



**Genetic Diversity of Ethiopian Tef (*Eragrostis tef* (Zucc.) Trotter)  
Varieties as Revealed by Morphological and Microsatellite  
Markers**

**By**

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**DECLARATION**

I, the undersigned, declare that this thesis is my original work. It has never been submitted in any institution and all sources of materials obtained from other sources have been duly acknowledged in the thesis.

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## ABSTRACT

*Tef (Eragrostis tef (Zucc.) Trotter) is a gluten free cereal crop mainly grown in Ethiopia for food, feed and cash. This study was designed to assess the genetic diversity using morphological and molecular marker, and investigate trait association among tef varieties of Ethiopia. The experiment was conducted at two locations (viz. Debre Zeit and Alem Tena) for morphological study and National Agricultural Biotechnology Research Center, Holetta for molecular study in 2014/15 using 49 and 51 tef varieties based on 23 phenotypic traits and 9 microsatellites, respectively. In morphological study, combined analysis of variance over location revealed highly significant variation ( $P \leq 0.01$ ) among the tested varieties for all recorded traits except for plant height and genotype  $\times$  location interact significantly ( $P \leq 0.01$ ) on 11 of the traits. The phenotypic coefficient of variation ranged from 3% for days to maturity to 30% for grain yield per plant, and the genotypic coefficient of variation ranged from 0% for second basal culm internode diameter to 21% for number of fertile tiller per plant. Estimates of broad sense heritability varied from 0% for second basal culm internode diameter to 95% for days to heading. The expected genetic advance as percent of the mean varied from 0% for second basal culm internode diameter to 32% for number of spikelets per main shoot panicle. The cluster analysis grouped the varieties into seven major groups consisting of one to 29 varieties. The first six principal components with eigenvalues greater than one explained 76% of the total variation. Grain yield showed significant positive correlation with panicle length, culm length, number of fertile tillers per plant, number of spikelets and primary branches per main shoot panicle, thousand seed weight, grain yield per plant and biomass yield. On the other set of experiment, the molecular study detected a total of 52 alleles with a mean of six alleles per locus ranged from five to seven. With a mean 0.69 the polymorphic information content (PIC) ranged from 0.59 for marker CNLTs27 to 0.77 for CNLTs86. The gene diversity ranged from 0.65 to 0.80 with a mean of 0.73. The degree of polymorphism among the groups varies from 67% for improved varieties obtained from hybridization to 57% for wild relatives with the average of 63%. The lowest level of genetic differentiation and high level of gene flow were observed among the varieties obtained through hybridization and direct selection. Molecular analysis of variance higher percentage of variation within individuals (60%) and the variation among groups was the least (10%). Cluster analysis based on SSR data grouped the varieties into three major cluster groups. Principal Co-ordinate Analysis showed that the first three most informative principal coordinates accounted for 29% of the genetic variation. Generally, the present studies showed the existence of low genetic diversity among the released varieties which calls for timely action to widen the genetic diversity of Ethiopian released tef varieties. Moreover, both marker systems identified distinct varieties such Simada, Gola, Tsedey and Were Kiyu in the early maturing category that is suitable for terminal drought-prone area, and Etsub in late maturing category that is meant for optimum rainfall areas using both marker systems. The incorporation of these selected varieties and other divergent varieties as parent materials will strengthen the future tef breeding program through developing distinct varieties in the respective agro-ecologies.*

**Key Words:** Tef, *Eragrostis tef*, Genetic diversity, Morphological traits, Microsatellite markers, Trait Association.

**DEDICATION**

**This thesis is dedicated to my beloved Mother and Father ABEBECH TEKLEYES  
and FIKRE MULABACHEW, respectively, with gratitude.**

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**LIST OF ACRONYMS**

AARC	Adet Agricultural Research Center
AFLP	Amplified Fragment Length Polymorphism
AGRA	Alliance for Green Revolution in Africa
AMOVA	Analysis of molecular variance
ANOVA	Analysis of variance
ATA	Agricultural Transformation Agency
BARC	Bako Agricultural Research Center
BODA	British Overseas Development Association
CA	Cluster analysis
CSA	Central Statistics Agency
CTAB	Cetyltriethylammonium bromide
DANIDA	Danish International Development Agency
DAP	Diammonium phosphate
DNA	Deoxyribo nucleic acid
DZARC	Debre Zeit Agricultural Research Center
EIAR	Ethiopian Institute of Agricultural Research
ENS	Ethiopian National Survey
EST-SSR	Express Sequence Tag derived Simple Sequence Repeats
FAS	Foreign Agricultural Service
GA	Genetic advance
GCV	Genotypic coefficient of variation
GD	Gene diversity
HARC	Holetta Agricultural Research Center
IAEA	International Atomic Energy Agency
ISSR	Inter Simple Sequence Repeat
KBFA	Kansas Black Farmers Association
LSD	Least Significant Difference
MARC	Melkasa Agricultural Research Center

m.a.s.l	Meter above sea level
MF-CCRP	McKnight Foundation's Collaborative Crop Research Program
NABRC	National Agricultural Biotechnology Research Center
NJ	Neighbor Joining
PAGE	Polyacrylamide Gel Electrophoresis
PC	Principal component
PCoA	Principal Coordinate Analysis
PCR	Polymerase Chain Reaction
PCV	Phenotypic Coefficient of Variation
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length polymorphism
RIL	Recombinant inbreed lines
SARC	Sirinka Agricultural Research Center
SAS	Statistical Analysis System
SDS	Sodium Dodocayte Sulphate
SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeats
TEMED	Tetramethylethylenediamine
TILLING	Targeting Induced Local Lesion IN Genomics
TTU	Texas Tech University

## 1. INTRODUCTION

### 1.1. Background

Tef, *Eragrostis tef* (Zucc.) Trotter, is a member of the grass family Poaceae and genus *Eragrostis*. The genus *Eragrostis* constitutes about 350 species of which only tef is cultivated for human consumption (Watson & Dallwitz, 1992). Tef is an allotetraploid species with a base chromosome number of 10 ( $2n=4x=40$ ) with genome size of 730 Mbp (Mulu *et al.*, 1996). It is self-pollinated with chasmogamous and hermaphroditic flowers. It has very low degree of out-crossing, that ranges from 0.2% - 1.0% (Seyfu, 1997).

Tef is believed to have originated and diversified in Ethiopia (Vavilov, 1951). It represents a unique biodiversity component in the agriculture and food security systems of millions of poor farmers in Ethiopia. Its domestication is considered as one of the legacies of Ethiopian farmers to the world (Seyfu, 1997). The crop is grown outside Ethiopia only in few countries including Eritrea, Kenya (near Marsabit), Yemen, Malawi, and India. Tef is also grown as a fodder crop in other countries including USA, South Africa and Australia. Since very recently, however, the use of tef as a cereal for humans is exceeding the boundaries of Ethiopia and commercial production has begun in the United States and South Africa, whereas some farmers in Netherlands, Spain, Israel and Australia have also experimented with it (Kebebew *et al.*, 2011). It is considered as the “latest super food of the 21<sup>st</sup> century” such that its international popularity is rapidly growing (Collins, 2013).

Tef is produced for different purposes including food and feed, cash and foreign currency earnings. In Ethiopia it is the leading crop accounting for 29.71% of the total acreage and 20.12% of the gross grain production of all cereals grown in the country (CSA, 2015). The long continued wide cultivation of tef by the Ethiopian farmers has been accentuated by its versatile adaptation with good resilience to both drought and waterlogged soil conditions (Kebebew *et al.*, 2011). It is suitable for long-term storage without much damage by insect pests (such as weevils) and diseases. Tef performs excellently in areas with altitudes of 1700-2200 m.a.s.l, annual rainfall of 750 - 850 mm (growing season rainfall of 450-550 mm) and temperature ranges of 10-27°C (Seyfu, 1993).

The primary use of tef grain is for grinding into flour to make *injera* (a fermented, pancake-like, soft, sour, circular flatbread), a major staple food in Ethiopia. Nutritionally, tef contains 11% protein, 80% complex carbohydrate and 3% fat. It is an excellent source of essential amino acids, especially lysine, the amino acid that is most often deficient in grain foods (Piccinin, 2002). Naturally, tef being gluten-free has gained popularity worldwide as an alternative grain for persons with gluten sensitivity and for patients of celiac disease (Saturni *et al.*, 2010).

In spite of the indispensable importance of the crop, scientific tef improvement research program in Ethiopia was started only after the late 1950's to increase production and productivity through genetic improvement and appropriate cultural practices (Fuffa, 1998). Over the years, 36 improved varieties have so far been released for production by different Federal and Regional Agricultural Research Centers of the country (MoARD, 2016). As tef is native to Ethiopia, the source of genetic variation for effective breeding is limited to landrace collections and crossing of selected parents from the landraces with little or no opportunities of introduction and acquisition of breeding materials and other germplasm from foreign sources. Excessive reliance on genetic resources from the same origin as starting material in plant breeding may result in releases of varieties with a narrow genetic base having a uniform genetic background. Relying on varieties with a uniform genetic background, in turn, may make the crops vulnerable to harmful diseases, insects and adverse climatic changes (Singh, 2002). Information on the magnitude and pattern of phenotypic and genetic diversity among the released varieties should, therefore, be periodically assessed in order to put effective and efficient breeding strategies in place (Dale and Schatz, 2002).

Although a number of studies were done on the extent and pattern of genetic diversity in tef at morphological level (Melak-Hail *et al.*, 1965; Tadesse, 1975; Costanza *et al.*, 1979; Hailu *et al.*, 1990, 2003, 2008; Kebebew *et al.*, 1999, 2000, 2001a, b, c, 2003b; Tiruneh *et al.*, 2000; Temsgen *et al.*, 2005; Solomon *et al.*, 2006, 2009; Habtamu *et al.*, 2011; Ayalneh *et al.*, 2012; Plaza-Wüthrich *et al.*, 2013), and other few studies measured genetic diversity at the molecular level using various types of DNA markers RAPD (Bai *et al.*, 2000), AFLP (Bai *et al.*, 1999; Mulu *et al.*, 1999), ISSR (Kebebew *et al.*, 2003a) and SSR (Zeid *et al.*,

2012), most such studies were based on both germplasm collection, cultivars and selected released tef varieties. Indeed, knowledge on the extent and pattern of diversity encompassing all released tef varieties thus far is generally rare and was not assessed using both marker systems complementarily. In addition, the relationships between grain yield and its morpho-agronomic components that have been the basis for the genetic improvements achieved in varieties released so far have not been studied. This calls for a more comprehensive genetic diversity study involving all released varieties and both morphological and molecular markers so that it would be possible to generate useful information for designing effective and efficient future tef breeding strategies. Hence, this research was initiated with the following objectives:-

## **1.2. Objectives of the Study**

### **General objective**

- To study the extent of genetic diversity and pattern of distribution using both morphological traits and SSR markers, and investigate trait association among tef varieties of Ethiopia.

### **Specific objectives**

- To assess the extent and pattern of morphological diversity present in tef varieties in Ethiopia;
- To identify the major traits that contributed to the overall genetic diversity;
- To assess the extent and pattern of SSR diversity present in tef varieties in Ethiopia;
- To identify traits and their combination that are helpful to set the selection criteria for higher grain yield in tef breeding.

## 2. LETRATURE REVIEW

### 2.1. Botany, Nutritive Value and Uses of Tef

#### 2.1.1. Taxonomy

The word tef comes from Amharic word "*tefa*" meaning 'lost' since the grains are so small such that they get lost if dropped on the ground. It is also known by the vernacular names "*tafi*" in Oromifa and "*tahf*" in Tigrigna. The common vernacular name of tef is written in three forms: tef, teff and t'ef. But the form 'tef' is most widely used in scientific writings and is preferred as such since it is similar to the species epithet "tef" in the scientific name of the crop and there by avoids confusion (ANON,1887).

According to Costanza *et al.*, (1979), Attilio Zuccagni was the first to publish a botanical description of tef as a species and named it *Eragrostis tef* in 1775. In 1851, however, Jacquin described this same species as *Poa abyssinica* (Costanza *et al.*, 1979) in the genus *Poa*. The word *abyssinica* is historically referring to Ethiopia. Later, tef was thought to belong to the love grass genus, *Eragrostis* and accordingly named *Eragrostis abyssinica*. In 1918, Trotter rediscovered the original description of Zuccagni, hence, the current scientific name *Eragrostis tef* (Zucc.) Trotter featuring the two authors (Costanza *et al.*, 1979).

Tef belongs to the grass family Poaceae (formerly Gramineae), sub-family Eragrostoideae or Chloridoideae, tribe Eragrostidae, Sub-tribe Eragrostae and genus *Eragrostis*. It is the only cultivated cereal in the genus *Eragrostis* (Watson & Dallwitz, 1992), and together with finger millet (*Eleusine coracana* (L.) Gaertn.), they constitute the only two species in the sub-family Chloridoideae cultivated as a cereal crop.

#### 2.1.2. Morphology, physiology and genetics of tef

Tef is a C<sub>4</sub>, self-pollinated, chasmogamous annual cereal. It has a fibrous root system with mostly erect stems, although some cultivars are bending or elbowing types. The sheaths of tef are smooth, glabrous, open and distinctly shorter than the internodes. Its ligule is very

short and ciliated while its lamina is slender, narrow and nearly linear with elongated acute tips. It has a panicle type of inflorescence showing different forms from very loose, loose, semi loose, semi-compact to compact. The semi-compact to compact type is appearing like a spike. Its spikelet's have 2-12 florets. The caryopsis is 0.9-1.7 mm in length, and 0.7-1.0 mm in diameter, which is very small, and its color varies from white to dark brown (Tadesse, 1975).

The flowers of tef are hermaphroditic. Each floret has a lemma, palea, three stamens, an ovary and mostly two but in exceptional cases three feathery stigmas, and two lodicules that assist in flower opening. The degree of out-crossing in tef is very low, 0.2-1.0% (Seyfu, 1997). Study of many ovules before and after fertilization showed absence of any apomictic type of embryo formation (Melak-Hail and Guard, 1966). Fertilization was found to occur in the basal floret of a spikelet when that floret was at the base of the flag leaf blade. The maturation of flowers is basipetal on panicle and on each branch bases, while it is acropetal on the spikelet basis (Melak-Hail and Guard, 1966).

Tef is an allotetraploid with a chromosome number of  $2n=4x=40$ , and the basic chromosome number of the genus *Eragrostis* is  $x=10$  (Tavassoli, 1986). In a karyotype study made on 15 *Eragrostis* spp., it was shown that the chromosomes of tef are very small even by the standards of the genus. When two accessions of tef were observed, measurements of the largest chromosome were 1.6-2.9  $\mu\text{m}$  and of the smallest were 0.8-1.1  $\mu\text{m}$  with the range within each measurement group attributed to differences in condensation (Tavassoli, 1986).

### **2.1.3. Origin and distribution**

Tef (*Eragrostis tef* (Zucc.) Trotter) is a crop for which Ethiopia is the center of origin and diversity (Vavilov, 1951). Tef is endemic to Ethiopia and its major diversity is found only in that country. As with several other crops, the exact date and location for the domestication of tef is unknown. However, there is no doubt that it is a very ancient crop in Ethiopia, where domestication took place before the birth of Christ (Seyfu, 1997). According to Ponti (1978) tef was introduced to Ethiopia well before the Semitic invasion

of 1000 to 4000 BC. It was probably cultivated in Ethiopia even before the ancient introduction of wheat and barley (Shaw, 1976).

In the genus *Eragrostis*, 43% of the species seem to have originated in Africa, 18% in South America, 12% in Asia, 10% in Australia, 9% in Central America, 6% in North America and 2% in Europe (Costanza *et al.*, 1979). According to Cufodontis (1974), 54 species are found in Ethiopia, out of which 14 (or 26%) are said to be endemic. Recent estimates indicated that only 44 of the 350 *Eragrostis* species are found in Ethiopia (Phillips, 1995).

As tef is considered an allotetraploid crop (Tavassoli, 1986). However, there is no definite information regarding the diploid putative parents that contributed to the origin of tef. There are a number of close relatives of tef but the molecular-based studies suggested that *Eragrostis pilosa* is an allotetraploid species of tef closest relative and possibly the immediate wild progenitor of tef (Ingram and Doyle, 2003) The close relationship between tef and *Eragrostis pilosa* is also evidenced by the successful hybridization of these two species (Hailu *et al.*, 2003a).

Tef has been introduced to different parts of the world through diverse institutions and individuals. However, the sources differ about the date of tef's international footmark. In his monograph, Seyfu (1997) reported that the Royal Botanical Gardens, Kew, London, United Kingdom, obtained tef seeds from Ethiopia in 1866 and 1886 and distributed it to some countries in the British colonies, India, Australia, United States of America, South Africa and British Guyana. According to Tadesse, (1975), Burt Davy in 1916 introduced tef to California (USA), Malawi, Zaire, India, Sri Lanka, Australia, New Zealand and Argentina. Skyes in 1911 introduced it to Zimbabwe, Mozambique, Kenya, Uganda, and Tanzania. In 1940, Horwitz introduced tef to Palestine.

Currently, in the United States, the Kansas black farmers association is experimenting tef by both its link to Africa and its market potential (KBFA, 2014). Tef is also being grown in South Africa, India, Australia, the Netherlands, Spain, Israel and Canada for both human consumption and animal feed (Seyfu, 1997; Stallknecht, 1997; Roseberg *et al.*, 2005).

#### **2.1.4. Agro- ecology**

Tef is adapted to a wide range of environments, and is presently cultivated under diverse agro-climatic conditions. It can be grown from sea level up to 2800 m.a.s.l, under various rainfalls, temperature and soil regimes. However, according to experience gained so far from national yield trials, conducted at different locations across the country, tef performs excellently at an altitude of 1700-2200 m.a.s.l, annual rainfall of 750 - 850 mm, growing season rainfall of 450-550 mm and a temperature range of 10°C-27°C (Seyfu, 1993).

Tef grows in 11 of the 18 major agro ecological zones of Ethiopia (Kebebew *et al.*, 2001a). It suffers less from diseases, gives better grain yield and possesses higher nutrient contents, especially protein, when grown on Vertisols rather than on Andosols (Seyfu, 1993). Tef plants cannot compete with weeds especially during the young growing stage (Seyfu, 1997). It is best to start with a weed-free, clean field that has been ploughed frequently during the appropriate season in order to kill the weeds. The work should also start with clean tef seeds that are free of weed seeds (Seyfu, 1997). Depending on variety, tef is ready for harvest three to five months after sowing (Fuffa *et al.*, 2001).

#### **2.1.5. Nutritional value and use**

Tef is highly nutritious and is an important part of Ethiopia's cultural heritage and national identity. It is an excellent source of essential amino acids especially lysine, the amino acid that is most often deficient in grain foods. Tef contains more lysine than barley, millet, and wheat and slightly less than rice or oats (Jansen *et al.*, 1962). It is an important source of water soluble vitamins especially vitamins B1, B2, B3 and B6, and in contrast to other cereals tef also contains vitamin C (Kaleab, 2014). Tef is also an excellent source of fiber and high in mineral contents like Fe, Ca, Cu, Zn and Mg (Melak - Hail, 1966). Moreover, it is gluten-free and preferred food for persons with celiac disease, diabetics (slow release carbohydrates) and anaemia (Saturni *et al.*, 2010).

It is the smallest grain in the world, and it takes 150 grains of tef to equal the size of one kernel of wheat. The grain also gives high returns in flour of 99% compared to 60-80%

from wheat (Tadesse, 1969). There are three types of tef grains known as white, brown and mixed (brown and white) in the market.

In Ethiopia tef is traditionally used to make *injera*, which is a soft, porous, thin pancake, with slightly sour taste. It is commonly consumed with various meat and/or pulse sauces called *wot*. The flour is also used for the preparation of tef porridge, and un-raised bread called *Kitta* or *anebabero* (two over-laid *injeras*). Sometimes, the grain is also brewed into a native beer called *Tella* or *Fersso* and a more alcoholic traditional liquor, locally known as *arakie*, or *katikalla*. Tef straw is used as animal feed, binder of mud used for plastering local houses or huts, and to make local grain storage silos called *goteras* (Seyfu, 1997). There are several recipes that fit western palates have been developed from tef flour, particularly in the United States and Europe, where it has found niches in the health food market as a gourmet food. Tef flour is used as a thickening agent in a range of products including gravies, casseroles, soups and stews. It is also used as an ingredient in puddings, smoothie drinks and in baked goods such as cookies, muffins and crackers. In addition, tef grain, owing to its high mineral content, is now being used in mixture with soybean, chickpea and other grains in the baby food industry (Seyfu, 1997).

According to Seyfu (1993), Tef has remained an important crop to Ethiopian farmers for several reasons, namely: the prices for its grain and straw are higher than other major cereals; the crop performs better than other cereals under moisture stress and waterlogged conditions; its grain can be stored for a long period of time without being attacked by weevils and the straw is a nutritious and highly preferred feed for cattle compared to other cereals.

## **2.2. Distribution and Yield Constraints of Tef in Ethiopia**

Tef is the dominant cereal in Ethiopia ranking first in area coverage (accounting for 28% of the area) and second to maize in terms of volume of production (Table 1).

Table 1. Area planted and production of the main cereals grown in Ethiopia

Crop	Area (1000 ha)	Production (1000 MT)
Tef	3760	3769
Maize	2150	5500
Sorghum	1780	3570
Wheat	1510	3200
Barley	1015	1620
Total	10215	17,659

Source: FAS (2014).

There are 19 major tef producing zones in Ethiopia. The central and south Tigray zones are the major tef producing zones in Tigray. Within the Amhara region, East Gojjam, West Gojjam, North Gonder, South Gonder, North Wollo, South Wollo, North Shewa and Awi Zones are the major producers of tef. Likewise, in the Oromia region, the major tef producing zones include East Shewa, West Shewa, South West Shewa, North Shewa, East Wellega, Horo Guduru Wellega, Jimma, Illubabor and Arsi Zones. In addition, tef is also produced in Southern Nations, Nationalities and Peoples major producing areas were North Omo, South Omo, Gurage, Silitie, Hadiya, Wolayita, Kambata, Alaba-Tembaro and Keficho-Shekicho. Similarly in Benishanul-Gumuz, includes Matakkel, Assosa and Mao Komo zones of the country (Table 2).

Table 2. Area planted, production and yield of tef in Ethiopia

Region	Area (ha)	Production (t)	Yield (t/ha)
Tigray	184,848.49	2,636,01.790	1.426
Amhara	1,143,115.98	18,093,67.086	1.583
Oromia	11,420.74	159,87.522	1.400
SNNP	238,539.40	3,268,60.266	1.370
Benishangul-Gumuz	21,409.26	246,59.144	1.152
Average	319,866.77	488,095.1616	1.526

Source: CSA (2014).

Despite its indispensable importance in the Ethiopian agriculture, the production and productivity of tef is low with the national average standing at 1.5 t/ha (CSA, 2014). The major yield limiting factors are lack of cultivars tolerant to lodging, drought and pests (Kebebew *et al*, 2011). Besides, the grains are also often lost in the harvesting and threshing process because of their minute size (Tadesse, 1975). Tef possesses tall, weak stems that

easily succumb to lodging due to wind or rain. In addition, Lodging hinders the use of high input husbandry since the application of increased amounts of nitrogen fertilizer to boost the yield results in severe lodging (Kebebew *et al.*, 2015). It is found to reduce grain yield by about 30% (Bekabil *et al.*, 2011). When this occurs, both the yields the quality of the grain and the straw are severely reduced, and both manual and mechanical harvesting is hampered (Kebebew *et al.*, 2015). Using lower seed rates and late sowing dates relatively decreases the problem of lodging. Although, various attempts have been made by the research community to develop lodging-resistant tef cultivars (Kebebew *et al.*, 2011; Zerihun and Kebebew, 2012) no cultivar with reasonable lodging resistance has been obtained to-date.

### **2.3. Overview of Tef Research and Development in Ethiopia**

Tef is an “orphan” crop meaning that it has not been subject of much research and development work. Scientific tef improvement research in Ethiopia was started in the late 1950's in Jimma Agricultural Technical High School, and later moved to Debre Zeit Agricultural Research Center (DZARC). DZARC began tef research in 1956-57 and presently it is the center of excellence for tef research in the Ethiopian Institute of Agricultural Research (EIAR). However, it has not been considered as an important crop by the international scientific community or funding agencies for a long period of time. The crop began gaining some attention at the beginning of the mid-1990s through the McKnight Foundation's Collaborative Crop Research Program (MF-CCRP) and International Atomic Agency (IAEA). Several other international and foreign institutions that have been supporting tef research in Ethiopia include the University of Bern, Texas Tech University (TTU), Cornell University through McKnight foundation collaborative crop research program (MF-CCRP), Alliance for Green Revolution in Africa (AGRA), British Overseas Development Association (BODA), and the World Bank and more recently Danish International development Agency (DANIDA) through the Ethiopian Agricultural Transformation Agency (ATA) (personal communication).

In the overall history of tef breeding, three major inter-related phases are documented. The first phase (1956-1974) which was described by an emphasis on germplasm enhancement

(collection/acquisition, characterization and evaluation, and conservation) and the genetic improvement work. This depended entirely upon mass or pure-line selection directly from the existing germplasm and initiation of induced mutation techniques. The second phase (1975-1995) was characterized by the incorporation of intra-specific hybridization into the already pre-existing breeding methods following the discovery of the chasmogamous floral opening behavior of tef flowers (from about 6:45–7:30 AM) and there by the artificial hybridization technique by Tareke (1975). The third phase (1995- until now) has been featuring initiation of molecular approaches (development of molecular markers and genetic linkage maps, analyses of molecular genetic diversity), further incorporation of *in vitro* culture techniques and inter-specific hybridization (Hailu *et al.*, 2003) along with re-appraisal of induced mutagenesis particularly for lodging and leaf rust disease resistance and introduction of participatory breeding approaches in the overall tef genetic improvement schemes (Getachew *et al.*, 2006, 2008).

Beginning from 1970, a total of 36 improved tef varieties have been officially released to the farming community in Ethiopia mainly based on their grain yield performance (MoARD, 2016). From those improved tef varieties, 21 of them have been released from the Debre Zeit Agricultural Research Center. Of which, 11 varieties were obtained from the hybridization program while the remaining ten resulted from direct selection from the indigenous germplasm accessions. Of the remaining released varieties, six varieties were from Srinika Agricultural Research Center, three varieties were from Adet Agricultural Research Center, two varieties each were from Holetta and Bako Agricultural Research Centers, and one variety each were from Melkasa and Areka Agricultural Research Centers. Of the total of 36 improved varieties released in Ethiopia, only 13 were developed through hybridization technique and the remaining were developed through pure line and/or mass selection. Among the 13 varieties resulting from crossing, only one variety (Simada) developed through inter-specific crossing between a selected tef line (DZ-01-2785) and *E.pilosa* (Personal Communication).

## 2.4. Genetic Diversity Study

### 2.4.1. Perception of genetic diversity in crop plants

Genetic diversity refers to both the enormous number different species as well as the diversity within a species. It is defined as the variety of alleles and genotypes present in a population and this is reflected in morphological, molecular, physiological and behavioral differences between individuals and populations (Farook and Azam, 2002). It is also broadly referred to as variation in the nucleotide, genes, chromosomes, or genomes of a species at a level of individual, population, species, or region for a given time.

It is basically the first step of plant breeding program and the information generated on the genetic diversity within and among closely related crop species is essential for a rational use of genetic resources. The analysis of genetic diversity can be a useful tool to get information about the genetic diversity of the varieties/stocks and possibly change the direction of breeding programs (Khleshtkina *et al.*, 2004). It is particularly useful in characterizing populations, plant varieties and species, in detecting duplications of genetic materials in germplasm collections, and for studying the evolutionary ecology of populations. Similarly, genetic diversity is essential to meet the diversified goals of plant breeding such as breeding for increasing yield, wider adaptation and desirable quality. The greater the genetic diversity within a species implies the greater that species chance of long-term survival and flourishing (Frankel *et al.*, 1995).

Evaluating the level and structure of genetic diversity in crops is a prerequisite for plant breeding and genetic resource conservation program. The major breeding efforts of the twentieth century have been a strong force in the reduction of crop genetic diversity (Gepts, 2006). It is generally thought that continuous selection among crosses of genetically related cultivars led to a narrowing of the genetic base of the crops on which modern agriculture is based, contributing to the genetic erosion of the crop gene pools on which breeding is based (Plucknett *et al.*, 1987). In the past, genetic uniformity of crops has led to several devastating attacks of pests and diseases (van de Wouw *et al.*, 2010).

Generally, several research results indicated that studying the extent and patterns of distribution of genetic variation of a crop species is essential for effective utilization of germplasm in plant breeding programs (Endashaw, 1983; Abebe and Bjornstrand, 1996).

#### **2.4.2. Measurement of genetic diversity**

Measurements techniques of genetic diversity were initially based on co-ancestry and pedigree records (Kim and Ward, 1997). Then, measurements were done on morphological markers, mostly agro-morphological markers; these were complemented by proteins (isozymes) which, in turn, have been supplemented partly by molecular markers (Griffiths *et al.*, 1996).

There are now different techniques that can be used to assess genetic diversity. The most widely employed measurement on morphological marker was partitioning the observed overall phenotypic variation into heritable and non-heritable components which enables to know whether the superiority of selection is inherited by the progenies or not (Firas *et al.*, 2012). Information regarding the genetic parameters such as variation coefficient, heritability and expected genetic advance are of permanent significance in exploiting the inherent diversity in the experimental materials (Firas *et al.*, 2012). Moreover, the most commonly employed measurements on molecular assessments are gene diversity or heterozygosity, the number of alleles per locus, the proportion of polymorphic loci, allele and genotypic frequencies. For any ploidy level and reproductive system, the Nei (1972) gene diversity index can be used to estimate the diversity that exists in a population.

#### **2.4.3. Studies of tef genetic diversity**

Most of the studies to-date were on selected tef genotypes have been based on the diversity of agro-morphological traits (Melak- Hail *et al.*, 1965; Tadesse, 1975; Costanza *et al.*, 1979; Hailu *et al.*, 1990, 2003, 2008; Kebebew *et al.*, 1999, 2000, 2001a, b, c, 2003b; Tiruneh *et al.*, 2000; Temsgen *et al.*, 2005; Solomon *et al.*, 2006, 2009; Habtamu *et al.*, 2011; Ayalneh *et al.*, 2012; Plaza-Wüthrich *et al.*, 2013), and these studies showed wide agro-morphological diversity of the different traits. However, environmental influence is

the limitation of this measurement. Complement to morphological markers, little protein (isozyme) studies have been applied on selected tef varieties (Endashaw and Lester, 1981; Endashaw *et al.*, 1995) and seed proteins revealed wide variation in tef. Some other studies measured genetic diversity on selected tef genotypes at the molecular level using various types of DNA markers such as RAPD (Bai *et al.*, 2000), AFLP (Bai *et al.*, 1999; Mulu *et al.*, 1999), these studies indicated low level of polymorphism among tef cultivars and accessions. In contrast, utilization of ISSR (Kebebew *et al.*, 2003a) and SSR (Zeid *et al.*, 2012) markers revealed promising level of polymorphism among tef genotypes. Those studies concluded that low level of polymorphism observed in the previous studies was marker related issue and SSR would provide better information for future tef diversity study (Zeid *et al.*, 2012).

Generally, molecular markers provide better estimate of genetic diversity among tef genotypes, and increase the resolution of measurement of genetic variation. All the markers that were used previously in the tef diversity study have shed light on the structure of allelic diversity within the selected genotypes (Dejene *et al.*, 2014). The genetic similarity estimated using RAPD ranged from 84-96%, AFLP from 73-99%, ISSR from 26-86% and SSR from 0.21-0.99. Correspondingly, comparison of AFLP, EST-SSR, ISSR, and SSR markers for polymorphisms in tef recombinant inbred lines depicted that EST-SSR and ISSR makers had as much polymorphism as AFLP markers (Solomon *et al.*, 2007).

#### **2.4.4. Heritability and genetic advance**

Heritability refers to the proportion of variation observed for a particular trait between individuals in a given population that is due to genetic factors. Depending on the number of variance used as a numerator in the calculation, heritability could be grouped into two; namely: broad-sense; and narrow-sense heritability. Broad-sense heritability is the degree to which a trait is genetically determined, and it is expressed as the ratio of the total genetic variance (additive and non-additive) to the phenotypic variance (Burton and Devane, 1953). Narrow-sense heritability is the degree to which a trait is passed from parent to offspring, and is expressed as the ration of additive genetic variance to the total phenotypic variance (Burton and Devane, 1953). Heritability is of interest to plant breeders primarily

as a measure of the value of selection for a particular trait in various types of progenies and as an index of transmissibility (Hayes, 1955). According to Singh (2001), if heritability of a trait is very high being around 80% or more, selection for such trait would be fairly easy since there is close correspondence between genotype and phenotype due to relatively small influence of the environment on the phenotype. Although, for characters with low heritability, say 40% or less, selection may be considerably difficult or virtually impractical due to the masking effect of environment. According to Allard (1960), if a trait has high heritability, it indicates that the influence of the environment on the trait is less. Heritability values vary with the nature of the test materials and the area where the experiment is conducted (Habtamu *et al.*, 2011).

Genetic advance refers to the improvement of mean genotypic value of a population for a particular trait towards the desired path as a result of selection (Bello *et al.*, 2012). It measures the genetic gain that would result from selecting the best performing genotype for a given trait (Hamdi *et al.*, 2003). The success of genetic advance depends on genetic variability, heritability, and selection intensity (Allard, 1960). If heritability associated with equally high genetic advance is chiefly due to the additive gene effect but if heritability is mainly due to dominance and epistasis the genetic gain would be low (Panes, 1957)

Mostly, genetic variability, heritability and genetic advance are pre-requisites for breeding program, and offer opportunity to plant breeders for selecting high yielding genotypes or to combine or transfer genes having desirable traits (Khorgade, 1985). Heritability and genetic advance are important aspects to determine the success of selection in breeding programs (Dagnachew *et al.*, 2012). Heritability estimates along with expected genetic gain is more useful than the heritability value alone in predicting the resultant effect for selecting the best genotypes (Johnson *et al.*, 1955). High estimates of heritability with relatively high genetic advance value can be used as an indicator for the ease of phenotype-based selection (Kebebew *et al.*, 2001a). However, this does not mean that high heritability and genetic advance values guarantee success in selection because resemblance between relatives is controlled by the proportion of the additive genes, not by all of the genetic variation (Falconer and Mackay, 1996).

Based on field evaluation of selected tef genotypes various scholars investigated high heritability estimates along with greater values of genetic advance. Hailu *et al.*, (1990, 2003) reported for number of spikelets per main panicle, panicle seed weight, panicle weight and grain yield while Kebebew *et al.*, (2000, 2001a) described for panicle length and number of fertile florets per spikelet. In other studies were noted for grain yield, aboveground plant biomass and panicle seed weight (Solomon *et al.*, 2009), for culm length (Habtamu *et al.*, 2011), and for days to heading (Ayalneh *et al.*, 2012). Frequently, lodging and lodging related traits such as culm length, length and diameter of the culm internodes showed relatively low heritability and genetic advance estimates than the other traits (Kebebew *et al.*, 2015). This suggested that breeding for lodging resistance in tef would be a demanding task (Kebebew *et al.*, 2000, 2001a; Hailu *et al.*, 2003; Solomon *et al.*, 2009).

#### **2.4.5. Trait association**

In addition to heritability and genetic advance, phenotypic and genotypic correlations are also the key parameters in the selection of superior genotypes, and to evaluate alternative breeding strategies (Falconer, 1989). Trait association or correlation is a technique that determines the interrelationship between various traits, and gives a better understanding of the contribution of each trait in the genetic makeup of the crop (Kimani, 2000). If two plant traits are measured to represent crop response, response in one trait may affect the other, or treatment effects may simultaneously influence both traits (Falconer, 1989). Any component of trait does not act independently; sometimes it reacts parallel to other component, sometimes controls each other, and acts in paradox compensating for either an increase or decrease in the other component (Matusuhima, 1970). Correlation analysis provides a measure of the degree of association between the traits or the goodness of fit of a prescribed relationship to the data at hand (Gomez and Gomez, 1984). Phenotypic correlation measures how different traits co-vary across phenotypes whereas genetic correlation measures the degree to which different traits are controlled by the same gene or genes that are close linked (Balcha *et al.*, 2003). Genetic and phenotypic correlations are used to forecast how selection for one trait influences response in another trait (Hardner *et al.*, 2001).

In tef, correlation studies have been made by various scholars. Hailu *et al.*, (2003) reported that grain yield showed a positive and significant correlation with main panicle seed weight, loose panicle form, panicle length, plant height, panicle weight, tiller number, biomass yield and lodging index. Besides grain yield, panicle weight and grain yield/plant showed strong positive correlation with panicle length and plant height. Lodging index exhibited a strong negative correlation with days to heading and maturity. Likewise, Yifru and Hailu (2005) reported that grain yield was significantly and positively correlated with biomass yield, number of spikelets/panicle and panicle yield. Conversely seeds/spikelet showed a weak negative correlation with grain yield. Habite *et al.*, (2015) reported highly significant and positive genetic correlation of grain yield with shoot biomass and harvest index. They also noted highly significant and positive phenotypic correlations of grain yield with phenologic traits, panicle length, shoot biomass and harvest index. On the other hand, plant height and culm length showed negative and highly significant association with grain yield. Lodging index showed positive and significant phenotypic association with culm length while it had significant and negative association with phenologic traits, plant height, culm length, grain yield and harvest index.

## **2.5. Genetic Markers in Crop Improvement**

Genetic markers can be used to study population, species or individuals and allow us to characterize accession of crop species. Traits that serve as genetic markers are by definition polymorphic; the more polymorphic the trait, the greater its potential value to germplasm management (Seetharam *et al.*, 2004). Markers are entities that are heritable as simple Mendelian traits and are easy to score (Schulman *et al.*, 2004). Three types of marker systems could be recognized as morphological, biochemical and molecular markers systems. Each of these has got different attributes that makes it more or less desirable to use in certain applications.

### **2.5.1 Morphological markers**

Morphological markers are the earliest of all markers that are used for assessment of genetic variation and still have great importance. Morphological markers are a marker

system based on phenotypic appearance. Moreover, morphological traits used as markers had several advantages these includes they are easily describable and facilitate enumeration, their detection and use are simple and inexpensive (Chittaranjan and Albert, 2008). Unlikely, morphological marker systems also have several drawbacks including the delay of marker expression until late into the development of organism, dominance which makes it impossible to distinguish homozygous dominant and heterozygous individuals because of dominant intra-allelic interactions (Chittaranjan and Albert, 2008). The few number of characters that can be detected (lower polymorphism), and the confounding effects of genes unrelated to the gene of interest and environmental factor which also affect the morphological marker. Moreover, some morphological traits are observed to be very plastic in nature, which could be easily influenced by environmental change (Vithanage *et al.*, 1995). Despite their limitations, morphological markers are well established tools for taxonomy, variety classification and breeding. The limitation to their application can be overcome by supplementation with the use of biochemical and molecular markers (Shabir *et al.*, 2013).

### **2.5.2. Biochemical markers**

Biochemical markers are markers based on protein polymorphisms through electrophoretic separation of protein molecules (Markert and Moller, 1959). The most known protein markers are isozyme/allozyme markers. As early as the 1970s, protein markers (enzymes) were mainly used as molecular markers since they represent enzyme variant. The analyses require the extraction of proteins from plant tissues, followed by their separation using gel electrophoresis (Schulman *et al.*, 2004). Depending on the number of loci, their state of homo-or heterozygosity, and the enzyme configuration (i.e. the number of separable units), from one to several bands are visualized (Schulman *et al.*, 2004). The position of these bands can be polymorphic and can be considered as informative loci. The main advantages of protein markers are their co-dominant inheritance with high reproducibility and low cost of the assay (Weising *et al.*, 2005). The weakness of biochemical markers includes the relatively small number of genetic loci that can be assayed in any population (low level of polymorphism), technical complexity (since each protein system requires its own electrophoretic and staining protocol) and problems associated with specificity to tissue

type, physiological age or developmental state of the tissue (Chittaranjan and Albert, 2008). Moreover, biochemical markers are intermediate between morphological and molecular markers and they may be affected by environmental factors (Parker *et al.*, 1998). Perhaps, the banding profile obtained for particular allozyme marker may change depending on the type of tissue used for assay such that the result of the gene that is being expressed in one tissue might not be expressed in another tissue (Krieger and Ross, 2002). In other words, the major shortcomings of this marker system are lower polymorphism, lower genome coverage and technical difficulties (Schulman *et al.*, 2004).

### **2.5.3. Molecular/DNA markers**

Molecular markers are markers that are working based on naturally arising polymorphism in DNA sequence. It contains a large variety of DNA molecular markers, which can be employed for analysis of variation. They are better than both morphological and biochemical markers because of their frequent occurrence throughout the genome, highly polymorphic nature, independence from environmental effects, and possibility of detection at any stage of plant development (O' Neill *et al.*, 2003). They provide an opportunity to characterize varieties and to measure genetic relationships more precisely than other markers (Soller and Beckmann, 1983). In addition, molecular markers can be used in the identification of cultivars and clones, genetic mapping, marker assisted selection, and molecular systematics (Weising *et al.*, 2005). There are certain desirable features of ideal molecular marker, and these are polymorphism, co-dominance, no phenotypic plasticity, frequent occurrence in the genome, even distribution throughout the genome, high reproducibility, easy and fast assay, and easy to exchange data between laboratories (Karp *et al.*, 1997; Weising *et al.*, 2005). Unfortunately, it is difficult to find molecular markers which could adequately meet all the ideal properties of the molecular markers (Lowe *et al.*, 2004). However, based on the particular type of study, one can still choose among the different molecular marker systems to find the one that best fits to the desired study.

A vast array of molecular markers has been discovered since the 1980s and new marker types are being developed continually. They arise from different classes of DNA mutations such as substitution mutation, point mutation, rearrangements, insertion and deletion; or

errors in replication of tandem repeated DNA. Molecular markers are generally classified as hybridization-based (non-PCR) markers and (PCR) based markers (Weising *et al.*, 2005).

#### **2.5.3.1 Non-PCR based molecular markers**

The major widely used non-PCR based molecular marker is restriction fragment length polymorphism (RFLP). This was developed by Botstein *et al.*, (1980). It is based on the differential hybridization of cloned DNA to DNA fragments in a sample of restriction enzyme digested DNA. The marker is specific to a single clone/restriction enzyme combination. RFLP is reliable marker in linkage analysis, genetic mapping, genetic fingerprinting and variation analysis. The RFLP marker system has got certain advantages including the need for no prior sequence information, abundant distribution in genome of most species, high reproducibility and co-dominance nature (Karp *et al.*, 1997). Conversely, their utility has been hampered due to the requirement of large amount of DNA for restriction enzyme and southern blotting, characterization of probe, the requirement of radioactive isotopes that makes the analysis relatively expensive and hazardous, the time-consuming and labor-intensive nature of the assay, and the fact that one out of several markers may be polymorphic, which is highly inconvenient especially for crosses between closely related species (Swati *et al.*, 1999).

#### **2.5.3.2 PCR-based molecular markers**

The development of polymerase reaction (PCR) has resulted in the development of a large number of molecular techniques. The technique could be classified into two main groups as arbitrary/ or semi-arbitrary primed sequence and site-targeted primed sequences (Karp *et al.*, 1997; Weising *et al.*, 2005).

The arbitrary /or semi-arbitrary (i.e. without the knowledge of flanking sequence of the region to be amplified) technique includes various types of markers such as random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and inter simple sequence repeats (ISSR). Inclusively, the afore-listed markers have

common approaches such that they have multi-locus, dominant and scattered throughout the entire genome (Lowe *et al.*, 2004).

**Random Amplified Polymorphic DNA (RAPD):** This method was described by Welsh & McClelland (1990) and Williams *et al.*, (1990). RAPD markers are decamer (10-nucleotide length) that a single oligonucleotide is used to prime the amplification of genomic DNA. It is a dominant marker and applicable for construction of linkage map, cataloguing of desirable traits for marker assisted selection and phylogenetic studies (Williams *et al.*, 1990). It has certain advantages comprising no sequence data requirement for random primer design, no probe development, no requirement of the use of radio-active material, features of being simple, fast and cheap, high genomic abundance and random distribution throughout the genome (Williams *et al.*, 1990). Inversely, RAPD has got serious limitations such as its dominant nature, being not-locus specific as well as not suitable for across species, low reproducibility due to mismatch annealing, differing results according to the source of tissue analyzed, and chance co-migration of bands at different loci (Black, 1993).

**Amplified Fragment Length Polymorphism (AFLP):** This technique was developed in the mid-1990s by Zabeau and Vos (1993). AFLP marker is generated by a combination DNA fragments (80-500 bp) digested with restriction enzyme and PCR amplification (Chittaranjan and Albert, 2008). It is a dominant marker and used for assessing genetic relationships, genome mapping, and DNA fingerprinting, and parentage analysis. It is more laborious and time consuming than RAPD method, but more reliable and again had higher efficiency in detecting polymorphism than RAPD (Garcia-Mas *et al.*, 2000). AFLP has certain significance which includes ability to generate high level of polymorphism and no sequence data requirement for selective primer construction (Rafalski *et al.*, 1996). It's reliable and reproducible (Jones *et al.*, 1997). However, it is technically demanding, requires high quality DNA, has got dominant nature, and may not be totally distributed throughout the genome because of clustering in certain genomic regions such as centromeres, and high number and different intensity of bands per primer combination that needs to take on certain strict but subjectively determined criteria for acceptance of bands in the analysis which limits the application of the technique (Vos *et al.*, 1995).

**Inter Simple Sequence Repeats (ISSR):** It was first reported by Zietkiewicz *et al.*, (1994). ISSR markers are DNA fragments of about 100-300 bp located between adjacent, oppositely oriented microsatellites region which involves PCR amplification of fragments (Zietkiewicz *et al.*, (1994). It is a dominant marker, and has various applications, including, for instance, studies of genetic variation, DNA fingerprinting, analyses of phylogenetic and taxonomic relationships, clone and strain identification, and gene mapping of closely related species. ISSR markers have their own advantages including does not require genomic sequence information, high level of polymorphism and cost effectiveness per polymorphism, reproducibility, random distribution throughout the genome, and the provision of less and ideal demanding methodology especially for organisms whose genetic information is lacking when compared to other dominant markers (Mishra *et al.*, 2003). However, it has some drawbacks including dominant nature, the possibility of non-homology of similar sized fragments, and reproducibility problems (Zietkiewicz *et al.*, 1994).

The techniques in the second classification of PCR-based markers depend on primers that are designed by targeting a single known site, such as a gene. These are alternative approaches to multi-locus profiling and they give information on as single locus when heterozygote data is needed (Karp *et al.*, 1997). The major and commonly used target sequence molecular markers include microsatellite (SSR) and single nucleotide polymorphism (SNP).

**Microsatellite (SSR):** The term "microsatellites" was first developed by Akkaya *et al.*, (1992). They are short tandem repeats, usually composed of 1-6 bp with the variation being the number of repeats. The PCR amplification products resolved on gels to reveal polymorphism are detected based on size differences on account of differences in the number of repeats. It has got wide range of applications including uses for detecting differences between and within species, DNA fingerprinting, marker-assisted selection, genetic linkage mapping, studying the structure of populations and determination of hybrids. The advantages of SSR markers include Mendelian inheritance as co-dominant marker, high reproducibility and polymorphism at each locus, high abundance and wide distribution throughout the genome, locus-specificity, revealing of high allelic diversity

and allowing rapid generation of data from relatively small amount of plant tissue (Tautz, 1989; Mohan *et al.*, 1997). Despite their advantage, SSR markers are expensive to generate and very laborious in certain species, and sequence information is required and may not be suitable across species due to species-specific primers.

**Single Nucleotide Polymorphism (SNP):** This is the most recently defined type of marker which is developed by Jordan and Humphries (1994). It detects variation at a single base change in a DNA sequence, that occur when a single nucleotide in the sequence is altered at least in one percent of the population (Syvanen, 2001) Single base changes and short insertion/deletion represent the most abundant source of DNA polymorphisms and they are both generally categorized as single nucleotide polymorphisms or SNPs (Cho *et al.*, 1999). They have got various applications in plant improvement programs such as construction of genetic maps, linkage disequilibrium based association mapping, studies of genetic diversity and phylogenetic analysis (Gupta *et al.*, 1999). SNP markers have certain advantages including amenable to automation, stable, efficient, and possibility to find in all parts of the genome as micro-array procedures have been developed for automatically scoring hundreds of SNP loci simultaneously at low cost per sample (Edwards and Batley, 2010). The major downsides of SNP markers are the need for sequence trace files which are rarely complete for large sequence data sets collected from a variety sources and the high level of sequence error associated with the reverse transcription process (Gary *et al.*, 2002)

Table 3. Comparison of most commonly used molecular/DNA marker systems

Feature	RFLPs	RAPDs	AFLPs	ISSRs	SSRs	SNPs
DNA required ( $\mu\text{g}$ )	10	0.02	0.5-1.0	0.05	0.05	0.05
DNA quality	High	High	Moderate	Moderate	Moderate	High
PCR based	No	Yes	Yes	Yes	Yes	Yes
Ease of use	Not easy	Easy	Easy	Easy	Easy	Easy
Amenability	Low	Moderate	Moderate	Moderate	High	High
Reproducibility	High	Unreliable	High	High	High	High
Development cost	Low	Low	Moderate	Moderate	High	High
Cost per analysis	High	Low	Moderate	Low	Low	Low

### 3. MATERIALS AND METHODS

#### 3.1. Experiment 1:- Genetic Diversity of Ethiopian Tef Varieties as Revealed by Morphological Markers

##### 3.1.1. Experimental plant materials

The experimental materials comprised 49 tef varieties. That include 36 released varieties in Ethiopia by various federal and regional agricultural research centers until the year 2016, three elite lines of which one was introduced from University of Bern, and 10 farmers' varieties widely grown in different areas, and commonly used as local checks in national tef yield trials in the country (Table 3). The experimental materials were supplied by different federal and regional agricultural research centers in Ethiopia.

Table 4. Description of the tef varieties used in the experiment

No.	Local Name	Variety Name	Source	Maintainer	Year of release
1	Asgori	DZ-01-99	Selection	DZARC	1970
2	Magna	DZ-01-196	Selection	DZARC	1978
3	Enatite	DZ-01-354	Selection	DZARC	1970
4	Wellenkomi	DZ-01-787	Selection	DZARC	1978
5	Menagesha	DZ-Cr-44	Hybridization	DZARC	1982
6	Melko	DZ-Cr-82	Hybridization	DZARC	1982
7	Tsedey	DZ-Cr-37	Hybridization	DZARC	1984
8	Gibe	DZ-Cr-255	Hybridization	DZARC	1993
9	Dukem	DZ-01-974	Selection	DZARC	1995
10	Ziquala	DZ-Cr-358	Hybridization	DZARC	1995
11	Gerado	DZ-01-1281	Selection	DZARC	2002
12	Koye	DZ-01-1285	Selection	DZARC	2002
13	Key Tena	DZ-01-1681	Selection	DZARC	2002
14	Gimbichu	DZ-01-899	Selection	DZARC	2005
15	Dega Tef	DZ-01-2675	Selection	DZARC	2005
16	Amarach	HO-Cr-136	Hybridization	DZARC	2006
17	Quncho	DZ-Cr-387/ RIL 355	Hybridization	DZARC	2006
18	Simada	DZ-Cr-385/ RIL 295	Hybridization	DZARC	2009
19	Boset	DZ-Cr-409/ RIL50d	Hybridization	DZARC	2012
20	Ambo Toke	DZ-01-1278	Selection	HARC	1999/00
21	Holetta Key	DZ-01-2053	Selection	HARC	1998/99
22	Yilmana	DZ-01-1868	Selection	AARC	2005

Table 4. *Continued*

No.	Local Name	Variety Name	Source	Maintainer	Year of release
23	Dima	DZ-01-2423	Selection	AARC	2005
24	Etsub	DZ-01-3186	Selection	AARC	2008
25	Gola	DZ-01-2054	Selection	SARC	2003
26	Genete	DZ-01-146	Selection	SARC	2005
27	Zobel	DZ-01-1821	Selection	SARC	2005
28	Mechare	Acc. 205953	Selection	SARC	2007
29	Laketch	RIL273	Hybridization	SARC	2009
30	Guduru	DZ-01-1880	Selection	BARC	2006
31	Kena	23-Tafi-Adi-72	Selection	BARC	2008
32	Ajora	PGRC/E 205396	Selection	Areka ARC	2004
33	Gemechis	DZ-Cr-387/RIL127	Hybridization	MARC	2007
34	Kora	DZ-Cr-438/RIL133B	Hybridization	DZARC	2014
35	Werekiyu	Acc.214746	Selection	SARC	2014
36	Dagem	DZ-Cr-438 /RIL91A	Hybridization	DZARC	2016
37	Elite lines	RIL-129A(DZ-Cr-387 X Kaye Murri)	Hybridization	DZARC	NA
38	Elite lines	RIL-27 (DZ-Cr-387 X DZ-Cr-37)	Hybridization	DZARC	NA
39	Elite lines	GA-10-3	Introduction	University of Bern	NA
40	Local check			Debre Zeit	NA
41	Local check			Minjar	NA
42	Local check			Alem Tena	NA
43	Local check			Holetta	NA
44	Local check			Adadi Mariam	NA
45	Local check			Adet	NA
46	Local check			Motta	NA
47	Local check			Enewary	NA
48	Local check			Melkassa	NA
49	Local check			Sirinka	NA

\*NA= Not applicable, RIL= that were developed from hybridization.

### 3.1.2. Description of experimental sites

The field experiment was carried out at two locations (*viz.*, Debre Zeit and Alem Tena) in the central parts of Ethiopia during the 2014 main cropping season. Debre Zeit located 47km and Alem Tena 109 km in southeast of Addis Ababa. The two locations represent two different agro-ecologies of the country. Debre Zeit shows relatively constant rainfall

with about 73% of the annual total rainfall (832 mm) received during the main growing season from June to September. The experimental field at this site is characterized by heavy black soil described as very fine Montmorillonitic Typic Pellustert or Pellic Vertisol (Tamirat, 1992) with very high moisture retention capacity. In contrast, Alem Tena often receives more than half of the annual total rainfall (500 mm) in only two months (July and August). The poor rainfall distribution coupled with relatively high temperature and the experimental field at this site is characterized by very light sandy soil with low moisture holding capacity. The weather conditions during the growing season were favorable and the experiments were received sufficient amount of rainfall for normal growth of tef crop at each of the test sites. Geographical position, climatic and soil-related data of the two locations during the growing period have been summarized and presented on Table 5.

Table 5. Geographical coordinates, weather data and soil physio-chemical properties of the test locations

Parameter	Trail sites	
	Debre Zeit	Alem Tena
Latitude	8°44' N	8° 20' N
Longitude	38°58' E	39° E
Altitude (m.a.s.l)	1860	1575
Rain fall (mm)	832	500
Soil type	Vertisols	Light soil
Maximum mean daily temperature (°C)	24.3	29.8
Minimum mean daily temperature (°C)	8.9	8.0
<b>Soil physico-chemical properties</b>		
Clay (%)	65	50
Silt (%)	29	18
Sand (%)	6	32
Organic carbon (%)	2.6	1.4
Nitrogen (%)	0.1	0.1
Carbon/Nitrogen ratio	26	14
Phosphorus (ppm)	41	3.9
pH (1:1 H <sub>2</sub> O)	6.97	7.4
CEC (meq/100 g soil)	42.7	25

Source: EIAR (2013).

### 3.1.3. Experimental design and management

The field experiment was conducted using 7x7 simple lattice designs with two replications. Each plot (1 m x 1 m) consisted of five rows of 1 m length with an inter-row spacing of 0.2 m. The distances were 1 m both between plots and incomplete blocks, and 1.5 m between replications. The varieties were allotted to plots at random within each replication. As per the research recommendations of 15 kg/ha, 1.5 g/plot of seeds were hand broadcasted along the surface of each row. The experiment was planted at Alem Tena (light soil) and Debre Zeit (black soil) in the middle and end of July, respectively. Fertilizers used were 40 kg N and 60 kg P<sub>2</sub>O<sub>5</sub> per hectare for light soil at Alem Tena, and 60 kg N and 60 kg P<sub>2</sub>O<sub>5</sub> per hectare for black soil at Debre Zeit. DAP was applied all at planting, while urea was applied two weeks after sowing and top dressed at tillering stage. Hand weeding was made three times during the crop growth stage.

Data were taken on twenty three quantitative traits on plot basis and from randomly selected five plants of tef from the central rows of each plot.

The following data were taken on whole plot basis.

- Days to heading (DTH): The number of days from sowing up to the emergence of the tips of the panicles from the flag leaf sheath in 50% of the plot stands.
- Days to maturity (DTM): The number of days from sowing up to 50% of the plants in the plot reaching physiological maturity stage (as evidenced by eye-ball judgment of the plant stands when the color of the vegetative parts changed from green to color of straw).
- Grain filling period (GFP): Number of days from 50% heading to 50% maturity of the stands in each plot obtained by subtracting the former from the latter.
- Lodging index (LOGI): It is the value measured from the whole plot according to the method of Caldicott and Nuttall, (1979). The method was taken as the product sum of the lodging degree taken on a 0-5 scale (0 being erect plant and 5 completely lodged) and the lodging severity as percent of the stand.
- Thousand seed weight (TSW): The weight of thousand kernels in milligram sampled from the entire plot.

- Biomass yield (BY): above ground total (shoot plus grain) biomass in gram for the entire plot.
- Grain yield (GY): The weight of seeds harvested in gram from each plot.
- Harvest index (HI): It is the ratio of grain yield to shoot biomass sampled from the entire plot expressed in percent.
- Economic growth rate (EGR): It is the ratio of grain yield to grain filling period considered from the entire plot expressed in percent.

The following observations were recorded on the basis of measurements made on five randomly selected and pre-tagged plants from the three central rows of each plot.

- Plant height (PLH): The length from the base of the stem of the main tiller to the tip of the main shoot panicle at maturity, recorded as the average of five plants per plot and measured in centimeter.
- Panicle length (PaL): The length from the base of the main shoot panicle where the first branch emerges to the tip of the panicle at maturity, recorded as the average of five plants per plot and measured in centimeter.
- Culm length (CL): The length of the main shoot culm from the ground level to the point of emergence of the panicle branches at maturity, recorded as the average on five of plants per plot and measured in centimeter
- Peduncle length (PDL): The length from the last culm node to the base of the panicle, recorded as the average on five plants per plot and measured in centimeter.
- Number of total tillers per plant (TT): It is recorded as the number of all tillers produced per plant assessed as the mean of five random plants per plot.
- Number of fertile tillers per plant (FT): It is recorded as the number of panicle bearing (fertile) tillers per plant assessed as the mean of five random plants per plot.
- Numbers of primary branches per main shoot panicle (PBMPa): The average number of primary branches that emerged from the rachis of the main panicle.
- Number of spikelet per main shoot panicle (SpPa): It is the average number of spikelets on the main shoot panicle of five plants measured in millimeter.

- First basal culm internode diameter (FBCD): The girth of the stem of the first internode from the ground level taken using caliper and recorded as the average of five plants measured in millimeter.
- Second basal culm internode diameter (SBCD): The girth of the stem of the second culm internode from ground level taken using caliper and recorded as the average of five plants measured in millimeter.
- Main shoot panicle weight (PaW): The average weight of the main panicle of five plants at harvest measured in gram.
- Main shoot panicle seed weight (PaSW): The average weight of the seeds harvested from the main panicle of five plants measured in gram.
- Total plant weight (PW): The average weight of single plant including tillers harvested at the level of the ground of five plants measured in gram.
- Grain yield per plant (Gy/P): The average weight of seeds for a single plant including all tillers yield of five plants measured in gram.

### 3.1.5. Data analyses

#### 3.1.5.1. *Methods of data preparation for analyses*

Data on lodging index was subjected to arcsine transformation and data on plant weight and plant seed weight were subjected to square root transformation as per the standard procedure set by Gomez and Gomez (1984) prior to performing statistical analysis of ANOVA in order to normalize the distribution.

For combined analysis of variance over locations, the homogeneity of error variance was tested using F-max test method of Hartley (1950), which is based on the ratio of the larger mean square of error (MSE) from the separate analysis of variance to the smaller mean square of error given by the following formula:

$$F_{\text{-max}} = \frac{\text{Largest MSE}}{\text{Smallest MSE}}$$

If the calculated value of  $F_{\text{-max}}$  was less than three, it means that the ratio of the highest error mean square is not three fold larger than the smallest error mean square, and this

indicates that the variance was considered homogenous thereby making it possible to proceed with the combined analysis of variance (Gomez and Gomez, 1984). Then pooled analysis of variance over locations was done using SAS statistical package (SAS, 2002). For multivariate analysis such as cluster, distance analyses and principal component analysis mean records on all traits were pre standardized to means of zero and variances of unity to avoid bias due to the differences in measurement scales (Manly, 1986).

The varieties were grouped into three according to their genetic background. The varieties resulting from direct selection from the landraces in one group, the varieties resulting from hybridization program which was employed to effect gene recombination in other group and the local varieties also in the other set.

### 3.1.5.2. Analysis of variance

The ANOVA for individual location followed the following model:

$$P_{ijk} = \mu + g_i + b_{k(j)} + r_j + e_{ijk}$$

Where,  $P_{ijk}$  = phenotypic value of  $i^{\text{th}}$  genotype under  $j^{\text{th}}$  replication and  $k^{\text{th}}$  incomplete block within replication  $j$ ;  $\mu$  = grand mean;  $g_i$  = the effect of  $i^{\text{th}}$  genotype;  $b_{k(j)}$  = the effect of incomplete block  $k$  within replication  $j$ ;  $r_j$  = the effect of replication  $j$ ; and  $e_{ijk}$  = the residual or effect of random error.

For pooled analysis of variance over location was conducted to measure the total variation among the varieties using the following model:

$$P_{ijks} = \mu + g_i + b_{k(j)(s)} + r_{j(s)} + l_s + (gl)_{is} + e_{ijks}$$

Where,  $P_{ijks}$  = phenotypic value of  $i^{\text{th}}$  genotype under  $j^{\text{th}}$  replication at  $s^{\text{th}}$  location and  $k^{\text{th}}$  incomplete block within replication  $j$  and location  $s$ ;  $\mu$  = grand mean;  $g_i$  = the effect of  $i^{\text{th}}$  genotype;  $b_{k(j)(s)}$  = the effect of incomplete block  $k$  within replication  $j$  and location  $s$ ;  $r_{j(s)}$  = the effect of replication  $j$  within location  $s$ ;  $l_s$  = the effect of location  $s$ ;  $(gl)_{is}$  = the interaction effects between genotype and location; and  $e_{ijks}$  = the residual or effect of random error.

Table 6. Analysis of variance for varieties tested at two locations

Source of variation	Degree of freedom	Mean square (MS)	Expected mean square (EMS)
Location (L)	L-1	M1	$\sigma_e^2 + R\sigma_{gl}^2 + G\sigma_l^2$
Replication	R-1	M2	$\sigma_e^2 + G\sigma_r^2$
Blocks within replication	R(K-1)	M3	$\sigma_e^2 + R\sigma_{gl}^2 + R\sigma_g^2$
Varieties (G)	G-1	M4	$\sigma_e^2 + R\sigma_{gl}^2 + RL\sigma_g^2$
G x L interaction	(G-1)(L-1)	M5	$\sigma_e^2 + R\sigma_{gl}^2$
Error	LG(R-1)-(RK-1)	M6	$\sigma_e^2$

Where, K represent blocks;  $\sigma_g^2$  = genotypic variance,  $\sigma_e^2$  = environmental variance,  $\sigma_l^2$  = location variance,  $\sigma_r^2$  = replication variance, and  $\sigma_{gl}^2$  = genotype x location interaction variance, L = number of locations, G = number of varieties and R = number of replications.

Mean separation was done using ANOVA protected LSD (least significant difference) at 5% probability level.

#### 3.1.5.4. Estimation of variance components

Partitioning of the total variance into components due to genotype ( $\sigma_g^2$ ), genotype by location interaction ( $\sigma_{gl}^2$ ) and environment ( $\sigma_e^2$ ) variances was performed by assuming observed mean squares equal to their expected mean squares as suggested by Singh and Chaudhary (1985) and Sharma (1998).

Estimates of the variance components were computed as follows.

- 1) Genotype variance ( $\sigma_g^2$ ) =  $[(\sigma_e^2 + R\sigma_{gl}^2 + RL\sigma_g^2) - (\sigma_e^2 + R\sigma_{gl}^2)]/RL = (MS4-MS5)/RL$
- 2) Genotype x location interaction variance ( $\sigma_{gl}^2$ ) =  $[(\sigma_e^2 + R\sigma_{gl}^2) - (\sigma_e^2)]/R = (MS5-MS6)/R$
- 3) Environmental variance ( $\sigma_e^2$ ) =  $\sigma_e^2 = MS6$
- 4) Phenotypic variance ( $\sigma_p^2$ ) =  $(\sigma_e^2 + R\sigma_{gl}^2 + RL\sigma_g^2) + (\sigma_e^2 + R\sigma_{gl}^2)/L + (\sigma_e^2)/LR = MS4+MS5/L+MS6/LR$ .

**Coefficients of variations at phenotypic and genotypic levels:** These were expressed as percentage of the corresponding phenotypic and genotypic standard deviations as described by Johnson *et al.*, (1955) and Kumar *et al.*, (1985) and expressed as follows:

$$\text{Phenotypic coefficient of variation (PCV)} = (\sigma_p / \bar{x}) \times 100$$

$$\text{Genotypic coefficient of variation (GCV)} = (\sigma_g / \bar{x}) \times 100$$

Where,  $\sigma_p$  = phenotypic standard deviation,  $\sigma_g$  = genotypic standard deviation, and  $\bar{x}$  = grand mean for the character x.

**Heritability ( $h_b^2$ ):** Heritability in broad sense for all characters was computed as the percentage of genotypic to phenotypic variance (Fehr, 1987):

$$h_b^2 = \sigma^2_g / \sigma^2_p \times 100$$

Where,  $\sigma^2_g$  = genotypic variance, and  $\sigma^2_p$  = Phenotypic variance

**Genetic advance (GA) and Genetic advance as percent of the mean (GAM):** The predicted response to selection using the method illustrated by Fehr (1987) assuming selection intensity of 5% was estimated as:

$$GA = k \sigma_p h_b^2$$

$$GAM = (GA / \bar{x}) \times 100$$

Where, k = the selection differential at 5% selection intensity (k = 2.063),  $\sigma_p$  = phenotypic standard deviation,  $h_b^2$  = heritability in broad sense ratio, and  $\bar{x}$  = Grand mean of the respective trait.

### 3.1.5.5. *Multivariate analyses*

For each of 23 traits, the standardized means of 49 varieties were used in all succeeding multivariate statistical analysis, including cluster analysis (CA), distance analysis and principal component analysis (PCA).

#### 3.1.5.5.1. Cluster and distance analyses

Agglomerative hierarchical cluster analysis approach was used to examine the assembling pattern of the 49 tef varieties based on their similarity with respect to the corresponding means of all the 23 traits studied. Cluster analysis was done by average linkage method

and the number of cluster was determined based on Pseudo-F and  $-t^2$  values. The points where local peaks of the pseudo F-statistic join with small values of the pseudo- $t^2$  statistic followed by a larger pseudo- $t^2$  for the next cluster combination was used to determine the number of clusters by using SAS statistical package (SAS Institute, 2002). The dendrogram was constructed based on the average linkage and Euclidean distance used as a measure of dissimilarity (the distance) technique using the MINITAB 14 statistical package.

Genetic distances between clusters as standardized were calculated using Mahalanobis's  $D^2$  statistics (Mahalanobis, 1936) as:  $D^2_{ij} = (x_i - x_j)' \text{cov}^{-1}(x_i - x_j)$

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  $\text{cov}^{-1}$  = 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 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 (Singh and Chaudhary, 1985).

#### 3.1.5.5.3. Principal component analysis

The principal components (PC) analyses were employed to identify the traits contributing large part of the total variation among the varieties. The characters with larger absolute values closer to unity within each principal component influence the clustering more than those with lower absolute values closer to zero (Chahal and Gosal, 2002). In this analysis, only PCs with eigenvalues greater than one are considered as important. As suggested by Johnson and Wichern (1988) trait coefficient or eigenvector greater than half divided by the standard deviation (square root) of the eigenvalue of the respective PC was employed as general guideline for weighing the relative significance of traits constituting the PCs.

### 3.1.5.6. Correlations among traits

Phenotypic and genotypic correlation coefficients were computed from variance and covariance components based on the method described by Singh and Chaudhury (1996). The Pearson and CANDISC correlation test were applied for phenotypic and genotypic correlation coefficients respectively using SAS statistical package (SAS Institute, 2002).

In this procedure, data were first subjected to statistical analysis using SAS statistical package for obtaining cross product sum of squares that were used to estimate the corresponding covariance components. To estimate phenotypic and genotypic correlation coefficient, covariance analysis between all pairs of traits was first calculated as:

$$\text{Genotypic covariance } (\sigma_{g_{xy}}) = \frac{(MSP_g - MSP_e)}{r}$$

$$\text{Phenotypic covariance } (\sigma_{p_{xy}}) = \sigma_{g_{xy}} + \frac{\sigma_{e_{xy}}}{r}$$

$$\text{Environmental covariance } (\sigma_{e_{xy}}) = MSP_e$$

Where,  $MSP_e$  = error mean variance,  $MSP_g$  = mean square product for varieties, and  $r$  = number of replications.

Phenotypic and genotypic correlation coefficients were then estimated using the standard procedure suggested by Miller *et al.*, (1958) from the corresponding variance and covariance components as:

$$\text{Phenotypic correlation coefficient } (r_{p_{xy}}) = \frac{\sigma_{p_{xy}}}{\left(\sqrt{\sigma^2_{p_x} * \sigma^2_{p_y}}\right)}$$

$$\text{Genotypic correlation coefficient } (r_{g_{xy}}) = \frac{\sigma_{g_{xy}}}{\left(\sqrt{\sigma^2_{g_x} * \sigma^2_{g_y}}\right)}$$

Where,  $r_{p_{xy}}$  = phenotypic correlation coefficient between characters x and y,  $r_{g_{xy}}$  = genotypic correlation coefficients between characters x and y.

The level of significance of correlation coefficients was determined from r-table (correlation table) at the appropriate degrees of freedom and probability level (Gomez and Gomez, 1984).

## **3.2. Experiment 2:- Genetic Diversity of Ethiopian Tef Varieties as Reveled by Microsatellite Markers**

### **3.2.1. Experimental plant materials**

The varieties used for morphological markers (section 3.1; Table 4) were used for this study. In addition, for comparison one accession from *Eragrostis curvula* and *Eragrostis pilosa* were included.

### **3.2.2. DNA extraction and purification**

About 50 seeds of each genotype were planted in plastic pots comprising the combination of sand, forest soil and compost in 2:2:2 ratios. The plants were grown under growth chamber at National Agricultural Biotechnology Research Center (NABRC), Holetta. Approximately equal amount of leaf tissues were collected from five seedlings and bulked from three weeks-old seedlings followed by allowing the leaves to dry using silica gel. After the leaves were totally dried, about 50 mg of the dried leaves were placed in 2 ml autoclaved Eppendorf tubes and ground using a mixer mill (Retsch RM 200). The genomic DNA was then extracted from the pulverized leaf samples using the modified cetyltrimethylammonium bromide (CTAB) extraction technique as described by Borsch *et al.*, (2004) with some modification. Double extraction was employed for each sample tissue so as to get optimum quality and quantity of genomic DNA.

### **3.2.3. Genomic DNA quality and quantity measurement**

The quality of genomic DNA was tested using 1% (w/v) agarose gel. Electrophoresis was carried out on horizontal electrophoresis set up (JYSPBT) using a standard DNA ladder HyperLadder™ IV with known reference band to quantify the DNA amount and visualized using gel documentation, 3UV- transilluminator (Bio-Doc). The quantification of genomic DNA was also measured using spectro-nano drop (ND-8000). The one with high band intensity, lesser smear and high concentration were selected for PCR analysis.

### **3.2.4. Microsatellite primer selection and optimization**

A total of 12 microsatellite primers-pairs previously developed by Zeid *et al.*, (2010) were used for initial testing for amplification, polymorphism and specificity to target loci. Out of the 12 tested primer pairs, nine of them were successfully amplified the genomic DNA and showed polymorphism across tested tef varieties. These markers were also reported to be polymorphic using different tef genotypes (Solomon *et al.*, 2007; Zeid *et al.*, 2012) and selected for PCR analysis in this study (Table 7).

The primers were supplied in lyophilized powdered form. According to the manufacturer's instruction they were dissolved using nuclease-free water to a final concentration of 100 mol/ $\mu$ l.

### **3.2.5. Microsatellite PCR analysis**

DNA amplification was performed in 25  $\mu$ l reaction volume containing 6.25  $\mu$ l, GoTaq® Green Mater Mix (supplemented with all PCR reaction components such as MgCl<sub>2</sub>, PCR buffer, dNTPs, and Taq DNA polymerase), 3.75  $\mu$ l genomic DNA, 1.25  $\mu$ l forward and reverse primer and 12.5  $\mu$ l nuclease-free water. PCR was carried out using thermal cycler Gene Amp® PCR system 9700 (Applied Biosystem, USA) programmed at an initial denaturation at 94 °C for 2 minutes followed by denaturation at 94 °C for 30 seconds, annealing at 51 °C up to 57.4 °C (depending on the primer used) for 30 seconds, initial extension at 72 °C for 1 minute and final elongation at 72 °C for 10 minutes. Prior to fragment analysis, amplification of the PCR products was checked on a 2% (w/v) agarose gel and visualized using gel documentation, 3UV-transilluminator (Bio-Doc).

### **3.2.6. Polyacrylamide gel electrophoresis (PAGE)**

After amplification, the samples were loaded on 40% polyacrylamide gel containing 70 ml ddH<sub>2</sub>O, 20 ml 40% polyacrylamide gel, 5 ml 10X TBE, 1100  $\mu$ l APS (1 g APS for 10 ml ddH<sub>2</sub>O,) and 60  $\mu$ l TEMED. Electrophoresis was carried out on a vertical electrophoresis set up CS-500V at a constant voltage of 300 V for 1:30 hour using a standard DNA ladder HyperLadder™ IV with known reference band to estimate the molecular weight of each amplified product. Amplification was visualized under UV using the same transilluminator used in the above procedure to confirm successful amplification of PCR products.

Table 7. Description (sequence, repeats, fragment size and annealing temperature) of polymorphic microsatellite makers used in the present analysis of tef varieties

No.	Microsatellite	Primer sequence		Repeat	Fragment size (bp)	Annealing temperature (°C)
		Forward	Reverse			
1	<i>CNLTs 17</i>	CAGTTGCCTCGTTCTTCCTC	AAGGGGGCAGGCTGATCT	(GA)8	152	54.4
2	<i>CNLTs 27</i>	TTGGAATGAGATGGCATTG	GAAGCGGGGTAAGATTTGAA	(TC)14	174	54.4
3	<i>CNLTs 31</i>	TGCAGCTCGAGTGTTGACTT	CTAGGGACCGTACGGATTGA	(CT)8	211	57.4
4	<i>CNLTs 56</i>	AAGGTTGAGACGTCCCCTCT	GTGAGCTTCGTCCGGTAACA	(TC)22	157	54.7
5	<i>CNLTs 60</i>	AGGGTGATAGCTGCCAGAC	CCCGAGTAATTGGTCGCTAA	(TC)20 & (CA)7	297	51
6	<i>CNLTs 61</i>	CCGCGTGACTCTGAGTAGTG	AGGGATGCATTCACGTATGG	(GA)6	217	56.4
7	<i>CNLTs 62</i>	GATCAGACCGAGGTCGATTAG	ACACACGCGCATACAAAGAG	(TC)9	100	56.3
8	<i>CNLTs 86</i>	ATTACAGCACCTGCAGCTC	TCGTCGGTGAAGTGTGGATA	(TC)18	167	56.4
9	<i>CNLTs 116</i>	AGCCCATGAAAGCAGATAAGA	CCCTATCTCCTTCGACACCA	(GA)7	205	54

Source Zeid *et al.*, 2011

### **3.2.7. Fragment scoring and data analysis**

Based on the number of alleles detected for that locus SSR fragments with clearly resolved with unambiguous bands were scored using binary numbers (1 for presence and 0 for absence of band (Warburton and Crossa, 2002; Saeed *et al.*, 2011). Fragments with the same mobility were considered as identical fragments and treated as a unit character. The data from all entries was combined for statistical analysis. Different statistical analyses described below were performed on SSR data.

#### **3.2.7.1. Diversity parameters and analysis**

Genetic diversity parameters of the 51 tef varieties was analyzed using GenAlex version 6.5 (Peakall and Smouse, 2015) software package. Genetic diversity parameters considered in this study were: number of effective alleles per locus ( $N_e$ ), Shannon information index (I), fixation index (F) (Nei's, 1978), gene flow (Nm) and percent polymorphism (% P). The number of alleles per locus ( $N_a$ ), polymorphic information content (PIC), observed ( $H_o$ ) and expected ( $H_e$ ) heterozygosity were analyzed using power marker v3,25 (Liu and Muse, 2005) software package.

#### **3.2.7.2. Analysis of molecular variance (AMOVA)**

The analysis of molecular variance was performed to estimate population genetic structure and differentiation among and within varieties based on their genetic source of origin. AMOVA uses the estimated F-statistics such as genetic differentiation ( $F_{ST}$ ), fixation index or inbreeding coefficient ( $F_{IS}$ ), and overall fixation index ( $F_{IT}$ ) to compare the genetic structure among and within populations. The AMOVA procedures were done using GeneAlex version 6.5 (Peakall and Smouse, 2015) software package.

#### ***3.2.7.4. Genetic distance and cluster analysis***

To examine the degree of population differentiation among the study materials the Nei unbiased genetic distance were computed according to Nei's (1978) using GenAlex version 6.5 (Peakall and Smouse, 2015) software package. Pair-wise  $F_{st}$  and  $N_m$  values were also computed using the same software.

Cluster analysis was carried out by using neighbor-joining (NJ) algorithm in DARwin version 6 software (Perrier and Jacquemoud-Collet, 2010). A dendrogram for 51 varieties was then generated based on the dissimilarity matrix to visualize pattern of cluster within and among the selected tef varieties.

#### ***3.2.7.5. Principal coordinate analysis (PCoA)***

Principal Coordinate Analysis (PCoA) was performed using the Jaccard's index to further validate the complementarity of clustering pattern revealed by the dendrogram using GenAlex version 6.5 (Peakall and Smouse, 2015) software package. The first three axes were used to plot the three dimensional PCo with STATISTICA version 6.0 software (Hammer *et al.*, 2001; Stastica soft, Inc. 2001).

## 4. RESULTS AND DISCUSSION

### 4.1. Experiment 1:- Phenotypic Diversity in Ethiopian Tef Varieties as Revealed by Morphological Markers

#### 4.1.1. Analysis of Variance

Separate analysis for each of the two test site showed consistently large variation among the tef varieties for all the traits studied except plant height. For some traits like grain yield lower values were recorded at Alem Tena and higher values were recorded at Debre Zeit. This indicates that the locations had significant effects on the performance of tef varieties (Table 8). This is not unexpected on the basis of their distinct agro-climatic classification of the test locations (Kebebew *et al.*, 2003b).

Table 8. Mean performances of 49 tef varieties for 23 traits over two locations

Traits	Alem Tena		Debre Zeit	
	Mean	SEM ( $\pm$ )	Mean	SEM ( $\pm$ )
Days to heading (days)	44.00	0.38	48.00	0.33
Days to maturity (days)	84.00	0.19	105.00	0.47
Grain filling period (days)	40.00	0.26	57.00	0.49
Plant height (cm)	106.82	1.11	106.00	1.16
Panicle length (cm)	43.68	0.65	41.00	0.47
Culm length (cm)	63.13	0.82	65.51	0.88
Peduncle length (cm)	16.80	0.29	19.22	0.26
No. of total tillers per plant	8.48	0.28	12.23	0.43
No. of fertile tillers per plant	6.13	0.20	10.48	0.41
No. of primary branches /main shoot panicle	26.94	0.60	28.94	0.38
No. of spikelets / main shoot panicle	489.33	12.57	565.21	15.17
Lodging index	91.26	0.61	75.04	0.91
First basal culm internode diameter (mm)	0.16	0.01	0.22	0.01
Second basal culm internode diameter (mm)	0.16	0.01	0.23	0.01
Thousand seed weight (mg)	0.30	0.01	0.32	0.01
Main shoot panicle weight (g)	1.65	0.07	1.36	0.04
Main shoot panicle seed weight (g)	0.49	0.03	0.85	0.03
Single plant weight (g)	6.48	0.26	17.37	0.71
Grain yield per plant (g)	0.92	0.06	5.10	0.27
Biomass yield (g/plot)	987.76	26.32	511.22	22.08
Grain yield (g/plot)	192.43	5.21	215.19	8.37
Harvest index (%)	9.97	0.33	16.38	0.37
Economic growth rate (%)	482.10	12.41	380.01	15.07

SEM=Standard error of mean

Prior to executing analysis of variance, tests were made to confirm the homogeneity of error variances, all of the traits showed homogeneity of error variances. Consequently, the data were pooled across locations and analyzed. The results of the combined analysis of variance across the two locations are presented on Table 9.

The mean square from the pooled analysis of variance over the two locations showed statistically significant location ( $P \leq 0.01$ ) effects for all traits recorded except plant height. These results are similar to the findings of Solomon *et al.*, (2009).

The mean square from the pooled analysis of variance over the two locations showed statistically significant ( $P < 0.01$ ) effects of genotype for all traits assessed except lodging index (Table 9). The basic cause of differences in stability between varieties is a wide occurrence of genotype x location interactions which is corresponding to genetic and non-genetic effects on growth. This interaction causes variation of yield and other traits over locations.

The mean squares due to genotype x location interaction were statistically significant ( $P < 0.01$ ) effect for all traits evaluated except for panicle length, culm length, primary branch per main shoot panicle, lodging index, first basal culm internode diameter, thousand seed weight, total plant weight and grain yield per plant (Table 9). Similar significant results were also reported by earlier investigators (Kebebew *et al.*, 1999, 2000, 2001a, b, 2003; Solomon *et al.*, 2009; Habite *et al.*, 2015). However, Similar to the current finding panicle length (Kebebew *et al.*, 1999; Solomon *et al.*, 2009) and first basal culm internode diameter (Kebebew *et al.*, 1999) showed statistically non-significant effect.

For those traits which showed significant genotype x location interaction, indicates differential performances of varieties at the two test locations. For panicle length, culm length, number of primary branches per main shoot panicle, lodging index, first basal culm internode diameter, thousand seed weight, total plant weight and grain yield per plant which showed no significant genotype x location interaction effects, it implies that the varieties performed consistently over locations with regard to these traits. This show varieties selected for those traits at one location may exhibit a similar relative performance

at other location. If varieties perform consistently for those traits over locations, breeders may be able to reduce the cost of extensive varietal evaluation by eliminating unnecessary testing sites and by modifying the breeding programs. To effectively assess varietal performance for significant traits it's essential to consider wide location interaction and identify traits performance in relation to location effect

Table 9. Mean squares from the combined analysis of variance on 23 traits of 49 tef varieties tested in 2014 main cropping season over two locations.

Traits	MSL(1)	MSG(48)	MSLG (48)	MSE	CV (%)
DTH	617.88**	38.63**	2.48*	1.56	2.72
DTM	22457.16**	21.58**	22.19**	1.56	1.32
GFP	14382.86**	27.10**	19.77**	4.27	4.27
PLH	6.80 <sup>ns</sup>	292.96**	79.16**	41.78	6.06
PaL	371.66**	54.89**	15.35 <sup>ns</sup>	16.24	9.53
CL	277.94**	137.60**	53.98 <sup>ns</sup>	38.66	9.67
PDL	285.36**	11.33**	6.99*	4.55	11.85
TT	690.94**	13.68*	16.91**	9.44	29.67
FT	925.90**	10.61**	14.07**	7.33	32.61
PBMPa	196.40**	33.97**	18.82 <sup>ns</sup>	18.93	15.57
SpPa	282097.56**	38813.23**	13094.72*	7977.96	16.94
LOGI	8796.70**	48.40 <sup>ns</sup>	32.89 <sup>ns</sup>	40.79	9.50
FCBD	0.166**	0.004**	0.002 <sup>ns</sup>	0.002	23.35
SBCD	0.264**	0.005**	0.006**	0.002	22.71
TSW	0.028**	0.006*	0.005 <sup>ns</sup>	0.004	19.55
PaW	4.11**	0.528**	0.302**	0.148	25.66
PaSW	6.600**	0.116**	0.113**	0.044	31.19
PLW	121.37**	0.389**	0.541 <sup>ns</sup>	0.512	21.78
PLSW	77.04**	0.223**	0.240 <sup>ns</sup>	0.200	29.00
BY	11126989.80**	96676.77**	64489.80**	29780.01	23.02
GY	25394.70**	6897.40**	5891.13**	2798.35	25.96
HI	2009.47**	22.71**	11.09**	5.69	18.11
EGR	510701.14**	27661.33**	19011.94*	11751.53	25.15

\*, \*\* Significant at  $p \leq 0.05$ , and  $p \leq 0.01$  probability level respectively and <sup>ns</sup> non-significant, <sup>β</sup> Figures in parenthesis indicate degrees of freedom. MSL= Mean Squares of locations, MSG= Mean squares of varieties, MSLG = Mean square of genotype x location interaction, MSE = Mean squares of error, CV = Coefficient of variation. DTH=days to heading, DTM = days to maturity, GFP = grain filling period, PLH= Plant height, PaL=panicle length, CL= culm length, PDL= peduncle length, TT = no. of total tiller per plant, FT =no. of fertile tiller per plant, PBMPa = no. of primary branches per main shoot panicle, SpPa = no. of spikelet per main shoot panicle, LOGI =lodging index. FBCD = first basal culm internode diameter, SBCD = second basal culm internode diameter, TSW = thousand seed weight, PaW = main shoot panicle weight, PaSW = main shoot panicle seed weight, PLW = single plant weight, PLSW = grain yield per plant, GY= grain yield, BY = biomass yield, HI = harvest index, and EGR= economic growth rate.

#### **4.1.2. Range of parameters**

Based on the average data over the two test locations, wide ranges between the maximal and minimal mean values were observed for the traits evaluated (Table 10). The ranges of days to heading, days to maturity and grain filling period were 38-53 days, 88-100 days and 43- 56 days, respectively. Likewise, wide ranges were also noted for all the traits assessed.

The results of the current investigation were in agreement with the range values reported previously in other tef studies (Kebebew *et al.*, 2001 b). Nevertheless, the range values for the three phenologic traits are relatively low as compared to those reported by Kebebew *et al.*, (2001b). This might be due to differences in the experimental plant materials and locations used in the different studies.

Though, plant height, panicle length, culm length, peduncle length, total tiller, fertile tiller, primary branches per main shoot panicle, lodging index, first and second basal culm internode diameter, thousand seed weight, main shoot panicle weight, main shoot panicle seed weight, total plant weight, grain yield per plant and harvest index found in range of the previous investigation of Kebebew *et al.*, (2001b) and the varieties showed not extensive range between the minimum and the maximum value for those traits indicating that the total span of the data is midway since the experimental plant materials used were improved varieties.

Table 10. Minimum and maximum values, means and standard errors of means (SEM) for 23 traits (average over two locations) of 49 tef varieties.

Traits	Minimum value	Genotype	Maximum value	Varieties	Mean	SEM ( $\pm$ )
Days to heading (days)	38.00	DZ-Cr-285	53.00	DZ-Cr-438	46.00	0.82
Days to maturity (days)	88.00	GA-10-3	100.00	DZ-Cr-438	95.00	0.81
Grain filling period (days)	43.00	GA-10-3	56.00	Holetta	48.00	0.67
Plant height (cm)	80.54	Enewary	126.99	DZ-Cr-82	106.63	0.80
Panicle length (cm)	31.80	Enewary	49.62	DZ-Cr-438	42.31	0.41
Culm length (cm)	48.74	Enewary	77.64	DZ-Cr-82	64.32	0.60
Peduncle length (cm)	14.19	DZ-01-2053	21.97	DZ-Cr-255	18.01	0.21
No. of total tillers per plant	6.54	DZ-01-1281	16.10	Holetta	10.36	0.29
No. of fertile tillers per plant	5.53	DZ-01-899	13.61	RIL 27 (Elite)	8.30	0.27
No. of primary branches /main shoot panicle	20.62	DZ-01-2053	34.15	DZ-01-1880	27.94	0.36
No. of spikelets / main shoot panicle	234.84	Enewary	740.97	DZ-Cr-387 (Quncho)	527.27	10.19
Lodging index	75.99	DZ-Cr-438	93.99	Holetta	83.15	0.80
First basal culm internode diameter(mm)	0.12	Sirinka	0.27	DZ-01-196	0.19	0.004
Second basal culm internode diameter (mm)	0.13	Holetta	0.34	Debre Zeit	0.20	0.005
Thousand seed weight (mg)	0.22	Motta	0.45	DZ-Cr-387 (Gemechis)	0.31	0.005
Main shoot panicle weight (g)	0.39	Enewary	2.43	DZ-01-438	1.50	0.04
Main shoot panicle seed weight (g)	0.28	Enewary	1.17	DZ-01-974	0.67	0.03
Single plant weight (g)	6.33	Enewary	16.39	DZ-01-1821	11.93	0.54
Grain yield per plant (g)	0.53	Adadi	4.90	DZ-01-1821	3.01	0.20
Biomass yield (g/plot)	307.36	Enewary	1153.97	DZ-Cr-82	749.49	24.18
Grain yield (g/plot)	76.11	DZ-01-2675	299.63	DZ-01-3186	203.81	4.98
Harvest index (%)	6.47	DZ-01-2675	18.60	DZ-Cr-285	13.17	0.34
Economic growth rate (%)	193.12	DZ-01-2675	602.01	DZ-01-3186	431.1	10.40

### 4.1.3. Variance components

#### 4.1.3.1. *Genotypic and phenotypic coefficients of variation*

Estimates of phenotypic coefficients of variation (PCV) were relatively high for grain yield per plant (30%) followed in that diminishing order by number of fertile tiller (29%), main shoot panicle seed weight (27%), main shoot panicle weight and total number of tillers per plant (26%), biomass yield (22%), grain yield and single plant weight, and economic growth rate (21%) and number of spikelets per main shoot panicle (20%) (Table 11). Intermediate PCV values were obtained for harvest index, and second basal culm internode diameter (19%), first basal culm internode diameter (16%), thousand seed weight (12%), number of primary branches per main shoot panicle (11%), panicle length, peduncle length and culm length (10%). On the other hand, phenotypic coefficient of variation was low for plant height (9%), days to heading (7%), grain filling period (6%), lodging index (5%) and days to maturity (3%) (Table 11).

Relatively high genotypic coefficients of variation (GCV) were observed in decreasing order for number of fertile tillers per plant (21%) followed by main shoot panicle weight (20%), number of total tillers per plant and number of spikelet per main shoot panicle (18%), biomass yield (15%) and harvest index, economic growth rate, main shoot panicle seed weight (14%) (Table 11). Intermediate GCV estimates were noted for grain yield and first basal culm internode diameter (11%), panicle length (9%), number of primary branches per main shoot panicle, culm length and plant height (8%), then days to heading, grain yield per plant and total plant weight (7%). In contrast, low GCV values were found for peduncle length (6%), thousand seed weight (4%), grain filling period (3%), lodging index (2%), days to maturity (1%) and second basal culm internode diameter (0.00%) (Table 11).

Generally, environmental variance was greater than the genetic variance for all the traits studied indicating high environmental effects in estimating these traits. The magnitude of genetic variation is better evaluated from GCV, improvement effort usually focus on traits with high GCV estimates. In this study, high GCV values were recorded for number of fertile and total tillers per plant, main shoot panicle weight, number of spikelets per main

shoot panicle, biomass yield, harvest index, economic growth rate and main shoot panicle seed weight showed a relatively high GCV indicating their amenability for improvement. This results are generally in line with the previous reports of Kebebew *et al.*, (1999, 2001b), Hailu *et al.*, (2008) and Solomon *et al.*, (2009).

#### **4.1.3.2. Broad sense heritability**

Heritability determines the efficiency of selection. The efficiency of selection for a trait depends on the relative significance of genetic and environmental factors in the expression of phenotypic difference among varieties. The broad sense heritability values of the different traits based on the combined analyses of across the two test locations ranged from 0% for second basal culm internode diameter to 95% for days to heading (Table 11).

Hence, high heritability estimates were found for traits such as days to heading (95%), panicle length (79%), plant height (77%), number of spikelet per main shoot panicle (77%) and culm length (64%). These traits may respond effectively for phenotypic selection, and selection for such traits is fairly easy because there would be a close correspondence between genotype and phenotype due to a relatively smaller contribution of environment to the phenotype. Likewise, intermediate heritability values were observed for harvest index (57%), main shoot panicle weight (54%), number of primary branches per main shoot panicle (53%), number of fertile tillers per plant (51%), number of total tiller per plant (50%), first basal culm internode diameter (48%), economic growth rate (47%), biomass yield (44%), peduncle length (41%) and grain filling period (35%). On the other hand, low value of broad sense heritability were estimated for grain yield (28%), main shoot panicle seed weight (27%), lodging index (25%), thousand seed weight (13%), total plant weight and days to maturity (10%) and grain yield per plant (5%) (Table 11). However, no heritability was noted for second basal culm internode diameter (Table 11). The latter indicates limited possibility of improvement for the trait through selection and almost it is impractical due to the masking effect of the environment on genotypic effect (Singh, 2002).

Hence, most heritable traits in all of the tested genotype in this study were days to heading, panicle length, plant height, number of spikelet per main shoot panicle and culm length.

However, heritability estimates alone are not sufficient, and estimates of heritability and genetic advance should always be considered simultaneously to increase the efficiency of selection (Johnson *et al.*, 1955). The present findings are in agreement with respect to high heritability estimates reported earlier for days to heading (Hailu *et al.*, 2003; Solomon *et al.*, 2009; Habtamu *et al.*, 2011; Ayalneh *et al.*, 2012) and panicle length (Kebebew *et al.*, 2000, 2001a). Similar to the present results, Kebebew *et al.*, (2000) found low heritability estimates for second basal culm internode diameter.

#### **4.1.3.3. Genetic advance**

Genetic advance as percent of mean varied from 0% for second basal culm internode diameter to about 32% for number of spikelets per panicle (Table 11). Likewise, relatively high genetic advance estimates as percent of the mean were recorded for number of fertile tillers per plant (31%), main shoot panicle weight (30%), number of total tillers per plant (26%) and harvest index (22%). Moderate genetic advance values as percent of the mean were obtained for economic growth rate and biomass yield (20%), panicle length and first basal culm internode diameter (16%), main shoot panicle seed weight (15%), days to heading and plant height (14%), culm length (13%), and grain yield and number of primary branches per main shoot panicle (12%). In contrast, low estimates were obtained for peduncle length (8%), total plant weight (5%), grain filling period (4%), thousand seed weight (3%), grain yield per plant (3%) and lodging index (3%), days to maturity (1%) and second basal culm internode diameter (0%).

According to Johnson *et al.*, (1955) suggestion number of spikelet per main shoot panicle, panicle length, plant height and days to heading had relatively high estimates of both heritability and genetic advance as compared to other yield contributing traits. This indicates that they may serve as important traits in indirect selection for higher grain yield. The estimate of genetic advance as percent of mean for number of fertile tillers per plant was in agreement with the former reports of Kebebew *et al.*, (2000, 2001a). The lowest genetic advance reported for second basal culm internode diameter was similar to the earlier findings of Kebebew *et al.*, (2000) and the estimates days to maturity were in line with the previous reports of Kebebew *et al.*, (2001a) and Solomon *et al.*, (2009).

Overall, genotypic and phenotypic coefficient of variation along with heritability plus genetic advance are very essential to improve traits of interest (Denton and Nwangburuka, 2011). Accordingly, in the present study, relatively high phenotypic and genotypic coefficient of variance along with heritability and genetic advance estimates were obtained for number of spikelets per main shoot panicle, harvest index, number of total and fertile tillers per plant, and main shoot panicle weight. This indicates the significance of those traits for genetic improvement of the crop.

Table 11. Estimates of phenotypic (PCV) and genotypic (GCV) coefficients of variations, broad sense heritability ( $h^2$ ), genetic advance (GA) and GA as percent of mean (GAM) for 23 traits of 49 tef varieties based on analysis of variance over two locations

Traits	PCV (%)	GCV (%)	$h^2$ (%)	GA	GAM
Days to heading (days)	7.17	6.99	95.07	6.47	14.10
Days to maturity (days)	2.63	0.83	10.03	0.51	0.54
Grain filling period (days)	5.61	3.31	34.71	1.94	4.02
Plant height (cm)	8.64	7.60	77.39	14.71	13.80
Panicle length (cm)	9.81	8.71	78.71	6.74	15.94
Culm length(cm)	9.53	7.65	64.33	8.14	12.65
Peduncle length(cm)	9.57	6.09	40.46	1.44	7.99
No. of total tiller per plant	25.64	18.07	49.66	2.73	26.27
No. of fertile tiller per plant	29.40	21.04	51.24	2.58	31.08
No. of primary branch/main shoot panicle	11.25	8.19	52.99	3.43	12.30
No. of spikelet/ main shoot panicle	20.16	17.66	76.74	168.27	31.91
Lodging index	4.96	2.46	24.62	2.09	2.52
First basal culm internode diameter (mm)	15.53	10.78	48.22	0.03	15.45
Second basal culm internode diameter (mm)	18.74	0.00	0.00	0.00	0.00
Thousand seed weight (mg)	12.37	4.43	12.84	0.01	3.28
Main shoot panicle weight (g)	26.37	19.45	54.36	0.44	29.58
Main shoot panicle seed weight (g)	26.48	13.72	26.85	0.10	14.67
Single plant weight (g)	21.15	6.78	10.28	0.53	4.48
Grain yield per plant (g)	29.65	6.80	5.27	0.10	3.22
Biomass yield (g/plot)	21.94	14.46	43.46	147.41	19.67
Grain yield (g/plot)	21.33	11.28	27.97	25.08	12.31
Harvest Index (%)	18.86	14.27	57.31	2.94	22.29
Economic growth rate (%)	20.48	14.00	46.72	85.12	19.74

#### **4.1.4. Multivariate analyses**

##### ***4.1.4.1. Cluster analysis***

Cluster analysis grouped the varieties into seven clusters based on their similarity (Figure 1). The first cluster (C<sub>1</sub>, n=29) had the largest number of varieties resulting from direct selection, hybridization and local varieties but dominated by varieties resulting from direct selection. The varieties were tend to form their own hierarchical sub-group as well. The second cluster comprised eight of the test tef varieties originating from direct selection and hybridization but dominated by hybridization. The fourth cluster comprised six of the test tef varieties originating from direct selection, hybridization and local varieties in equal proportion. Though, in this cluster varieties resulting from inter-specific hybridization and elite line resulting from TILLING were included. Local varieties remain solitary and form their own distinct group in cluster three, five, six and seven (Figure 1).

The result showed that, in most cases the varieties originating from direct selection and hybridization clustered together while local varieties remained distinct and ungrouped. This may be attributed to an exchange of genetic materials between the two breeding approaches. This may also be related to the use of some of the varieties developed through direct selection as parents for the varieties developed through hybridization. The local varieties grouped separately due probably to the presence of distinct farmer varieties in different areas. The current cluster analysis indicated that the diversity presented in tef genotypes cannot be reduced into a few number of groups as was done in earlier studies (Melak-Hail *et al.*, 1965; Kebebew *et al.*, 2003b; Habite *et al.*, 2015).

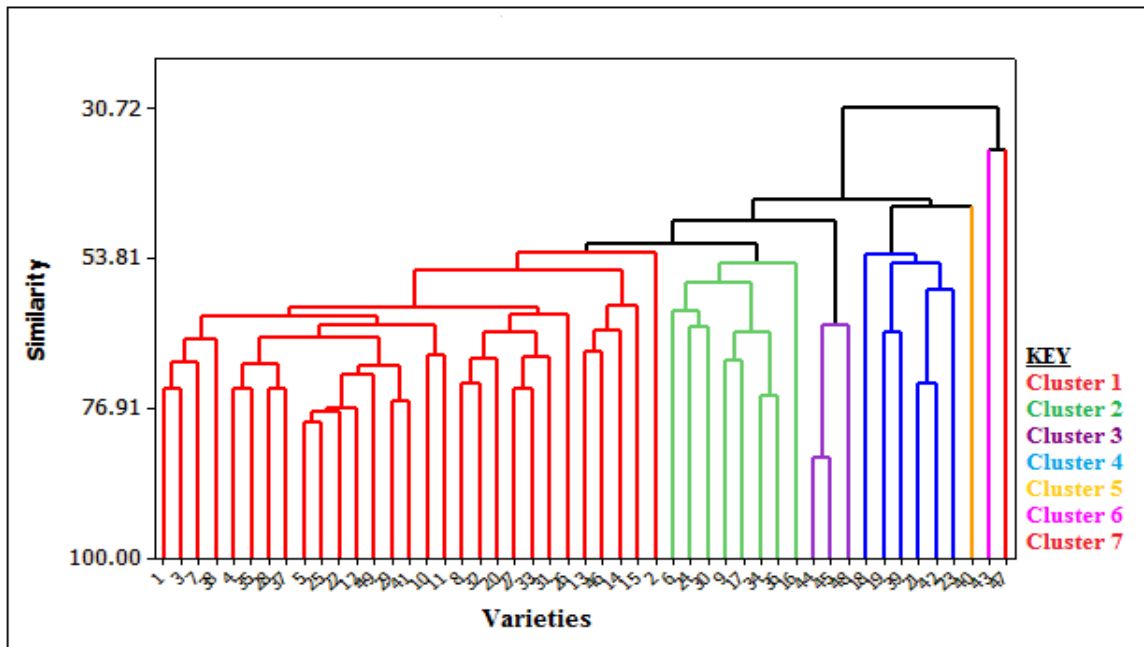


Figure 1. Dendrogram showing relationships among 49 tef varieties based on average linkage and Euclidean distance using the mean of 23 quantitative traits.

#### 4.1.4.2. Distance among clusters

The standardized Mahalanobis  $D^2$  statistics showed existence of high genetic distance among the seven clusters, and out of 21 pairs, the genetic divergences between 20 pairs were highly significant at  $P < 0.01$ . The maximum distance was found between clusters seven and five with  $D^2 = 589$  followed by cluster six and five with  $D^2 = 543$  which was presumably due to the distinct nature of the solitary grouped farmers' varieties. The minimum distance was found between cluster two and one  $D^2 = 30$  followed by cluster four and one with  $D^2 = 41$ . It should be noted that both clusters that showed least genetic divergence were comprised of predominantly improved varieties resulting from i.e. direct selection and hybridization (Table 12).

In view of that, the high inter-cluster distance values obtained in this study is largely due to the inclusion of the local varieties. Parents for hybridization could be selected on the basis of large inter-cluster distance for isolating useful recombinants in the segregating generations. Therefore, crosses involving cluster five may exhibit high heterotic expression in the first filial generation and broad-spectrum of variability in segregating populations as were also cited in Gashaw *et al.*, (2007).

Table 12. Pair-wise generalized square distance ( $D^2$ ) between seven clusters constructed from 49 tef varieties

Clusters	C <sub>1</sub>	C <sub>2</sub>	C <sub>3</sub>	C <sub>4</sub>	C <sub>5</sub>	C <sub>6</sub>	C <sub>7</sub>
C <sub>1</sub>	0	29.67 <sup>ns</sup>	73.75**	40.70**	361.63**	191.19**	181.61**
C <sub>2</sub>		0	89.42**	114.87**	425.91**	289.40**	308.35**
C <sub>3</sub>			0	80.94**	536.08**	259.99**	143.15**
C <sub>4</sub>				0	403.55**	171.94**	92.80**
C <sub>5</sub>					0	543.01**	589.21**
C <sub>6</sub>						0	269.16**
C <sub>7</sub>							0

<sup>ns</sup>, \* and \*\* non-significant, significant, highly significant respectively.

#### 4.1.4.3. Comparison of genotype performances among clusters

The cluster mean performance showed significant variation among all the seven clusters for all of the traits studied except peduncle length (Table 13).

The first cluster (C<sub>1</sub>) was showed average performance for all traits studied. This cluster had higher performance for total plant weight and grain yield per plant and low mean for lodging index.

The second cluster (C<sub>2</sub>) was characterized by longest days to heading and highest means for plant height, panicle length, culm length, number of primary branches per main shoot panicle and number of spikelet per main shoot panicle, first basal culm internode diameter, main shoot panicle weight, main shoot panicle seed weight and biomass yield. This cluster also showed lowest mean for lodging index but had shortest grain filling period and lower peduncle length and number of total tillers. Compared to the other clusters this cluster generally showed highest performance in most of desirable traits and this cluster were dominated by varieties resulting from hybridization. Hence, hybridization might be good approach for accumulating of the disable trait in a single variety.

The third cluster (C<sub>3</sub>) was characterized by shorter grain filling period and lowest means for peduncle length, number of total and fertile tillers, grain yield per plant, grain yield and harvest index .It also showed lower total plant weight, economic growth rate and lodging index.

The fourth cluster (C<sub>4</sub>) was characterized by highest grain yield, harvest index and economic growth rate, high grain yield per plant and better response for total plant weight, but it exhibited shortest maturity and short grain filling period.

The fifth cluster (C<sub>5</sub>) exhibited relatively average performance for most traits. It is characterized by long peduncle length, and highest second basal culm internode diameter and thousand seed weight but had shorter heading period and low number of primary branches per main shoot panicle.

The genotype in the sixth cluster (C<sub>6</sub>) exhibited longest maturity and grain filling periods, and highest number of total and fertile tiller per plant, high total plant weight and grain yield per plant, and higher peduncle length. But it also depicted highest lodging index and lowest second basal culm internode diameter, and lower number of primary branches per main shoot panicle.

Cluster seven (C<sub>7</sub>) contain varieties with high peduncle length, but having shortest heading period and lowest plant height, short panicle and culms, and lowest values of primary branches per main shoot panicle, number of spikelets / main shoot panicle, first basal culm internode diameter, thousand seed weight, main shoot panicle weight, main shoot panicle seed weight, total plant weight, biomass yield and economic growth rate.

The solitary un-grouped lines appear very interesting in various aspects (Kebebew *et al.*, 2003b). Similar to this finding the current study revealed interesting features of solitary lines (cluster five, six and seven) that showed peculiar features in some traits. Accordingly, cluster five showed highest thousand seed weight, cluster six depicted highest lodging index and cluster seven inferior performance in most of the trait studied.

Table 13. Differences among the seven clusters of 49 tef varieties for mean performance of 23 traits

Traits	Clusters							Grand Mean	LSD (0.05)
	C <sub>1</sub>	C <sub>2</sub>	C <sub>3</sub>	C <sub>4</sub>	C <sub>5</sub>	C <sub>6</sub>	C <sub>7</sub>		
DTH	45.65ab	50.08a	48.45a	42.53bc	40.26c	41.50bc	40.22c	44.10	4.56
DTM	94.61abc	96.50ab	95.21abc	91.64c	92.92bc	97.03a	91.67c	94.23	4.11
GFP	48.75bc	46.61c	46.99c	47.43c	52.97ab	55.63a	51.41abc	49.97	4.94
PLH	108.24ab	117.89a	101.62bc	93.88c	99.29bc	94.89c	80.54d	99.48	11.51
PaL	42.97ab	46.48a	42.93ab	36.17cd	37.02c	40.54bc	31.80d	39.70	5.14
CL	65.27ab	71.41a	58.69bcd	57.71bcd	62.27abc	54.35cd	48.74d	59.78	10.32
PDL	18.38a	16.87a	16.84a	17.41a	20.05a	19.73a	19.64a	18.42	3.55
TT	10.53bcd	8.76cd	8.02d	11.72bc	12.43b	16.10a	9.16bcd	10.96	3.55
FT	8.49bcd	7.11cd	6.04d	9.13bc	10.48ab	13.35a	7.00cd	8.80	3.07
PBMPa	28.50ab	30.73a	29.36a	23.87bc	22.24c	21.92c	21.45c	25.44	4.75
SpPa	539.23ab	647.25a	513.13ab	419.71bc	380.32bcd	348.00cd	234.84d	440.4	163.58
LOGI	82.74bc	80.14c	81.00c	87.30abc	89.47ab	93.99a	83.59bc	85.46	7.31
FBCD	0.19ab	0.23a	0.19ab	0.18ab	0.16ab	0.16ab	0.14b	0.18	0.07
SBCD	0.19bc	0.23b	0.18bcd	0.16cd	0.34a	0.13d	0.15cd	0.20	0.05
TSW	0.31b	0.32b	0.27b	0.31b	0.42a	0.26b	0.24b	0.30	0.09
PaW	1.53b	2.05a	1.16bc	1.15bc	1.15bc	0.70cd	0.39d	1.16	0.47
PaSW	0.67ab	0.90a	0.55bc	0.57bc	0.41bc	0.46bc	0.28c	0.55	0.32
PLW	12.88a	11.94a	7.16bc	10.57ab	11.36a	12.90a	6.33c	10.45	4.07
PLSW	3.36ab	2.74ab	0.95c	2.98ab	2.04abc	3.44a	1.76bc	2.47	1.64
BY	746.39abc	972.48a	734.23abc	578.61bc	791.32ab	526.69cd	307.36d	665.3	257.28
GY	205.58a	217.59a	115.12b	241.98a	207.86a	159.20ab	120.08b	181.1	85.14
HI	13.23ab	12.14bc	8.41c	16.88a	13.42ab	10.99bc	13.78ab	12.69	4.00
EGR	430.37ab	472.30ab	265.61c	516.71a	400.55abc	312.61bc	252.44c	378.7	162.24

Figures sharing similar letter within rows are not statistically significant different. DTH=days to heading, DTM=days to maturity, GFP= grain filling period, PLH= Plant height, PaL=panicle length, CL= culm length, PDL= peduncle length, TT= no. of total tillers per plant, FT=no. of fertile tillers per plant, PBMPa= no. of primary branches per main shoot panicle, SpPa=no. of spikelets per main shoot panicle, LOGI =lodging index. FBCD= first basal culm internode diameter, SBCD=second basal culm internode diameter, TSW=thousand seed weight, PaW= main shoot panicle weight, PaSW= main shoot panicle seed weight, PLW= total plant weight, PLSW =grain yield per plant, GY= grain yield, BY = biomass yield, HI= Harvest index and EGR=economic growth.

#### **4.1.4.4. Principal component analysis (PCA)**

The principal component analysis showed that the first six principal components with eigenvalues greater than unity altogether explain about 76% of the total variation among 49 tef varieties evaluated for 23 traits (Table 14).

The first principal component alone accounted for about 31% of the total variation which is similar to the findings of Kebebew *et al.*, (2001b). Among the 23 traits studied, 11 of them had high contribution effect to the first PC, and these traits included grain yield, plant height, main shoot panicle weight, culm length, number of spikelets per main shoot panicle, days to heading, biomass yield, panicle length and number of primary branches per main shoot panicle. The second component accounting for about 13% of the total variation predominantly illustrates variation in harvest index, grain yield per plant, single plant weight, number of fertile and total tillers per plant, economic growth rate, and thousand seed weight. The third principal component accounted 12% of the total variation and it was chiefly accounted by variation in economic growth rate, single plant weight, harvest index, grain yield per plant and number of total tillers per plant. The fourth principal component accounted 8% of the total variation and was indicated with high variation in grain filling period, fertile tiller per plant, peduncle length, total tiller per plant, second basal culm internode diameter and thousand seed weight. The fifth principal component that accounted for about 6% of the total variation was due mainly to high variation in days to maturity, grain filling period, panicle length, lodging index, second basal culm internode diameter and first basal culm internode diameter. The sixth principal component also accounted for about 6% of the total variation, and its major contributors are variations in grain yield per plant, culm length, number of fertile tiller per plant, thousand seed weight and main shoot panicle seed weight (Table 14).

Generally, the presence of phenotypic diversity among the varieties is also reflected in the principal component analysis which revealed that the entire variation cannot be explained in terms of few PCs. In the current study, 76 % of the variation among 49 varieties with more number of PCs from the previous other studies of Kebebew *et al.*, (1999, 2000) who reported that about 71-74% of the variation in 320 tef germplasm lines was explained by

five PCs. On the other hand, only five PCs were reported to have explained about 81% of the gross variation in 1080 (Kebebew *et al.*, 2001b) and 60 (Kebebew *et al.*, 2003b) tef germplasm lines.

Table 14. Eigenvectors, eigenvalues and percentage of total variance explained by the first six principal components (PC) for 23 traits in 49 tef varieties

Traits	PC1	PC2	PC3	PC4	PC5	PC6
Days to heading (days)	0.28	-0.18	0.07	0.26	-0.07	0.15
Days to maturity (days)	0.15	-0.05	0.11	-0.22	0.46	-0.03
Grain filling period (days)	-0.12	0.09	0.04	-0.44	0.46	0.04
Plant height (cm)	0.34	0.04	0.04	-0.03	0.16	0.10
Panicle length (cm)	0.27	-0.06	0.03	0.01	0.38	0.02
Culm length (cm)	0.29	0.13	0.03	-0.04	-0.03	0.29
Peduncle length (cm)	-0.04	0.24	0.09	-0.29	0.07	0.06
No. of total tillers per plant	-0.14	0.29	0.28	0.28	0.09	0.16
No. of fertile tillers per plant	-0.11	0.30	0.21	0.35	0.13	0.28
No. of primary branches /main shoot panicle	0.26	-0.15	0.11	0.16	0.09	-0.01
No. spikelets/ main shoot panicle	0.29	-0.01	-0.03	0.06	0.18	-0.19
Lodging index	-0.20	0.11	-0.20	0.16	0.33	0.07
First basal culm internode diameter (mm)	0.24	0.13	0.16	-0.20	-0.25	0.01
Second basal culm internode diameter (mm)	0.19	0.17	0.15	-0.27	-0.26	0.14
Thousand kernel weight (mg)	0.14	0.27	0.02	-0.27	-0.21	0.28
Main shoot panicle weight (g)	0.32	0.01	-0.03	0.14	-0.10	-0.09
Main shoot panicle seed weight (g)	0.23	-0.01	-0.16	0.20	0.00	-0.26
Single plant weight (g)	0.08	0.32	0.34	0.17	0.03	-0.18
Grain yield per plant (g)	0.04	0.34	0.29	0.13	0.08	-0.33
Biomass yield (g/plot)	0.28	-0.01	-0.21	0.03	0.16	0.23
Grain yield (g/plot)	0.44	0.11	0.19	0.02	0.04	0.09
Harvest index (%)	-0.09	0.35	-0.31	0.06	-0.02	-0.16
Economic growth rate (%)	0.10	0.30	-0.43	0.08	-0.04	0.03
Eigenvalue	7.36	3.15	2.93	1.91	1.48	1.32
Total variance explained (%)	30.65	13.12	12.19	7.94	6.16	5.52
Cumulative total variance explained (%)	30.65	43.77	55.96	63.91	70.06	75.58

#### **4.1.5. Correlation among traits**

Phenotypic and genotypic correlation coefficients between all possible pairs of the 23 traits recorded from the 49 tef varieties in this study are presented on Tables 15 and 16.

##### ***4.1.5.1. Relationship of grain yield with other traits***

Generally, increasing grain yield is the breeding objective in tef improvement program (Kebebew *et al.*, 2011). However, direct selection based on crop yields is often a paradox in breeding programmes because yield is influenced by its component traits (Mustafa and Elsheikh, 2007). Hence, most of the economic traits including yield are polygenically controlled and are much influenced by environmental factors, an understanding of inheritance and study of association between yield and its components is necessary for planning an effective selection program in identifying high yielding varieties (Firas *et al.*, 2012). Accordingly, it is important to select indirectly for other traits in order to improve grain yield. In this study, the phenotypic and genotypic association of grain yield with the rest of the traits were examined and presented below.

Significant positive phenotypic correlations were detected for grain yield with panicle and culm length, number of fertile tillers per plant, number of primary branch per main shoot panicle, number of spikelet per main shoot panicle, thousand seed weight, main shoot panicle seed weight, grain yield per plant, biomass yield, harvest index and economic growth rate (Table 15).

Significant positive genotypic associations of grain yield were observed with panicle length, number of fertile tillers per plant, number of spikelet per main shoot panicle, main shoot panicle weight, and main shoot panicle seed weight, grain yield per plant, biomass yield, harvest index and economic growth rate (Table 16).

Similar to the results in the current study, Yifru and Hailu (2005) found significant and positive association of grain yield with number of spikelets per main shoot panicle, main shoot panicle seed weight and biomass yield. In addition, Hailu *et al.*, (2003) also reported high and positive direct effect of grain yield with plant height, panicle length, number of

spikelet and number of primary branches per main shoot panicle, main plant weight and seed yield per main plant. This indicates that prolific varieties with high number of spikelets per panicle are likely to have high panicle seed weight as well as high above ground biomass which ultimately leads to high seed yield. Likewise, the significant positive correlation of grain yield with harvest index and economic growth rate in the present study also suggests that prolific varieties are likely to have good harvest index and high economic growth rate.

#### ***4.1.5.2 Relationship of phenologic traits with other traits***

Days to heading showed significant positive phenotypic association with days to maturity, grain filling period, plant height, panicle and culm length, number of primary branches per main shoot panicle, number of spikelets per main shoot panicle, first and second basal culm internode diameter, main shoot panicle weight, main shoot panicle seed weight and biomass yield. In contrast, days to heading exhibited significant negative phenotypic relationship with lodging index, number of total and fertile tillers per plant and harvest index (Table 15).

Days to heading showed significant positive genotypic association with days to maturity, grain filling period, plant height, panicle and culm length, number of primary branches per main shoot panicle, number of spikelets per main shoot panicle, first and second basal culm internode diameter, main shoot panicle weight, main shoot panicle seed weight and biomass yield. On the other hand, days to heading showed significant negative association with number of total and fertile tillers per plant, lodging and harvest index (Table 16).

Days to maturity revealed positive significant phenotypic association with grain filling period, plant height, panicle and culm length, number of primary branches per main shoot panicle, number of spikelets per main shoot panicle, and first and second basal culm internode diameter, main shoot panicle weight and biomass yield. In contrast, days to maturity showed significant negative relationship with number of total and fertile tillers per plant, lodging and harvest index (Table 15).

Days to maturity showed positive significant genotypic relationship with grain filling period, plant height, panicle and culm length, number of primary branches per main shoot panicle, number of spikelets per main shoot panicle, first basal culm internode diameter, main shoot panicle weight, single plant weight, and biomass yield. In contrast, it showed negative correlation with lodging and harvest index (Table 16).

Grain filling period exhibited positive significant phenotypic association with peduncle length. But it showed negative significant association with number of primary branches per main shoot panicle, number of spikelets per main shoot panicle, main shoot panicle weight and main shoot panicle seed weight (Table 15).

Grain filling period showed positive significant genotypic association with peduncle length while it depicted negative significant correlation with number of primary branches per main shoot panicle, number of spikelets per main shoot panicle and main shoot panicle weight (Table 16).

The current results showing negative association of lodging with days to heading and maturity are in line with the previous report of Hailu *et al.*, (2003).

#### ***4.1.5.3 Relationship of lodging with other traits***

Lodging index showed positive significant phenotypic association with number of total and fertile tillers per plant and harvest index. On the other hand, it depicted negative significant phenotypic relationship with plant height, panicle and culm length, number of primary branches per main panicle, spikelets per main shoot panicle, first basal culm internode diameter and main shoot panicle weight (Table 15). Lodging index also showed positive significant genotypic association with number of total and fertile tillers per plant and harvest index. In contrast, it showed negative significant genotypic correlation with plant height, panicle and culm length, number of primary branches and spikelets per main shoot panicle, first and second basal culm diameter, main shoot panicle weight, main shoot panicle seed weight, single plant weight, and biomass and grain yield (Table 16).

In agreement with the current finding, Habite *et al.*, (2015) reported substantial negative correlation of grain yield with lodging index. Generally, the existence of a strong correlation is the indication that those traits are conditioned by the same set of gene, be it in the positive or negative direction (Falconer, 1989). Consequently, selection for one trait can indirectly introduce changes in the other trait in positive or negative direction due to either genetic linkage or presence of pleiotropic gene effect or both (Falconer, 1989).

Table 15. Estimates of correlation coefficient (r) at phenotypic level for 23 traits of 49 tef varieties

Traits	DTH	DTM	GFP	PLH	PaL	CL	PDL	TT	FT	PBMPa	SpPa	LOGI
DTH	1											
DTM	0.41**	1										
GFP	0.61**	0.28*	1									
PLH	0.65**	0.48**	-0.17	1								
PaL	0.58**	0.52**	-0.09	0.79**	1							
CL	0.57**	0.36*	-0.18	0.93**	0.52**	1						
PDL	-0.20	0.02	0.28*	0.08	-0.03	0.13	1					
TT	-0.34*	-0.34*	0.13	-0.29*	-0.34*	-0.20	0.13	1				
FT	-0.29*	-0.29*	0.12	-0.23	-0.27	-0.16	0.16	0.94**	1			
PBMPa	0.60**	0.35*	-0.30*	0.59**	0.69**	0.42**	-0.19	-0.39**	-0.29*	1		
SpPa	0.56**	0.32*	-0.30*	0.71**	0.78**	0.53**	-0.04	-0.22	-0.20	0.57**	1	
LOGI	-0.45**	-0.28*	0.26	-0.46**	-0.37**	-0.43**	-0.01	0.36*	0.36*	-0.35*	-0.30*	1
FBCD	0.46**	0.34*	-0.19	0.57**	0.36*	0.59**	0.08	-0.08	-0.10	0.31*	0.42**	-0.39**
SBCD	0.34*	0.28*	-0.07	0.48**	0.29*	0.50**	0.19	-0.11	-0.06	0.26	0.31*	-0.26
TSW	-0.05	0.13	0.14	0.32*	0.10	0.39**	0.15	-0.03	-0.01	0.02	0.07	-0.10
PaW	0.67**	0.40**	-0.38**	0.72**	0.58**	0.67**	-0.09	-0.37**	-0.27	0.60**	0.60**	-0.36*
PaSW	0.53**	0.24	-0.32*	0.46**	0.42**	0.40**	-0.19	-0.29*	-0.27	0.41**	0.57**	-0.15
PLW	0.12	0.20	-0.01	0.27	0.15	0.30*	0.18	0.44**	0.46**	0.17	0.17	-0.22
PLSW	-0.13	0.01	0.03	0.01	0.01	0.02	0.23	0.46**	0.47**	0.06	0.11	-0.10
GY	-0.14	-0.10	0.03	0.25	0.47**	0.31*	0.08	-0.02	0.33*	0.46**	0.28*	0.24
BY	0.63**	0.41**	-0.21	0.75**	0.61**	0.70**	-0.07	-0.41**	-0.31*	0.54**	0.54**	-0.18
HI	-0.54**	-0.39**	0.08	-0.27	-0.30*	-0.21	0.13	0.20	0.13	-0.36*	-0.12	0.36*
EGR	-0.02	-0.18	-0.19	0.25	0.06	0.32*	0.02	-0.03	-0.05	-0.01	0.21	0.17

Table 15. Continued

Traits	FBCD	SBCD	TSW	PaW	PaSW	PLW	PLSW	GY	BY	HI	EGR
FBCD	1										
SBCD	0.66**	1									
TSW	0.42**	0.55**	1								
PaW	0.47**	0.47**	0.27	1							
PaSW	0.37**	0.27	0.31*	0.72**	1						
PLW	0.27	0.27	0.24	0.39**	0.15	1					
PLSW	0.03	-0.01	0.15	0.16	0.03	0.82**	1				
GY	0.05	0.02	0.28*	0.22	0.45**	-0.03	0.39**	1			
BY	0.30*	0.38**	0.18	0.68**	0.50**	-0.04	-0.24	0.44**	1		
HI	-0.16	-0.20	0.13	-0.18	-0.05	-0.05	0.24	0.75**	-0.18	1	
EGR	0.10	0.02	0.22	0.27	0.33*	-0.03	0.09	0.97**	0.44**	0.73**	1

Table 16. Estimates of correlation coefficient (r) at genotypic level for 23 traits of 49 tef varieties

Traits	DTH	DTM	GFP	PLH	PaL	CL	PDL	TT	FT	PBMPa	SpPa	LOGI
DTH	1	0.31*	0.58**	0.75**	0.57**	0.72**	-0.24	-0.43**	-0.32*	0.65**	0.59**	-0.55**
DTM		1	0.28*	0.47**	0.46**	0.38**	0.06	-0.25	-0.18	0.34*	0.31*	-0.28*
GFP			1	-0.19	-0.06	-0.25	0.33*	0.23	0.21	-0.30*	-0.29*	-0.26
PLH				1	0.83**	0.92**	-0.05	-0.39**	-0.29*	0.69**	0.69**	-0.57**
PaL					1	0.53**	-0.02	-0.38**	-0.31*	0.73**	0.80**	-0.51**
CL						1	-0.05	-0.31*	-0.21	0.52**	0.47**	-0.49**
PDL							1	0.08	0.06	-0.24	-0.07	-0.09
TT								1	0.94**	-0.35*	-0.24	0.38**
FT									1	-0.24	-0.21	0.32*
PBMPa										1	0.62**	-0.54**
SpPa											1	-0.51**
LOGI												1

Table 16. *Continued*

Traits	FBCD	SBCD	TSW	PaW	PaSW	PLW	PLSW	GY	BY	HI	EGR
DTH	0.61**	0.39**	-0.05	0.68**	0.40**	0.18	-0.05	-0.12	0.69**	-0.57**	-0.00
DTM	0.37**	0.25	0.08	0.40**	0.26	0.31*	0.09	-0.15	0.30*	-0.37**	-0.23
GFP	-0.23	-0.09	0.09	-0.31*	-0.19	0.10	0.12	-0.04	-0.26	0.06	-0.26
PLH	0.68**	0.45**	0.11	0.73**	0.46**	0.31*	0.07	0.10	0.73**	-0.44**	0.12
PaL	0.56**	0.32*	0.05	0.66**	0.49**	0.28*	0.12	0.41**	0.56**	-0.30*	0.03
CL	0.62**	0.44**	0.14	0.62**	0.34*	0.27	0.02	0.12	0.70**	-0.44**	0.16
PDL	0.03	0.09	0.09	-0.11	-0.16	0.13	0.18	-0.08	-0.21	0.06	-0.13
TT	-0.28*	-0.21	-0.02	-0.42**	-0.30*	0.29*	0.39**	-0.13	-0.43**	0.10	-0.16
FT	-0.15	-0.08	0.01	0.31*	-0.22	0.37**	0.47**	0.44**	-0.30*	0.01	-0.17
PBMPa	0.59**	0.28*	-0.07	0.66**	0.46**	0.35*	0.22	-0.14	0.47**	-0.44**	-0.07
SpPa	0.49**	0.23	-0.10	0.63**	0.59**	0.32*	0.24	0.38**	0.44**	-0.21	0.06
LOGI	-0.45**	-0.28*	0.03	-0.53**	-0.39**	-0.36*	-0.20	-0.29*	-0.32*	0.37**	0.14
FBCD	1	0.67**	0.20	0.58**	0.36*	0.28*	0.06	-0.13	0.40**	-0.38**	-0.08
SBCD		1	0.45**	0.39**	0.11	0.25	0.02	-0.11	0.38**	-0.35*	-0.10
TSW			1	0.13	-0.01	0.10	-0.00	0.05	0.06	-0.03	0.04
PaW				1	0.77**	0.51**	0.32*	0.28*	0.62**	-0.27	0.19
PaSW					1	0.42**	0.33*	0.39**	0.34*	-0.07	0.22
PLW						1	0.85**	0.18	0.03	-0.24	-0.16
PLSW							1	0.32*	-0.15	0.07	-0.00
GY								1	0.52**	0.71**	0.97**
BY									1	-0.31*	0.37**
HI										1	0.69**
EGR											1

\*, \*\* Significant at 0.05 and 0.01 probability level respectively, DTH=days to heading, DTM=days to maturity, GFP= grain filling period, PLH= Plant height, PaL=panicle length, CL= culm length, PDL= peduncle length, TT= no. of total tillers per plant, FT=no. of fertile tillers per plant, PBMPa= no. of primary branches per main shoot panicle, SpPa=no. of spikelets per main shoot panicle, LOGI =lodging index. FBCD= first basal culm internode diameter, SBCD=second basal culm internode diameter, TSW=thousand seed weight, PaW= main shoot panicle weight, PaSW= main shoot panicle seed weight, PLW= single plant weight, PLSW =grain yield per plant, GY= grain yield, BY =biomass yield, HI= Harvest index and EGR=economic growth rate

## 4.2. Experiment 2:- Genetic Diversity of Ethiopian Tef Varieties as Reveled by Microsatellite Markers

### 4.2.1. Polymorphism levels and utility of SSR markers

The difference between the longest and shortest amplified fragment size ranged from 110 bp to 490 bp. The highest variation in fragment size was observed for primer *CNLTs86* (175-470 bp) and the lowest for primer *CNLTs27* (120-190 bp). All the nine SSR primer-pairs were polymorphic, and detected a total of 52 alleles. The number of alleles per locus ranged from five for *CNLTs27*, *CNLTs31* and *CNLTs116* to seven for *CNLTs86* with in an average of six alleles per locus (Table 18). This was in contrary to Zeid *et al.*, (2012) who reported 8-23 alleles per locus. The number of alleles detected in the present study was lower than the number of alleles for each of these loci reported by Zeid *et al.*, (2012). The difference might be due to few individuals analyzed per genetic sources in the present study and sample types (natural population vs released varieties) and primer used. The number of effective alleles ( $N_e$ ) ranged from three for marker *CNLTs116* to six for marker *CNLTs86* with a mean of four.

The observed heterozygosity ( $H_o$ ) varied from 0.75 for marker *CNLTs116* to 1.00 for *CNLTs27*, *CNLTs31*, *CNLTs56*, *CNLTs60*, *CNLTs61*, *CNLTs62*, and *CNLTs86* with a mean of 0.96. Expected heterozygosity or gene diversity ( $H_e$ ) of the markers ranged from 0.65 for marker *CNLTs27* to 0.80 for marker *CNLTs56* and *CNLTs86*, with a mean of 0.73 (Table 17). However, Zeid *et al.* (2012) documented relatively higher  $H_e$  value ranging from 0.75 to 0.91 this could be explained by sample size and the number of loci used in the present study.

The polymorphic information content (PIC) values are usually calculated to assess the level of polymorphism of a marker. As described by Botstein *et al.*, (1980), a marker with PIC >0.5 is said to be highly informative, a marker with PIC between 0.5 and 0.25 is considered as reasonably informative, and those with values below 0.25 are slightly informative. In the present study, all the nine microsatellite loci used had PIC values greater than 0.5 (Table 17). The PIC of loci ranged from 0.59 for marker *CNLTs27* to 0.77 for *CNLTs86* with a mean of 0.69. This indicates the high discrimination ability of the microsatellites for the

studied tef varieties. These are useful markers for further analysis of genetic diversity in tef. The high value of PIC could also indicate the genetic heterogeneity of the sampled populations (Rehman and Khan, 2009). The markers used in this study were also found to show higher PIC value as compared to the previous study done using various tef varieties (Solomon *et al.*, 2007; Zeid *et al.*, 2012).

Table 17. Values of genetic parameters in tef and its relatives using nine SSR markers

Locus	Genetic parameters							
	Na	Ne	I	Ho	He	F	Nm	PIC
<i>CNLTs17</i>	6	4	1.25	0.90	0.73	0.35	2.24	0.68
<i>CNLTs27</i>	5	4	1.02	1.00	0.65	0.68	8.63	0.59
<i>CNLTs31</i>	5	5	1.43	1.00	0.74	0.36	8.21	0.69
<i>CNLTs56</i>	6	4	1.26	1.00	0.80	0.51	1.43	0.76
<i>CNLTs60</i>	6	4	1.12	1.00	0.68	0.61	4.32	0.63
<i>CNLTs61</i>	6	5	1.16	1.00	0.67	0.62	7.32	0.62
<i>CNLTs62</i>	6	5	1.36	1.00	0.77	0.42	2.71	0.73
<i>CNLTs86</i>	7	6	1.49	1.00	0.80	0.33	3.00	0.77
<i>CNLTs116</i>	5	3	0.99	0.75	0.77	0.32	0.39	0.73
Mean	5.78	4.28	1.23	0.96	0.73	0.48	4.25	0.69
SE	0.67	0.24	0.06	0.03	0.02	0.04	1.02	0.06

Na=number of alleles; Ne=number of effective alleles; I=Shannon's information index; Ho=observed heterozygosity; He=unbiased expected heterozygosity; F=fixation index; Nm=gene flow; PIC=polymorphic information content; SE=standard error

#### 4.2.2. Pattern of diversity and genetic relationship

Knowledge of frequency and distribution of alleles is important to identify genetically distinct parents (Iskakova AN *et al.*, 2014). The numbers of observed and effective alleles were higher for improved varieties resulting from direct selection and hybridization than the local varieties and wild relatives (Table 18). Likewise, the highest Shannon diversity index of 1.38 was recorded for improved varieties that came from hybridization and the lowest value of 0.85 was noted for wild relatives. Similarly, Kebebew *et al.*, (2003a) reported a mean value of 1.23 which was higher than the mean Shannon diversity index of 0.73.

In this study, the observed heterozygosity was 0.96, 0.99, 1.00 and 0.89 for direct selection, hybridization, local varieties and wild relatives, respectively (Table 18). The expected

heterozygosity was equally 0.71 for varieties from direct selection and hybridization, while the local varieties and wild relatives exhibited 0.69 and 0.53, respectively. Since, high genetic variation was observed among local varieties, the incorporation of these varieties in the tef breeding program would serve as important sources of genes and also benefit from heterotic effect. Moreover, the highest variation was noted within the tested improved varieties that came from hybridization program, and this might be due to genetically distant parents used in the development of the varieties. Likewise, the high variation noted within the tested improved varieties resulting from direct selection imply that the tef breeding program in Ethiopia has been utilizing diverse gene sources for developing new varieties.

The present results are in agreement with genetic diversity reported by Kebebew *et al.*, (2003a) and Zeid *et al.*, (2012). In contrast to Bai *et al.*, (1999b, 2000), Mulu *et al.*, (1999), and Mulu and Nguyen (2000) reported high genetic similarity estimates in selected tef genotypes using AFLP or RAPD markers. This deviation could be explained due to the difference in discrimination power of the genetic marker used in the study and followed by sampling strategy. SSR markers are more powerful and informative for genetic diversity analysis than AFLP and RAPD markers (Zeid *et al.*, 2012).

The overall mean observed heterozygosity ( $H_o$ ) of 0.96 was higher than the corresponding average expected heterozygosity ( $H_e$ ) of 0.66, which might indicate the presence of overall gain in heterozygosity within the examined tef varieties (Table 18). The degree of polymorphism among the groups varied from 67% for improved varieties obtained via hybridization to 57% for wild relatives with the average of 63% (Table 18). This can be confirmed by visual observation on the gels of the specific markers which revealed the existence of more polymorphism for the improved varieties obtained via hybridization than the rest of the varieties studied (Figure 2). Overall, the National Breeding Program is working towards developing tef varieties having wider gene pool. This strategy should be further strengthened to divergent varieties of released varieties resilient to changing environment.

Table 12. Summary of genetic parameters for four groups using nine selected SSR markers

Populations	Genetic parameters							
	N	Na	Ne	I	Ho	He	F	%p
Direct selection	23	5	4	1.37	0.96	0.71	0.36	61.03
Hybridization	16	5	4	1.38	0.99	0.71	0.4	67.05
Local varieties	10	5	3	1.32	1.00	0.69	0.45	65.89
Wild relatives	2	2	2	0.85	0.89	0.53	0.73	57.39
Mean	12.75	4.23	3.22	1.23	0.96	0.66	0.49	62.84
SE		0.24	0.16	0.06	0.03	0.02	0.04	2.78

N=number of varieties; Na=number of alleles; Ne=number of effective alleles; I=Shannon's information index; Ho=observed heterozygosity; He=expected heterozygosity; F=fixation index; %P=percentage of polymorphic loci; SE=standard error

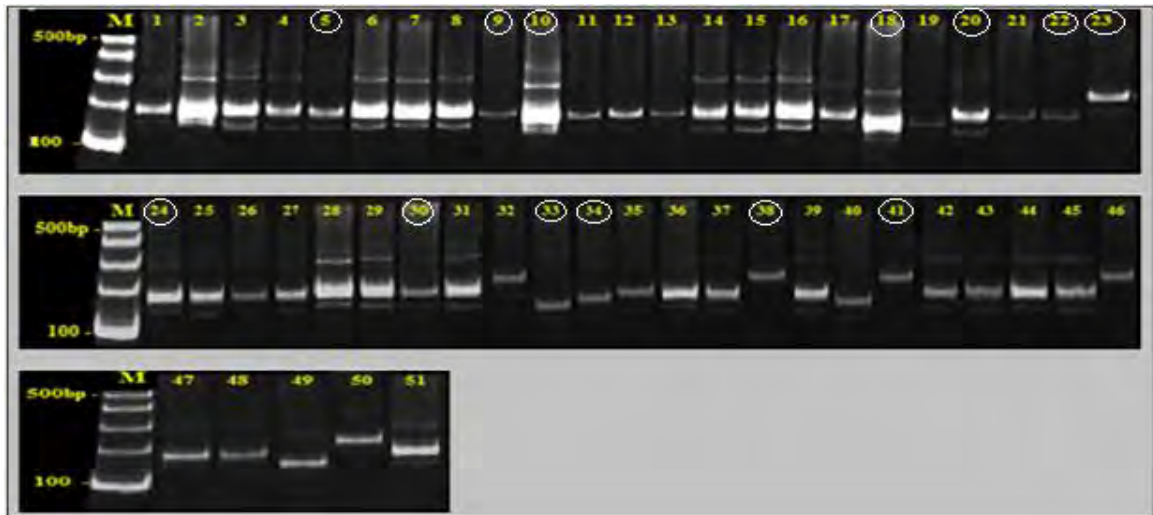


Figure 2. Electrophoretic bands of tef DNA of 51 varieties as revealed by polymorphic SSR marker CNLT<sub>S</sub> 61. The lane numbers identify serial number of varieties as designated on Table 4, M stands for DNA ladder. Some lane numbers circled are released varieties

#### 4.2.3. Analysis of molecular variance and partitioning the genetic diversity

Analysis of molecular variance (AMOVA) partitioned the total molecular variance into within and among the sets of varieties evaluated based on their source of genetic origins (Nyaligwa *et al.*, 2015). There were highly significant ( $P < 0.001$ ) molecular variances among populations, among individual within populations and within individuals. The largest genetic variation (60%) was attributed to variation within individuals, while 30% of the total variation was explained by variation among individuals within populations, and 10% of variation was explained by variations among populations (Table 19). This signifies

the small sources of varieties but significant contribution to the total molecular variance detected, and that the maximum variation was within individuals. This might be due to the presence of wild relatives in the study (*E.curvula* and *E.pilosa*). Some reports indicated that the level of polymorphism depends on the type of genotypes (He *et al.*, 2011), marker used (Baraket *et al.*, 2011), primers selected (Kong *et al.*, 2011; Sharma *et al.*, 2011) and sampling strategy (Kong *et al.*, 2011).

The magnitude between and within population differentiation were quantified using F-statistics ( $F_{IT}$ ,  $F_{IS}$  and  $F_{ST}$ ), also known as fixation indexes. The latter term was coined by Wright (1951) to describe the properties of subdivided populations. The genetic differentiation among the populations was low ( $F_{ST}=0.028$ ), with low effect of non-random mating among individuals within population ( $F_{IS}=0.328$ ) (Table 19). The low genetic differentiation among population may be due to the high degree of inbreeding and high gene flow. According to Wright (1951),  $F_{ST}$  value below 0.05 is generally considered as a very low level of genetic differentiation. Thus, the varieties under study showed low level of genetic differentiation because of the high variability within individuals, which might be due to gene flow (Gepts and Papa, 2003). The exchange of genes through hybridization program, the continuing replacement of farmer's local varieties by improved varieties and the incorporation of wild relatives in tef hybridization program (use of limited varieties for crossing program; eg. *E.pilosa*), this could be one of causes of the current low level of genetic differentiation observed in the studied populations.

Table 13. Analysis of molecular variance (AMOVA) among 51 tef genotype assembled from four source of origin using nine SSR markers.

Source of variation	Df	SS	MS	Variance		F-statistics	P
				Estimated	%		
Among population	3	21.08	7.03	0.20	10	$F_{st}=0.028$	>0.001
Among individual within population	47	116.66	2.48	0.61	30	$F_{is}=0.328$	>0.001
Within individual	51	64.00	1.26	1.26	60	$F_{it}=0.394$	>0.001
Total	101	201.74	-	2.07	100	-	-

\*df = degree of freedom, SS=sum of squares and MS=mean squares

#### 4.2.4. Genetic differentiation and gene flow between populations

The pair-wise genetic differentiation among varieties within the source of origin ranged from 0.010 between direct selection and hybridization to 0.178 between hybridization and local varieties (Table 20). The low value of  $F_{ST}$  implies that there is high frequency of identical alleles among varieties from hybridization and direct selection. This could be due to the use of identical sources population for hybridization and possibly genetic admixture among the two groups. The lowest  $F_{ST}$  value can be explained by high level of gene flow, which leads to genetic homogeneity (Amelework *et al.*, 2014).

Gene flow ( $Nm$ ) or gene migration varied considerably among the sources of origin with consistently low values between local varieties and hybridization than with the rest of the sources (Table 20). The lowest level of genetic differentiation and high level of gene flow were observed among those from hybridization and direct selection sources of origin of the varieties. This considerable amount of gene flow existed among hybridization and direct selection probably due to the use of some of the varieties developed through direct selection as parents for the varieties developed through hybridization. This may also be attributed to an exchange of genetic materials between the two breeding approaches. In addition, the varieties from both breeding approaches were selected based on their morphological accumulation of desirable traits, which in turn implies the accumulation of high frequency of identical alleles.

Table 20. Pair-wise genetic differentiation ( $F_{ST}$ ) below the diagonal and pair-wise estimate of gene flow ( $Nm$ ) above the diagonal between *tef* genetic sources

Genotype Source	Direct selection	Hybridization	Local varieties	Wild relatives
Direct selection		4.977	1.451	1.532
Hybridization	0.010		1.154	1.208
Local varieties	0.147	0.178		1.985
Wild relatives	0.140	0.171	0.112	

#### 4.2.5. Genetic distance and relationship between populations

The genetic relationship among populations can be evaluated using genetic distance measurements. There are so many methods developed so far to examine genetic distance between populations (Cavalli-Sforza and Edwards, 1967; Nei, 1972, 1978; Takezaki and Nei, 1996). In the current study, Nei's (1978) method was used to estimate the genetic distance between the test tef varieties.

Based on the magnitude of the genetic distance (GD), more differentiation was revealed between the different varieties from different genetic sources and wild relatives (GD range=0.363-0.454) (Table 21). The second largest genetic distance (GD=0.194) was observed between varieties from direct selection and local varieties. The highest value of genetic distance (GD=0.454) was recorded between local varieties and the wild relatives. The smallest genetic distance (GD=0.101) was observed between varieties from direct selection and hybridization. Wild relatives were also found to be consistently distant from the other three genetic sources followed by local varieties.

The results revealed that the group of wild varieties is consistently distant from the other populations as it is classified as other species of *Eragrostis* whereas the others share similar *Eragrostis* tef genetic background. It has also been shown that the genetic distance between varieties from direct selection and hybridization was found to be the smallest one (0.101). This might be due to relatively similar genetic background as some parents for crossing were from the varieties developed through direct selection. This might also be due to the existence of relatively related genetic makeup (Table 21).

Table 21. Pair-wise population Nei's genetic distance showing the magnitude of genetic differentiation between tef populations

Genotype Source	Direct selection	Hybridization	Local varieties	Wild relatives
Direct selection	0.000			
Hybridization	0.101	0.000		
Local varieties	0.194	0.166	0.000	
Wild relatives	0.367	0.363	0.454	0.000

#### 4.2.6. Clustering analysis and relationship among varieties

The 51 varieties were grouped into three major clusters, consisting of 47%, 37% and 16% of the total populations in clusters I, II and III, respectively forming different hierarchical sub-group (Table 22 and Figure 3). Some clusters constituted genotypes mostly from the same genetic sources while others had genotypes from more than one sources showing that they had evolved from different lines of ancestry or derived from independent events of evolutionary forces (genetic drift, mutation, migration, selection and in flux/out flux of genes in the form of germplasm exchange) that separated them into related but different gene pools (Gemechu *et al.*, 2012). These also indicated that varieties from different seed types might have similar genetic background for microsatellite markers. The first and third clusters constituted varieties mainly from direct selection. The second cluster constituted varieties from all origins mainly from hybridization and local varieties. Varieties resulting from direct selection were relatively dispersed all over the three clusters.

Cluster analysis indicated that the first cluster constituted Simada a variety derived from inter-specific cross DZ-01-2785 x *E.pilosa* were clustered together with its parent *E.pilosa* in the same sub-group within same cluster. Again the two varieties Quncho and Gemechis are sister lines that are derived from cross of Dukem x Magna (personal communication). These varieties were grouped in the same cluster as a similar trend was reported by Zeid *et al.*, (2012). Likewise, the elite line GA-10-3 which is obtained through Targeting Induced Local Lesions IN Genomics from the variety Tseday were clustered together with its parent Tseday in same cluster. Similarly the two varieties Kora and Dagem are sister lines and they were grouped in the same sub-group within the same cluster. This indicates that SSR markers are powerful to show genetic relatedness between varieties.

The local varieties dispersed in the three clustering groups and revealed poor clustering pattern with geographical proximities of improved varieties originating from the particular production areas of the local varieties. This shows that there is genetic dissimilarity between improved and local varieties irrespective of their geographic origin. This trend was similar with the past study reported by Kebebew *et al.*, (2003a). In the latter study, the 10 improved varieties released until 1995 were grouped in one cluster indicting their

genetic similarity, whereas the present study distributed these varieties into three clusters, which could be explained by the discriminator power of SSR markers used in this study.

The cluster analysis based on the polymorphic markers surfaced varieties such as Simada, Gola and Were Kiyu in cluster I whereas, Tsedey was grouped in cluster II. These varieties are characterized by early maturing and display good grain yield performance in moisture stress areas. However, the genotype Etsub grouped in cluster I is a late maturing entry with high grain yield, tall plant height and longest panicle length in optimum rainfall area. The unique agronomic attributes of the varieties were consistent with the present genetic analysis using SSR markers and the incorporation of this varieties would be useful for future tef breeding program.

Table 22. Estimated probabilistic memberships of tef populations from different genetic sources to the three clusters

Genetic source of origin	No. of entries	Cluster		
		C1	C2	C3
Direct selection	23	0.652	0.131	0.217
Hybridization	16	0.312	0.625	0.063
Local varieties	10	0.300	0.600	0.100
Wild relatives	2	0.500	0.000	0.500

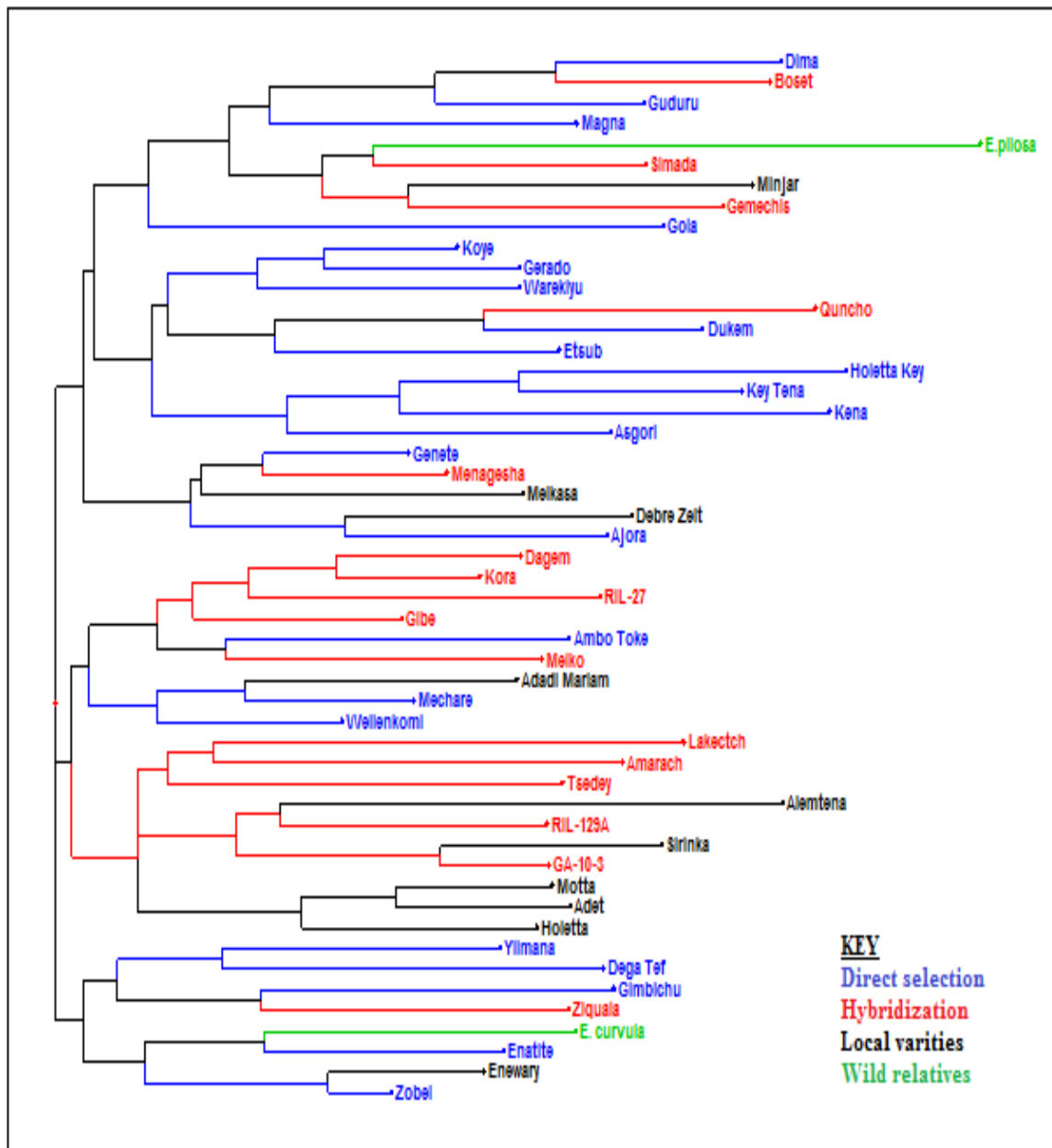


Figure 3. Neighbor Joining (NJ) dendrogram showing genetic relationship of 51 tef varieties using nine microsatellites

#### 4.2.7. Principal coordinate analysis (PCoA)

Principal Co-ordinate Analysis (PCoA) showed that the first three most informative principal coordinates accounted for about 29% of the genetic variation present in SSR molecular data derived from tef varieties in the study. The first, second, and third principal coordinates respectively explained about 12%, 10% and 7% of the gross variation (Table 23).

The pattern of distribution of varieties in the PCoA plot revealed three major clusters in the two-dimensional and three dimensional coordinates (Figures 4 and 5). The groupings identified through PCoA analysis were also similar to those identified in the NJ cluster analysis thereby illustrating the conformity of results obtained from the study. The complementarity of this result suggests the representative nature of the used markers.

Table 23. Percentage of variation explained by the first three axes using SSR markers in Principal Coordinate Analysis

Axis	SSR Markers		
	1	2	3
Percentage of individual variation	12.30	10.27	7.31
Percentage of cumulative variation	12.30	22.57	29.88

In the two-dimensional plot, most individuals of the respective populations were observed to form three major cluster groups with some sub-clustering group and few inter-mixing from different populations. Thus, in the two-dimensional (2D) axis, most of varieties from direct selection were isolated from the other populations making a separate group of their own. In the three dimensional axes (3D) and NJ, almost all individuals of each population were spread all over the plot. Moreover, 2D showed better resolution as compared to 3D and NJ, respectively. Lack of association of the individual varieties into their respective populations in case of 3D and NJ may indicate the exchange of seeds between populations.

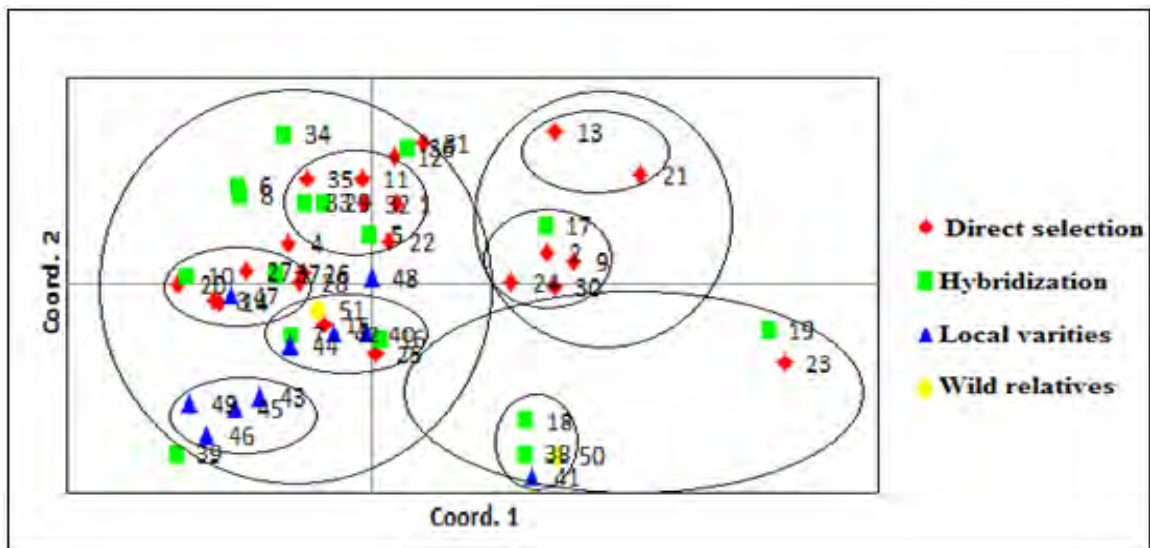


Figure 4. Two dimensional representation of PCoA analysis of 51 tef varieties based on SSR data

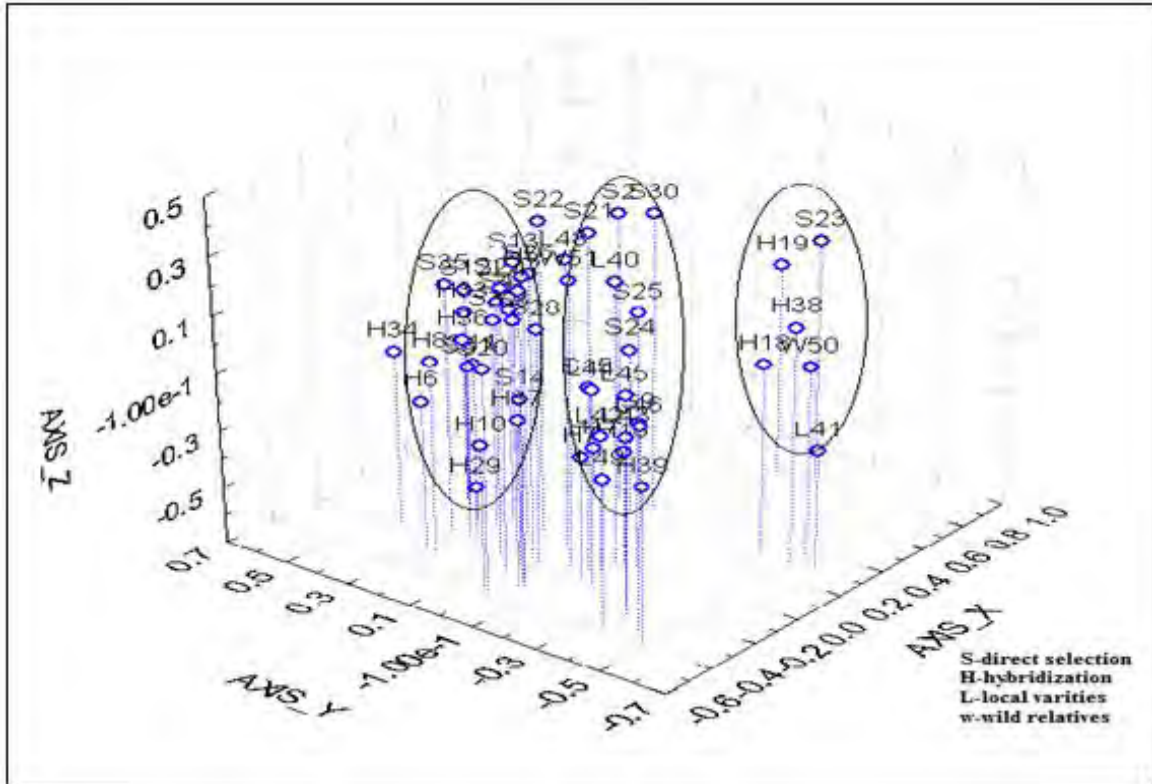


Figure 5. Three dimensional representations of PCO analysis of 51 teff varieties based on SSR data

## 5. CONCLUSIONS AND RECOMMENDATION

### 5.1. Conclusion

Ethiopia is considered as the center of origin for tef. Tef research in Ethiopia started over five decades, and has registered commendable achievements invaluable for enhancing the production and productivity of the crop. Tef has been an orphan crop little touched by scientific research, but currently it has received due emphasis among other crops and its international popularity is rapidly growing. Over the years, a total of 36 improved tef varieties have been released by federal and regional research centers of the country. Information on the extent and pattern of diversity present in the varieties and trait interrelationships is essential for designing effective and efficient future tef improvement strategies.

The current study focuses on detailed assessment on extent and pattern of genetic diversity of tef varieties using agro-morphological traits along with nine polymorphic SSR markers. The results revealed low genetic diversity among the Ethiopian tef varieties. This indicated that the varieties are closely related and probably few parents were used in the breeding program. Specially, the varieties obtained from using different breeding approach (*i.e* direct selection and hybridization) were greatly related, while the local varieties had better divergence from each other as well as from the improved varieties. This, in turn, indicates that the potential of Ethiopian tef landraces has been underexploited by the hitherto tef breeding efforts in Ethiopia.

Using agro-morphological traits and molecular data it was observed that a limited number of the varieties appeared distinct and could serve as genetic source for multiple attributes. For instance, Simada, Gola, Tseday and Were Kiyu from the early maturing categories that are suitable for terminal low moisture stress areas, and the variety Etsub from the late maturing categories suitable for optimum rainfall areas. In the efforts towards developing appropriate varieties series of multiple crossing may be required in order to bring desirable traits into a single variety.

Determination of the association of agro-morphological traits in tef is very important for further study and improvement of the crop. Thus, to improve grain yield a positive selection for panicle length, culm length, number of fertile tillers per plant, number of spikelet per main shoot panicle, number of primary branches per main shoot panicle, thousand seed weight, grain yield per plant, biomass yield, harvest index and economic growth rate may aid in the identification of best candidates lines in the breeding program.

## **5.2. Recommendation**

The low genotypic variation observed in the present study implies the Ethiopian tef breeding program should revise its tef breeding approach in a way that assists in broadening the existing low genetic variation of the varieties in the country so as to reduce vulnerability to disease insect pest and harsh growing condition caused by climate change. This could be achieved through ingenious utilization of the existing tef genetic resources including the wild relatives and, when and if necessary, induced mutation techniques along with simultaneous use of modern biotechnological tools to broaden the genetic base and hasten the breeding program as a whole.

In addition, the convectional breeding approach has been playing a decisive role for the last five decades of tef breeding activities. However, in order to bring about breakthrough, the convectional varietal breeding efforts should be supported by modern molecular tools and scientific techniques. Furthermore, identifying the pedigree relationships of the existing improved varieties may have a significant role to design efficient and effective future tef breeding strategy.

Moreover, additional studies should be carried out to evaluate the Gx $\times$ E interaction as well as delimit the genetic relationship among varieties. To have full scale data, further study should be carried out and include:

- Multi-location trial at various environments to determine narrow sense heritability;
- Additional landraces need to be assessed representing major tef growing region along with major gene donors varieties;

- More SSR markers should be used to see the potential application of this marker system in markers-assisted breeding;
- SNPs markers should also be incorporated to fingerprint and refine the pattern

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**APPENDIXES**

Appendix 1. Mean performance of 49 improved and local tef varieties for 23 traits tested at two locations (Average over two locations)

Varieties	DTH	DTM	GFP	PLH	PaL	CL	PDL	TT	FT
DZ-01-99	43.65n-q	91.15pq	47.60g-m	99.07o-u	37.78n-q	61.29g-p	15.77lm	12.08d-k	9.81c-g
DZ-01-196	46.31i-l	95.69d-i	49.36b-h	114.94b-j	39.92g-o	75.02a-d	21.74ab	10.08d-k	7.85c-g
DZ-01-354	45.65j-m	94.40i-n	48.85c-i	105.22l-r	43.73d-m	61.49g-p	16.64j-m	11.83d-k	8.76c-g
DZ-01-787	48.12f-i	96.79c-f	48.63b-h	112.82c-l	48.33a	64.49e-o	19.98a-e	11.44b-j	9.46b-g
DZ-Cr-44	45.98i-m	95.98d-i	49.40b-g	109.75d-l	41.70b-k	68.05b-j	20.52a-e	9.19f-k	7.11e-g
DZ-Cr-82	49.59c-e	95.71e-j	45.74k-o	126.99a	49.34a	77.64a	20.75b-f	8.43g-k	6.86g
DZ-Cr-37	44.28l-o	90.30q-r	46.02j-o	104.39m-t	8.10j-p	66.29f-p	18.14g-m	10.07d-k	7.58c-g
DZ-Cr-255	44.02m-p	95.88e-j	51.86b-f	103.28h-p	43.55a-i	59.73h-p	21.97a	10.09d-k	8.48b-g
DZ-01-974	48.94c-e	95.29f-l	47.00k-o	113.08b-g	46.39a-e	66.69a-i	14.25j-m	7.00g-k	5.66fg
DZ-Cr-358	44.33k-n	96.71c-g	52.98b-d	103.42m-t	41.3e-n	62.09j-q	18.51c-j	9.13d-k	7.31c-g
DZ-01-1281	44.13k-o	95.36g-m	48.95g-n	103.72l-r	47.89a-d	55.82m-q	17.22e-l	6.54k	6.34g
DZ-01-1285	42.27p-s	95.63f-l	50.86b-g	105.48e-m	44.75a-h	60.73f-p	19.09b-f	9.29d-k	6.53fg
DZ-01-1681	41.92q-s	90.94qr	49.59d-j	96.60p-u	41.78c-m	54.82o-q	15.95f-m	11.44a-i	8.83b-g
DZ-01-899	46.61g-j	96.85c-f	50.82b-h	103.52l-t	43.12a-j	60.40q	17.07d-j	8.19e-k	5.53fg
DZ-01-2675	45.84i-m	95.44e-j	49.78b-h	104.86h-n	39.54i-o	65.33b-k	17.94c-i	9.90c-k	8.24b-g
HO-Cr-136	52.52a	96.96ab	45.85l-o	122.78ab	43.32a-e	73.16ab	18.79c-j	11.49d-k	9.07c-g
DZ-Cr-387RIL355	50.09cd	97.44b-d	47.53g-n	118.36a-c	46.14a-f	72.23a-c	15.07j-m	8.95d-k	7.04c-g
DZ-Cr-285	38.07u	95.22a	46.12l-o	84.78wx	32.88qr	51.91q	17.17e-l	11.80a-i	8.87b-g
DZ-Cr-409	44.51k-o	88.50rs	44.10o-q	99.55n-u	35.76o-r	63.79e-o	18.32c-j	11.14a-i	9.16a-e
DZ-01-1278	45.23i-m	94.61g-m	49.51c-i	110.26h-o	43.02c-m	67.23d-n	19.13c-j	11.73a-h	9.64a-f
DZ-01-2053	41.26q-s	92.25n-p	51.10b-f	93.25u-w	36.16o-r	57.09n-q	14.19m	12.44a-e	9.71a-c
DZ-01-1868	45.39h-k	94.75g-m	48.94f-l	108.2i-p	42.92e-m	65.29d-n	18.39h-m	8.45i-k	6.65g
DZ-01-2423	44.59i-m	93.09k-n	48.47g-m	101.81q-v	37.90lq	63.91j-q	19.08f-m	8.59g-k	6.67fg
DZ-01-3186	46.12g-i	96.48b-e	50.32b-g	119.76a-e	45.79a-h	73.97a-e	17.84g-m	9.10f-k	6.89c-g
DZ-01-2054	45.45i-l	94.93e-j	49.49c-i	111.07c-l	44.97a-h	66.10c-l	18.37d-k	9.71d-k	6.92c-g
DZ-01-146	46.62f-i	94.48f-l	47.82g-n	112.96e-l	45.14a-i	67.82c-m	17.61h-m	15.40a	12.19a
DZ-01-1821	44.93j-m	96.83b-e	51.83b-c	108.74h-o	42.56c-m	66.18d-n	18.04e-l	11.40c-k	8.95c-g
Acc. 205953	46.79f-i	93.52k-n	46.10j-o	108.82e-m	45.77a-g	63.05f-p	17.91g-m	10.61d-k	7.69c-g
RIL273	47.63d-g	95.95c-h	47.81g-m	116.04b-i	42.68c-m	73.37a-d	17.61g-m	9.14e-k	7.50d-g

Appendix 1. *Continued*

Varieties	DTH	DTM	GFP	PLH	PaL	CL	PDL	TT	FT
DZ-01-1880	48.07c-f	96.30b-e	48.09g-l	123.25a-d	48.51ab	74.74a-f	16.05k-m	7.38jk	6.06g
23-Tafi-Adi-72	48.36c-f	97.94ab	49.44b-h	109.50f-m	40.25g-o	69.24b-k	17.38e-l	11.69a-g	9.59a-d
PGRC/E 205396	42.07o-r	93.80h-m	51.59b-d	111.20f-n	43.66a-j	67.54e-o	21.80a-c	10.48d-k	10.06a-d
DZ-Cr-387 RIL127	44.67k-o	94.03h-m	49.21b-h	111.88d-l	45.12a-h	66.76c-l	17.48d-k	10.59d-k	8.04c-g
DZ-Cr-438	53.35a	100.00a-c	44.23n-q	106.30f-n	49.62a-i	62.97e-o	16.08i-m	7.70j-k	7.79c-g
Acc.214746	45.38i-l	94.20h-m	48.31f-l	116.19b-h	46.33a-h	69.87a-l	19.11c-j	10.04d-k	8.25c-g
RIL-91A	51.96ab	93.85k-n	44.10o-q	112.60c-k	42.69c-k	69.91a-q	16.13i-m	10.04d-k	7.50d-g
RIL-129A	48.38bc	97.02n-p	49.05pq	117.97c-l	43.92k-q	74.05a-c	19.80b-h	10.13g-k	7.91g
RIL-27	50.63j-m	92.77q	43.05l-o	113.24g-n	40.77i-o	72.47b-k	17.36h-m	14.73a-c	13.61a
GA-10-3	44.82i-m	91.32s	46.44q	108.99v-x	41.28p-r	67.70pq	18.55d-k	15.18a-d	11.81a-d
Debre Zeit	45.88st	88.27m-o	42.80b	89.67t-v	36.82n-q	52.85k-q	20.05b-h	12.43a-f	10.48a-d
Minjar	40.26d-g	92.92c-f	52.97f-l	99.29b-f	37.02f-o	62.27ab	16.80i-m	9.28j-k	7.61g
Alemtena	40.87r-t	92.50n-p	51.99b-e	94.20s-v	37.48k-q	56.71lq	17.13e-l	11.14a-i	8.58b-g
Holetta	41.50st	97.03c-f	55.63a	94.89r-v	40.54m-q	54.35k-q	19.73b-g	16.10ab	13.35ab
Adadi Mariam	50.24c-e	95.02f-l	44.82m-p	100.92k-r	42.41d-m	58.52f-p	15.84g-m	8.56e-k	6.47d-g
Adet	48.49e-h	93.77j-n	45.32l-o	100.42l-s	43.26c-l	57.17h-p	17.59c-i	7.31h-k	6.12f-g
Motta	46.57i-l	95.11g-m	47.93e-k	101.87j-q	41.36f-o	60.51d-n	18.99b-h	11.61a-i	8.95b-g
Enewary	40.22tu	91.67o-q	51.41b	80.54x	31.80r	48.74q	19.64a-d	9.16e-k	7.00g
Melkasa	48.22f-i	94.67h-m	46.41h-n	105.74h-o	41.90h-o	63.84c-k	16.99d-k	9.86d-k	8.55c-g
Sirinka	46.18i-l	93.59l-o	47.02h-n	102.71l-t	46.06a-j	56.65j-q	16.87h-m	9.30d-k	7.75d-g
Mean	45.86	94.63	48.41	106.63	42.31	64.32	18.01	10.36	8.30

Appendix 1. *Continued*

Varieties	PBMPa	SpPa	LOGI	FBCD	SBCD	TSW	PaW	PaSW	PLW
DZ-01-99	28.87a-i	485.74j-r	86.70a-f	0.16h-l	0.17f-k	0.28c-h	1.50g-n	0.78d-l	13.44a-f
DZ-01-196	22.99g-n	396.58p-s	78.21d-f	0.27a-d	0.26b-d	0.38a-c	2.02a-c	0.88bc	14.94a-c
DZ-01-354	25.92e-l	573.99c-n	86.20a-f	0.18e-l	0.18d-k	0.31b-f	1.67d-l	0.74f-l	14.52a-e
DZ-01-787	31.21a-f	648.73ab	78.05d-f	0.18e-l	0.18d-k	0.31b-f	1.78b-i	0.85b-f	15.36a-d
DZ-Cr-44	28.61a-j	506.27d-o	85.51b-f	0.18f-l	0.19e-k	0.31c-h	1.32g-n	0.69d-l	11.26a-e
DZ-Cr-82	30.36a-h	693.94a-d	85.13a-f	0.23a-c	0.23b-d	0.32c-f	2.20a-d	0.77c-l	9.87d-f
DZ-Cr-37	27.55c-l	473.51i-r	81.31d-f	0.19a-j	0.19b-i	0.32c-h	1.73c-j	0.85b-f	14.14a-d
DZ-Cr-255	29.04a-k	572.73b-g	81.08d-f	0.19c-k	0.20c-k	0.28c-h	1.69b-h	0.89b-d	14.88a
DZ-01-974	31.02a-f	601.65b-f	76.78e-f	0.19a-j	0.19c-j	0.27c-h	2.26a	1.17a	9.83a-e
DZ-Cr-358	27.73d-l	620.12b-f	85.39a-f	0.15g-l	0.17c-k	0.26h	1.58d-l	0.70b-i	11.93a-e
DZ-01-1281	31.19a-f	617.40b-l	85.21a-f	0.20a-g	0.23b-e	0.33c-f	1.61e-m	0.73c-l	9.84b-f
DZ-01-1285	27.64d-l	598.73b-f	85.83b-f	0.19c-k	0.20c-k	0.31c-h	1.26g-n	0.60e-l	10.41a-f
DZ-01-1681	28.88c-k	401.89m-s	79.63d-f	0.14j-l	0.16i-k	0.28c-h	1.08l-o	0.33l-o	11.47a-f
DZ-01-899	31.91a-d	496.43f-p	82.29b-f	0.24a-i	0.21c-j	0.34c-h	1.33e-n	0.65b-k	7.75b-f
DZ-01-2675	30.12a-e	449.92i-r	78.24d-f	0.21a-i	0.22b-h	0.31c-g	1.35e-n	0.68b-j	14.54ab
HO-Cr-136	29.96a-e	697.97a-c	86.44f	0.26ab	0.27b	0.30c-h	2.04b-f	0.66g-l	15.54a-e
DZ-Cr-387 RIL 355	27.82a-i	740.97a	78.49d-f	0.26a	0.23b-f	0.33b-f	1.82b-e	1.16a	12.24a-e
DZ-Cr-285	21.74k-n	350.86q-t	85.59a-e	0.16k-l	0.15jk	0.31c-h	1.15m-o	0.47g-n	13.44a-f
DZ-Cr-409	24.07d-l	470.91h-r	82.50a-f	0.22g-l	0.18h-k	0.31c-h	1.39e-n	0.52e-l	12.76a-e
DZ-01-1278	22.73i-n	585.04b-k	77.85d-f	0.14k-l	0.15h-k	0.33c-h	1.44h-n	0.48g-m	11.57a-f
DZ-01-2053	20.62j-n	424.51n-s	90.50a-c	0.17l	0.15k	0.31c-h	1.10k-o	0.65b-j	9.52a-f
DZ-01-1868	25.28f-n	527.28j-r	81.52d-f	0.17b-k	0.17c-k	0.27f-h	1.22k-n	0.63j-o	8.60e-f
DZ-01-2423	25.82e-l	411.60p-t	83.95c-f	0.16g-l	0.15f-k	0.38b-e	1.01m-o	0.46k-o	9.05c-f
DZ-01-3186	28.31a-f	631.02b-f	76.61e-f	0.25a-g	0.24b-f	0.33c-h	1.66b-g	0.77b-h	11.43a-f
DZ-01-2054	29.21a-g	596.43b-g	82.09c-f	0.20a-i	0.18c-j	0.28d-h	1.55b-i	0.82b-g	10.90a-e
DZ-01-146	30.56a-d	645.22a-d	81.36d-f	0.25a-g	0.22b-i	0.31d-h	1.18g-n	0.53g-m	15.40a-c
DZ-01-1821	30.58a-f	477.68g-q	79.45d-f	0.21a-h	0.23b-f	0.34b-e	1.56c-k	0.53h-n	16.39a-d
Acc. 205953	27.09c-l	667.62ab	85.15c-f	0.24a-d	0.23b-g	0.36b-e	1.41d-l	0.78b-k	11.39a-f
RIL273	29.11a-j	664.87b-h	84.31a-f	0.18a-i	0.20b-g	0.31c-h	1.86b-i	0.66h-n	12.25a-f
DZ-01-1880	34.15a	592.54b-l	76.74f	0.22a-c	0.20b-f	0.34c-h	1.79b-i	0.57g-l	11.90a-e
23-Tafi-Adi-72	27.34a-j	429.06o-s	80.9d-f	0.25a-e	0.24b-d	0.35c-h	1.54c-k	0.50h-n	15.09a

*Appendix I. Continued*

Varieties	PBMPa	SpPa	LOGI	FBCD	SBCD	TSW	PaW	PaSW	PLW
PGRC/E 205396	27.65c-l	559.94d-o	84.99a-f	0.21a-e	0.19b-h	0.37c-f	1.51e-n	0.53g-n	12.76a-e
DZ-Cr-387 RIL127	29.21a-j	571.47b-k	83.24a-f	0.18c-k	0.19c-j	0.45a	1.63c-k	0.56g-l	13.35a-e
DZ-Cr-438	33.81ab	589.51b-i	75.99a-f	0.22a-f	0.24bc	0.33c-h	2.43a	1.00b	12.72a-d
Acc.214746	33.76a-c	654.62b-j	85.56a-f	0.19a-h	0.20b-g	0.29e-h	1.90b-i	0.78c-l	12.24a-f
RIL-91A	30.41a-j	630.43b-e	84.92c-f	0.19b-k	0.21c-j	0.31c-g	2.18ab	1.06b-e	11.95a-e
RIL-129A	29.67a-k	557.15e-p	79.12d-f	0.22a-f	0.24b-d	0.30c-f	1.72e-m	0.78i-o	10.81c-f
RIL-27	30.45a-k	543.05h-r	86.29a-f	0.17b-k	0.18c-j	0.29c-h	1.26n-o	0.67l-o	12.35a-f
GA-10-3	25.27i-n	503.15l-s	87.87a-e	0.19b-k	0.18d-k	0.25c-h	1.16n-o	0.74k-o	11.57a-f
Debre Zeit	22.24mn	380.32r-t	89.47a-d	0.16f-l	0.34a	0.42ab	1.15n-o	0.41m-o	11.36a-f
Minjar	28.22d-l	437.20p-t	87.37a-f	0.17f-l	0.18d-k	0.27c-h	1.48i-n	0.76j-o	11.19b-f
Alemtena	25.68h-n	357.20r-t	93.36ab	0.17d-k	0.15f-k	0.27c-h	1.10k-n	0.56l-o	7.06b-f
Holetta	21.92l-n	348.00st	93.99a	0.16f-l	0.13i-k	0.26c-h	0.70op	0.46n-o	12.90a-f
Adadi Mariam	27.78c-l	514.00d-n	83.98a-f	0.17b-k	0.16c-k	0.24c-h	1.13i-n	0.47l-o	7.05b-f
Adet	28.38b-k	528.95b-m	76.73e-f	0.16d-k	0.17c-j	0.23d-h	1.02j-n	0.53j-o	6.67d-f
Motta	26.96e-m	550.68b-j	83.54d-f	0.15h-l	0.16f-k	0.22g-h	1.29f-n	0.50l-o	15.74a
Enewary	21.45n	234.84t	83.59a-f	0.14i-l	0.15g-k	0.24d-h	0.39p	0.28o	6.33f
Melkasa	29.90a-j	329.24q-t	79.09d-f	0.17d-k	0.18c-j	0.32a-d	1.91b-e	0.50j-o	13.97a-e
Sirinka	29.05b-k	495.35k-r	86.16a-f	0.12j-l	0.16c-j	0.25d-h	1.46h-n	0.80d-l	12.68a-f
Mean	27.94	527.27	83.15	0.19	0.20	0.31	1.50	0.67	11.93

Appendix 1. *Continued*

Varieties	PLSW	BY	GY	HI	EGR
DZ-01-99	3.14 a-h	826.49d-k	231.59a-f	13.70d-l	492.36a-e
DZ-01-196	2.88a-g	786.79e-k	219.72a-i	12.42f-q	462.45a-l
DZ-01-354	3.44a-h	651.49i-o	217.09a-h	16.05a-d	441.39a-l
DZ-01-787	3.95a-h	807.17c-j	183.97c-m	12.45e-o	387.70b-k
DZ-Cr-44	3.42a-g	777.62d-k	240.33a-d	15.20a-f	513.00a-d
DZ-Cr-82	1.56g-h	1153.97a	233.73a-f	11.68g-q	491.12a-f
DZ-Cr-37	3.77a-c	512.07l-o	165.26g-n	13.42d-m	377.58e-l
DZ-Cr-255	4.45a	723.60f-l	216.64a-h	14.23a-h	427.61a-h
DZ-01-974	1.98a-h	1043.25a-d	214.61a-h	11.86e-o	484.50a-e
DZ-Cr-358	3.63a-d	683.05h-n	170.32f-n	12.42e-n	330.82g-l
DZ-01-1281	2.66a-h	649.95j-o	177.29d-m	13.32d-l	367.17d-l
DZ-01-1285	2.68a-h	748.60e-k	253.64ab	17.01a-c	518.80ab
DZ-01-1681	3.39a-h	628.15i-o	173.78d-m	14.13a-g	357.58d-l
DZ-01-899	1.25b-h	757.78f-l	142.28l-o	9.27o-r	298.02j-m
DZ-01-2675	3.63a-e	529.16j-o	76.11o	6.47s	193.12m
HO-Cr-136	3.62a-h	833.39b-h	155.15k-n	9.77p-s	343.82g-l
DZ-Cr-387 RIL 355	2.31a-h	829.16a-g	199.36a-m	11.68j-q	428.53a-j
DZ-Cr-285	4.48a-h	339.06no	221.03a-i	18.60a	485.90a-g
DZ-Cr-409	4.19ab	593.68h-n	247.78a-g	16.73a-e	569.36a-c
DZ-01-1278	3.85a-e	718.96e-k	207.82b-m	12.96f-q	430.82b-k
DZ-01-2053	2.00a-h	618.68g-m	234.28a-i	15.22d-k	462.48a-j
DZ-01-1868	2.20f-h	929.83a-f	275.63a-d	15.90c-j	562.92a-e
DZ-01-2423	2.44a-h	862.93a-g	291.41a-c	16.21a-h	591.31a-d
DZ-01-3186	3.13a-h	1012.66a-c	299.63a-c	16.31c-j	602.01a-d
DZ-01-2054	3.08a-h	723.14e-k	244.70a-h	15.57c-j	511.59a-h
DZ-01-146	4.54a	662.66g-l	179.13j-o	12.22m-r	374.30i-m
DZ-01-1821	4.90a-f	658.03f-l	204.88d-m	13.50f-q	407.86f-l
Acc. 205953	3.19a-h	728.48d-k	197.48e-m	13.02h-q	434.81d-l
RIL273	2.31b-h	909.40b-h	242.59a-i	12.47j-q	480.00a-j
DZ-01-1880	2.17a-h	842.50c-j	199.87e-n	12.02k-r	437.86c-k
23-Tafi-Adi-72	2.84a-e	817.23d-k	193.59g-n	11.56n-r	402.51g-l
PGRC/E 205396	3.61a-c	792.50e-k	236.37a-l	15.08d-l	456.94b-k
DZ-Cr-387 RIL127	4.04a-g	637.60h-n	214.34a-m	14.16d-n	439.24b-k
DZ-Cr-438	3.67a-d	1033.05a-c	198.69d-m	12.57i-q	440.58b-k
Acc.214746	3.50a-h	1009.40a-e	264.34a-e	13.69e-p	536.83a-f
RIL-91A	3.48a-g	1031.87ab	239.68a-d	11.26l-r	550.01a
RIL-129A	2.14d-h	830.75c-i	218.45a-h	13.21e-n	510.35a-e
RIL-27	3.32a-h	808.22c-j	212.33a-j	13.29e-o	464.51a-h
GA-10-3	3.43a-h	430.75m-o	205.20a-k	18.10ab	488.90a-f
Debre Zeit	2.04a-h	791.32c-j	207.86a-m	13.42e-n	400.55c-k
Minjar	2.33c-h	880.75a-g	214.95a-i	11.91g-q	437.12a-i

Appendix 1. *Continued*

Varieties	PLSW	BY	GY	HI	EGR
Alemtena	1.34b-h	626.53g-m	252.15a-c	16.43a-d	502.31a-e
Holetta	3.44a-h	526.69k-o	159.20e-n	10.99i-q	312.61h-m
Adadi Mariam	0.53h	822.46a-g	119.16m-o	8.60q-s	275.37j-m
Adet	1.08e-h	622.46g-l	83.91no	7.37rs	223.45lm
Motta	4.13a-d	627.81f-l	124.93k-o	10.16n-r	276.48j-m
Enewary	1.76h	307.36o	120.08m-o	13.78d-k	252.44k-m
Melkasa	3.34a-h	807.36a-g	145.83k-n	9.62n-r	330.59g-l
Sirinka	3.22a-h	779.16c-j	258.59a	14.49b-i	554.20a
Mean	3.01	749.50	203.81	13.17	431.057

Figures sharing similar letter within columns are not statistically significant different. DTH=days to heading, DTM=days to maturity, GFP= grain filling period, PLH= Plant height, PaL=panicle length, CL= culm length, PDL= peduncle length, TT= number of total tillers per plant, FT= number of fertile tillers per plant, PBMPa= number of primary branches per main shoot panicle, SpPa=number of spikelets per main shoot panicle, LOGI =lodging index. FBCD= first basal culm internode diameter, SBCD=second basal culm internode diameter, TSW=thousand seed weight, PaW= main panicle weight, PaSW=main panicle seed weight, PLW= total plant weight, PLSW =grain yield per plant, GY= grain yield, BY =biomass yield, HI= Harvest index and EGR=Economic growth rate.

## Appendix 2. Clustering of 49 tef varieties into seven cluster group using mean of 23 response traits

Cluster	Number of genotype	Varieties include in this cluster	Source
C <sub>1</sub>	29	DZ-01-99, DZ-01-354, DZ-01-787, Acc.214746, Acc.205953, DZ-01-2054, DZ-01-1868, DZ-01-1285, DZ-01-1281, PGRC/E 205396, DZ-01-1278, DZ-01-1821, 23-Tafi-Adi-72, DZ-01-146, DZ-01-1681, DZ-01-2675, DZ-01-196, DZ-01-899.	Direct selection
		DZ-Cr-37, RIL-27 (Elite), RIL-129A (Elite), DZ-Cr-44, RIL273, DZ-Cr-358, DZ-Cr-255, DZ-Cr-387 /RIL127	Hybridization (intra specific)
		Sirinka, Minjar, Motta.	Local variety
C <sub>2</sub>	8	DZ-01-3186, DZ-01-1880, DZ-01-974.	Direct selection
		DZ-Cr-82, DZ-Cr-387/RIL355, DZ-Cr-438/RIL133B, DZ-Cr-438/ RIL91A, HO-Cr-136.	Hybridization (intra specific)
C <sub>3</sub>	3	AdadiMariam, Adet, Melkasa.	Local variety
C <sub>4</sub>	6	DZ-Cr-285	Hybridization (inter specific)
		DZ-Cr-409	Hybridization (intra specific)
		DZ-01-2053, DZ-01-2423, GA-10-3 Alemtena	Direct selection Introduction Local variety
C <sub>5</sub>	1	Debre Zeit	Local variety
C <sub>6</sub>	1	Holetta	Local variety
C <sub>7</sub>	1	Enewary	Local variety

Appendix 3. Pictures taken during the experimental period in the field and laboratory



A. Field data collection



B. Experimental field visit by Advisors



C. Samples taken for DNA extraction



D. Varieties in the DNA extraction technique