



**GENETIC VARIABILITY AND TRAIT ASSOCIATIONS IN SOME  
SELECTED SEMI-DWARF TEF [*Eragrostis tef* (Zucc.)Trotter]  
RECOMBINANT INBRED LINES IN CENTRAL ETHIOPIA**

**M.SC. THESIS**

**BY**

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**OCTOBER, 2018  
JIMMA, ETHIOPIA**

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SELECTED SEMI - DWARF TEF [*Eragrostis tef* (Zucc.)Trotter]  
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*M.Sc. Thesis*

*Submitted to the School of Graduate studies, Jimma University College of  
Agriculture and Veterinary Medicine, in Partial fulfillment of the  
Requirements for the Degree of Master of Science in Plant Breeding*

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**October, 2018  
Jimma, Ethiopia**

**Jimma University College of Agriculture and Veterinary Medicine**

Thesis Submission Request Form (F-05)

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## **DEDICATION**

I dedicate this piece of work to my dearly loved father and mother Bekana Wedajo and DaktuOljera as well as to my young sister Atsede Bekana and my friend Birahne Lakew, who were willing to see my success and instructing me to grasp the level I have reached now.

## STATEMENT OF THE AUTHOR

First, I declare that this thesis is my authentic work and that all sources of materials used for this thesis had acknowledged properly. The thesis submitted to in fulfillment of the requirements for M.Sc. degree in Plant Breeding at Jimma University then deposited at library of the University to make available to borrowers under the rules of the library. I solemnly declare that this thesis was not submitted to any other institution anywhere for the award of any academic degree, diploma or certificate.

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## **BIOGRAPHICAL SKETCH**

Getahun was born in Abdeta kebele, Gutogidda district (Nekemte), East Wollega Zone, Oromia Region, Ethiopia on 21 March 1987. He started education in Mesera Georges Elementary School and concluded at Nekemte Comprehensive Preparatory School. He joined Mada Walabu University, College of Agriculture; Bale Robe Campus in 2011 and graduated with B.Sc. Degree in Plant Science on 29 June 2013. Then, he was hired by the Ethiopian Institute of Agricultural Research on 4 May 2014 and served as Junior Researcher for two years at Holetta Agricultural Research Center and worked in the tef research commodity until September; 2016. He was n't married. He joined the School of Graduate Studies at Jimma University to pursue his education leading to M.Sc. degree in Plant Breeding Program.

## ACKNOWLEDGEMENTS

First of all, I thank my Almighty God who reached me at this time and brought me to this success. I also express my heartfelt love and respect to the mother of the God, Virgin Mary.

Then, I would like to express my sincere gratitude to my major advisor Dr. Mulugeta Seyoum for his guidance and support at the beginning of the research project. I am also grateful to my co-advisor Dr. Kebebew Assefa for his advice and valuable contribution throughout the research work. He was also always there with me whenever I needed his help. I also appreciate Dr. Zarihun Tadele for his kindness and encouragement to do this study. My sincere appreciation also extended to the Tef Project staff of Debre Zeit Agricultural Research Center who (DZARC) provided me with all available resources including plant materials, especially Mr. Nigusu Hussein for his support in availing of different facilities. I am also deeply indebted to Holetta Research Center (HARC) workers of the research program in general for their endless support on different activities of field works.

The admirable assistance with friendly and kind-hearted treatment received from the staff of DZARC and HARC in general and particularly Mr. Yazachew Genet, Mrs. Tsion Fikre, Mr. Nigusu Hussein, Mr. Nigus Belay and Mr. Kitaw Assefa are highly acknowledged. Specially, the help of Mr. Nigusu Hussein and Mr. Kitaw Assefa was ever unforgettable. I also greatly wish to express my sincere appreciation to my Sisters and friend for their support and encouraging me. Furthermore, my great appreciation also goes to my families and relatives, friends as well as to all those who were directly and indirectly involved in this study for my success for their unreserved support and encouragement.

It is my pleasure to acknowledge the Ethiopian Institute of Agricultural Research (EIAR) for sponsoring my M.Sc. study. I am also highly thankful to Jimma University for hosting my study and facilitating all the academic processes. I extend my gratefulness to my instructors in the Horticulture and Plant Sciences Department of the College, and to the indispensable guest lecturers for their genuine guidance that I benefited much from. In addition, I would like to appreciate the School of Graduate Studies for supporting me to the best of its capacity.

## ACRONYMS AND ABBREVIATIONS

AGRA	Alliance for Green Revolution in Africa
ANOVA	Analysis of variance
ATA	Agricultural Transformation Agency
BODA	British Overseas Development Association
CSA	Central Statistics Agency
DANIDA	Danish International Development agency
DZARC	Debre Zeit Agricultural Research Center
EIAR	Ethiopian Institute of Agricultural Research
EMS	Ethyl-Methane Sulphonate
GA	Genetic advance
GAM	Genetic Advance as percent of the Mean
GCV	Genotypic coefficient of variation
HARC	Holetta Agricultural Research Center
IAEA	International Atomic Energy Agency
IBC	Institute of Biodiversity Conservation
m.a.s.l	Meters above sea level
Mbp	Million base pairs
NPS	Nitrogen, Phosphorous and Sulphur blended Fertilizer
PCA	Principal Component Analysis
PCV	Phenotypic Coefficient of Variation
RILs	Recombinant Inbred Lines
TILLING	Targeted Induced Local Lesion IN Genome
TTU	Texas Tech University
USDA	United States Department of Agriculture



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**GENETIC VARIABILITY AND TRAIT ASSOCIATIONS IN SOME SELECTED SEMI-DWARF TEF  
[*Eragrostis tef* (Zucc.) Trotter] RECOMBINANT INBRED LINES IN CENTRAL ETHIOPIA**

**ABSTRACT**

*A field experiment was conducted to determine the extent of genetic variability and trait association among semi-dwarf tef recombinant inbred lines for yield and yield components with emphasis on lodging and thereby generate information as well as identify superior recombinant inbred lines. A total of forty nine recombinant inbred lines were evaluated for 16 traits using simple lattice design at Holetta and Debre Zeit in 2017 cropping season. All the traits measured over the locations showed highly significant differences among the recombinant inbred lines except fertile tiller per plant, while the inbred lines x location interaction effect was highly significant for most of the traits measured. Grain yield showed the highest phenotypic coefficients of variation (26.36%) followed by above ground biomass (23.16%), while the remaining traits showed low (<10%) to moderate (10-20%). No highest genotypic coefficients of variation (>20%) recorded. However, moderate (10-20%) genotypic coefficient of variation was recorded for above ground biomass. Plant height and panicle length showed high heritability (>60%), whereas half of the remained traits showed low (<30%) and moderate (30% to 60%) heritability. Genetic advance as percent of the mean was the highest for above ground biomass (>17.02%) and least for number of branches per panicle (0.09%). Both the genotypic and phenotypic correlation coefficients showed positive association of grain yield with most traits. Lodging index showed negative phenotypic correlation with most lodging related traits and positive with grain yield as well as phenological traits. Path coefficient analysis revealed that above ground biomass exerted the highest positive genotypic and phenotypic direct effect on grain yield. Cluster analysis grouped the recombinant inbred lines into four clusters based on their similarity. The highest inter-cluster distance noted between clusters II and IV while the lowest was between clusters III and IV. Principal component analysis showed that about 77.6% of the gross variance among recombinant inbred lines explained by five Principal components with eigenvalues greater than unity. Traits, which are closely associated with lodging such as plant height, culm length and diameters of the basal culm internodes have special interest in the improvement of tef. Of all the traits evaluated in this study, plant height, panicle length showed high  $H^2$  and above ground biomass performs relatively high values of GCV, PCV and GAM. Therefore, these traits are important for selection and further improvements. This study revealed that four recombinant inbred lines had higher yield than local and standard checks. RIL#14 showed the highest grain yield and low lodging index, longer panicle, higher number of spikelets per panicle, as well as the highest above ground biomass than all recombinant inbred lines, which could be the base for future tef breeding program.*

**Key Words:** Correlation, Genetic variation, Heritability, Inbred lines, Tolerance, Traits

# 1 INTRODUCTION

Tef [*Eragrostis tef* (Zucc.)Trotter] belongs to the family *Poaceae*, subfamily *Chloridoideae*, genus *Eragrostis* with binomial nomenclature of *Eragrostis tef* (Zucc.)Trotter. It is an allotetraploid ( $2n=4X=40$ ), self-pollinated with bisexual florets of chasmogamous pollination behavior, and C<sub>4</sub>plant (Stallknecht *et al.*, 1993; Yu *et al.*, 2006). Its center of origin and diversity is in Ethiopia (Vavilov, 1951). Fifty-four of the 350 *Eragrostis* species, including the 14 endemic species were found in Ethiopia where they believed to been domesticated by pre–Semitic inhabitants between 4000 and 1000 BC (Seyfu,1997; Habtamu *et al.*, 2011; Alganesh, 2013).

Tef is the main cereal crop widely produced and consumed in Ethiopia and favored by millions of local smallholder farmers (Seyfu, 1997). In terms of area of cultivation, it is the leading cereal crop followed by maize and wheat. According to the Central Statistical Agency (CSA, 2018), the area covered by tef during the 2017/2018 cropping season was over 3.02 million hectares or 30% of the total area occupied by cereals in the country.

Despite being a staple food for many people in Ethiopia for centuries, tef has gained prominence as a food crop in other parts of the worldvery recently. This interest is mainly associated with its gluten-free grains and its nutritive value that is generally comparable with other common cereals (Hailu *et al.*, 2001; Spaenij-Dekking *et al.*, 2005; USDA, 2015; Cheng, 2017). However, it is also grow as a pasture crop in several countries (Kebebew *et al.*, 2011).The straw from tef is a valuable source of livestock feed because it is more palatable and nutritious than that from wheat and barley (Alemu, 2013).

Tef is a highly versatile crop with respect to adaptation to different agro-ecologies being widely grown from sea level up to 2800 m.a.s.l. with reasonable resilience to both drought and water logging (Kebebew *et al.*, 2010). The national average yield of tef is about 1.75 ton per hectare (CSA, 2018), but it has a potential of yielding four to five tons of grain per hectare if the lodging problem is resolved (Yifru and Hailu, 2005).The major yield limiting factors are lack of cultivars that are tolerant to lodging and shortage of improved varieties(Kebebew *et al.*,2015).



Besides, the grains are also often lost in the harvesting and threshing process because of their minute size and traditional cultural practices (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 practices since the application of increased amounts of nitrogen fertilizer to boost the yield results in severe lodging (Kebebew *et al.*, 2015).

Lodging greatly reduces both yields and quality of the grain as well as the straw. It is reported to decrease tef grain yield by approximately 15 to 45% (Zhu *et al.*, 2012) depending on the weather condition and inherent nature of the variety used; it also hampers both manual and mechanical harvesting (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; Kebebew and Zarihun, 2012), no cultivar with reasonable lodging resistance has been obtained to-date except a novel tef mutant named *kegne*, and GA-10-3 which have a semi-dwarf phenotype, resulting in increased lodging tolerance (Jöst *et al.*, 2015).

The tef germplasm accessions showed wide genetic variability in phenologic, morphologic and agronomic traits (Hailu *et al.*, 2001; Solomon, 2007 and Kebebew *et al.*, 2001, 2011). In spite of this, there has been lack of sufficient variability in the tef germplasm for some valuable traits such as lodging and shattering resistance. Since recent past, a chemical mutagen, ethyl methane sulphonate (EMS), has been successfully utilized to induce semi-dwarf tef variants with lodging resistance as well as tolerance to aluminum toxicity and other acidity-related soil fertility problems (Mesfin, 2007; Esfeld *et al.*, 2009 and Ermias *et al.*, 2017). The first semi-dwarf lodging-tolerant tef line, called *kegne* developed from an ethyl methane sulphonate-mutagenized population (Jöst *et al.*, 2015).

Some important works have also reported based on morphological, molecular and biochemical markers. According to Tareke *et al.* (2011), many efforts made in the past to implement different techniques and tools in order to improve tef. Some of them are such as inter-specific crossing that made between tef (*Eragrostis tef*) and *Eragrostis curvula* in an attempt to transfer the lodging tolerant trait of *Eragrostis curvula* to tef. However, so far, no viable hybrid obtained

from the crosses. In attempts to develop double haploids using gynogenesis technique, some promising tef lines were obtained (Likyelesh, 2006). The variations noted in panicle length (14-65 cm), culm length (11-82 cm), plant height (31-155 cm), culm thickness (1.2-4.5 mm) all indicate the potential for developing lodging-resistant genotypes through gene re-combination as suggested by Seyfu (1993).

Efforts made so far have enabled the development and release of over 42 improved varieties to the farming communities in Ethiopia (MoA, 2017). However, development of high yielding and lodging tolerant tef varieties, adapting to the changing climate remains to be the primary focus of tef research (Solomon, 2009; Solomon *et al.*, 2013). Especially, semi-dwarf tef types did not studied much yet on measuring correlations among traits and path analysis of agronomic traits affecting grain yield using recombinant inbred lines and there is no lodging resistant tef (Habte *et al.*, 2017). Therefore, the current study conducted with the following objectives. I have greatly acknowledged DZARC for their generous of all the plant materials.

### **General objective**

- ✚ To estimate the extent of genetic variability among selected semi-dwarf tef recombinant inbred lines with emphasis on lodging tolerance, yield and yield components, and thereby generate information as well as identify superior inbred lines.

### **Specific objectives**

- To determine the magnitude and pattern of genetic variability in semi-dwarf tef recombinant inbred lines with respect to yield and lodging tolerance.
- To measure the associations among yield and its component traits then partition the correlation coefficients into direct and indirect effects to the grain yield.
- To identify major traits that contribute to the overall genetic variability among semi-dwarf tef recombinant inbred lines to emphasize on these traits for further tef breeding.

## 2 LITERATURE REVIEW

### 2.1 Botanical Overview of Tef

Taxonomically, tef belongs to the grass family, *Poaceae*, sub-family *Chloridoideae* (*Eragrostoideae*), tribe *Eragrostidae*, sub-tribe *Eragrostae*, and genus *Eragrostis*. The genus *Eragrostis* comprises about 350 species (Watson and Dallwitz, 1992). 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 1918, Trotter rediscovered the original description of Zuccagni, hence, the current scientific name [*Eragrostis tef* (Zucc.) Trotter] featuring the two authors and the word ‘tef’ is most widely used in scientific writings (Costanza *et al.*, 1979). In cultivation as a cereal, tef is the only species in the genus *Eragrostis* and together with finger millet (*Eleusine crochana* L.); they constitute the sole two species in the sub-family *Chloridoideae* cultivated for human consumption of the grains (Jones *et al.*, 1979; Costanza *et al.*, 1980; Endeshawa and Lester, 1981).

Morphologically, the tef plant root system is thin and fibrous (thread-like) rarely emerging from nodes above the base, and growing 4–8 cm deep under field conditions (Tadesse, 1969). The stems are mostly erect, except there is bending in some cultivars and jointed with hollow internodes separated by nodes. Each culm internode, except the most basal one, bears one leaf consisting of a sheath and a blade. It has a panicle type of inflorescence showing different forms from very loose, loose, semi loose, semi-compact to compact (Kebebew *et al.*, 2011). The panicles ramify into primary, secondary and tertiary branches bearing spikelets. 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 0.7-1.0mm in diameter, which is very small, and its color varies from white to dark brown (Tadesse, 1975; Stallknecht, 1993).

Tef seeds are very minute (hundred kernel mass = 0.18– 0.38 mg; (hundred kernel mass of *Arabidopsis* = 0.17–0.21 mg) and vary in the outer caryopsis colour ranging from dark brown to yellowish or orange white. The height of the plant ranges from about 20–155 cm with the culm

(11–72 cm) and the panicle (10–65 cm) accounting for about 47–65% and 35– 53% of the total aboveground height, respectively (Kebebew *et al.*, 2001).

Physiologically, tef is an herbaceous annual plant requiring 60–140 days to attain physiological maturity (Kebebew *et al.*, 2001a). It is a C<sub>4</sub> plant having 4-carbon compounds (malic or aspartic acid) as the first photosynthesis product (Kebebew *et al.*, 2011). In addition, the leaf anatomy of tef is Kranz type having vascular bundles surrounded by bundle sheath cells in a circular manner (Abuhay *et al.*, 2001). The flowers of tef are hermaphroditic. Each floret has a lemma, 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% (Seyfu, 1997; Kebebew *et al.*, 2017). Fertilization is 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-Haile and Guard, 1966).

The genus *Eragrostis* is generally a complex taxon characterized by the prevalence of polyploidy (about 69%) and common presence of cytological races. The species in the genus range from diploids ( $2n = 2x = 20$ ) to hexaploids ( $2n = 6x = 60$ ). 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, the largest chromosomes 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). The largest tef chromosome is smaller than the smallest (1D) wheat chromosome (Likyelesh *et al.* 2001). The average nuclear genome size is 730 Mbp according to Mulu *et al.* (1996) and Fufa *et al.* (2000), that is comparable to sorghum and greater than rice genome by half.

## 2.2 Origin, Distribution and Agro-Ecology of Tef

Tef is one of the crops which their center of origin and diversity in Ethiopia (Vavilov, 1951). It is endemic to Ethiopia and its major diversity found only in this country. Similar to several other crops, the strict 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 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 found in Ethiopia (Phillips, 1995).

As tef 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* have evidenced by the successful hybridization of these two species (Hailu *et al.*, 2003).

However, based on morphological data the following species have been identified, by different researchers, as the ancestors and contributors to the origin of tef or as species closely related to tef:- Species suggested as ancestors of tef (Costanza 1974) are *Eragrostis pilosa*, *E. macilentata*, *E. aethiopica*, *E. pseudo tef*, *E. longifolia* and *E. atrovirens*. Species suggested as contributors to the origin of tef (Endeshaw 1978) are *E. pilosa*, *E. curvula*, *E. aethiopica*, *E. cilianensis*, *E. mexicana* and *E. bicolor*. Species suggested as very closely related to tef (Ponti 1978) are *E. pilosa* and *E. aethiopica*; *E. mexicana*, *E. cilianensis*, *E. minor* and *E. barrelieri* sufficiently

related; while *E. macilenta* and *E. aegyptica* are suspected to be close enough but need further investigation. Among perennials, *E. papposa*, *E. heteromera* and *E. bicolor* are more closely related to tef than others. Species suggested as closely related to tef based on cytological evidence (Tavassoli 1986) are *E. aethiopica* 2x, *E. pilosa* 2x, *E. mexicana* 6x, *E. barrelieri* 6x, *E. minor* 2x, 4x and *E. cilianensis* 2x, 4x, 6x as reported by (Seyfu, 1997; Dejene *et al.*, 2018).

Tef was been introduced to different parts of the world through diverse institutions and individuals (Abraham, 2015). 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 distributed it to some countries in the British colonies, India, Australia, United States of America, South Africa and British Guyana. According to Tadesse (1975), tef first introduced to California (USA), Malawi, Zaire, India, Sri Lanka, Australia, New Zealand and Argentina. It also introduced to Zimbabwe, Mozambique, Kenya, Uganda, and Tanzania. Tef being grown in South Africa, India, Australia, the Netherlands, Spain, Israel and Canada for both human consumption and animal feed (Stallknecht, 1993; Seyfu, 1997; Roseberg *et al.*, 2005).

Tef is adapted to a wide range of environments, and is presently cultivated under diverse agro-climatic conditions (Guta, 2015). It can grow 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 largely in 11 of the 19 major agro ecological zones of Ethiopia (Thion, 2016).

Tef grain yield in the US averages from 0.7 t/ha dry land to 1.4 t/ha irrigated (Stallknecht *et al.*, 1993). In Ethiopia, the national average grain yield of tef is about 1.75 t/ha (CSA, 2018). However, improved varieties of tef produced a grain yield of 1700-2200 kg/ha on farmers' fields and 2200-2800 kg/ha on research fields and well managed large farms (Anteneh *et al.*, 2014; Seyfu, 1997). 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).

Tefplants cannot compete with weeds especially during the young growing stage. It is best to start with a weed-free, clean field that 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 (Fufa *et al.*, 2001).

### **2.3 Use and Nutritional Value of Tef**

The use of tef traced back to about 3359 B.C (Melak-Haile, 1965). In contrast to amaranth, which utilized by early civilizations throughout the world, tef production and uses have been primarily restricted to the countries of Ethiopia, India and its colonies, and Australia (Stallknecht *et al.*, 1993). While tef grain still provides over two-thirds of the human nutrition in Ethiopia, it is relatively unknown as a food crop elsewhere. Late 20th century publications in the United States describes tef grain as being marketed as a health food product, or used as a late planted emergency forage for livestock (Goerge and Weibye, 1991).

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 cereal tef 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 - Haile, 1966). Moreover, it is gluten-free and preferred food for persons with celiac disease, diabetics (slow release carbohydrates) and anemia (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 traditionally used to make *injera*, which is a soft, porous, thin pancake, with slightly sour taste. Tef commonly consumed with various meat and/or pulse sauces called *wot*. Its flour also used for the preparation of tef porridge, and un-raised bread called *Kitta* or *anebabero* (two over-laid *injera*). Sometimes, the grain brewed into a native beer called *Tella* or *Fersso* and a more alcoholic traditional liquor, locally known as *arakie*, or *katikalla*. Tef straw also 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 was 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 used as a thickening agent in a range of products including gravies, casseroles, soups and stews. It 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, now used in mixture with soybean, chickpea and other grains in the baby food industry (Seyfu, 1997; USDA, 2015).



According to Seyfu (1993) Tef remained an important crop to Ethiopian farmers because of the prices for its grain and straw are higher than other majorcereals. The crop performs better than other cereals under moisture stress and waterloggedconditions; its grain can be stored for a long period without attacked byweevils and the straw is a nutritious and highly preferred feed for cattle compared to othercereals.

#### **2.4 Genetic Resources of Tef in Ethiopia**

There are about 350 *Eragrostis* species of which *Eragrostis tef* is the only species cultivated for human consumption. Recently the gene bank in Ethiopia holds over five thousand tef accessions collected from geographical regions diverse in terms of climate and elevation. These germplasm accessions appear to have huge variability with regard to key agronomic and nutritional traits. In order to utilize properly that variability for developing new tef cultivars various techniques have implemented to catalog the extent and unravel the patterns of genetic diversity (Kebebew *et al.*, 2015).

Large number of variants had observed within the existing tef genetic resources. Among the traits depicting huge variability are days to maturity (60 to 120 days), number of grains per plant (9,000 to 90,000), plant height (31 to 155cm), tillering capacity (5 to 35 tillers per plant), panicle type (very loose open to very compact), and flag leaf area (2 to 26 cm<sup>2</sup>), culm diameter (1.2 to 5 mm). The Institute of Biodiversity Conservation (IBC) of Ethiopia makes regular collection of tef accessions from diverse agro-ecological regions in the country in order to reduce genetic erosion and conserve the native genetic resources. So far, 5169 tef accessions and 10,000 tef genotypes are available at the institute (Seyfu, 1993, Solomon *et al.*, 2009).

From these genotypes, 114 types of panicle forms were identified of which 94 were present in rare frequency (<1%). Five variants contribute for lodging resistance. Since the tef landraces have particularly in recent years been under increasing threats of replacement by high-yielding and improved varieties, appropriate conservation measures should be taken in order to harness

the valuable and unique characters of the landraces. In general, the tef landrace collections of the IBC holdings made from regions with altitudes ranging from 800 to 3200 m. a. s.l and most Ethiopian farmers grow tef landraces (Temesgen *et al.*, 2005)

The main conservation strategy is in-situ conservation. However, the fate of this strategy has decided by the preference of traditional farmers. Therefore, to sustain the conservation, it is essential to complement with ex-situ conservation. Hence, the Ethiopian Institute of Biodiversity Conservation (IBC) made some efforts to explore and collect the tef germplasm. So far, 5164 accessions of tef landraces and 5 accessions of wild relatives were have been conserved in the national gene bank of Ethiopia. The accessions collected from the former twelve administrative regions representing diverse agro-ecological zones. All accessions are stored at -10 °C under long-term storage (Kebebew *et al.*, 2011).

## 2.5 Constraints of Tef production in Ethiopia

Tef is the dominant cereal in Ethiopia ranking first in area coverage (accounting for 30%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 in 2017/18(2010 E.C)

<b>Crop</b>	<b>Area in Million Hectares</b>	<b>Production Million Tones</b>	<b>Yield(t/ha)</b>
Tef	3.02	5.28	1.75
Maize	2.13	8.40	3.94
Sorghum	1.96	5.17	2.73
Wheat	1.76	4.64	2.74
Barley	0.95	2.05	2.16

Finger millet	0.46	10.31	2.26
Total	10.28	35.85	15.68

Source: CSA (2018)

Despite its indispensable importance in the Ethiopian agriculture, the production and productivity of tef is low with the national average standing at 1.75  $\text{tha}^{-1}$  (CSA, 2018). The major yield limiting factors are lack of cultivar that 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).

Lodging (permanent displacement of the stem from the upright position) is the major production constraint in tef. Lodging is a key agronomic problem in tef production (Yu *et al.*, 2007) and up to 23% yield loss is accountable to lodging under natural conditions (Seyfu, 1993) i.e. with minimal or no fertilizer condition. Even with good crop management practices, lodging is a major limitation to sustainable improvement of the crop.

Tef possesses tall, weak stems and fibrous root that easily surrender 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 (Pinthus, 1973; Kebebew *et al.*, 2017; Cannarozzi *et al.*, 2018). When this occurs, both the yields and quality of the grain as well as the straw severely reduced; both manual and mechanical harvesting is hampered (Kebebew *et al.*, 2015). Most of the above characteristics appear to be typical making the crop very susceptible to lodging due to weak stem-base having insufficient strength to hold the shoot up against leverage.

While studying the lodging phenomenon with tef, Seyfu identified the following major types of lodging (Seyfu, 1993): i) Transient lodging is a temporary situation occurring before heading with the plants often capable of recovering into the upright position. ii) Permanent lodging is a permanent displacement from the upright position often manifested after heading. It comprises three sub-categories: a) Root lodging involves uprooting of the whole plant while the stems still appear intact. b) Break lodging involves breakage of the stem usually near the base of the

peduncle. c) Bend lodging is characterized by loss of plant elasticity leading to bending of stems while the roots are still secure in the soil.

In practical husbandry, bend lodging is by far the commonest, most prevalent and economically most important type of lodging in tef (Seyfu, 1993). While bend lodging is the most significant, break lodging is of minor concern, and root lodging is relatively unimportant. In contrast, Van Delden and co-workers (Van Delden *et al.*, 2010), using biomechanical models with two tef cultivars in field trials in the Netherlands, reported that tef is most sensitive to root lodging and that given its current morphology, lodging of free-standing plants is inevitable in the tested environments. If the root lodging that the latter workers meant is similar to that described earlier, it may be unexpected especially on the sandy soil conditions and particularly under low plant density.

However, under Ethiopian conditions, tef is predominantly a heavy clay soil crop, and even on light soils the crop is grown in densest and such that root lodging is not the most important type of lodging. In tef, lodging reduces grain yield by 11–22% (average = 17%), 1000-kernel weight by 35%, grain yield per panicle by 51%, and percentage and rate of seed germination by 41 and 44%, respectively (Seyfu, 1993). This, therefore, indicates the economic significance of the problem of lodging in tef, and the urgent need for finding means for combating the problem.

Using lower seed rates and late sowing dates relatively decreases the problem of lodging. Although, various attempts were made by the research community to develop lodging-resistant tef cultivars, however no cultivar with reasonable lodging resistance was obtained so far (Kebebew *et al.*, 2011; Kebebew and Zarihun, 2012).

## **2.6 Tef Research 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 started in the late 1950's in Jimma Agricultural Technical High School and later moved to Debre

Zeit Agricultural Research Center. 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. However, it has not been considered as an important crop by the international scientific community or funding agencies for a long period. Tef gained some attention at the beginning of the mid-1990s through the McKnight Foundation's Collaborative Crop Research Program and several international and foreign institutions such as TTU, AGRA, BODA, DANIDA, ATA and University of Bern, that have been supporting tef research in Ethiopia according to Thion (2016).

In the overall history of tef breeding, three major inter-related phases have been documented. The first phase (1956-1974) 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 thereby the artificial hybridization technique by Tareke (1975).

The third phase from 1995 to now was initiation of molecular approaches, development of molecular markers and genetic linkage maps, analyses of molecular and genetic

diversity(Hailu *et al.*, 2003). Further incorporation of *invitro* culture techniques and inter-specific hybridization along with reappraisal of induced mutagenesis particularly for lodging and leaf rust disease resistanceand introduction of participatory breeding approaches in the overall tef geneticimprovement schemes (Getachew *et al.*, 2006).

Beginning from 1970 totally 42 improved tef varieties were officially released to the farming community in Ethiopia mainly based on their grain yield performance (MoARD, 2017). From these improved tef varieties, 18 of them released from the hybridization program while the remaining 24 resulted from direct selection from the indigenous germplasm accessions. From over head released varieties, 24 of them were from Debre Zeit Agricultural Research Center, while six varieties each of them were from Srinika and Adet Agricultural Research Centers. Two varieties each of them were from Holetta, Bako and Areka Agricultural Research Centers, and one variety from Melkasa Agricultural Research Center. Among the 18 varieties resulting from crossing, only one variety (Simada) developed through inter-specific crossing between a selected tef line (DZ-01-2785) and *E.pilosa* (Thion, 2016).

The main aim of the research development is to boost the productivity of tef by tackling major production constraints through developing cultivars with desirable agronomic and nutritional traits(Cannarozzi *et al.*, 2018). Hence, it is focuses on problem-oriented or demand driven research with the following Specific objectives: (i) To develop tef cultivars with desirable traits using diverse improvement techniques. Priority has been given to lodging and drought, both of which contribute to significant yield loss in tef production. (ii) To sequence the genome and

transcriptomicsof tef for use in marker-assisted breeding and high throughput screenings.(iii) To study diversity in tef accessions with the aim of identifying natural variation in relevant traits.(iv) To disseminate new tef varieties with improved traits to the Ethiopian farming community. (v) To contribute to the human capacity building of the Ethiopian Agricultural Research System.

## **2.7 Semi-dwarf Tef Development**

Tef is adapted to a wide range of environments, and is presently cultivated under diverse agro-climatic conditions (Guta, 2015). Despite its versatility in adapting to adverse environmental conditions and being the staple food for about 70 million people in the Horn of Africa, seed yield of tef is low. The national average yield is ~1.75 ton per hectare, in contrast to 3.94 ton per hectare for maize (CSA, 2018). Provided with optimal fertilizer, well management, and a mesh to prevent lodging, a yield of 3.4–4.6 ton per hectare could be achieved (Yifru and Hailu, 2005). Nevertheless, such agricultural inputs are expensive and time-consuming, and therefore not desirable for agricultural practice.

A major cause of low productivity of tef is lodging, the permanent displacement of the stem from the upright position. Tef has a tall and slender stem, which is susceptible to lodging caused by wind and rain. In addition, when fertilizer applied to increase yield, stems of tef grow taller and become even more susceptible to lodging, resulting in significantly reduced quantity and quality of grain and straw. Moreover, lodging makes harvesting by hand difficult and mechanical harvesting nearly impossible. The average yield reduction due to lodging estimated at 17% (Seyfu, 1993).

Major yield improvements in rice and wheat have achieved in the 1960s through intensive breeding, known as the ‘Green Revolution’. One important trait of these improved varieties was their semi-dwarf phenotype, which resulted in increased standing ability and resource reallocation into grain rather than above ground biomass. According to Endale (2012) cited several studies have shown morphological traits that are related to the lodging in *Eragrostis tef* to be related to plant height, stem diameter of lower internodes, panicle length, biomass and seed weight (Melak-Haile *et al.* 1965; Seyfu, 1993; Fufa *et al.* 1999; Solomon, 2009). Considerable efforts have been made more than 50 years to incorporate by conventional breeding desirable

agronomic traits into tef. However, no lodging resistance traits, such as reduced height and stiff straw reported using conventional breeding so far (Kebebew *et al.*, 2010).

According to Habte *etal.*(2017) reported tef researchers are also doing their best to tackle lodging through semi-dwarf tef development, by employing both conventional and modern molecular tools such as TILLING (Targeted Induced Local Lesion in Genome) (Tadele *et al.*, 2010; Esfeld *et al.*, 2013). To this end, tef mutant lines showing promising results regarding lodging tolerance (for instance, *Kegne* and *Kindemutant* tef lines) developed in collaboration between the University of Bern and the Ethiopian Institute of Agricultural Research (EIAR). *Kegne* linked to the mutation in the *Alpha-tubulin-1* gene and characterized by a right-hand twisting phenotype and resistance to microtubule-related drugs like oryzalin (Jöstet *al.*, 2015). On the other hand, *Kinde* identified as a promising line, having semi-dwarf stature, increased numbers of tillers, tolerance to lodging, larger leaf size and deep green phenotype.

## **2.8 Genetic Variability**

Genetic variability is different from genetic diversity in a way that the former measures how much the trait or the genotype will tend to vary whereas the latter measures the number of the actual variation of species in a population. Genetic variability is the tendency of individual genetic characteristics in a population to vary from one another or the potential of a genotype to change or deviate when exposed to environmental or genetic factors. However, genetic diversity refers to both the vast numbers of different species as well as the diversity within a species (Rao and Hodgkin, 2002; Mahoney and Springer, 2009).

Genetic variability has vast importance to the breeders, as it is prerequisite for any improvement in crop plants and identification of superior genotype (Welsh, 2008). The primary source of variability for the genetic improvement of tef is the indigenous germplasm resource. Ethiopia is the center of both diversity and origin for the tef crop species (Vavilov, 1951). Previously, the Institute of Biodiversity Conservation (IBC) of Ethiopia maintains over 5000 tef germplasm accessions in its genebank. Generally, the tef germplasm accessions showed wide genetic variability in phenologic, morphologic and agronomic traits (Kebebew *et al.*, 2001, 2011, 2013).



In spite of this, there has been lack of sufficient variability in the tef germplasm for some valuable traits such as seed size, and lodging, shattering and leaf rust disease resistance.

It is the basic step of plant breeding program and the information generated on the genetic variability within and among closely related crop species is essential for a rational use of genetic resources. The analysis of genetic Variability can be a useful tool to get information about the genetic diversity of the varieties/lines 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 species implies the greater chance of that species to long-term survival and flourishing (Frankel *et al.*, 1995).

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 (Endeshaw, 1983; Abebe and Bjorstrand, 1996). There are now different techniques that can be used to assess genetic Variability. The most widely employed measurement on morphological marker was partitioning the observed overall phenotypic variation into heritable and non-heritable components that enables to know whether the superiority of selection inherited by the progenies or not.

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 to express genetic Variability (Al-Ayshet *et al.*, 2012).

## **2.9 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 is grouped into two namely: broad sense and narrow-sense heritability. Broad-sense heritability is the degree to which a trait is genetically determined and 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 expressed as the ratio 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 and Chaudhary (1985), 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 due to selection. 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 could use 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 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 annotation 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, culm internode length and diameter of the culm internodes showed relatively low heritability and genetic advance estimates than the other traits (Demeke, 2013 and 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).

## 2.10 Trait Association

In addition to heritability and genetic advance, phenotypic and genotypic correlations are also the key parameters in the selection of superior genotypes 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; Demeke, 2013; Chekole, 2016).

If two plant traits 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. 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, genotypic 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 relation of traits may result from pleiotropic effects of a gene, linkage of two genes, chromogema and regimental affiliation or due to the environmental influences (Sgro and Hoffmann, 2004). Generally, genetic

and phenotypic correlations used to forecast how selection for one trait influences response in another trait (Hardner *et al.*, 2001).

Hailu *et al.* (2003) reported that grain yield showed a positive and significant genotypic correlation with main panicle seedweight, loose panicle form, panicle length, plant height, panicle weight, tiller number, biomass yield and lodging index. Likewise, Yifru and Hailu (2005) reported that grain yield significantly and positively correlated with biomass yield, number of spikelets per panicle and panicle yield. Solomon *et al.* (2009) also reported a positive and significant phenotypic association between lodging index and grain yield. This indicates that the problem of lodging is more severe in high yielding than in low yielding genotypes since the heavy weight of the panicles in high yielders contributes to the lodging inducing force.

In other way, lodging index exhibited a strong negative phenotypic correlation with days to heading and maturity plant height, culm length, grain yield and harvest index in according to Habte *et al.* (2015) reported. This contrast between lodging index and other traits is due to the differences in the type of tef varieties used and the environmental condition they grown in during the experimentation. Varieties with long heading time are more vulnerable to lodging due to longer period of exposure to wind and rain while those with shorter heading time score lower degree of lodging.

## **2.11 Path Coefficient Analysis**

Path coefficient analysis carried out using the phenotypic correlation coefficients as well as genotypic correlation coefficients to determine the direct and indirect effects of the yield components and other morphological characters on grain yield (Dewey and Lu.,

1959). Determination of the interrelationships between various agronomic characters and their direct and indirect effect on grain yield provide information necessary for breeders in improving the productivity of crops.

As reported by Ayalneh *et al.* (2012) Phenotypic path coefficient analysis combined over the two locations revealed that number of fertile tillers showed positive correlation coefficient with grain yield (0.83) and had negative direct phenotypic path coefficient (-0.179). At genotypic path coefficient analysis combined over the two locations revealed, that thousand-kernel weight and grain yield per plant had the highest direct effects (0.393 and 0.307, respectively) and positive significant correlation coefficient with grain yield. Previously, Habtamu *et al.* (2011) reported biomass yield, number of productive tiller per plant and harvest index for their highest direct effect and their correlation with grain yield of tef landraces. The authors suggested that selecting for these traits indirectly selects for grain yield. Similarly, Ayalneh *et al.* (2012) and Abele *et al.* (2012) reported that harvest index and biomass yield had a strong direct effect and positive correlation with grain yield in tef landraces.

This indicated that attention should be given for these traits, which have positive correlation with grain yield in the process of selection, as these traits are helpful for indirect selection. Trait association among yield components and grain yield with its component in this particular study indicated various magnitude of association, which can be carefully looked into while exploiting in selection to improve traits of interest in tef breeding.

## **2.12 Cluster and Distance Analyses**

Different multivariate approaches are available for analyzing the dissimilarity or similarity of genotypes based on variables recorded; cluster analysis (CA), Principal coordinate analysis (PCoA), Principal component analysis (PCA), Canonical Correlation and Multidimensional Scaling (MDS) (Aremu, 2012). CA and PCA are, however, the two commonly used approaches. Cluster analysis is a group of multivariate techniques whose primary purpose is to group individuals or objects based on their characteristics, so that individuals with similar descriptions are mathematically gathered into the same cluster (Aremu, 2005).

The resulting clusters of individuals would then exhibit high internal (within cluster) homogeneity and high external (between clusters) heterogeneity. Thus, with a successful classification, individuals within a cluster are similar or related to one another and different or unrelated to those in other groups. Distance-based clustering methods can either be hierarchical or nonhierarchical. The former is more commonly used in analysis of genetic diversity in crop species. Among various hierarchical methods, the UPGMA (Unweighted Paired Group Method using Arithmetic averages) is the most commonly adopted clustering method. Cluster analysis used to group genotypes into homogenous sets based on their response to the environments considered (Ramburan *et al.*, 2012).

Genetic distance is “the extent of gene differences between individuals, populations or species or genotypes that is measured by some numerical quantity.” It may be at sequence or allele frequency level (Beaumont *et al.*, 1998). Euclidean or straight-line measure of distance is the most commonly used statistic for estimating genetic distance (GD) between individuals (genotypes or populations) by morphological data as suggested by (Mohammadi and Prasanna, 2003). Euclidean distance between two individuals  $i$  and  $j$  having observations on morphological traits ( $p$ ) denoted by  $x_1, x_2, \dots, x_p$  and  $y_1, y_2, \dots, y_p$  for  $i$  and  $j$ , respectively, can be calculated by the following formula as described by (Mohammadi and Prasanna, 2003) on his study.

$$D(i, j) = [(x_1 - y_1)^2 + (x_2 - y_2)^2 + \dots + (x_p - y_p)^2]^{1/2}$$

On the basis of data obtained by measurement of quantitative traits in inbred lines, Smith *et al.* (1991) applied another measure of genetic distance as follows:

$$D(i, j) = [(T_1(i) - T_2(i))^2 / \text{var}T(i)]^{1/2}$$

Where  $T_1$  and  $T_2$  are the values of the  $i$ th trait for inbred lines 1 and 2, respectively, and the  $\text{var}T(i)$  is the variance for the  $i$ th trait over all inbreds.

### 2.13 Principal Components Analysis

Principal component analysis was performed to identify the traits that contributed to the large part of the total variation among the genotypes (Garg and Choudhary, 2012). It is done using standardized values to explore the contribution of each trait to the total variability (Obeng-



Antwiet *al.*, 2011). The goal of PCA is to extract important information from a table and represent it as a set of new orthological variables. The first step in PCA is to calculate Eigen values which explain the amount of total variation displayed on the component axes as suggested (Nelimor, C., 2015).

Eigen values greater than one are worthy of interpretation. The rationale is that an Eigen value less than one implies that the scores on the component would have negative reliability. It is expected that the first 3 axes will explain a large sum of the variations captured by the genotypes. The first PC summarizes most of the variability present in the original data relative to all remaining PCs. The second PC explains most of the variability not summarized by the first PC and uncorrelated with the first PC and so on. Generally, each PC reveals different properties of the original data and as such is interpreted independently (Nelimor, C., 2015).

### **3 MATERIALS AND METHODS**

#### **3.1 Descriptions of Experimental Locations**

The field experiment was carried out at two locations (Debre Zeit and Holetta) in the central parts of Ethiopia during the 2017 cropping season (July to December). Debre Zeit is located at 47 km to south east of Addis Ababa, while Holetta is located at 42 km to the west of Addis Ababa. DZARC found at (8° 44' N, 38° 58' E and 1860 m.a.s.l) whereas, HARC found at (9° 03' N, 38° 30' E and 2400 m.a.s.l) latitude, longitude and altitude, respectively. The two locations represent two different agro-ecologies of the country. Debre Zeit receives mean annual rainfall of 832 mm during the main growing season with maximum and minimum mean annual temperature of 24.3 °C and 8.9 °C, respectively. The experimental field at Debre Zeit characterized by heavy black soil (Vertisol) with a pH of 6.9 and described as very fine montmorillonitic typic pellustert with very high moisture retention capacity (Tamirat, 1992; Habteet *al.*, 2015).

In contrast, Holetta often receives annual total rainfall 1100 mm with maximum and minimum mean annual temperature of 24.1 °C and 6.6°C, respectively. The experimental field at this location characterize by light red soil (Andosol) with a pH of 6.3 and good moisture holding capacity. The weather conditions during the growing season were favorable and the experiment

received sufficient amount of rainfall for normal growth of tef crop at each of the test locations. The mean monthly rainfall and maximum as well as minimum mean monthly temperatures during the crop-growing season in relation to the two locations (Appendix I).

### 3.2 Planting Materials

These experimental plant materials comprised 49 semi-dwarf tef recombinant inbred lines including local and standard checks. These included 45 recombinant inbred lines (RIL) derived from the crosses of DZ-01-192 x GA-10-3, the two parents (pure lines), one standard and one local check (Table 2). The RILs are descendants of the intra-specific cross through continuous maintenance of progenies up to the seventh filial generation (F7) through selfing using F2-derived single-seed-decent breeding method. The tef cultivar DZ-01-192 is late maturing, thick culmed, tall, has loose panicle and white seed color. GA-10-3 is a mutant line developed through mutation breeding by using Ethyl methane sulphonate (EMS) assisted by Targeted Induced Local Lesions IN Genomes (TILLING) method and introduced from university of Bern (Switzerland). It has lodging tolerance characters, early maturity, semi-dwarf structure and pale white seed color. The materials kindly supplied by Debre Zeit agricultural research center, in Ethiopia. I have duly acknowledged DZARC for their kindness.

Table 2 Experimental materials

<b>No.</b>	<b>Recombinant Inbred Lines SD-Tef</b>	<b>No.</b>	<b>Recombinant Inbred Lines SD-Tef</b>
1	DZ-01-192 x GA-10-3 (RIL # 1)	26	DZ-01-192 x GA-10-3 (RIL # 58)
2	DZ-01-192 x GA-10-3 (RIL # 2)	27	DZ-01-192 x GA-10-3 (RIL # 68)
3	DZ-01-192 x GA-10-3 (RIL # 4)	28	DZ-01-192 x GA-10-3 (RIL # 75)
4	DZ-01-192 x GA-10-3 (RIL # 5)	29	DZ-01-192 x GA-10-3 (RIL # 160)
5	DZ-01-192 x GA-10-3 (RIL # 6)	30	DZ-01-192 x GA-10-3 (RIL # 161)
6	DZ-01-192 x GA-10-3 (RIL # 8)	31	DZ-01-192 x GA-10-3 (RIL # 162)
7	DZ-01-192 x GA-10-3 (RIL # 12)	32	DZ-01-192 x GA-10-3 (RIL # 166)
8	DZ-01-192 x GA-10-3 (RIL # 14)	33	DZ-01-192 x GA-10-3 (RIL # 169)
9	DZ-01-192 x GA-10-3 (RIL # 15)	34	DZ-01-192 x GA-10-3 (RIL # 171)
10	DZ-01-192 x GA-10-3 (RIL # 16)	35	DZ-01-192 x GA-10-3 (RIL # 172)
11	DZ-01-192 x GA-10-3 (RIL # 19)	36	DZ-01-192 x GA-10-3 (RIL # 174)
12	DZ-01-192 x GA-10-3 (RIL # 20)	37	DZ-01-192 x GA-10-3 (RIL # 175)
13	DZ-01-192 x GA-10-3 (RIL # 21)	38	DZ-01-192 x GA-10-3 (RIL # 178)
14	DZ-01-192 x GA-10-3 (RIL # 22)	39	DZ-01-192 x GA-10-3 (RIL # 179)

<b>15</b>	DZ-01-192 x GA-10-3 (RIL # 24)	40	DZ-01-192 x GA-10-3 (RIL # 180)
<b>16</b>	DZ-01-192 x GA-10-3 (RIL # 25)	41	DZ-01-192 x GA-10-3 (RIL # 182)
<b>17</b>	DZ-01-192 x GA-10-3 (RIL # 27)	42	DZ-01-192 x GA-10-3 (RIL # 185)
<b>18</b>	DZ-01-192 x GA-10-3 (RIL # 28)	43	DZ-01-192 x GA-10-3 (RIL # 195)
<b>19</b>	DZ-01-192 x GA-10-3 (RIL # 33)	44	DZ-01-192 x GA-10-3 (RIL # 203)
<b>20</b>	DZ-01-192 x GA-10-3 (RIL # 41)	45	DZ-01-192 x GA-10-3 (RIL # 262)
<b>21</b>	DZ-01-192 x GA-10-3 (RIL # 44)	46	Boset (standard check)
<b>22</b>	DZ-01-192 x GA-10-3 (RIL # 45)	47	DZ-01-192 (parental check)
<b>23</b>	DZ-01-192 x GA-10-3 (RIL # 48)	48	GA-10-3 (parental check)
<b>24</b>	DZ-01-192 x GA-10-3 (RIL # 52)	49	Local Check
<b>25</b>	DZ-01-192 x GA-10-3 (RIL # 57)		

\*SD: - Semi-dwarf tef; DZ-01:-Debre Zeit tef cultivar released through selection; GA-10-3: - - Mutant elite tef line. Source of all material were from cross of (DZ-01-192 x GA-10-3) and F<sub>7</sub> (2016) progeny at DZARC

### 3.3 Experimental Design, Layout and Management

The field experiments conducted using 7x7 simple lattice designs with two replications at both locations. 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 are 1 m, both between plots and incomplete blocks and 1.5 m between replications. The tef recombinant inbred lines allotted to plots at random within each replication. Sowing were done on 13 August, 25 July 2017 at Debre Zeit and Holetta, respectively. As per the research recommendations, 15 kg/ha seed rate was used for both locations.

The fertilizer rate used for each location recommended depending on the type of soil. The fertilizers used for Holetta (light red soil) were 40kg N, 60kg P<sub>2</sub>O<sub>5</sub>, and 11kgS per hectare, as well as 60kg N, 60kg P<sub>2</sub>O<sub>5</sub> and 11 kg S per hectare for Debre Zeit (Vertisol). All NPS were applied at planting with a rate of 158 kg/ha and the remaining urea applied at the rate of 22 kg/ha for HARC and 65 kg /ha for DZARC. Half of the urea applied at sowing, while the remaining half applied at tillering. Hand weeding and other management practices were performed as required for both locations.

### 3.4 Data Collected

Data collected from sixteen quantitative traits including seven traits taken on plot basis and nine traits assessed on randomly taken five plants of tef from the central rows of each plot. For individual plant trait sampled, averages of data from the five random samples of plants per plot used for statistical analyses.

**The following data taken from plot basis:**

**Days to heading/ panicle emergence (DH):** Number of days from seedling emergence to the appearance of the tips (about 5 cm) of the main shoot panicle on 50% of the plants in a plot. Note that tef panicle appears without showing the booting stage, which is unlike the other small cereals like wheat and barley, but similar to that in rice.

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

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

**Above ground biomass yield (ABM):** The total dry weight in kilogram of the above ground biomass per plot before threshing

**Grain yield (GY):** The entire plot of grains weight in kilogram after threshing and sun drying.

**Harvest index (HI):** The ratio of grain yield to the total biomass in percent.

**Lodging index (LI):** lodging assessment was performed as suggested by Caldicott and Nuttall (1979) as follows:

$$\text{Lodging index} = \frac{\text{Sum (lodging scores * respective percentage of area lodged)}}{5}$$

Lodging score was recorded on a 0-5 scale as the degree of leaning from the upright position and whereby zero=completely upright non-lodged plants and five=completely flat on the ground. The severity of lodging for each degree assessed as the proportion in percent of plants in a plot manifesting each degree of lodging. Finally, the lodging index for each plot was computed as the average of the product sum of each degree of lodging and the corresponding severity as indicated in the formula above.

The following observations recorded based on measurements made on five randomly taken and pre-tagged plants from the three central rows of each plots.

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

**Panicle length (PL):-** The length in centimeter from the node where the first panicle branch starts to the tip of the panicle.

**Culm length (CL):-** The length in centimeter from ground level to the node where the first panicle branch starts.

**Peduncle length (PDL):-** The length in centimeter of the top most culm internode spanning from the last culm node until the start of the first panicle branch. It stretches from the node where the flag leaf starts to where the first panicle branch starts.

**Second basal culm internode length (SCIL):-** The length in centimeter of the second basal culm internode.

**Second basal culm diameter (SCID):** The diameter in millimeter of the second basal culm internode measured using caliper.

**Fertile tiller number per plant (NFT):-** Counts of the panicle-bearing tillers of pre tagged main plants that have produced a fertile panicle.

**Numbers of branches per main shoot panicle (NBP):-** Counts of the total number of branches per main panicle from bottom to top.

**Number of spikelets per panicle (NSP):-** It is the number of spikelets counted on the panicle.

### 3.5 Statistical Analyses

Tests of homogeneity and normality of error variances were done mainly using relationships of predicted means and residuals for all traits. ANOVA were done for single location as well as for the combined over locations. For combined analysis of variance over locations, the homogeneity of error variance were tested using F-max test method of Hartley (1950), which requires independent random samples of the same size from normally distributed populations (Ott & Longnecker, 2015). It 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:

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

If the calculated value of  $F_{\max}$  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).

Estimates of coefficients of phenotypic and genotypic variances, heritability and genetic advance done from mean square value and grand mean for each traits. For multivariate analysis such as cluster, distance and principal component analysis mean records on all traits are pre-standardized to mean zero and variance unity to avoid bias due to the differences in measurement scales (Manly, 1986).

### 3.5.1 Analysis of variance

All measured traits using simple lattice design were subjected to analysis of variance (ANOVA) of SAS software version 9.3 (SAS institute, 2011). Total variability present among the recombinant inbred lines for each of the traits were partitioned into known (treatment) and unknown (residual) effects following the standard procedures of ANOVA using the following model according to Gomez and Gomez (1984) indicated. After two error terms (Mean square error of block ( $E_b$ ) and Mean square of Experimental error ( $E_e$ )) calculated from combined ANOVA analysis.

Comparing  $E_b$  with  $E_e$ ; If  $E_b > E_e$  an adjustment of the treatments were carried out, otherwise if  $E_b < E_e$  no need of an adjustment of the treatments and the block effect is negligible then the data can be analyzed by RCBD, using replication as block. The SAS program for analyzing lattice design consists of two parts. In the first, PROC GLM was used to calculate unadjusted block SS (TYPE I SS–Sequential SS), adjusted block SS (TYPE III SS), unadjusted treatment SS, and intra-block error. To calculate the unadjusted block SS from TYPE I SS, the order in which variables were entered into the model statement is important. The block was entered before the treatment in the model statement. These estimates were used in the second part of the program to calculate the adjusted treatment SS, adjusted means, and the average effective error, respectively (Gomez and Gomez, 1984).

The comparison of mean performance of genotypes was done following the significance of mean squares using Duncan's Multiple Range Test (DMRT). Genotypic, environmental and phenotypic variances were estimated according to Falconer (1981) as follows:

$$\text{Genotypic variance for single location } \sigma^2_g = \frac{MSg - MSe}{r} ; \quad \text{Interaction variance } \sigma^2_I = \frac{MSI - Mse}{r}$$

$$\text{Over locations genotypic variance } \sigma^2_g = \frac{MSg - MSI}{rl} ; \quad \text{Environmental variance } \sigma^2_e = \frac{Mse}{r}$$

$$\text{Phenotypic variance } \sigma^2_p = \sigma^2_g + \sigma^2_e$$

Where,  $\sigma^2_g$ -Genotypic variance; MS<sub>g</sub>-Mean square of genotype; MS<sub>e</sub> - Mean square of error;  $\sigma^2_I$ - Interaction variance; MS<sub>I</sub> – Mean square of interaction variance;  $\sigma^2_p$  – phenotypic variance;  $\sigma^2_e$ -Error variance; r-Number of replication and l - Number of location.

**Model of the experiment:**

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  $i^{th}$  genotype under  $j^{th}$  replication and  $k^{th}$  incomplete block within replication j;  $\mu$ =grand mean;  $G_i$ = the effect of  $i^{th}$  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 combined analysis of variance over locations, the total variations among the inbred lines measured using the following model:

$$P_{ijkz} = \mu + G_i + B_{k(j)(z)} + R_{j(z)} + L_z + (GL)_{iz} + E_{ijkz}$$

Where,  $P_{ijkz}$ = phenotypic value of  $i^{th}$  genotype under  $j^{th}$  replication at  $z^{th}$  location and  $k^{th}$  incomplete block within replication j and location z;  $\mu$ =grand mean;  $G_i$  = the effect of  $i^{th}$  genotype;  $B_{k(j)(z)}$ = the effect of incomplete block k within replication j and location z;  $R_{j(z)}$ =the effect of replication j within location z;  $L_z$ = the effect of location z;  $(GL)_{iz}$ =the interaction effects between genotype and location; and  $E_{ijkz}$ = the residual or effect of random error.

Table 3 ANOVA skeleton for individual locations (HARC and DZARC) in simple lattice design

Source of variation	Degree of freedom	Sum of Squares(SS)	Mean Squares(MS)
Replications(r)	r-1	SS <sub>r</sub>	
Genotypes( $g_{unadjusted}$ )	g-1	SS <sub>g</sub>	
Block in rep(adjusted)	r(b-1)	SS <sub>B</sub>	$E_b$
Intra block error	(b-1)(rb-b-1)	SS <sub>E</sub>	$E_e$
Total(T)	rb-1	SST	



\*  $g$  = Number of genotypes,  $b$  = Number of plots in a block or block size / intra block

\* $E_b$  – Error for block =  $SSB/r (b-1)$  and  $E_e$  – Experimental error =  $SSE/ ((b-1) (rb-b-1))$

Table 4 Analysis of variances for combined over locations in simple lattice design

Source of variation	Degree of freedom	Mean square (MS)	Expected mean square (EMS)
Location (L)	L-1	MSL	$\sigma^2 e + r\sigma^2 gi + g\sigma^2 L$
Replication with in location(r)	L(r-1)	MSr	$\sigma^2 e + g \sigma^2 rL$
Blocks within replication(b)	r(b-1)	MSb	$\sigma^2 e + r\sigma^2 gi + r\sigma^2 g$
Genotypes (g)	g-1	MSg	$\sigma^2 e + r\sigma^2 gi + rL\sigma^2 g$
g x L interaction (i)	(g-1) (L-1)	MSi	$\sigma^2 e + r\sigma^2 gi$
Error (e)	Lg(r-1)-(rb-1)	MSe	$\sigma^2 e$

Where,  $b$ - represent intra blocks;  $\sigma^2 g$ = genotypic variance,  $\sigma^2 e$  = environmental variance,  $\sigma^2 L$  =location variance,  $\sigma^2 r$  = replication variance, and  $\sigma^2 gi$  = genotype x location interaction variance,  $L$  = number of locations,  $g$  = number of genotypes and  $r$  = number of replications.

Appropriate mean separation will be done if there is significance.

- Comparing  $E_b$  with  $E_e$ : - If  $E_b \leq E_e$ , Adjustment of treatment means will have no effect and analyze as if it were an RCBD using replications as blocks
- If  $E_b > E_e$  then compute an adjustment factor  $A$
- $A = (E_b - E_e) / (b(r - 1)E_b)$ , used to compute adjusted treatment means

✚ **Relative Efficiency:** -Estimate the error mean square of an RCBD

$$E_{RCBD} = (SSB + SSE) / ((g-1)(r-1)), \text{ Then the relative efficiency of the lattice is } RE = E_{RCBD} / E_e$$

From the analysis of variances of data from each locations efficiency of simple lattice design over RCBD was calculated depending on the above formula and simple lattice have 26.2% efficient than randomized complete block design (RCBD).

### 3.5.2 Estimation of variance components

Phenotypic (PCV) and genotypic (GCV) coefficients of variation were calculated according to the method suggested by Burton (1953) as:

#### Phenotypic Coefficient of Variation:-

$$PCV = \left[ \frac{\sqrt{\delta^2 p}}{\mu} \right] \times 100, \text{ Where; PCV= phenotypic coefficient of variation; } \mu = \text{Population mean}$$

#### Genetic coefficient of Variation:-

$$GCV = \left[ \frac{\sqrt{\delta^2 g}}{\mu} \right] \times 100, \text{ Where; GCV= phenotypic coefficient of variation; } \mu = \text{Population mean}$$

PCV and GCV values > 20% is regarded as high, 10 - 20% is considered as medium and < 10% is considered as low (Kherdade *et al.*, 1985).

### 3.5.3 Estimates of broad sense heritability

Heritability in broad sense ( $H^2$ ) was calculated according to Allard (1960) as –  $H^2 = \frac{\sigma^2 g}{\sigma^2 p} \times 100\%$   
Where,  $\sigma^2 g$  and  $\sigma^2 p$  are genetic and phenotypic variance, respectively.

According to Robinson *et al.* (1949), broad sense heritability in cultivated plants can be categorized into low for values of 0-30%, medium for estimates of 30-60%, and high for values above 60%.

### 3.5.4 Estimates of genetic advance

Genetic Advance (GA) was estimated using the formula of Johnson *et al.* (1955) as follows.  $GA = H^2 k \sigma_p$ , Where, GA = Genetic advance,  $H^2$  is broad sense heritability,  $k (= 2.056)$  was the selection differential expressed in phenotypic standard deviation depending on the selection intensity of 5%,  $\sigma_p$  is the phenotypic standard deviation. Where Genetic advance as percent of mean as follows according to Falconer and Mackay (1996):

$$GAM = \frac{GA}{\text{Mean}} \times 100\% \quad \text{where 0-10\% is low, 10-20\% is moderate and 20\% and above is high.}$$

### 3.5.5 Estimation of correlation

Phenotypic and genotypic correlation coefficients were computed from the components of variance and covariance based on the method described by Singh and Chaudhary (1996), using the CANDISC procedure of SAS software(SAS, 2011).

### 3.5.6 Path coefficient analysis

Path coefficient analysis was carried out using the phenotypic correlation coefficients as well as genotypic correlation coefficients. This analysis computed as suggested by Dewey and Lu (1959) with the following formula.

$$r_{ij} = P_{ij} + \sum r_{ik} P_{kj}$$

Where,  $r_{ij}$  is the mutual association between independent trait (i) and dependent variable (j),  $P_{ij}$  is component of direct effect of the independent (i) on the dependent (j) and  $\sum r_{ik} P_{kj}$  is sum of components of indirect effect of a given independent trait (i) on the dependent variable (j) *via* all other independent traits (k).

The residual effect (U) calculated using the formula suggested by Dewey and Lu (1959).  $U = \sqrt{1 - R^2}$  Where;  $R^2 = \sum r_{ik} P_{kj}$ , U= the residual; unexplained variation of the dependent variable.

### 3.5.7 Cluster and distance analyses

The use of established multivariate statistical algorithms is important in classifying breeding materials from germplasm, accessions, lines, and other races into distinct and variable groups depending on the genotype performance. Before subjecting to statistical grouping techniques, it is advisable to transform units of measurements of characters (agronomic) into standardized units. This will eliminate the impact of the unit in differences of measurement of each variable on variances and covariance.

Hierarchical cluster analysis approach used to examine the assembling pattern of the 49-tef lines based on their similarity with respect to the corresponding means of all the 15 traits studied. A

cluster analysis was done to group the tested tef genotypes into genetically distinct classes using SAS Statistical Software Version 9.3 (SAS, 2011), following the average linkage cluster analysis. The numbers of clusters were determined based on the Pseudo-F and Pseudo-t<sup>2</sup> options resulted from SAS procedure of cluster data analysis. The dendrogram constructed based on the average linkage and Euclidean distance used as a measure of dissimilarity.

Genetic distances between clusters as standardized were calculated using Mahalanobis's D<sup>2</sup> statistics (Mahalanobis, 1936) as  $D^2_{ij} = (x_i - x_j)' \text{cov}^{-1}(x_i - x_j)$ , where D<sup>2</sup><sub>ij</sub> = the distance between cases i and j, x<sub>i</sub> and x<sub>j</sub> = vectors of the values of the variables for cases i and j and cov<sup>-1</sup> = the pooled within groups' variance-covariance matrix. The D<sup>2</sup> values come from pairs of clusters were considered as the calculated values of Chi-square (X<sup>2</sup>) and tested for significance both at 1% and 5% probability levels against the tabulated value of X<sup>2</sup> for 'P' degree of freedom, where P is the number of traits considered (Singh and Chaudhary, 1996).

### **3.5.8 Principal component analysis**

Principal component analysis was performed using Minitab Statistical software, release 17 for windows (Minitab, 2007) to identify the traits that contributed to the large part of the total variation among the genotypes (Garg and Choudhary, 2012). In principal component analysis, eigenvalues greater than one were considered important to explain the observed variability.

## 4 RESULTS AND DISCUSSIONS

### 4.1 Mean Performance

Mean squares of the 16 traits from analysis of variance (ANOVA) at individual location and combined over the two locations are presented in Tables 5, 6 and 7. From the separate analysis, at Holetta highly significant differences among inbred lines ( $p < 0.01$ ) were observed for all traits except number of fertile tillers per plant. At Debre Zeit, significant differences among lines ( $p < 0.01$ ) were observed for all traits except peduncle length, second culm internode length, Second culm internode diameter, number of branches per panicle and fertile tillers per plant.

For some traits like grain yield, harvest index, lodging index, days to heading and maturity lower mean values were recorded at Debre Zeit and higher values recorded at Holetta. In the case of remaining traits such as plant height, panicle length, culm length, second culm internode length, second culm internode diameter, number of fertile tillers, number of branches and number of spikelets per panicle the highest value recorded at Debre Zeit whereas the lowest value at Holetta. This indicates that the locations had significant effects on the performance of semi-dwarf tef recombinant inbred lines (Table 5 and 6). This expected based on the distinct agro-climatic classification of the test locations (Kebebew *et al.*, 2003b).

The combined analysis of variance over the two locations of the 49 semi-dwarf tef recombinant inbred lines showed highly significant ( $P < 0.01$ ) genotype effects for all 16 traits, except for number of fertile tillers per plant (Table 7). Locations revealed highly significant ( $P \leq 0.01$ ) effects on 13 of the traits and significant ( $P \leq 0.05$ ) effects on two traits (peduncle length and grain yield), while number of branches per main panicle was not significantly affected by locations. Genotype and locations interacted highly significantly on eleven of the traits, while one trait (panicle length) showed significant interaction and four traits (peduncle length, second culm internode length, second culm internode diameter and number of fertile tillers) showed no statically significant interaction effects. This indicate that the two location environmental conditions highly different.

Comparisons of the mean performances of each traits of combined locations presented on (Appendix Table III). From grain yield traits RIL-14, RIL-45, RIL-28 and RIL-41 in this order had mean grain yields of 2.52, 2.29, 2.21 and 2.19 t ha<sup>-1</sup>, which were higher than that of the standard check Boset (1.83 t ha<sup>-1</sup>) and the local check (2.14 t ha<sup>-1</sup>). This indicates that grain yield potential of these semi-dwarf tef were different; thus, indicating that the opportunity for breeders to further improvement of tef yield through the existing breeding strategy. In line with the present findings, Yifru and Hailu (2005) also reported the grain yield potential in tef improvement.

In lodging index traits RIL-19(39.5%), RIL-75(44.5%), RIL-8(47.0%), RIL-169(50.5%), RIL-22(51.1%), RIL-14(54.0%) and DZ-01-192(53.0%) have the least lodging index than local and standard checks as well as the parent checks except RIL-14, which more than DZ-01-192 parental check by one percent. This indicates that there is high potential to increase grain yield by decreasing the loss exposed by lodging. From the main lodging related traits second basal culm internode diameter have the highest mean performance for the following recombinant inbred lines such as RIL-169(2.13 mm), RIL-14(2.08 mm), RIL-57(1.96), RIL-45(1.92 mm), RIL-175(1.91 mm) and parental check (DZ-01-192) which have 1.98 mm, while the standard and local checks shown lower in diameter. As indicated above the highest in grain yield have highest culm diameter and lower lodging index, this finding in line with (Habte *et al.*, 2017).

This indicates that as the second basal internode diameter increases the lodging become decrease and grain yield increase even if the other traits may averagely affect their association non – significant in this study. RIL-14(115.95 cm) also exhibited the longest plant height and length of the culm, panicle and second basal culm in addition to culm diameter, next to RIL-169, which have highest diameter. However, the parental line DZ-01-192 also had the longest better than the checks. Generally, all the recombinant inbred lines have shown clearly different mean performance in each traits comparing with each other and checks (Appendix Table III).

Table 5 Analysis of variance for the 16 traits of 49 semi-dwarf tefrecombinant inbred lines evaluated at Holetta

Traits	Rep(df=1)	Intra blocks (df=12 )	Inbred lines(df=48)	Error (df=36)	CV (%)	Mean	R <sup>2</sup>
DH	16.33**	3.85ns	35.19**	2.29	2.49	60.69	96.04
DM	1489.02**	74.53**	68.55**	15.16	3.25	119.94	92.53
GFP	1193.51**	80.26**	58.46**	13.31	6.16	59.24	92.64
PH	18.17ns	125.85**	186.06**	9.98	3.52	89.76	96.73
PL	19.39*	7.34*	35.10**	3.22	5.59	32.09	94.23
CL	0.02ns	75.35**	87.20**	6.56	4.44	57.68	95.70
PDL	6.99ns	3.18ns	12.97**	2.32	6.50	23.41	90.00
SCIL	1.69ns	4.07**	4.09**	0.74	10.00	8.61	90.00
SCID	0.06ns	0.04ns	0.05**	0.02	8.32	1.78	76.26
NFT	0.06 ns	0.86 ns	0.62ns	0.73	25.91	3.29	61.81
NBP	12.93 ns	7.62 ns	11.77**	5.16	8.77	25.89	80.99
NSP	318.24 ns	3976.57 ns	8016.64**	2371.36	11.24	433.21	84.02
ABM	344680.1 ns	2295049.5**	2586895.7**	281746.60	9.26	5733.10	95.85
GY	251709.31**	317362.28**	158861.96**	40207.54	12.57	1595.56	93.58
HI	20.88 ns	16.20 ns	34.37**	12.86	12.77	28.09	81.91
LI	35.52 ns	9.53 ns	48.56**	12.46	5.54	63.72	88.93

\*, \*\* Significant at  $p \leq 0.05$  and  $p \leq 0.01$  respectively, while ns- non-significant, DH= days to heading, DM = days to maturity, GFP= grain filling period, PH= plant height, PL= panicle length, CL= culm length, PDL= peduncle length , SCIL=second culm internode length , SCID= second basal culm internode diameter, NFT= no. of fertile tillers per plant, NBP= no. of branches per panicle, NSP= no. of spikelets per panicle, ABM = above ground biomass yield(kg/ha), GY= grain yield(Kg/ha), HI= harvest index, LI= lodging index, df = degree of freedom and CV = coefficient of variation (%).

Table 6 Analysis of variance for the 16 traits of 49 tef recombinant inbred lines evaluated at Debre Zeit

Traits	Rep (df= 1)	Intra-blocks (df=12)	Inbred lines (df= 48)	Error (df = 36)	CV (%)	MEAN	R <sup>2</sup>
DH	5.39 ns	9.03 ns	16.67**	6.99	5.55	92.74	79.71
DM	0.83 ns	9.19 *	16.56**	4.34	2.25	45.06	86.64
GFP	10.45 ns	10.74 ns	25.25**	11.24	7.44	102.79	79.66
PH	34.33 ns	24.84 ns	127.72**	23.79	4.74	39.91	90.30
PL	19.39 ns	4.48 ns	18.54*	9.57	7.75	62.89	74.75
CL	2.12 ns	36.84 ns	88.09**	34.40	9.33	22.81	82.21
PDL	28.88*	7.06 ns	5.66 ns	6.14	10.86	12.2	67.74
SCIL	9.43*	4.62**	2.08 ns	1.78	10.93	1.83	74.01
SCID	0.06 ns	0.02 ns	0.03 ns	0.03	8.74	6.93	64.16
NFT	5.49*	1.40 ns	0.74 ns	0.90	17.35	25.69	65.10
NBP	1.30 ns	7.44 ns	7.97 ns	6.71	10.08	453.35	66.20
NSP	9035.52*	4254.33*	16735.10 **	2169.42	10.27	4.32	92.38
ABM	3594830.4**	661930 ns	6456901.7**	380149.40	7.96	1534.61	96.20
GY	186602.9**	25269.68 ns	446728.35**	14970.99	7.97	19.85	97.83
HI	4.67 ns	5.44 ns	21.12**	4.43	10.61	3403.12	88.37
LI	0.09 ns	64.83*	158.46**	32.70	9.70	47.68	88.81

\*, \*\* Significant at  $p \leq 0.05$  and  $p \leq 0.01$  respectively, while ns- non-significant, DH= days to heading, DM = days to maturity, GFP= grain filling period, PH= plant height, PL= panicle length, CL= culm length, PDL= peduncle length , SCIL=second culm internode length , SCID= second basal culm internode diameter, NFT= no. of fertile tillers per plant, NBP= no. of branches per panicle, NSP= no. of spikelets per panicle, ABM = above ground biomass yield(kg/ha), GY= grain yield(Kg/ha), HI= harvest index, LI= lodging index, df = degree of freedom and CV = coefficient of variation (%).



Table 7 Analysis of variance for 16 traits of 49 semi-dwarf tef recombinant inbred lines over the two locations

Traits	Locations(L) (df=1)	Replications(r) (df=1)	Intra Block(b) (df=12)	Inbred lines(I) (df=48)	I x L (df=48)	Error(e) (df=85)	CV (%)	R <sup>2</sup>
DH	8294.01**	1.47ns	9.22*	42.09**	11.83**	4.69	4.00	96.57
DM	36235.84**	780.01**	59.95**	54.80**	38.75**	19.96	4.20	96.20
GFP	9857.65**	713.65**	63.37**	56.21**	39.14**	20.07	8.59	90.71
PH	8320.05**	51.22ns	63.36**	267.03**	50.21**	26.64	5.36	91.95
PL	2996.78**	38.80**	7.44ns	44.67**	8.92*	6.04	6.83	91.96
CL	1330.17**	0.86ns	44.40ns	134.06**	46.59**	26.93	8.61	83.40
PDL	17.34*	32.15**	5.79ns	13.12**	5.79ns	4.25	8.92	76.48
SCIL	631.52**	1.57ns	4.49**	3.99**	2.18ns	1.77	12.79	86.85
SCID	0.17**	0.04ns	0.04*	0.06**	0.02ns	0.02	8.31	68.44
NFT	231.04**	2.21ns	1.24ns	0.77ns	0.69ns	0.87	21.33	81.09
NBP	1.90 ns	11.22 ns	11.90*	10.60**	10.50**	5.51	9.10	72.36
NSP	19872.94**	6372.6 ns	4382.45 ns	13649.28**	12226.54**	2501.54	11.28	86.54
ABM	197799029**	3082888.8**	1531599.9**	6604503.8**	3131395.8**	491640.4	10.41	94.79
GY	182640.23*	435881.02**	159891.98**	358714.8**	322262.61**	49196.92	14.17	91.21
HI	3330.69**	22.65 ns	6.88 ns	26.19**	34.37**	9.44	12.82	85.95
LI	1098.45**	19.61 ns	42.03 ns	130.43**	94.53**	23.88	7.96	87.05

\*, \*\* Significant at  $p \leq 0.05$  and  $p \leq 0.01$  respectively, while ns- non-significant, DH= days to heading, DM = days to maturity, GFP= grain filling period, PH= plant height, PL= panicle length, CL= culm length, PDL= peduncle length , SCIL=second culm internode length , SCID= second basal culm internode diameter, NFT= no. of fertile tillers per plant, NBP= no. of branches per panicle, NSP= no. of spikelets per panicle, ABM = above ground biomass yield(kg/ha), GY= grain yield(Kg/ha), HI= harvest index, LI= lodging index, df = degree of freedom and CV= coefficient of variation (%).

Relative efficiency of the simple lattice design compare to that of a randomized complete block design where done as follows:-

- First by computing MSE for the RCBD as:  $E_{RCBD} = (SSB+SSE)/(k^2- 1)(r -1)$ .
- Then % relative efficiency =  $(E_{RCBD} / E_e' )100$  while,  $E_e' = (1+(rkA)/(k+1))E_e$  and

- $A = (E_b - E_e) / (k(r - 1)E_b)$ . where  $E'_e$ - effective error mean square, A- adjusted treatment,  $k^2$ - number of treatments, k- number of plot in block, r- number of replications,  $E_e$ = pooled error and  $E_b$ - block error
- Therefore there is a 26.2% gain in efficiency from using the lattice as this study.

#### **4.2 Genotypic and Phenotypic Coefficients of Variation**

The value of genotypic coefficients of variation (GC) and phenotypic coefficients of variation (PCV) were grouped to High >20%, intermediate 10-20% and low <10% (Kherdade *et al.*, 1985). Depending on this for the current study, the GCV ranged from 0.61% for number of branches per panicle to 13.83 % for above ground biomass. All traits grouped in the low GCV value except above ground biomass, which grouped under the intermediate GCV value (Table 8). Similarly, Solomon *et al.* (2009) reported that plant height, days to maturity and harvest index had low GCV values. Correspondingly, Habtamu *et al.* (2011) and Habte *et al.* (2015) also reported that days to maturity and days to grain filling had low GCV values, respectively. This might be attributing to high influence of the environment on the inbred lines. Low values of GCV suggest less scope of improvement for these traits by selection. The magnitude of genetic variation better assessed from genotypic coefficients of variation (Solomon *et al.*, 2013). Therefore, selecting the tef recombinant inbred lines having higher harvest index and lower lodging index could help enhancing the productivity of tef.

Phenotypic coefficient of variation values ranged from 4.55% for days to maturity to 26.36% for grain yield (Table 8). The grain yield and above ground biomass were categorized into high PCV (>20%). However, panicle length, culm length, second basal culm internode length, number of spikelets per main panicle, harvest index and lodging index were grouped into intermediate PCV values (10-20%). The third group of PCV had a low (0-10%) value, which computed for days to heading days to maturity, grain filling period, plant height, peduncle length and second basal culm internode diameter and number of branches per main panicle. Phenotypic coefficient of variation is usually the reflection of the effects of inbred lines and environment. If the PCV is greater than GCV it means the environment contributes more than the genes' effect for phenotypic expression of the trait. Previous findings by different researchers were also similar to the present study results (Kebebew *et al.*, 2001b; Habtamu *et al.*, 2011 and Habte *et al.*, 2015).

### **4.3 Heritability**

The broad sense heritability ranged from 68.35% for plant height to 0.47% for number of branches per main panicle (Table 8). In addition to plant height, panicle length (66.71%) also had high heritability values >60% (Robinson *et al.*, 1949). This indicates less influence of environment as compared to the genetic factors in controlling the traits and it suggested that the progenies would have a higher chance to perform the same as the parent. Days to heading, culm length, peduncle length, second basal culm internode length, second basal culm internode diameter, above ground biomass had estimates categorized under moderate heritability (30<60%).

Whereas days to maturity, grain filling period, number of branches per main panicle, number of spikelets per main panicle, grain yield, harvest index and lodging index categorized into low heritability values (<30%). Low heritability indicates the non- predictable of the

phenotype range of environments. This showed that these traits are highly influenced by environment. This suggestion is supported with the findings of several authors who conducted studies on tef (Kebebew *et al.*, 2000, 2001; Solomon *et al.*, 2009; Habtamu *et al.*, 2011; Abel *et al.*, 2012; Habte *et al.*, 2015).

#### 4.4 Expected Genetic Advance

The expected GA, expressed as a percentage of the mean, ranged from 0.09% for number of branches to 17.02% for above ground biomass (Table 8). Similarly, moderate expected GA observed for plant height (13.02%) and panicle length (13.97%) and culm length (11.12%). All the rest of the traits showed low genetic advance values as a percentage of mean between 0.09% and 8.05%. Similar findings with this also study reported by (Abel *et al.*, 2012; Kebebew *et al.*, 2001 and Solomon *et al.*, 2009).

Low heritability and genetic advance estimated for the traits suggest that breeding for those traits would be a difficult task. Johnson *et al.* (1955) in soybean suggested that heritability estimate with genetic gain are more useful for effective improvement. In addition to high heritability along with high genetic advance as percentage of mean implies the role of additive genes for the expression of the characters, and thus it could be very effective in improvement upon selection. In general, high GCV, heritability and genetic advances for traits could be an excellent tool for improving through selection of high performing genotypes. In the current study even if no high GCV recorded, high heritability (plant height and panicle length) and high above ground biomass genetic advance were displayed as also as reported by (Nigus *et al.*, 2016).

Table 8 Phenotypic and Genotypic coefficients of variation, Heritability, Genetic advance and Genetic advance as percent of means for 15 traits in 49 recombinant inbred lines of semi-dwarf tef at Holetta and Debre Zeit.

Traits	$\sigma^2_g$	$\sigma^2_e$	$\sigma^2_{gl}$	$\sigma^2_p$	GCV	PCV	H <sup>2</sup>	GA	GAM
DH	7.57	2.34	3.57	13.48	5.08	6.78	56.12	4.24	7.83
DM	4.01	9.98	9.40	23.39	1.88	4.55	17.16	1.71	1.61

GFP	4.27	10.04	9.54	23.84	3.96	9.36	17.90	1.80	3.45
PH	54.21	13.32	11.79	79.31	7.65	9.25	68.35	12.54	13.02
PL	8.94	3.02	1.44	13.40	8.30	10.17	66.71	5.03	13.97
CL	21.87	13.47	9.83	45.16	7.76	11.15	48.42	6.70	11.12
PDL	1.83	2.13	0.77	4.73	5.86	9.41	38.76	1.74	7.51
SCIL	0.45	0.89	0.20	1.54	6.47	11.94	29.34	0.75	7.22
SCID	0.01	0.01	0.001	0.02	5.52	7.81	50.00	0.15	8.05
NBP	0.02	2.75	2.50	5.28	0.61	8.88	0.47	0.02	0.09
NSP	355.69	1250.77	4862.50	6468.96	4.25	18.14	5.50	9.11	2.06
ABM	868277.00	245820.20	1319877.70	2433974.90	13.83	23.16	35.67	1146.48	17.02
GY	9113.06	24598.46	136532.85	170244.36	6.10	26.36	5.35	45.50	2.91
HI	2.05	4.72	12.46	19.23	5.97	18.29	10.63	0.96	4.01
LI	8.98	11.94	35.33	56.24	4.88	12.22	15.96	2.47	4.02

$\sigma^2_g$ - genotypic variance,  $\sigma^2_e$ - environmental variance,  $\sigma^2_{gl}$ - Genotypic by location interaction variance,  $\sigma^2_p$ - phenotypic variance(, GCV- genotypic coefficients of variation(%), PCV- phenotypic coefficients of variation (%),  $H^2$ - Broad sense heritability (%), GA – genetic advance and GAM- Genetic advances as percent of means(%). DH= days to heading, DM=days to maturity, GFP=grain filling period, PH=plant height, PL=panicle length, CL=culm length, PDL=peduncle length, SCIL= second culm internode length, SCID=second culm internode diameter, NBP= no of branches per panicle, NSP= no. of spikelets per panicle, ABM= above ground biomass (kg/ha), GY=grain yield (kg/ha), HI=harvest index, LI=lodging index.

## 4.5 Association of Traits

The phenotypic and genotypic correlations of the different traits combined over the locations were presented in (Table 9).

### 4.5.1 Grain yield association with other traits

At genotypic level, grain yield showed highly significant ( $p \leq 0.01$ ) positive correlation with above ground biomass ( $r=0.87$ ), days to maturity (0.57), plant height (0.47), culm length (0.43), second culm internode length (0.43), panicle length (0.40) and grain filling period (0.36). It also showed significant ( $p \leq 0.05$ ) positive correlation with harvest index (0.31). These indicate that all the traits governed by additive gene action and these findings in line with (Habte et al., 2017).

Similarly, Solomon (2009) reported positive genotypic correlation of grain yield with the majority of the traits tested, while no negative correlations were been recorded for grain yield with all traits tested. The positive correlation could be due to linkage or pleiotropic genetic effects

causing the traits to change in the same direction (Falconer and Mackay, 1996). However, lodging index and other traits such as days to heading, peduncle length, second culm internode diameter, number of branches per panicle and number of spikelets per panicle were haven't correlated with grain yield statically in this study. The breeding implications of positive significant association provide that improvement for one trait could improve the others because they governed by one gene.

At phenotypic level, grain yield (0.25) positively correlated with above ground biomass (0.66), harvest index (0.39), grain filling period (0.30) culm length (0.27), plant height (0.26), days to maturity (0.23), panicle length (0.14) and lodging index (0.20) corresponding to (Habte *et al.*, 2017). The remained traits have no correlation with grain yield.

#### **4.5.2 Lodging index association with other traits**

Lodging index had positive phenotypic coefficient of correlation with days to heading, days to maturity, grain filling period, plant height, grain yield, and harvest index and negatively correlated with panicle length, and second culm internode length. However, it did not show significant correlation with the rest of the traits. The negatively correlated traits indicate that the pleiotropic effects of one gene on the other. This improved by selecting other secondary traits to improve that trait indirectly. Phenotypic positive association of lodging index with phenological traits depicted in the current result

revealed that the shorter time to heading, maturity and grain filling might help to reduce lodging of tefas well as the longer the time to give higher grain may causes higher lodging in line with the results of (Hailu *etal*,2001; Habte *et al*,2015 andNigus *et al*, 2016).

Dagnachew and Girma (2014)also reported that there was a positive phenotypic association of harvest index with grain yield but a negative association with biomass yield in tef landraces collected from different zones of Ethiopia. The current study results contradict with the previous findings of Nigus *et al*. (2016), who reported that high harvest index have high grain yield and high grain yield in turn correlated negatively with high lodging index. However, the present result showed lodging correlated positively with harvest index as well as grain yield.This indicated that the high yielders are likely vulnerable to lodging and *vice versa*.

#### **4.5.3 Phenological traits association with other traits**

In the case of phenological traits association with others, days to heading showed significant positive genotypic association with days to maturity, plant height, panicle,second culm internode diameter and aboveground biomass. On the other hand, days to heading showed significant negative genotypic associationwith grain filling period and lodging index, while no significant with the rest traits including grain yield, similar to (Habte *etal*, 2017). Days to maturity also exhibited positive genotypic correlation with grain yield,days to heading, aboveground biomass, plant height, panicle

length, culm length and second culm internode diameters, while not correlated with the remaining traits.

#### **4.5.4 Morphological traits association with other traits**

Most of the morphological traits such as showed positive phenotypic associations with grain yield, above ground biomass but negative correlations with harvest and lodging index as well as phenological traits, while not correlated with some of the traits. This finding corresponding to previous study of (Habte *et al.* 2017). At genotypic level plant height, panicle length, culm length, second basal culm internode length and diameter showed positive correlation coefficients with grain yield, above ground biomass and phenological traits, while not correlated with other traits such as harvest index and lodging index (Ayalneh *et al.*, 2012). Similar to present findings Fufa *et al.* (2000) reported positive correlation of shoot biomass with plant height, panicle length; while Solomon *et al.* (2010) also reported that above ground plant biomass was strongly correlated with grain yield, plant height and panicle length.

For traits with highly significant and negative association, the improvement of one trait would result in the reduction of another trait. Interestingly, this may enhance low above ground biomass and reduce lodging, which had reported as important traits of semi-dwarf varieties of small cereals such as tef, wheat, barley and rice (Wondewosen *et al.*, 2012).

Relatively tall tef varieties are desired by farmers because of tef is highly valued for its straw yield as a major source of animal feed (Yami, 2013). However, tall tef varieties have relatively thin stems and shallow root system that are sensitive to lodging (Van Delden *et al.*, 2010). Late maturing and tall tef varieties possess deeper root systems than early maturing genotypes that have shorter plant heights (Ayele *et al.*, 1999). Therefore, breeding tef varieties with a good stem thickness and improved root depth could offer high adoption rate of tef varieties by farmers than breeding dwarf varieties to reduce lodging. However, tef genotypes with long days to maturity have tall plant height and longer panicle, consequently, more photosynthetic



products is not used for the seed setting. As a result, development of considerably semi-dwarf tef varieties have been the goal of tef breeding to enhance grain yield and reduce effect of lodging without affecting multipurpose income of tef producers (Esfeld and Tadele, 2010; Kebebew *et al.*, 2011).

Generally, correlation may arise from different factors of gene action (additive or non-additive) and the other factors such as pleiotropy expresses the extent to which two traits are influenced by the same gene, but the correlation resulting from pleiotropy is the overall effect of all segregating genes that affect both traits some genes may increase both traits, while others increase one and reduce the other; the former tend to cause a positive correlation while, the later a negative correlation (Welsh, 2008).

Table 9 Genotypic (below) and phenotypic (above) diagonal correlation coefficients of the 15 traits in 49 semi-dwarfs tef recombinant inbred lines combined over the two locations

Traits	DH	DM	GFP	PH	PL	CL	PDL	SCIL	SCID	NBP	NSP	ABM	GY	HI	LI
DH		0.83**	0.50**	-0.38**	-0.46**	-0.23**	-0.03 ns	-0.60**	0.03 ns	0.07 ns	-0.03 ns	-0.33**	0.08 ns	0.48**	0.16 *
DM	0.40**		0.90**	-0.36**	-0.52**	-0.17*	0.07 ns	-0.62**	-0.07 ns	-0.01 ns	-0.09 ns	-0.29**	0.23**	0.62**	0.25**
GFP	-0.41**	0.67**		-0.27**	-0.44**	-0.08 ns	0.13 ns	-0.49**	-0.12 ns	-0.07 ns	-0.11 ns	-0.19**	0.30**	0.59**	0.25**
PH	0.33**	0.53**	0.26**		0.77**	0.90**	0.23**	0.68**	0.35**	0.12 ns	0.11 ns	0.63**	0.26**	-0.47**	-0.18**
PL	0.41**	0.48**	0.15**	0.80**		0.43**	0.05 ns	0.66**	0.45**	0.17**	0.19**	0.56**	0.14*	-0.53**	-0.22**
CL	0.23 ns	0.47**	0.28*	0.94**	0.56**		0.29**	0.53**	0.19**	0.06 ns	0.02 ns	0.52**	0.27**	-0.31**	-0.10 ns
PDL	-0.27 ns	-0.03 ns	0.19ns	0.44**	0.16 ns	0.53**		0.09 ns	-0.03 ns	0.19**	-0.09 ns	0.03 ns	0.10 ns	0.08 ns	-0.02 ns
SCIL	0.16 ns	0.22 ns	0.09ns	0.61**	0.49**	0.58**	0.30*		0.24**	0.17*	0.21**	0.52**	0.12 ns	-0.52**	-0.19**
SCID	0.52**	0.44**	0.01ns	0.48**	0.50**	0.39**	0.01 ns	0.34**		0.11 ns	0.29**	0.20**	0.04 ns	-0.22**	-0.08ns
NBP	0.09 ns	-0.13 ns	-0.20 n	0.23 ns	0.30*	0.16 ns	0.08 ns	0.41**	0.15 ns		0.19**	-0.07 ns	-0.01 ns	0.04 ns	0.04 ns
NSP	0.17 ns	0.04 ns	-0.10 ns	0.01 ns	0.05 ns	-0.02 ns	-0.13 ns	0.20 ns	0.27 ns	0.08 ns		0.08 ns	0.01ns	-0.13 ns	0.11 ns
ABM	0.36**	0.59**	0.30*	0.62**	0.45**	0.61**	0.16 ns	0.43**	0.24 ns	-0.04 ns	0.07 ns		0.66**	-0.41**	-0.04 ns
GY	0.26 ns	0.57**	0.36**	0.47**	0.40**	0.43**	0.10 ns	0.43**	0.19 ns	0.01 ns	0.07 ns	0.87**		0.39**	0.20**
HI	-0.23 ns	-0.03 ns	0.15 ns	-0.28*	-0.10 ns	-0.33**	-0.07 ns	0.01 ns	-0.10 ns	0.08 ns	-0.05 ns	-0.16 ns	0.31*		0.29**
LI	-0.27*	-0.13 ns	0.09 ns	-0.17 ns	-0.14 ns	-0.16 ns	-0.06 ns	0.09 ns	-0.13 ns	0.05 ns	0.23 ns	0.13 ns	0.23 ns	0.30*	

\*, \*\* Significant at  $p \leq 0.05$ , and  $p \leq 0.01$ , respectively and ns- non-significant among the traits, DH= days to heading, DM= days to maturity, GFP= grain filling period, PH= plant height, PL= panicle length, CL= culm length, PDL=peduncle length, SCIL= second culm internode length, SCID= second culm internode diameter, NBP= no of branches per panicle, NSP= no of spikelets per panicle, ABM= above ground biomass (kg/ha), GY=grain yield(kg/ha), HI=harvest index(%), LI=lodging index.

## **4.6 Path Coefficient Analysis**

Path coefficient analysis measures the direct influence of one variable upon the other, and permits separation of correlation coefficients into components of direct and indirect effects. Path analysis allows identification of direct and indirect effects association and measure the relative importance of each trait. Combined over locations, the phenotypic and genotypic correlations were partitioned into direct and indirect effects using grain yield as a dependent variable as shown in Tables 10 and 11, respectively for genotypic and phenotypic path coefficient correlations. Only those traits having significant correlations with grain yield were included in the path coefficient analyses at each of the genotypic and phenotypic level.

### **4.6.1 Phenotypic path coefficient analysis**

At phenotypic level, aboveground biomass (0.965) had highest positive direct effect on grain yield followed by harvest index (0.777) and days to maturity (0.096) (Table 10). This showed that the strong correlations of above ground biomass yield and harvest index with grain yield were largely due to the additive gene effect of the traits. Therefore, direct selection of the high performing genotypes for these traits will improve the mean grain yield of the selected genotypes. Aboveground biomass as well as harvest index and days to maturity can be considered as good contributors to grain yield and suggesting important traits for selection in a breeding program for higher grain yield of tef. However, traits with negative indirect effect through above ground biomass yield need to be managed during selection because the selection of traits might have reducing effect on yield, this finding also agree with (Nigus *et al.*, 2016).

Similarly, Abel *et al.* (2012) as well as Dagnachew and Girma (2014) found highest direct effect on grain yield of harvest index and above ground biomass. Habtamu *et al.* (2011) findings also showed that biomass had the higher direct effect on grain yield. The residual factor for phenotypic level was 0.192 thus indicating that the traits included in the analysis explained 80.8% of the total variation in the grain yield per hectare whereas; the remaining 19.2% was out of the path. The maximum value of residual factor in phenotypic path analysis indicates that higher environmental factor influence on grain yield at phenotypic level rather at genotypic level.

Table 10 Estimate of direct (bold/diagonal) and indirect (off diagonal) correlation of grain yield with eight yield correlated traits at phenotypic level.

Traits	DM	GFP	PH	PL	SCIL	ABM	HI	LI	GY <sub>p</sub>
DM	<b>0.096</b>	-0.024	0.005	-0.009	-0.042	-0.280	0.482	0.003	0.23**
GFP	0.086	<b>-0.027</b>	0.003	-0.007	-0.034	-0.183	0.459	0.003	0.3**
PH	-0.034	0.007	<b>-0.013</b>	0.013	0.047	0.608	-0.365	-0.002	0.26**
PL	-0.050	0.012	-0.010	<b>0.017</b>	0.045	0.540	-0.412	-0.002	0.14*
SCIL	-0.059	0.013	-0.009	0.011	<b>0.068</b>	0.502	-0.404	-0.002	0.12ns
ABM	-0.028	0.005	-0.008	0.009	0.036	<b>0.965</b>	-0.319	0.000	0.66**
HI	0.059	-0.016	0.006	-0.009	-0.036	-0.396	<b>0.777</b>	0.003	0.39**
LI	0.024	-0.007	0.002	-0.004	-0.013	-0.039	0.225	<b>0.010</b>	0.2**

Residual effect = 0.192 for dependent variable.

When \*\*=represents highly significance genotypic correlation coefficient of grain yield with all traits at ( $P \leq 0.01$ ) except two traits. DM-Days to Maturity (days); GFP- Grain Filling Period (days); PH- Plant Height(cm); PL-Panicle Length(cm); SCL-Second culm internode length(cm); ABM-Above ground biomass kg per hectare and HI- Harvest Index(%), Lodging index(%) and GY<sub>p</sub> – Grain yield of phenotypic correlation coefficient.

#### 4.6.2 Genotypic path coefficient analysis

Under genotypic path coefficient, above ground biomass yield had the highest positive direct effect on grain yield followed by harvest index, 0.908 and 0.455 (Table 11). The residual factor for tef at genotypic level was 0.183 implying that the characters included in the path analysis explained 81.7% of the total variation in grain yield; while, the remaining 18.3% was contributed by other factors not included in the path analysis. Therefore, selection for these characters would give good responses to yield improvement. Corresponding to current results, Habtamu *et al.* (2011) reported biomass yield and harvest index for their highest direct effect and their correlation with grain yield of tef landraces. The authors suggested that selecting for these traits indirectly selects for grain yield. Similarly, Abel *et al.* (2012) and Ayalneh *et al.* (2012) also reported that harvest index and biomass yield had a strong direct effect and positive correlation with grain yield in tef.

However, plant height (0.112, days to maturity (0.059) and second culm internode length (0.031) had weak positive direct effect on the grain yield (Table 11). Conversely, grain filling period, culm length and panicle length showed weak negative direct effects of (-0.019, -0.038 and -0.098) on grain yield trait of tef. This indicates that late maturity tends to decrease grain yield performance by increasing only the above ground biomass or vegetative parts. Similarly, Sintayehu and Getachew (2011) found better grain yield performance of early maturing recombinant inbred lines than late maturing types in a moisture stressed environment.

In addition to its direct effect, days to maturity showed relatively strong negative indirect effects via grain filling period, plant height, panicle length, number of productive tillers per plant. Generally, the genotypic path analysis indicated that selection for high above ground biomass yield, harvest index and long maturity could provide increased grain yield as the result of this study revealed. While in moisture stressed environments, yield improvement can be achieved through selection for reduced days to maturity, high biomass yield and harvest index as reported by (Nigus *et al.*, 2016).

Table 11 Estimates of direct (bold/diagonal) and indirect (off diagonal) effects at genotypic level in semi-dwarf recombinant inbred lines of tef.

Traits	DM	GFP	PH	PL	CL	SCIL	ABM	HI	GY <sub>gr</sub>
DM	<b>0.059</b>	-0.013	0.059	-0.018	-0.046	0.007	0.536	-0.014	<b>0.57**</b>
GFP	0.040	<b>-0.019</b>	0.029	-0.006	-0.027	0.003	0.272	0.068	<b>0.36**</b>
PH	0.031	-0.005	<b>0.112</b>	-0.030	-0.092	0.019	0.563	-0.127	<b>0.47**</b>
PL	0.028	-0.003	0.090	<b>-0.038</b>	-0.055	0.015	0.409	-0.046	<b>0.40**</b>
CL	0.028	-0.005	0.105	-0.021	<b>-0.098</b>	0.018	0.554	-0.150	<b>0.43**</b>
SCL	0.013	-0.002	0.068	-0.019	-0.057	<b>0.031</b>	0.390	0.005	<b>0.43**</b>
ABM	0.035	-0.006	0.069	-0.017	-0.060	0.013	<b>0.908</b>	-0.073	<b>0.87**</b>
HI	-0.002	-0.003	-0.031	0.004	0.032	0.001	-0.145	<b>0.455</b>	<b>0.31**</b>

Residual effect = 0.183 for dependent variable.

\*\*= highly significant genotypic correlation coefficient of grain yield with all traits at ( $P \leq 0.01$ ). DM-days to maturity, GFP- grain filling period, PH- plant height, PL- panicle Length CL- culm length, SCL- second culm internode length, ABM- above ground biomass kg per hectare and HI- harvest index and GY<sub>gr</sub> – Grain yield of genotypic correlation coefficient.

#### 4.7 Cluster Analysis and Inter-Cluster Distance Analysis

Cluster analysis grouped the 49 semi-dwarf tef recombinant inbred lines into four clusters based on the pooled mean values (Appendix Table III). The dendrogram showed their similarity by using SAS version 9.3 average linkage clustering methods (Fig. 1). The numbers of recombinant inbred lines in each cluster varied from nineteen in cluster one; fifteen in cluster two, thirteen in cluster three and only two in the last cluster four (Appendix Table II). The different recombinant inbred lines grouped with in each clusters assumed more closely related in terms of the studied traits than those recombinant inbred lines grouped into different clusters as suggested by (Singh *et al.*, 2006; Pandey *et al.*, 2017).

Cluster four had higher mean values for days to heading, days to maturity, grain filling period, plant height, panicle length, culm length, second basal culm internode length, above ground biomass, grain yield and harvest index when compared to the other clusters. In contrast to this, cluster two consisted of inbred lines, which had the lower values for traits such as days to

maturity, grain filling period, plant height, culm length, peduncle length; second basal culm internode length, above ground biomass, grain yield and lodging index (Table 12). Recombinant inbred lines in cluster two were the earliest, the shortest in plant height, culm length, and second culm internode lengths and peduncle length and the least yielding ones in grain and biomass.

The current cluster analysis indicated that the variability presented in these recombinant inbred lines were similar to earlier studies of Habte (2008), who grouped 21 tef varieties and landraces into four clusters and that of three clusters reported by Costanza *et al* (1979) using 39 accessions. It is also in agreement with Ebba (1975) who reported six major clusters from 35 cultivars, Kebebew *et al* (2001) six main clusters at 75% similarity from 36 tef germplasm populations and other groups (Habte *et al.*, 2015; Kebebew *et al.*, 2003b; Melak -Haile *et al.*, 1965).

Table 12 Mean values for traits of the four clusters of tef recombinant inbred lines evaluated at HARC and DZARC.

Traits	Clusters				Means
	I	II	III	IV	
Days to heading	52.12	54.13	56.62	58.50	55.34
Days to maturity	104.64	104.62	109.73	113.38	108.09
Grain filling period (days)	52.53	50.48	53.12	54.88	52.75
Plant height (cm)	96.36	90.07	101.35	109.05	99.21
Panicle length (cm)	34.85	35.18	37.83	41.10	37.24
Culm length (cm)	61.51	54.89	63.53	67.95	61.97
Peduncle Length (cm)	24.36	21.95	22.53	23.76	23.15
Second culm internode length(cm)	10.51	9.79	10.89	10.88	10.52
Second culm internode diameter (mm)	1.77	1.80	1.84	2.00	1.85
No of Branches per main panicle	25.82	25.84	25.87	24.70	25.56

No Spikelets per main panicles	435.07	440.95	460.29	428.18	441.12
Above ground biomass (kg/ha)	6702.1	5091.23	8238.26	9670.18	7425.44
Grain yield (kg/ha)	1540.52	1236.75	1850.15	2406.71	1758.53
Harvest index (%)	24.06	24.75	22.82	24.81	24.11
Lodging index	63.21	58.8	61.98	58.88	60.72

All the recombinant inbred lines were grouped into four clusters by estimating genetic divergence of fifteen quantitative traits using the hierarchical Euclidean cluster analysis which showed the highest and significant inter-cluster distance between cluster IV and cluster II ( $D^2 = 108.87$ ) followed by cluster III and II ( $D^2 = 52.9$ ) and then cluster IV and I ( $D^2 = 52.77$ ) (Table 13). The genotypes having high mean performance for grain yield per plant and several other yield components were found to be concentrated in cluster IV and III which merit showed due consideration for selection of parents (Singhet *al.*, 2006).

Thus, crosses between promising lines belonging to cluster pair having higher inter-cluster distances may be attempted for isolating transgressive segregants as these cluster pair were also separated by high inter-cluster distances. This indicated existence of high degree of genetic variability in the tef semi-dwarf recombinant inbred lines. Therefore, these materials may serve as valuable source for selecting diverse parents for use in hybridization programme. The minimum and non-significant inter-cluster distances were found between cluster III and I ( $D^2 = 13.93$ ) followed by IV and III ( $D^2 = 20.67$ ) as well as II and I ( $D^2 = 21.75$ ) which indicating that the genotypes in these two clusters were relatively close to each other (Pandey *et al.*, 2017).

Table 13 Pair wise generalized squared distances ( $D^2$ ) values between clusters constituting 49 semi-dwarf tef recombinant inbred lines

Clusters	I	II	III	IV
I	0			
II	21.75 ns	0		
III	13.93 ns	52.90**	0	
IV	52.77**	108.87**	20.67 ns	0

\*Significant at  $p < 0.05$  for  $\chi^2 = 23.68$ ; \*\* significant at  $p < 0.01$  for  $\chi^2 = 29.14$  and ns = non-significant



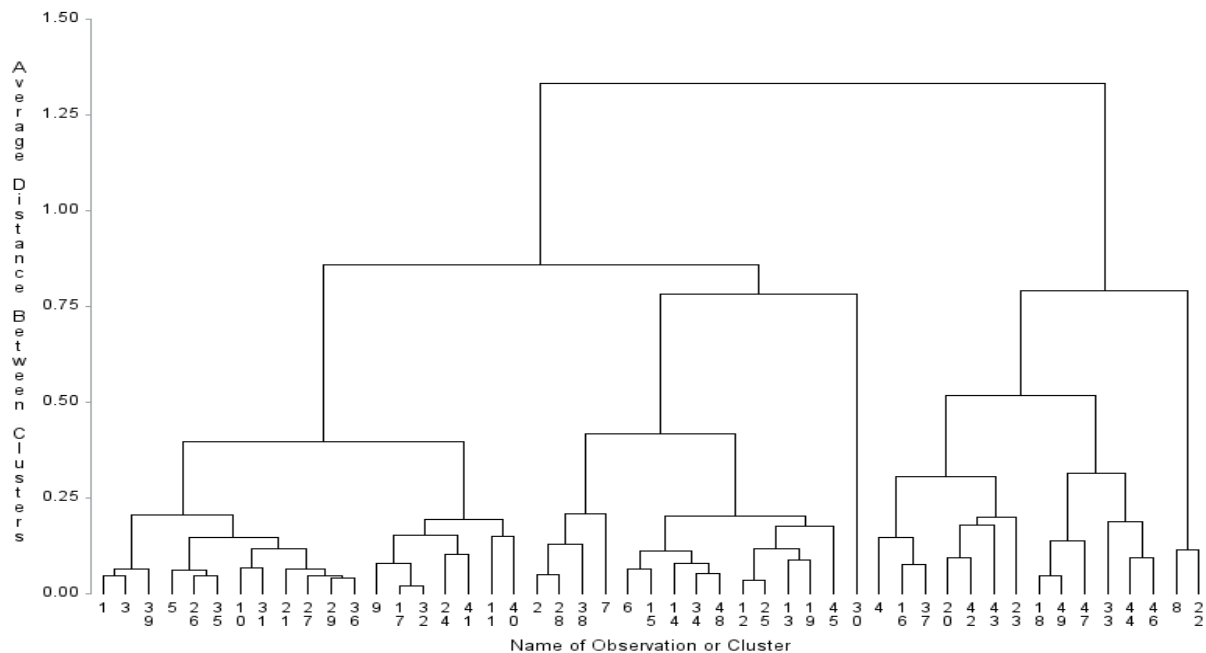


Figure 1. Dendrogram showing relationship among 49 semi-dwarf tef recombinant inbred lines based on average linkage and Euclidean distance using the mean of 15 quantitative traits

#### 4.8 Principal Component Analysis.

In the principal component analysis (PCA), to estimate the relative contribution of traits towards the variation in the 49 tef recombinant inbred lines, 77.6% explained by the first five PCs with eigenvalues greater than one out of the fifteen PCs employed for all the 15 traits. Therefore, five PCs retained to explain the observed variation without losing a substantial variability explained (Table 13).

The first PC explained about 34%, the second 14%, and the third 11.7%, the fourth 10.9% and the fifth 6.9% of the variation. Plant height, culm length, panicle length, aboveground biomass, days to maturity and grain yield showed greater loadings in the first PC. Similarly, grain filling period, days to heading, harvest index, lodging index and grain yield contributed in the second PC; while peduncle length, days to heading and number of spikelet per panicle were displayed significant load in the third PC. In the fourth PC, number of branches per panicle,

lodging index, second basal culm internode length and days to maturity were chief contributors, while in the fifth PC number of spikelets per panicle, harvest index, number of branches per panicle and lodging index accounted for much of the observed gross variation.

The percentage contribution of the first five principal components to gross genetic variation obtained in the current study (77.6%) is slightly different from Kebebew *et al* (2003) 81% and Temsgenet *et al* (2005) 80.6%, while it is far greater than Kebebew *et al* (1999) 71%. This indicates that the variation depends on the type of material used in the study. There was a sharp decline in contribution from PC1 to PC2 and then from PC2 to PC3 in that order while the rate of decrease in contribution became lower and lower for the remaining PCs. This shows that the first few principal components had the greatest contribution to the overall variation in the recombinant inbred lines and for the 15 traits considered in this study.

Generally, the contribution of PC1 (34%) obtained in this study is somewhat in line with Kebebew *et al.* (2003) 40% while, it is relatively higher than Kebebew *et al.* (1999) 28% and slightly lower than Temsgenet *et al.* (2005) 55%.

Table 14 Eigenvectors, eigenvalues and percentage of total variance explained by the first six principal components (PC) for 16 traits in 49 tef recombinant inbred lines.

<b>Traits</b>	<b>PC1</b>	<b>PC2</b>	<b>PC3</b>	<b>PC4</b>	<b>PC5</b>
Days to Heading (days)	0.200	-0.428	0.354	0.157	-0.063

Days to Maturity(days)	0.317	0.166	0.215	0.349	-0.131
Grain Filling Period(days)	0.154	0.511	-0.071	0.221	-0.080
Plant Height(cm)	0.411	-0.067	-0.212	-0.014	0.002
Panicle length(cm)	0.344	-0.136	-0.009	-0.055	-0.253
Culm Length(cm)	0.380	-0.017	-0.290	0.011	0.144
Peduncle Length(cm)	0.143	0.140	-0.547	-0.150	0.143
Second culm internode length(cm)	0.294	-0.022	-0.072	-0.404	0.019
Second culm internode diameter(mm)	0.256	-0.272	0.188	0.006	-0.103
N <sub>o</sub> of Branches per main panicle	0.088	-0.207	-0.087	-0.529	-0.351
N <sub>o</sub> Spikelets per main panicles	0.049	-0.133	0.338	-0.265	0.527
Above ground biomass (kg/ha)	0.349	0.172	0.160	0.072	0.242
Grain yield (kg/ha)	0.305	0.311	0.285	-0.067	-0.027
Harvest index (%)	-0.074	0.344	0.254	-0.291	-0.539
Lodging index	-0.040	0.325	0.248	-0.414	0.326
Eigenvalue	5.100	2.100	1.750	1.640	1.040
Proportion of variance explained (%)	34.000	14.000	11.700	10.900	6.900
Cumulative variance explained (%)	34.000	48.000	59.700	70.600	77.600

## 5 SUMMARY AND CONCLUSIONS

Studying the extent and pattern of genetic variability among semi-dwarf tef recombinant inbred lines special for lodging and yield related traits is very important. It can also provide valuable information for plant breeders who are interested in introgression of agronomically desirable traits into establish cultivars or to select recombinant inbred lines from the existing diversity for high yield and lodging tolerance. The current experiment carried out on 49 semi-dwarf tef recombinant inbred lines that selected from GA-10-3 X DZ-01-192 crosses of F<sub>7</sub> single seed descent developed inbred lines at DZARC.

The results of this study indicate that highly significant difference among the recombinant inbred lines for all traits evaluated except for number of fertile tiller per plant. Genotypes by locations interactions were highly significant for 10 traits and significant for one trait (panicle length), while the remaining traits showed no significant interaction effects. Averaged over the two test locations results revealed RIL-14, RIL-45, RIL-28 and RIL-41 in these order exhibited mean grain yields of 2.52, 2.29, 2.21 and 2.19 ton per hectare respectively; which were higher than that of the standard check Boset (1.83 ton per hectare) and the local check (2.14 ton per hectare) as well as their parent materials.

Generally, the analysis of variance results showed the presence of considerable variations among the 49-semi-dwarf tef recombinant inbred lines almost for all the traits thereby suggesting higher chance of selecting recombinant inbred lines for traits of interest. The results of analysis of variance allow carrying out further genetic analyses for all traits, except number of fertile tillers per plant, which was not significant. Grain yield showed the maximum PCV (26.36%) followed by above ground biomass (23.16%), while moderate PCV (20 > 10%) estimates were recorded for number of spikelets per main panicle, harvest index, lodging index, second culm internode length, culm length and panicle length. The remaining traits showed low PCV values. Moderate GCV (10-20%) was recorded for above ground biomass while, GCV was low for the rest of the traits.

Two of the traits (i.e. plant height and panicle length) showed high heritability ( $> 60\%$ ), while days to heading, culm length, peduncle length, second culm internode diameter, second culm internode length and above ground biomass showed intermediate heritability estimates. The remaining traits showed low heritability ( $<30\%$ ). Genetic advance as percentage of mean were maximum for above ground biomass ( $>17.02\%$ ) and lower for number of branches per panicle ( $0.09\%$ ). Both genotypic and phenotypic correlation coefficient analyses showed positive association of grain yield with most traits. Lodging index showed positive phenotypic correlation with phenological and agronomical traits except above ground biomass, while not statistically correlated with most morphological traits except plant height, panicle length and second culm internode length, which negatively correlated with lodging.

However, path analysis revealed that effect of above ground biomass on grain yield had high and positive genotypic and phenotypic path coefficient correlation. The rest of the traits showed consistently low positive or negative effect. This indicated that attention should be given for those traits, which have which have positive correlation with grain yield in the process of selection, as these traits are helpful for indirect selection. Trait association among yield components and grain yield with its component in this particular study indicated various magnitude of association, which can be carefully looked into while exploiting in selection to improve traits of interest in tef breeding.

Cluster analysis grouped the recombinant inbred lines into four clusters based on their similarity. The highest inter-cluster distance occurred between clusters two and four while the lowest one was between clusters one and four. Principal components analysis showed that about  $77.6\%$  of the gross variance among recombinant inbred lines laid in  $PC_1$  to  $PC_5$  and the total variance loaded largely by traits like plant height, panicle length and days to maturity.

Generally, genetic variability got supreme importance to the breeders, as it is prerequisite for any improvement in crop plants and identification of superior recombinant inbred lines. This study also revealed that four recombinant inbred lines from the studied recombinant inbred lines had

higher yield than local and standard checks. There were differences in the performance of the recombinant inbred lines as there were statistically significant differences among recombinant inbred lines for most of the traits studied at both locations.

However, the level of genetic variations for many traits including grain yield might be not sufficient to expect progress in selection and showed moderate to low genetic coefficient of variation that made improvement through selection a difficult task. Aboveground biomass showed maximum genetic advance as percent of mean, as well as positive direct effect correlation compared to other traits. Hence, it will be a useful trait for indirect selection to increase grain yield, even though negatively correlated with harvest index. Plant height and panicle length showed high heritability, relatively better genetic advance as percent of mean and positive correlation coefficient and direct effect on grain yield. This implies that these characters may be included as a component of indirect selection.

To this end, the results revealed the existence of considerable variations for most traits of the test inbred lines, thus indicating the possibility of exploiting the variability in further tef breeding. Thus, recombinant inbred lines like RIL-14 have significantly low lodging index, longer panicle, higher number of spikelets per panicle, as well as the highest above ground biomass and grain yield. Genotypes identified with better grain yield related traits and reasonable lodging tolerance require further evaluation and eventual release to the farming communities in tef growing environments in Ethiopia.

Finally, since it is one season experiment, the research needs additional two or more years across wide range of environments to arrive at concrete recommendations for maximization of tef yields. Molecular marker assisted selection in combination with field evaluation of this semi-dwarf tef inbred recombinant inbred lines traits based on conventional breeding under different environmental conditions also comprehensively recommended.

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## **7 APPENDICES**

Appendix Table I. Mean monthly rainfall and temperature during cropping season at both locations of the experiments

Locations	Weather parameters		Months of the cropping season						Means
			July	Aug	Sept	Oct	Nov	Dec	
Holeta	Rain Fall (mm)		172.8	311.4	244	29	0	0	126.2
	Temp.°C	max	22.1	21.7	22.6	24.2	24	23.1	23.1
		Min	8.8	10.4	8.3	7.8	2.8	6.5	6.5
Debre Zeit	Rain Fall (mm)		262.3	200.2	115.2	19	0	0	99.5
	Temp.°C	max	23.9	21.8	24.5	26.5	26.2	24.9	24.9
		Min	14.6	14.3	14	11.1	8.3	11.6	11.6

Source: DZARC and HARC (2017)

Appendix Table II. Clustering of 49 semi-dwarf tef recombinant inbred lines into four cluster using mean of 15 traits

Clusters	NoRIL <sub>s</sub>	RIL <sub>s</sub> exist under each clusters								Source	
C1	19	RIL <sub>s</sub> NO-1,	4,	6,	15,	16,	19,27,	44,	52,	F <sub>7</sub> of DZ-01-192 x GA-10-3	
		58,	68,	160,	162,	166,	172,	174,	179,		
									180, and 182		
C2	15	RIL <sub>s</sub> NO-2,8,12,20,21,22,24,33,57,75,161,171,178,262 and	GA-							F <sub>7</sub> of DZ-01-192 x GA-10-3	
			10-3								
C3	13	RIL <sub>s</sub> NO-5,25,28,41,48,169,175,185,195, 203 and	Local check, DZ-Cr-				F <sub>7</sub> of DZ-01-192 x GA-10-3 and				
			192, Boset				Checks				
C4	2	RIL <sub>s</sub> NO-14 and 45								F <sub>7</sub> of DZ-01-192 x GA-10-3	

C-represent cluster numbers, RIL<sub>s</sub>-recombinant inbred lines, DZ-Cr-192 and Ga-10-3-parental lines, Boset is Standard check and Local check is the farmer cultivar for respective locations. All materials were taken from DZARC.

Appendix Table III. Mean performance of fifteen traits of 49 semi-dwarf tefrecombinant inbred lines evaluated over two locations.

RILs	DH	DM	GFP	PH	PL	CML	PDL	SCL	SCD	BP	SP	SBM	GY	HI	Li
<b>RIL # 1</b>	44.0n	105.5a-m	61.5a	93.7i-o	35.9a-n	57.8e-l	23.4a-h	11.5a-e	1.7f-h	24.3e-i	408.6h-o	7165.9e-i	1888.8c-k	27.1a-d	67.5a-c
<b>RIL # 2</b>	52.0g-m	104.8f-m	52.8c-k	75.0s	29.5p	45.5m	17.9l	8.5f-h	1.8b-g	25.4b-i	493.7a-h	4742.0op	1059.3t-v	23.2d-j	66.0a-d
<b>RIL # 4</b>	49.3m	102.8j-m	53.5b-k	93.8i-o	33.9g-o	59.9c-k	23.4a-h	11.1a-e	1.6gh	25.4b-i	450.8c-l	7154.0e-i	1806.2e-m	26.4a-f	61.3a-g
<b>RIL # 5</b>	52.0g-m	104.5f-m	52.5c-k	89.2n-q	34.0g-o	55.2j-l	21.8e-k	10.5a-g	1.7f-h	24.1f-i	518.2a-d	7436.1d-h	1521.8k-r	20.9g-k	67.5a-c
<b>RIL # 6</b>	51.8h-m	105.8e-m	54.0a-k	100.6c-k	37.4d-i	63.3a-j	23.2b-h	11.1a-e	1.9b-g	29.2ab	475.2a-k	6790.5f-k	1557.8i-o	24.6a-i	68.8a
<b>RIL # 8</b>	57.5cd	106.8c-m	49.3g-m	110.6ab	41.6bc	69.1ab	22.5c-i	11.1a-e	1.9a-f	28.2a-f	403.7i-p	5434.4m-p	1309.4l-r	24.5a-i	47.0jk
<b>RIL # 12</b>	55.8c-g	102.0lm	46.3k-m	78.1rs	31.5op	46.6m	19.3i-l	7.7h	1.8c-g	24.7c-i	377.6l-q	4332.0pq	1138.2r-v	27.0a-e	58.8d-h
<b>RIL # 14</b>	62.5a	115.5a	53.0c-k	116.0a	46.5a	69.5a	24.0a-h	11.1a-e	2.1ab	24.5a-i	438.3d-l	9671.4a	2523.7a	26.1a-g	54.0f-j
<b>RIL # 15</b>	50.5k-m	101.8lm	51.3e-m	98.3d-n	34.7f-o	63.6a-i	23.7a-h	11.0a-e	1.9b-g	27.2a-g	475.4a-k	6091.9i-n	1335.5o-u	21.5f-k	60.0b-g
<b>RIL # 16</b>	53.5e-k	103.5h-m	50.0f-m	96.4g-o	34.1g-o	62.3a-j	24.9a-k	9.2a-h	1.8b-g	24.2f-i	343.0m-q	6914.4e-j	1446.1m-s	22.3d-k	63.0a-e
<b>RIL # 19</b>	52.3f-m	109.5a-l	57.3a-f	99.3d-l	32.9j-p	66.4a-f	23.7a-h	10.1a-g	1.7c-g	22.5i	331.8o-q	6160.6i-n	1067.6s-v	17.9k	39.5l
<b>RIL # 20</b>	54.5d-j	103.3i-m	48.8i-m	92.9j-o	35.6e-o	57.3j-l	24.9a-f	10.7a-f	1.9b-g	26.0a-i	488.8a-i	5166.0n-p	1212.6q-u	25.3a-h	60.5a-g
<b>RIL # 21</b>	55.8c-g	104.0g-m	48.3i-m	82.2q-s	32.5n-p	49.7lm	20.6h-l	7.7h	1.7d-h	23.0hi	450.7c-l	5409.8m-p	1146.8r-v	21.5f-k	62.0a-f
<b>RIL # 22</b>	52.8f-m	105.8a-m	53.0c-k	93.6i-o	35.3e-o	58.3d-l	23.9a-h	9.4e-h	1.7f-h	25.4b-i	489.3a-i	5733.0j-o	1371.4n-u	23.9b-j	51.5h-k

Appendix Table III.(Continued)

<b>RIL<sub>s</sub></b>	<b>DH</b>	<b>DM</b>	<b>GFP</b>	<b>PH</b>	<b>PL</b>	<b>CML</b>	<b>PDL</b>	<b>SCL</b>	<b>SCD</b>	<b>BP</b>	<b>SP</b>	<b>SBM</b>	<b>GY</b>	<b>HI</b>	<b>Li</b>
<b>RIL # 24</b>	56.8c-e	106.0d-m	49.3g-m	97.4g-o	38.6b-f	58.8d-k	20.4h-l	11.8a-d	1.8c-g	28.7a-d	485.7a-j	5537.0l-o	1309.9o-u	23.7c-j	60.8a-g
<b>RIL # 25</b>	58.8bc	103.3i-m	44.5lm	101.6c-j	39.5b-e	62.1a-j	20.4h-l	11.0a-e	1.9b-g	26.6a-i	433.7d-l	7653.2d-g	1491.5l-r	19.7i-k	65.5a-d
<b>RIL # 27</b>	52.8f-m	108.0a-m	55.3a-i	93.4i-o	33.1e-p	60.3b-k	24.3a-g	9.2e-h	1.7e-h	26.1a-i	378.8l-q	6159.8i-n	1431.3m-t	26.1a-g	60.3a-g
<b>RIL # 28</b>	61.5ab	113.5a-d	52.0c-l	106.6b-f	42.0bc	64.6a-i	20.8g-l	11.0a-e	1.9b-g	28.8a-c	394.3k-p	9039.3ab	2208.1a-c	24.4a-i	62.3a-f
<b>RIL # 33</b>	51.0i-m	102.0lm	51.0a-m	97.6g-o	37.0d-k	60.7a-j	22.9b-h	9.4d-h	1.8c-g	26.5a-i	338.5n-q	5264.7m-p	1138.8r-v	23.5c-j	61.8a-f
<b>RIL # 41</b>	54.3d-k	104.0g-m	49.8f-m	98.8d-l	38.8b-f	60.0b-k	22.8b-i	12.2a	1.8b-g	29.6a	398.8j-p	7917.3b-f	2191.9a-d	28.8a-c	64.3a-e
<b>RIL # 44</b>	53.5e-k	103.3i-m	49.8f-m	106.9b-d	40.8b-d	66.1a-g	25.5a-d	10.8a-e	1.8c-g	25.2b-i	503.5a-g	7090.0e-i	1629.6h-p	24.0a-j	61.8a-f
<b>RIL # 45</b>	54.5d-j	111.3a-h	56.8a-h	102.2b-i	35.8e-o	66.4a-f	23.5a-h	10.6a-f	1.9a-f	24.9c-i	418.1g-n	9669.0a	2289.7ab	23.6c-j	63.8a-e
<b>RIL # 48</b>	54.5d-j	106.0d-m	51.5d-m	103.8b-h	37.2d-i	66.7a-e	24.5a-f	10.1a-g	1.7c-h	25.1b-i	534.5a-c	8044.5b-e	1726.7f-n	21.6e-k	56.8e-i
<b>RIL # 52</b>	55.5c-h	108.0a-m	52.5c-k	105.0b-g	36.8d-l	68.2a-c	24.8a-f	10.1a-g	1.9b-g	25.7a-i	462.5a-l	6418.3h-m	1353.0n-u	21.1f-k	68.8a
<b>RIL # 57</b>	55.8c-g	102.8j-m	47.0j-m	95.5h-o	37.9c-h	57.7e-l	25.0a-f	11.2a-e	2.0a-d	26.1a-i	504.8a-g	5167.7n-p	1281.1p-u	25.1a-i	62.8a-e
<b>RIL # 58</b>	50.8j-m	110.0a-k	59.3a-d	97.8f-o	37.1d-j	60.8a-j	24.3a-g	11.1a-e	1.9b-g	27.5a-f	471.5a-k	6690.2g-l	1641.1g-p	24.9a-i	65.5a-d
<b>RIL # 68</b>	51.5i-m	103.3i-m	51.8c-l	98.0e-n	37.3d-i	60.7a-j	26.2ab	9.9a-h	1.7d-h	26.5a-i	494.3a-h	6914.7a-g	1670.1g-o	24.0b-j	64.8a-e
<b>RIL # 75</b>	54.0d-k	102.3k-m	48.3i-m	89.4m-q	32.6l-p	56.8h-l	25.0a-f	10.2a-g	1.8c-g	28.5a-e	428.4a-m	4786.2op	1004.8uv	20.8g-k	44.5kl

<b>RIL # 160</b>	54.8d-i	101.3m	46.5j-m	95.3h-o	32.2n-p	63.1a-j	22.1c-k	10.7a-f	1.7d-h	27.6a-f	548.8a	6992.2e-i	1621.8h-p	22.6d-k	66.3a-d
<b>RIL # 161</b>	49.5lm	103.0j-m	53.5b-k	99.0d-l	37.2d-i	61.8a-j	23.9a-h	10.5a-g	1.8b-g	25.1b-i	377.0l-q	3665.9q	896.4v	24.4a-i	62.3a-f
<b>RIL # 162</b>	52.8f-m	101.8lm	49.0h-m	91.5l-f	35.9e-n	55.7i-l	23.5a-h	9.7b-h	1.8c-g	25.6a-i	420.8f-n	7027.7e-i	1456.9l-r	22.0d-k	63.5a-e

Appendix Table III. (Continued)

<b>RILs</b>	<b>DH</b>	<b>DM</b>	<b>GFP</b>	<b>PH</b>	<b>PL</b>	<b>CML</b>	<b>PDL</b>	<b>SCL</b>	<b>SCD</b>	<b>BP</b>	<b>SP</b>	<b>SBM</b>	<b>GY</b>	<b>HI</b>	<b>Li</b>
<b>RIL # 166</b>	53.5e-k	101.8lm	48.3i-m	91.1l-k	32.9j-p	58.3d-l	25.7a-c	11.3a-e	1.7d-h	28.4a-e	393.9k-p	6161.1i-n	1468.4l-r	26.2a-g	63.5a-e
<b>RIL # 169</b>	62.5a	114.5ab	52.0c-l	108.9a-c	41.5bc	67.4a-d	21.7e-k	10.9a-e	2.1a	24.8c-i	520.9a-d	8542.7b-d	1548.6j-k	19.0jk	50.5i-k
<b>RIL # 171</b>	52.5f-m	112.0a-f	59.5a-c	98.9d-l	38.1c-g	60.8a-j	23.9a-h	10.5a-g	1.8b-g	26.4a-i	399.9j-p	5664.1k-o	1406.5n-t	25.7a-h	61.3a-g
<b>RIL # 171</b>	52.5f-m	112.0a-f	59.5a-c	98.9d-l	38.1c-g	60.8a-j	23.9a-h	10.5a-g	1.8b-g	26.4a-i	399.9j-p	5664.1k-o	1406.5n-t	25.7a-h	61.3a-g
<b>RIL # 172</b>	51.0i-m	102.8j-m	51.8c-l	88.9o-q	31.8n-p	57.2g-l	21.6f-k	9.5c-h	1.8b-g	25.0c-i	449.5c-l	6667.0g-l	1551.4i-k	26.4a-f	67.8a-c
<b>RIL # 174</b>	54.8d-i	104.3f-m	49.5f-m	97.0g-n	34.6f-o	62.4a-j	25.4a-e	12.0ab	1.9b-g	23.2g-i	466.6a-k	6990.1a-i	1637.5g-p	24.0b-j	65.8a-d
<b>RIL # 175</b>	55.5c-h	112.8a-e	57.3a-f	106.8b-a	42.5b	64.4a-i	22.8b-i	11.9a-c	1.9a-f	27.1a-h	501.0a-g	7790.7c-g	1460.7l-r	18.8jk	67.5a-c
<b>RIL # 178</b>	58.8bc	102.5j-m	43.8m	78.1rs	33.6h-p	44.5m	18.8kl	10.3a-g	1.8c-g	25.1b-i	513.1a-e	4669.9o-q	1274.6p-u	27.1a-d	58.5d-h
<b>RIL # 179</b>	52.0g-m	110.3a-j	58.3a-e	99.6d-l	34.8f-o	64.9a-h	25.5a-d	11.5a-e	1.9b-g	25.4b-i	427.1e-m	7092.5e-i	1954.0b-h	29.4a	66.0a-d
<b>RIL # 180</b>	53.0e-l	101.8lm	48.8i-m	92.2k-o	32.5m-p	59.7c-k	27.0a	9.7b-h	1.7d-h	24.5e-i	302.9q	6446.3h-m	1186.8q-v	20.6h-k	59.3c-h
<b>RIL # 182</b>	53.3e-k	103.3i-m	50.0f-m	92.3k-o	33.9g-o	58.4d-l	24.9a-f	10.2a-g	1.7c-g	27.6a-f	461.7b-l	6413.0h-m	1566.0i-q	26.2a-g	68.0ab
<b>RIL # 185</b>	57.5dc	114.3a-c	56.8a-h	97.8f-o	32.7k-p	65.1a-h	21.9d-k	10.9a-e	1.9a-f	24.2f-i	446.3d-l	8062.1b-e	2069.4b-f	25.4a-h	61.0a-g
<b>RIL # 195</b>	53.0e-l	113.8a-c	60.8ab	102.3b-i	36.8d-m	65.6a-h	23.5a-h	10.2a-g	1.9a-f	24.2f-i	545.0ab	7720.4d-g	1924.6b-j	25.9a-h	66.3a-d



<b>RIL # 203</b>	59.0bc	107.3b-m	48.3i-m	96.2g-o	34.1g-o	62.1a-j	23.0b-h	11.3a-e	1.9a-e	25.0c-i	506.5a-f	8422.5b-d	2006.0b-g	24.0a-j	62.3a-f
<b>RIL # 262</b>	51.3i-m	109.5a-k	58.3a-e	79.7rs	35.1f-o	44.6m	21.5f-k	8.2g-h	1.8c-g	24.6d-i	452.8c-l	5223.1n-p	1536.5k-q	29.2ab	61.0a-g
<b>Boset</b>	57.5dc	111.5a-g	54.0a-k	98.8d-l	33.4i-p	65.5a-h	23.1b-h	10.8a-f	1.8c-g	25.8a-i	473.0a-k	8502.2b-d	1830.7d-l	21.7d-k	64.8a-e
<b>DZ-01-192</b>	56.0c-f	110.3a-j	54.3a-j	108.8a-c	41.5bc	67.3a-d	24.4a-g	11.0a-e	2.0a-c	28.1a-f	389.2k-p	8919.1a-c	1927.1b-i	21.9d-k	53.0g-j

Appendix Table III. (Continued)

<b>RILs</b>	<b>DH</b>	<b>DM</b>	<b>GFP</b>	<b>PH</b>	<b>PL</b>	<b>CML</b>	<b>PDL</b>	<b>SCL</b>	<b>SCD</b>	<b>BP</b>	<b>SP</b>	<b>SBM</b>	<b>GY</b>	<b>HI</b>	<b>Li</b>
<b>GA-10-3</b>	54.3d-k	102.8j-m	48.5i-m	83.5p-r	32.0n-p	51.5k-m	18.9j-l	9.8b-h	1.8c-g	24.4a-i	410.5h-o	5572.8l-o	1465.1l-r	26.4a-f	63.5a-e
<b>Local</b>	54.0d-k	111.0a-i	57.0a-g	98.3d-m	38.2c-g	60.2b-k	22.3c-j	9.9a-h	1.5h	23.3g-i	322.7pq	9047.4ab	2145.0b-e	24.7a-i	64.3a-e
<b>Mean</b>	<b>54.2</b>	<b>106.3</b>	<b>52.2</b>	<b>96.3</b>	<b>36.0</b>	<b>60.3</b>	<b>23.1</b>	<b>10.4</b>	<b>1.8</b>	<b>25.8</b>	<b>443.3</b>	<b>6737.7</b>	<b>1565.0</b>	<b>24.0</b>	<b>61.4</b>
<b>CV</b>	<b>4.0</b>	<b>4.2</b>	<b>8.6</b>	<b>5.4</b>	<b>6.8</b>	<b>8.6</b>	<b>8.9</b>	<b>12.8</b>	<b>8.3</b>	<b>9.1</b>	<b>11.3</b>	<b>10.4</b>	<b>14.2</b>	<b>12.8</b>	<b>8.0</b>
<b>Duncan</b>	<b>0.6</b>	<b>1.3</b>	<b>1.3</b>	<b>1.5</b>	<b>0.7</b>	<b>1.5</b>	<b>0.6</b>	<b>0.4</b>	<b>0.0</b>	<b>0.7</b>	<b>14.2</b>	<b>199.2</b>	<b>63.0</b>	<b>0.9</b>	<b>1.4</b>

\*Means with the same letter are not significantly different, DH= days to heading, DM= days to maturity, GFP= grain filling period, PH= plant height, PL= panicle length, CL= culm length, PDL=peduncle length, SCIL= second culm internode length, SCID= second culm internode diameter, NBP= no of branches per panicle, NSP= no of spikelets per panicle, ABM= above ground biomass (kg/ha), GY=grain yield(kg/ha), HI=harvest index(%), LI=lodging index, CV = coefficient of variation (%) and RIL<sub>s</sub>= Recombinant inbred lines.