments and the stability of genotypes. The mean grain yield across test environments ranged from 1.7 t/ha for Alem Tena-2 to 4.29 t/ha for Debre Zeit-1 while, the genotypic mean yield ranged from 2.49 t/ha for Simada to 3.33 t/ha for Melko. The AMMI, GGE and Eberhart Russell model confirmed Melko as the highest yielding and relatively stable variety. GGE biplot analysis grouped the nine test environments and the 35 genotypes into four mega environments and seven genotypic groups, respectively. Thus, Melko was the highest yielding and ideal genotype to evaluate and rank different test environments relative to it while Debre Zeit-1 was the highest yielding and ideal environment to evaluate and rank different genotypes relative to it. Both the relationship between environments and GGE biplot revealed existence of four Mega environments: group-I (Debre Zeit-1), Group-II (Adet, Axum & Debre Zeit-2), Group-III (Alem Tena-1, Alem Tena-2, Holetta-1 & Holetta-2) and group-IV (Shambu). Besides, Debre Zeit-1 & 2, and Holetta 1 & 2 having the longest vector length were the most discriminating of all environments while, Adet and Axum having the smallest angle with the average environmental axis were the most representative of all environments. The association of grain yield with second culm length and shoot biomass were significant and positive while, that with lodging index was significant ($P < 0.01$) and negative. Cluster analysis formed seven clusters whereby high yielding and early maturing genotypes appeared into their respective cluster. In principal component analysis, the first three PCs with eigenvalue greater than unity accounted for 82.9% of the total variations. This study generally enabled to identify genotypes with better yield and moderate stability, and discriminating and representative environments for future tef breeding.

Key words: AMMI; Cluster; discriminating power; Genotype x Environment; GGE, Eberhart Russell model; Genotype plus Genotype x Environment; Mega environment; PCA; Tef

5.1 ***Introduction***

Crop performance is a function of genotype, environment, and genotype by environment interactions (GEI) and hence, the importance of understanding crop management and its growing environments has, therefore, been suggested by various researchers to increase agricultural production (Tolessa et al., 2013; Yan and Kang, 2003; Basford and Cooper, 1998). This is possible by eliminating unnecessary spatial and temporal replication of yield trials and by establishing additional testing environment only when the existing ones are underrepresented (Basford and Cooper, 1998).

Tef, a crop widely cultivated in Ethiopia, adapts to wide range of environments which include soil, altitude, rainfall and temperature conditions (Ketema, 1997; Assefa *et al.*, 2011). G x E study is useful in tef crop breeding and varietal development particularly for releasing variety for different agro-ecologies, effective allocation of resources and characterization of genotype responses to variable productivity levels (Ketema, 1993; Kefyalew et al, 2001). Such information enables breeders to determine optimum breeding strategy so as to make informed choices of the locations and input systems to be used in the breeding efforts (Gruneberg *et al.*, 2005). However, there are very limited studies on G x E in tef crops suggesting to conduct more studies across major tef growing areas in Ethiopia (Kefyalew, 1999; Kefyalew et al., 2000, 2001; Ashamo and Belay, 2012).

This study was, therefore, conducted to (1) assess the magnitude of GE interaction and stability; (2) examine the possible existence of different mega-environments; and (3) determine the discriminating ability and representativeness of the environments.

5.2 *Materials and Methods*

5.2.1 Plant materials

Thirty-five improved varieties released by seven research centers in Ethiopia between 1970 and 2014 were evaluated. About 35% and 65% of these varieties were developed through hybridization and selection from landraces, respectively. Furthermore, 77.1%, 17.1% and 5.7% of these varieties respectively were released for high potential environments, moisture deficit areas and waterlogged areas in the country. Detail descriptions of the 35 varieties investigated are given in Table 30.

5.2.2 Description of the study environment

The study environments include Holetta (2015 & 2016), Debre Zeit (2015 & 2016), Alem Tena (2015 & 2016), Adet, Axum and Shambu in 2015 (Table 31). All study environments are located in the highland high potential areas except for Alem Tena a moisture deficit environment situated in the Central Rift Valley of Ethiopia. Holetta and Shambu are located at 39 and 310 km west of Addis Ababa, respectively while Debre Zeit and Alem Tena are located at 47 and 109 Km in the southeast direction from Addis Ababa, respectively. Adet and Axum, however, are located in the Northern parts of Ethiopia at 533 and 1025 Km in that order from Addis Ababa. Detail descriptions of the studied environments are given in Table 31.

Table 30. Description of the tef varieties considered for GXE interaction studies

Varietal name		Code	Release Year	Seed color	Techniques used	Environmental Adaptation
Common name	Pedigree					
Enatit	DZ-01-354	G1	1970	P. white	Selection	High potential
Asgori	DZ-01-99	G2	1970	Brown	Selection	High potential
Magna	DZ-01-196	G3	1978	v. white	Selection	High potential
Wolenkomi	DZ-01-787	G4	1978	P. white	Selection	High potential
Menagesha	DZ-Cr-44	G5	1982	White	Hybridization	High potential
Melko	DZ-Cr-82	G6	1982	White	Hybridization	High potential
Tseday	DZ-Cr-37	G7	1984	White	Hybridization	Low moisture
Gibe	DZ-Cr-255	G8	1993	White	Hybridization	High potential
Ziquala	DZ-Cr-358	G9	1995	White	Hybridization	High potential
Dukem	DZ-01-974	G10	1995	White	Selection	High potential
Holeta Key	DZ-01-2053	G11	1999	Brown	Selection	High potential
Ambo-Toke	DZ-01-1278	G12	2000	White	Selection	High potential
Gerado	DZ-01-1281	G13	2002	White	Selection	High potential
Koye	DZ-01-1285	G14	2002	White	Selection	High potential
Key Tena	DZ-01-1681	G15	2002	Brown	Selection	High potential
Gola	DZ-01-2054	G16	2003	P. white	Selection	High potential
Ajora	PGRC/E 205396	G17	2004	P. white	Selection	High potential
Genet	DZ-01-146	G18	2005	P. white	Selection	High potential
Zobel	DZ-01-1821	G19	2005	P. white	Selection	High potential
Dima	DZ-01-2423	G20	2005	Brown	Selection	High potential
Yilmana	DZ-01-1868	G21	2005	P. white	Selection	High potential
Dega Tef	DZ-01-2675	G22	2005	P. white	Selection	Highland vertisols
Gimbichu	DZ-01-899	G23	2005	White	Selection	Highland vertisols
Amarach	Ho -Cr-136	G24	2006	P. white	Hybridization	Low moisture
Quncho	DZ-Cr-387 (RIL 355)	G25	2006	v. white	Hybridization	High potential
Guduru	DZ-01-1880	G26	2006	White	Selection	High potential
Gemechis	DZ-Cr-387 (RIL-127)	G27	2007	v. white	Hybridization	Low moisture
Mechare	Acc. 205953	G28	2007	P. white	Selection	High potential
Kena	23-Tafi Adi-72	G29	2008	White	Selection	High potential
Etsub	DZ-01-3186	G30	2008	White	Selection	High potential
Laketch	RIL 273	G31	2009	V. white	Hybridization	High potential
Simada	DZ- CR-385 (RIL-295)	G32	2009	White	Hybridization	Low moisture
Boset	DZ-Cr-409 (RIL 50d)	G33	2011	V. white	Hybridization	Low moisture
Kora	DZ-Cr-438 (RIL 133B)	G34	2014	v. white	Hybridization	High potential
Worekiyu	214746A	G35	2014	White	Selection	Low moisture

Table 31. Description of the test environments*

Environment name	Location code	Altitude	latitude	longitude	Annual RF(mm)	Temperature		Soil type
						Min °C	Max °C	
Adet	E1	2240	11°17' N	37°43' E	921.3	7.3	31.3	Nitosol
Alem Tena1	E2	1580	8°20' N	39°E'	500	8.0	29.8	Light sandy
Alem Tena 2	E3	1580	8°20' N	39° E	500	8.0	29.8	Light sandy
Axum	E4	2100	14°6' N	38°48' E	700	12.2	26.8	Vertisol
Debre Zeit 1	E5	1900	8°44' N	38° 58' E	851	8.9	28.3	Pellic Vertisol
Debre Zeit 2	E6	1900	8° 44' N	38° 58' E	851	8.9	28.3	Pellic Vertisol
Holetta1	E7	2400	9°44'N	38°30'	1100	6	22	Nitosol
Holetta2	E7	2400	9°44' N	38°30'	1100	6	22	Nitosol
Shambu	E9	2503	9°57'N	37°10' E				Nitosol

*The weather and edaphic informations were obtained from their respective research centers.

5.2.3 Experimental Design and other practices

The experiment was laid out in Randomized Complete Block Design with three replications. Five rows of one-meter length were used with spacing of 0.2m, 1m and 1.5m between rows, plots and replications, respectively. All agronomic and cultural practices were applied during the crop growth stage as per the recommendation for each location. Data on days to 50% panicle emergence and maturity, length of plant, culm, panicle and peduncle, diameter of the second culm internode, number of total and fertile tillers shoot biomass, grain yield, 1000-kernel weight and lodging index were recorded. Harvest index was calculated as the ratio of grain yield to biomass yield.

5.3 *Statistical analysis for phenotypic traits*

All quantitative traits data were subjected to analysis of variance (ANOVA) as per the procedure suggested by (Gomez and Gomez, 1984) using (SAS, 2002), Minitab 17.1 (2013) statistical software packages. Test of homogeneity and combined analysis of variance was also performed using the general linear model (PROC GLM) while mean separation and test of significance was performed using Duncan's multiple range test at 5% probability level. Additive main effects and multiplicative interactions (AMMI), and genotype plus genotype-by-environment (GGE) were performed using the Genotype by Environment Analysis with R (GEA-R) version 4.0 (Pacheco *et al.*, 2016). AMMI and GGE biplot were used to analyze the multi-environment trial (MET) data, to graphically represent the GE interaction, and to identify the rank of the studied genotypes and environments (Yan *et al.*, 2000). The extracted principal component axes (PCAs) were statistically tested by Gollob's F-test procedure (Gollob, 1968). The first two components were used to construct a biplot based on the no-scaling and tester-centered model for the E and $G \times E$ interaction biplot analysis.

Genotypic and phenotypic correlation of major quantitative traits were analyzed using multi environment trial analysis with R for windows (META-R) version 6.0 developed by CIMMYT (Alvarado *et al.*, 2016). Principal component and cluster analyses were conducted using MINITAB software version 17.1 (MINITAB, 2013).

Genetic distances between clusters as standardized Mahalanobis's D^2 statistics were calculated as:

$$D^2_{ij} = (\mathbf{x}_i - \mathbf{x}_j)' \text{cov}^{-1}(\mathbf{x}_i - \mathbf{x}_j)$$

Where, D^2_{ij} = the distance between cases i and j ; x_i and x_j = vectors of the values of the variables for cases i and j ; and cov^{-1} = the pooled within groups variance-covariance matrix.

The D^2 values obtained for pairs of clusters were considered as the calculated values of Chi-square (X^2) and were tested for significance both at 1% and 5% probability levels against the tabulated value of X^2 for 'P' degree of freedom, where P is the number of characters considered in each study (Singh and Chaudhary 1985).

5.4 RESULTS

5.4.1 Analysis of variance

Combined analysis of variance for the major quantitative traits of released tef varieties across environment is given in Table 32. Thus, almost all traits in the combined analysis were highly significant ($P \leq 0.01$) due to genotype, environment and genotype by environment interaction effects. Grain yield performance of some genotypes was affected by lodging at Adet (E_1) due to excess rainfall whereas moisture deficit exhibited a critical problem at Alem Tena (E_2 and E_3) and partly at Axum (E_4). The highly significant variation among genotypes revealed existence of high inherent variability among the tef varieties (Fig. 22). Significant variation for genotype x environment interaction observed in this study clearly showed unstable performance of the studied tef varieties across testing environments. Such conditions were reported to complicate selection and recommendation of stable genotype across environments (Hundera *et al.*, 2000a; Kefyalew *et al.* 2000, 2001).

Table 32. Analysis of variance for major quantitative traits of tef varieties evaluated at nine environments

Source	Loc (8)	Rep (Loc) (18)	Geno (34)	Loc*Geno (272)	Error (612)	Mean	CV
DH	12707.14***	8.67***	201.74***	11.50***	2.69	51.83	3.17
DGF	34265.40***	7.57**	173.51***	44.32***	3.83	59.06	3.31
DM	75815.65***	11.54***	375.39***	41.71***	3.81	110.89	1.75
PH	8241.74***	8.28*	893.14***	68.38***	4.51	93.08	2.28
PL	6428.83***	7.70***	311.60***	42.96***	3.68	55.37	3.46
CL	1540.36***	5.012ns	251.53***	23.88***	4.09	37.72	5.36
PdL	657.00***	3.57***	28.14***	5.62***	1.35	18.48	6.29
SCL	465.37***	1.33*	21.61***	3.86***	0.73	12.43	6.88
SCD	19.10***	0.26***	0.36***	0.10***	0.064	1.87	13.47
TT	953.67***	1.06***	11.92***	4.35***	0.49	5.11	13.73
FT	582.05***	0.98***	9.22***	2.83***	0.28	4.39	12.15
SBM	2054.44***	0.71ns	26.09***	9.63***	0.45	12.28	5.49
GY	104.93***	0.011ns	1.35***	0.62***	0.014	2.89	4.08
HI	4157.36***	3.21*	36.10***	20.77***	1.795	24.58	5.45
TKW	189.90***	13.01ns	15.06**	10.51**	8.12	28.43	10.02
LG	13925.21***	486.69***	1081.49***	150.29***	92.17	71.77	13.38

Abbreviations: DH: days to heading; DM: days to maturity; PH: plant height; PL: panicle length; CL: culm length; Pdl: peduncle length; SCD: second culm diameter; TT: number of total tillers; FT: number of fertile tillers; SPK: number of spikelet; SBM: shoot biomass; GY: grain yield; HI: harvest index; TKW: thousand kernel weight; LG: lodging index.



Figure 22. The performance of tef genotypes at Holetta Agric. Research Center (October 2015). Photo: Zerihun Tadele.

5.4.2 AMMI analysis of variance for grain yield

AMMI analysis revealed highly significant ($p \leq 0.01$) differences for grain yield (t ha^{-1}) of 35 tef varieties due to genotypes, environments and their interaction. Thus, the environment accounted for 79.7% of the total variations followed by the GEI (16.2%) and genotype (4.1%). In this case, the variation explained by the environment was about four times higher than what was explained by G plus GE revealing the differential performance of genotypes across the test environments. The AMMI analysis, furthermore, partitioned the $G \times E$ variance into principal component axes (IPCA) and the results are presented in Table 33. In this study, the first eight of the nine possible IPCA of the AMMI model were found to capture the entire pattern in the $G \times E$ interaction whereas the ninth was pooled into the residual. Even though all the eight PCs were significant ($P < 0.01$) in the AMMI analysis, substantial percentage of GEI was explained by PC_1 (53.04%) followed by PC_2 (19.41%)

and PC₃ (13.12) whereas less than 15% was explained by the remaining five PCs. In current study, the first two PCs which contributed for over 70% of the G x E interaction were used to create a biplot.

Table 33. AMMI analysis of variance for grain yield (tha⁻¹) of 35 tef genotypes grown at nine environments.

Source of variation	DF	SS	MS	F value	Explained % of SS
Environment	8	745.80	93.23	7581.71***	79.66
Genotype	34	40.80	1.20	97.60***	4.34
GEI	272	149.65	0.55	44.74***	15.98
PC1	41	79.38	1.94	157.20***	53.04
PC2	39	29.04	0.74	60.46***	19.41
PC3	37	19.63	0.53	43.08***	13.12
PC4	35	9.32	0.27	21.61***	6.23
PC5	33	4.58	0.14	11.26***	3.06
PC6	31	4.48	0.144	11.73***	2.99
PC7	29	1.64	0.056	4.58***	1.09
PC8	27	1.59	0.059	4.78***	1.06
PC9	25	0	0	0	0
Residuals	630	7.75	0.012	NA	0

GEI= Genotype by Environment interaction; DF= Degrees of freedom; SS= Sums of square; MS= Mean square.

5.4.2.1 Mean grain yield and stability

The mean grain yield across the nine test environments ranged from 1.7 t ha⁻¹ at Alem Tena in 2016 to 4.29 t ha⁻¹ at Debre Zeit in 2015 with a grand mean of 2.89 t ha⁻¹ (Table 34 & Fig. 23 & 24). Thus, Debre Zeit 1 & 2 were among the top yielding environments whereas Alem Tena 1 & 2 remained the lowest yielding environments in both seasons. In this study, Debre Zeit-1 followed by Holetta-1, Debre Zeit 2 & Holetta-2, respectively were identified to be

the best environments with a mean grain yield higher than the grand mean unlike the remaining test environments.

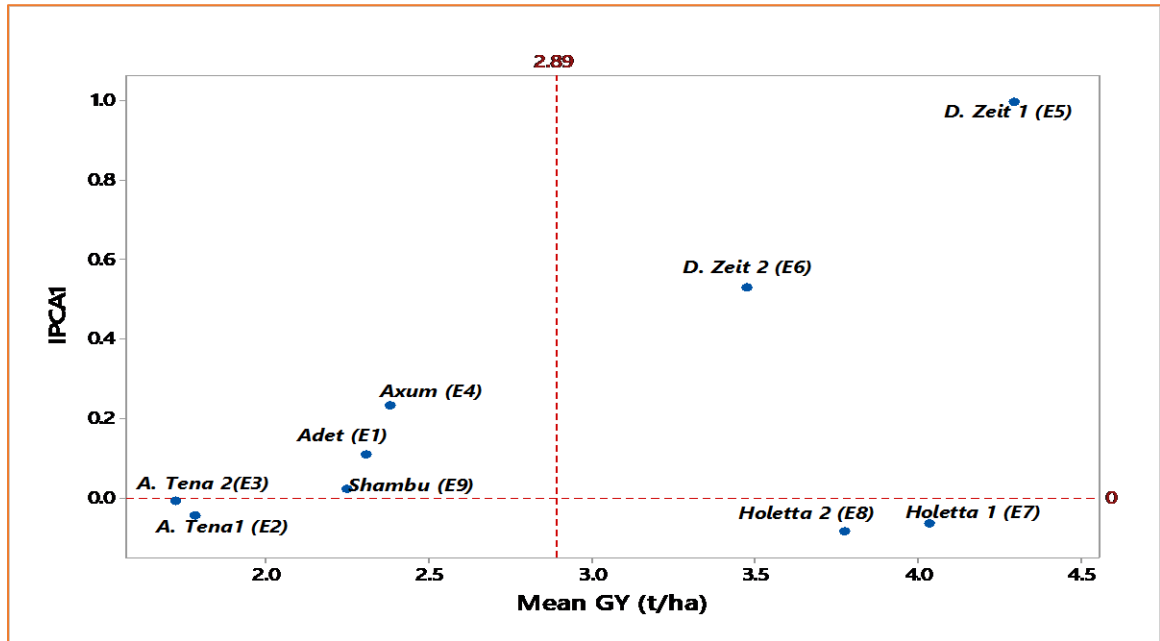


Figure 23. Plot showing environment focused mean grain yield versus IPCA-1 score. The reference line on the x-axis is the grand mean for grain yield of genotypes across environments (2.89-ton ha⁻¹) and on the y-axis is the IPCA1 value indicating genotype stability (IPCA1=0)

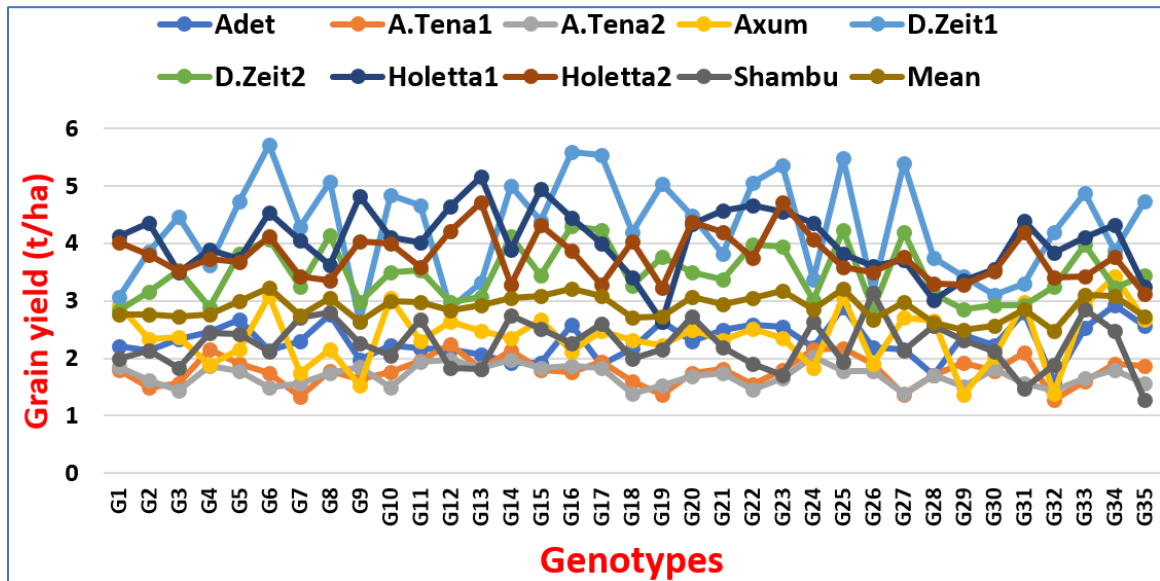


Figure 24. Interaction of mean grain yield performance for 35 tef genotypes evaluated across nine environments

On the other hand, mean grain yield among the 35 studied tef genotypes at nine environments ranged from 2.49 t ha⁻¹ for Simada (G₂₉) to 3.33 t ha⁻¹ for Melko (G₆). In this study, Melko (3.33t/ha) followed by Kora (3.27t/ha), Quncho (3.22t/ha), Gimbichu (3.18t/ha) had the highest mean yield. Thus, G₆ which have the highest mean grain yield and IPCA-1 value of 0.46 was moderately adaptable and high yielding (Table 34, Fig 23; Fig 25a, b, c) whereas G₂₁, which had the lowest IPCA-1 score (-0.01) was found to be stable and having consistent performance across locations but with lower grain yield than G₆.

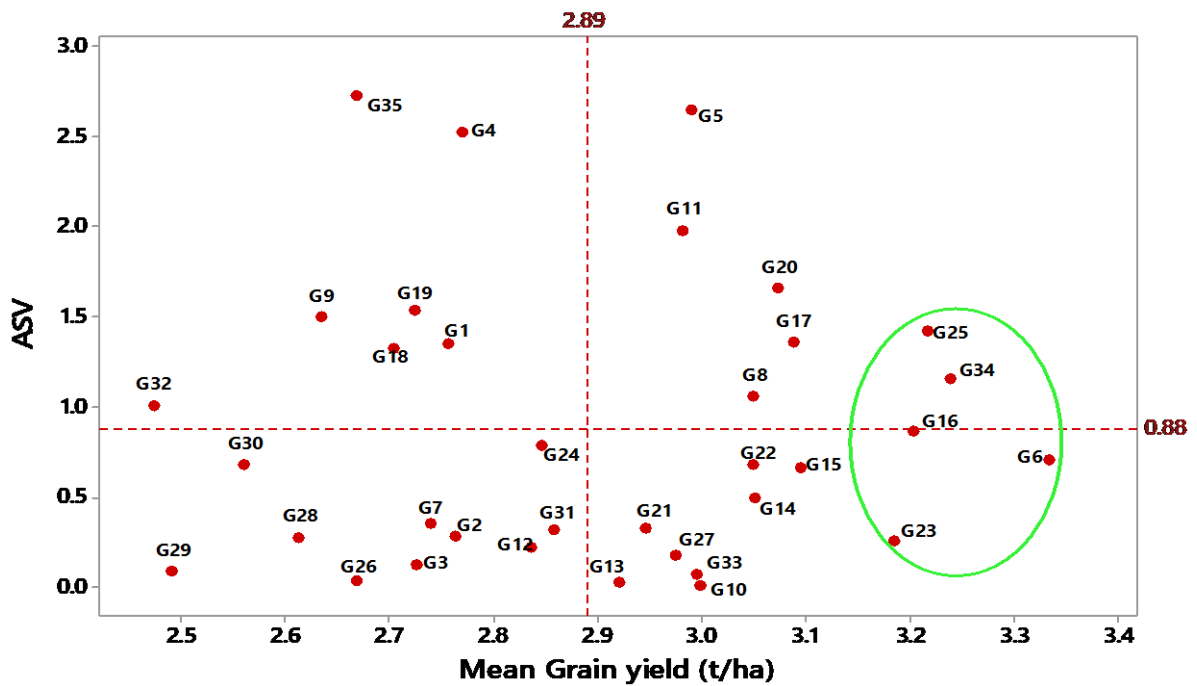
5.4.2.2 AMMI stability value (ASV)

Dukem (G₁₀) had the lowest ASV (0.03) and moderately high grain yield (3.0 t/ha) whereas G₆ had the highest yield (3.33t/ha) with relatively lower ASV (0.71) followed by Kora (G₃₄) having grain yield of 3.27 t/ha and ASV=1.16 (Table 34, Fig. 25a). Hence, when considering yield, Melko, Kora and Qunchowhich had the highest mean yield and relative stability could be a variety of choice instead of G₁₀ or G₂₀ which were stable and moderately low yielding (Table 34, Fig 25a, b&c).

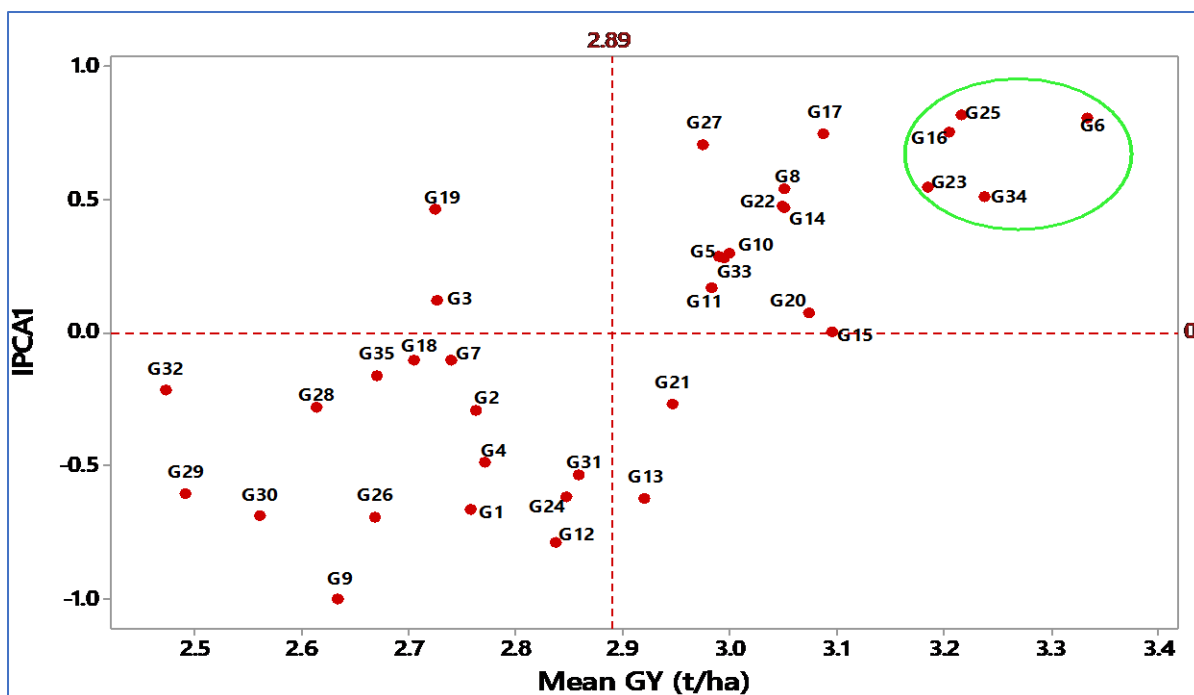
5.4.2.3 Analysis based on Eberhart and Russell Regression Model

In the present study the five high yielding genotypes Melko (G₆), followed by Kora (G₃₄), Quncho (G₂₅), Gola (G₁₆) and Gimbichu (G₂₃) had a regression coefficient (b_i) value of 1.27, 1.06, 1.09, 1.34 and 1.44, respectively. Among these, especially, Kora and Quncho are the second-highest yielding but with regression coefficient closer to unity followed by Melko which was identified to be high yielding and more stable genotypes in this study (Table 34, Fig. 26c).

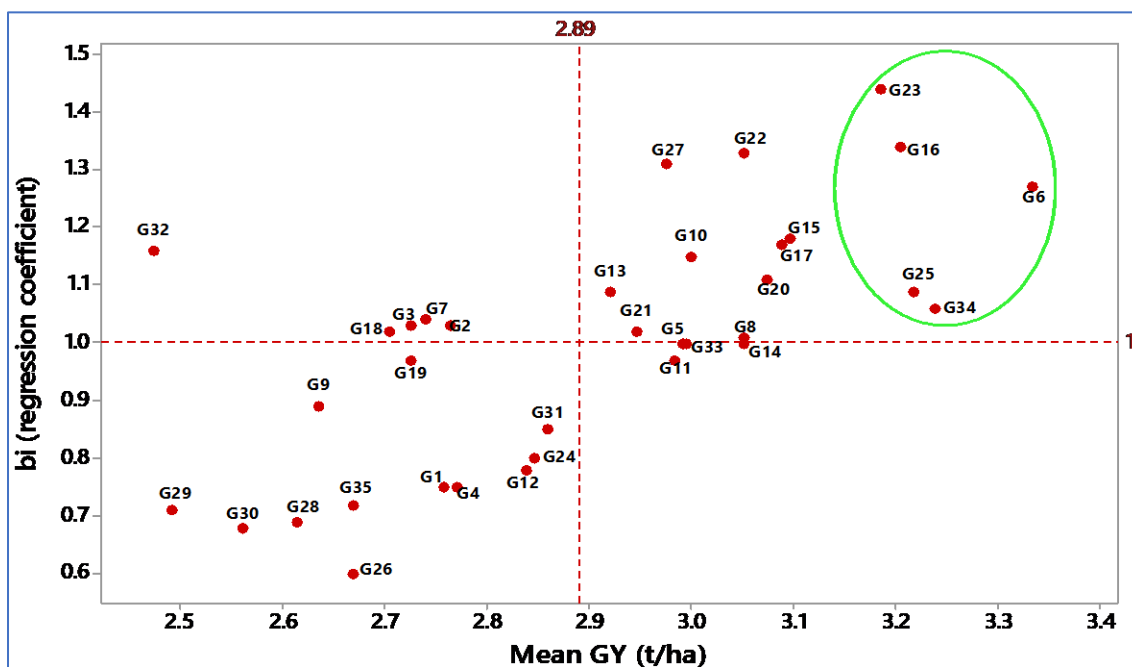
Ingeneral, Melko, Kora, Quncho, Gola and Gimbich which had a mean grain yield of 3.33, 3.27, 3.22, 3.20 and 3.18 t/ha, with ASV value of 0.71, 1.16, 1.42, 0.26 and 0.26), IPCA value of 0.46, 0.58, 0.67, 0.31 and 0.31 as well as a regression coefficient (bi) value of 1.27, 1.06, 1.09, 1.34 and 1.44, respectively were identified as the highest yielding and adaptable genotypes (Fig. 25a, b & c).



a) Genotypes mean grain yield (t/ha) versus AMMI stability value (ASV).



b) Scatter plot of IPCA vs Mean grain yield (t/ha)



c) Genotypes mean grain yield (t/ha) versus regression coefficient (bi).

Figure 25. Plot showing genotypes mean grain yield (t/ha) versus IPCA1 (a), versus ASV(b) and versus regression coefficient (c). (The reference line on the x-axis is the average grain yield (2.89 t/ha) whereas that on the y-axis is the IPCA1 value (IPCA1= 0), ASV is (ASV=0.88) and the regression coefficient (bi=1) indicating stability of genotype).

Table 34.Mean grain yield (t/ha) of tef varieties evaluated at nine environments in Ethiopia

Genotype	Environments									Mean	bi	S ² di	ASV	ICPA ₁	ICPA ₂
	E1	E2	E3	E4	E5	E6	E7	E8	E9						
G1	2.21	1.80	1.85	2.93	3.07	2.84	4.12	4.01	1.99	2.76	0.75	0.24	1.35	-0.70	-0.11
G2	2.14	1.50	1.61	2.33	3.86	3.15	4.35	3.81	2.13	2.76	1.03	0.05	0.29	0.22	-0.39
G3	2.33	1.56	1.43	2.37	4.47	3.54	3.49	3.51	1.84	2.73	1.03	0.06	0.13	0.15	0.26
G4	2.46	2.15	1.87	1.88	3.63	2.89	3.89	3.73	2.44	2.77	0.75	0.09	2.53	-0.95	-0.25
G5	2.68	1.91	1.77	2.15	4.74	3.82	3.73	3.68	2.43	2.99	1.00	0.08	2.65	-0.88	-0.73
G6	2.13	1.75	2.49	3.09	5.71	4.07	4.54	4.12	2.11	3.33	1.27	0.21	0.71	0.46	0.36
G7	2.30	1.33	1.57	1.74	4.29	3.24	4.06	3.43	2.71	2.74	1.04	0.11	0.36	-0.25	-0.43
G8	2.77	1.78	1.75	2.16	5.08	4.14	3.63	3.35	2.80	3.05	1.01	0.24	1.06	0.60	-0.25
G9	1.98	1.63	1.85	1.52	2.63	2.98	4.82	4.03	2.27	2.63	0.89	0.55	1.50	0.74	0.10
G10	2.22	1.76	1.50	3.05	4.84	3.49	4.10	4.00	2.05	3.00	1.15	0.08	0.01	0.06	-0.03
G11	2.18	1.96	1.94	2.30	4.66	3.53	4.02	3.58	2.68	2.98	0.97	0.05	1.98	0.80	0.47
G12	2.16	2.24	1.98	2.63	2.87	2.97	4.64	4.21	1.84	2.84	0.78	0.41	0.23	-0.27	-0.20
G13	2.07	1.82	1.84	2.47	3.31	3.06	5.17	4.72	1.82	2.92	1.09	0.50	0.03	-0.09	-0.10
G14	1.93	2.14	1.98	2.36	5.00	4.13	3.90	3.28	2.74	3.05	1.00	0.22	0.50	-0.41	-0.22
G15	1.92	1.79	1.83	2.68	4.39	3.44	4.95	4.32	2.52	3.10	1.18	0.11	0.67	0.36	-0.55
G16	2.59	1.76	1.86	2.12	5.60	4.31	4.44	3.88	2.27	3.20	1.34	0.13	0.87	0.31	-0.78
G17	1.88	1.94	1.84	2.47	5.54	4.24	4.00	3.29	2.61	3.09	1.17	0.30	1.36	-0.68	0.30
G18	2.17	1.60	1.39	2.31	4.20	3.26	3.41	4.03	1.99	2.70	1.02	0.06	1.33	0.69	-0.14
G19	2.63	1.36	1.53	2.22	5.03	3.76	2.63	3.22	2.15	2.73	0.97	0.43	1.54	-0.53	0.88
G20	2.30	1.75	1.69	2.50	4.49	3.50	4.34	4.38	2.72	3.07	1.11	0.04	1.66	0.76	-0.29
G21	2.49	1.82	1.74	2.32	3.82	3.38	4.57	4.19	2.19	2.95	1.02	0.10	0.33	-0.01	0.57
G22	2.59	1.55	1.46	2.51	5.06	3.98	4.66	3.75	1.90	3.05	1.33	0.07	0.68	-0.28	0.68
G23	2.54	1.80	1.66	2.36	5.36	3.95	4.56	4.71	1.71	3.18	1.44	0.08	0.26	0.31	-0.03
G24	2.19	2.15	2.03	1.83	3.37	3.01	4.35	4.07	2.63	2.85	0.80	0.23	0.79	-0.48	0.40
G25	2.89	2.17	1.78	3.06	5.49	4.23	3.82	3.58	1.94	3.22	1.09	0.30	1.42	-0.67	-0.44
G26	2.19	1.91	1.77	1.91	3.15	2.86	3.60	3.50	3.14	2.67	0.60	0.18	0.04	0.12	-0.06
G27	2.15	1.36	1.38	2.71	5.39	4.20	3.71	3.76	2.13	2.98	1.31	0.20	0.18	0.26	0.06
G28	1.71	1.71	1.70	2.66	3.74	3.12	3.02	3.30	2.57	2.61	0.69	0.11	0.28	0.28	-0.26
G29	2.40	1.92	1.51	1.37	3.42	2.86	3.35	3.28	2.31	2.49	0.71	0.12	0.09	0.06	0.28
G30	2.23	1.78	1.81	2.03	3.10	2.92	3.56	3.52	2.11	2.56	0.68	0.05	0.68	-0.43	0.43
G31	2.80	2.10	1.57	2.98	3.30	2.92	4.39	4.20	1.48	2.86	0.85	0.39	0.32	0.30	0.27
G32	1.60	1.28	1.44	1.38	4.19	3.25	3.84	3.41	1.88	2.47	1.16	0.06	1.01	0.51	-0.55
G33	2.53	1.60	1.65	2.92	4.74	3.45	4.07	3.43	2.27	2.96	1.00	0.10	0.08	0.05	0.28
G34	2.92	1.93	1.64	3.42	4.88	3.99	4.35	3.76	2.57	3.27	1.06	0.15	1.16	0.58	0.49
G35	2.57	1.84	1.71	2.67	3.88	3.22	3.24	3.13	1.77	2.67	0.72	0.08	2.73	-1.00	-0.03
Mean	2.31	1.78	1.70	2.38	4.29	3.48	4.04	3.78	2.25	2.89	1.00	0.18	0.88	0.00	0.00

Key: G1 to G35 name of genotypes; E1= Adet, E2=Alem Tena (2015), E3= Alem Tena (2016), E4= Axum, E5= Debre Zeit (2015), E6= Debre Zeit (2016), E7=Holetta (2015), E8= Holetta (2016), E9= Shambu. IPCA = Interaction Principal Component Axis, bi = Regression coefficient, s²di = Squared deviation from regression, ASV = AMMI Stability Value.

5.4.3 Analysis of GGE biplot

GGE biplot is visualized on the basis of results explained for the first two principal components (Yan, 2001). Thus, in the present study, the first two principal components of GGE biplot explained 72.8 % (PC₁=49.8 and PC₂=23.0%) of the total variations in GEI (Fig. 27). Smaller values of PC₂ for genotypes indicate high stability of genotypes while smaller values of PC₂ for environment indicate more representativeness of the overall environment. In this study, therefore, higher PC₁ (49.8%) and lower PC₂ (23.0%) values were obtained for G₆, G₁₆, G₂₅, G₃₄ and G₂₃ indicating high mean yield for the studied genotypes. Similarly, E₄ and E₅ had also high PC₁ and lower PC₂ values and hence they are representative of the test environments.

5.4.3.1 Genotype performance and mega environment determination

GGE biplot enables to classify the study genotypes and environments based on the performance of genotypes and the response of the growing environments. In the present study, a polygon view was created by connecting the vertex genotypes (G₁₉, G₆, G₁₃, G₉ and G₂₉) which were found furthest away from the origin. These genotypes were the highest yielding in their respective environments or sector. The lines emanating from the origin which are perpendicular to the line connecting the furthest genotypes divided the test environments and genotypes into different groups. Based on this grouping, the nine test environments were grouped into four mega environments whereas, the 35 test genotypes were grouped into seven genotypic groups (Fig. 26). Thus, G₆ was the highest yielding genotype in the environment where E₁, E₄ and E₆ exist. Similarly, G₁₃ was the highest yielding genotype in the environment where E₇ and E₈ exist while, G₁₉ was the highest yielding at E₉. E₅ was identified to be the fourth mega environment though it had no vertex genotype. The other vertex genotypes (G₉ and G₂₉) had no corresponding environment and

hence have the lowest mean grain yield across environments. On the other hand, the sector consisting G_{17} , G_{33} and G_{27} had no vertex genotype and corresponding environment. Nevertheless, the mean yields of these genotypes were substantially higher than the grand mean and were among the top yielding genotypes in their neighboring environments (Fig. 26).

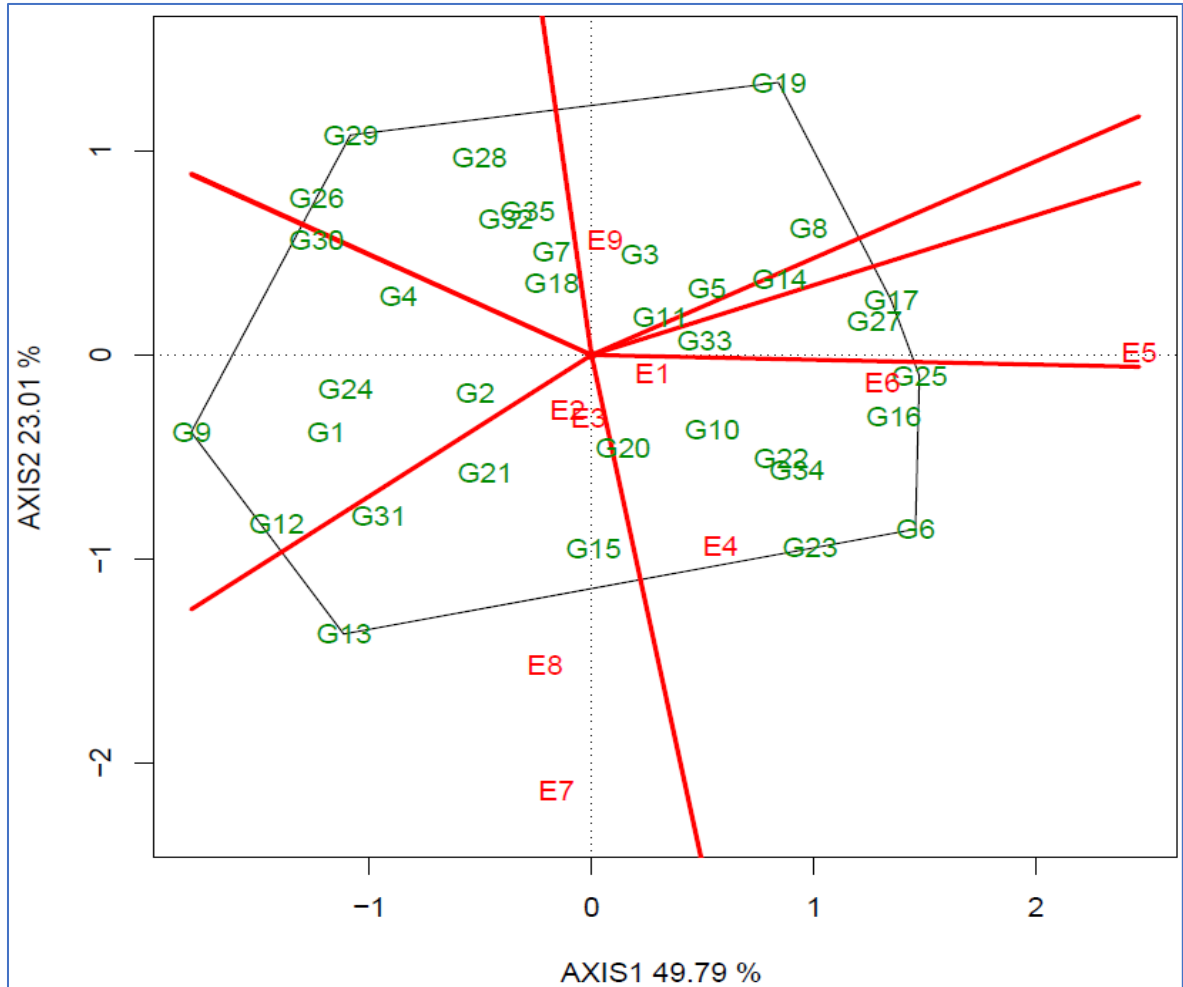


Figure 26. Polygon view of the GGE biplot showing “which-won-where”

5.4.3.2 Relationship among environments

In the present study, the relationships among the nine test environments is presented in Fig. 27. The smallest angle was observed between E_5 & E_6 followed by those between E_7 &

E₈ indicating the existence of high correlation between them. This shows that genotypes performing best at E₅ can repeat the same performance at E₆, and those performing best at E₇ can also perform similarly at E₈ and vice versa. The angles between E₇ and E₆, and E₈ and E₁, on the other hand, were closer to 90° showing that they have no correlation or indicating that each environment has independent genotypic performance. Furthermore, the angle between E₉ and that of E₇ and E₈ is greater than 90° showing that they have negative correlations. Hence, genotypes performing best at E₉ does not repeat the same performance at E₇ & E₈ and vice versa. The nine test environments in the present study were grouped into three based on the relationship among environments. Thus, group-I consisted of E₁, E₄, E₅ and E₆; group two consisted of E₂, E₃, E₇ and E₈ whereas the third group consisted of E₉ only. The grouping based on relationship among environments is in line with the environmental grouping of the polygon view. In both cases, however, the grouping of high potential environments (E₇ & E₈) along with the moisture deficit environments (E₂ & E₃) is unexpected.

Figure 27, furthermore, presented the variation in vector length of the different environments. E₅, E₇ and E₆ had the longest vector length and, therefore, are the most discriminating environments in the present study. Similarly, E₁, E₂ and E₃ had the shortest vector length which indicates that they are the least discriminating of all the test environments.

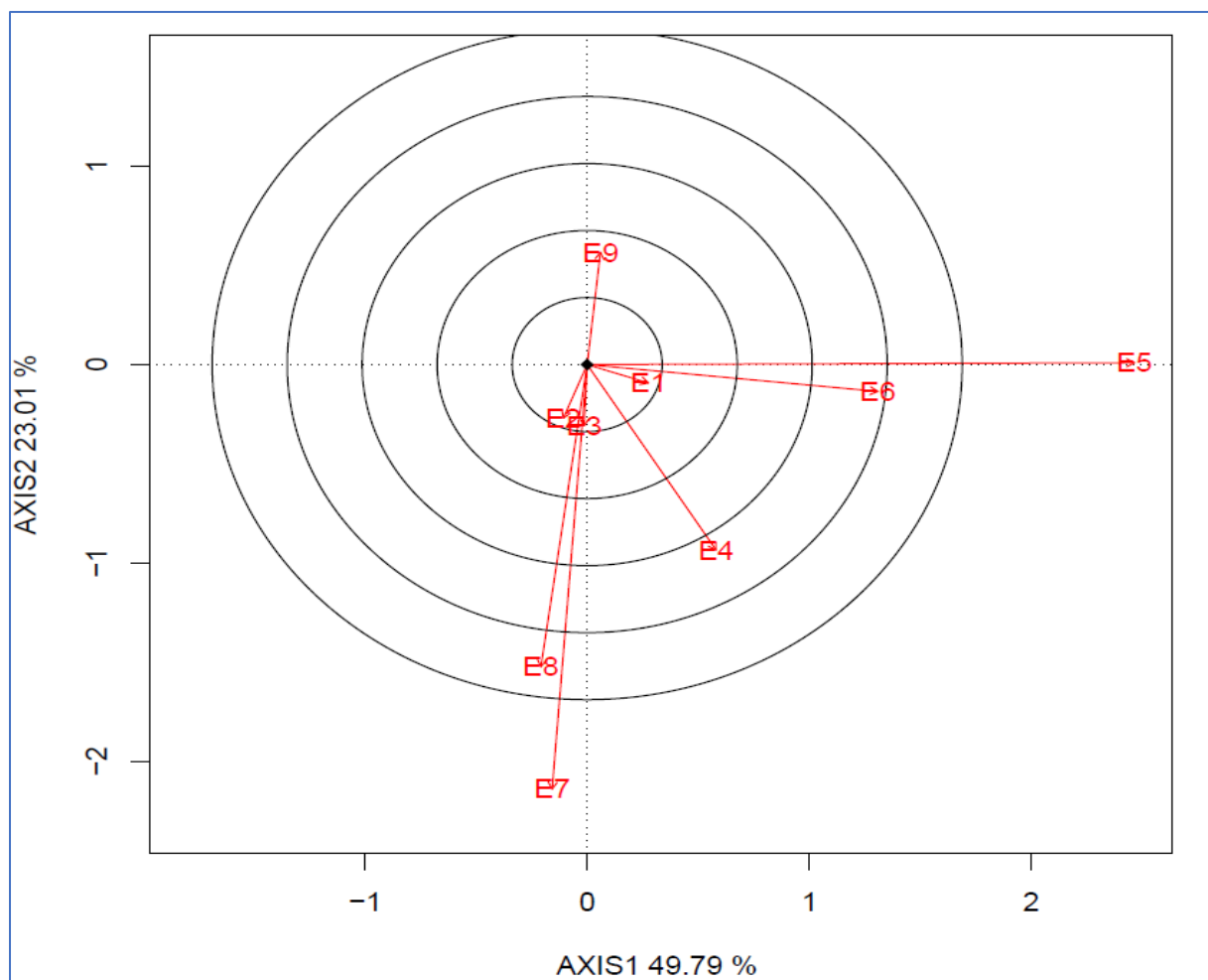


Figure 27. GGE biplot showing the relationship among test environments

5.4.3.3 Discriminativeness vs representativeness

Figure 28 also illustrated ranking based on both discriminating ability and representativeness of the test environments. It is known that the GGE biplot is also useful to assess how much a test environment is capable of generating unique information about the differences among genotypes and how representative the mega-environment is. In this study, E₅, E₆ and E₄ were closest to the small circle on the average environmental axis and, therefore, are the most discriminating environments whereas E₉ is the poorest for selecting cultivars adapted to the whole region. On the other hand, E₄ and E₁ which had the smallest

angle with average environmental axis were identified to be the most representative environment whereas, E_9 having the largest angle is the least representative of all studied environments (Fig.28).

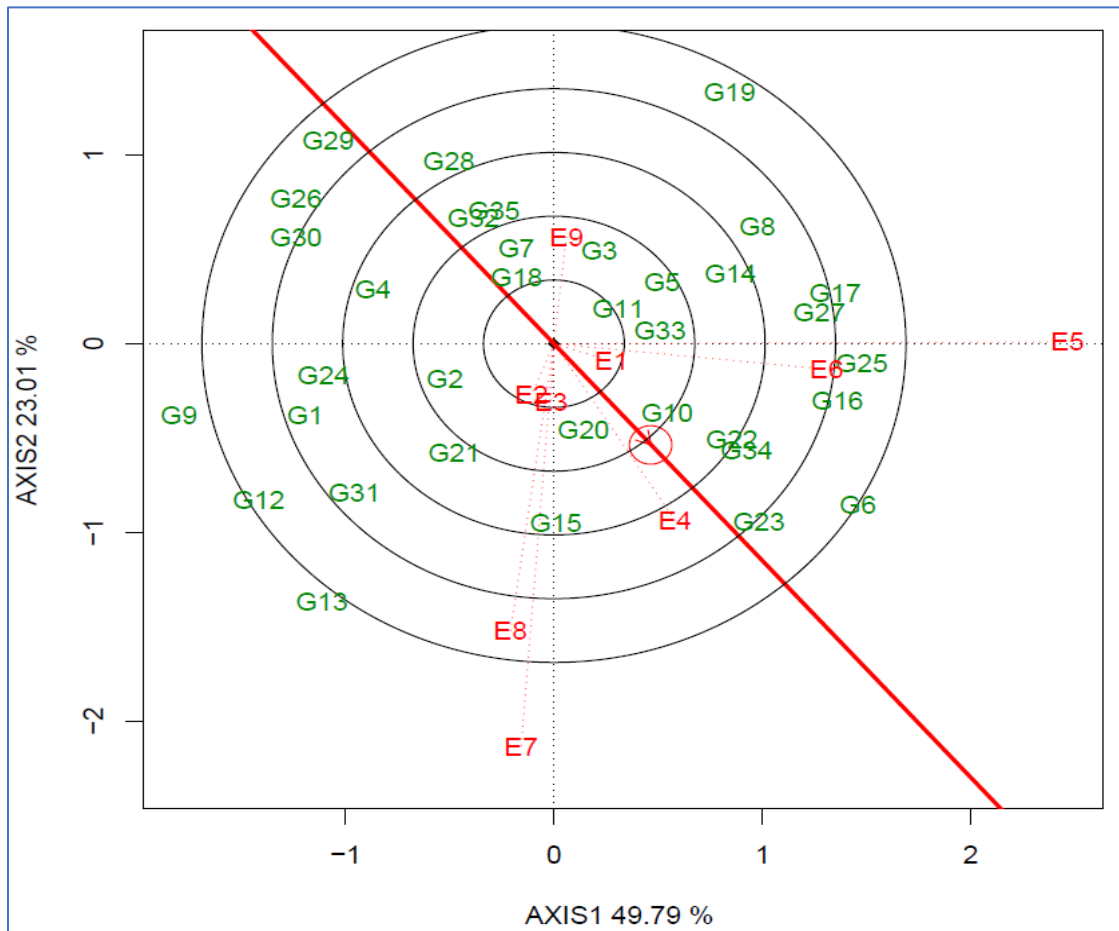


Figure 28. Discriminativeness vs Representativeness

5.4.3.4 Genotypes mean yield and their stability

Based on Fig. 30, G_6 , G_{34} , G_{22} and G_{10} had the shortest vector length from the average environmental coordination (AEC) and, therefore, are identified to be the most stable. G_{13} , G_{19} , G_{12} and G_9 , which had the longest vector length from the AEC, however, were the most unstable ones. G_6 followed by G_{23} , G_{34} , G_{16} and G_{25} exhibited were identified to have the

highest mean yield whereas G₂₉, G₂₆, G₃₀ and G₂₈ performed far below the mean in all environments (Fig. 29). An ideal genotype for a specific environment has the highest mean yield and responds best to the particular environment while it is less stable in the other environments. Thus, the vertex genotypes which have the highest mean yield in their respective sectors need to be recommended for their specific sector.

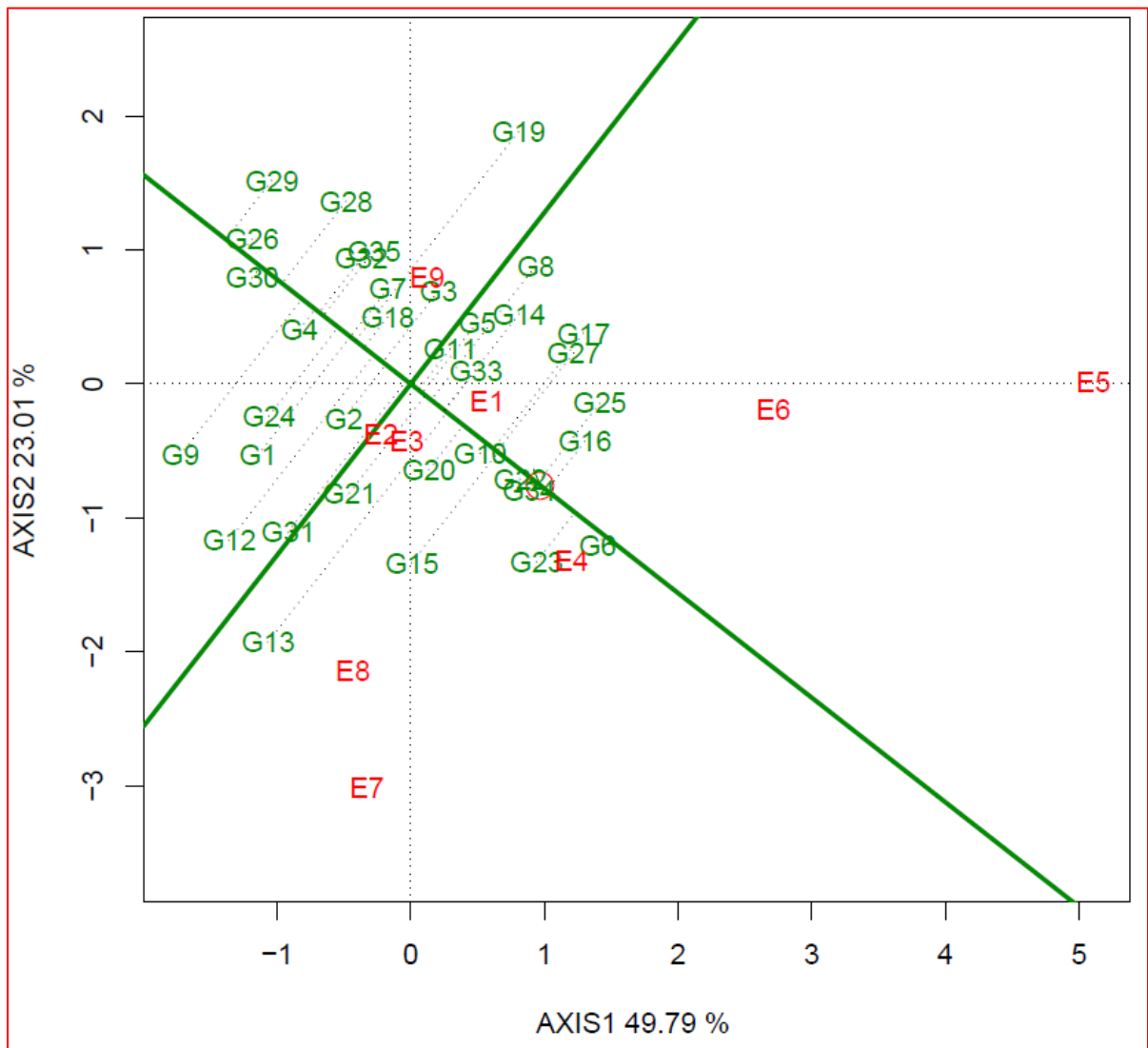


Figure 29. Ranking biplot based on mean yield performance and stability of the genotypes.

5.4.3.5 Ranking genotypes based on ideal environment

In this study, E₅ (Debre Zeit-1) was the highest yielding environment to evaluate and rank the performance of different genotypes relative to it (Fig.30). Those genotypes appearing on the right side of the perpendicular line with E₅ performed above the mean unlike those in the opposite side of E₅. Thus, G₂₅, G₂₇, G₁₆, G₁₇, G₆, G₂₂, G₃₄, G₂₃ appeared on the same side with E₅ had above average performance whereas G₉, G₁₂, G₂₉, G₂₆, G₃₀, etc. which appeared on the opposite side of the perpendicular line to E₅ performed below average at E₅. Hence, those genotypes performing above the grand mean and occurred on the right side of the perpendicular are suitable genotypes for E₅ whereas the remaining ones are not.

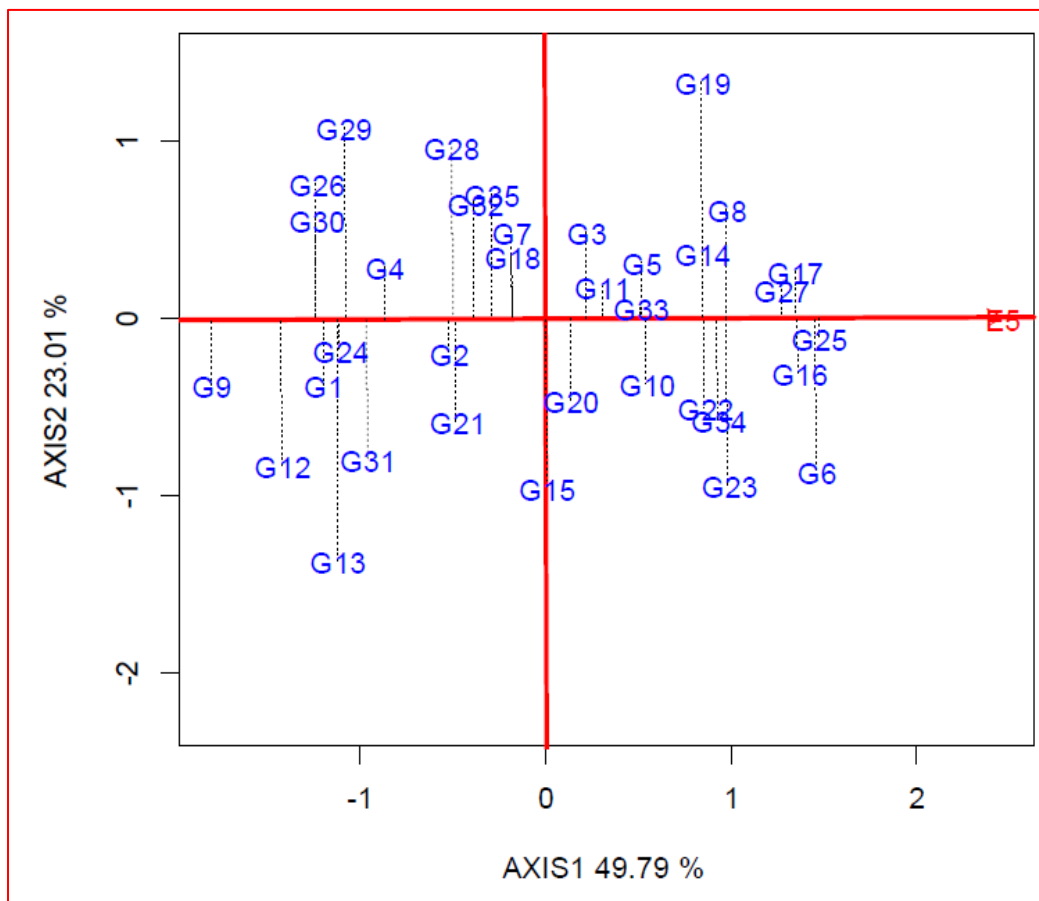


Figure 30. Ranking the performance of different genotypes relative to the best environment (E₅).

5.4.3.6 Ranking genotypes relative to the ideal genotypes

Figure 31 shows the ranking of genotypes based on the performance of an ideal genotypes in the present study. The length of environmental projections appeared onto this genotype axis indicate the performance of the best genotype at different environments relative to the other genotypes. As Fig 31 clearly shows, E₅ followed by E₇, E₆ and E₈ had the longest projection from the genotype axis (G₆ axis). Hence, E₅, E₇, E₆ and E₈ are the environment where G₆ performed the best compared to the remaining environments.

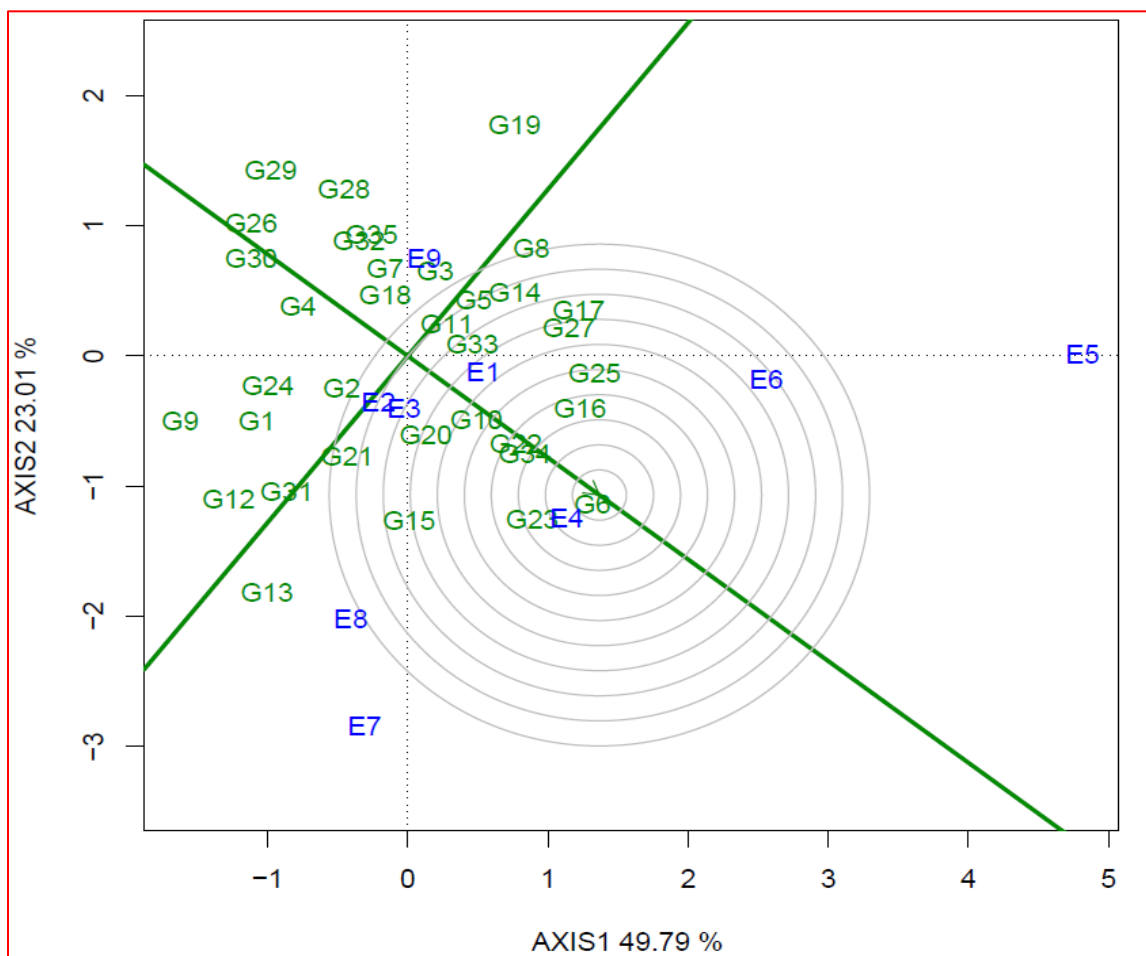


Figure 31. Ranking Environments based on the performance of an ideal Genotype ('G6')

5.4.4 Association of traits

Table 35 presents the association among major traits of improved tef varieties evaluated across nine environments. Grain yield showed significant ($P < 0.01$) association with the second culm diameter, shoot biomass and lodging index while, its association with the all remaining traits were not significant at both phenotypic and genotypic level. Shoot biomass exhibited positive and significant ($P < 0.01$) genotypic association with all traits except peduncle length and culm diameter. The same holds true with all traits except for fertile tillers, peduncle length and culm diameter. Lodging index, on the other hand, had negative and significant ($P < 0.01$) association with all traits other than fertile tillers and grain yield at both genotypic and phenotypic levels. Thus, its association with number of fertile tillers was significant ($P < 0.01$) and positive at both genotypic and phenotypic levels while it had no association with grain yield at both levels. The association of number of fertile tillers was significant and negative at phenotypic level with all traits other than grain yield and lodging index. It also showed the same association with all traits other than shoot biomass, grain yield and lodging index at genotypic level (Table 35).

Table 35. Genotypic (lower diagonal) and Phenotypic (upper diagonal) association of major quantitative traits in 35 tef varieties evaluated at nine environments

Traits	DH	DM	PH	PL	Pdl	SCL	CD	FT	SBM	GY	LG
DH	1	0.74**	0.89**	0.84**	0.42**	0.77**	0.75**	-0.59**	0.45**	0.11ns	-0.85*
DM	0.79**	1	0.72**	0.83**	0.60**	0.68**	0.56**	-0.52**	0.33*	0.01ns	-0.72**
PH	0.96**	0.81**	1	0.89**	0.57**	0.89**	0.77**	-0.74**	0.52**	0.18ns	-0.87**
PL	0.92**	0.92**	0.91**	1	0.50**	0.80**	0.63**	-0.54**	0.52**	0.17ns	-0.72**
Pdl	0.48**	0.70**	0.61**	0.57**	1	0.49**	0.51**	-0.61**	0.16ns	0.03ns	-0.56**
SCL	0.88**	0.81**	0.95**	0.90**	0.59**	1	0.71**	-0.66**	0.58**	0.37*	-0.81**
CD	0.88**	0.68**	0.90**	0.73**	0.65**	0.8**	1	-0.67**	0.22ns	0.05ns	-0.79**
FT	-0.74**	-0.68**	-0.95**	-0.71**	-0.84**	-0.88	-0.98**	1	-0.28ns	-0.01ns	0.70*
SBM	0.58**	0.42**	0.60**	0.62**	0.18ns	0.65**	0.31ns	-0.45**	1	0.76**	-0.43**
GY	0.15ns	0.01ns	0.13ns	0.17ns	-0.01ns	0.37*	0.04ns	-0.02ns	0.79**	1	-0.14ns
LG	-0.95**	-0.82**	-1.00**	-0.82**	-0.71**	-0.99**	-0.98**	0.94**	-0.57**	-0.25ns	1

Abbreviations: DH: days to heading; DM: days to maturity; PH: plant height; PL: panicle length; Pdl: peduncle length; SCL: second culm diameter; FT: number of fertile tillers; SPK: number of spikelet; SBM: shoot biomass; GY: grain yield; TKW: thousand kernel weight; LG: lodging index.

5.4.4.1 Cluster Analysis

Cluster analysis based on data from the combined means across nine environments for 12 major quantitative traits grouped the 35 tef varieties into seven distinct classes (Fig.32). The number of varieties in each cluster ranged from one variety in cluster-VII to 12 varieties in cluster-I. Cluster-II consisted of nine varieties whereas cluster-III, IV & VI each consisted of three varieties. Cluster-V, on the other hand, consisted of four varieties including two brown seeded and one extra white seeded variety released for the moisture stress areas. Cluster-IV, consisted of three high yielding genotypes (Dukem, Quncho and Kora) which were released for the high potential areas whereas, cluster-VI consisted of three varieties released for the moisture stress areas of the country. Cluster-I, II and III consisted of a mix of varieties with various agro- ecological adaptation, seed color, maturity groups. Analysis of distance between clusters revealed that the best recombination is expected between C₅ & C₆ followed by C₄ & C₅, C₆ & C₇, and C₄ & C₇ which had the highest inter cluster distance respectively (Table 36).

The traits mean for the seven clusters in the present study are shown in Table 37. Cluster I consisted of 12 tef varieties that have the longest days to maturity and peduncle length. Cluster II and V, however, had moderate values for all the traits evaluated. Cluster-VI, on the other hand, were found to have the highest values for about 70% of the studied traits with the smallest value for lodging percent whereas cluster-VII was solitary and that include a genotype with the highest number of fertile tillers, 1000-kernel weight and lodging index along with the shortest length of peduncle and second culm and the thinnest second culm diameter. The genotype in cluster VI had the shortest days to heading and maturity, plant height, panicle length as well as the smallest values for shoot biomass and

grain yield. Cluster VI composed of three varieties with the shortest days to heading and maturity that were originally released for moisture deficit environments. In general, the observed clustering pattern among the studied tef varieties is in line with their distribution in a biplot graph (Fig. 33).

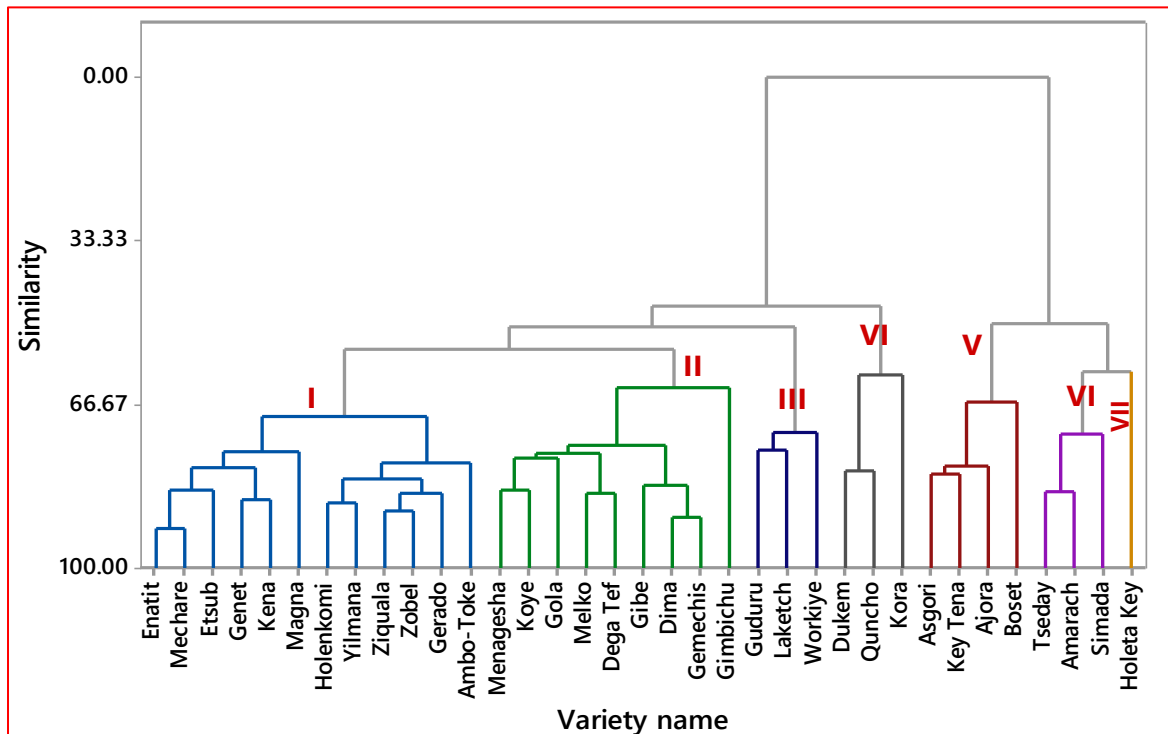


Figure 32. Dendrogram based on complete linkage Euclidean distance showing the clustering pattern of 35 tef varieties evaluated for major quantitative traits at nine environments in Ethiopia.

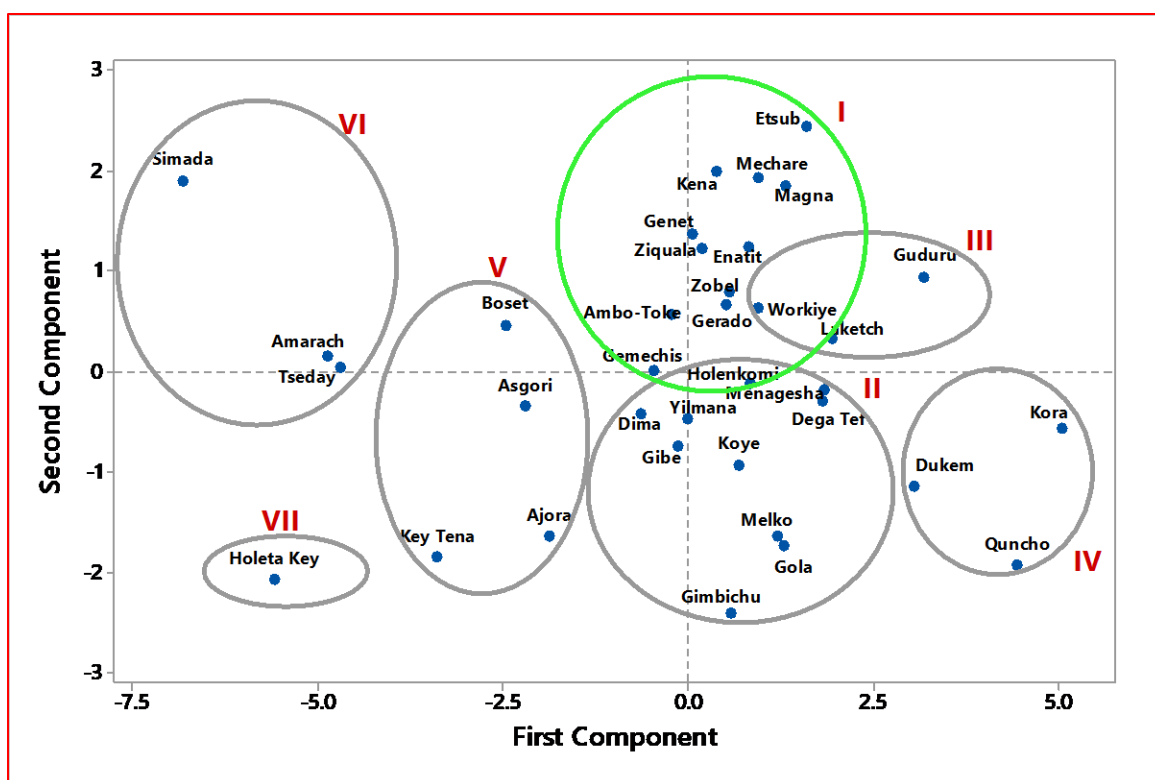


Figure 33. A biplot graph showing the distribution of the studied tef varieties using the first two principal components

Table 36. Distance between Cluster Centroid

	Cluster1	Cluster2	Cluster3	Cluster4	Cluster5	Cluster6	Cluster7
Cluster1	0						
Cluster2	3.76	0					
Cluster3	2.31	3.30	0				
Cluster4	6.22	3.51	6.5	0			
Cluster5	4.51	6.72	3.8	9.88	0		
Cluster6	7.21	3.96	6.9	3.98	10.06	0	
Cluster7	2.67	5.16	3.3	7.76	3.49	8.47	0

Table 37. Means for 12 different traits of 35 released tef varieties grouped into seven clusters

Traits	Cluster means						
	C1	C2	C3	C4	C5	C6	C7
Days to panicle emergence	52.71	51.58	54.35	56.30	49.40	46.09	49.44
Days to maturity	113.28	111.98	112.15	112.98	106.35	103.04	104.26
Plant height (cm)	94.06	93.67	99.02	102.24	88.73	81.62	82.39
Panicle length (cm)	38.82	38.34	39.44	41.28	35.36	30.57	33.93
Peduncle length (cm)	18.89	18.90	18.07	19.20	17.61	17.49	15.27
Second culm length (cm)	12.44	12.69	12.81	14.11	11.81	10.97	10.53
Second culm diameter (mm)	1.90	1.88	1.99	2.03	1.75	1.72	1.60
Fertile tillers (number)	4.36	4.29	3.66	3.85	4.77	5.10	5.76
Shoot biomass yield (t/ha)	11.66	13.04	12.45	13.87	12.23	10.73	12.55
Grain yield (t/ha)	2.72	3.10	2.73	3.16	2.98	2.69	2.98
1000- kernel weight (mg)	28.48	28.62	27.40	28.22	28.61	28.53	28.92
Lodging index (%)	70.00	71.41	66.53	62.24	77.10	82.78	86.26

5.4.5 Principal component analysis

The first three principal components (PCs) with eigenvalue greater than one contributed for 82.9% of the entire variation among 35 tef varieties evaluated at nine environments in Ethiopia (Table 38; Fig. 34). PC₁ accounted for 58.3 % of the variation among the test varieties mainly due to variation in all traits other than grain yield and 1000-kernel weight which contributed for less than 10%. PC₂, on the other hand, accounted for 14.2% of the total variations among the tested varieties mainly due to the largest contribution from shoot biomass and grain yield. PC₃ contributed for 10.4% of the total variation mainly due to 1000-kernel weight, peduncle length and grain yield.

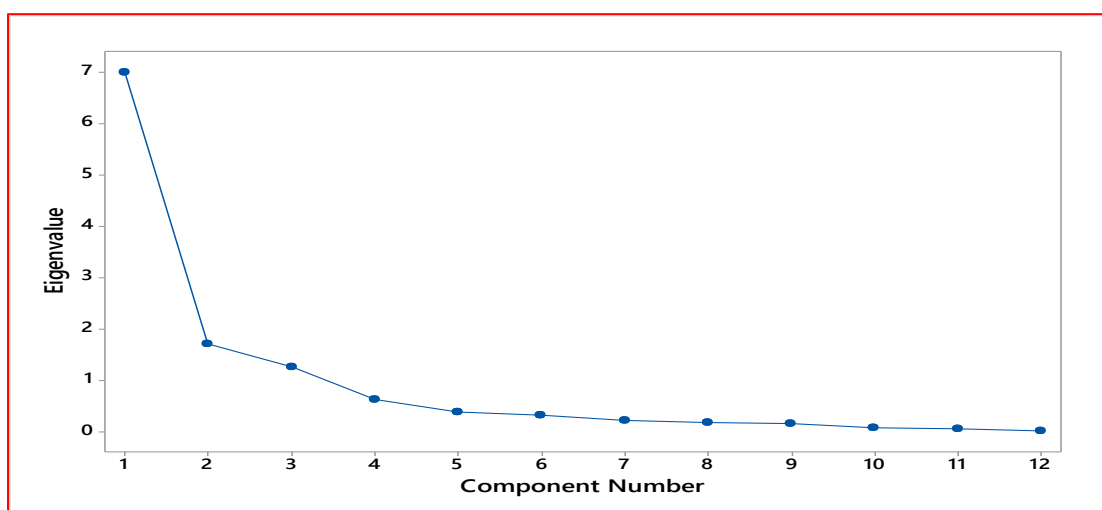


Figure 34. Scree plot showing the Eigen value for each component number

Table 38. Eigenvectors and values of the first three principal components for 35 released tef test varieties evaluated at nine environments in Ethiopia

Variable	PC1	PC2	PC3
Days to panicle emergence	0.344	0.033	0.171
Days to maturity	0.316	0.112	0.023
Plant height (cm)	0.364	0.008	0.077
Panicle length (cm)	0.343	-0.046	0.119
Peduncle length (cm)	0.258	0.201	-0.439
Second culm length	0.346	-0.103	-0.037
Second culm diameter (mm)	0.314	0.207	-0.057
Fertile tillers (number)	-0.265	-0.209	0.044
Shoot biomass yield (t/ha)	0.208	-0.609	-0.004
Grain yield (t/ha)	0.094	-0.669	-0.327
100- kernel weight (mg)	-0.071	0.147	-0.802
Lodging index (%)	-0.347	-0.085	0.012
Eigenvalue	6.99	1.706	1.254
Proportion	0.583	0.142	0.104
Cumulative	0.583	0.725	0.829

5.5 *Discussion*

5.5.1 **Analysis of variance**

The highly significant ($P < 0.01$) variation detected among genotypes, environments and genotype by environment interaction for almost all traits in the current study is in line with the previous reports in tef (Kefyalew *et al.*, 2000). The existence of significant variation among testing environments might be attributed due to variation in climatic element and soil condition across the testing environments. Such significant genotype by environment interaction complicates selection and recommendation of stable genotype across environments (Hundera *et al.*, 2000a; Kefyalew *et al.* 2000, 2001).

5.5.2 **AMMI Analysis**

AMMI analysis which revealed the existence of significant mean grain yield variation among genotype, environment and GE interaction is in line with the previous works (Kefyalew, 1999; Kefyalew *et al.*, 2000, 2001). Besides, the variation explained by the environment was very high and it was about four times what was explained by the genotype and the genotype plus genotype by environment interactions. Similar results were also reported in the previous findings (Gauch and Zobel, 1997; Tolessa *et al.*, 2013). PC₁ and PC₂, respectively explained 53.0% and 19.4% of the variation for GEI. Kefyalew *et al.* (2001) also reported the PC₁ value of 52.1% while Lule (2015) obtained the PC₁ value of 66.1%. However, according to Crossa *et al.* (1990), 93% of the variance of GxE interaction was from the first IPCA alone. The use of the first two PCs which contributed for more than 70% of the G x E interaction in this study is in line with several previous works in tef (Kefyalew *et al.*, 2000; 2001), Faba beans (Tolessa *et al.*, 2013) and Finger Millet (Lule, 2015).

AMMI Stability Value (ASV) was suggested to aid selection of relatively stable & high yielding genotypes in many crops whereby an ideal genotype has high mean yield and small ASV value (Lule, 2015). According to Eberhart and Russell (1966) model, however, the best yielding genotype is expected to have high mean yield and a regression coefficient (bi) value of unity. As Eberhart and Russell (1966) suggested high yielding genotype having squared deviation from regression (S^2di) different from zero should be considered as stable and adaptable ones. Hence, in this study, consideration of Melko which had bi value of 1.27 and S^2di value of 0.21 is justifiable.

5.5.3 Analysis of GGE Biplot

One of the most important features of a GGE biplot is its ability to show the which-won-where pattern of a genotype by environment dataset (Yan and Tinker, 2005; Farshadfar *et al.*, 2011). In a GGE biplot, the polygon view is created by connecting the markers of the furthest genotypes and thus all other genotypes are included in the polygon indicating that the best genotype(s) in each environment and groups of environments are represented at the vertex (Farshadfar *et al.*, 2011; Yan, 2002). In other words, the the vertex genotypes had the highest yield for the environments falling within that quadrant (Farshadfar *et al.*, 2011; Yan, 2002). In the present study, therefore, G6, G13, G9, G29 and G19 were the vertex genotypes having the highest mean yield in their respective environments.

The angle between the vectors of two environments has a meaningful relation with the correlation coefficient between them (Kroonenberg, 1995; Yan, 2002; Yan and Kang, 2003)

and such relationship is used to group the test environments. Thus, if two environments are positively correlated, the best yielding genotypes in one environment will perform best in the other environment. Contrary to this, if two environments are negatively correlated, the best yielding genotypes in one environment perform the least in the other environment and vice versa (Yan, 2002; Yan and Kang, 2003). In the present study, Debre Zeit-1, Debre Zeit-2, Adet and Axum which had an angle less than 90^0 are positively correlated with each other. Similarly, the angles between Axum, Holetta-1, Holetta-2, Alem Tena-1 and Alem Tena-2 were also less than 90^0 indicating that they have a positive correlation with each other. On the other hand, the angle between Axum and all environments other than Shambuis nearly 90^0 and hence these environments have no correlation with each other. The angle between Shambu & all environments other than Adet, Debre Zeit-1 & 2 had greater than 90^0 and hence they have negative correlations.

A vector length is the absolute distance between the marker of an environment and the plot origin (Yan, 2001; Yan *et al.*, 2002; Yan and Tinker, 2006) and it is used to measure the discriminating ability of an environment. Thus, the longer the vector length, the better the discriminating power of an environment. An ideal environment is a point on the average environmental axis (AEA) in the positive direction of the biplot origin and is equal to the longest vector of all environments (Yan and Tinker, 2006). It is represented by small circle with an arrow pointing to it and is the most discriminating of the genotypes across all other test environments. In the present study, therefore, E₅, E₆, E₇ and E₈ which had the longest vector length were identified to be the most discriminating of all test environments. On the other hand, the average environmental axis (AEA) is a line passing through the origin and

pointing to the positive direction with its distance equal to the longest vector. Thus, a test environment having a smaller angle with that of AEA is said to be more representative of other test environments (Yan and Tinker, 2006). In the present study, therefore, E₁ and E₄ which had the smallest angle with the average environmental axis were identified to be the most representative of all test environments.

The average environment coordination (AEC) is a line that passes through the origin and points to the higher mean yield across environments and shows the increase in rank of genotypes towards the positive end (Yan *et al.*, 2000). AEC was reported to be important to evaluate mean yield and stability of genotypes (Yan, 2001; Yan, 2002; Yan and Hunt, 2002) based on genotypes performance across environments. According to this evaluation, genotypes considered to be stable are those appeared closer to the origin with the shortest vector length from the AEC. Thus, in the present study, G33 which had minimal distance from the AEC was the most stable, but lower yielding genotypes whereas G6, G25 and G34 were the highest yielding but moderately stable genotypes (Table 27).

According to Farshadfar *et al.*, 2011), an ideal environment or the best environment is represented by an arrow pointing to it. According to the same author, an environmental axis is a line passing through the origin and connected to the best environment. A perpendicular to the environmental axis that passes through the biplot origin is then used to separate genotypes yielding above and below the mean in the ideal environment. Thus, E5 was identified to be the best environment and G6, G34, G25, G27, G16, G17, G23 and

G22 which appeared on the same direction with E5 were found to perform above average in this environment.

As suggested by Yan *et al.* (2000), the relative adaptation of the ideal genotype was evaluated by drawing a line passing through the biplot origin and the best genotype marker. A genotype axis is a line passing through the biplot origin and connected to the best genotype (Yan *et al.*, 2000). In the present study, G₆ which had the highest average yield was identified to be the ideal genotype to evaluate the performance of test environments relative to it. Hence, all environments other than E9 were found to be best for the performance of G6 (Melko). In general, according to Yan *et al.* (2001), ideal cultivars have large PC₁ scores (high mean yield) and small PC₂ scores (high stability) while ideal environments are expected to have more power of discriminating genotypes and more representative of the overall environments (Yan *et al.*, 2001; Yan and Rajcan, 2002). Thus, in the present study, genotypes such as G6, G25, G16, G23 and G34 which had larger PC₁ and smaller PC₂ scores were identified to be high yielding and stable.

The fact that no association was observed between grain yield and all traits other than shoot biomass and second culm diameter at both phenotypic and genotypic level is contrary to the positive and significant association previously reported for the same traits (Jifar *et al.*, 2015; Jifar *et al.*, 2017). However, the positive and significant association obtained between fertile tillers and lodging index in the present study is in line with the previous report (Jifar *et al.*, 2017).

5.5.4 Cluster Analysis

The result of cluster analysis in the present finding is in line with Jifar *et al* (2015) who reported the grouping of 36 brown tef genotypes into seven clusters whereas Plaza-Wüthrich *et al.* (2013) obtained six clusters based on 10 traits of 18 genotypes. The results of cluster analysis and that of the plotting of genotypes across the first and second PCs revealed the formation of seven distinct clusters. For instance, the shavy brown seeded, Holetta Key, variety was solitary and grouped alone in cluster seven whereas the early maturing Tsedey, Amarach and Simada were grouped together in cluster six. Surprisingly, the extra white seeded and high yielding genotypes (Kora and Quncho) were grouped in cluster four along with Dukem which is the female parents of Quncho.

5.5.5 Principal Component Analysis

The value obtained for the principal component analysis (82.9%) in the present study is in line with the earlier reports on the same crop ranging from 79 to 85% (Assefa *et al.*, 2003; Adinew *et al.* 2005; Plaza-Wüthrich *et al.*, 2013; Jifar *et al.* 2015). On the other hand, the contribution of PC₁ is substantially higher than some previous reports (Assefa *et al.*, 2003; Plaza *et al.*, 2013; Jifar *et al.*, 2015), but in line with Adinew *et al.* (2005). The contribution of PC₂ (14.2%) in the present study, however, is much lower than that of earlier report (Jifar *et al.*, 2015).

Chapter 6

6. Variability and Association of Food-feed traits in Tef [*Eragrostis tef* (Zucc.) Trotter] varieties

Abstract

The importance of tef as food and feed, the critical decline in grazing land, and the fact that farmers have started to worry about crop residues and its grain require to identify tef genotypes with better grain and straw yield and quality. Thus, field and laboratory studies were conducted to assess the variability and association of food-feed traits in 36 tef genotypes evaluated at two locations for two years in RCBD with three replications. Grain yield (GY), total biomass (TBM) and straw yield (STY) were recorded from the field experiment while dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), metabolic energy (ME), in vitro organic matter digestibility (IVOMD) contents were determined through lab analysis. Combined ANOVA revealed that the food-feed traits of improved tef varieties were significantly ($P<0.01$) affected by the genotypes, year, locations and their interactions. The highest mean value of CP, IVOMD, ME, TBM and STY and the lowest NDF, ADF and ADL was obtained at Debre Zeit while GY was not significantly affected due to locations. PCA-biplot of the studied traits revealed the formation of three groups of traits whereby group-I, II and III consisted of food-feed yield traits, traits affecting feed intake negatively and traits affecting feed intake positively. Thus, traits in group-III (CP, IVOMD and ME) showed significant and positive association among themselves but had negative and significant ($P<0.05$) relationship with the remaining traits. Similarly, TBM, GY and STY showed positive and significant correlation with each other and with traits in group-II whereas their associations with the remaining traits were negative and significant ($P<0.01$). The PCA-biplot, furthermore, grouped the genotypes into four categories: i) genotypes with high feed quality traits, ii) genotypes with high food-feed yield traits, iii) genotypes with better yield and quality (dual purpose), and iv) genotypes having less contribution for food-feed traits. Hence, Dukem, Gimbichu, Dega Tef, Gola, and Qunchovarieties represent genotypes with high yield and are recommended for farmers and breeders for high yield of food-feed. Kena, Genet, Ambo Toke-Zobel, and Ziquilavarieties represent genotypes with high feed quality traits. Breeders or farmers interested in dual-purpose genotypes, on the other hand, should consider genotypes like Melko, Koye, Key Tena, and Gerado which were identified to have reasonable yield and quality traits.

Key words: *Eragrostis tef*; feed quality; Food-feed traits; tef; straw quality; straw yield

6.1 ***Introduction***

Tef [*Eragrostis tef* (Zucc.) Trotter] is a major cereal crop domesticated and widely grown in Ethiopia. It is a gluten free (Spaenij-Dekking *et al.*, 2005), nutritious human food (Ketema, 1993; Assefa *et al.*, 2011) and livestock feed (Bediye and Fekadu, 2001; Yami, 2013). It is annually grown by seven million households in Ethiopia covering over three million hectares of land and providing five million tons of grain (CSA, 2017). Though tef has wider adaptations, its major production is in the mid and highland areas having the largest human and livestock population.

Natural pastures, crop residues, agro-industrial by-products, and to some extent improved forage and pasture are the major sources of animal feeds in Ethiopia (Geleti *et al.*, 2013). The contribution of natural pasture is, however, declining continuously due to continued increase of crop farming into grazing lands to nourish the rising human population (Tolera *et al.*, 1999). Hence, feed shortage is becoming a serious constraint in Ethiopia. As livestock is the major components of the farmingsystem in Ethiopian, designing a comprehensive strategicaloption to solve such feed shortage problem is very essential. Proper use of crop residue is, therefore, among theoptions to be implemented. Tef straw provides nearly half of the total annual cereal straws in the central highlands (Yami, 2013) and about a quarter of the total straw for the country (Bediye *et al.*, 1996). Hence, itisone of the most important feed resources to alleviate the feed shortage through better use of cultivars with higher fodder yield and higher straw quality. To date, however, studies conducted on food-feed traits of improved tef varieties are limited and key food-feed traitswere not addressed properly.

The importance of tef as food and feed, and farmers worry about crop residues and its grain require to identify genotypes with better grain and straw yield and quality. Conducting feed quality analysis enables to identify tef genotypes suitable for food-feed traits and to incorporate such traits into future variety development. Hence, the objectives of this study were: to assess the variability for food-feed traits among released varieties; to reveal the relationships among the food and feed traits; and to provide recommendations regarding food and feed value of the crop to the tef breeding.

6.2 *Materials and methods*

6.2.1 Field Study

Thirty-five improved tef varieties (Table 30) and a local check were grown for two years (2015 and 2016) at two locations (Debre Zeit and Holetta) using randomized complete block design (RCBD) with three replications. A plot size of 1m x1m was used with a spacing of 0.2m between rows, 1m between plots and 1.5mbetween replications. Total biomass was weighed and recorded before threshing whereas the grain yield and straw yieldwere recorded after threshing. The tef straw samples for laboratory analysis were taken after threshing and partitioning of shoot biomass into grain and straw.

6.2.2 Laboratory Study

Four hundred thirty-two straw samples from 35 improved tef varieties and a local check grown in three replications at two locations for two years (36 genotypes x 3 replications x 2 locations x 2 years = 432) were collected and evaluated. Each sample was ground to pass through 1 mm sieve using Christu lab mill at the International Livestock Research Institute (ILRI), Ethiopia. Dry matter (DM), ash, nitrogen (N), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) and *in vitro* organic matter digestibility

(IVOMD) were determined using the Near Infrared Reflectance Spectroscopy (NIRS) according to the manufacturer's protocol.

6.2.2.1 Analysis of feed quality using NIRS

Scanning of samples was performed using NIRS machine (Foss NIRS 5000 Win II project version 1.5, Intra Soft International, LLC) on 3 g ground samples. Before scanning, all samples were pre-dried in an oven at 105⁰ C overnight to standardize moisture conditions. In this study, a wave length range of 1100 to 2498 was used for the NIRS machine at 2 nm intervals.

6.2.2.2 Wet Chemistry Analysis

After NIRS scanning, 108 selected samples were used for the wet chemistry analysis to develop and validate a tef straw equation at ILRI Feed Nutrition Laboratory in Addis Ababa, Ethiopia and ICRISAT, India. Thus, 82 of these samples were used to develop and calibrate the equation whereas the remaining 26 were used to validate the developed equation. Thus, the prediction of all samples scanned by NIRS were done after developing and validating a new tef straw equation.

Dry matter (DM), ash and nitrogen were analyzed according to AOAC (1990), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) following the Goering and Van Soest (1970) method and *in vitro* organic matter digestibility (IVOMD) by two-stage *in vitro* technique (Tilley and Terry, 1963). The determination of crude protein was performed following the Kjeldahl method procedure (AOAC, 2000). All chemical analyses were performed in duplicate and expressed on a dry weight basis whereby 2 g, 1 g, 0.5 g and 0.3 g were used for determination of DM, ADF, NDF and N, respectively.

6.2.2.3 Developing the tef straw equation

Predictive equations were developed using the modified partial least squares (MPLS) (Shenk and Wester Haus, 1993) regression with internal cross-validation and scatter correction using standard normal variates and detrend (Barnes et al., 1989). The mathematical treatment applied in the present study was (1, 4, 4, 1); where the first number indicates the order of derivative (one is first derivative of $\log 1/R$), the second number is the gap in nm over which the derivative is calculated; the third number is the number of nm used in the first smoothing and the fourth number refers to the number of nm over which the second smoothing is applied. Standard error of calibration (SEC), the coefficient of determination in calibration (R^2), the standard error of cross validation (SECV) and the coefficient of determination in cross validation (1-VR) were calculated as calibration statistics (Shenk and Wester Haus, 1993). The optimum calibrations were selected on the basis of minimizing the standard error of cross validation (SECV). The standard error of cross validation to standard deviation of the constituent data was calculated to evaluate the calibrations (Murray, 1993).

6.3 *Analysis of variance*

Descriptive statistics was used to summarize all the data on food-feed traits. The GLM procedure of SAS (SAS, 2002) was employed for analysis of variance and the test of significance was declared at $P \leq 0.05$ using the least significance differences (LSD). Associations among the various food-feed traits were assessed using the SAS software (SAS, 2002). A biplot showing the distribution of nine traits and 36 tef genotypes were analyzed using MINITAB version 17.1 (Minitab, 2013).

6.4 RESULTS

6.4.1 Variation in major food-feed traits of tef

The chemical composition of feed quality traits and their descriptive statistics like mean, standard deviation (SD), range, and coefficient of variation (CV%) for individual locations were estimated and are presented (Table 39). At Debre Zeit, the measurements for key traits ranged from 6.95 to 9.3 for CP, 74.9 to 78.8% for NDF, 41.3 to 45.0% for ADF, 6.6 to 7.5% for ADL, 6.7 to 7.2 for ME and 46.8 to 50.1% for IVOMD. Similarly, at Holetta, the values ranged from 2.78 to 3.96% for CP, 82.3 to 84.7% for NDF, 50.1 to 51.8% for ADF, 8.0 to 8.5% for ADL, 6.2 to 6.4 for ME, and 42.3 to 43.6% for IVOMD. The yields of total biomass, grain and straw, on the other hand, ranged from 11.98 to 21.90 t/ha, 2.90 to 4.94 t/ha and 9.06 to 16.96 t/ha, respectively at Debre Zeit whereas the same yield traits in the same order ranged from 10.70 to 16.78 t/ha, 3.17 to 4.78 t/ha and 7.53 to 12.72 t/ha at Holetta (Table 39).

Combined mean, SD, range, and CV% of the chemical composition of feed quality traits of tef are presented in Table 39. This result showed that the mean CP content was 5.69%, with values ranging from 5.04 for G₂₅ to 6.26 % for G₁₉ (SD = 0.30; CV = 10.24%). The NDF, on the other hand, ranged from 79.08% for G₁₈ to 81.2% for G₂₀, with a mean of 80.2% (SD = 0.57; CV = 1.68%). The ADF content, similarly, ranged from 46.05 for G₂₉ to 47.9% for G₁₆ with a mean of 47.1% (SD = 0.54; CV = 2.41%). Likewise, the ADL ranged from 7.32% for G₂₉ to 7.96% for G₂₇, with a mean of 7.69% (SD = 0.15; CV = 5.05%). Furthermore, the ME content (MJ Kg⁻¹ DM) also ranged from 6.51 for G₇ to 6.70 for G₂₆, with a mean of 7.69 (SD = 0.05; CV = 2.01%). Combined mean of IVOMD %, on the other hand, was 45.6% with values ranging from 44.9 for G₂₅ to 46.30% for G₉ (SD =

0.42; CV = 2.01%). On the other hand, the combined mean for biomass yield was 15.80 t/ha, with values ranging from 13.30 t/ha for G₂₀ to 18.94 t/ha for G₁₀ (SD = 1.48; CV = 10.68 %). Besides, the mean for grain yield was 3.88 t/ha with values ranging from 3.23 t/ha G₃₀ to 4.63 t/ha for G₁₇ (SD = 0.37; CV = 7.66 %). Furthermore, the combined mean for tef straw yield was 11.92 t/ha, with values ranging from 9.7 t/ha for G₁₂ to 14.83 t/ha for G₁₀.

Combined analysis variance revealed that the food-feed traits of improved tef varieties were significantly ($P < 0.01$) affected by the genotypes, year, locations and their interactions (Table 40). Thus, all studied traits were significantly affected by the genotypes and by the location except for grain yield while the year effect significantly ($P < 0.01$) affected all food-feed traits except metabolic energy. On the other hand, the genotype by location effect was significant ($P < 0.01$) for all traits other than ADL. Similarly, the year by genotype interaction was significant for all traits other than ADL, SBM and STY while, all traits other than ADL were significant ($P < 0.01$) affected by the interaction of the three factors (Table 40).

Table 39. Simple descriptive statistics for major feed quality traits at Debre Zeit and Holetta

Location	Variable	Mean	Minimum	Maximum	Std. Dev	CV (%)
Debre Zeit	CP	8.1	7.0	9.3	0.54	13.82
	NDF	76.7	74.9	78.8	0.96	1.43
	ADF	43.0	41.3	45.0	0.97	2.14
	ADL	7.1	6.6	7.5	0.24	4.74
	ME	6.9	6.7	7.2	0.10	1.67
	IVOMD	48.3	46.8	50.1	0.82	1.72
	TB	17.7	12.0	21.9	2.49	8.57
	GY	3.9	2.9	4.9	0.60	6.67
	STY	13.9	9.1	17.0	1.96	10.63
Holetta	CP	3.3	2.8	3.8	0.25	8.47
	NDF	83.7	82.3	84.7	0.60	1.94
	ADF	51.1	50.1	51.8	0.43	2.73
	ADL	8.3	8.0	8.5	0.15	5.42
	ME	6.3	6.2	6.4	0.04	2.24
	IVOMD	42.9	42.3	43.6	0.29	2.21
	TB	13.9	10.7	16.8	1.40	11.64
	GY	3.9	3.2	4.8	0.38	8.54
	STY	10.0	7.5	12.7	1.13	13.82
Combined	CP	5.7	5.0	6.3	0.30	10.24
	NDF	80.2	79.1	81.2	0.57	1.68
	ADF	47.1	46.1	47.9	0.54	2.41
	ADL	7.7	7.3	8.0	0.15	5.05
	ME	6.6	6.5	6.7	0.05	2.01
	IVOMD	45.6	44.9	46.3	0.42	2.01
	TB	15.8	13.3	18.9	1.48	10.68
	GY	3.9	3.2	4.6	0.37	7.66
	STY	11.9	10.1	14.3	1.19	12.70

CP=crude protein, NDF=neutral detergent fiber, ADF=acid detergent fiber, ADL=acid detergent lignin, ME=metabolic energy, IVOMD=in vitro organic matter digestibility, TB=total biomass, GY= grain yield, STY=straw yield

Table 40. Combined ANOVA across two locations and two years

Source	DF	CP	NDF	ADF	ADL	ME	IVOD	SBM	GY	STY
Entry	35	1.08***	3.92***	3.55***	0.25*	0.03***	2.10***	26.12***	1.66***	16.91***
Loc	1	2556.91***	52951.10	7134.16***	141.76***	37.87***	3179.07***	1634.41***	0.10ns	1659.61***
Entryx Loc	35	1.06***	3.71***	3.23***	0.215ns	0.04***	2.45***	22.77***	1.36***	13.8***
Year	1	171.59***	339.02***	107.60***	16.57***	0.06ns	24.94***	1152.10***	33.36***	1577.57***
Entryx Year	35	0.97***	5.19***	3.02***	0.22ns	0.03***	1.87***	3.93ns	0.375***	2.89ns
Locx Year	1	19.20***	2.26ns	81.32***	0.0003ns	0.45***	13.2***	1389.27***	8.80***	1619.17***
Entryx LocxYear	35	0.88***	3.35***	3.66***	0.20ns	0.03**	2.04***	7.08***	0.56***	5.28***
Error	288	0.34	1.82	1.29	0..15	0.018	0.84	2.84	0.088	2.29
Mean		5.69	80.18	40.07	7.69	6.60	45.58	15.80	3.88	11.92
CV (%)		10.24	1.68	2.41	5.05	2.01	2.01	10.67	7.66	12.70
R ²		0.97	0.92	0.95	0.81	0.90	0.94	0.88	0.88	0.90

*, *** significant at 5 and 0.01%, respectively.

This study showed that the performance of all food-feed traits was significantly ($P \leq 0.01$) affected by genotypes at Debre Zeit whereas only CP, SBM, and STY were significantly ($P \leq 0.01$) affected by genotypes at Holetta. On the other hand, the year effect was significant for all traits at Holetta whereas only seven traits were significantly affected at Debre Zeit. Furthermore, the interaction of year by genotypes were significant for all traits at Debre Zeit while only one feed quality and three yield related traits were significantly affected by such interactions at Holetta. The lowest value of CP, ME and IVOMD, and the straw and biomass yield were obtained at Holetta whereas Debre Zeit exhibited the lowest value for all fiber related traits (Table 41). The extent and pattern of variations for major food-feed traits and their various interactions are presented in Appendix Fig. 3 & 4. The highest value of IVOMD and ME for most of genotypes were obtained at Debre Zeit in 2015 whereas that of NDF, ADF and ADL were the highest at Holetta in 2015 with minimum interaction (Appendix Fig. 3) followed by Holetta 2016, Debre Zeit 2015 and Debre Zeit 2016, respectively (Appendix Fig. 4).

Table 41. Variation in major food-feed traits of tef across different locations and years

Traits	Years		Locations		LSD (5%)
	2015	2016	Holetta	D/Zeit	
DM (%)	92.82a	92.71b	93.48a	92.05b	0.051
OM (%)	92.33a	92.00b	93.38a	90.95b	0.111
CP (%)	5.06b	6.32a	3.24b	8.13a	0.11
NDF (%)	81.06a	78.29b	83.68a	76.67b	0.26
ADF (%)	47.57a	46.57b	51.14a	43.01b	0.22
ADL (%)	7.89a	7.50b	8.27a	7.12b	0.074
ME (MJ/Kg)	6.58a	6.60 a	6.30b	6.89a	0.025
SBM (t/ha)	14.16b	17.43a	13.85b	17.74a	0.32
GY (t/ha)	4.16a	3.60b	3.89a	3.87a	0.056
STY (t/ha)	10.01b	13.83a	9.96b	13.88a	0.29

NB. Mean in same row followed by the same letter are not significantly differ from each other. OM=organic matter; CP= crude protein, ME= metabolic energy

6.4.2 Heritability of food-feed traits in Tef

The heritability values of various food-feed traits were estimated based on data from the two locations (Debre Zeit and Holetta) and combined data across locations (Table 42). Based this, the highest heritability value was estimated at Debre Zeit followed by Holetta and combined data of the two locations. Among the nine investigated traits, eight, six and five traits had sufficient heritability at Debre Zeit, Holetta and at combined level, respectively. Surprisingly, in all the three cases, the highest heritability was obtained for yields of shoot biomass, grain and straw whereas feed quality traits had the least or zero values. Thus, no heritability was observed for CP, NDF, ME and IVOMD values for combined data while ADF, ADL, ME and IVOMD showed no heritability at Holetta and NDF at Debre Zeit.

Table 42. Heritability (%) of major food-feed traits of tef

State	CP	NDF	ADF	ADL	ME	IVOMD	SBM	GY	STY
Debre Zeit	15.5	0	8.2	14.9	18.12	22.93	79.31	73.23	74.38
Holetta	8.51	14.13	0	0	0	0	71.6	58.0	71.33
Combined	0	0	6.81	5.73	0	0	12.83	18.60	18.25

6.4.3 Association of food-feed traits of tef

In the present study, the association among major food-feed traits of tef was highly significant for most of the studied traits (Table 43). Thus, the association among CP%, ME and IVOMD % was positive and significant while their association with all remaining traits was negative and significant ($P < 0.01$). Similarly, the association of SBM, GY and STY was significant and positive with all traits except CP%, ME and IVOMD%. In general, a negative association was observed between yields of food-feed traits and feed quality traits while the association of food-feed yield and other traits was moderately significant (Table

43). All studied parameters, however, showed no significant association between Debre Zeit and Holetta (Table 44).

Table 43. Correlation between major food-feed traits of tef data across locations and years

Traits	CP	NDF	ADF	ADL	ME	IVOMD	SBM	GY	STY
CP (%)	1								
NDF (%)	-0.79***	1							
ADF (%)	-0.90***	0.84***	1						
ADL (%)	-0.54***	0.75***	0.75***	1					
ME ()	0.73***	-0.82***	-0.83***	-0.84***	1				
IVOMD (%)	0.82***	-0.88***	-0.89***	-0.81***	0.98***	1			
SBM (t/ha)	-0.44**	0.43***	0.45***	0.44***	-0.48**	-0.52***	1		
GY (t/ha)	-0.45**	0.40*	0.47***	0.47***	-0.48**	-0.51**	0.83***	1	
STY (t/ha)	-0.40*	0.41*	0.42*	0.40*	-0.45**	-0.48**	0.98***	0.71***	1

CP=crude protein, NDF=neutral detergent fiber, ADF= acid detergent fiber, ADL= acid detergent lignin, ME= metabolic energy, IVOMD= invitro organic matter digestibility, SBM= shoot biomass, GY= grain yield, STY= straw yield

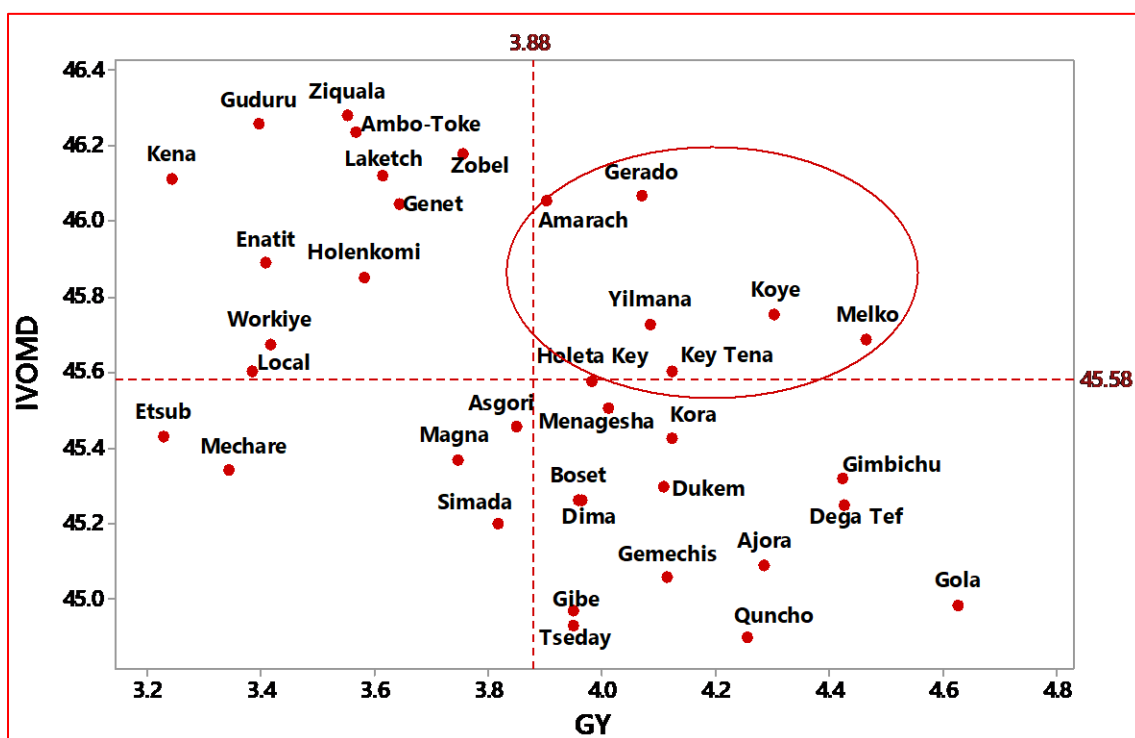
Table 44. Correlation between locations for all parameters of tef (the middle diagonal)

Traits	CP_DZ	NDF_DZ	ADF_DZ	ADL_DZ	ME_DZ	IVOMD_DZ	sbm_DZ	gy_DZ	sty_DZ
CP_HT	0.009ns	-0.001ns	-0.105ns	0.013ns	0.011ns	0.006ns	0.001ns	0.006ns	-0.001ns
NDF_HT	0.016 ns	0.031 ns	0.071 ns	0.059 ns	0.026 ns	0.042 ns	-0.049 ns	-0.086 ns	-0.036 ns
ADF_HT	-0.109 ns	0.031 ns	0.064 ns	0.008 ns	0.009 ns	-0.001 ns	0.111 ns	0.101 ns	0.110 ns
ADL_HT	-0.037 ns	0.017 ns	0.012 ns	0.093 ns	-0.006 ns	0.006 ns	0.179 ns	0.157 ns	0.180 ns
ME_HT	-0.045 ns	0.067 ns	0.099 ns	0.116 ns	-0.143 ns	-0.156 ns	-0.021 ns	0.079 ns	-0.050 ns
IVOMD_HT	0.002 ns	0.017 ns	0.046 ns	0.103 ns	-0.115 ns	-0.122 ns	-0.048 ns	0.049 ns	-0.076 ns
SBM_HT	0.079 ns	-0.168 ns	-0.100 ns	-0.184 ns	0.100 ns	0.104 ns	0.080 ns	0.072 ns	0.080 ns
GY_HT	0.116 ns	-0.138 ns	-0.110 ns	-0.130 ns	0.064 ns	0.082 ns	-0.014 ns	0.110 ns	-0.051 ns
STY_HT	0.058 ns	-0.161 ns	-0.087 ns	-0.183 ns	0.102 ns	0.100 ns	0.104 ns	0.052 ns	0.116 ns

CP=crude protein, NDF=neutral detergent fiber, ADF= acid detergent fiber, ADL= acid detergent lignin, ME= metabolic energy, IVOMD= invitro organic matter digestibility, SBM= shoot biomass, GY= grain yield, STY= straw yield, HT=Holetta, DZ= Debre Zeit

6.4.4 Scatterplot showing the relationships among food-feed traits of tef genotypes

Scatterplots showing the relative position of various genotypes on a biplot of major food-feed traits of tef are presented (Fig 35a-h). Thus, scatter plot of grain yield versus *invitro* organic matter digestibility revealed that Koye, Gerado, Melko, Amarach, Yilmana, Key Tena and Holetta Key varieties had a mean value above the mean for both the grain yield and *invitro* organic matter digestibility (36a).

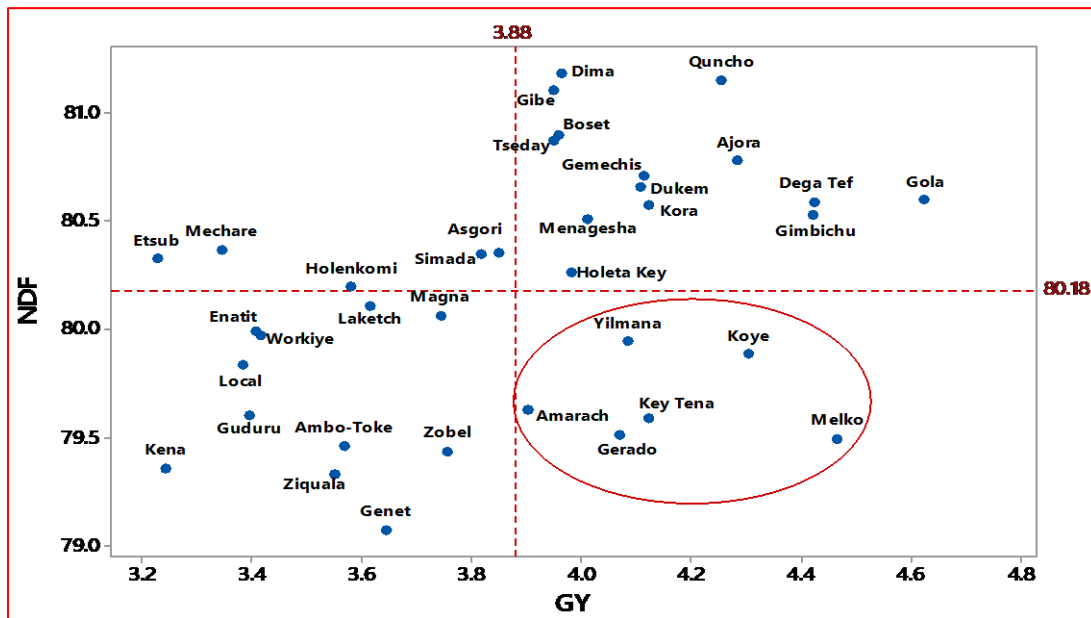


35a. Scatter plot of GY vs IVOMD

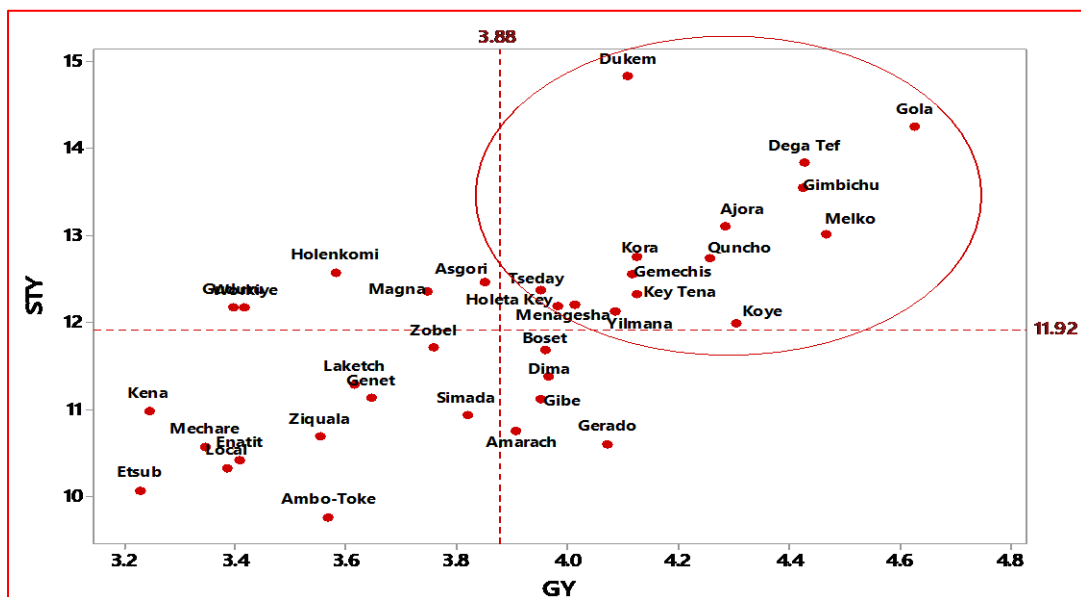
Figure 35a-h. Scatter plots showing the the distribution of genotypes evaluate for food feed.

On the other hand, scatter plot of grain yield versus NDF revealed that Melko, Koye, Key Tena, Amarach, Yilmana and Gerado varieties were identified to have lower NDF value

coupled with high grain yield which is one indication of feed quality traits (Fig 35b). Furthermore, scatterplot of GY vs STY showed that Dukem, Gimbichu, Gola, Quncho, Melko, Dega Tefa, Kora, Gemechis, Key Tena, Koye, Yilmana, Tseday, Menagesha and Holetta Key varieties had above average grain and straw yield (Fig. 35c).

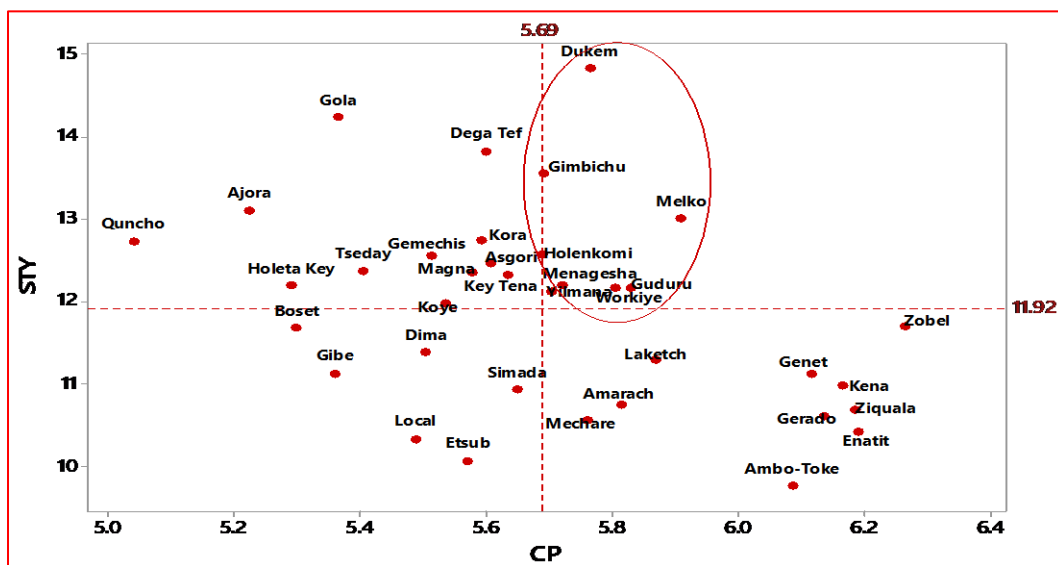


35b. Scatter plot of GY vs NDF

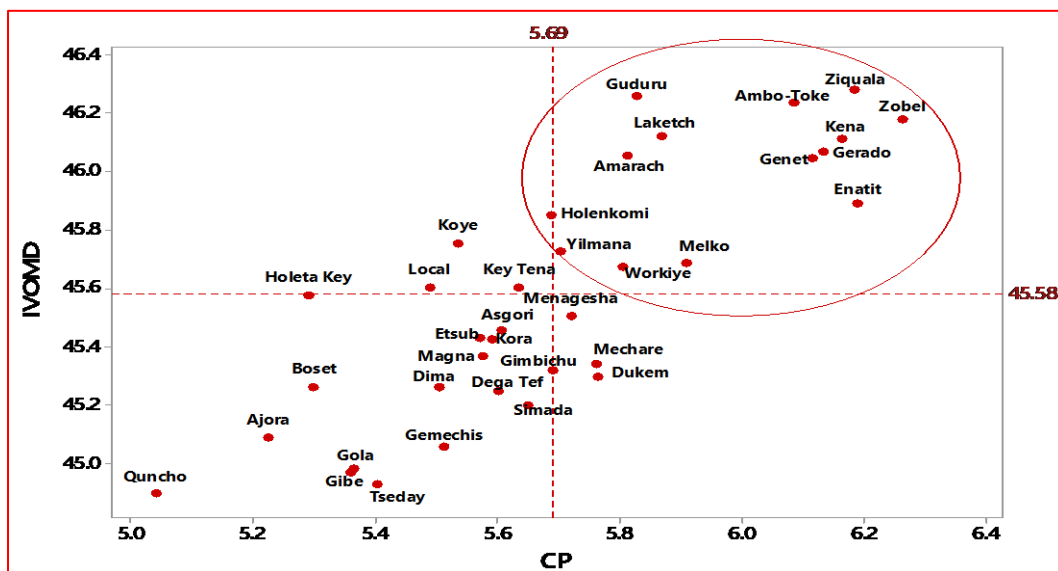


35c. Scatter plot of GY vs STY

Scatter plot of CP vs STY showed that Melko, Dukem, Gimbichu, Wolenkomi, Guduru, Menagesha, Yilmana and Worekiyu varieties had values greater than the mean for the two traits (Fig.35d). Scatterplot of CP vs IVOMD, on the other hand, indicated that Kenna, Ambo Toke, Guduru, Zobel, Genet, Laketch, Gerada, Ziquala, Melko, Enatit, Amarach, Wolenkomi, Yilmana and Workiyu varieties scored the highest values of both CP % and IVOMD % (Fig. 35e).

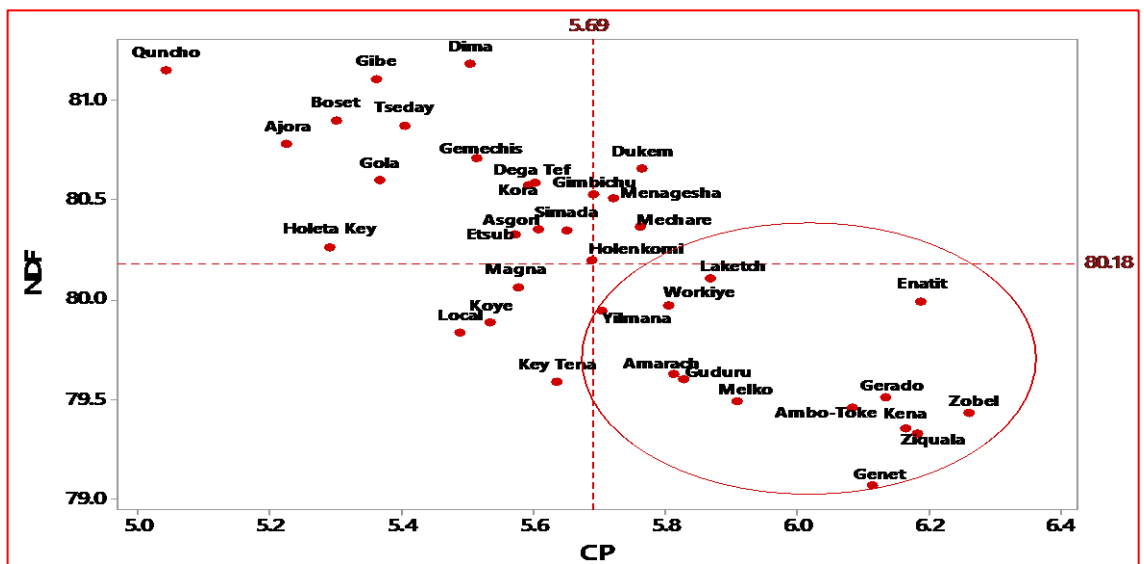


35d. Scatter plot of STY vs CP



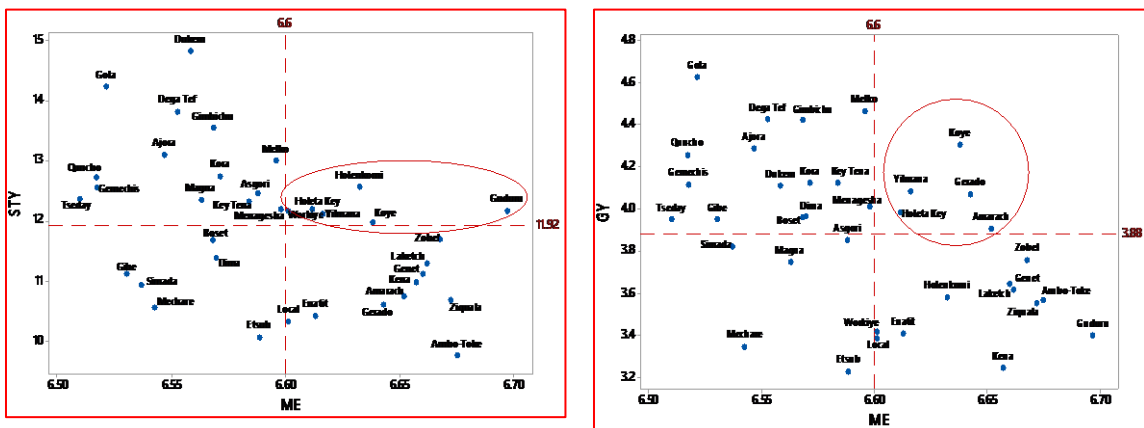
35e. Scatter plot of IVOMD vs CP

As shown in Fig 35f, Genet, Kenna, Workiyu, Gerado, Ambo-Toke, Melko, Zobel, Ziquala, Guduru, Amarach, Yilmana, Laketch and Koye varieties scored high CP coupled with the smallest NDF value. These genotypes are, therefore, have better feed quality traits. Furthermore, Guduru, Wolenkomi, Koye, Worekiyu, Holetta Key and Yilmana varieties had above average value of ME and straw yield (Fig. 35g) whereas Koye, Gerado, Amarach, Yilmana and Holetta Key varieties had the highest ME and grain yield values (Fig. 35g & h).



h).

35f. Scatterplot of NDF vs CP



35g & h. Scatterplot of STY vs ME (g) and grain yield vs ME (h)

6.4.5 Distribution of traits and genotypes across PC1 and PC2

The studied food-feed traits of improved tef varieties was plotted on PC₁ (68.2%) and PC₂ (18.2%) which accounted for 86.4% of the total variations (Fig.36). Based on this figure three major groups of traits were formed. Thus, group-I consisted of feed quality traits positively correlated with feed intake (ME, IVOMD and CP) whereas the traits in group-II include SBM, STY and GY which are related to food-feed traits yield. On the other hand, the third group of traits include NDF, ADL and ADF which are known to affect the feed intake negatively.

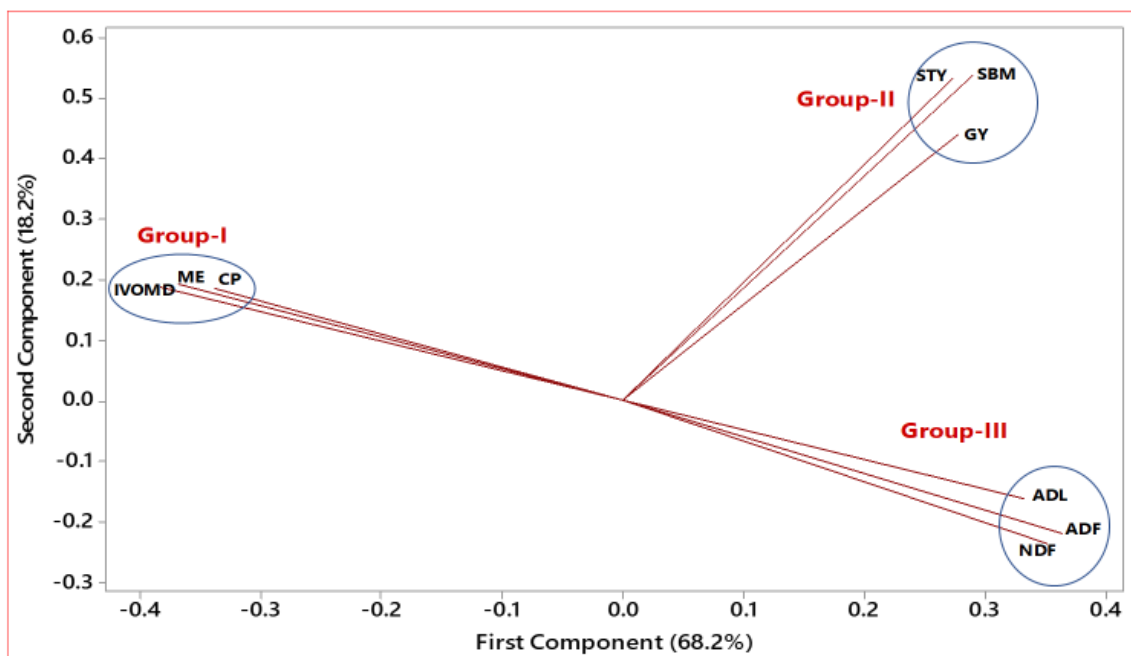


Figure 36. Distribution of major food-feed traits of tef across PC₁ and PC₂

Like the food-feed traits, the 36 studied varieties were also plotted on PC₁ vs PC₂ which resulted in the formation of four distinct groups (Fig. 37). Thus, the first group consisted of tef genotypes having better yield of both food-feed traits and they include Melko, Dukem, Gola, Gimbichu, Quncho, Kora, Dega Tef, Koye, Key Tena, Yilmana, Menagesha, Tsedey,

Gemechis and Ajora. Group two, on the other hand, consisted of tef genotypes having better feed quality traits such as: Kenna, Genet, Zobel, Ambo-Toke, Gerado, Ziquala, Enatit, Laketch, Guduru, Worekiyu, Amarach and Wolenkomi. Genotypes in Group III, however, were known to have above average food-feed trait yield and feed quality traits. The genotypes in group III were partly overlapped with both group-I and group-II and are encircled with green colour. Hence, the genotypes in group-III include Melko, Koye, Key Tena, Yilmana and Holetta Key from Group-I and Wolenkomi, Worekiyu, Guduru, Gerado and Amarach from Group-II. Genotypes in group-IV, however, were not identified to be good for any of the nine studied traits (Fig. 37).

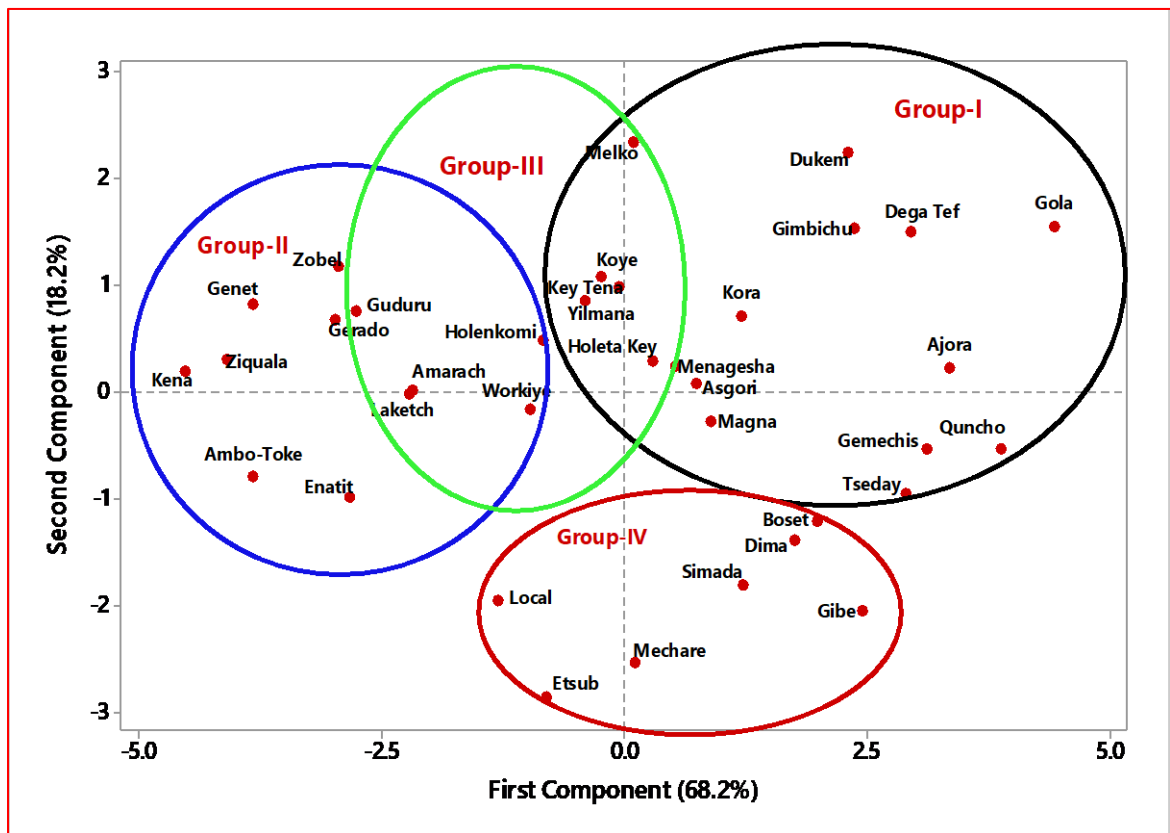


Figure 37. Distribution of 36 tef varieties evaluated for major food-feed traits across PC₁ and PC₂

6.4.6 Economic return of growing tef varieties from various groups

In this study, the economic advantages of growing selected tef genotypes from the identified four categories were assessed and presented (Table 45). For this purpose, four genotypes from each category were used to assess the expected economic return from growing a given variety. Dukem, Gola, Gimbichu and Quncho from group-I; Kenna, Genet, Guduru and Ambo-Toke from group-II; Melko, Koye, Yilmana and Key Tena from group-III; and local, Dima, Boset and Etsub from group-IV were selected. The market price list for both tef grain and straw was based on data collected from east Shewa zone (Personal communication with and unpublished socio-economic data from Dr. Abate Bekele, socio-economist at Debre Zeit Agricultural Research). Based on that data, the price for a kilo gram of tef straw, very white, creamy white, mixed and brown grain color was 1.00, 23.0, 21.0, 20.0 and 18.0 Ethiopian Birr, respectively. The results of such economic analysis revealed that a farmer growing Quncho variety gets the highest economic return of 98,900.00 Birr/ha followed by Gimbichu (98,700 Birr /ha), Gola (96,600.00 Birr/ha) and Melko (96,600 Birr /ha) from the grain sales. With respect to sales from tef straw, however, growing Dukem variety gave the highest return of 15,000 Birr followed by Gimbichu (14,500.00 Birr), Gola (14,400 Birr), Quncho (13,800 Birr) and Melko (13,600 Birr). Regarding both grain plus straw, the highest economic return was obtained from Gimbichu (113,200.00Birr) followed by Quncho (112,700.00Birr), Gola (111, 000.00Birr) and Melko (110,200.00 birr) respectively. Besides, the economic advantage of growing the above varieties over the local variety was 45.7% for Gimbichu, 45.1% for Quncho, 42.9% for Gola and 41.8% for Melko.

Table 45.Analysis of the economic benefits of producing tef with various traits(ha)

Parameters	Genotypes	Quality	Yield (t/ha)		Price*		
		IVOMD	GY	STY	GY	Straw	Total
Genotypes selected for yield	Dukem	45.60	4.1	15.00	86100.0	15000.0	101100.0
	Gola	45.20	4.6	14.40	96600.0	14400.0	111000.0
	Gimbichu	45.40	4.7	14.50	98700.0	14500.0	113200.0
	Quncho	45.10	4.3	13.80	98900.0	13800.00	112700.0
Genotypes selected for quality traits	Kenna	46.40	3.2	11.60	67200.0	11600.0	78800.0
	Genet	46.10	3.7	10.60	77700.0	10600.0	88300.0
	Guduru	46.30	3.3	12.10	69300.0	12100.0	81400.0
	Ambo-Toke	46.30	3.7	9.70	77700.0	9700.0	87400.0
Genotypes selected for dual purpose	Melko	45.90	4.6	13.60	96600.0	13600.0	110200.0
	Koye	46.10	4.1	11.80	86100.0	11800.0	97900.0
	Yilmana	45.40	4.0	11.90	84000.0	11900.0	95900.0
	Key Tena	45.30	4.3	12.00	77400.0	12000.0	89400.0
Genotypes with little yield and quality traits	Local	45.50	3.4	9.70	68000.0	9700.0	77700.0
	Simada	45.30	3.7	10.00	77700.0	10000.0	87700.0
	Etsub	45.30	3.3	9.70	69300.0	9700.0	79000.0
	Boset	45.20	3.9	11.40	89700.0	11400.0	101100.0

* The average price for very white, pale white and brown tef grain and tef straw for all varieties in east Shewa is 22.00birr/ kg and 1birr/kg respectively. GY = grain yield, STW = Straw yield

Table 46. Combined mean of major food-feed traits for 36 tef varieties evaluated at Debre Zeit and Holetta for two years

Entry	DM (%)	OM (%)	CP (%)	NDF (%)	ADF (%)	ADL (%)	ME (MJ/Kg)	IVOM D (%)	SBM (t/ha)	GY (t/ha)	STY (t/ha)
Enatit	92.7	91.9	6.0	80.3	46.5	7.7	6.6	45.8	14.2	3.5	10.7
Asgori	92.8	92.2	5.6	80.0	47.1	7.7	6.6	45.6	16.5	3.8	12.7
Magna	92.8	92.2	5.6	80.5	47.5	7.9	6.5	45.1	15.5	3.8	11.7
Wolenkomi	92.7	92.2	5.6	80.3	46.8	7.5	6.7	46.0	16.2	3.5	12.7
Menagesha	92.8	92.4	5.7	80.2	47.4	7.7	6.6	45.4	16.0	4.0	12.0
Melko	92.7	91.8	6.0	79.5	46.7	7.6	6.6	45.9	18.2	4.6	13.6
Tseday	92.9	92.3	5.4	80.8	47.7	7.9	6.5	45.0	15.5	3.8	11.7
Gibe	92.8	92.4	5.4	81.0	47.4	7.8	6.5	45.0	15.6	4.1	11.5
Ziquala	92.7	92.2	6.0	79.9	46.9	7.7	6.6	45.9	14.3	3.6	10.7
Dukem	92.7	92.2	5.9	80.5	46.8	7.7	6.6	45.6	19.1	4.1	15.0
Holeta Key	92.9	92.3	5.1	80.3	47.2	7.5	6.6	45.5	16.6	4.0	12.6
Ambo-Toke	92.6	92.1	6.4	79.5	46.4	7.6	6.7	46.3	13.4	3.7	9.7
Gerado	92.7	92.0	5.9	79.4	46.7	7.5	6.7	46.0	14.4	4.1	10.4
Koye	92.7	92.1	5.7	79.2	46.4	7.5	6.7	46.1	15.9	4.1	11.8
Key Tena	92.8	92.1	5.5	80.3	47.3	7.8	6.6	45.3	16.3	4.3	12.0
Gola	92.9	92.2	5.5	80.5	47.9	7.9	6.5	45.2	19.0	4.6	14.4
Ajora	92.9	92.3	5.1	80.7	47.9	7.8	6.5	45.0	17.3	4.3	13.0
Genet	92.6	92.0	6.2	78.8	46.0	7.5	6.7	46.1	14.4	3.7	10.6
Zobel	92.6	92.1	6.3	79.9	46.3	7.7	6.7	46.1	15.8	3.7	12.2
Dima	92.8	92.4	5.4	81.1	47.5	7.8	6.6	45.3	16.2	4.2	12.0
Yilmana	92.8	92.4	5.7	80.1	47.2	7.8	6.6	45.4	15.9	4.0	11.9
Dega Tef	92.8	92.3	5.8	80.1	47.3	7.6	6.6	45.6	17.6	4.4	13.3
Gimbichu	92.7	92.2	5.7	80.3	47.1	7.8	6.6	45.4	19.2	4.7	14.5

Table 46. continued.

Entry	DM (%)	OM (%)	CP (%)	NDF (%)	ADF (%)	ADL (%)	ME (MJ/Kg)	IVOMD (%)	SBM (t/ha)	GY (t/ha)	STY (t/ha)
Amarach	92.8	92.2	5.6	80.4	47.4	7.8	6.6	45.6	13.2	3.7	9.5
Quncho	92.9	92.2	5.1	81.0	47.5	7.7	6.5	45.1	18.1	4.3	13.8
Guduru	92.7	92.5	5.8	79.4	47.0	7.6	6.7	46.3	15.3	3.3	12.1
Gemechis	92.8	92.2	5.6	80.9	47.5	7.9	6.5	45.1	16.5	4.3	12.2
Mechare	92.8	92.0	5.7	80.3	47.2	7.9	6.5	45.2	13.6	3.3	10.3
Kena	92.6	91.9	6.2	79.3	46.1	7.3	6.7	46.4	14.8	3.2	11.6
Etsub	92.8	92.3	5.6	80.6	47.4	7.8	6.6	45.3	13.0	3.3	9.7
Laketch	92.7	92.1	5.8	80.0	46.6	7.6	6.7	46.1	15.8	3.7	12.1
Simada	92.9	92.2	5.5	80.1	47.9	7.9	6.6	45.3	13.7	3.7	10.0
Boset	92.9	92.5	5.4	81.0	47.6	7.7	6.6	45.2	15.3	3.9	11.4
Kora	92.7	92.1	5.6	80.8	47.0	7.9	6.6	45.4	17.6	4.2	13.4
Workiye	92.7	91.7	6.0	79.4	46.5	7.6	6.6	45.8	16.0	3.4	12.6
Local	92.8	92.1	5.5	80.0	47.3	7.6	6.6	45.5	13.0	3.4	9.7
Mean	92.76	92.2	5.7	80.2	47.1	7.69	6.60	45.58	15.80	3.88	11.92
Max.	92.94	92.5	6.43	81.1	47.9	7.91	6.71	46.35	19.19	4.65	15.03
Min.	92.58	91.7	5.05	78.8	46.0	7.28	6.52	45.01	12.98	3.23	9.54
LSD (5%)	0.22	0.47	0.73	1.18	1.22	0.30	0.10	0.80	0.60	0.10	0.50

6.4.7 Near Infrared Reflectance Spectroscopy (NIRS) for determination of feed quality parameters

Calibration and validation of the chemical entities (DM, Ash, OM, CP, NDF, ADF, ADL, ME and IVOMD) as well as the equation statistics for all the studied parameters are summarized (Table 47). Based on this, the coefficients of determination in calibration (R^2) and the errors in cross validation (SECV) were 0.99 (0.48), 0.67 (0.63), 0.98 (0.45) 0.98 (1.13) and 0.94 (1.45) for crude protein, dry matter, ash, neutral detergent fiber and acid detergent fiber on the basis of a dry weight respectively. The R^2 obtained in the present study is high and consistent for all the studied parameters except for dry matter per cent.

Table 47. NIRS calibration statistics for tef straw feed quality parameters on dry weight basis.

Parameters/ Traits	N	Mean	SD	Range (%)	SEC	RSQ	SECV	1-VR
DM (%)	76	92.518	1.036	89.41-95.6	0.60	0.67	0.63	0.63
Ash (%)	78	8.317	1.745	3.08-13.6	0.25	0.98	0.45	0.93
OM (%)	78	91.683	1.745	86.45-96.9	0.25	0.98	0.45	0.93
N (%)	81	1.094	0.508	0.000-2.6	0.06	0.99	0.08	0.98
CP (%)	81	6.837	3.180	0.00-16.4	0.35	0.99	0.48	0.98
NDF (%)	78	78.347	4.556	64.7-92.0	0.63	0.98	1.13	0.94
ADF (%)	77	45.436	5.077	30.2-60.7	1.24	0.94	1.45	0.92
ADL (%)	79	7.480	0.904	4.8-10.2	0.34	0.86	0.44	0.77
ME (MJ/Kg)	76	6.692	0.382	5.55-7.8	0.061	0.97	0.091	0.943
IVOMD (%)	77	46.529	3.389	36.36-56.7	0.41	0.99	0.651	0.963

N= Number of samples in calibration, R²= Coefficient of determination in calibration, SEC= Standard error of calibration, 1-VR= Coefficient of determination of cross validation, SECV= error of cross validation, ME= Metabolic energy, IVOMD=Invitro organic matter digestibility

As it can be seen from Table 48 the ratio of SECV/SD is less than 0.30 for all parameters other than DM and ADL. Hence, the equation is acceptable for predicting the chemical parameters suggesting that the models built are very robust and not overfitted. Because, all parameters had the best equations except for the dry matter ($R^2 = 0.67$) and ADL ($R^2 = 0.86$). In this study, in general, high coefficients of determination and low SEC or SECV were obtained for almost all of the analyzed components suggesting comprehensive calibrations detect less outlier samples and probably being more robust and efficient.

Table 48. SECV/SD ratio

Traits	STD	SEP	RPD	SECV/SD
ASH (%)	1.452	0.497	2.92	0.26
OM (%)	1.463	0.488	3.00	0.26
CP (%)	2.877	0.317	9.08	0.15
NDF (%)	4.12	1.178	3.50	0.25
ADF (%)	4.954	1.012	4.90	0.29
ADL (%)	0.874	0.348	2.51	0.49
ME (MJ/Kg)	0.391	0.088	4.44	0.24
IVOMD (%)	3.32	0.558	5.95	0.19

The relationships of NIRS data versus chemical data for crude protein, OM%, *in vitro* organic matter digestibility %, NDF%, ADF% and ADL % ash exhibited similar graphing patterns (Fig. 38- 43). The high correlation observed between NIRS and chemical analysis in the present study, therefore, shows the potential use of NIRS for prediction of tef straw quality.

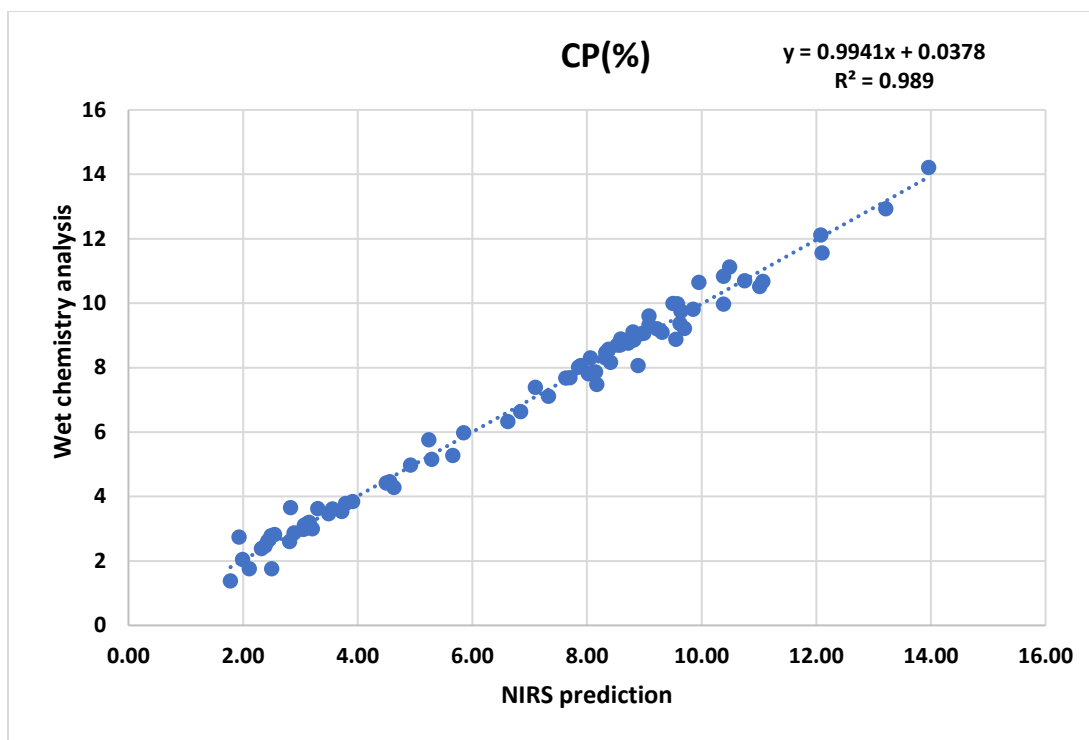


Figure 38. Relationship between chemical analysis and NIRS results for CP % from tef straw

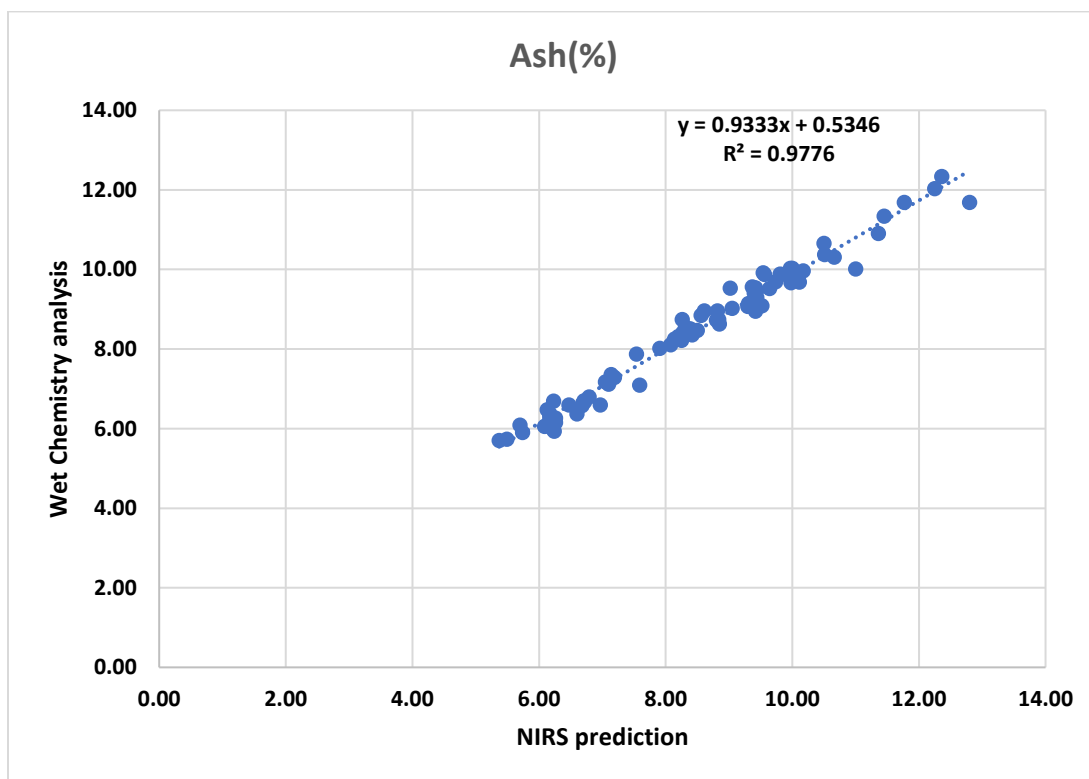


Figure 39. Relationship between chemical analysis and NIRS results for Ash% from tef straw

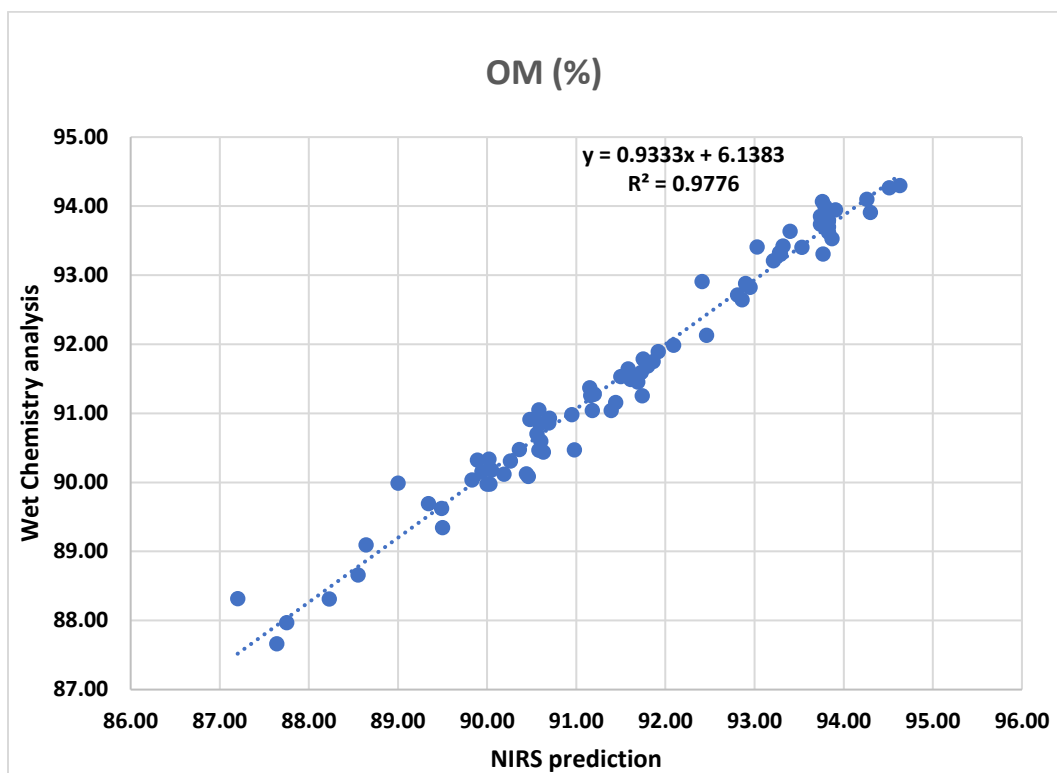


Figure 40. Relationship between chemical analysis and NIRS results for OM % from tef straw

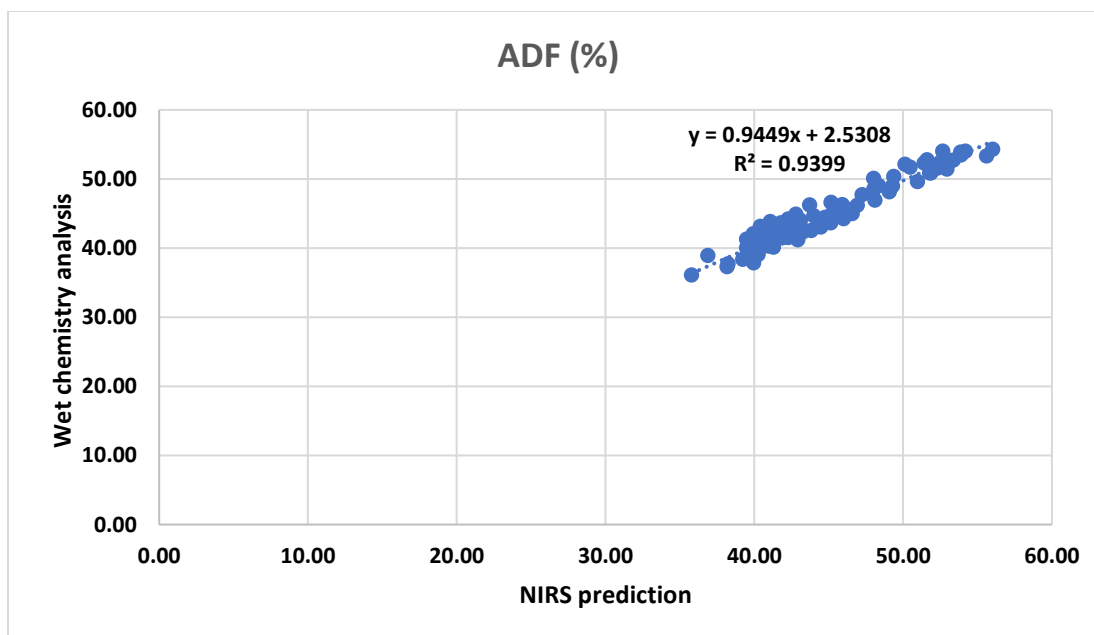


Figure 41. Relationship between chemical analysis and NIRS results for ADF % from tef straw

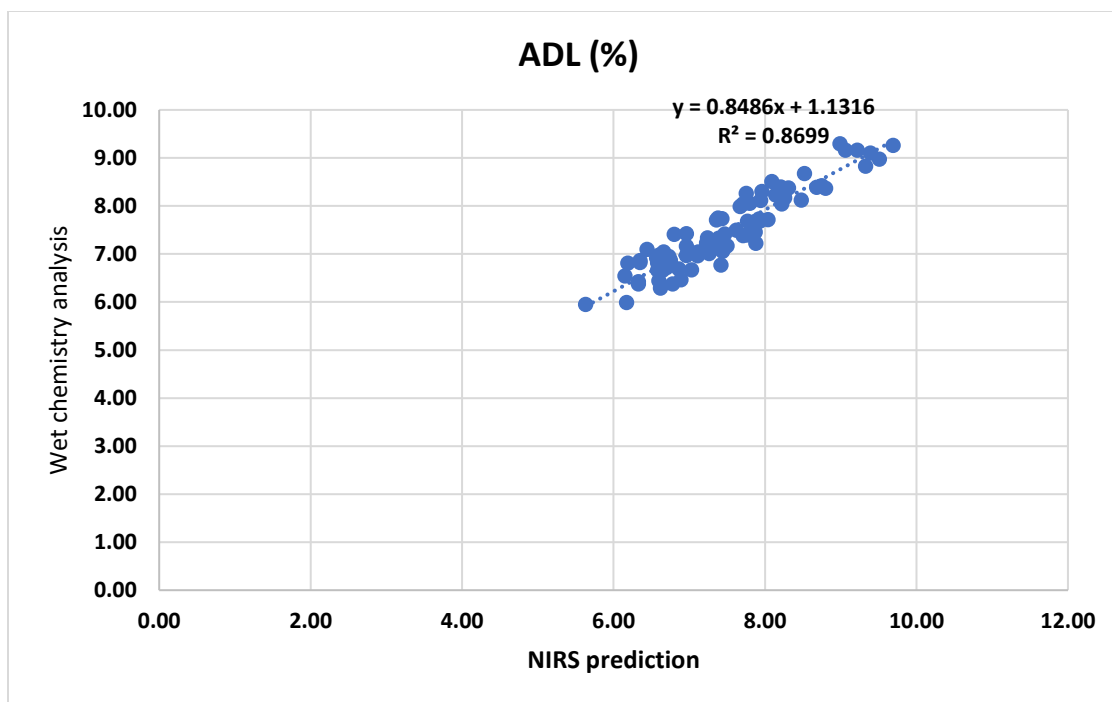


Figure 42. Relationship between chemical analysis and NIRS results for ADL % from tef straw

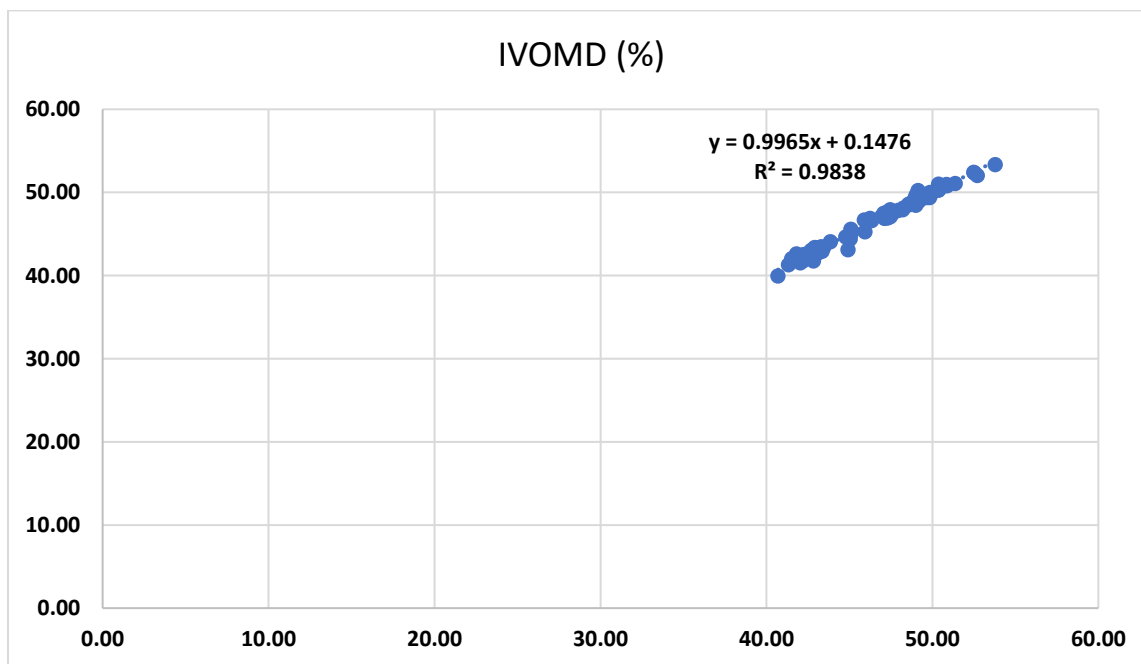


Figure 43. Relationship between chemical analysis and NIRS results for IVOMD % from tef straw

6.5 *Discussions*

Study on food-feed traits variation in 36 improved tef varieties revealed existence of significant variation among genotypes, location, year and their interactions. The range of values for crude protein (5.04 -6.26%) in the current study is far below the 9-14% reported previously (Miller, 2009) whereas, the combined mean crude protein (5.7%) is higher than the 3.9% reported earlier (Geleti *et al.*, 2013). In the present study, a wide range of variation was generally observed for food-feed yield traits compared to the feed quality traits may be due to the fact that the breeders were focusing mostly on improving grain yield related traits with little or no attention to feed quality traits (Table 39).

Kassier (2002) reported that the protein, amino acid and mineral content of tef are affected by altitude showing that samples from low altitude contain more lysine than those from mid and high altitudes. The high altitudes are, therefore, more deficient than the mid altitudes. Furthermore, the same study reported that the proline level at mid altitude is twice that of the low altitude and three times that of the high-altitude samples. This could probably be a reason for the low protein content observed in the present study for samples collected from Holetta which is located at high altitude compared to Debre Zeit which is situated in the mid altitude. On the other hand, Debre Zeit location was also found to favor tef feed yield and quality traits compared to Holetta mainly due to substantial differences in soils and climate conditions.

The heritability values observed for feed quality traits in the present study were generally very low compared to that of the yield related traits. Thus, the highest heritability was

obtained for yields of shoot biomass, grain and straw whereas feed quality traits had the least or zero values at Holetta, Debre Zeit and for the combined data from both locations. In general, no heritability value was observed for the location combined CP, NDF, ME and IVOMD values, and location specific ADF, ADL, ME and IVOMD at Holetta and NDF at Debre Zeit. This might be partially due to the previous varietal development strategy of breeders that did not give due attention to improvement of feed quality traits. In the present study, in general, various locations were observed to have different heritability value indicating the need to design a location specific strategy to develop the feed quality traits.

The association between yield traits and feed quality traits in the present study was found to be negative and significant. This is in agreement with the negative association previously reported between grain yield and crude protein content (Tolera *et al.*, 1999). Such negative correlations between feed quality and yield related traits indicates the difficulty to simultaneously improve both yield and quality traits in tef without affecting the other. However, the moderately significant association observed between food-feed yield and quality traits shows the possibility of improving feed quality traits without significantly affecting the yield traits (Table 43). The development of suitable variety for food-feed yield and feed quality traits could, therefore, be either for high yield, for high quality or for dual purpose (yield and quality traits). The absence of significant association between Holetta and Debre Zeit locations in the present study (Table 44), on the other hand, shows that each location needs an independent action for the development of various food-feed traits.

Based on the current study results, some genotypes were identified for high grain and straw yield, others for better feed quality traits while few genotypes combined high yield and feed quality traits whereas some others had no significant contribution for the studied food-feed traits. Hence, this study revealed the formation of four groups of genotypes. These include: those genotypes having high yield of grain and straw; better feed quality traits; dual purpose (yield and quality traits); and no significant importance for the studied food-feed traits. Based on this, Melko, Gerado, Koye, Yilmana, Key Tena, Amarach and Worekiyu varieties exhibited more than four desirable food-feed traits and hence, are varieties of choice for breeders and consumers interested in both food-feed yield and quality traits. Breeders and consumers interested in high yield of food-feed traits, however, should focus on Gola, Dukem, Dega Tefa, Quncho, Kora and Gemechis varieties which showed high food-feed yield traits. Furthermore, those interested in feed quality traits should focus on Zobel, Ziquala, Kenna, Genet, Gerado, Laketch and Goduru varieties which gave better feed quality traits. The economic return obtained from growing tef varieties from the four categories were also analyzed and the result enable to identify four varieties with the highest economic returns based on sales of both grain and straw (Table 45). The four varieties identified to have high economic return per hectare include Gimbichu (113,200.00 Eth. birr), Quncho (112,700.00 Eth. birr), Gola (111, 000.00 Eth. birr) and Melko (110,200.00 Eth. birr).

Regarding feed quality analysis using NIRS, the calibration equations for all studied feed quality traits in the present study have shown high coefficient of determination (R^2), except for DM which is still relatively high (0.67). Besides, the detected low standard errors of

calibration (SEC) and standard errors of cross validation (SECV) indicates that all parameters were among the traits that could be predicted with good precision (Table 48). Previously, Fekadu *et al.* (2010) also reported similar results using NIRS for the determination of chemical entities of natural pasture from Ethiopia. The observed scatter plot in this study also showed that the data points were distributed evenly about the line for almost all studied traits. Hence, it confirms the usefulness of NIRS for predicting the indicated nutrient contents compared to the conventional chemical method of analysis which is highly accurate, but expensive and time consuming.

Chapter 7

7. Summary, Conclusions and Recommendations

7.1 *Summary*

Despite the fact that tef is the most important indigenous cereal with various uses, its productivity is far below its expected genetic potential and that of other major cereal crops grown in Ethiopia. Exhaustive characterization, refining and establishing non-redundant core collections, assessing variation in food feed traits and tef growing environment characterizations are priority research areas in tef improvement. This section of the thesis, therefore, summarizes the overall findings of the four experimental chapters accordingly.

Chapter I and II dealt with the general introduction and literature review, respectively. In chapter-III, the genetic diversity was studied based on different phenotypic traits of tef genotypes obtained from various origins. The results from chapter-III revealed existence of wide ranges of qualitative and quantitative variations among individual genotypes, origins, and zones of collection, altitude and woredas. In the assessment of qualitative traits, variation in seed colour, lemma colour, panicle form, basal stalk colour and number of internodes were observed. Based on panicle form, for instance, five groups were observed with the loose panicle type covering the vast majority. Among the three categories, the white seeded group occupied the largest proportion followed by the brown type. As to lemma colour, the yellowish and the variegated group occupied the majority of the colour. The fact that the majority of the studied genotypes were of loose panicle form

gives two important advantages for breeders: 1) the chance of developing high yielding and adapted genotypes for future use; and 2) the chance of developing early maturing varieties to combat the problem of climate change. The wide range of quantitative traits variations were also observed for yield and yield related traits, phenological traits and morphological traits. Thus, genotypes with the highest mean grain yield, 1000-kernel weight, number of spikelets per panicle and number of fertile tillers per plant as well as shoot biomass were identified especially among collections from West Shewa and Central Tigray zones.

Chapter IV dealt with molecular diversity study of 189 tef genotypes using SSR markers. The result revealed that all studied markers were observed to be highly polymorphic (mean PIC = 0.80) to discriminate the test tef genotypes. Thus, the number of alleles per locus ranged from 2 for SSR 3.3 to 26 for CNLT 216. Markers like CNLT 538, CNLT 42 and CNLT 216 were found to have the largest number of alleles, PIC and gene diversity while SSR 3.3 had the least value for all genetic parameters. Populations having high mean gene diversity (North Shewa and North Gonder) and allelic richness (improved varieties) and number of private allele (East Tigray) were identified while the breeding lines had the least value for all genetic parameters. Clustering based on individual genotypes and population revealed formation of four clusters. Similarly, the model-based approach of structure software has also inferred four gene pools. Thus, the observed pattern of genetic diversity at population and individual genotypes level does not strictly follow the geographic origin, collection zones, altitudes or woredas. Regarding establishment of non-redundant core collections, 64 genotypes were found to be sufficient to retain 100% of the SSR diversity in the 189 studied tef genotypes. In general, both phenotypic and molecular markers based genetic

diversity study confirmed the existence of huge diversity among tef genotypes from diverse origins that can be utilized for further genetic improvement and conservation of tef.

Chapter V, on the other hand, dealt with the genotype by environment interactions, stability analysis and characterization of tef growing environments. Thus, environmental mean grain yields were ranged from 1.7 t/ha for E₃ (Alem Tena 2016) to 4.29 t/ha for E₅ (Debre Zeit 2015) whereas the genotypic mean grain yield ranged from 2.49 t/ha for Simada (G₂₉) to 3.33 t/ha for Melko (G₆). Among genotypes, variety Melko was identified as the highest yielding, relatively stable and ideal genotype to evaluate and rank performance of different environments relative to it. Regarding environment, Debre Zeit 2015 (E₅) was the highest yielding and ideal environment identified to evaluate and rank performance of different genotypes relative to it. The pattern of GGE biplot and the relationship between environments grouped the nine test environments into three mega environments whereas the 35 studied genotypes were grouped into seven genotypic groups. Thus, mega environment one consisted of E₁, E₄, E₅ & E₆, the second mega environment consisted of E₂, E₃, E₇ & E₈ while the third environment consisted of E₉ only. In this study, E₅, E₆, E₇ and E₈, which had the longest vector length, were identified to be the most discriminating of all environments whereas E₁ and E₄, which had the smallest angle with average environmental axis, were the most representative of all environments. Regarding genotypes, G₆, G₃₄, G₂₂ and G₃ were identified to be high yielding and stable while G₁₃, G₁₉, G₁₂ and G₉ were unstable.

Chapter VI dealt with the study of food-feed traits of tef and thus, the studied food-feed traits were significantly affected by genotypes, locations, years and their interactions. Besides, NDF, ADF and AD that negatively affect feed intake, CP, ME and IVOMD that positively correlated with feed intake and GY, STY and SBM which are related to food-feed yield were identified to appear in three distinct groups. The association among traits within each group was found to be positive and significant whereas the association between traits in group-I and that of the second and third group was negative and significant. However, the association between the traits in 2nd and 3rd group were positive and significant. The values of CP, ME, IVOMD, SBM and STY at Debre Zeit were higher than that of Holetta. The genotypes in the present study were generally grouped into four clusters such as those having better yield of food-feed traits; having better feed quality traits; having dual purpose; and those having less significance to food-feed traits. The economic analysis of growing representative genotypes from each group has revealed that Quncho followed by Gimbichu, Gola and Melko gave the highest overall economic return.

7.2 Conclusions

- This PhD thesis was conducted to analyze the phenotypic and molecular diversity, genotype by environment interactions and the food-feed traits variation among tef genotypes from various sources.
- Genetic diversity assessment based on phenotypic and molecular markers revealed the existence of significant genetic variation among the studied genotypes.
- Genotypes from central Tigray and west Shewa were identified to have the highest value for most yield related traits in tef.

- Besides, most studied genotypes were observed to have loose panicle form which is a good character to identify genotypes with high yield, earliness and adaptation.
- In the molecular diversity study, the employed SSR markers were found to have high polymorphic information content with a mean PIC ≥ 0.80 .
- North Shewa and North Gonder zones were identified to have high mean gene diversity whereas those from East Tigray zone had the highest mean number of private alleles.
- Besides, the molecular study has also enabled to establish a core set representing 33.9% of the studied genotypes and 100% of the entire allele without any redundancy.
- Stability analysis has revealed that variety Melko had the highest mean yield and relatively wider adaptation across a wide range of environment.
- This study, furthermore, enabled the identification of three mega environments for consideration in future variety development and release.
- The food feed traits variation study revealed existence of sufficient variation for food-feed traits among improved varieties.
- It was also possible to identify genotypes having better yield, better feed quality traits, dual purpose and no important food-feed traits.

7.3 Recommendations

The following recommendations are suggested based on the findings of phenotypic and molecular genetic diversity, genotype by environment interactions and food-feed traits study.

- The wide range of genetic variation observed in the present study is useful to bring the improvement expected in tef breeding.
- Genotypes identified to have highest yield related traits (Central Tigray and West Shewa) are useful for the improvement aiming at increasing tef productivity.
- SSR markers with high mean PIC ($PIC > 0.80$) are useful in future tef germplasm characterization and conservation ventures.
- Zones with high mean gene diversity (North Shewa and North Gonder) and high mean number of private allele (East Tigray) are very useful for future germplasm collection and *in situ* conservation.
- The established core set in the present study is a step forward for proper conservation and utilization of tef germplasm. However, future research should use more number and kind of markers especially in establishing core germplasm collections.
- Melko, a variety identified for better mean yield and wider adaptation, is a good resource in future development of better yielding and wider adapting genotypes.
- The four identified mega environments are useful for consideration in future variety development and release. However, the more the number of years studied, the more reliable conclusion and recommendation will be attained.
- The grouping of improved varieties into four categories based on the study of food feed traits is very useful for farmers and breeders to use them accordingly.
- Eventhough the present markers detected sufficient level of variation for the studied genotypes, future studies should use more number and kind of markers in germplasm characterization and establishment of core collections.

- Future studies should also include more accessions from the present study area as well as those un addressed areas in Ethiopia.
- Future tef breeding strategies should consider the development of both food-feed yield and quality traits to address the problem of both food and feed shortage.
- As the demand for tef is increasing at national and international level, future studies should focus on the identification of genotypes suitable for the non-traditional tef growing environments.

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APPENDICES

Appendix 9. Tables

Appendix Table 1. Regional, zonal and altitudinal distribution of the studied tef germplasm

Region	Zones	Altitudinal classes				Total
		<1500m	1501-2000m	2001-2500m	>2500m	
Tigray	Central Tigray	1	4	3	4	12
	East Tigray	0	1	7	4	12
	West Tigray	6	3	3	0	12
	North Gonder	0	5	7	0	12
	South Gonder	0	3	7	2	12
	North Wello	0	9	0	3	12
	South Wello	0	5	4	3	12
	East Gojam	1	2	3	6	12
Amhara	West Gojam	0	3	7	2	12
	North Shewa	4	3	0	5	12
	East Shewa	0	6	6	0	12
Oromia	West Shewa	0	5	4	3	12
	Total	12	49	51	32	144

Appendix Table 2. Distribution of studied tef germplasm by regions, zones of collection

Region	Zones	Collection years		Total
		Before 2000	After 2000	
Tigray	Central Tigray	4	8	12
	East Tigray	3	9	12
	West Tigray	4	8	12
	North Gonder	0	12	12
	South Gonder	7	5	12
	North Wello	11	1	12
	South Wello	5	7	12
Amhara	East Gojam	8	4	12
	West Gojam	2	10	12
	North Shewa	5	7	12
	East Shewa	7	5	12
	West Shewa	4	8	12
Oromia	Total	60	84	144

Appendix Table 3. Private allelic richness per locus & populations for 189 tef genotypes from various sources

Locus	CT	EG	ES	ET	NG	NS	NW	SG	SW	WG	WS	WT	Imp	Mut	Mean
CNLT 133	0.00	0.00	0.00	0.00	2.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.29
CNLT 216	0.00	0.00	0.00	2.00	1.00	0.00	1.00	1.00	0.00	1.00	0.00	0.00	0.00	0.00	0.43
CNLT 27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CNLT 538	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.00	1.00	1.00	1.00	0.00	0.00	0.36
CNLT 60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.07
CNLT 33	0.00	1.00	0.00	2.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.29
SSR 9.4	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07
CNLT 42	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.14
CNLT 150	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CNLT 157	0.00	0.00	0.00	2.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.14
SSR 3.3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CNLT 136	0.00	0.00	0.00	1.00	2.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.43
CNLT 315	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.14
CNLT 416	1.00	0.00	2.00	1.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	1.00	0.00	0.50
CNLT 438	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07
CNLT 484	0.00	0.00	0.00	2.00	0.00	1.00	0.00	0.00	2.00	2.00	1.00	0.00	0.00	0.00	0.57
Mean	0.06	0.13	0.13	0.63	0.44	0.31	0.13	0.06	0.25	0.31	0.19	0.25	0.19	0.00	0.22

CT=Central Tigray, EG= East Gojam, ES= East Shewa, ET=East Tigray, NG=North Gondar, NS= North Shewa, NW=North Wello, SG= South Gondar, SW= South Wello, WG= West Gojam, WS=West Shewa, WT=West Tigray, Imp= Improved variety, Mut= Mutant lines

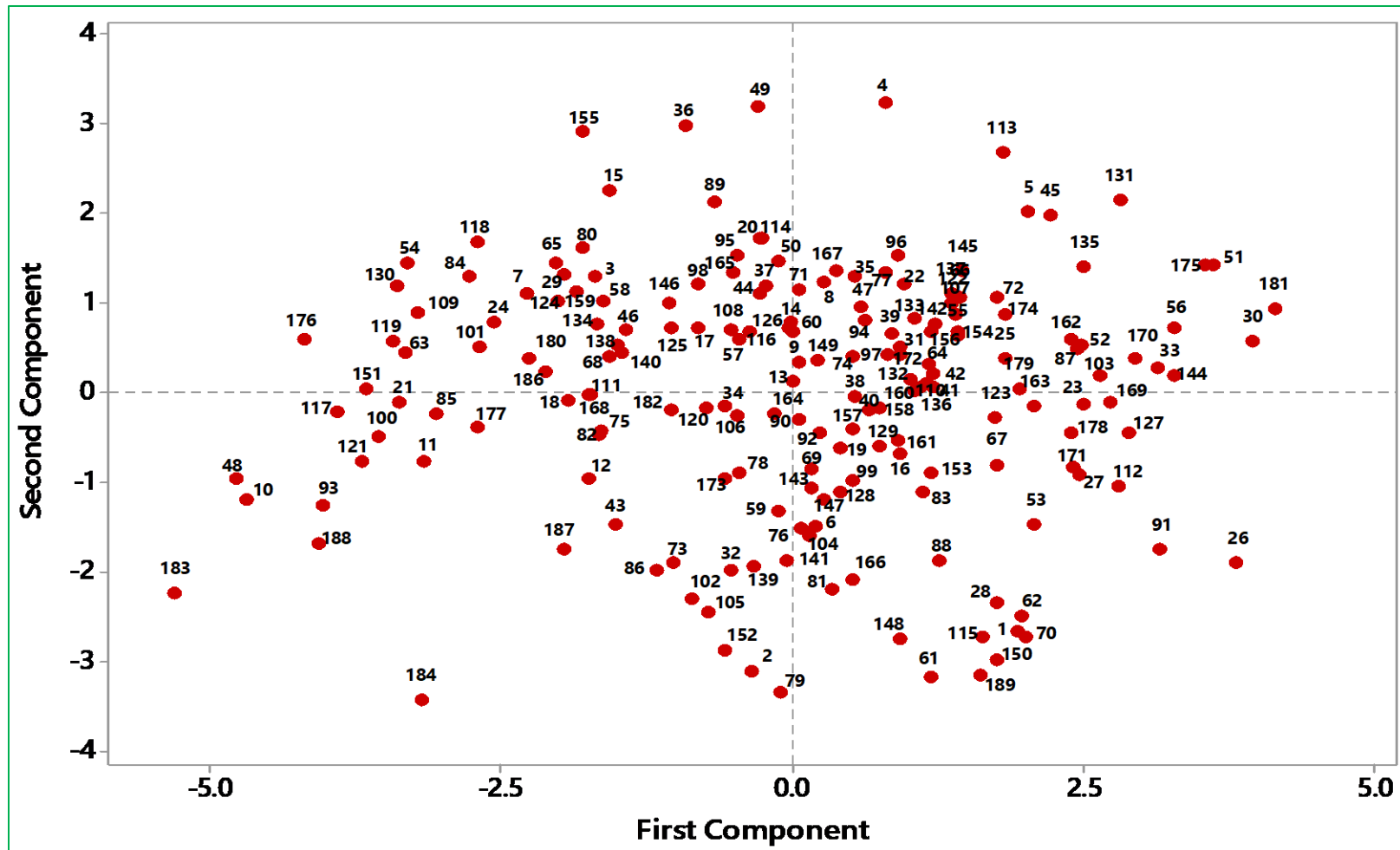
Appendix Table 4. Allelic frequency of each marker for 189 tef genotypes from various sources

Allele/Locus	CNLT_133	CNLT_216	CNLT_27	CNLT_538	CNLT_60	CNLT_33	SSR_9.4	CNLT_42
A	0.0029	0.011	0.1108	0.0028	0.0057	0.006	0.0054	0.0299
B	0.0029	0.0746	0.2243	0.0221	0.0575	0.0569	0.1102	0.0978
C	0.0143	0.1878	0.0595	0.0773	0.0603	0.0778	0.3306	0.0054
D	0.0086	0.0387	0.2784	0.047	0.0776	0.0479	0.3172	0.0136
E	0.0057	0.047	0.0946	0.1354	0.1121	0.0988	0.2124	0.0299
F	0.0171	0.0055	0.0676	0.0829	0.1552	0.2246	0.0242	0.0272
G	0.1514	0.0552	0.1162	0.1077	0.1609	0.1976		0.019
H	0.2543	0.0829	0.0486	0.1519	0.2184	0.006		0.019
I	0.1657	0.1436		0.011	0.1063	0.0928		0.0598
J	0.0486	0.0718		0.0221	0.023	0.0569		0.0027
K	0.0229	0.0497		0.0746	0.0172	0.0389		0.1467
L	0.0171	0.0138		0.0331	0.0057	0.015		0.1033
M	0.0086	0.0249		0.0028		0.015		0.0571
N	0.02	0.0304		0.0193		0.015		0.0543
O	0.0371	0.0304		0.0055		0.021		0.0516
P	0.0571	0.011		0.0166		0.012		0.038
Q	0.0943	0.0055		0.0055		0.009		0.0679
R	0.0429	0.0221		0.0028		0.003		0.0815
S	0.0171	0.0055		0.0525		0.006		0.0489
T	0.0057	0.0552		0.0663				0.019
U	0.0057	0.0249		0.0193				0.0136
V		0.0028		0.011				0.0136
W		0.0028		0.011				
X		0.0028		0.0055				
Y				0.011				
Z				0.0028				

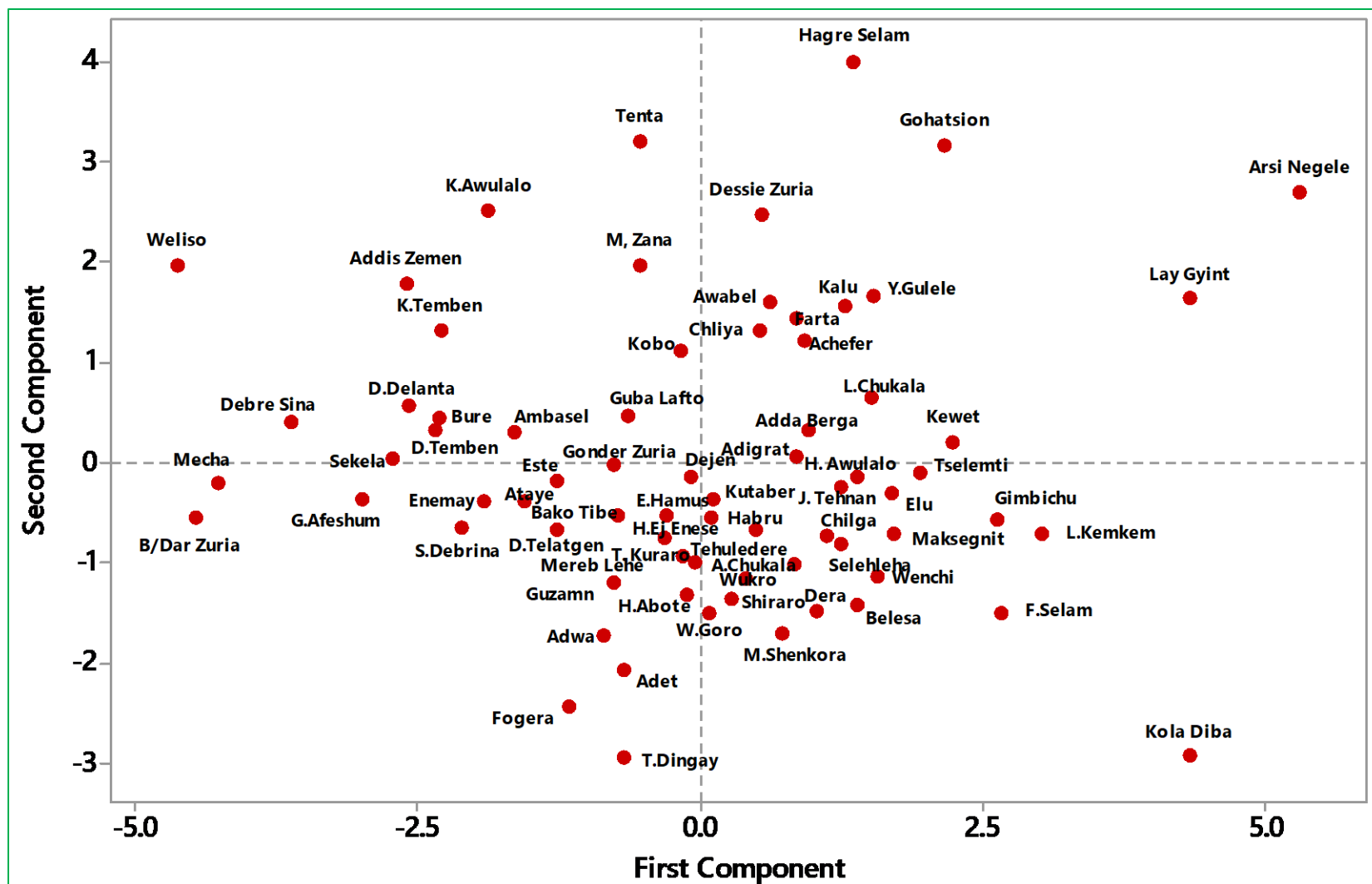
Appendix Table 4. Continued.

Allele/Locus	CNLT_150	CNLT_157	SSR_3.3	CNLT_136	CNLT_315	CNLT_416	CNLT_438	CNLT_484
A	0.1485	0.0056	0.2358	0.0068	0.5239	0.003	0.0806	0.0027
B	0.0333	0.1854	0.7642	0.0135	0.0133	0.1152	0.0548	0.0027
C	0.0061	0.0758		0.1453	0.0293	0.0152	0.1516	0.0108
D	0.0758	0.0674		0.0203	0.2101	0.0061	0.0194	0.2081
E	0.2424	0.191		0.0541	0.0186	0.0212	0.0194	0.0027
F	0.0394	0.3483		0.0135	0.1463	0.2364	0.0129	0.2054
G	0.0303	0.0646		0.0068	0.0106	0.003	0.0774	0.0703
H	0.0121	0.0393		0.098	0.0186	0.1879	0.0129	0.0135
I	0.0212	0.0169		0.0068	0.0027	0.0606	0.0323	0.0216
J	0.1121	0.0056		0.1014	0.0186	0.003	0.0935	0.0189
K	0.0091			0.0135	0.0053	0.0273	0.0226	0.0324
L	0.0364			0.1351	0.0027	0.0152	0.0516	0.0622
M	0.0121			0.0304		0.0061	0.1387	0.1135
N	0.2212			0.2669		0.0242	0.0903	0.0378
O				0.0608		0.1	0.0903	0.0027
P				0.0135		0.0273	0.029	0.0595
Q				0.0135		0.0061	0.0161	0.0676
R						0.003	0.0065	0.0162
S						0.003		0.0027
T						0.0091		0.0108
U						0.0121		0.0297
V						0.0091		0.0027
W						0.0091		0.0027
X						0.0909		0.0027
Y						0.0061		

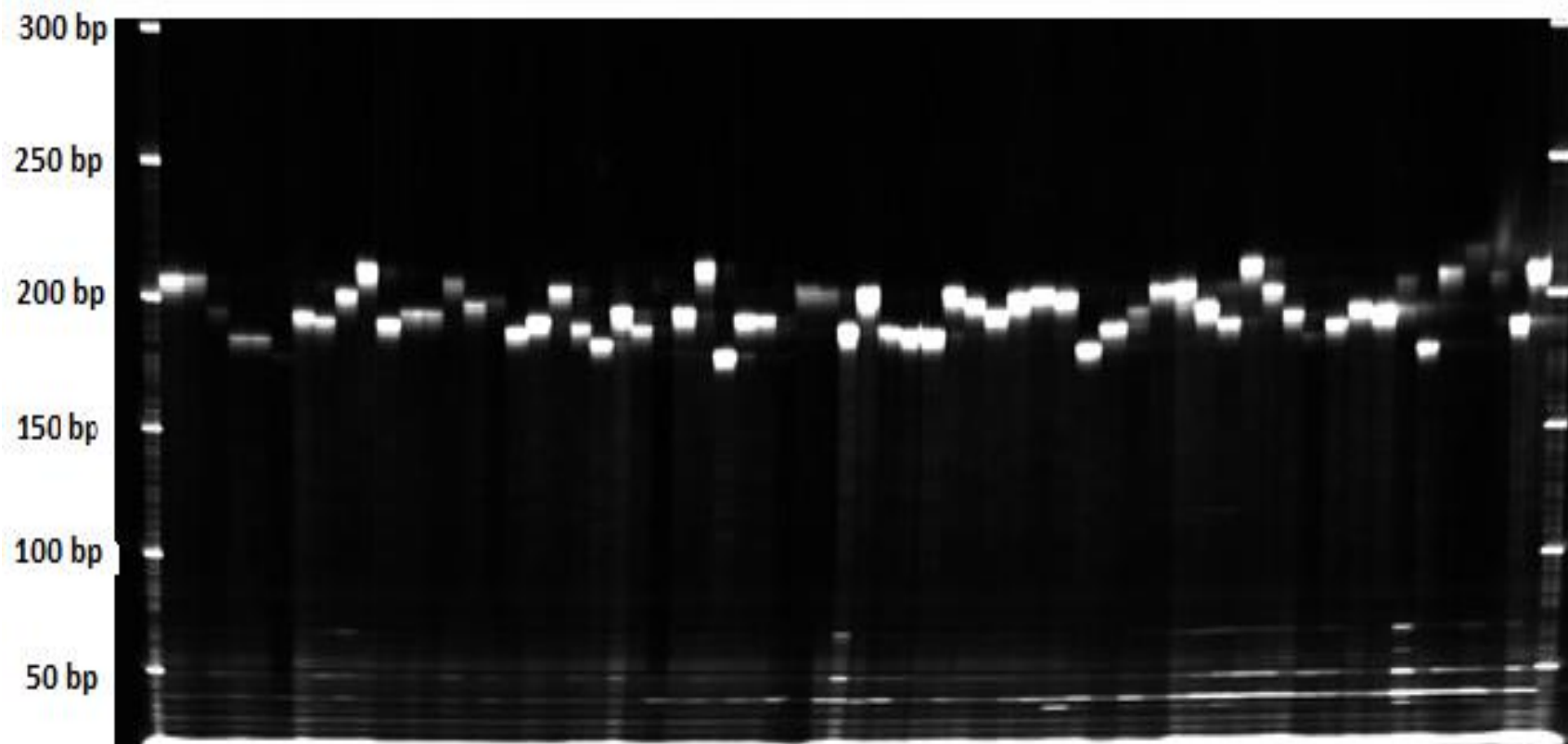
Appendix 9. Figures



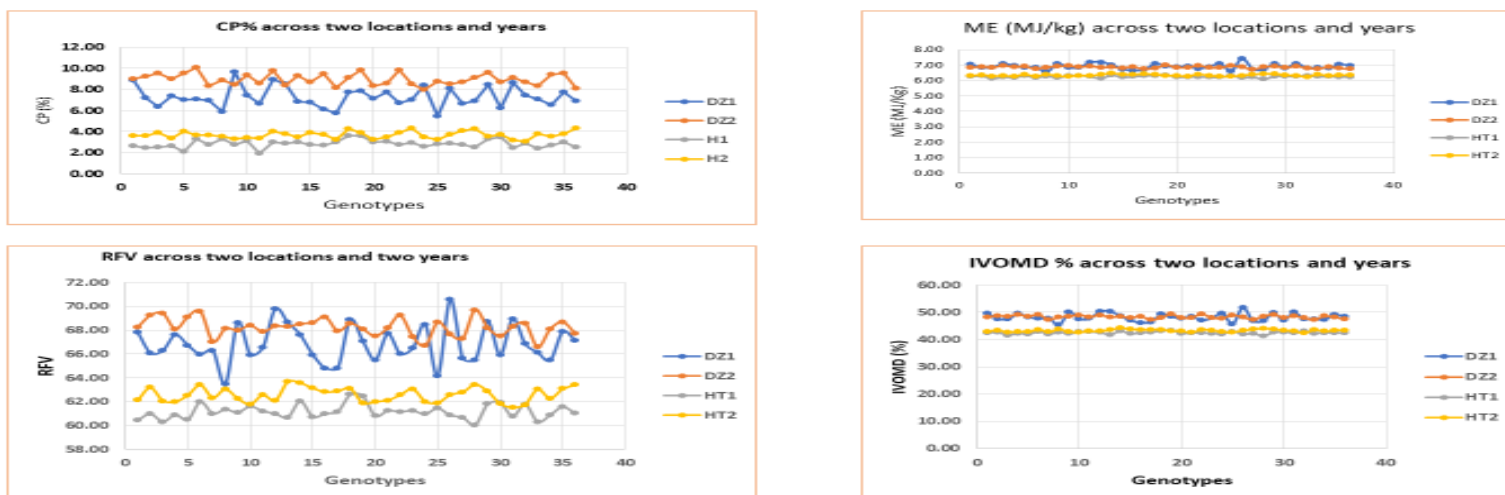
Appendix Figure 1. Distribution of 188 tef genotypes across the first two PCs



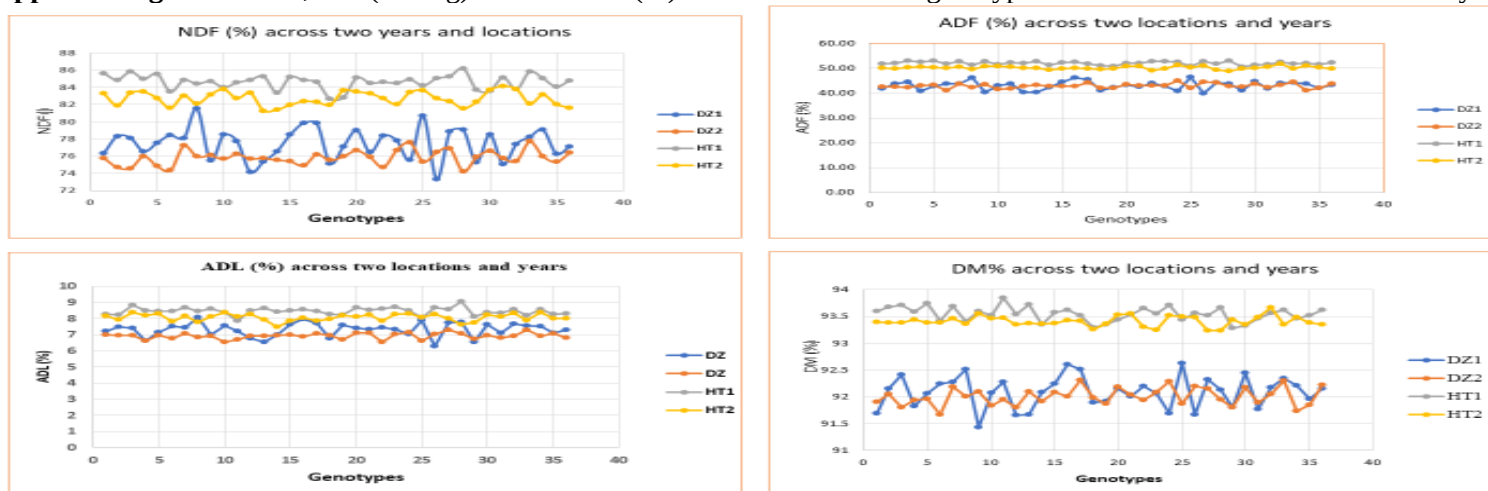
Appendix Figure 2. Biplot showing distribution of 72 Woredas of tef germplasm collection on PC₁ & PC₂



Appendix Figure 3. Partial view of LI-COR gel electrophoretic bands of DNA from 189 tef genotypes as revealed by polymorphic SSR marker, CNLT 42. Lane number 2 to 65 represent 64 samples of tef genotypes while lane 1 and 65 represent the DNA ladder with 50bp.



Appendix Figure 4. CP%, ME (MJ/Kg) and IVOMD (%) of tef straw from 36 genotypes evaluated at two locations for two years



Appendix Figure 5. NDF, ADF, ADL and DM% of tef straw from 36 genotypes evaluated at two locations for two years



Biography of the author

Habte Jifar Daba was born on July 12, 1970 in Illubabor Zone, Ethiopia. Initially, he graduated in Diploma from Addis Ababa University Awassa Junior College of Agriculture in Plant Science and Technology in July 1989. Upon graduation, he was assigned to Bako Agricultural Research Center as a Technical Assistant by the then Institute of Agricultural Research (IAR). He competed and got full scholarship opportunity from the then Alemaya University, now Haramaya University in 1998 to continue his study as an advanced standing student and obtained a BSc degree in Plant Sciences in 2001. After graduating with B. Sc. Degree, he was employed back to the Ethiopian Institute of Agricultural Research (EIAR) and assigned as a junior researcher to Jimma Agricultural Research Center in December 2001. After serving for five years, he allowed to pursue his post graduate study and obtained MSc degree in Biology (Applied Genetics) in 2008 from Addis Ababa University, Ethiopia. Habte resumed his career as a researcher again in EIAR where he is still working. Thus, Habte has served as a technician and researcher at various positions and capacities in EIAR. His research work mainly deals with the improvement of cereal crops like tef, maize and sorghum. Habte along with his research colleagues have released several improved varieties of maize and tef crops for the farmers. He has been among the tef improvement research team awarded the Gold Medal at the national level for outstanding accomplishment in 2013. He authored and

coauthored various peer reviewed research articles, conference and workshop proceedings. Habte is a breeder and member of the national tef research team in Ethiopia.

Publications

A. Peer reviewed journal published during this PhD Study

1. **Habte Jifar**, Kifle Dagne, Kassahun Tesfaye, Kebebew Assefa and Zerihun Tadele (2018). Agro-Morphological Traits Diversity in Tef [*Eragrostis Tef* (Zucc.) Trotter] Genotypes from Various Sources Ethiop. J. Agric. Sci, **28(3)**: 131-148.
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6. Mosisa W., Legesse W., Berhanu T., Girum A., Wonde A., Solomon A., Tolera K., Dange W., Girma D., Temesgen C., Leta Tulu, Habtamu Z., **Habte J.**, Alemu T., Fitsum S., Andualem W. and Belayneh A. Genetic Improvement of maize for Mid-Altitude and Lowland Sub-Humid Agro-Ecology of Ethiopia. **In:** Worku, M., Twumasi-Afriyie, S., Wolde, L., Tadesse, B., Demisie G., Bogale, G., Wegary, D. and Prasanna, B.M. (Eds.) (2012). *Meeting the Challenges of Global Climate Change and Food Security through Innovative Maize Research*. Proceedings of the Third National Maize Workshop of Ethiopia April 18-20, 2011; Addis Ababa, Ethiopia.
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